MilliporeSigma

Race through your Separations on Any System

Chromolith®

Monolithic Silica HPLC Columns

The cost and time saving solution

Supelco®
Analytical Products

Table of Contents

Introducing monolithic silica technology

Revolutionary, monolithic silica for rapid and robust separations

To truly accelerate chromatographic separations, there is no better choice than Chromolith® HPLC columns. Due to their revolutionary, monolithic technology, Chromolith® columns provide excellent and rapid separations with extremely high robustness and matrix tolerance compared to particulate columns.

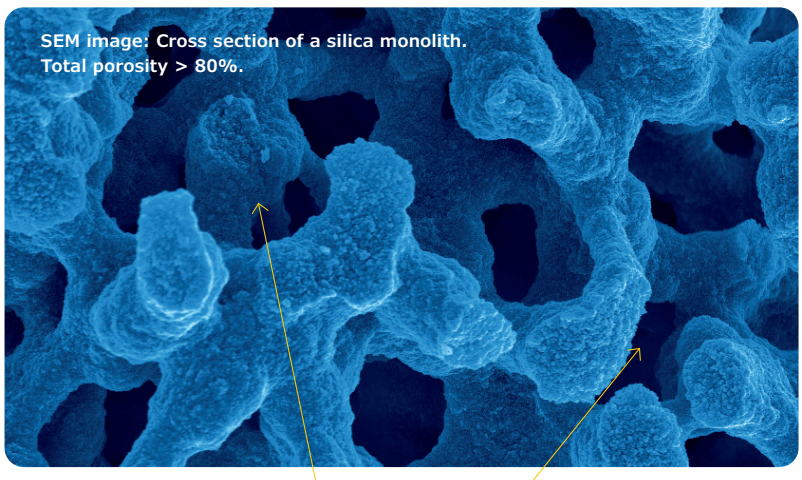
The secret to the speed of Chromolith® columns is their exceptionally low back pressure. Produced from a continuous piece of porous silica using a sol-gel process, Chromolith® columns possess a defined, bimodal pore structure with macro and mesopores in the micro and nanometer range. The high permeability 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.

Several key benefits result directly from the monolithic structure of the silica gel:

- Rapid separations at very low column back-pressure
- Standard HPLC instruments are fully compatible with all Chromolith® columns and UHPLC instruments are fully compatible with Chromolith® 2mm i.d. columns.
- Matrix-rich samples (such as food or biological samples) can be analyzed without the need for sophisticated and time-consuming sample preparation. Guard column cartridges are also available.
- Cost-savings are achieved as the column lifetimes are much longer than for particulate HPLC columns, when analyzing matrix-rich samples.
- Complex, multi-component samples can be separated either by using Chromolith® HighResolution (HR) columns or by using long, high-efficiency columns formed by connecting two or more Chromolith® columns together. The low column backpressure makes this coupling possible.
- Easy transfer of methods from a particulate column to a Chromolith® column.

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

Learn more about monolithic silica technology



Mesopores: Average 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 1.5 µm for Chromolith® 2 mm I.D. , 1.15 µm for Chromolith® HR, and 2 µm for all others.
Allows rapid flow of the mobile phase at low back pressure.

Perfect Fit for All HPLC, UHPLC and UPLC® Instruments

Chromolith® columns are instrument friendly and can be used with HPLC, UHPLC and UPLC® systems from all vendors. The end-fittings of Chromolith® columns are standard Parker designed 1/16'' fittings with a port depth of 2 mm (0.09 in.) Short, capillary tubing is recommended to minimize extra-column volumes.

Connection of Chromolith® Columns of Different Dimensions to HPLC, UHPLC and UPLC® Instruments of Select Vendors

Agilent 1100

Shimadzu LC-40

Thermo Scientific Vanquish™ Flex

Waters ACQUITY UPLC H-Class Bio

Rapid and Robust separations for small and large molecules

Chromolith® columns are available with several surface modifications such as octadecyl (RP-18e) or octyl (RP-8e) endcapped, Phenyl, CN (nitrile), Diol and NH_2 (amino) as well as an unmodified, pure silica. The available column dimensions range from capillary (nano) columns to preparative HPLC columns with 25 mm I.D.

	Column dimensions (I.D.)
HighResolution RP-18e	2 and 4.6 mm I.D. and Capillary Columns
HighResolution RP-8e	4.6 mm I.D.
RP-18e	2, 3, 4.6, 10, 25 mm I.D. and Capillary Columns
RP-8e	150-0, 1 mm I.D. and Capillary Columns
Phenyl	4.6 mm I.D.
CN	4.6 mm I.D.
Diol	4.6 mm I.D.
NH_2	4.6 mm I.D.
Si	4.6, 10 and 25 mm I.D.

Chromolith® columns have shown great potential for the analysis of proteins, antibodies and large peptides where columns with good permeability, along with better mass transfer and selectivity, are required. Chromolith® columns remove back pressure as a 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 matrix rich sample analysis

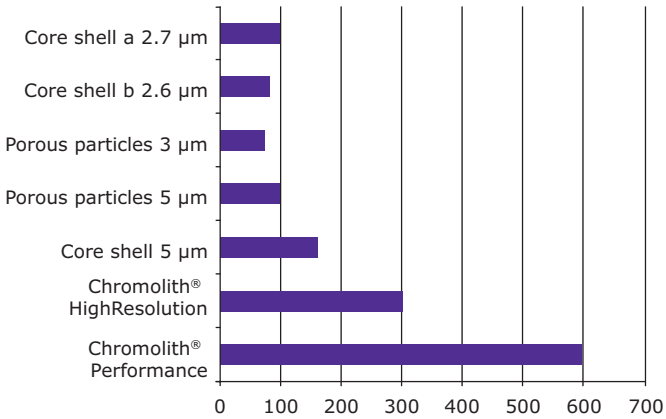
	Molecular Weight	**Column dimensions (I.D.)**
WP 300 RP-18	< 5 kDa	2 and 4.6 mm I.D.
WP 300 RP-8	< 20 kDa	4.6 mm I.D.
RP-4	> 20 kDa	4.6 mm I.D.
WP 300 Protein A	Affinity	2 and 4.6 mm I.D.
WP 300 Epoxy	Immobilisation	2 and 4.6 mm I.D.

Benefits of Monolithic Silica Columns

Tired of congestion?

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 superficially porous particles (SPP) such as Fused Core® or Core-shell particulate columns, which are designed for lower resistance, still exhibit unacceptable back pressure, as column back-pressure is directly depended on particle size. Hence, the task is to minimize back pressure in order to maximize speed.

Plates per pressure (N/bar)



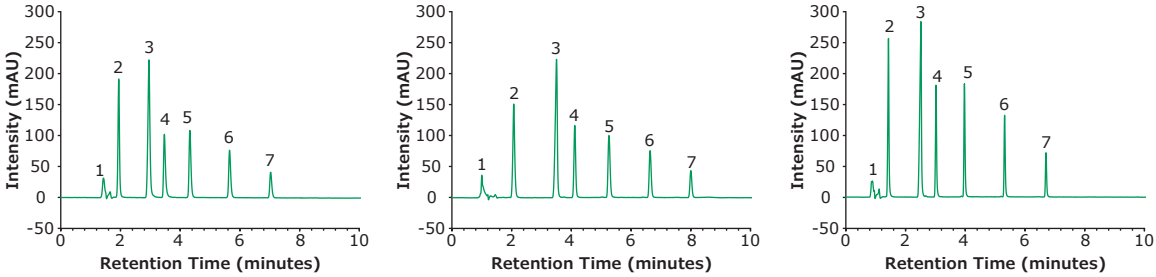
All columns are C_{18} modified, 100-4.6 mm. Sample: anthracene, eluted isocratically using acetonitrile/water (60/40) at 2 mL/min flow rate. Injection volume: 5 µL, detection at 254 nm UV. All analyses performed at room temperature.

Chromolith® HR columns deliver similar results to 3 µm fully porous particles (FPP) or 2.7 µm superficially porous particles (SPP) columns – however at much lower back pressures.

Silica Monolith
Chromolith® HighResolution RP-18e
Pressure = 36 bar

Fully porous silica particle (FPP)
C18, 3 µm
Pressure = 146 bar

Superficially porous silica particle (SPP)
C18, 2.7 µm
Pressure = 170 bar



Chromatographic conditions

Column:	Silica monolith: Chromolith® HighResolution RP-18e 100-4.6 mm 1.52022.0001 FPP: Purospher™ STAR RP18e 3 µm, 100-4.6mm SPP: Ascentis® Express C18, 2.7 µm 100-4.6 mm		
Mobile phase:	A: 100% acetonitrile		
	B: 20 mM Phosphate buffer pH 4.5		
Gradient:	Time/min	%A	%B
	0	20	80
	12.0	80	20
Flow rate:	1.0 mL/min		

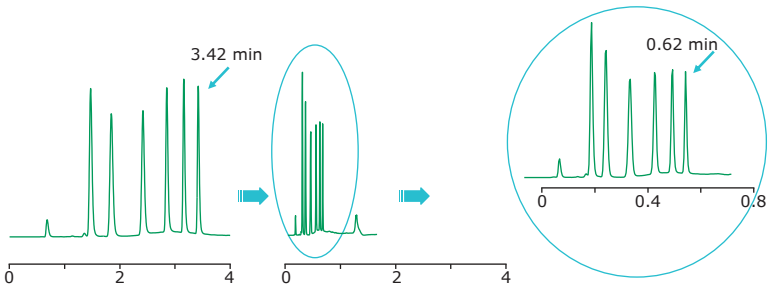
Detection:	UV 230 nm
Detection cell:	Standard 11 µL
Temperature:	22 °C
Injection volume:	2.0 µL
Sample:	1. Ascorbic acid 2. 4-Hydroxybenzoic acid 3. Benzoic acid 4. Sorbic acid 5. Methyl 4-hydroxy benzoic acid 6. Methyl 4-hydroxy benzoic acid 7. Methyl 4-hydroxy benzoic acid

Enabling rapid separations and high throughput

Thanks to laboratory automation of HPLC systems the sample throughput improved significantly during the last decades 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 blocking. 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, if needed. Increase sample troughtput by flow rate while the solvent consumption per sample stays the same.

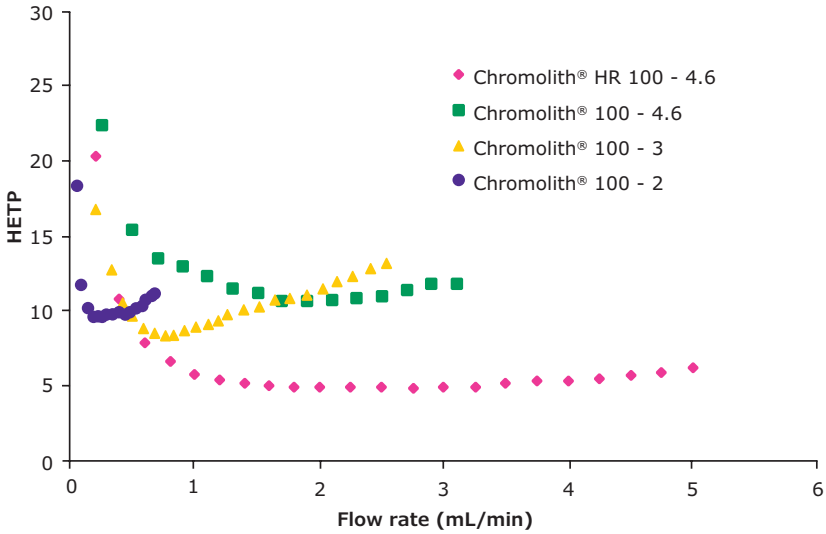
High throughput analysis with increased flow and temperature



Column: Chromolith® FastGradient RP-18e 50-2 mm; Mobile phase: [A] Water, [B] Acetonitrile; Gradient;
Flow rate: 0.2 mL/min → 1 mL/min; temperature: 25 °C → 50 °C;
Sample: uracil, toluene, ethyl-, propyl-, butyl- pentyl- and hexylbenzene

High separation efficiency

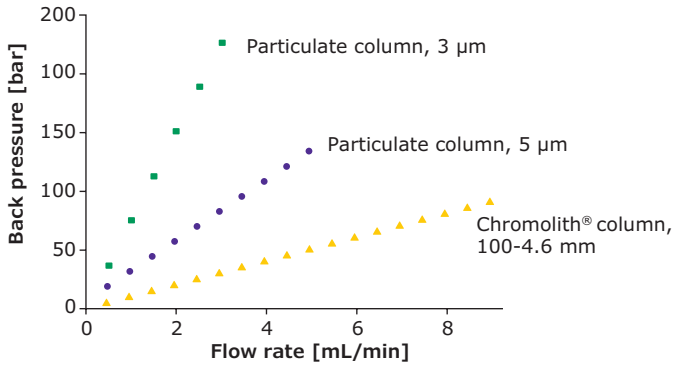
The traditional plate-count method of measuring quality shows that the separation efficiency of Chromolith® columns is better than standard 5 µm particulate columns, and just as good as 3.5 µm columns, but with the ability to continue up to 9 mL/min without reaching HPLC system pressure limits. The van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



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

Rapid separations

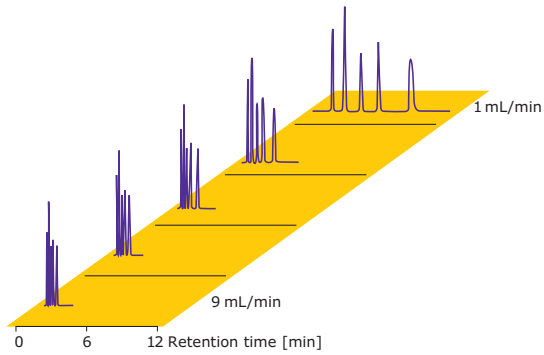
Chromolith® columns owe their rapid separation speed to their unique bimodal pore structure of macro and mesopores. The macropores reduce column back pressure and allow the use of faster flow rates, thereby considerably reducing analysis time. The mesopores form a fine porous structure, which creates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



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.

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100-4.6 mm 1.02129.0001	
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

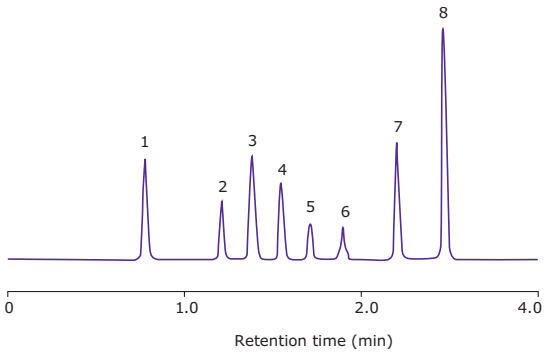


Flow programming

Chromolith® columns respond quickly to changes in flow rate, giving you maximum flexibility in flow programming. Flow 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 modification enables improved 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.

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100-4.6 mm 1.02129.0001			
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		5. Chloro-3-methylphenol	
	2. 2-Chlorophenol		6. 2,4-Dinitro-6-methylphenol	
	3. 2-Nitrophenol		7. 2,4,6-Trichlorophenol	
	4. 2,4-Dinitrophenol		8. Pentachlorphenol	



The challenge with matrix-rich samples

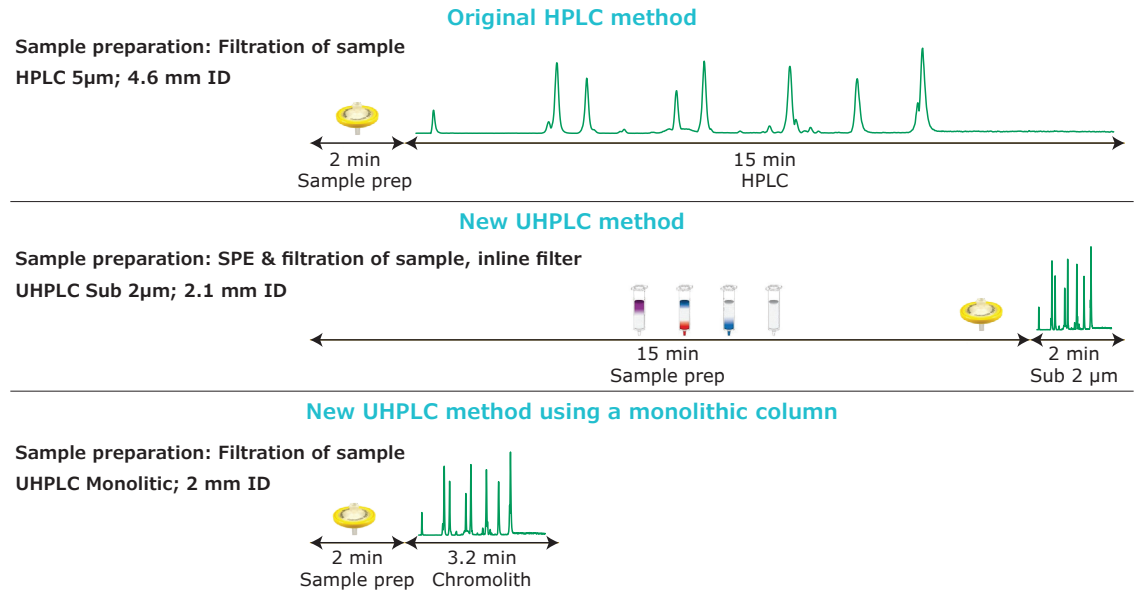
Matrix-rich samples often require extensive sample preparation when analyzed with HPLC and UHPLC. This requirement is a substantial time and cost factor. A robust column material enabling a simplified sample preparation regime will save time and improve lab productivity.

The performance of HPLC columns has improved dramatically in recent years, particularly in terms of separation power as measured by the number of theoretical plates per meter. The improvement in performance has been achieved primarily by a reduction in particle size.

Not all samples have benefited equally from this development: Clean samples with a relatively simple matrix are ideally suited for this new generation of columns. But for samples with a more difficult and complex matrix, the benefits are often accompanied by significant limitations. Essentially, the smaller the particle size and the higher the sample amount of matrix-rich samples injected, the more easily a column plugs or blocks. Therefore, it becomes necessary to perform a rigorous sample preparation before injection. This fact demands additional time and involves addition cost.

A typical scenario is the transfer of HPLC methods to UHPLC in order to increase lab efficiency. UHPLC allows for rapid separations. A decrease from 15 minutes separation time down to 2 minutes using short, UHPLC columns with small particles is a typical case. For the UHPLC analysis of matrix-rich samples, the consequence is an increased need for sample clean-up.

HPLC columns based on monolithic silica are an excellent alternative for cost efficient separations of matrix-rich samples.



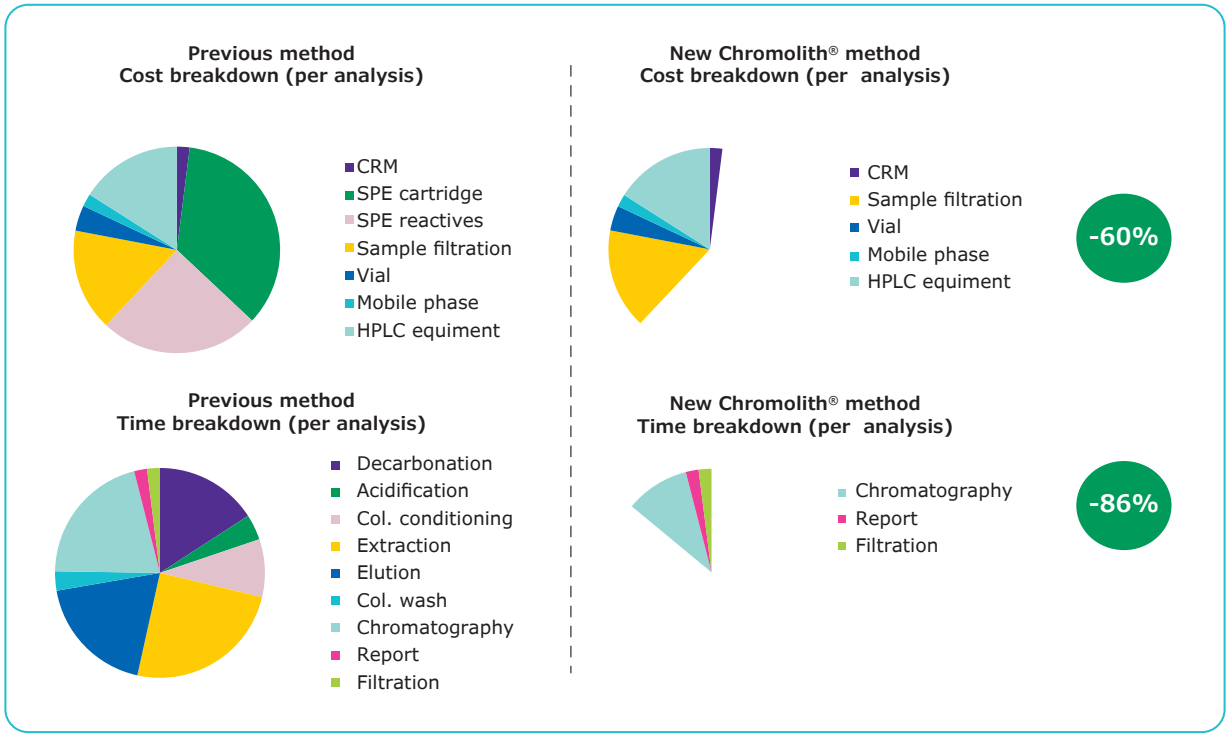
Effect on time for HPLC and UHPLC separation of matrix-rich samples when SPE is needed for UHPLC to extend the column lifetime. The column lifetime for the original HPLC method using a 5 µm particulate column was 7000 Injections of a matrix-rich sample. The column lifetime dropped to less than 100 Injections using the UHPLC method with a sub-2 µm particulate column without sample preparation. After adding SPE for sample preparation the column lifetime increased to 5000 Injections, but the time saving on UHPLC was fully compromised.

The UHPLC method using a monolithic 2 mm I.D. column allowed a very fast separation with simple filtration and increased column lifetime (about 9000 Injections)

Enhance lab effectiveness - save time and money

Chromolith® columns offer excellent robustness and unsurpassed column lifetimes. This quality 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.

When analyzing challenging, matrix-rich samples with traditional HPLC, the benefits of monolithic silica columns are significant. Sample preparation is time consuming and costly. The transfer from particulate columns to monolithic columns can, therefore, save a substantial amount of cost and time.



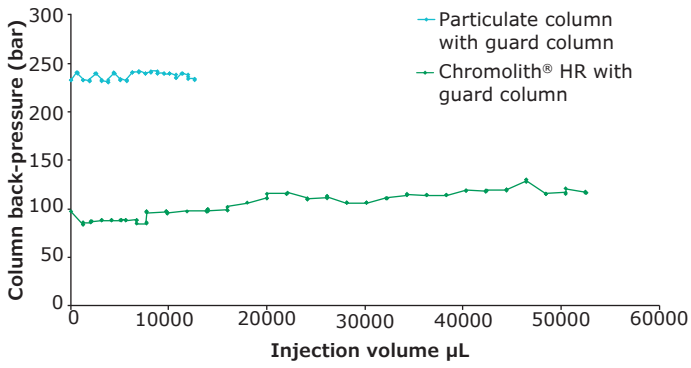
Time and cost per analysis for the determination of Iso-alpha-acids in Beer using a particulate and a monolithic column.

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 only min 3 column volumes. These features allow high-throughput analysis without loss of separation efficiency or peak capacity.

The significantly longer column lifetime of Chromolith® monolithic HPLC columns, in comparison to particulate HPLC columns, is over-compensating the higher cost of this column material in comparison to particulate columns, especially if matrix-rich samples are used with simplified sample preparation.

Therefore, the cost per sample can be significantly lower in direct comparison under equal conditions and the same sample.



Beyond high resolution – Make your column longer

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 phenomenon 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.

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 of columns results in a theoretical plate count per column/available for the separation, 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 critical separation problems in which resolution is a limiting factor. This fact 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 fully porous 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

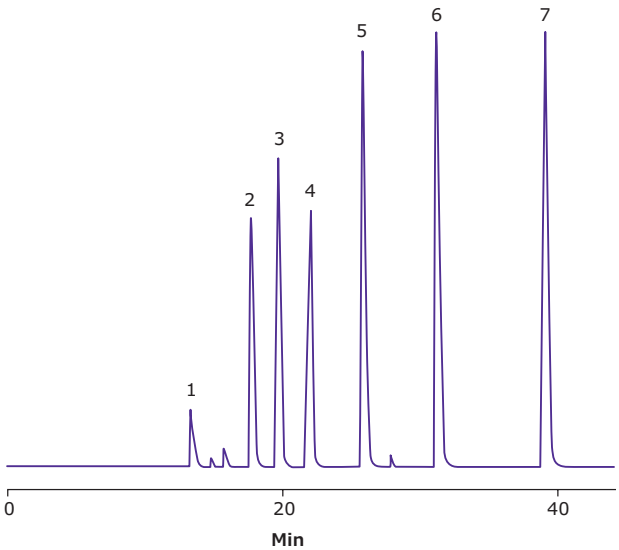
Pressure* = 3 mL/min 75% acetonitrile, 25% water

Application of Chromolith® column coupler

Chromatographic conditions

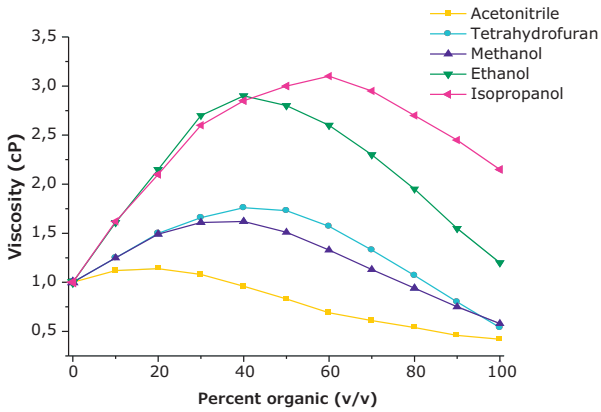
81,000 plates at 85 bar pressure

Column:	10 columns of Chromolith® Performance RP-18 endcapped 100-4.6 mm 1.02129.0001
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



High flexibility with mobile phases

The most common organic solvent in HPLC/UHPLC is acetonitrile because of low UV absorbance > 200 nm and low viscosity which helps to minimize column back pressure. When UV detection is set to above 220 nm, 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 pressures could be up to 1.7 times higher, which could breach the maximum instrument back pressure limit as well as shorten column lifetime. Since Chromolith® columns have such a low resistance to 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, than acetonitrile; isopropanol tends to be less denaturing to biomolecules than methanol; ethanol is “greener” and less dangerous to chromatographers; and buffer solubility is much better in methanol containing mobile phases compared to acetonitrile.



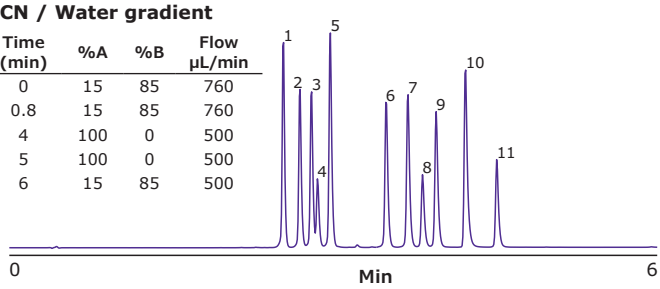
Doping drug separation using ACN and MeOH

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100 - 2 mm 1.52006.0001
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

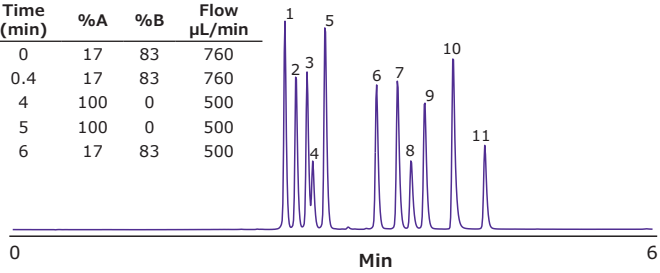
ACN / Water gradient

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



MeOH / Water gradient

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

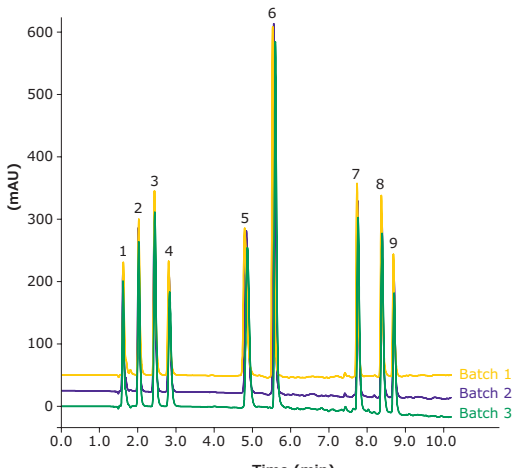


Excellent batch-to-batch reproducibility

Long-term reproducibility is a key factor for the quality of an HPLC column robot based state of the art production minimizing human type of errors. The batch-to-batch reproducibility of Chromolith® HPLC columns are strictly controlled and fulfill the requirements of QA and QC laboratories. Chromolith® columns for small and large molecule separation enable long-term reliable results.

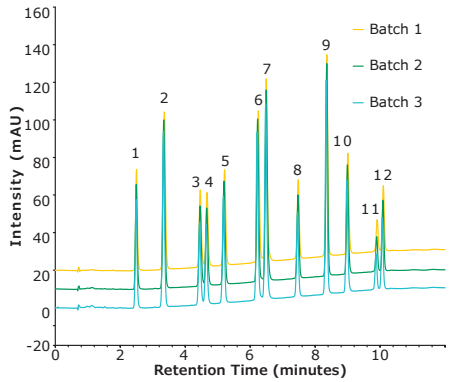
Chromatographic conditions

Column:	Chromolith® HighResolution RP-18 endcapped 100-4.6 mm 1.52022.0001	
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



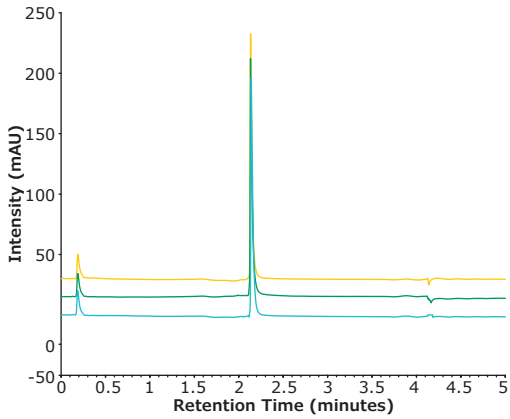
Chromatographic conditions

Column:	Chromolith® HighResolution RP18e 100-2 mm (1.52322.0001)		
Mobile phase:	A: 0.1% (v/v) Phosphoric acid, pH 2.2 B: Methanol		
Gradient:	**Time**	**%A**	**%B**
	0	28	72
	0.1	28	72
	7.1	10	90
	12.0	10	90
Flow rate:	0.380 mL/min		
Pressure:	130 bar		
Detection:	UV 228 nm		
Temperature:	25 °C		
Injection volume:	0.2 µL		
Sample:	1. CBDV Cannabidivarin 2. CBDVA Cannabidivarinic Acid 3. CBD Cannabidiol 4. CBG Cannabigerol 5. CBDA Cannabidiolic acid 6. CBGA Cannabigerolic acid	7. CBN Cannabinol 8. CBL (±)-Cannabicyclol 9. CBC Cannabichromene 10. CBNA Cannabinolic Acid 11. CBLA Cannabicyclolic Acid 12. CBCA Cannabichromenic Acid	



Chromatographic conditions

Column:	Chromolith® WP 300 Protein A 25-2 mm (1.52358.0001)		
Eluent:	A: 100 mM disodium hydrogen phosphate, pH 7.4 B: 100 mM disodium hydrogen phosphate, pH 2.5		
Gradient:	**Time**	**%A**	**%B**
	0	100	0
	0.5	100	0
	0.55	0	100
	2.6	0	100
	2.65	100	0
	5.0	100	0
Flow rate:	0.380 mL/min		
Pressure:	16 bar		
Detection:	UV 280 nm		
Temperature:	25 °C		
Injection volume:	2.0 µL		
Sample:	1.8 mg IgG 1 mg/mL		

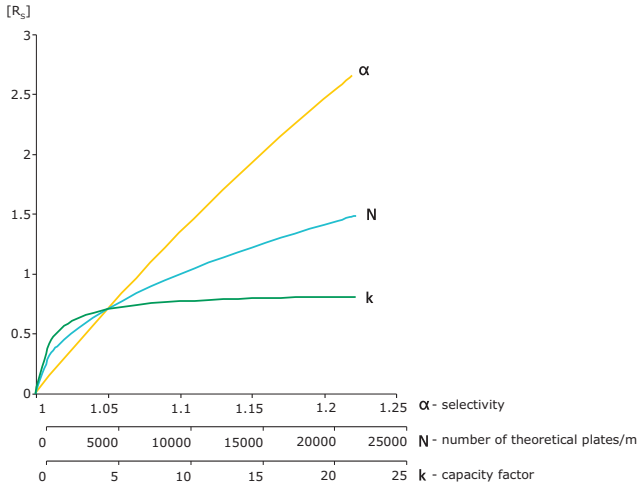


Chromolith® HPLC columns

Selection of Column Chemistry / Selectivity

Chromatographic resolution is influenced by the selectivity (α) (when k>2 and N> 3000). Changing the mobile phase composition or the stationary phase is the most powerful way of optimizing selectivity whereas the particle size, pore size, length of the column, temperature, and mobile phase strength have much less effect. Therefore, if satisfactory results are not met, or no retention is achieved, it is better to change to another selectivity using a different column type and/or a different mobile phase.

Selecting the most suitable column chemistry is highly depended upon the sample undergoing analysis. While compounds can often be separated using various column chemistries, some column selectivities are better than others. The table on page 15 shows a selection of compound classes typically analyzed by HPLC methods.



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 130 Å/150 Å 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 for small molecule separations

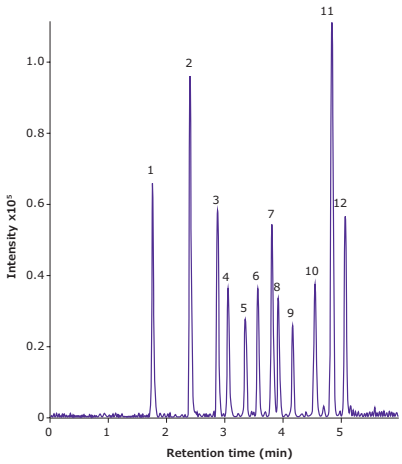
Chromolith® columns are available in column dimensions for Nano-LC (Capillary columns, see page 38), analytical columns from typical UHPLC/LC-MS dimensions to HPLC classical dimensions (2, 3 and 4.6 mm I.D. columns see page 40) and semi-preparative as well as preparative columns (10 and 25 mm I.D., see page 43).

Chromolith® Performance HPLC columns provide the highest matrix-tolerance due to their large marcopores (2 µm) and are available with several column chemistries for a broad selectivity range.

Chromolith® columns with 2 mm internal diameter are ideal for use with UHPLC or UPLC instruments, thanks to their very small internal volumes. A particular benefit is the very fast analysis, reduced sample-preparation and the low column backpressure. Ultra-high performance and extremely low operating pressure make Chromolith® 2 mm I.D. columns truly unique.

Chromolith® and Chromolith® HighResolution columns are perfectly suitable for LC-MS use; however, the column dimension of 2 mm I.D. or below is preferred to be used in LC-MS methods.

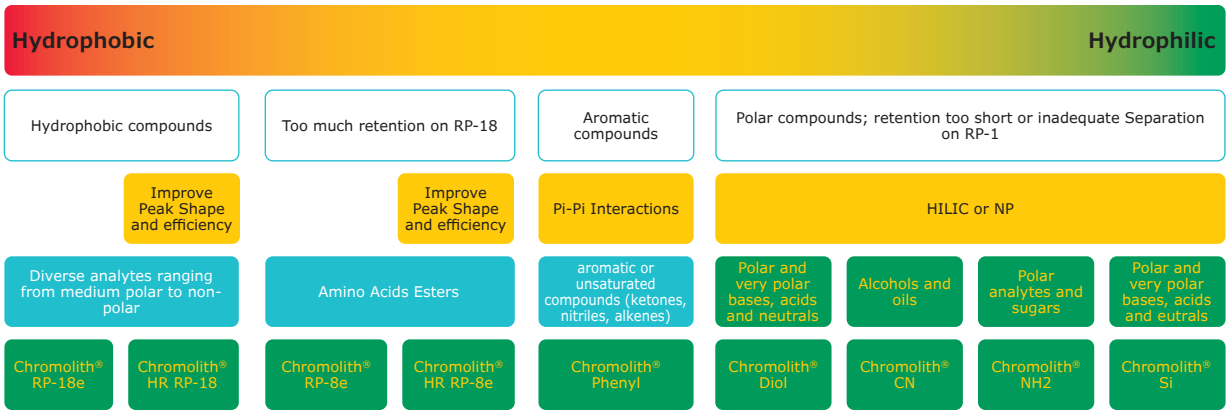
Excellent, ultra-fast results are obtained, not only in UHPLC and UPLC® instruments, but equally well in all standard HPLC systems with low dead volume. Chromolith® 2 mm columns have macropores of 1.5 µm in diameter, resulting in a column efficiency that exceeds 100,000 plates/meter.



Chromatographic conditions

Column:	Chromolith® FastGradient RP-18 endcapped 50-2 mm (1.52007.0001)		
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

Chromolith® columns are available with a broad range of column modifications enabling the selection of the best suitable selectivity for hydrophobic and hydrophilic compounds. C18 is usually the first choice for starting a new method. However, when a C18 doesn't give the desired separation, or your sample contains compounds that are known to be difficult to retain or resolve on a C18, then you should consider changing the stationary phase, modification or both.



Chromolith® RP-18 endcapped phases

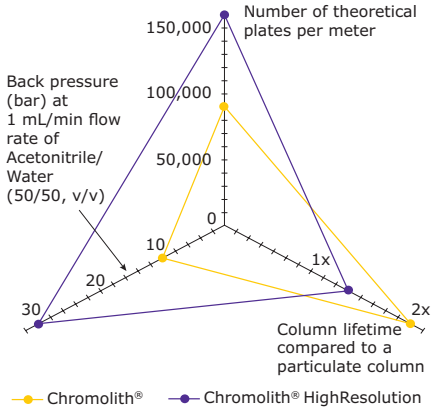
The chemical basis of Chromolith® RP-18 endcapped columns – from starting materials to surface modifications — is the same as high-end particulate columns. Thus, their selectivity is comparable to high-quality C18 endcapped reversed-phase particle packed columns. This trait allows the use of standard methods when developing new protocols. The columns are based on high-purity silica; hence they minimize the negative effect of trace metals. Furthermore, the silica rods 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-18e phase characteristcis

Chromolith®	RP-18 endcapped 3, 4.6, 10, 25 mm I.D.	RP-18 endcapped 2 mm I.D.	HighResolution RP-18e 2, 4.6 mm I.D.
Base material:	Monolithic silica Type B	Monolithic silica Type B	Monolithic silica Type B
Phase chemistry:	Octadecylsilane	Octadecylsilane	Octadecylsilane
USP classification:	L1	L1	L1
Macropore size:	2 µm	1.5 µm	1.1 µm
Mesopore size:	130 Å	130 Å	150 Å
Mesopore Volume:	1 mL/g	1 mL/g	1 mL/g
Porosity:	80%	80%	80%
Surface Area:	300 m²/g	300 m²/g	250 m²/g
Carbon load:	18%	18%	15%
Endcapped:	Yes	Yes	Yes
Minimum Efficiency (N/m):	80000 N/m	100000 N/m	140000 N/m
Typical Efficiency (N/m):	95000 N/m	120000 N/m	165000 N/m
pH range:	2.0–7.5	2.0–7.5	2.0–7.5
Max Temperature:	50 °C	50 °C	50 °C
Shipping solvent:	Acetonitrile / Water 60:40	Acetonitrile / Water 60:40	Acetonitrile / Water 60:40
Storing	Acetonitrile	Acetonitrile	Acetonitrile

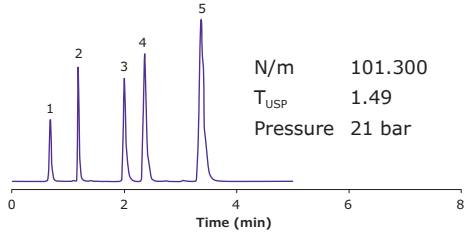
Chromolith® HighResolution RP-18e

Chromolith® HighResolution (HR) 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.

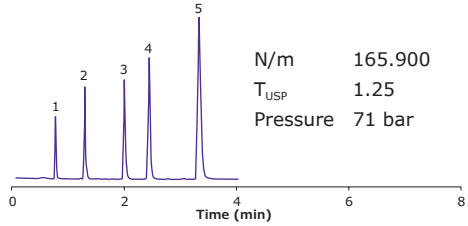


Our classical Chromolith® columns are recommended for analyzing matrix-rich samples, as this type of column will have a longer lifetime. Also, lower back pressure would allow column coupling if necessary.

Chromolith® Performance RP-18e 100-4.6 mm (1.02129.0001)



Chromolith® HR RP-18e 100-4.6 mm (1.52022.0001)



Chromatographic conditions

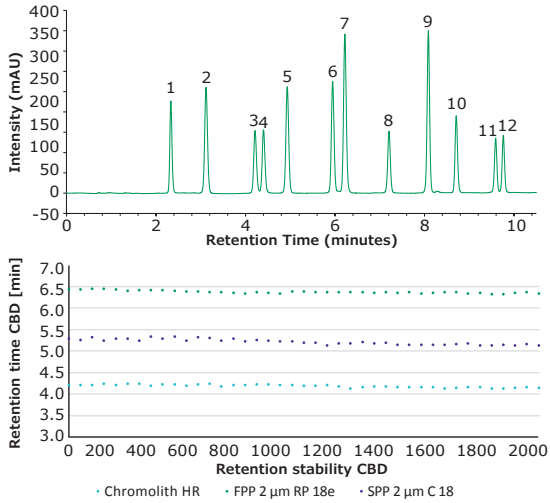
Mobile phase:	Acetonitrile / water 60/40
Flow rate:	2 mL/min
Detection:	UV 254 nm
Temperature:	ambient
Injection volume:	5 µL
Sample:	1. Urea 2. Biphenyl-2-ol 3. Progesterone 4. Hexanophenon 5. Anthracene

The separation of matrix-rich samples, such as herbs, food, or biological samples, tends to reduce the column lifetime of particulate columns if no sufficient sample preparation was performed before HPLC separation. Chromolith® columns allow the separaion of matrix-rich samples with extended column life with no, or very reduced sample preparation before separation.

Cannabinoid separation on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

Column:	Chromolith® HighResolution RP18e 100-2 mm (1.52322.0001)		
Mobile phase:	A: 0.1% (v/v) Phosphoric acid, pH 2.2 B: Methanol		
Gradient:	**Time**	**%A**	**%B**
	0	28	72
	0.1	28	72
	7.1	10	90
	10.0	10	90
Flow rate:	0.38 mL/min		
Pressure:	148 bar		
Detection:	UV 228 nm		
Sample:	1. CBDV Cannabidivarin	7. CBN Cannabinol	
	2. CBDVA Cannabidivarinic Acid	8. CBL (±)-Cannabicyclol	
	3. CBD Cannabidiol	9. CBC Cannabichromene	
	4. CBG Cannabigerol	10. CBNA Cannabinolic Acid	
	5. CBDA Cannabidiolic Acid	11. CBLA Cannabicyclolic Acid	
	6. CBGA Cannabigerolic Acid	12. CBCA Cannabichromenic Acid	



The analysis of cannabinoids from hemp and cannabis is of increased interest because of regulatory requirements for tetrahydrocannabinol (THC) content and the therapeutic benefits of minor cannabinoids in different formulations. This fact leads to a variety of difficult matrices and the need for robust columns and high-throughput methods.

In this application, industrial hemp leaves were extracted with methanol and the sample was filtered prior to injection. Twelve cannabinoid standards were spiked into the sample, and a stability test was performed on three different column materials: silica monoliths, fully-porous particles (FPP) and superficially-porous particles (SPP).

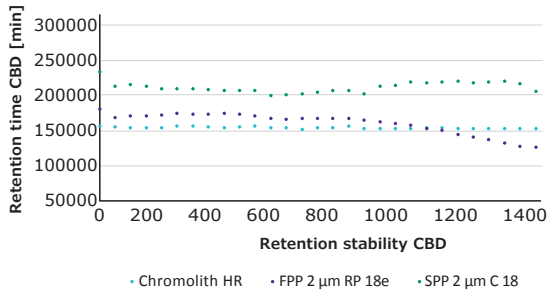
In between the cannabinoid sample analysis, the separation efficiency of the columns was tracked with a performance test with anthracene as the analyte.

As can be seen in the graphics and table, no significant change in the retention time of CBD is observed after 2000 injections. The efficiency for the Chromolith® HR stays nearly constant for 1200 injections, while for the FPP and SPP, a reduction of about 10% and 30% can be noticed.

These results show how robust the bimodal pore structure and the rigid monolithic silica skeleton performs over an extended period of time.

Chromatographic conditions Efficiency Test

Column:	Chromolith® HighResolution RP18e 100-2 mm (1.52322.0001)	
Mobile phase:	A: Water B: Acetontrile	
Gradient:	A/B 60/40	
Flow rate:	0.38 mL/min	
Pressure:	148 bar	
Detection:	UV 254 nm	
Temperature:	25 °C	
Injection volume:	0.1 µL	
Sample:	Thiourea	2.1 mg
	Progesterone	11.6 mg
	Anthracene	1.8 mg
	in 100 mL A/B 60/40	



Column	Separation efficiency anthracene			Retention time CBD		
	Initial (N/m)	After 1400 injections (N/m)	Relative Change	Initial (min)	After 2000 injections (min)	Relative Change
Chromolith® HR RP18e	165990	161500	-2.7%	4.19	4.13	-1.4%
FPP 2 µm RP18e	189450	135370	-28.5%	6.42	6.32	-1.6%
SPP 2 µm C18	241690	214310	-11.3%	5.28	5.11	-3.2%

Chromolith® RP-8 endcapped and Phenyl phases

Chromolith® RP-8 endcapped

With its shorter alkyl chain, Chromolith® and 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.

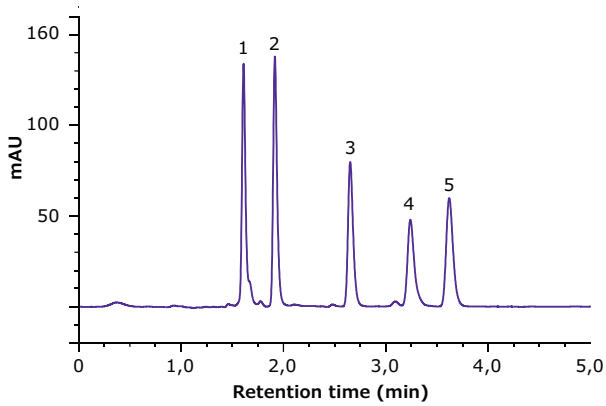
Chromolith® Phenyl

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, PAHs, preservatives, purines and pyrimidines.

Chromolith®	RP-8 endcapped 3 and 4.6 mm I.D.	HighResolution RP-8e 4.6 mm I.D.	Phenyl 4.6 mm I.D.
Base material:	Monolithic silica Type B	Monolithic silica Type B	Monolithic silica Type B
Phase chemistry:	Octylsilane	Octylsilane	Phenylsilane
USP classification	L7	L7	L11
Macropore size:	2 µm	1.1 µm	2 µm
Mesopore size:	130 Å	150 Å	130 Å
Pore Volume:	1 mL/g	1 mL/g	1 mL/g
Porosity:	80%	80%	80%
Surface Area:	300 m²/g	250 m²/g	300 m²/g
Carbon load:	11%	11%	11%
Endcapped:	Yes	Yes	Yes
Minimum Efficiency (N/m):	80000 N/m	140000 N/m	80000 N/m
Typical Efficiency (N/m):	95000 N/m	165000 N/m	90000 N/m
pH range:	2–7.5	2 – 7.5	2–7.5
Max Temperature:	50 °C	50 °C	50 °C
Shipping solvent:	Acetonitrile / Water 80:20	Acetonitrile / Water 80:20	Acetonitrile / Water 50:50
Storing	Acetonitrile	Acetonitrile	Acetonitrile

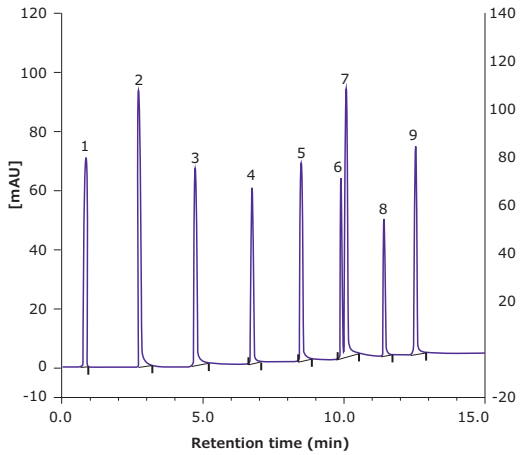
Chromatographic conditions

Column:	Chromolith® HighResolutionRP-8 endcapped 100x4.6 mm (1.52064.0001)
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:	1. Gly-Tyr 2. Val-Tyr-Val 3. Met enkephalin 4. Leu enkephalin 5. Angiotensin II 0.5 mg/mL in Mobile Phase



Chromatographic conditions

Column:	Chromolith® Phenyl 100x4.6 mm (1.52058.0001)	
Mobile phase:	A: ACN / B: H_2O	
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® CN, NH_2, Diol and Si Phases

Chromolith® columns with polar modifications (CN, NH_2 and Diol) as well as plain silica (Si) phases are suitable for the separation of polar compounds. These column chemistries can be used for Normal-phase conditions as well as with Reversed phase molble phases (HILIC -mode).

Chromolith®	CN 4.6 mm I.D.	NH_2 4.6 mm I.D.	Diol 4.6 mm I.D.	Si 4.6, 10- and 25-mm I.D.
Base material:	Monolithic silica Type B	Monolithic silica Type B	Monolithic silica Type B	Monolithic silica Type B
Phase chemistry:	Cyanosilane	Aminopropyl	Diolsilane	unbonded
Usp classification	L10	L8	L20	L3
Macropore size:	2 µm	2 µm	2 µm	2 µm
Mesopore size:	130 Å	130 Å	130 Å	130 Å
Mesopore volume:	1 mL/g	1 mL/g	1 mL/g	1 mL/g
Porosity:	80%	80%	80%	80%
Surface area:	300 m²/g	300 m²/g	300 m²/g	300 m²/g
Carbon load:	6%	5%	9%	N/A
Endcapped:	No	No	No	No
Minimum efficiency (n/m):	80000 N/m	80000 N/m	80000 N/m	80000 N/m
Typical efficiency (n/m):	90000 N/m	90000 N/m	90000 N/m	85000 N/m
Ph range:	2–7.5	2–7.5	2–7.5	2–7.5
Max temperature:	50 °C	50 °C	50 °C	50 °C
Shipping solvent:	Acetonitrile / Water 30:70	Acetonitrile/Water 90:10	n-Heptane/Dioxane (95:5)	n-Heptane/Dioxane (95:5)
Storing	Acetonitrile / Water 30:70*	Acetonitrile*	2-propanol**	n-Heptane/Dioxane (95:5)**

* RP storing conditions, if used in NP use 2-Propanol or n-Heptane/Dioxane for storing
** NP storing conditions, if used under HILIC conditions use Acetonirile/Water for storing

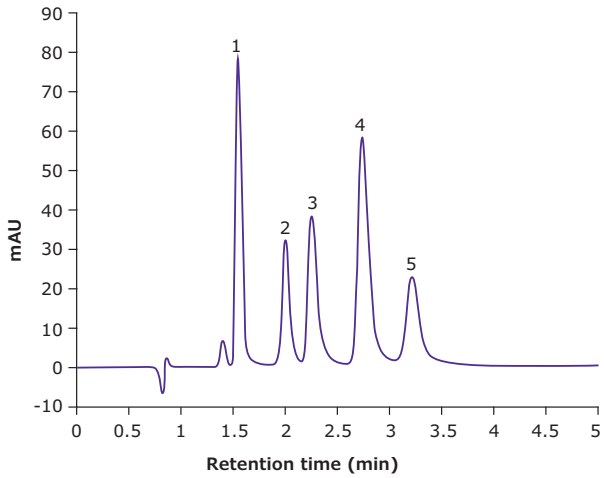
Chromolith® CN

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

Chromatographic conditions

Column:	Chromolith® CN 100-4.6 mm (1.52048.0001)	
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 2. Estradiol 3. Testosterone 4. Ethynylestradiol 5. Estrone Solved in 100 mL ACN/$H_2$0 5/5	9.4 mg/mL 8.7 mg/mL 11.6 mg/mL 7.9 mg/mL 13.3 mg/mL



Chromolith® NH_2

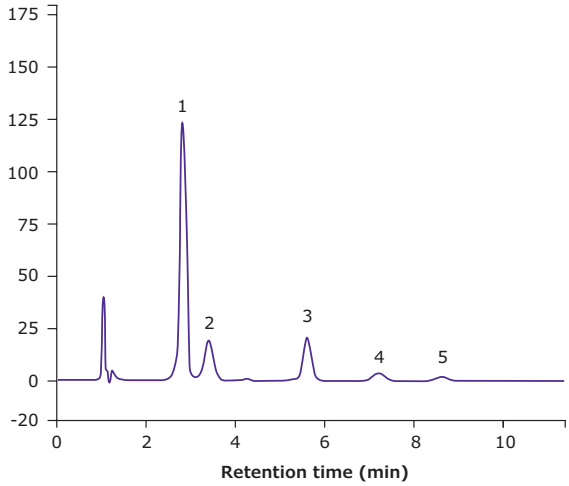
Chromolith® aminopropyl-modified columns possess medium polarity, between those of bare (normal-phase) silica and reversed-phase silica. Consequently, these columns display hydrophilic as well as hydrophobic properties, and can be used under both reversed-phase and normal-phase conditions. However, retention is weaker than on silica or reversed-phase 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 of sugars

Chromatographic conditions

Column:	Chromolith® Performance NH_2 100-4.6 mm (1.52028.0001)
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



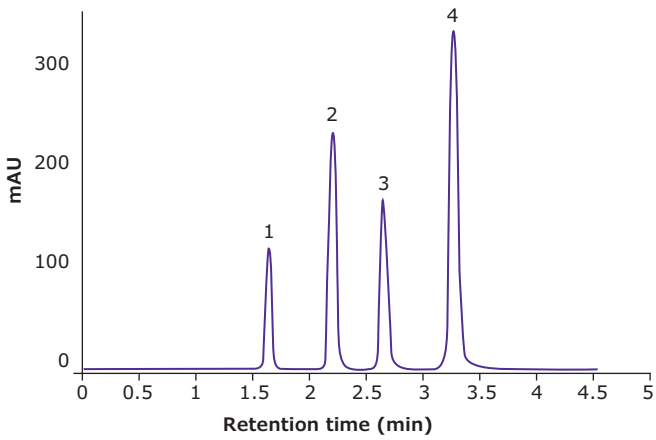
Chromolith® Diol

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 trait 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

Chromatographic conditions

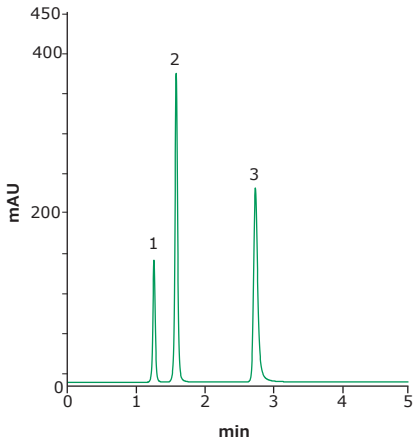
Column:	Chromolith® Diol 100-4.6 mm (1.53172.0001)
Mobile phase:	n-Heptane / Dioxane 95/5 v/v
Flow rate:	1.3 mL/min
Detection:	254 nm
Cell volume:	11 µL
Temperature:	ambient
Injection volume:	5 µL
Sample:	1. Anisole 390 µg/mL 2. 3-Nitroanisole 70 µg/mL 3. 4-Nitroanisole 260 µg/mL 4. 2-Nitroanisole 180 µg/mL



HPLC Analysis of nitro-aromatic compounds on Chromolith® Performance Diol 100-4.6mm

Chromatographic conditions

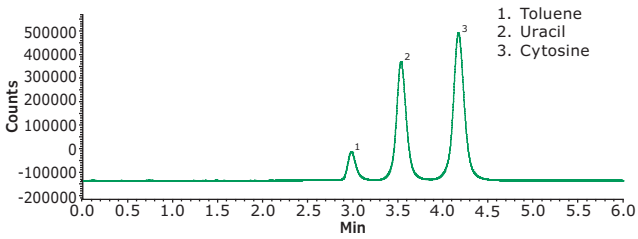
Column:	Chromolith® Performance DIOL, 100 x 4.6 mm (1.53172.0001)	
Temperature:	25 °C	
Mobile phase:	[A] n-Heptane; [B] Dioxane (99:1, A:B)	
Flow rate:	1.3 mL/min	
Pressure:	10 bar	
Injection:	5 µL	
Detector:	254 nm	
Sample:	1.) 135,5 mg 2.) 12,4 mg 3.) 28,4 mg In 100mL n-Heptane/Dioxane (95/5)	Toluene 2-Nitrotoluene 2-Nitroanisole



HPLC Analysis of Toluene, Uracil and Cytosine on Chromolith® Performance Diol 100-4.6mm

Chromatographic conditions

Column:	Chromolith® Performance DIOL, 100 x 4.6 mm (1.53172.0001)
Temperature:	23 °C
Mobile phase:	[A] acetonitrile; [B] 5 mM Ammonium acetate buffer, pH 6.8; (80:20, A:B)
Flow rate:	0.5 mL/min
Pressure:	87 psi (6 bar)
Injection:	10 µL
Detector:	254 nm
Sample:	Toluene 0.67 mg/mL, Uracil 0.04 mg/mL, Cytosine 0.06 mg/mL (Diluent: Mobile phase)

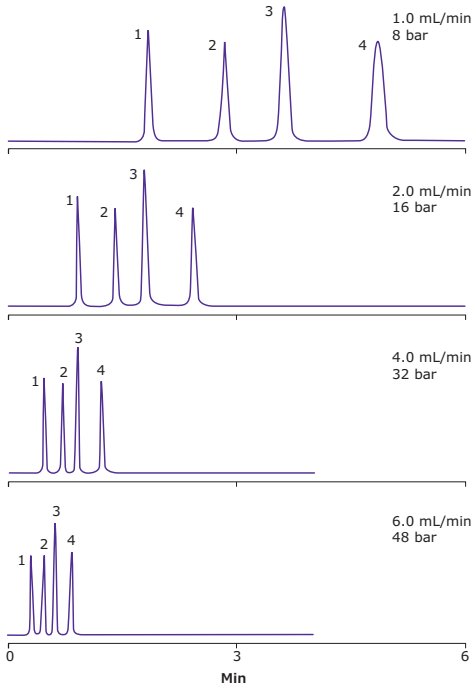


Chromolith® Si

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

Separation examples

Column:	Chromolith® Performance Si 100-4.6 mm (1.51465.0001)	
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 2. 3-Nitroanisole 3. 4-Nitroanisole 4. 2-Nitroanisole	0.39 mg/mL 0.07 mg/mL 0.26 mg/mL 0.18 mg/mL



Chromolith® Columns for Biomolecule Separation

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. 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, particle-packed 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, Protein A, and Epoxy depending on the use of the column.

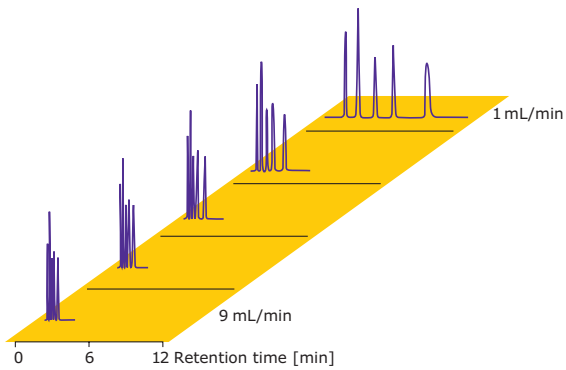
Monolithic columns remove back pressure as a consideration in method development and allow flow rate flexibility for much higher throughput, a choice of column lengths for superior resolution, and more solvent options for optimum selectivity. With no individual particles to shift or break, column performance is consistent over a much longer lifetime, making them ideal for matrix rich sample analysis. High permeability also makes them forgiving of less rigorously prepared samples, in addition to making it easier to aggressively flush out for re-equilibration. Finally, 2 mm I.D. geometries are available for the C18, Protein A, and Epoxy columns for improved sensitivity of low concentration analytes and complete MS-compatibility.

Why choose Chromolith® WP 300 columns

- Completely bioinert column hardware
- High biorecovery
- Tailored selectivities for biomolecules
- Low column back pressure
- High-speed separations possible
- Substantially longer column lifetime
- High resistance to column blockage
- Cost savings from higher sample throughput and column durability

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.

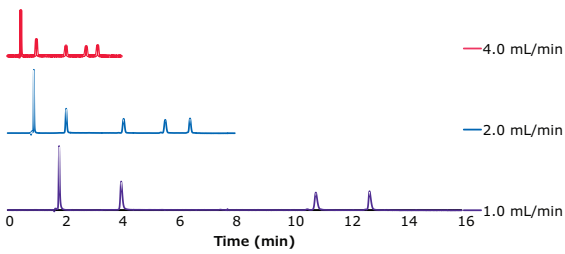


Column back pressure at different flow rates. Comparison of a Chromolith® Widepore column vs. equivalent classical particulate HPLC columns.

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, 100-4.6 mm 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



Chemistry Options Tailored for Your Separation Challenges

Chromolith® WP 300 RP18	Chromolith® WP 300 RP8	Chromolith® WP 300 RP4	Chromolith® WP 300 Epoxy	Chromolith® WP 300 Epoxy
Selectivity for peptides	**Selectivity for peptides or proteins**	**Selectivity for proteins**	**Immobilization of ligands**	**Selectivity for antibodies**
MW < 5 kDa	MW < 20 kDa	MW < 20 kDa	Depending on Ligand, different selectivty	High Affinity and specific selectivity

The characterization of biomolecules is a multi-pronged, analytical challenge requiring several columns to determine all the critical quality attributes (CQAs) required for new drug applications (NDAs). The Chromolith® WP 300 line of HPLC columns can aid you in determining some of these CQAs in a quick, and efficient, manner.

Chromolith® WP 300 RP18

The most hydrophobic of the Chromolith® WP 300 line, the RP18 column is useful for the resolution of peptides and smaller proteins. One CQA required by regulatory bodies is the peptide map of a biotherapeutic. Peptide maps generated by RP-HPLC provide valuable information about protein structure, stability, and purity. To be effective, the RP-HPLC column must be able to resolve a high percentage of the peptides in the sample. The more peptides, the better the information. Chromolith® WP 300 RP18 column gives unsurpassed RP-HPLC resolution of peptide maps from enzymatic digests. The improvements in silica and bonded-phase chemistry incorporated into the Chromolith® WP 300 line improve resolution by increasing efficiency and reducing the peak tailing.

Chromolith® WP 300 RP8

The Chromolith® WP 300 RP8 column exhibits hydrophobicity intermediate between the Chromolith® WP 300 RP4 and the Chromolith® WP 300 RP18 column. The difference in hydrophobicity gives it unique selectivity relative to these other phases. The column is ideal for peptide mapping because it provides complementary information compared to a C18 separation. Because of its intermediate hydrophobicity, it is also recommended for method development or scouting work. As with all Chromolith® WP 300 phases, the C8 phase gives efficient, symmetrical peaks, exceptional stability, long column lifetime, and LC-MS compatibility.

Chromolith® WP 300 RP4

The Chromolith® WP 300 RP4 column was designed for the efficient and reliable separation of proteins and peptides, especially hydrophobic peptides, by RP- HPLC. Long-chain phases, like C8 or C18, are often too hydrophobic for proteins and can cause excessively long retention time or even irreversible binding to the column. For this reason, short-chain phases, typically C3 or C4, are often used for RP-HPLC of proteins.

Chromolith® WP 300 Epoxy

The Chromolith® WP 300 Epoxy column is a unique addition to the Chromolith® WP 300 line of HPLC columns in that this column enables facile bonding of your own ligand to the column for do-it-yourself (DIY) affinity chromatography. Several different applications have been developed with this column including unique affinity chromatographic separations, enzymatic reactors, fast chiral HPLC, and many more.

Chromolith® WP 300 Protein A

The Chromolith® WP 300 Protein A column is a specific, affinity chromatography column for immunoglobulin G (IgG) titer determination. Titer determination is an important parameter to monitor in the expression of biotherapeutics, especially in cell-culture supernatants. Utilizing a monolithic format, Chromolith® WP 300 Protein A column enables rapid and accurate in-process checks of reactors with little to no sample preparation.

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. In addition, the 2 mm I.D. format for the Chromolith® WP 300 RP-18 column enables improved sensitivity and complete MS-compatibility.

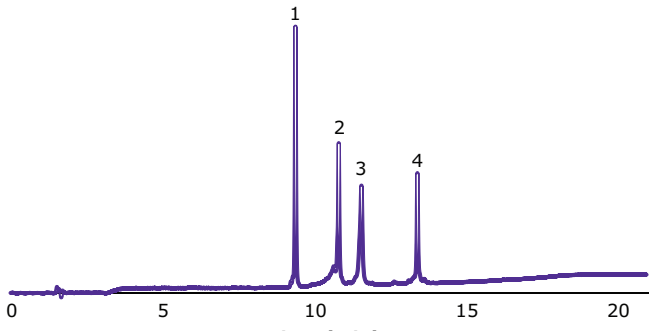
Chromolith®	WP 300 RP-18 2 and 4.6 mm I.D.	WP 300 RP-8 4.6 mm I.D.	WP 300 RP-4 4.6 mm I.D.
Base material:	Monolithic silica Type B	Monolithic silica Type B	Monolithic silica Type B
Phase chemistry:	Octadecylsilane	Octylsilane	Butylsilane
Usp classification	L1	L7	L26
Macropore size:	2 µm	2 µm	2 µm
Mesopore size:	300 Å	300 Å	300 Å
Mesopore volume:	1 mL/g	1 mL/g	1 mL/g
Porosity:	80%	80%	80%
Surface area:	120 m²/g	120 m²/g	120 m²/g
Carbon load:	8 – 9 %	4 – 5 %	3 – 4 %
Endcapped:	No	No	No
Minimum efficiency (n/m):	80000 N/m	80000 N/m	80000 N/m
Typical efficiency (n/m):	90000 N/m	90000 N/m	90000 N/m
Ph range:	1.5–7.5	1.5–7.5	1.5 – 7.5
Max temperature:	60 °C	60 °C	60 °C
Shipping solvent:	Acetonitrile / Water 60:40	Acetonitrile / Water 60:40	Acetonitrile
Storing	Acetonitrile*	Acetonitrile*	Acetonitrile*

* prefered solvent for storing. Methanol, Isopropanol or Ethanol are suitable as well

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

Chromatographic conditions

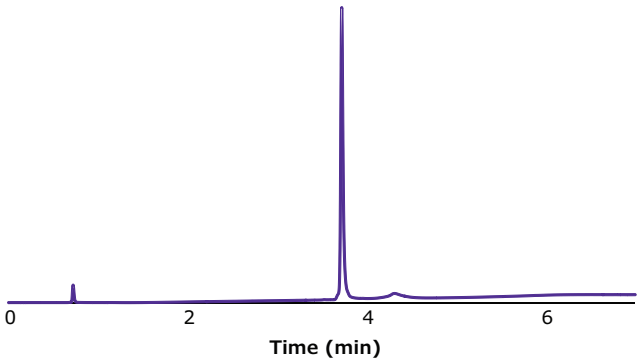
Mobile phase:	A 0.1% TFA in water B 0.1% TFA in acetonitrile
Flow rate:	1.0 mL/min
Detection:	220 nm
Peak Identification:	1. Angiotensin II 2. Neurotensin 3. Ribonuclease 4. Myoglobin
Gradient:	1 min 4% B 15 min 4 – 60% B 5 min 60% B



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

Chromatographic conditions

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

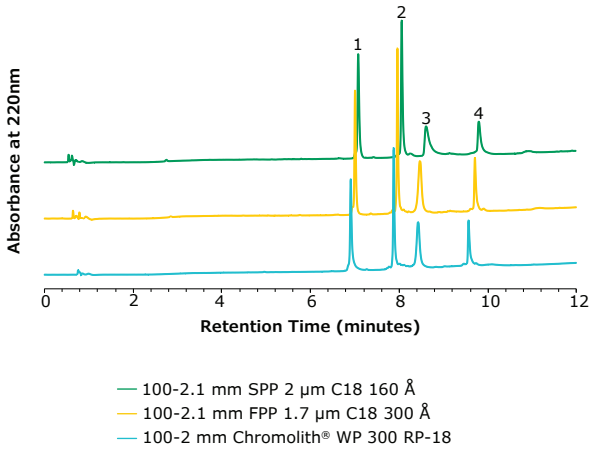


Protein Analysis on Chromolith® WP 300 RP-18

For large molecule separations, high efficiency separations are necessary in order to achieve resolution and good peak shape. Moving to sub-2 µm FPP-packed columns or 2.0 µm SPP-packed columns can deliver that desire; however, this comes at the cost of elevated backpressure. Chromolith® WP 300 RP-18, 2 mm I.D. columns provide UHPLC efficiencies, but at nearly 1/10th the backpressure.

Chromatographic conditions

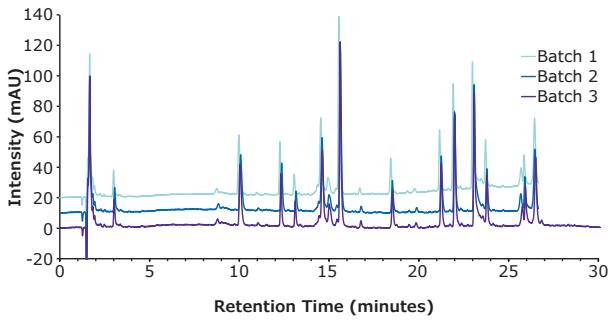
Columns:	Chromolith® WP 300 RP-18 100-2 mm (1.52370.0001) SPP, C18, 160 Å, 2.0 µm, 100-2.1 mm FPP, C18, 300 Å, 1.7 µm, 100-2.1 mm
Mobile phase:	A: water (0.1% TFA) B: acetonitrile (0.08% TFA)
Gradient:	4% B to 60% B in 10 minutes
Flow rate:	0.38 mL/min
Detection:	UV, 220 nm
Temperature:	30 °C
Injection volume:	0.5 µL
Sample:	HPLC Protein Mix 1 mg/mL, water 1) Ribonuclease 2) Cytochrome C 3) Holo-Transferrin 4) Apomyoglobin



Chromolith® WP 300 RP-18 columns exhibit excellent batch-to-batch reproducibility, as demonstrated below with the same peptide map generated for cytochrome C using three different Chromolith® WP 300 RP-18 columns across three different batches.

Chromatographic conditions

Columns:	Chromolith® WP 300 RP-18 100-2 mm (1.52370.0001)		
Mobile phase:	A: acetonitrile 0.08% (v/v) TFA B: water 0.1% (v/v) TFA		
Gradient:	**Time**	**%A**	**%B**
	0	5	95
	25.0	30	70
	30.0	30	70
Flow rate:	0.190 mL/min		
Pressure:	18 bar		
Detection:	Vanquish DAD 20 Hz, UV		
Detector cell:	LightPipe 10 mm		
Temperature:	30 °C		
Injection volume:	0.2 µL		
Sample:	Rapid Trypsin Digestion with SOLu-Trypsin Rapid Digestion Kit 2.5 mg Cytochrome C was added in a PCR vial and dissolved in 320 µL Rapid Trypsin Digestion Buffer. In the solution was 80 µL SOLu-Trypsin added and incubated at 60 °C for 1 hour in a Thermomixer. The digestion was quenched by adding 12 µL of hydrochloric acid 32 %.		



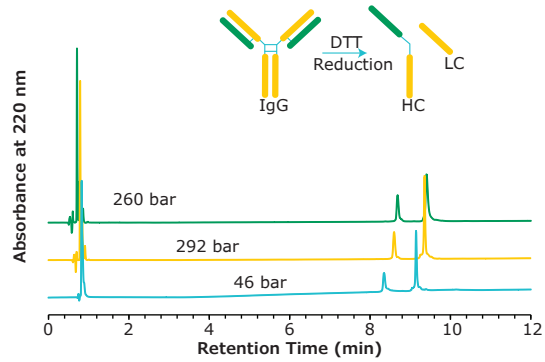
Antibody Fragment Analysis on Chromolith® WP 300 RP-18

Fragment analysis of a monoclonal antibody (mAb), also called middle-up analysis, is a useful technique in characterizing mAb domains without the inherent complexity of a peptide map. High efficiency is needed here to resolve subtle, structural variants of the mAb domains. The Chromolith® WP 300 RP-18 column is able to achieve the same separation efficiency and sensitivity as sub-2 µm FPP and 2.0 µm SPP-packed columns but at only 20% of the backpressure of those columns.

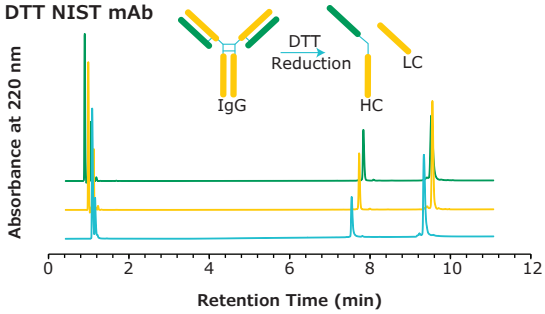
Chromatographic conditions

Columns:	Chromolith® WP 300 RP-18, 2 mm I.D. (1.52370.0001) SPP, C18, 160 Å, 2.0 µm, 100-2.1 mm FPP, C18, 300 Å, 1.7 µm, 100-2.1 mm
Mobile phase:	A: Water (0.1% (v/v) TFA) B: Acetonitrile (0.08% (v/v) TFA)
Gradient:	0 min 20% B 1 min 20% B 9 min 45% B
Detection:	UV, 220 nm
Temperature:	80 °C
Injection volume:	1.0 µL
Flow rate:	380 µL/min
Sample:	SigmaMAb, 2 mg/mL (SiLu™ Lite Universal Antibody) NISTmAb, NIST® RM 8671
Sample preparation:	DTT digest: 60 µL of 40 mM Dithiothreitol (DTT) solution was added in a PCR vial, 40 µL mAb was added and incubated at 37 °C for 30 minutes creating LC and HC parts of the antibody. IdeS digest: 4 µL IdeS-Protease and 56 µL water were added in a PCR vial, 40 µL mAb was added and incubated at 37 °C for 30 minutes creating F(ab')2 and scFc parts of the antibody.

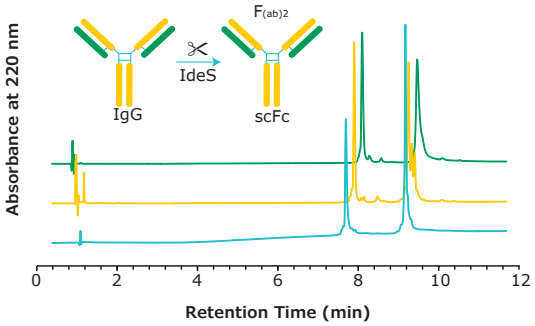
DTT SigmaMAb



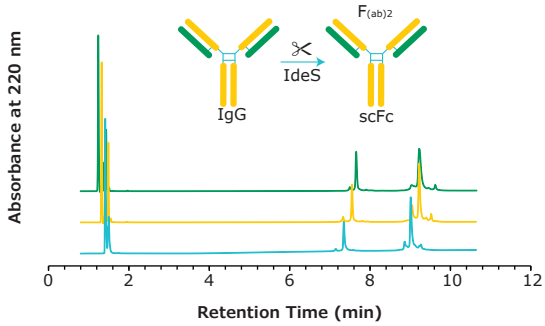
DTT NIST mAb



IdeS SigmaMAb



IdeS NIST mAb



— 100-2.1 mm SPP C18 160 Å
— 100-2.1 mm FPP 1.7 µm C18 300 Å
— 100-2 mm Chromolith® WP 300 RP-18

— 100-2.1 mm 2 µm SPP C18 160 Å
— 100-2.1 mm FPP 1.7 µm C18 300 Å
— 100-2 mm Chromolith® WP 300 RP-18

Chromolith® WP 300 Protein A – Fast Monoclonal Antibody Quantitation

Affinity chromatography is a selective technique which takes advantage of 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. The 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).

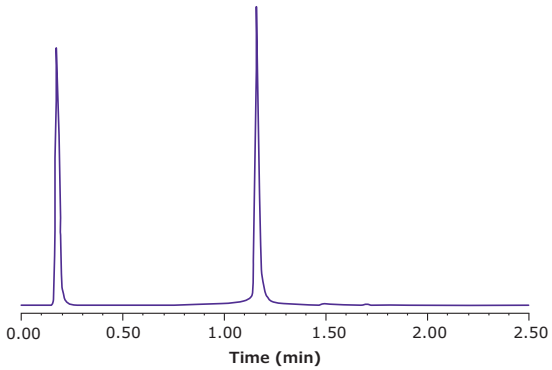
Columns provide extremely fast separations and could be used longer, minimizing analysis costs. In addition, the 2 mm I.D. format enables even more sensitivity for more accurate and precise measurements of titer concentration.

Chromolith®	WP 300 Protein A 2 and 4.6 mm I.D
Base material:	Monolithic silica Type B
Phase chemistry:	Protein A
Usp classification	N/A
Macropore size:	2 µm
Mesopore size:	300 Å
Mesopore volume:	1 mL/g
Porosity:	80%
Surface area:	120 m²/g
Carbon load:	NA
Endcapped:	No
Minimum efficiency (n/m):	n.a.
Ph rage:	2 - 7.5
Max temperature:	45 °C
Shipping solvent:	50 mM sodium phosphate + 150 mM sodium chloride pH7.4 +0.09% sodium azide
Storing	50 mM sodium phosphate + 150 mM sodium chloride pH 7.4 +0.09% sodium azide

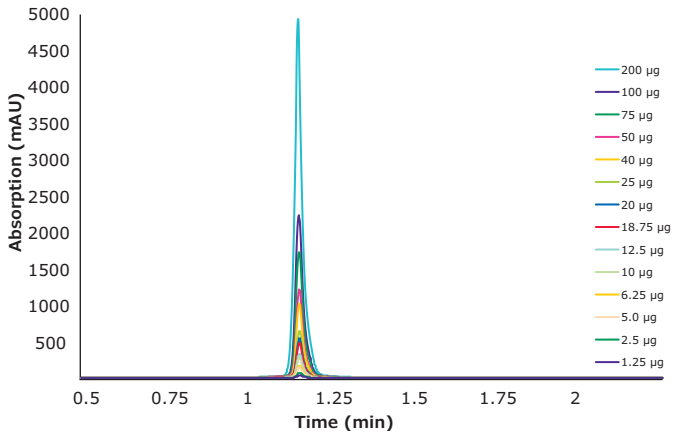
Separation of monoclonal antibodies

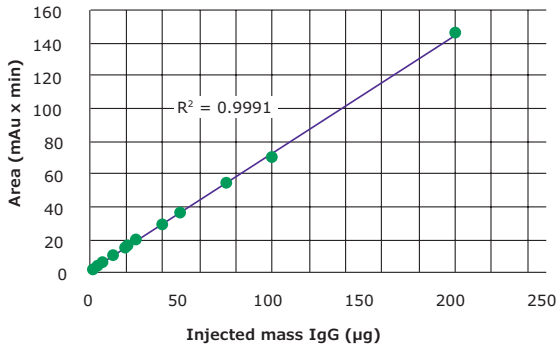
Chromatographic conditions

Eluent A:	100 mM sodium phosphate pH 7.4		
Eluent B:	100 mM sodium phosphate pH 2.5		
Flow rate:	2.0 mL/min		
Detection:	280 nm		
Temperature:	25 °C		
Injection volume:	10 µl		
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



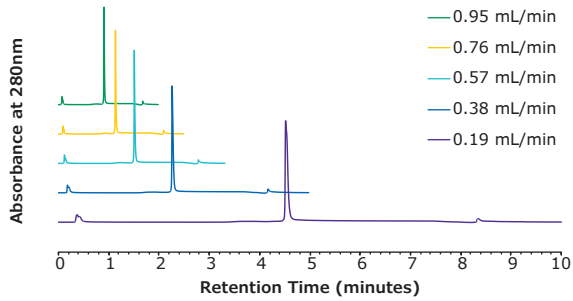


Constant binding efficiency at any flow rate

- 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

Monolithic column provides low column backpressure at high flow rates

Flow rate	Unbound area	IgG area	Pressure (bar)
0.19 mL/min	11%	89%	5 bar
0.38 mL/min	11%	89%	10 bar
0.57 mL/min	11%	89%	15 bar
0.76 mL/min	11%	89%	19 bar
0.95 mL/min	11%	89%	24 bar



Reproducibility and Stability

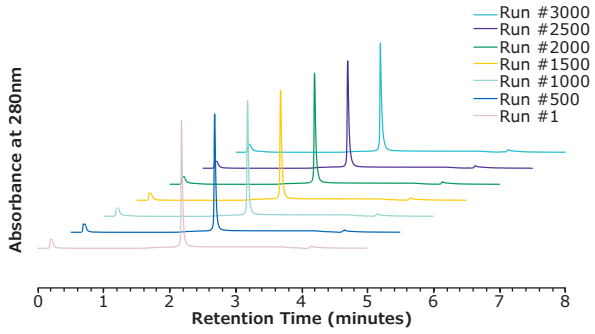
Chromolith® WP 300 Protein A column offers high stability and reproducibility

More than 80,000 column volumes of 100 mM sodium phosphate buffer including 6,000 pH shifts were applied to the column. 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.

Stability Chromolith® WP 300 Protein A 25-2 mm with IgG

Chromatographic conditions

Column:	Chromolith® WP 300 Protein A 25-2 mm (1.52358.0001)		
Mobile phase:	A. 100 mM Disodium hydrogen phosphate, pH 7.4 B. 100 mM Disodium hydrogen phosphate, pH 2.5		
Gradient:	Time	%A	%B
	0.00	100	0
	0.50	100	0
	0.55	0	100
	2.60	0	100
	2.65	100	0
	5.00	100	0
Detection:	UV, 280 nm		
Temperature:	25 °C		
Flow rate:	0.38 mL/min		
Injection Volume:	2 µL		
Sample:	1 mg/mL IgG (Gammanorm)		



Parameter	RSD
Retention time	< 0.3%
Peak Area	< 3.2%

Effect of instrument setup on bind/elute on Protein A 2 mm I.D. columns

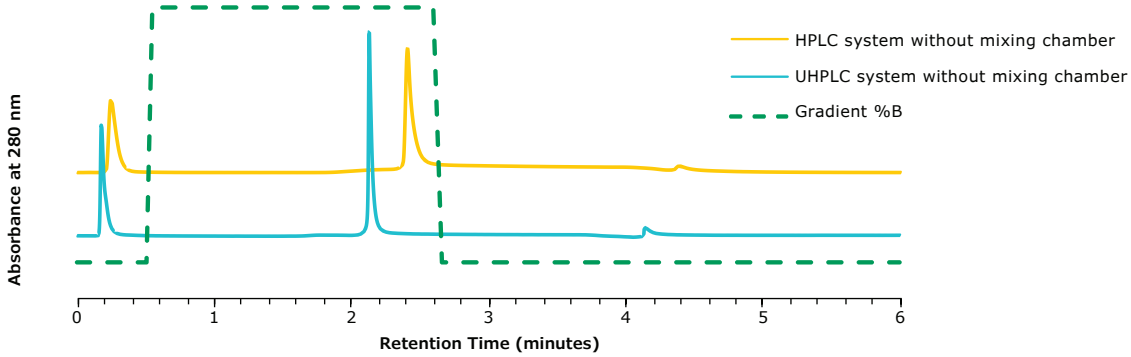
For a fast titer determination of monoclonal antibodies, a step gradient is used with a fast and precise pH change. Especially for small I.D. columns, instrument setup can have a big influence on peak shape and retention time.

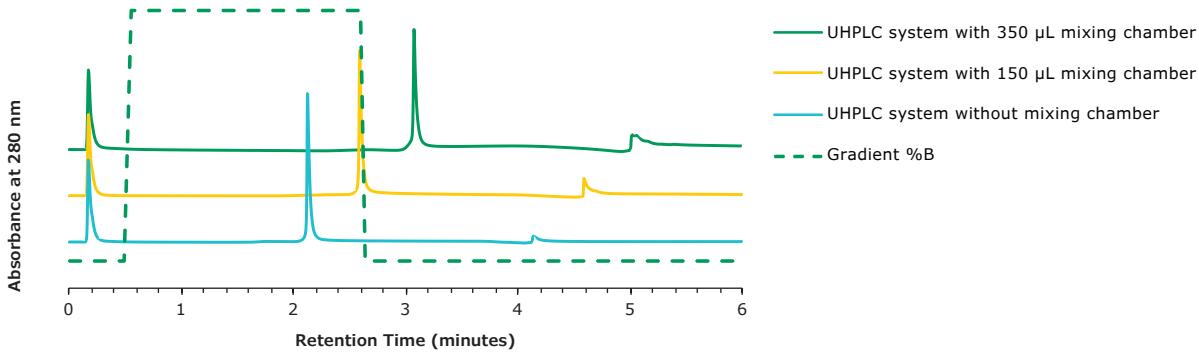
In this example, the separation is carried out on an HPLC and a UHPLC instrument with and without mixing chamber; all instruments are fully biocompatible.

Due to the step gradient, mixing is not required and hinders a fast elution.

Chromatographic conditions

Column:	Chromolith® WP 300 Protein A 25-2 mm (1.52358.0001)	
Mobile phase:	A: 100 mM Disodium hydrogen phosphate, pH 7.4 B: 100 mM Disodium hydrogen phosphate, pH 2.5	
Flow rate :	0.38 mL/min	
Gradient:	**Time**	**%A**
	0	100
	0.5	100
	0.55	0
	2.60	0
	2.65	100
	6.00	100
Detection	UV, 280 nm	
Temperature	25 °C	
Volume	2 µL	
Sample	IgG, 1 mg/mL gammanorm	





Comment	Peak Width 50%	Peak Width 10%	Area mAU*min	Height mAU	TUSP	Pressure bar
UHPLC system without mixer	0.022	0.061	5.0	161	2.12	11
UHPLC system with 150 µL mixer	0.022	0.065	5.4	157	2.06	11
UHPLC system with 350 µL mixer	0.023	0.076	5.5	131	1.46	11
HPLC system without mixer	0.047	0.149	13.0	98	4.90	11

Chromolith® WP 300 Epoxy – Create Your Own Column Chemistry

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. With the 2 mm I.D. column geometry, improved efficiency, sensitivity, and MS-compatibility can be realized.

Chromolith®	WP 300 Epoxy 2 and 4.6 mm I.D.
Base material:	Monolithic silica Type B
Phase chemistry:	Epoxy
Macropore size:	2 µm
Mesopore size:	300 Å
Mesopore volume:	1 mL/g
Porosity:	80%
Surface area:	120 m²/g
Carbon load:	NA
Endcapped:	No
Minimum efficiency (n/m):	60000 N/m
Typical efficiency (n/m):	80000 N/m
Ph rage:	2.0 – 8.0
Max temperature:	60 °C
Shipping solvent:	100% 2-Propanol; wash with 20 CV deionized water before immobilization.
Storing	2-Propanol

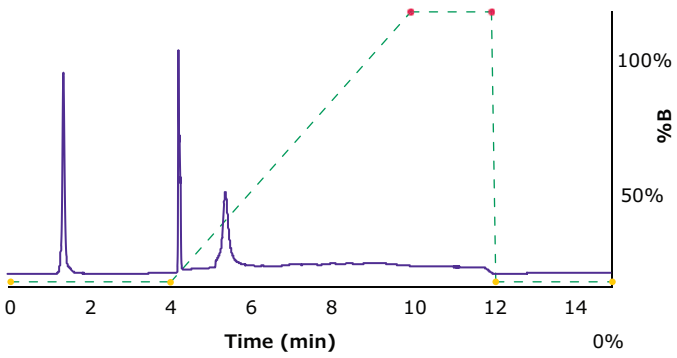
Immobilization of iminodiacetic acid

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-4.6 mm column
- 1 g imidodiacetic acid dissolved in 25 mL 50 mM Disodium hydrogen phosphate 1.9 M Ammonium sulfate, pH 8.0
- Immobilization for 72 hours at 0.2 mL/min
- No quenching of remaining epoxide functions
- Column was flushed with Copper (II) sulfate solution before separation

Immobilization of imidodiacetic acid– Affinity chromatography

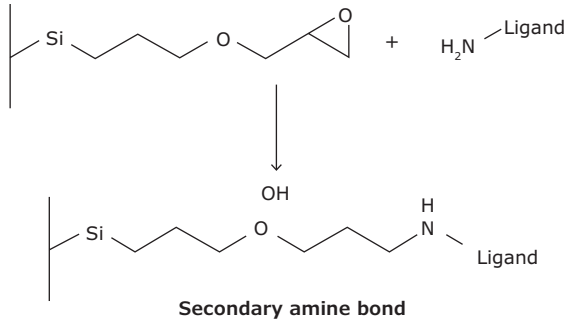
Chromatographic conditions

Eluent A:	20 mM sodium phosphate + 100 mM sodium chloride pH 7.0
Eluent B:	Eluent A + 200 mM imidazole
Flow rate:	1.0 mL/min
Detection:	280 nm
Temperature:	25 °C
Injection volume:	20 µL



Immobilization via epoxide functions

The reaction mechanism for a direct immobilization using the epoxide groups is shown below. 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.



Secondary amine bond

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

Additionally, other parameters influence the coupling yield of the 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.

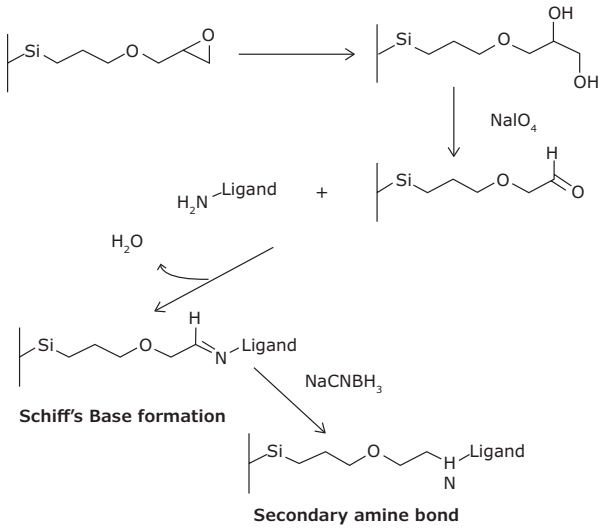
After coupling of the ligand, residual epoxide groups on the monolithic surface must be quenched to avoid undesired backbone interactions with the analytes. Suitable reagents for quenching are 1 M glycine or 1 M urea. If the used ligand is stable at low pH, it is possible to use 150 mM phosphoric acid 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 column volumes of 50 mM sodium phosphate + 1.9 M ammonium sulfate, pH 8.0, 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 of 50 mM sodium phosphate + 1.9 M 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 1 M 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 the starting mobile phase.

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.



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 column volumes of 2% sulfuric acid using a flow rate of 2.0 mL/min at room temperature. The column end is connected directly to waste.
- Wash the column with at least 15 column volumes of deionized water at a flow rate of 2.0 mL/min. The column end is still connected to waste.
- The resultant diol groups are oxidized by 100 column volumes of 100 mM 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 waste.
- Again, wash the column with at least 15 column volumes of deionized water at a flow rate of 2.0 mL/min. The column end is still connected to waste.
- • Dissolve the desired amount (1–10 mg/mL) of ligand and 5 mM sodium cyanoborohydride in 25 mL of 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 20 mM sodium cyanoborohydride, dissolved in 50 mM sodium phosphate, pH 3.0, for 60 column volumes at 2.0 mL/min and room temperature. The column end is directly connected to waste.
- Finally, equilibrate the column with starting mobile phase. 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

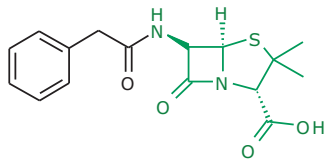
After immobilization, the column is ready for use for the desired purpose of the immobilized ligand. The type of required solvent or buffers depends on the type of ligand used. Chromolith® WP Epoxy columns can be used with all commonly used HPLC grade organic solvents, with the following restrictions. 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 if the appropriate pH range is not exceeded. Nevertheless, be careful not to expose the column to conditions which could cause denaturation of the 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 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, it is recommended to apply one or more sample preparation methods prior to separation (i.e. solid phase extraction, filtration, centrifugation, etc.). Make sure that the 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

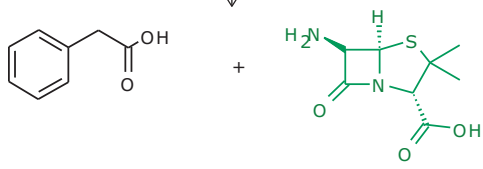
Immobilization of penicillin acylase

- 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 + 1.9 M ammonium sulfate pH 8.0
- Immobilization for 24 hours at 0.2 mL/min
- Quenching of remaining epoxide groups with glycine



Penicillin G

Penicillin G Acylase

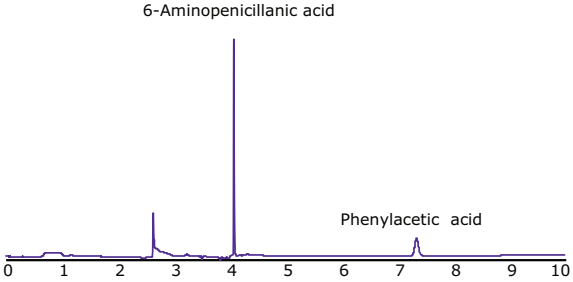


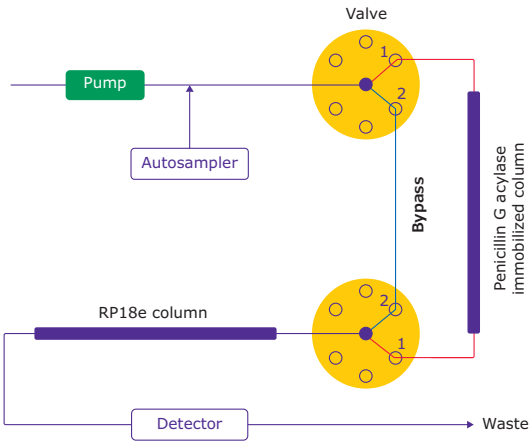
Phenylacetic acid + **6-Aminopenicillanic acid**

Immobilization of penicillin acylase – Enzymatic bioreactor

Chromatographic conditions

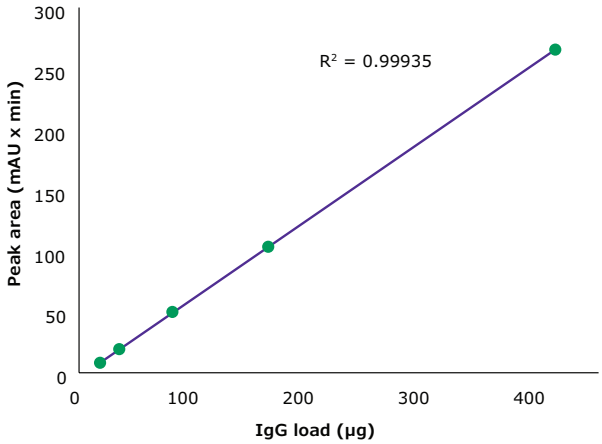
Flow rate:	1.0 mL/min				
Temperature:	23 °C				
Detection:	UV 225 nm				
Eluent A:	10 mM sodium phosphate pH 7.0				
Eluent B:	10 mM sodium phosphate pH 3.0				
Eluent C:	Acetonitrile				
Sample:	1.0 µL Penicillin G (3.5 mg/mL)				
Gradient:	**Time**	**Valve**	**A**	**B**	**C**
	0	1	100	0	0
	2	1	100	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

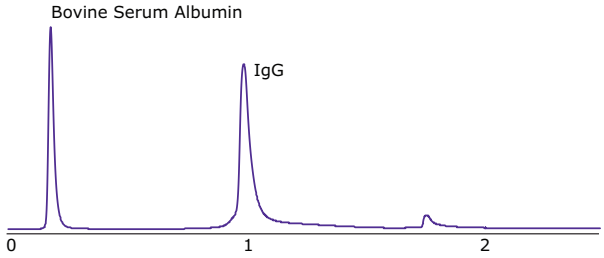
- According to Epoxy method
- Chromolith® WP 300 Epoxy 25-4.6 mm column
- 12.5 mg Protein G dissolved in 6.25 mL 50 mM sodium phosphate + 1.9 M ammonium sulfate pH 8.0
- Immobilization for 4 hours at 0.2 mL/min
- Quenching of remaining epoxide functions with glycine



Immobilization of protein G – Affinity chromatography

Chromatographic conditions

Eluent A:	100 mM sodium phosphate pH 7.4		
Eluent B:	100 mM sodium phosphate pH 2.5		
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



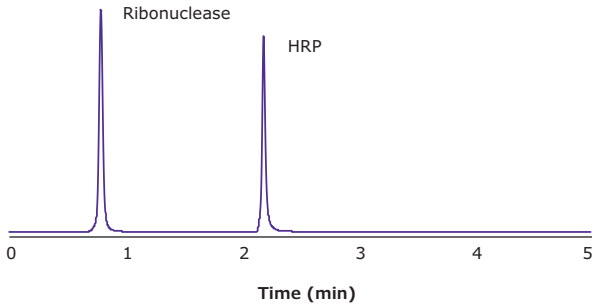
Immobilization of concanavalin A

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-4.6 mm column
- 50 mg concanavalin A from Jack bean dissolved in 25 mL 50 mM Disodium hydrogen phosphate, 1 mM Calcium chloride + 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 concanavalin A – Affinity chromatography

Chromatographic conditions

Eluent A:	50 mM sodium acetate, 200 mM sodium chloride, 1 mM calcium chloride pH 5.3		
Eluent B:	Eluent A + 100 mM 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 HSA

- According to Schiff Base mechanism
- Chromolith® WP 300 Epoxy 100-4.6 mm column

1. Hydrolysis

Epoxide groups were hydrolyzed to diols with 2% sulfuric acid using a flow rate of 2.0 mL/min at room temperature for 2 hours. Afterwards the column was washed with 20 mL water.

2. Oxidation

3.86 g Sodium periodate were diluted in 180 mL water:methanol (4:1). Resulting diol groups from step 1 were oxidized to aldehyde with the 100 mM sodium periodate solution with a flow rate of 2.0 mL/min in 85 min. Again, the column was washed with 20 mL water.

3. Immobilization

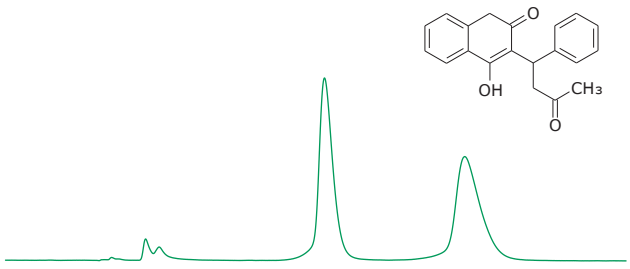
252.64 mg Human Serum Albumin and 8.32 mg sodium cyanoborohydride were dissolved in 25 mL of 0.05 M disodium hydrogen phosphate + 1.9 M ammonium sulfate, pH 8.0. The protein solution was pumped through the column, circulating at a flow rate of 0.2 mL/min for 24 hours.

4. Reduction

15.49 mg sodium cyanoborohydride are dissolved in 120 mL of 50 mM sodium hydrogen phosphate, pH 3.0, and is pumped through the column to reduce the remaining carbonyl groups at a flow rate of 2.0 mL/min for 50 min. Afterwards the column is washed with 0.1 M disodium hydrogen phosphate, pH 7.4.

Chromatographic conditions

Column:	Chromolith® WP 300 HSA 100-4. 6mm
Mobile phase:	10 mM Disodium hydrogen phosphate, pH 6.0/Acetonitrile 70/30
Flow rate:	1.0 mL/min
Detection:	UV, 214 nm
Temperature:	25 °C
Injection volume:	1.0 µL
Sample:	Warfarin 0.6 mg/mL in methanol

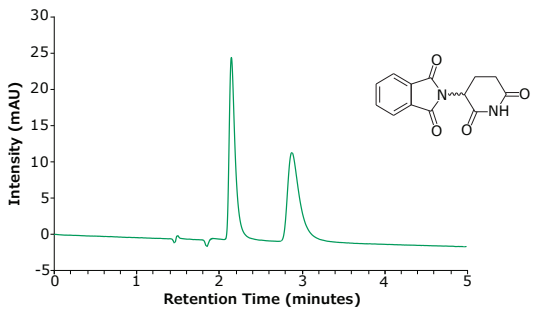


Immobilization of Vancomycin

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-2 mm column
- 50 mg Vancomycin are dissolved in 5 mL 50 mM disodium hydrogen phosphate + 1.9 M ammonium sulfate, pH 8.0, buffer (Concentration Ligand: 10 mg/mL)
- Immobilization for 24 hours at 0.1 mL/min
- Quenching of remaining epoxide functions with glycine

Chromatographic conditions

Column:	Chromolith® WP 300 Vancomycin 100-2 mm
Mobile phase:	10 mM Disodium hydrogen phosphate, pH 7.0
Flow rate:	0.19 mL/min
Detection:	UV, 214 nm
Temperature:	25 °C
Injection volume:	0.2 µL
Sample:	Thalidomide, 1 mg in 10 mL of 0.1 M hydrochloric acid



Selection of Column Chemistry / Selectivity

Chromolith® columns are available in Capillary column dimensions, as analytical columns suitable for UHPLC and HPLC applications and as (Semi) Preparative columns. Depending on the scale, sensitivity and/or efficiency required, the most appropriate column inner dimension and column length can be selected.

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
	-			Loadability				+
	+			Sensitivity				-
	+			Solvent saving				-

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

The table below can help you to choose the most suitable column dimension for your application need:

Column Dimension Length x I.D. in mm	Application	Reason
0.05 – 0.2 mm I.D.	Nano-LC (MS) Very high detection sensitivities / (OMICS)	Capillary column for very low injection volumes and low peak dispersion
5 x 2 / 3 / 4.6 10 x 4.6 / 10	Guard-columns	Protection from contamination (mechanical and sample)
25 / 50 x 2 / 3 / 4.6	Method development Rapid HPLC / UHPLC	Short run times Rapid separation and equilibration
50 / 100 - 2	High detection sensitivity (Mass selectivity) Low solvent consumption	Low injection volumes and low peak dispersion
50 / 100 - 3	Incresed detection sensitivity Reduced solvent consumption	Lower injection volumes
100 / 150 x 4.6	Standard column	Adequate performance for most applications
100 x 10	Semi-preparative use	Very high loadability for purification steps in mg range
100 x 25	Preparative use	Very high loadability for purification steps in g range

Chromolith® CapRod® Capillary columns
0.05 mm, 0.1 mm and 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 trait allows for higher flow rates, improving the speed and quality of biomolecule characterization. To answer the growing interest in micro and nano-HPLC, a wide range of outstanding monolithic silica capillaries with a variety of internal diameters, bonded phases, pore structures, and lengths are available.

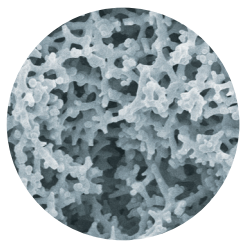
Why choose capillary Chromolith® columns?

- Higher flow rates than particle-packed capillary columns
- 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® 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

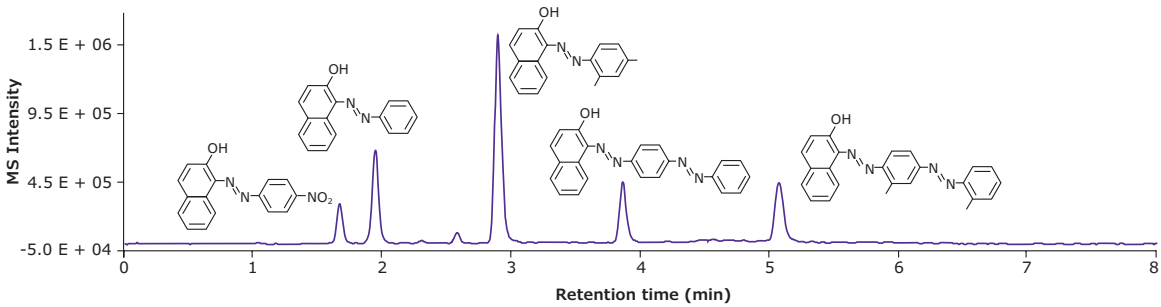
Recommended use	RP-18e 150 x 0.05 mm	RP-8e 150 x 0.1 mm	RP-18e 50 x 0.1 mm Trap	RP-18e 150 x 0.1 mm	RP-18e 300 x 0.1 mm	RP-18e 150 x 0.1 mm HR	RP-18e 50 x 0.2 mm Trap	RP-18e 150 x 0.2 mm	RP-18e 150 x 0.2 mm HR
Separation of small molecules	●		●	●	●	●	●	●	●
– of peptides	●	●	●	●	●	●	●	●	●
– of proteins		●							
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

Chromatographic conditions

Column:	Chromolith® CapRod® RP-18 endcapped 150 x 0.1 mm (1.50402.0001)
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

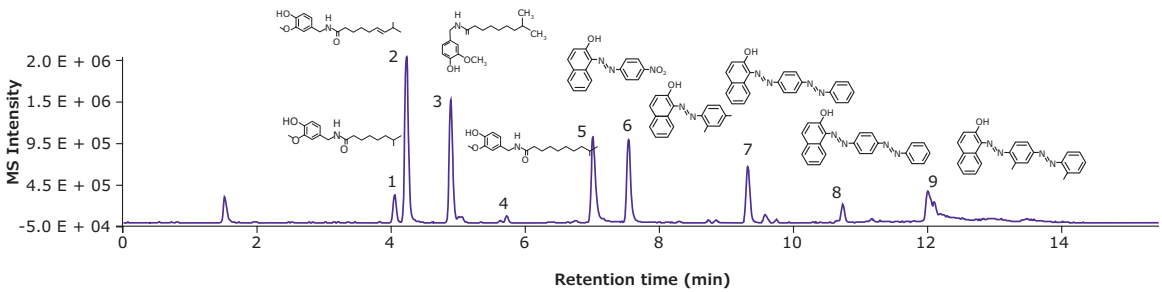


Separation example: Sudan dyes and capsaicinoids in hot chili sauce

Chromatographic conditions

Column:	Chromolith® CapRod® RP-18 endcapped 150 x 0.1 mm (1.50402.0001)
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 2 mm, 3 mm and 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 aspect allows for 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)
- Increased column performance by column coupling

Chromolith® 2 mm I.D. columns
UHPLC performance at low column backpressure

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 I.D. columns truly unique. Excellent, ultra-fast results are obtained, not only in UHPLC and UPLC® instruments, but equally well in all standard HPLC systems with low dead volume. Chromolith® 2 mm I.D. 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.

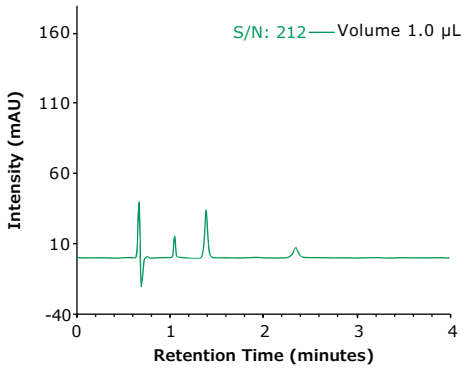
Increase sensitivity and save solvents with 2 mm I.D. Chromolith® HR columns

The use of smaller inner diameter (I.D.) columns results in decreased solvent usage and increase sensitivity.

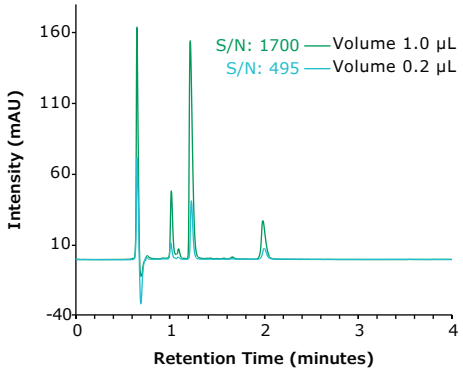
The peak response is increased with small I.D. columns – the peak height increases as the column inner diameter decreases. The peak response for 2 mm I.D. columns is almost three times higher in comparison to 4.6 mm I.D. columns. This trait is beneficial when analyzing mass limited samples, typically used in LC-MS applications.

The direct comparison of the same separation on Chromolith® HR 4.6 and a 2 mm I.D. columns demonstrate a significant improvement of the signal to noise ration (S/N)

column I.D.: 4.6 mm



column I.D.: 2 mm



Chromatographic conditions

Column:	Chromolith® HighResolution RP-18e 100-4.6 mm (1.52022.0001) Chromolith® HighResolution RP-18e 100-2 mm (1.52322.0001)
Mobile phase:	A: Acetonitrile B: 20 mM Sodium dihydrogen phosphate, pH 4.5 C: Methanol
Isocratic:	A/B/C 65/25/10 (v/v/v)
Flow rate 4.6 mm:	2.0 mL/min
Flow rate 2 mm:	0.380 mL/min
Pressure 4.6 mm:	58 bar
Pressure 2 mm:	66 bar
Detection:	Vanquish DAD 20 Hz, UV, 210 nm
Detection cell:	LightPipe 10 mm
Temperature:	23 °C
Injection volume 4.6:	1.0 µL
Injection volume 2:	0.2 µL (1.0 µL)
Sample:	Latanoprost 50 µg/mL Pfizer (without sample preparation)

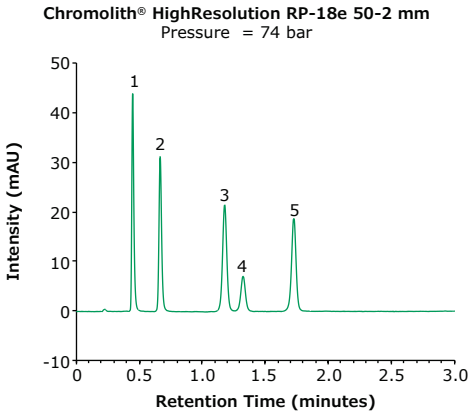
Simplifiy your UHPLC (MS) workflow

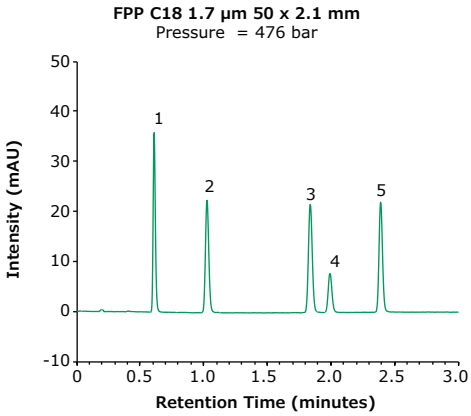
Chromolith® HighResolution 2 mm I.D. columns enable true UHPLC performance with very low column backpressure with the benefit of significantly extended column life-time and minimal need for sample preparation.

Fast separation of Profens on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18e 50-2 mm (1.52321.0001)		
	FPP C18, 1.7 µm, 50-2.1 mm		
Mobile phase:	A: 100% acetonitrile B: 20 mM Phosphate buffer, pH 4.5		
Gradient:	Time/min	%A	%B
	0	40	60
	0.1	40	60
	2.0	60	40
Flow rate:	600 µL/min		
Pressure:	74 / 476 bar		
Detection:	DAD 20Hz UV, 220 nm		
Detector cell:	LightPipe 10 mm		
Temperature:	23 °C		
Injection volume:	0.2 µL		
Sample:	Indoprofen	5.0 mg	
	Ketoprofen	5.9 mg	
	Fenoprofen	5.2 mg	
	Fluorbiprofen	3.4 mg	
	Ibuprofen	6.1mg	
	dissolved in 100 mL A/B 50/50		

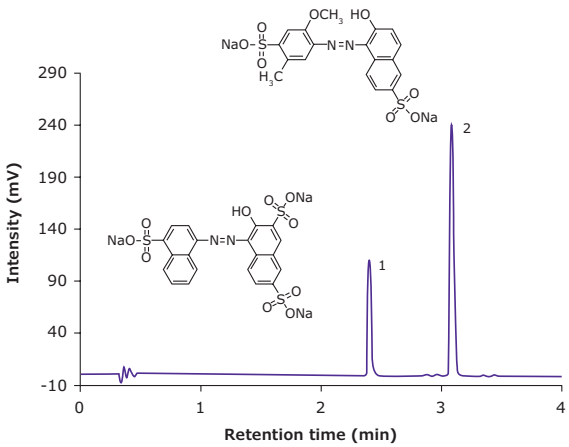




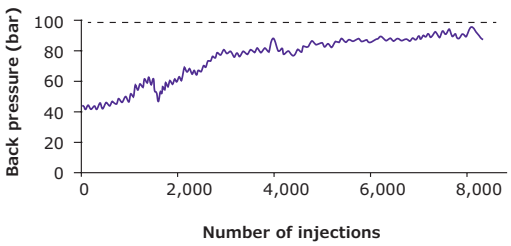
Food colorants in an alcoholic beverage

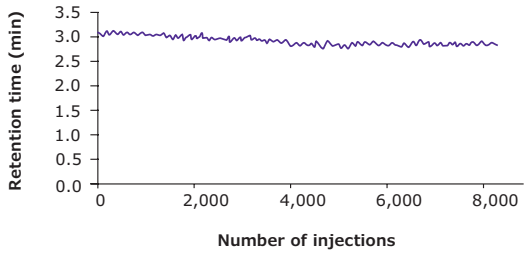
Chromatographic conditions

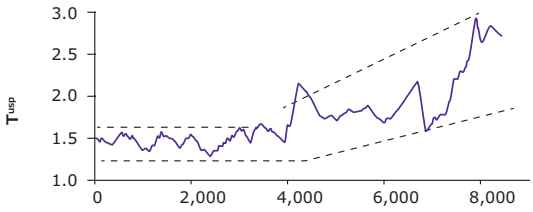
Column:	Chromolith® Performance RP-18 endcapped 50 x 2.0 mm (1.52007.0001)		
Mobile phase (v/v):	A: Acetonitrile B: 0.1 % Phosphoric acid in water		
Gradient program:	**Time**	**% A**	**% B**
	0.00 – 0.50 min	0	100
	0.50 – 4.50 min	0 → 50	100 → 50
	4.50 – 5.00 min	50 → 95	50 → 5
	5.00 – 6.00 min	95	5
	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.		

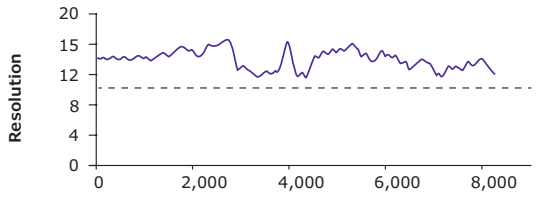


Analysis of colorants in rum. Two food colorants in rum, E 123 (Amaranth) and E 129 (Allura Red AC), were analyzed to illustrate the long-term performance and method robustness of Chromolith® columns. More than 8,000 samples (total volume of injected sample: 16 mL + 30 L mobile phase) were analyzed on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

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 trait 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
- Efficient 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.

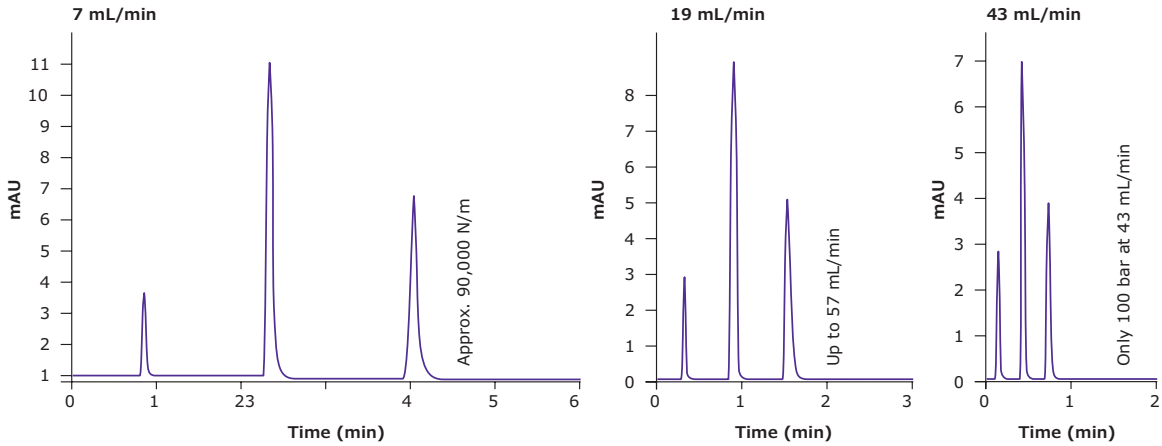
Chromolith® SemiPrep 10 mm i.d. columns combine high separation speed with excellent performance. This makes them the perfect alternative to particulate columns of 10 mm i.d. (and even 21.2 mm i.d.). They have the same bimodal porous silica rod structure as Chromolith® analytical columns with an internal 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 RP-18e, 100-10 mm

Chromatographic conditions

Column:	Chromolith(R) SemiPrep RP-18e 100-10 (1.52016.0001)
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 loading 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

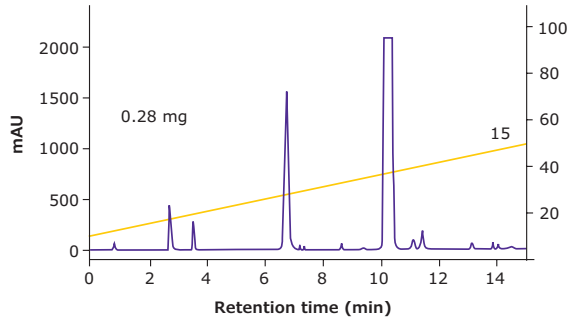
Columns	Column dimension (L/D)	Typical flow rate	Loading capacity	Loading volume
Analytical column	100 – 4.6 mm	2 mL/min	5 mg	5 – 50 µL
Preparative column	100 – 25 mm	60 mL/min	150 – 370 mg	100 – 1,500 µL

Analytical separation

Chromolith® Performance RP-18e 100-4.6 mm

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100-4.6 mm (1.02129.0001)
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

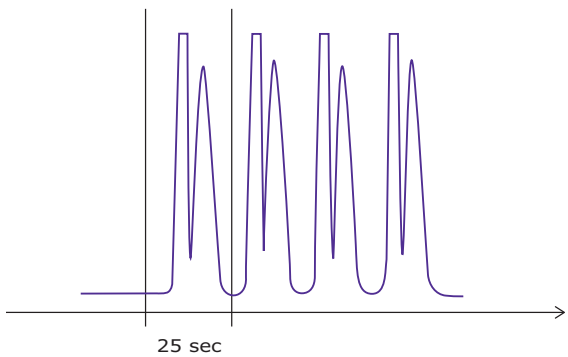


Separation of diastereomers with a productivity of 861 g/d

Chromolith® Prep Si 100-25 mm

Chromatographic conditions

Column:	Chromolith® Prep Si 100-25 mm (1.25252.0001)
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

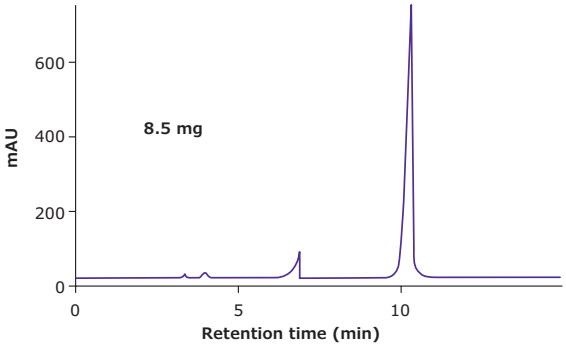


Preparative separation

Chromolith® Prep RP-18e 100-25 mm

Chromatographic conditions

Column:	Chromolith® Prep RP-18 endcapped 100-25 mm (1.25252.0001)
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

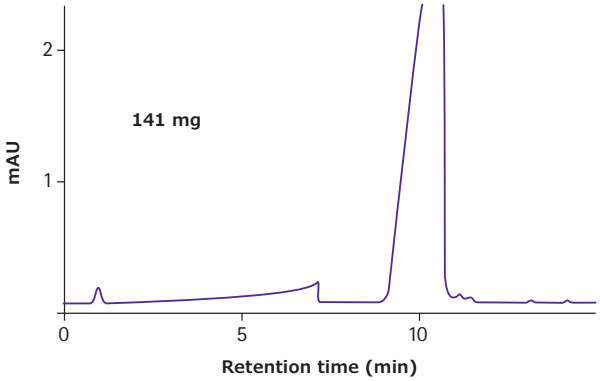


Preparative separation

Chromolith® Prep RP-18e 100-25 mm

Chromatographic conditions

Column:	Chromolith® Prep RP-18 endcapped 100-25 mm (1.25252.0001)
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

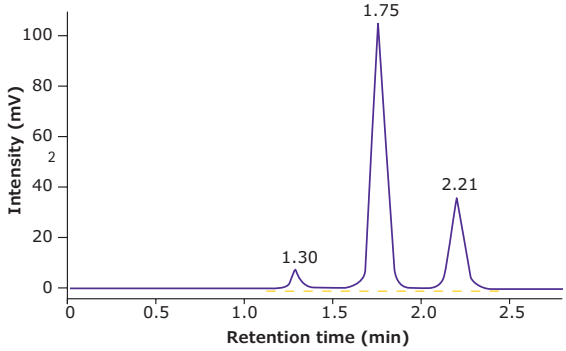
Chromolith® Prep columns can be operated at a flow rate of up to 400 mL/min, and pressures of up to 100 bar. This is a tenfold increase in flow rate compared to particulate columns of an equivalent size.

Separation at different flow rates 40 and 390 mL/min

Chromolith® Prep Si 100-25 mm

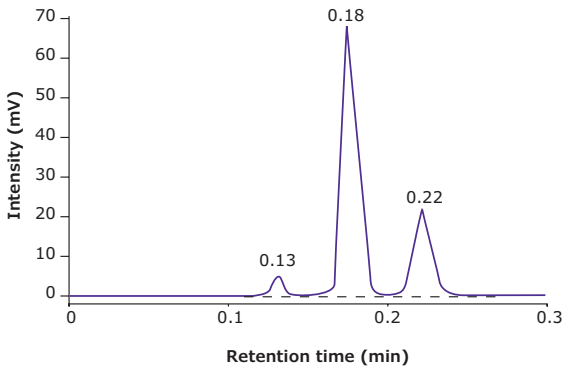
Chromatographic conditions

Column:	Chromolith® Prep Si 100-25 mm (1.25251.0001)
Solvent:	n-Heptane/Dioxane (80/20 v/v)
Flow rate:	40 mL/min
Sample:	1. Toluene 2. Dimethylphthalate 3. Dibutylphthalate



Chromatographic conditions

Column:	Chromolith® Prep Si 100-25 mm (1.25251.0001)
Solvent:	n-Heptane / Dioxane (80/20 v/v)
Flow rate:	390 mL/min
Sample:	1. Toluene 2. Dimethylphthalate 3. Dibutylphthalate



Application Notes

Food & Beverage

- HMF in Honey on Chromolith® HighResolution RP-18 endcapped
- Sulfonamides in Honey on Chromolith® HighResolution RP-18e
- Sulfonamides on Chromolith® HighResolution RP-18e
- Fast Separation of Sulfonamide on Chromolith® HighResolution RP-18e at high flow rate
- Neonicotinoids in Honey using a Chromolith® RP-18 HPLC column and UV Detection
- LC-MS/MS Analysis of Pesticides in Kidney on Chromolith® Performance RP-18e 50-2 mm
- LC-MS/MS Analysis of Pesticides in Kidney on Chromolith® CapRod® RP-18e
- Xylose, Fructose, Glucose and Saccharose on Chromolith® NH_2
- Monosaccharides - Ribose, Xylitol, Mannose on Chromolith® NH_2
- Disaccharides Turanose, Lactulose, and Melibiose on Chromolith® NH_2
- Linear Oligosaccharides on Chromolith® NH2
- Glyocoalkaloids - Solanine and Cacoline on Chromolith® NH2
- Disaccharides Turanose, Lactulose, and Melibiose on Chromolith® NH_2
- Bisphenol A in Spiked Milk Powder on Chromolith® HighResolution RP-18e
- Artificial Food Colorants Cochineal Red A and Azorubine in Yogurt on Chromolith® FastGradient RP-18 endcapped
- LC/MS Analysis of Artificial Food Colorants Cochineal Red A and Azorubine in Yogurt on Chromolith® FastGradient RP-18 endcapped
- Artificial Food Colorants (Tartrazine, Amaranth, Carmine Red, Sunset Yellow, Fancy Red) on Chromolith® HighResolution RP-18 endcapped
- Artificial Food Colorants (Amaranth, Carmine Red, Sunset Yellow, Fancy Red, Erythrosine) on Chromolith® HighResolution RP-18 endcapped
- LC/MS Analysis of Annato (or Roucou or Achiote) on Chromolith® FastGradient RP-18 endcapped
- Artificial Food Colorants in Alcopops on Chromolith® FastGradient RP-18 endcapped
- Artificial Food Colorants in Orange Soft Drink on Chromolith® HighResolution RP-18 endcapped
- Artificial Food Colorants in Dried Plums on Chromolith® HighResolution RP-18 endcapped
- Artificial Food Colorants on Chromolith® HighResolution RP-18 endcapped
- Malachite Green and Leucomalachite Green in Salmon Using Chromolith® HighResolution RP-18e with UV Detection
- Carotenoide of Salmon using Chromolith® HighResolution RP-18e
- Flavonoids in Red Wine on Chromolith® HighResolution RP-18e
- LC/MS Analysis of Flavonoids on Chromolith® FastGradient RP-18 endcapped
- LC/MS Analysis of Isoflavones on Chromolith® FastGradient RP-18 endcapped
- Polyphenols in Coffee on Chromolith® Performance 2 x 100-3 mm
- LC/MS Analysis of Green Tea Components on Chromolith® FastGradient RP-18 endcapped
- Capsaicinoids in Chili Extract using Chromolith® HighResolution RP-18e
- Magnesium Ascorbyl Phosphate (MAP) on Chromolith® NH_2 50 - 4.6 mm
- Folic Acid Syrup on Chromolith® HighResolution RP-18e
- B-Vitamins on Chromolith® NH_2 100 - 4.6 mm
- B-Vitamins on Chromolith® NH_2 50 - 4.6 mm
- B-Vitamins on Chromolith® NH_2 25 - 4.6 mm
- HPLC Analysis of Vitamin E on Chromolith HighResolution RP-18e
- Contents of Energy Drinks without SPE
- Caffeine and metabolite analysis on Chromolith® HighResolution RP-18e

Herbs and Nutrition

- UHPLC (Isocratic) Analysis of 14 Cannabinoids on Chromolith® HR RP-18e
- Cannabinoid analysis using Methanol – cost saving method
- Panax Notoginseng
- Radix Puerariae
- Radix Angelica
- Coptis Chinensis (Chinese Goldthread)
- Fructus Forsythiae
- Radix Glycyrrhizae (Liquorice)
- Radix Scrophulariae
- Rheum Palmatum
- Salvia Miltiorrhiza

Pharmaceuticals

- Hormones on Chromolith® HighResolution RP-18e
- Hormones on Chromolith® Performance 100-3 mm
- Sildenafil Citrate and Related Substances on Chromolith® HighResolution RP-18e
- Sildenafil and Impurities on Chromolith® Performance (4.6 mm I.D. columns)
- Sildenafil and Impurities on Chromolith® Performance (3 mm I.D. columns)
- Olmesartan Medoxomil, Amlodipine Besylate, and Hydrochlorothiazide Tablet on Chromolith® HighResolution RP-18e
- Alfuzosin and Related Substances on Chromolith® HighResolution RP-18 endcapped
- Itraconazole and Impurities on Chromolith® HighResolution RP-18e
- Albuterol Tablets - Assay on Chromolith® HighResolution RP-18 endcapped USP38-NF33
- Famotidine Tablets on Chromolith® HighResolution RP-18 Endcapped USP38-NF33
- Finasteride Tablets on Chromolith® HighResolution RP-18 Endcapped USP38-NF33
- Ganciclovir Injection Solution on Chromolith® HighResolution RP-18 endcapped USP38-NF33
- Pioglitazone Hydrochloride on Chromolith® HighResolution RP-18 endcapped USP38-NF33
- Metformin & Pioglitazone on Chromolith® HighResolution RP18e
- Spironolactone on Chromolith® HighResolution RP-18 endcapped USP38-NF33
- Vinblastine Sulfate on Chromolith® HighResolution RP-18 endcapped USP38-NF33
- Ibuprofen & Codeine Phosphate Tablet on Chromolith® HighResolution RP18e
- Albuterol Tablets - Assay on Chromolith® HighResolution RP-18 endcapped
- Diclofenac and Metabolites on Chromolith® High Resolution RP-18e
- USP Dissolution Testing Method (HPLC) for Folic Acid Tablets using a Monolith Column and UV detection
- Carbidopa on Chromolith® HighResolution RP-18e
- Biphenyles on Chromolith® High Resolution RP-18e

Biopharmaceuticals

- Tryptic Digest Analysis on Chromolith® WP 300 RP-18
- Insulin on Chromolith® Performance 100-2 mm
- Peptides on Chromolith® WP 300 RP-18
- Proteins on Chromolith® WP 300 RP-8
- Proteins in SigMatrix Serum Diluent on Chromolith® WP 300 RP-18
- Nucleosides on Chromolith® WP 300 Epoxy immobilized with Cysteine
- IgG on Chromolith® WP 300 Epoxy immobilized with Protein G
- Proteins on Chromolith® WP 300 Epoxy immobilized with Iminodiacetic Acid

Cosmetics

- Rutin in Anti-Aging Skin Cream Formulations using Chromolith® HighResolution RP-18 endcapped Column and UV Detection
- Parabenes in Sun Lotion on Chromolith® HighResolution RP-18e
- Parabenes in Sun Lotion on Chromolith® HighResolution RP-18e

Food & Beverage

Chromolith® columns are just as versatile and, in addition, provide several advantages over particle packed columns especially for “matrix-rich” sample analysis. The much higher tolerance for sample matrix makes monolith columns the preferred choice for Food & Beverage sample analysis. The analysis of matrix-rich samples using Chromolith® columns require much less sample preparation which results in significant time and cost savings. In addition, the simplified workflow is often less error-prone.

To meet customer demands for all compound classes, Chromolith® columns with cyano (CN), diol (OH), amino (NH2) and phenyl stationary phase chemistries are available in addition to the most widely used C18 (RP-18) and C8 (RP-8) phases.

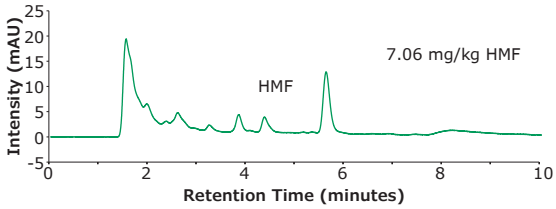
Chromolith® columns can be used with any HPLC instrument and detection technique, i.e. UV, fluorescence (FL), refractive index (RI), electrochemical (EC), evaporative light scattering (ELSD), and mass spectrometric (MS) detection. The choice of column dimension ultimately depends on the type of instrument used, the sample matrix, required sensitivity and separation efficiency (peak capacity) needs. Chromolith® columns are available with 25, 10, 4.6, 3.0 and 2.0 mm I.D. down to 50 µm, all with the intention to ease scaling of methods.

HMF in Honey on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18e 100-2 I.D. (1.52322.0001)
Mobile phase:	A: 10 mM Disodium hydrogen phosphate, pH 3 with phosphoric acid (85%) B: Methanol
Isocratic:	A/B 95/5 (v/v)
Flow rate:	190 µL/min
Detection:	Agilent 1100 VWD @ VIS = 284 nm (micro flow cell; 1.4 µL/7 mm)
Temperature:	25 °C
Injection volume:	2.0 µL
Sample:	Honey from local beekeeper Hydroxymethylfurfural (HMF) analytical standard
Standard solution (10 mg/ml):	10 mg of homogenized HMF was weighed into a 100 mL volumetric flask, dissolved in diluent and diluted up to mark with diluent
Sample preparation	Weigh 10 g of homogenized honey precisely to 0.1 g into a 100 mL beaker and dissolve in 50 mL diluent. Transfer quantitatively to a 100 mL volumetric flask. For protein precipitation and stabilization of HMF, 1 mL of Carrez I and 1 mL of Carrez II solutions were added. The volumetric flask was filled up to mark with diluent and shaken for 1 min. Subsequently, proteins were removed by filtration using a paper filter. 1 mL of the filtrate was re-filtered with a 0.45 µm syringe filter directly into a vial.

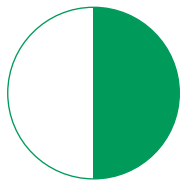
Honey sample after sample preparation



Maximum allowed concentration in the EU for non-tropical honey: 40 mg/kg

For tropical honey: 80 mg/kg

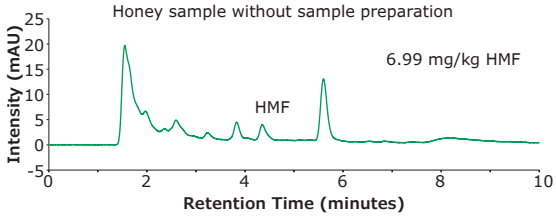
“Made in Germany”: 20 mg/kg



Save 10 min with skipping sample preparation

The Carrez clarification kit (Carrez I and II) is used to remove proteins or fats which might interfere with the analysis. However, with the monolithic column, this is not necessary and offers time savings and reduces labor steps.

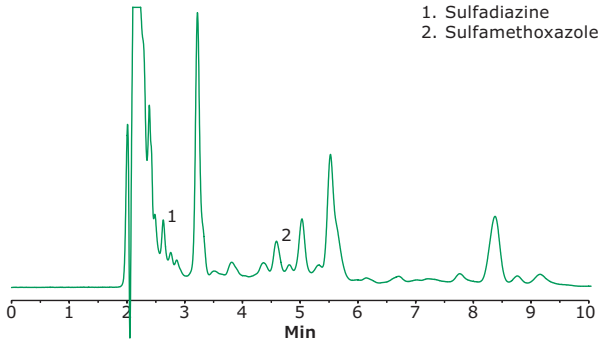
Honey sample without sample preparation



Sulfonamides in Honey on Chromolith® HighResolution RP-18e

Chromatographic conditions

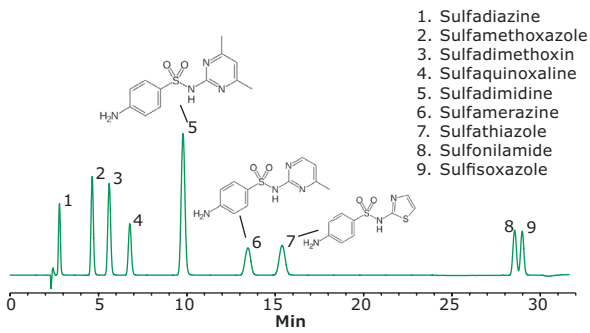
Column:	Chromolith® HighResolution RP-18 encapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] Acetonitrle / 20 mM Ammonium acetate, pH 4.75 (2/98 v/v); [B] Acetonitrle / 20 mM Ammonium acetate, pH 4.75, (32/68 v/v)
Gradient:	0 - 20.7 min, 22% B to 35% B; 20.7 - 27.3 min, 35% B to 75% B; 27.4 – 32 min, 95% B
Flow rate:	0.7 mL/min
Pressure:	522 psi (36 bar)
Column temp.:	40 °C
Injection volume:	5 µL
Detector:	270 nm (cell: 11 µL)
Sample:	Honey 2 g/10 mL (Diluent: Honey: 100% A)
Sample preparation:	2 g honey dissolved in 10 mL 100% mobile phase A acetonitrile:20 mM ammonium acetate, pH 4.75, 2:98 v/v), homogenized in an ultrasonic bath, filtered through 0.2 µm filter prior to analysis



Sulfonamides on Chromolith® HighResolution RP-18e

Chromatographic conditions

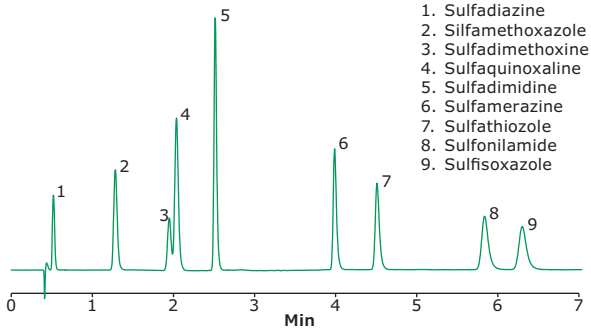
Column:	Chromolith® HighResolution RP-18 encapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] acetonitrile and 20 mM ammonium acetate, pH 4.75 (2:98 v/v); [B] acetonitrile and 20 mM ammonium acetate, pH 4.75 (32:68 v/v)
Flow rate:	0.7 mL/min
Pressure:	522 psi (36 bar)
Column temp.:	40 °C
Injection volume:	5 µL
Detector:	270 nm cell: 11 µL
Sample:	Sulfadiazine 1.1 mg/mL, Sulfamethoxazole 0.8 mg/mL, Sulfadimethoxyine, 0.8 mg/mL, Sulfaquinoxaline 1.0 mg/mL, Sulfadimidine 2.6 mg/mL, Sulfamerazine 1.0 mg/mL, Sulfathiazole 1.5 mg/mL, Sulfanilamide 0.7 mg/mL, Sulfisoxazole 1.0 mg/mL in diluent (Diluent: Acetonitrile/Water 50/50 v/v)



Fast Separation of Sulfonamides on Chromolith® HighResolution RP-18e at high flow rate

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] acetonitrile; [B] water + 0.1% (v/v) TFA
Gradient:	98% B held for 1 min; to 85% B for 3.2 min; held at 85% B for 6.8 min; (Initial composition: 0 – 1 min 98% B, 1 – 3.2 min 98% B to 85% B, hold 85% B until 10 min)
Flow rate:	4.0 mL/min
Column temp.:	ambient
Injection volume:	5 µL
Detector:	270 nm cell: 11 µL
Sample:	Sulfadiazine 1.1 mg/mL, Sulfamethoxazole 0.8 mg/mL, Sulfadimethoxine 0.8 mg/mL, Sulfaquinoxaline 1.0 mg/mL, Sulfadimidin 2.6 mg/mL, Sulfamerazine 1.0 mg/mL, Sulfathiazole 1.5 mg/mL, Sulfonilamid 0.7 mg/mL, Sulfisoxazole 1.0 mg/mL

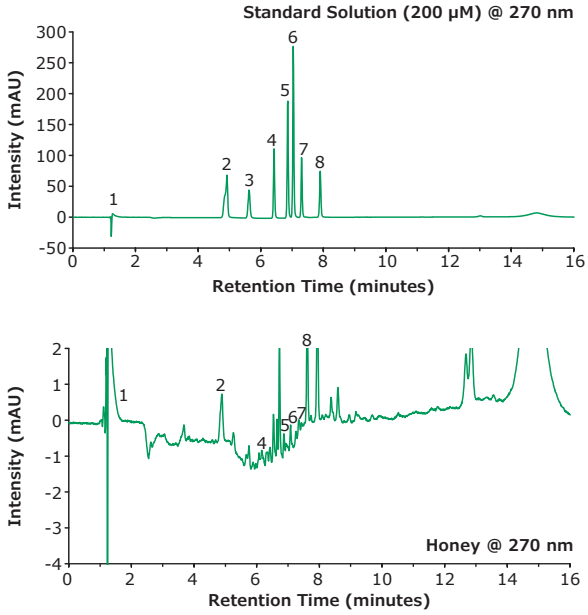


Neonicotinoids in Honey using a Chromolith® RP-18 HPLC column and UV Detection

Neonicotinoids are water soluble insecticides. These insecticides are used for crop protection and can migrate from treated soil into plant tissue and flower nectar. Neonicotinoids have a negative effect on brain cells of honey bees, and colony collapse can occur. [Clothianidin, Imidacloprid and Thiamethoxam are banned in Germany, (EU) No. 485/2013].

Chromatographic conditions

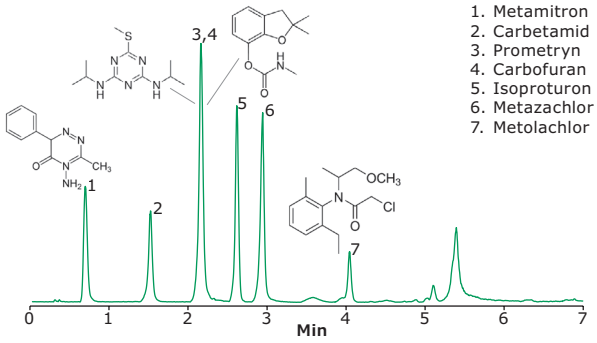
Column:	Chromolith® Performance RP-18 endcapped 100 x 2.0 mm (1.52006.0001)		
Detection:	Dionex™ Ultimate 3000 VWD-3400; Thiamethoxam, Acetamiprid and Thiacloprid @ UV = 254 nm; Dinotefuran, Nitenpyram, Imidacloprid and Clothianidin @ UV = 270 nm (micro flow cell; 1.4 µL/7 mm)		
Mobile phase:	A: Water + 0.1% formic acid B: Acetonitrile + 0.1% formic acid		
Gradient:	**Time**	**%A**	**%B**
	0	100	0
	3	90	10
	5	70	30
	12	60	40
	12.4	100	0
	16	100	0
Injection volume:	1 µL		
Flow rate:	250 µL/min		
Temperature:	Ambient		
Pressure drop:	27-32 bar (392-464 psi)		
Standard solution (200 µM):	Pipette 500 µL of each of the stock solutions into a 25 mL volumetric flask, dilute to volume with acetonitrile to obtain a 200 µMol standard solution.		
Sample solution:	Place 3 g of homogenized honey in a 50 mL extraction tube, add 10 mL deionized water and homogenize the sample. Add 15 mL acetonitrile and shake for 2 minutes at 2250 rpm. Combine with Supel QuE acetate tube and mix for 1 minute. Centrifuge at 3,400 rpm for 5 minutes. Add 1 mL supernatant to a 2 mL extraction tube, containing cleanup sorbent. Shake for 1 minute at 2250 rpm. Centrifuge for 3 minutes at 3,400 rpm. Place 1.5 mL of the supernatant into an Eppendorf vial end evaporate to dryness under nitrogen at 30 °C. Reconstitute the residue in 200 µL acetonitrile.		
Sample fortification:	Place 3 g of homogenized honey in a 50 mL extraction tube and spike with 5 µL of 200 µM neonicotinoid pesticide standard solution. After 1 hour, proceed with extraction and cleanup as described in sample preparation part.		



LC-MS/MS Analysis of Pesticides in Kidney on Chromolith® Performance RP-18e 50-2 mm

Chromatographic conditions

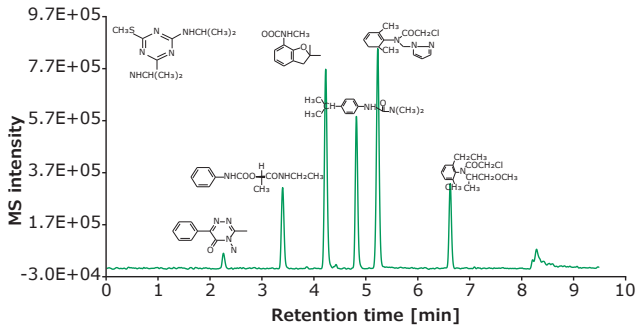
Column:	Chromolith® Performance RP-18e, 50 x 2 mm (1.52007.0001)
Mobile phase:	[A] Acetonitrile + 0.1% Formic acid; [B] Water + 0.1% Formic acid;
Gradient:	20% A; to 80% A in 6 min; to 70% A for 1 min; to 20% A in 1 min
Flow rate:	0.4 mL/min
Column temp.:	25 °C
Pressure:	420 psi (29 bar)
Injection volume:	1.0 µL
Detector:	Bruker ESI-MS, m/z 190-370, BPC (1.4 µL)
Sample preparation:	A mixture of eight pesticides was prepared utilizing ACN as a solvent (See Analysis Note for more information)
Sample:	Metamitron 159 mg/L, Carbetamid 487 mg/L, Prometryn 60 mg/L, Carbofuran 548 mg/L, Isoproturon 159 mg/L, Metazachlor 796 mg/L, Metolachlor 395 mg/L, Chlorfenvinphos 300 mg/L spiked in porcine kidney homogenate



LC-MS/MS Analysis of Pesticides in Kidney on Chromolith® CapRod® RP-18e

Chromatographic conditions

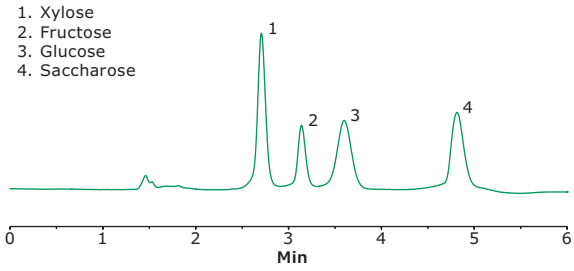
Column:	Chromolith® CapRod® RP-18e, 150 x 0.1 mm (1.50402.0001)
Mobile phase:	[A] water + 0.1% formic acid [B] acetonitrile + 0.1% formic acid
Gradient:	20% B to 90% B in 10 min
Flow rate:	1.25 nL/min
Pressure:	83 bar (instrument + column)
Temperature	25 °C
Injection volume:	2.50 nL
Detection:	Nano-ESI(+)-MS (m/z scan 100-600)
Sample preparation:	Metamitron, Carbetamid, Carbofuran, Prometryne, Isoproturan, Metazachlor, Metolachlor in acetonitrile/water 20/80



Xylose, Fructose, Glucose and Saccharose on Chromolith® NH_2

Chromatographic conditions

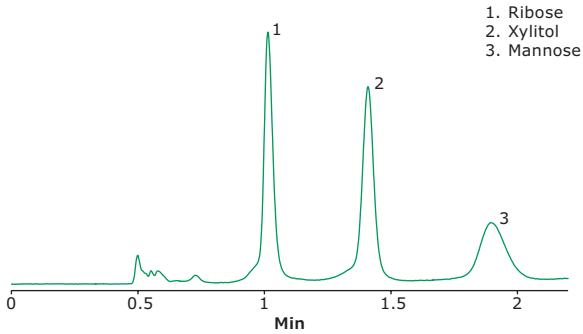
Column:	Chromolith® NH_2 100 x 4.6 mm (1.52028.0001)
Mobile phase:	[A] Acetonitrile; [B] Water; (75:25, A:B)
Flow rate:	1.0 mL/min
Pressure:	160 psi (11 bar)
Column temp.:	23 °C
Injection volume:	2 µL
Detector:	190 nm cell: 11 µL
Sample:	Xylose 2.28 mg/mL, Fructose 6.45 mg/mL, Glucose 9.20 mg /mL, and Saccharose 9.42 mg/mL diluted in mobile phase.



Monosaccharides - Ribose, Xylitol, Mannose on Chromolith® NH_2

Chromatographic conditions

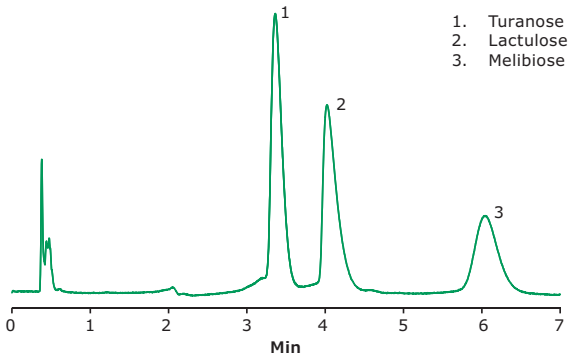
Column:	Chromolith® NH_2, 100 x 4.6 mm (1.52028.0001)
Mobile phase:	[A] Acetonitrile; [B] Water; (85:15, A:B)
Flow rate:	3.0 mL/min
Column temp.:	23 °C
Pressure:	682 psi (47 bar)
Injection volume:	5 µL
Detector:	190 nm
Sample:	Ribose 5.9 mg/mL, Xylitol 8.1mg/mL, Mannose 7.6 mg/mL in mobile phase



Disaccharides Turanose, Lactulose, and Melibiose on Chromolith® NH_2

Chromatographic conditions

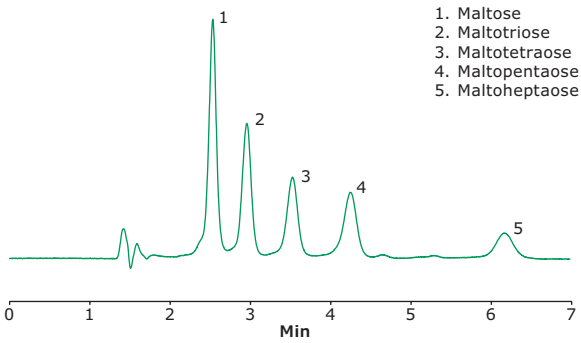
Column:	Chromolith® NH_2, 100 x 4.6 mm I.D. (1.52028.0001)
Mobile phase:	[A] Acetonitrile; [B] Water; (85:15, A:B)
Flow rate:	4 mL/min
Column temp.:	23 °C
Pressure:	1102 psi (76 bar)
Injection:	10 µL
Detector:	UV, 190 nm
Sample:	turanose 7.7 mg/mL, lactulose 8.0 mg/mL, melibiose 10.6 mg/mL in mobile phase



Linear Oligosaccharides on Chromolith® NH_2

Chromatographic conditions

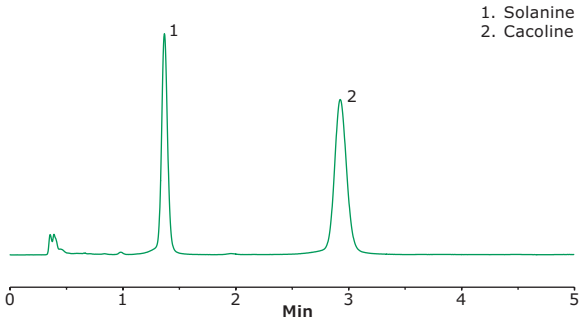
Column:	Chromolith® NH_2 100 x 4.6 mm (1.52028.0001)
Mobile phase:	[A] Acetonitrile; [B] Water; (65:35, A:B)
Flow rate:	1.0 mL/min
Column temp.:	23 °C
Pressure:	377 psi (26 bar)
Injection volume:	2 µL
Detector:	190 nm
Sample:	Maltose 21.6 mg/mL, Maltotriose 19.5 mg/mL, Maltotetraose 15.7 mg/mL, Maltopentaose 16.2 mg/mL, Maltoheptaose 10.6 mg/mL in mobile phase



Glyocoalkaloids - Solanine and Cacoline on Chromolith® NH_2

Chromatographic conditions

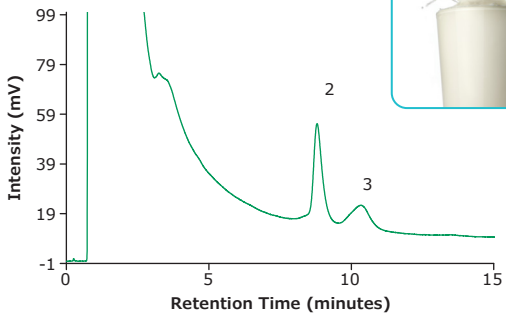
Column:	Chromolith® NH_2, 100 x 4.6 mm (1.52028.0001)
Mobile phase:	[A] Acetonitrile; [B] phosphate buffer, pH 6; (80:20, A:B)
Flow rate:	2.0 mL/min
Column temp.:	23 °C
Pressure:	174 psi (12 bar)
Injection volume:	8 µL
Detector:	208 nm
Sample:	200 ppm (0.2 mg/mL) of each Solanine and Cacoline in mobile phase

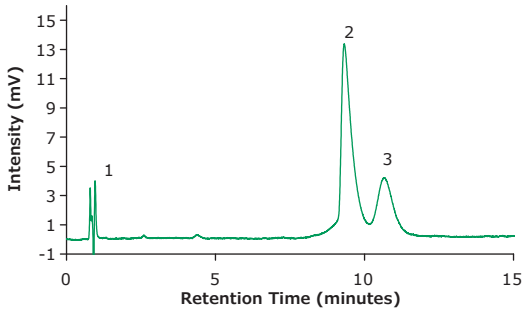


Lactulose + Lactose on Chromolith® NH_2 100 - 4.6 mm

Chromatographic conditions

Column:	Chromolith® Performance NH_2 100- 4.6 mm (1.52028.0001)
Mobile phase:	Acetonitrile / Water 88 / 12 v/v
Flow rate:	2.0 mL/min
Temperature:	23 °C
Injection volume:	5 µL
Pressure:	28 bar
Detection:	190 nm
Cellvolume:	11 µL
Sample:	1. Lactulose 180 mg/20mL 2. Lactose 270 mg/20mL Acetonitrile/Water 50/50



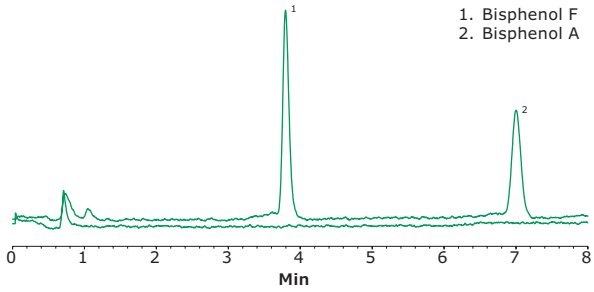


Bisphenol A in Spiked Milk Powder on Chromolith® HighResolution RP-18e

To facilitate the monitoring of BPA, we have developed a rapid and inexpensive method for the analysis of baby milk formulations. The data presented here illustrates the excellent long-term performance and method robustness obtained with Chromolith® columns. More than 2700 samples were analyzed on a single column

Chromatographic conditions

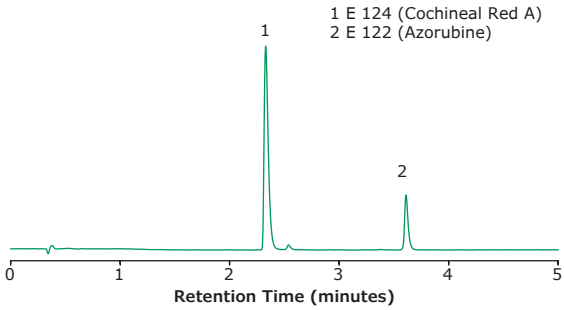
Column:	Chromolith® HighResolution RP-18 endcapped 100 x 4.6 mm I.D. (1.52022.0001)
Mobile phase:	[A] Water; [B] Acetonitrile; (75:25, A:B)
Flow rate:	2 mL/min (0 - 4 min), 4 mL/min (4.1 - 7.5 min), 2 mL/min (7.6 - 8 min)
Pressure:	90-185 bar (1305-2682.5 psi)
Column temp.:	35 °C
Detector:	fluorescence, λex=230 nm; λem=316 nm, 13 µL cell
Injection:	10 µL
Sample preparation:	12.1 g of milik powder dissolved in 100 mL hot water. Spike100 ppb of BPA to the solution, Add 0.1% of Hydrochloric acid and 10% acetonitrile. Add Internal standard BPF 60 ppb. Centrifuged and filter the sample with Millex® simplicity filter.
Sample:	Bisphenol A & 60 ppb Bisphenol F in Methanol (5.2 mg/10 mL)



Artificial Food Colorants Cochineal Red A and Azorubine in Yogurt on Chromolith® FastGradient RP-18 endcapped

Chromatographic conditions

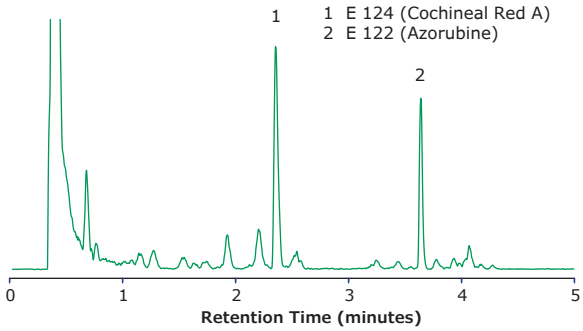
Column:	Chromolith® FastGradient RP-18, 50 x 2 mm (1.52007.0001)
Mobile phase:	[A] Acetonitrile; [B] 20 mM Ammonium acetate, pH 4.7 in water
Gradient:	2.5 to 50% A in 6 min; held at 50% A for 2 min
Flow rate:	0.4 mL/min
Pressure:	899 - 856 psi (62 - 59 bar)
Column temp.:	25 °C
Injection volume:	1 µL
Detector:	UV, 480 nm
Sample:	10 g of yogurt was mixed with 30 mL acetonitrile, stirred for 30 min and centrifuged for 20 min at 4500 rpm. Acetonitrile was removed (TurboVap II, 90 min, 40 °C, 1 bar nitrogen) and solution was filtered using 0.45 µm filter. Sample was spiked with E-124 (Cochineal Red A, 69.44 mg in 50 mL water) and E-122 (Azorubine, 3.46 mg in 10 mL water) stock solutions in a 10:2:3 ratio (v/v)



LC/MS Analysis of Artificial Food Colorants Cochineal Red A and Azorubine in Yogurt on Chromolith® FastGradient RP-18 endcapped

Chromatographic conditions

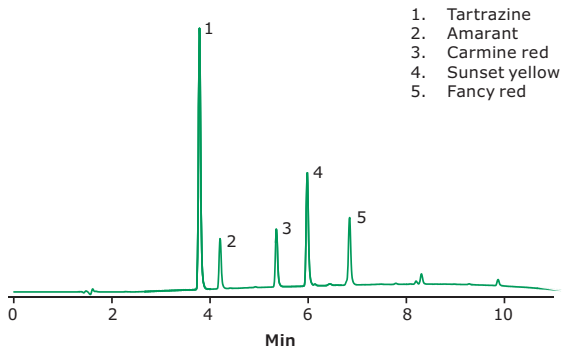
Column:	Chromolith® FastGradient RP-18, 50 x 2 mm (1.52007.0001)
Column temp.:	25 °C
Mobile phase:	[A] Acetonitrile; [B] 20 mM Ammonium acetate, pH 4.7 in water
Gradient:	2.5 to 50% A in 6 min; held at 50% A for 2 min
Flow rate:	0.4 mL/min
Pressure:	856-899 psi (59-62 bar)
Injection volume:	1 µL
Detector:	ESI-MS(-) (m/z range 100-550), BPC
Sample preparation:	10 g of yogurt was mixed with 30 mL acetonitrile, stirred for 30 min and centrifuged for 20 min at 4500 rpm. Acetonitrile was removed (TurboVap II, 90 min, 40 °C, 1 bar nitrogen) and solution was filtered using 0.45 µm filter. Sample was spiked with E-124 (Cochineal Red A, 69.44 mg in 50 mL water) and E-122 (Azorubine, 3.46 mg in 10 mL water) stock solutions in a 10:2:3 ratio (v/v)



Artificial Food Colorants (Tartrazine, Amaranth, Carmine Red, Sunset Yellow, Fancy Red) on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

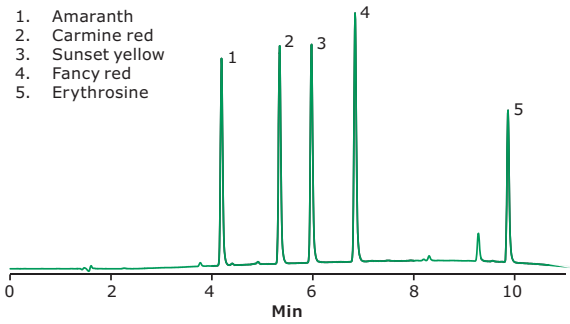
Column:	Chromolith® HighResolution RP-18, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 20 mM Ammonium acetate, pH 6.8 in water; [B] Methanol
Gradient:	5 to 50% B in 6 min; to 100% B in 4 min; held at 100% B for 1 min; to 5% B in 0.01 min
Flow rate:	1 mL/min
Column temp.:	30 °C
Pressure:	1526 psi (106 bar)
Injection volume:	20 µL
Detector:	UV, 428 nm
Sample:	Tartrazine 0.05 mg/mL, Amaranth 0.05 mg/mL, Carmine Red 0.05 mg/mL, Sunset Yellow 0.05 mg/mL, Fancy Red 0.05 mg/mL in methanol:water (50:50)



Artificial Food Colorants (Amaranth, Carmine Red, Sunset Yellow, Fancy Red, Erythrosine) on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

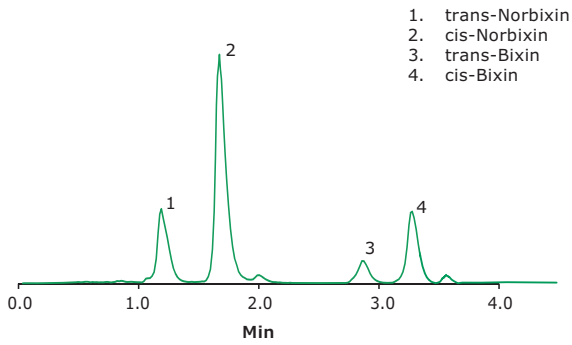
Column:	Chromolith® HighResolution RP-18, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 20 mM Ammonium acetate, pH 6.8 in water; [B] Methanol
Gradient:	5 to 50% B in 6 min; to 100% B in 4 min; held at 100% B for 1 min; to 5% B in 0.01 min
Flow rate:	1 mL/min
Column temp.:	30 °C
Pressure:	1526 psi (106 bar)
Injection volume::	20 µL
Detector:	UV, 510 nm
Sample:	Amaranth 0.05 mg/mL, Carmine Red 0.05 mg/mL, Sunset Yellow 0.05 mg/mL, Fancy Red 0.05 mg/mL, Erythrosine 0.05 mg/mL in methanol:water (50:50)



LC/MS Analysis of Annato (or Roucou or Achiote) on Chromolith® FastGradient RP-18 endcapped

Chromatographic conditions

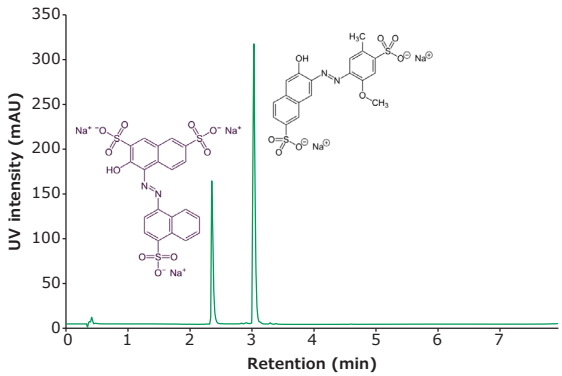
Column:	Chromolith® FastGradient RP-18, 50 x 2.0 mm (1.52007.0001)
Mobile phase:	[A] Water (0.1% Formic acid); [B] Acetonitrile (0.1% Formic acid)
Gradient:	55 to 64% B in 0.5 min, to 70% B in 3 min, to 90% B in 0.5 min, held at 90% B for 2 min, to 55% B in 0.5 min, held at 55% for 2.5 min
Flow rate:	0.4 mL/min
Column temp.:	25 °C
Pressure:	562-317 psi (39 - 22 bar)
Injection volume:	10 µL
Detector:	ESI-MS(+) (m/z range 378-396), BPC
Sample preparation:	Dissolve 2.2 mg/mL cis-norbixin and 0.12 mg/mL cis-bixin in diluent. After dissolution of the analytes, the solution was heated for 5.5 hours at 75 °C under reflux to speed up transformation of cis- to trans-isomers and thereafter diluted 1:10 with acetonitrile and water (50:50 v/v).



Artificial Food Colorants in Alcopops on Chromolith® FastGradient RP-18 endcapped

Chromatographic conditions

Column:	Chromolith® FastGradient RP-18, 50 x 2.0 mm (1.52007.0001)
Mobile phase:	[A] 20 mM ammonium acetate pH 4.7 [B] Acetonitrile
Gradient:	100% B for 0.5 min, to 50% B in 4 min, to 5% B in 0.5 min, held at 5% B for 1 min, to 100% B in 1 min
Flow rate:	0.4 mL/min
Column temp.:	25 °C
Pressure:	46 - 40 bar
Injection volume:	2 µL
Detector:	UV, 500 nm
Sample:	1.E123 (Amaranth) 2.E129 (Allura Red AC)
Sample preparation:	Heating of 10 mL of beverage to almost complete dryness at 40 °C for 12 hours (removal of ethanol) filtration of the beverage "Bacardi Watermelon" using a 0.45 µm syringe filter. Dissolution of the residue in water (making up to 5 mL) filtration of the solution using a 0.45 µm syringe filter



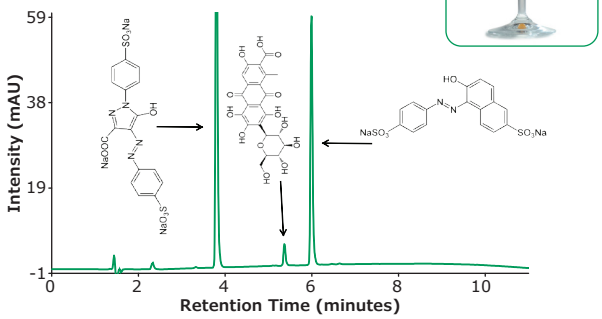
Chromatographic Data :

#	Compound name	RT [min]
1	E123 (Amaranth)	2.37
2	E 129 (Allura Red AC)	3.05

Artificial Food Colorants in Orange Soft Drink on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

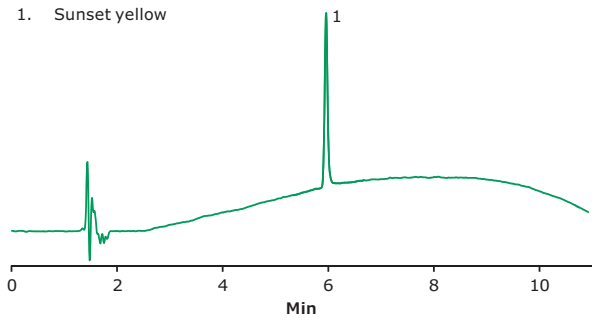
Column:	Chromolith® HighResolution RP-18, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 20 mM Ammonium acetate, pH 6.8 in water; [B] Methanol
Gradient:	5 to 50% B in 6 min; to 100% B in 4 min; held at 100% B for 1 min; to 5% B in 0.01 min
Flow rate:	1 mL/min
Pressure:	1526 psi (106 bar)
Injection volume:	20 µL
Detector:	UV, 428 nm
Sample preparation:	5 mL of the beverage was transferred to a 10 mL volumetric flask, sonicated for 5 minutes, then pure water was added to the volume. The solution was filtered by 0.45 µm PTFE syringe filter into a 2 mL autosampler vial prior to injection.



Artificial Food Colorants in Dried Plums on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

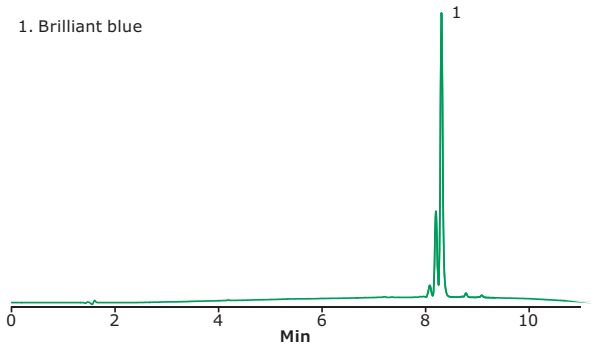
Column:	Chromolith® HighResolution RP-18, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 20 mM Ammonium acetate, pH 6.8 in water; [B] Methanol
Gradient:	5 to 50% B in 6 min; to 100% B in 4 min; held at 100% B for 1 min; to 5% B in 0.01 min
Flow rate:	1 mL/min
Column temp.:	30 °C
Pressure:	1526 psi (106 bar)
Injection volume:	20 µL
Detector:	UV, 510 nm



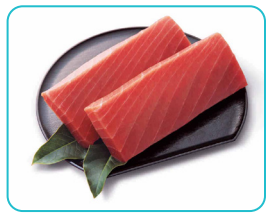
Artificial Food Colorants on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 20 mM Ammonium acetate, pH 6.8 in water; [B] Methanol
Gradient:	5 to 50% B in 6 min; to 100% B in 4 min; held at 100% B for 1 min; to 5% B in 0.01 min
Flow rate:	1 mL/min
Column temp.:	30 °C
Pressure:	1526 psi (106 bar)
Sample:	Brilliant Blue, 0.05 mg/mL in methanol:water (50:50)
Injection volume:	20 µL
Detector:	UV, 628 nm



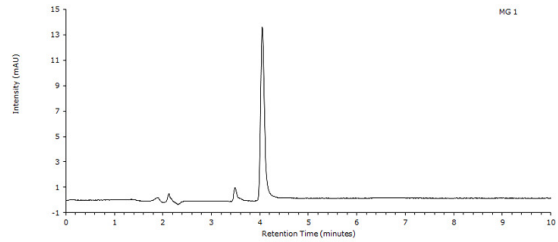
Malachite Green and Leucomalachite Green in Salmon using Chromolith® HighResolution RP-18e with UV Detection

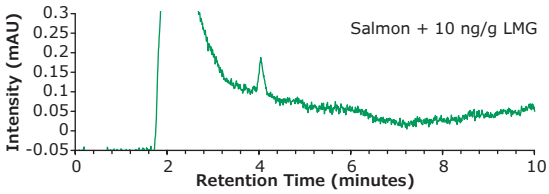


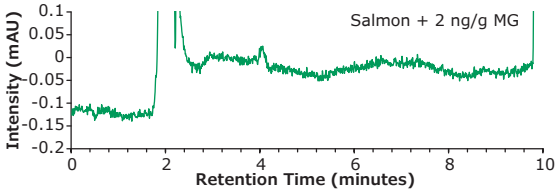
Malachite green (MG) is an effective topical fungicide used in the aquaculture industry. MG absorbed by fish tissue is metabolically reduced to leucomalachite green (LMG), which is lipophilic and can be stored in edible fish tissue for an extended period. The European Commission requires that methods be able to determine the sum of MG and LMG residues at the minimum performance limit of 2 ng/g.

Chromatographic conditions

Column	Chromolith® HighResolution RP-18 endcapped 150 x 4.6 mm (1.52023.0001)
Mobile phase:	[A] Ammonium acetate buffer/acetonitrile 1/1 (v/v) (filtered through a 0.45 µm PVDF membrane) Prepare a 0.1 M ammonium acetate solution by dissolving 7.7 g ammonium acetate in 1 L deionized (DI) water. Adjust 1 L of this solution to pH 4.5 by adding 8 mL of acetic acid and 5 mL of 1 M p-TSA (equivalent to 5 mM p-TSA); [B] Acetonitrile
Isocratic	A/B 95/5 (v/v)
Flow rate	1.0 mL/min
Temperature	35 °C
Injection volume	100 µL
Pressure drop:	53 bar (769 psi)
Detection:	Dionex™ Ultimate 3000 VWD-3400 @ VIS = 618 nm (analytical flow cell; 11 µL/10 mm)
Standard stock solutions 100 µg/mL:	Accurately weigh 10.0 mg of each reference standard (corrected for MG and LMG purity) into separate 100 mL volumetric flasks (for LMG use low actinic glass or wrap with aluminium foil to protect from light), dilute to volume with methanol and mix. Note: The mass of MG was also corrected for the MG oxalate product, which contains two molecules of MG for one molecule of the MG-oxalate dimer complex (7.109 mg MG per 10 mg of MG-oxalate dimer). MG-ICV: A second MG stock solution should be prepared as the Initial Calibration Verification (ICV). Stock solutions should be stored at room temperature, protected from light, and freshly prepared every six months.
Mg1, 1.0 Mg/mL:	Pipette 1 mL of the MG stock solution into a 100 mL volumetric flask, dilute to volume with methanol and mix. MG1-ICV: Prepare a second 1.0 µg/mL MG working standard from the MG-ICV stock solution. MG1 was freshly prepared monthly and stored at room temperature.
Salmon:	5.0 g of thawed tissue composite was extracted by an extraction procedure
Salmon spiked with lmg and mg:	To generate validation data, 5.0 g portions of thawed tissue composite were fortified by spiking with 50, 100 or 200 µL of LMG2 or 50 µL of LMG1 solutions to produce samples containing 1, 2, 4 or 10 ng/g of LMG, respectively. Samples fortified with MG at 2 ng/g were generated by spiking 5.0 g of tissue with 100 µL of the MG2 solution. Samples were allowed to sit at room temperature for at least 15 minutes before proceeding with extraction.



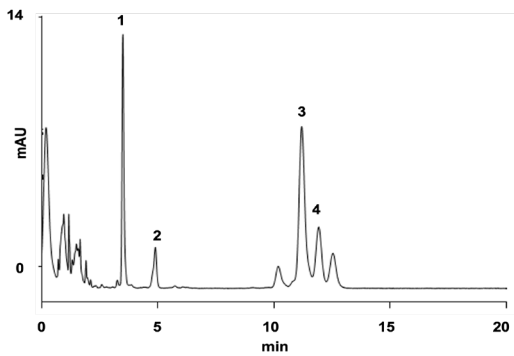




Carotenoide of Salmon using Chromolith® HighResolution RP-18e

Chromatographic conditions

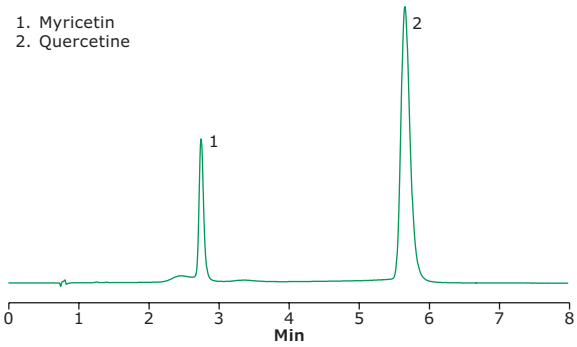
Column:	Chromolith® HighResolution RP-18e 100 x 4.6 mm (1.52022.0001)	
Mobile phase:	Acetonitrile / Water + 0.1% Formic acid 85/15 v/v	
Flow:	2 mL/min	
Temp.:	ambient	
Injection volume:	5.0 µL	
Pressure:	88 bar	
Detector:	436 nm	
Cell:	11 µL	
Sample:	1. Astaxanthin Isomer 1 2. Astaxanthin Isomer 2 3. Canthaxanthin Isomer 1 4. Canthaxanthin Isomer 2	1 µg/mL 1 µg/mL



Flavonoids in Red Wine on Chromolith® HighResolution RP-18e

Chromatographic conditions

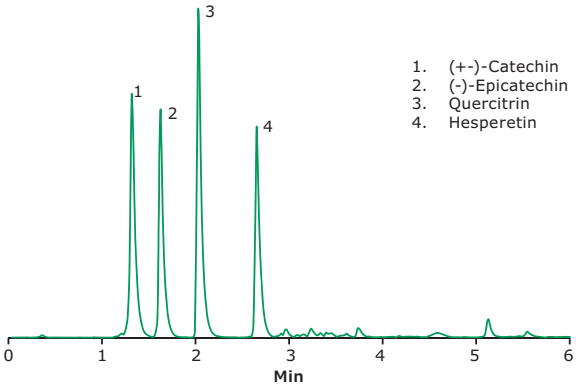
Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	19 mL Acetonitrile / 5 mL Methanol / 1 mL THF / 75 mL Water
Flow rate:	2.0 mL/min
Column temp.:	ambient
Pressure:	1406 psi (97 bar)
Injection volume:	1 µL
Detector:	360 nm (cell: 11 µL)
Sample:	Red wine



LC/MS Analysis of Flavonoids on Chromolith® FastGradient RP-18 endcapped

Chromatographic conditions

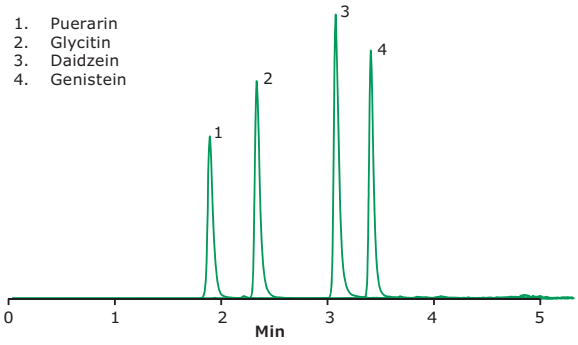
Column:	Chromolith® FastGradient RP-18, 50 x 2 mm (1.52007.0001)
Mobile phase:	[A] Water (0.1% Formic acid); [B] Acetonitrile (0.1% Formic acid)
Gradient:	5 to 95% B in 4 min, held at 95% B for 0.5 min, to 5% B in 0.5 min, held at 5% for 2 min
Flow rate:	0.4 mL/min
Column temp.:	25 °C
Pressure:	677-245 psi (47 - 17 bar)
Injection volume:	1 µL
Detector:	ESI-MS(+) (m/z range 100-455)
Sample:	catechin 317 ppb, epicatechin 270 ppb, quercitin 198 ppb, hesperetin 177 ppb in water



LC/MS Analysis of Isoflavones on Chromolith® FastGradient RP-18 endcapped

Chromatographic conditions

Column:	Chromolith® FastGradient RP-18, 50 x 2 mm (1.52007.0001)
Mobile phase:	[A] Water (0.1% Formic acid); [B] Acetonitrile (0.1% Formic acid)
Gradient:	5 to 15% B in 1.5 min, to 95% B in 4 min, held at 95% B for 1 min, to 5% B in 1 min, held at 5% for 1.5 min
Flow rate:	0.5 mL/min
Column temp.:	25 °C
Pressure:	821-317 psi (57 - 22 bar)
Injection volume:	1 µL
Detector:	ESI-MS(+) (m/z range 100-600), overlay of four EICs (m/z 271.0, 255.0, 447.1, 417.1)
Sample:	genistein 10 ppb, daidzein 10 ppb, glycitin 10 ppb, puerarin 20 ppb in methanol and water (50:50 v/v)



Polyphenols in Coffee on Chromolith® Performance 2 x 100-3 mm

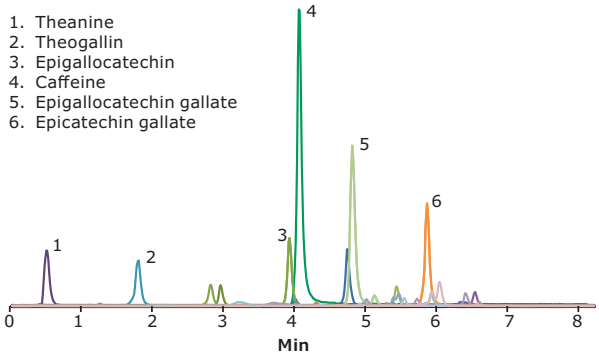
Chromatographic conditions

Column:	2x Chromolith® Performance 100 x 3 mm (1.52001.0001)
Column temp.:	ambient
Mobile phase:	[A] Acetonitrile; [B] Water + 0.1% Acetic acid (v/v)
Gradient:	0-3 min 10% A, 3-14 min 10% A to 50% A, hold 50% A for 6 min
Flow rate:	850 µL/min
Pressure:	11213 psi (670-780 bar)
Injection volume:	1.0 µL
Detector:	327 nm (cell: 1.4 µL)
Sample:	6 g Coffee powder in 200 mL water
Sample preparation:	6 g of coffee powder with 200 mL of boiling water in a coffee extractor. The extract was injected directly into the HPLC-System.

LC/MS Analysis of Green Tea Components on Chromolith® FastGradient RP-18 endcapped

Chromatographic conditions

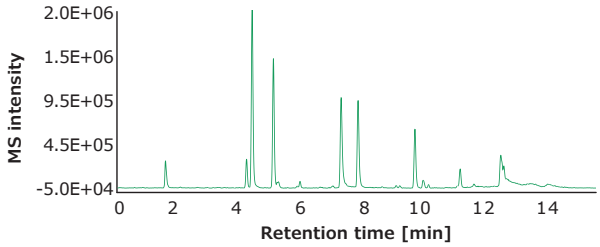
Column:	Chromolith® FastGradient RP-18, 5 cm x 2 mm (1.52007.0001)
Mobile phase:	[A] Acetonitrile (0.1% Formic acid); [B] Water (0.1% Formic acid)
Gradient:	100% B for 0.6 min, to 72.5% B in 7.4 min, held at 72.5% for 1 min
Flow rate:	0.4 µL/min
Column temp.:	25 °C
Pressure:	605 psi (42 bar)
Injection volume:	1 µL
Detector:	ESI-MS(+) (m/z range 150-950), BPC and overlay of EICs)
Sample Preparation:	1.75 g of green tea was extracted for three minutes using 100 mL of boiling water. Prior to injection, both samples were centrifuged at 4500 rpm for 10 min and cleaned using a 0.45 µm syringe filter.



Capsaicinoids in Chili Extract using Chromolith® HighResolution RP-18e

Chromatographic conditions

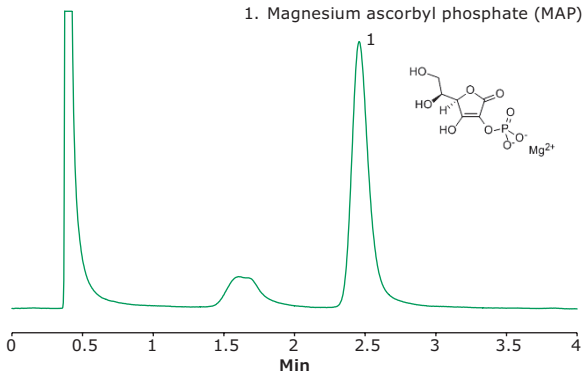
olumn:	Chromolith® CapRod RP-18 endcapped, 150x0.1 mm (1.50402.0001)
Mobile phase:	A: water + 0.1 % formic acid B: acetonitrile + 0.1 % formic acid
Gradient:	35 % B to 95 % B in 12 min
Flow Rate:	1.24 µL/min
Pressure Drop:	80 Bar (1160 psi)
Column temp.:	Ambient
Detection:	nano-ESI(+) 100-600 m/z
Injection:	2.5 nL
Diluent	Acetonitrile
Sample:	Sudan dyes and capsaicinoids in hot chili sauce



Magnesium Ascorbyl Phosphate (MAP) on Chromolith® NH_2 50 - 4.6 mm

Chromatographic conditions

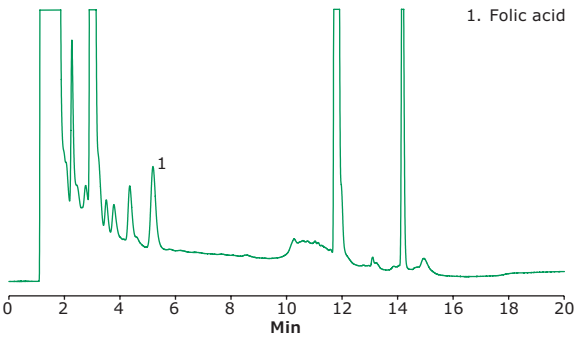
Column:	Chromolith® NH2, 50 x 4.6 mm (1.52027.0001)
Mobile phase:	[A] Acetonitrile; [B] 10 mM Sodium phosphate buffer, pH 4.0; (20:80, A:B)
Flow rate:	2 mL/min
Column temp.:	23 °C
Pressure:	421 psi (29 bar)
Injection volume:	1 µL
Detector:	255 nm cell: 11 µL
Sample:	Magnesium ascorbyl phosphate, 100 µg/mL



Folic Acid Syrup on Chromolith® HighResolution RP-18e

Chromatographic conditions

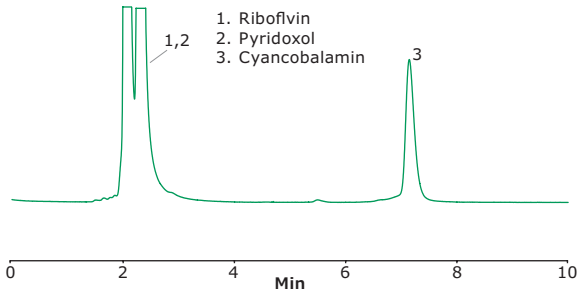
Column:	Chromolith® High Resolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 1.5 g of hexane 1-sulfonate in 1000 mL water containing 10 mL of glacial acetic acid; [B] acetonitrile;
Flow rate:	1.0 mL/min
Column temp.:	30 °C
Pressure:	1203 psi (83 bar)
Injection:	10 µL
Detector:	277 nm cell: 10 µL
Sample:	10 ppm in diluent (Diluent: Dissolve 6.8 g of Potassium dihydrogen phosphate in 1000 mL water. Adjust pH to 6.8 with 10% Sodium hydroxide solution)
Sample preparation:	Pipette out 15 mL of syrup in 50 mL volumetric flask. Add diluent to make up the volume. Sonicate for 30 min. Filter through 0.2 µm filter paper.



B-Vitamins on Chromolith® NH_2 100 - 4.6 mm

Chromatographic conditions

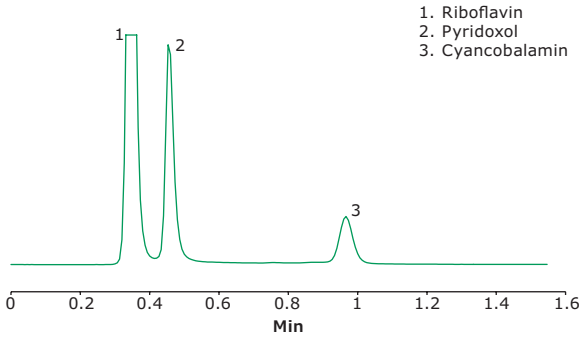
Column:	Chromolith® NH2, 100 x 4.6 mm (1.52028.0001)
Mobile phase:	[A] Acetonitrile; [B] Water; (20:80, A:B)
Gradient:	0-3 min 1 mL/min, 3.1-10 min 2 mL/min
Column temp.:	ambient
Pressure:	290 psi
Injection volume:	2 µL
Detector:	280 nm cell: 11 µL
Sample:	Riboflavin (8.8 mg/10 mL), Pyridoxin (5.5 mg/10 mL), Cyanocobalamin (5.2 mg/10 mL)



B-Vitamins on Chromolith® NH_2 50 - 4.6 mm

Chromatographic conditions

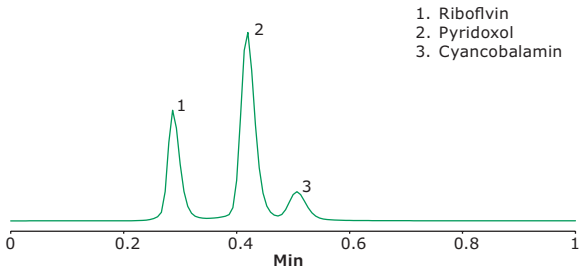
Column:	Chromolith® NH2 50 x 4.6 mm (1.52027.0001)
Column temp.:	ambient
Mobile phase:	[A] Acetonitrile; [B] Water; (74:26, A:B)
Flow rate:	2 mL/min
Pressure:	218 psi (15 bar)
Injection volume:	0.3 µL
Detector:	280 nm
Sample:	Riboflavin (8.8 mg/10 mL), Pyridoxin (5.5 mg/10 mL), Cyanocobalamin (5.2 mg/10 mL)



B-Vitamins on Chromolith® NH_2 25 - 4.6 mm

Chromatographic conditions

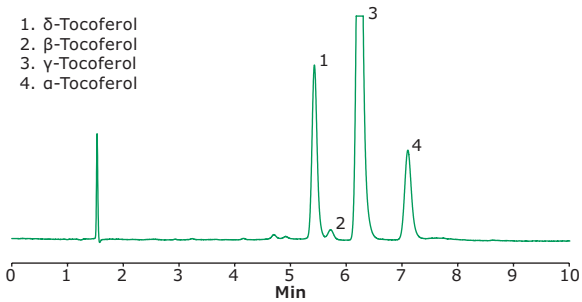
Column:	Chromolith® NH2 25 x 4.6 mm (1.52026.0001)
Mobile phase:	[A] Acetonitrile; [B] Water; (74:26, A:B)
Flow rate:	2 mL/min
Column temp.:	ambient
Pressure:	57 psi (3.9 bar)
Injection volume:	0.3 µL
Detector:	280 nm
Sample:	Riboflavin (8.8 mg/10 mL), Pyridoxin (5.5 mg/10 mL), Cyanocobalamin (5.2 mg/10 mL)



HPLC Analysis of Vitamin E on Chromolith® HighResolution RP-18e

Chromatographic conditions

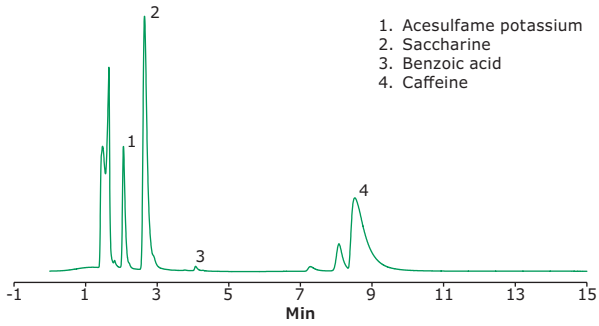
Column:	Chromolith® HighResolution RP-18 encapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] Methanol; [B] Acetonitrile; (92:8, A:B)
Flow rate:	1.0 mL/min
Column temp.:	25 °C
Injection volume:	5 µL
Detector:	230 nm cell: 10 µL
Sample:	1000 ppm sample in mobile phase



Contents of Energy Drinks without SPE

Chromatographic conditions

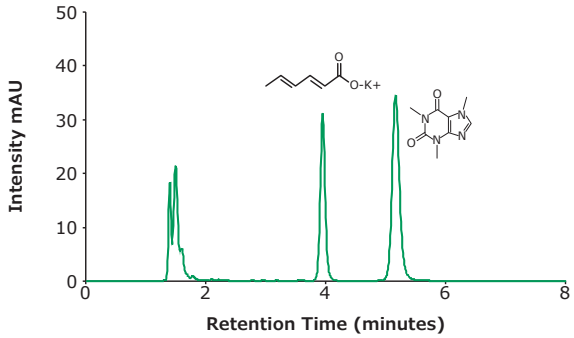
Column:	Chromolith® Performance 100 x 4.6 mm (1.52028.0001)
Mobile phase:	[A] Methanol; [B] 20 mM Phosphate buffer pH 5; (10:90, A:B)
Flow rate:	0-3 min 1 mL/min, 3.1-12 min 2.5 mL/min, 12.1-15 min 1 mL/min
Column temp.:	40 °C
Injection volume:	8 µL
Detector:	227 nm cell: 11 µL
Sample:	Energy drink (Red Bull)



Potassium Sorbate, Caffeine and metabolite analysis on Chromolith® HighResolution RP-18e

Chromatographic conditions

Column.	Chromolith® HighResolution RP-18 endcapped, 100x4.6 mm (1.52022.0001)
Mobile phase:	[A] 15 mM ammonium acetate and 50 mM potassium di-hydrogen phosphate 40:60 v/v [B] Acetonitrile [C] Methanol Composition: A:B:C= 85:3:12 (v/v)
Flow Rate:	1.0 mL/min
Pressure Drop:	47 Bar (681psi)
Column temp.:	30 °C
Detection:	UV, 220 nm
Cell:	1 µL/10 mm
Injection:	10 µL
Diluent:	water
Sample:	10mL of the beverage was sonicated for 5 min. 1 mL of the degassed beverage was diluted to 25 mL by water (sample is diluted 25 times).

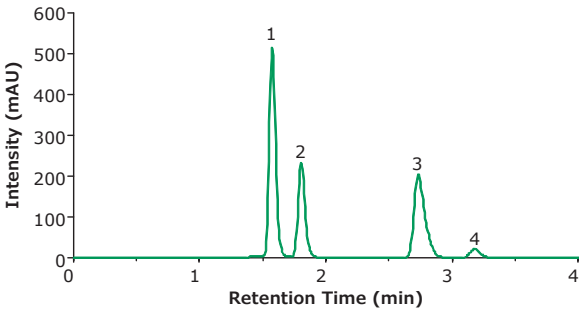


Analysis of Organic Acids in Beverages

Chromatographic conditions

Column.	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	10 mM Di-ammonium hydrogen phosphate solution (pH 2.7)
Flow Rate:	1.0 mL/min
Pressure Drop:	32 Bar (464psi)
Column temp.:	30 °C
Detection:	UV, 210 nm
Cell:	1 µL/10 mm
Injection:	20 µL
Diluent:	water
Sample:	5 mL of orangeade was sonicated for 5 minutes. Thereafter 0.2 mL of phosphoric acid solution (1M) was added. Finally, the solution was diluted to 10 mL by water.

No	Compound
1	Tartaric Acid
2	Malic Acid
3	Citric Acid
4	Succinic Acid



Herbs and Nutrition

Herbs and other natural products are used worldwide for preventive and therapeutic purposes. Identification and quality control of products of vegetal origin is thus required.

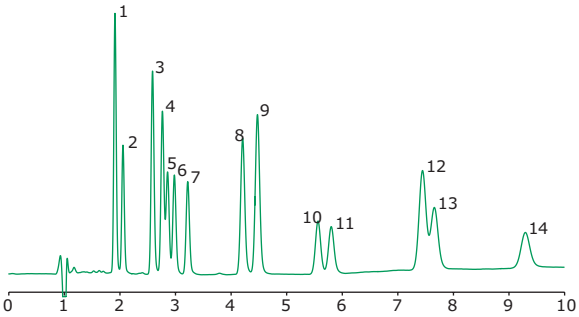
The chromatographic fingerprint approach is accepted by the World Health Organization (WHO) as a quality and identification evaluation technique. When developing a new method for fingerprinting, generally the first step is to create conditions maximizing the peak capacity. A rule of thumb in chromatography is that the longer the column – the higher the peak capacity (more theoretical plates are available). Therefore, long columns have traditionally been used. Monolithic columns are a suitable alternative to packed particle columns for maximizing the peak capacity. The low column backpressure offers the possibility of connecting several columns in series while still maintaining a low backpressure. In addition, the very high robustness and resistance towards sample matrix is a major advantage of monolithic columns for the analysis of herbal extracts of roots, dried berries or leaves.

UHPLC (Isocratic) Analysis of 14 Cannabinoids on Chromolith® HR RP-18e

Cannabinoids are a group of active compounds found in cannabis. These compounds reportedly provide relief to an assortment of symptoms in the treatment of pain, nausea, anxiety, mood disorders, and inflammatory diseases. Cannabinoids imitate naturally occurring compounds and interact with two specific types of receptors: CB1 and CB2, located in the nervous system and immune system, respectively. Shown here is an isocratic separation of fourteen cannabinoids in less than ten minutes on a Chromolith® HR RP-18e column. Cerilliant® cannabinoid CRMs provide reliable identification and quantification. These standards are used in testing methods by GC/MS, LC/MS, or HPLC for applications in clinical toxicology, testing of cannabis potency or impurity profiling by growers, pharmaceutical research, forensic analysis, and urine drug testing.

Chromatographic conditions

Column:	Chromolith® HR RP-18e, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] Water (0.1% Formic acid); [B] Acetonitrile (0.1% Formic acid)
Isocratic:	30/70 [A]/[B]
Flow rate:	1.5 mL/min
Pressure:	1100 psi (75.8 bar)
Column temp.:	30 °C
Detector:	UV at 230 nm
Injection volume:	5 µL
Sample:	25 µg/mL, 14 Cannabinoid mix in acetonitrile

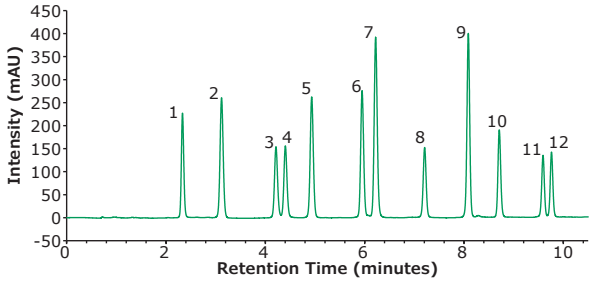


1. Cannabidivarinic acid (CBDVA)
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Tetrahydrocannabivarinic acid (THCVA)
9. Cannabinol (CBN)
10. Δ9-Tetrahydrocannabinol (Δ9-THC)
11. Δ8-Tetrahydrocannabinol (Δ8-THC)
12. Cannabichromene (CBC)
13. Δ9-Tetrahydrocannabinolic acid A (THCA-A)
14. Cannabichromenic acid (CBCA)

Cannabinoid analysis using Methanol – cost saving method

Chromatographic conditions

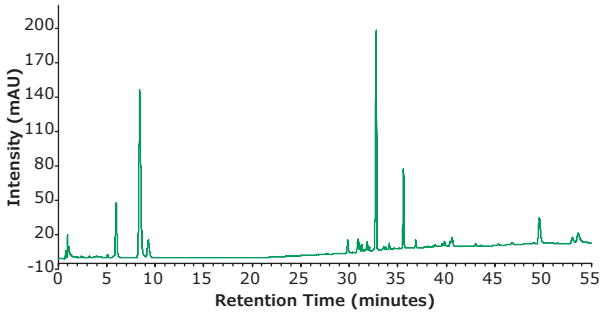
Column:	Chromolith® HighResolution RP18e 100-2 mm (1.52322.0001)		
Mobile phase:	[A] 0.1% (v/v) Phosphoric acid, pH 2.2; [B] Methanol		
Gradient:	**Time**	**%A**	**%B**
	0	28	72
	0.1	28	72
	7.1	10	90
	10.0	10	90
Flow rate:	0.38 mL/min		
Pressure:	148 bar		
Detector:	UV 228 nm		
Column temp.:	25 °C		
Injection volume:	0.2 µL		
Sample:	1. CBDV Cannabidivarin 2. CBDVA Cannabidivarinic Acid 3. CBD Cannabidiol 4. CBG Cannabigerol 5. CBDA Cannabidiolic acid 6. CBGA Cannabigerolic acid 7. CBN Cannabinol 8. CBL (±)-Cannabicyclol 9. CBC Cannabichromene 10. CBNA Cannabinolic Acid 11. CBLA Cannabicyclolic Acid 12. CBCA Cannabichromenic Acid		



Panax Notoginseng

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)		
Mobile phase :	A: Milli-Q® ultrapure water		
	B: Acetonitrile		
Gradient:	**Time (min)**	**% A**	**% B**
	0-20	19	81
	45	45	55
	55	55	45
	55.1	19	81
	60	19	81
Flow rate:	1.5 mL/min		
Temperature:	25 °C		
Pressure:	77 Bar (1165 psi) – initial pressure		
Injection volume:	10 µL		
Detection:	UV, 203 nm		
Diluent:	Mobile phase		
Sample:	The extract of panax notoginseng dissolved in methanol.		

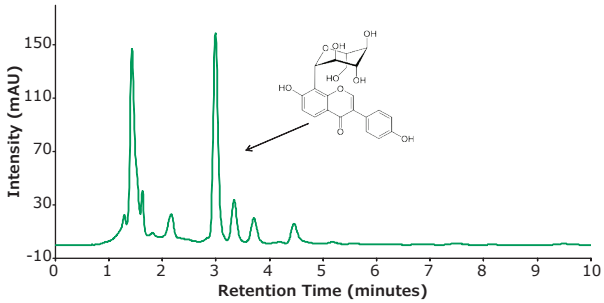


No	Compound	Retention Time (min)
1	R1	6.0
2	Rg1	8.5
3	Re	9.3
4	Rb1	32.8

Radix Puerariae

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase :	Methanol and Water 25:75 (v/v)
Flow rate:	1.0 mL/min
Temperature:	25 °C
Injection volume:	5 µL
Diluent	Mobile phase
Sample:	The extract of Radix Puerariae dissolved in methanol.
Pressure drop:	43 Bar (619 psi)
Detection:	UV 250 nm

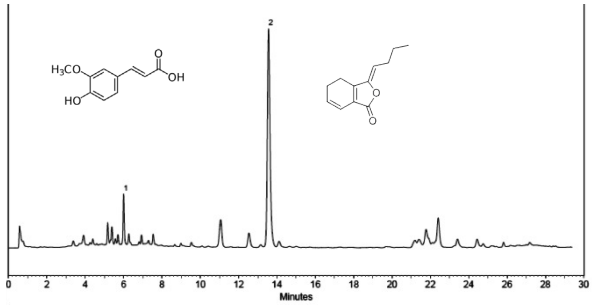


No	Compound	Retention Time (min)
1	Puerarin	3.0

Radix Angelica

Chromatographic conditions

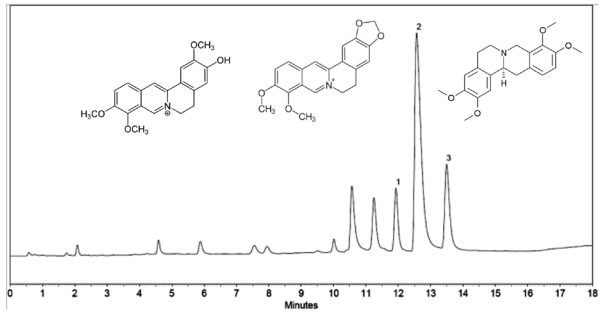
Column:	Chromolith® Performance RP-18 endcapped 100 x 4.6 mm (1.02129.0001)		
Mobile phase:	A: Acetonitrile B:0.1 % phosphoric acid		
Gradient:	**Time (min)**	**% A**	**% B**
	0	5	95
	1	5	95
	6	32	68
	16	43	57
	24	60	40
	26	90	10
Flow rate:	3.0 mL/min		
Temperature:	Ambient		
Injection:	5 µL		
Diluent	Mobile phase		
Detection:	UV, 278 nm		
Sample:	Alcohol extract of roots		



Coptis Chinensis (Chinese Goldthread)

Chromatographic conditions

Column:	Chromolith® Performance RP-8 endcapped 100 x 4.6 mm (1.02129.0001)		
Mobile phase:	A: Acetonitrile B:0.1 % phosphoric acid		
Gradient:	**Time (min)**	**% A**	**% B**
	0	7	93
	2	10	90
	3	17	83
	7	20	80
	8	27	73
	15	35	65
Flow rate:	3.0 mL/min		
Temperature:	30 °C		
Injection:	5 µL		
Diluent:	Mobile phase		
Detection:	UV, 280 nm		
Sample:	Alcohol extract of roots		

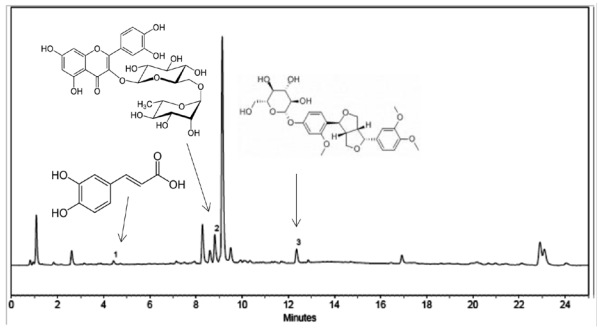


No	Compound	Retention Time (min)
1	Jatrorrhizine	11.9
2	Berberine	12.6
3	Tetrahydropalmatine (THP)	13.6

Fructus Forsythiae

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100 x 4.6 mm (1.02129.0001)		
Mobile phase:	A: Acetonitrile; B:0.1 % phosphoric acid		
Gradient:	**Time (min)**	**% A**	**% B**
	0	5	95
	10	23	77
	12	35	65
	22	60	40
	25	95	5
Flow rate:	2.0 mL/min		
Temperature:	Ambient		
Injection:	5 µL		
Diluent	Mobile phase		
Detection:	UV, 265 nm		
Sample:	Alcohol extract of seeds		

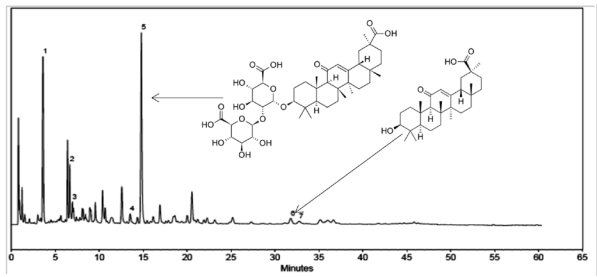


No	Compound	Retention Time (min)
1	Caffeic acid	4.4
2	Rutin	8.8
3	Forsythin	12.4

Radix Glycyrrhizae (Liquorice)

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100 x 4.6 mm (1.02129.0001)		
Mobile phase:	A: Acetonitrile B:Milli-Q® ultrapure water		
Gradient:	**Time (min)**	**% A**	**% B**
	0	15	85
	1	15	85
	6	28	72
	15	35	65
	18	40	60
	40	50	50
	50	80	20
Flow rate:	2.0 mL/min		
Temperature:	30 °C		
Injection:	5 µL		
Diluent:	Mobile phase		
Detection:	UV, 250 and 276 nm		
Sample:	Alcohol extract of roots		

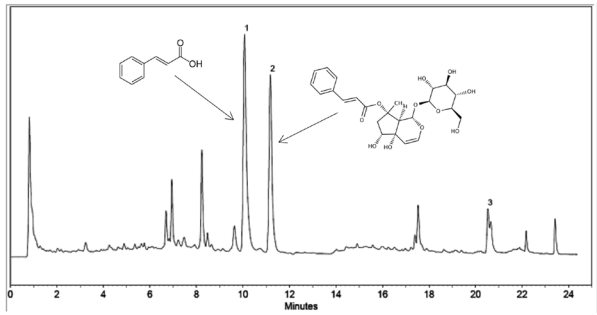


No	Compound	Retention Time (min)
1	Liquiritin	3.6
2	Isoliquiritin	6.7
3	Liquiritigenin	7.0
4	lquiritigenin	13.5
5	Glycyrrhizic Acid	15.0
6	Glycyrrhetinic acid	13.8
7	ß-Glycyrrhetinic Acid	32.8

Radix Scrophulariae

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100 x 4.6 mm (1.02129.0001)		
Mobile phase:	[A] Acetonitrile; [B]0.1 % phosphoric acid		
Gradient:	**Time (min)**	**% A**	**% B**
	0	5	95
	6	22	78
	12	25	75
	14	44	56
	20	66	34
	20.1	95	5
Flow rate:	2.0 mL/min		
Temperature:	30 °C		
Injection:	5 µL		
Diluent	Mobile phase		
Detection:	UV, 278 nm		
Sample:	Alcohol extract of roots		

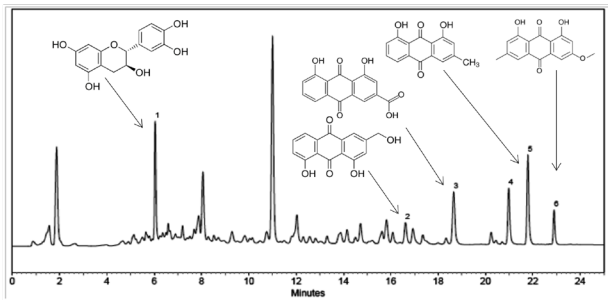


No	Compound	Retention Time (min)
1	Cinnamic acid	10.2
2	Harpagoside	11.2

Rheum Palmatum

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100 x 4.6 mm (1.02129.0001)		
Mobile phase:	A: Methanol B: Milli-Q® ultrapure water		
Gradient:	**Time (min)**	**% A**	**% B**
	0	5	95
	2	5	95
	4	25	75
	18	70	30
	22	95	5
Flow rate:	2.0 mL/min		
Temperature:	30 °C		
Injection:	5 µL		
Diluent	Mobile phase		
Detection:	UV, 254 nm		
Sample:	Alcohol extract of roots		

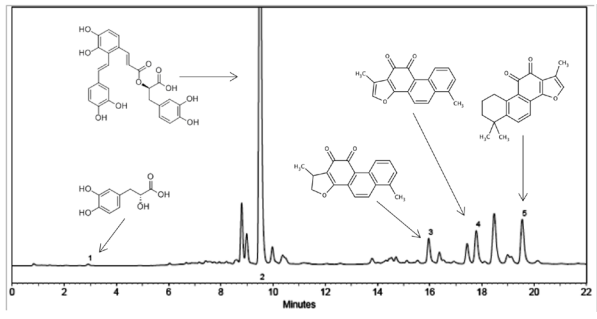


No	Compound	Retention Time (min)
1	Catechin	6.1
2	Aloe Emodin	16.6
3	Rhein	18.6
4	Emodin	21.0
5	Chrysophanol	21.8
6	Physcion (Parietin)	22.8

Salvia Miltiorrhiza

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped 100 x 4.6 mm (1.02129.0001)		
Mobile phase:	A: Acetonitrile B: 0.1 % phosphoric acid		
Gradient:	**Time (min)**	**% A**	**% B**
	0	3	97
	3	3	97
	5	20	80
	11	33	67
	12	46	54
	20	70	30
	22	95	5
Flow rate:	2.0 mL/min		
Temperature:	30 °C		
Injection:	5 µL		
Diluent	Mobile phase		
Detection:	UV, 254 nm		
Sample:	Alcohol extract of roots		



No	Compound	Retention Time (min)
1	Salvianic acid A	2.9
2	Salvianolic acid B	9.5
3	Dihydrotanshinone	15.9
4	Tanshinone I	17.8
5	Tanshinone IIA	19.6

Pharmaceuticals

Chromolith® monolithic silica columns provide an excellent solution for the separation of pharmaceutical compounds, including those pharmaceuticals analyzed by pharmacopeia methods.

Chromolith® HighResolution 4.6 and 2 mm I.D. columns offer a major advantage of providing high separation efficiency even at high linear velocities while still providing low column backpressures. You can vary the flow rate over a large range even when you analyze “matrix-rich” samples; i.e samples from dissolution testing which are only filtered. If you have sufficient chromatographic space, i.e. resolution between your peaks, you can easily increase the flow rate and save time. Monolithic columns can be used instead of particulate columns in regulated methods by using the calculation of N (within −25% to +50%, relative to the prescribed column) in the procedure.

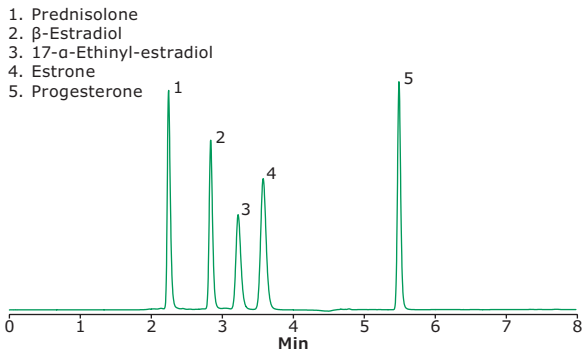
The following benefits can be attained in transferring from particle to monolithic column:

- The method runs faster. The retention is > 30% longer with the particle-packed column
- Lower solvent consumption with the monolithic column (the elution volume is 50% less for the last eluting peak)
- Lower backpressure on the monolithic column = less stress on HPLC system

Hormones on Chromolith® HighResolution RP-18e

Chromatographic conditions

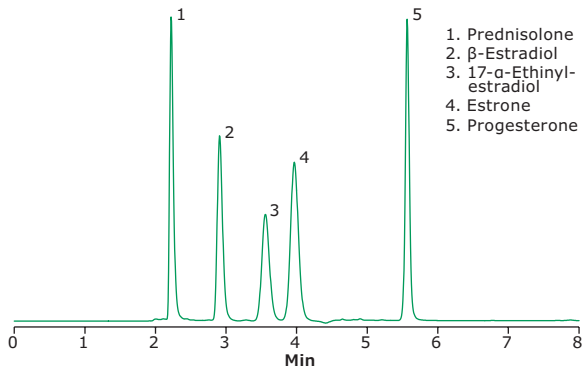
Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] Acetonitrile; [B] 90% 10 mM Phosphate buffer, pH 7.0 + 10% acetonitrile
Gradient:	Initial composition: 0 - 0.5 min 45% A 0.5 mL/min; 0.5-0.6 min 45% A to 35% A 0.5 mL/min; 0.6 – 3 min 35% A 0.5 mL/min to 1.5 mL/min; 3.0 - 3.1 min 35% A to 55% A 1.5 mL/min; 3.1 – 8 min 55% A 1.5 mL/min
Flow rate:	Flow gardient – see gradient
Column temp.:	30 °C
Injection volume:	1 µL
Detector:	220 nm cell: 11 µL
Sample:	Prednisolone 0.6 mg/mL, beta-Estradiol 0.4 mg/mL, 17-alpha-Ethinyl-estradiol 0.4 mg/mL, Estrone 0.5 mg/mL, Progesterone 0.8 mg/mL in Acetonitrile



Hormones on Chromolith® Performance 100-3 mm

Chromatographic conditions

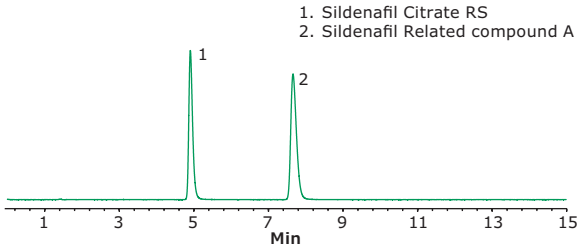
Column:	Chromolith® Performance, 100 x 3 mm (1.52001.0001)
Mobile phase:	[A] Acetonitrile; [B] 90% 10 mM Phosphate buffer, pH 7.0 + 10% Acetonitrile
Gradient and flow gardient:	Initial composition: 0 - 0.5 min, 45% A, 212 µL/min, 0.5 - 0.6 min, 45% A to 35% A , 212 µL/min, 0.6 – 3 min, 35% A, 212 µL/min to 637 µL/min, 3.0 - 3.1 min, 35% A to 55% A, 637 µL/min, 3.1 – 8 min, 55% A, 637 µL/min
Pressure:	653 psi
Column temp.:	30 °C
Injection volumne:	1 µL
Detector:	220 nm cell: 11 µL
Sample:	Prednisolone 0.6 mg/mL, beta-Estradiol 0.4 mg/mL, 17-alpha-Ethinyl-estradiol 0.4 mg/mL, Estrone 0.5 mg/mL, Progesterone 0.8 mg/mL in Acetonitrile



Sildenafil Citrate and Related Substances on Chromolith® HighResolution RP-18e

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 7 mL of triethylamine with water to 1 L. Adjust with phosphoric acid to a pH 3.0 ± 0.1; [B] methanol; [C] acetonitrile; (58:25:17, A:B:C)
Flow rate:	1.0 mL/min
Pressure:	971.5 psi (67 bar)
Column temp.:	30 °C
Injection volume:	20 µL
Detector:	290 nm (cell: 13 µL)
Sample:	Sildenafil Citrate RS 28 ppm, Sildenafil impurity A 28 ppm .

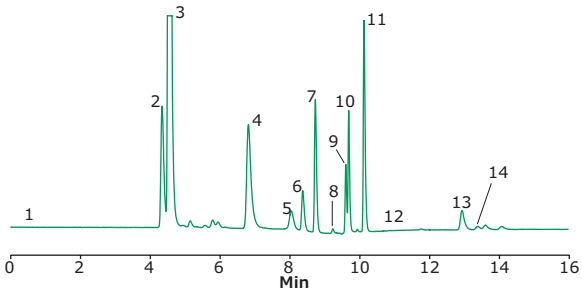


Sildenafil and Impurities on Chromolith® Performance (4.6 mm I.D. columns)

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped, 10 cm x 4.6 mm (2 columns in series) (1.02129.0001)
Mobile phase:	A] Acetonitrile; [B] 20 mM Sodium phosphate buffer, pH 3.0
Gradient and flow gradient:	Initial composition: 0 – 8 min 55% A 45% B isocratic, flow increases from 0.5 to 1 mL/min, 8.1 – 20 min 65% A 35%B isocratic, flow 3 mL/min.
Pressure:	1450 psi (100 bar)
Column temp.:	ambient
Injection volume:	0.5 µL
Detector:	254 nm cell: 11 µL
Sample:	Methoxyamin (1 mg/mL), Sildenafil Citrate (5 mg/mL), Amidin (1 mg/mL), Imidon 1 (0.5 mg/mL), Imidon 2 (0.5 mg/mL), Thiophenethylester (1 mg/mL), Acyl-BT-Ester (2 mg/mL),Aminacid (0.5 mg/mL), CHT-Ester (1 mg/mL), Acyl-CHT-Ester (2 mg/mL), Aminester (0.2 mg/mL), Imidochlorid 1 (0.3 mg/mL), Imidochlorid 2 (0.2 mg/mL)

Peak No.	Name	Retention time (min)	Peak No.	Name	Retention time (min)
1.	Void volume	0.8	8.	Acyl-BT-Acetate (impurity)	9.2
2.	Methoxyamine (impurity)	4.3	9.	Aminacid (impurity)	9.6
3.	Sildenafil Citrate	4.5	10.	CHT-Acetate (impurity)	9.7
4.	Amidin (impurity)	6.8	11.	Acyl-CHT-Acetate (impurity)	10.1
5.	Imidon 1 (impurity)	8.0	12.	Aminacetate (impurity)	11.0
6.	Imidon 2 (impurity)	8.4	13.	Imidochloride 1 (impurity)	13.0
7.	Thiophenethylacetate (impurity)	8.7	14.	Imidochloride 2 (impurity)	13.6

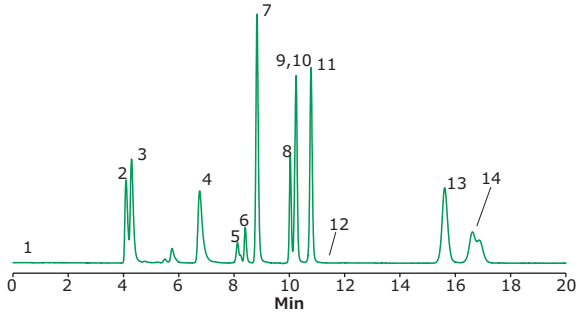


Sildenafil and Impurities on Chromolith® Performance (3 mm I.D. columns)

Chromatographic conditions

Column:	Chromolith® Performance RP-18 endcapped, 10 cm x 3 mm I.D. (2 columns in series) (1.52001.0001)
Mobile phase:	[A] Acetonitrile; [B] 20 mM Sodium phosphate buffer, pH 3.0
Gradient with flow gradient:	Initial composition: 0 – 8 min 55% A 45% B isocratic, flow increases from 213 µL/min to 425 µL/min, 8.1 – 20 min 65% A 35% B isocratic, flow 850 µL/min
Pressure:	8913 psi
Column temp.:	ambient
Injection volume:	0.5 µL
Detector:	254 nm cell: 1.4 µL
Sample:	Methoxyamin (1 mg/mL), Sildenafil Citrate (3 mg/mL), Amidin (1 mg/mL), Imidon 1 (0.5 mg/mL), Imidon 2 (0.5 mg/mL), Thiophenethylester (1 mg/mL), Acyl-BT-Ester (0.8 mg/mL), Aminacid (0.5 mg/mL), CHT-Ester (2 mg/mL), Acyl-CHT-Ester (2 mg/mL), Aminester (0.2 mg/mL), Imidochlorid 1 (0.5 mg/mL), Imidochlorid 2 (0.3 mg/mL)

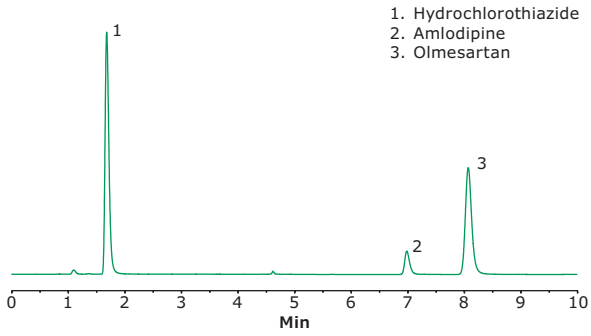
Peak No.	Name	Retention time (min)	Peak No.	Name	Retention time (min)
1.	Void volume (t0)	1.7	8.	Acyl-BT-Acetate (impurity)	10.0
2.	Methoxyamine (impurity)	4.1	9.	Aminacid (impurity)	10.2
3.	Sildenafil Citrate	4.3	10.	CHT-Acetate (impurity)	10.2
4.	Amidin (impurity)	6.8	11.	Acyl-CHT-Acetate (impurity)	10.8
5.	Imidon 1 (impurity)	8.1	12.	Aminacetate (impurity)	11.5
6.	Imidon 2 (impurity)	8.4	13.	Imidochloride 1 (impurity)	15.6
7.	Thiophenethylacetate (impurity)	8.8	14.	Imidochloride 2 (impurity)	16.6



Olmesartan Medoxomil, Amlodipine Besylate, and Hydrochlorothiazide Tablet on Chromolith® HighResolution RP-18e

Chromatographic conditions

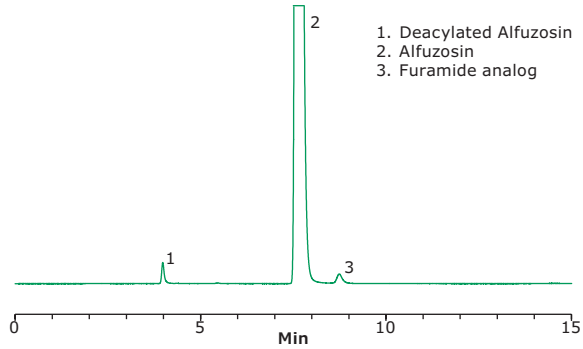
Column:	Chromolith® HighResolution RP-18 endcapped, 50 x 4.6 mm (1.52021.0001)
Mobile phase:	[A] 2.72 g of potassium dihydrogen phosphate in 1000 mL Milli-Q® ultrapure water - Add 1.0 mL triethylamine. Adjust pH to 3.0 with ortho-phosphoric acid ; [B] acetonitrile; (70:30, A:B)
Flow rate:	1 mL/min
Column temp.:	40 °C
Injection:	2 µL
Detector:	225 nm cell: 10 µL
Sample preparation:	To prepare standards: Dilute 40 mg olmesartan medoxomil, 10 mg amlodipine besylate, and 25 mg hydrochlorothiazide to 100 mL with mobile phase component A (buffer):acetonitrile (70:30). To prepare tablets: Crush 20 tablets. Weigh the quantity equivalent to 40 mg olmesartan, 10 mg amlodipine and 25 mg hydrochlorothiazide and transfer into a 100 mL volumetric flask. Add water and sonicate for 20 minutes. Dilute up to the mark with water. Filter through 0.45 µm filter. Resolution solution: Weigh 5.0 mg of amlodipine besylate in 5.0 mL volumetric flask. Add 5.0 mL hydrogen peroxide. Heat at 70 °C for 45 min in water bath.
Sample:	olmesartan medoxomil (0.4 mg/mL), amlodipine besylate (0.1 mg/mL), hydrochlorothiazide (0.25 mg/mL)



Alfuzosin and Related Substances on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

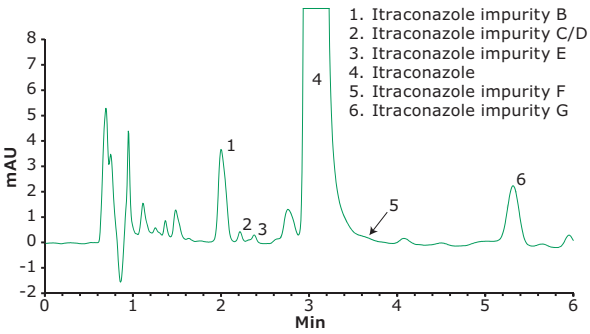
Column:	Chromolith® HighResolution RP-18 endcapped, 150 x 4.6 mm (1.52023.0001)
Mobile phase:	[A] 5 mL of perchloric acid in 900 mL Milli-Q® ultrapure water. Adjust pH to 3.5 with 2 M Sodium hydroxide. Dilute to 1000 mL with water; [B] acetonitrile; [C] THF; (80:20:1, A:B:C)
Flow rate:	1 mL/min
Column temp.:	25 °C
Pressure:	994 psi (69 Bar)
Injection volume:	10 µL
Detector:	254 nm cell: 10 µL
Sample preparation:	Dissolve 40.0 mg of the sample in 100 mL of mobile phase. 0.4 mg/mL (400 ppm) of USP Alfuzosin System Suitability Mixture RS in Mobile phase
Sample:	0.40 µg/mL of Alfuzosin Hydrochloride in Mobile phase from the Sample solution



Itraconazole and Impurities on Chromolith® HighResolution RP-18e

Chromatographic conditions

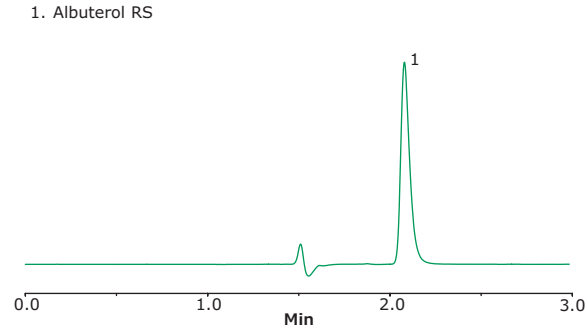
Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] Acetonitrile; [B] Methanol; [C] Water + 3.4 g Tetrabutylammonium hydrogen sulfate; (45:10:45, A:B:C)
Flow rate:	2 mL/min
Column temp.:	30 °C
Injection volume:	10 µL
Detector:	225 nm cell: 11 µL
Sample preparation:	50 mg itraconazol dissolved in 50 mL solution (5 mL 30% hydrochloric acid in 1000 mL of methanol)
Sample:	itraconazol (1 mg/mL)

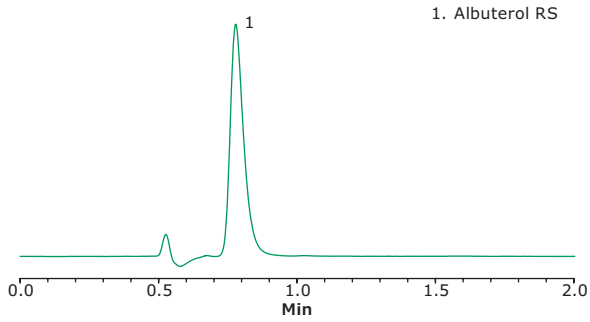


Albuterol Tablets - Assay on Chromolith® HighResolution RP-18 endcapped USP38-NF33

Chromatographic conditions

Column:	A) Chromolith® HighResolution RP-18 endcapped, 150 x 4.6 mm (1.52023.0001) B) Chromolith® HighResolution RP-18 endcapped, 50 x 4.6 mm (1.52021.0001)
Mobile phase:	[A] 10 mL/L of glacial acetic acid in water; [B] 1.13 g of sodium 1-Hexanesulfate in 1200 mL water, add 12 mL of glacial acetic acid; (36:64,:A:B)
Flow rate:	1.5 mL/min
Column temp.:	ambient
Pressure:	1189 psi (82 bar)
Injection volume:	A)25 µL B)8 µL
Detector:	276 nm cell: 11 µL
Sample preparation:	120 µg/mL of Albuterol Sulfate RS prepared as follows. Transfer USP Albuterol Sulfate RS to a suitable volumetric flask and add a volume of Solution A corresponding to 60% of the flask volume. Sonicate for 5 minutes and dilute with methanol to volume.
Sample:	Albuterol RS 30 µg/mL in diluent (30 µg/mL of Albuterol Sulfate RS in Diluent from Standard stock solution)

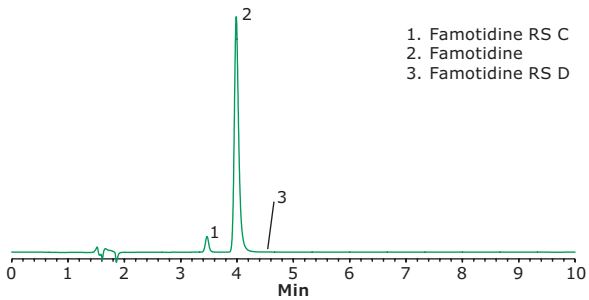




Famotidine Tablets on Chromolith® HighResolution RP-18 Endcapped USP38-NF33

Chromatographic conditions

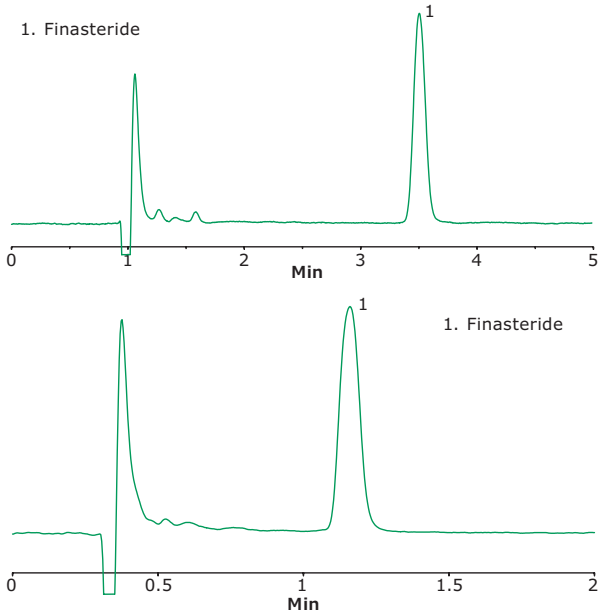
Column:	Chromolith® HighResolution RP-18 endcapped, 150 x 4.6 mm (1.52023.0001)
Mobile phase:	[A] 1 mL Triethylamine, adjust with glacial acetic acid to pH 6.0, dilute with water to 1 L.; [B] acetonitrile; (93:7,:A:B)
Flow rate:	1.4 mL/min
Column temp.:	40 °C
Pressure:	696 psi (48 Bar)
Injection volume:	50 µL
Detector:	275 nm cell: 11 µL
Sample preparation:	Dissolve 6.8 g of monobasic potassium phosphate in 750 mL of water. Adjust with 1 M potassium hydroxide to pH 6.0 and dilute with water to 1 L.
Sample:	System suitability solution



Finasteride Tablets on Chromolith® HighResolution RP-18 Endcapped USP38-NF33

Chromatographic conditions

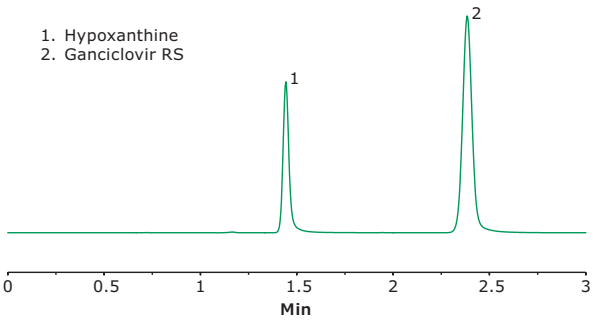
Column:	A) Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001) B) Chromolith® HighResolution RP-18 endcapped, 50 x 4.6 mm (1.52021.0001)
Mobile phase:	[A] Acetonitrile; [B] 2.5 mM Phosphoric acid in Water; (40:60,:A:B)
Flow rate:	A) 1.5 mL/min B) 2.25 mL/min
Column temp.:	45 °C
Pressure:	624 psi (43 bar)
Injection volume:	A) 20 µL B) 10 µL
Detector:	240 nm cell: 11 µL
Sample preparation:	Dissolve an accurately weighed quantity of USP Finasteride RS in Diluting solution, and dilute quantitatively, and step-wise if necessary, with Diluting solution to obtain a solution having a known concentration of about 100 µg/mL.
Sample:	Finasteride RS 100 µg/mL (100 ppm) (Diluent: Acetonitrile and water 70:30 (v/v))



Ganciclovir Injection Solution on Chromolith® HighResolution RP-18 endcapped USP38-NF33

Chromatographic conditions

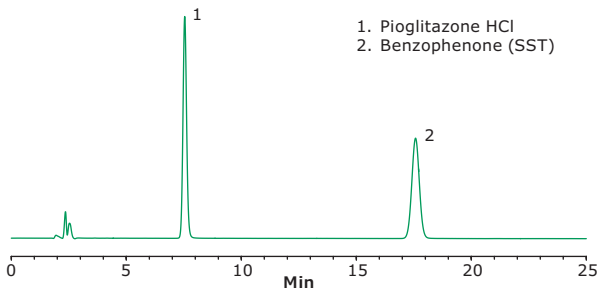
Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	1.4 g/L of monobasic ammonium phosphate and 2.0 g/L of o-phosphoric acid (85%) in water
Flow rate:	2.0 mL/min
Column temp.:	ambient
Pressure:	1407 psi (97 bar)
Injection volume:	10 µL
Detector:	254 nm cell: 11 µL
Sample preparation:	Transfer 20.0 mL of standard stock solution (250 µg/mL of USP Ganciclovir RS in water) and 10.0 mL of the Internal standard solution to a 100 mL volumetric flask. Dilute with mobile phase to volume.
Sample:	Ganciclovir RS, 0.05 mg/mL of USP Ganciclovir RS (Diluent: water)



Pioglitazone Hydrochloride on Chromolith® HighResolution RP-18 endcapped USP38-NF33

Chromatographic conditions

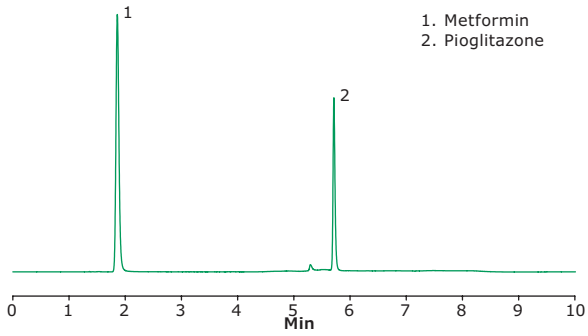
Column:	Chromolith® HighResolution RP-18 endcapped, 150 x 4.6 mm (1.52023.0001)
Mobile phase:	[A] Acetonitrile; [B] 0.1 M Ammonium acetate; [C] glacial acetic acid; (25:25:1, A:B:C))
Flow rate:	0.7 mL/min
Column temp.:	ambient
Pressure:	972 psi (67 bar)
Injection volume:	20 µL
Detector:	269 nm cell: 13 µL
Sample:	50 µg/mL of Pioglitazone HCl and 13 µg/mL of benzophenone (SST solution)



Metformin & Pioglitazone on Chromolith® HighResolution RP18e

Chromatographic conditions

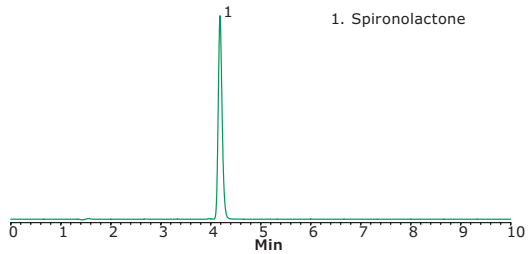
Column:	Chromolith® HighResolution RP18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 1.38 g Sodium hydrogen phosphate in 1800 mL water. Add 3.484 g of Sodium pentane sulfonate. pH adjusted to 3.0 with phosphoric acid and diluted to 2000 mL; [B] acetonitrile;
Gradient:	0 min 90% A, 2.7 min 90% A, 4.0 min 60% A, 6.7 min 60% A, 7.5 min 90% A, 10.0 min 90% A
Flow rate:	1.2 mL/min
Column temp.:	30 °C
Injection volume:	3 µL
Detector:	230 nm cell: 10 µL
Sample:	1. Metformin; 2. Pioglitazone

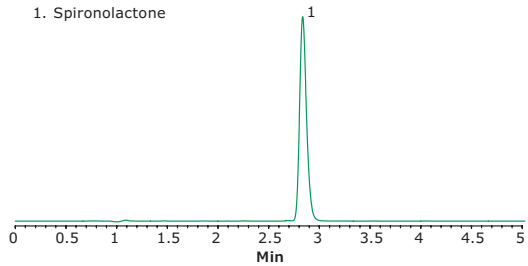


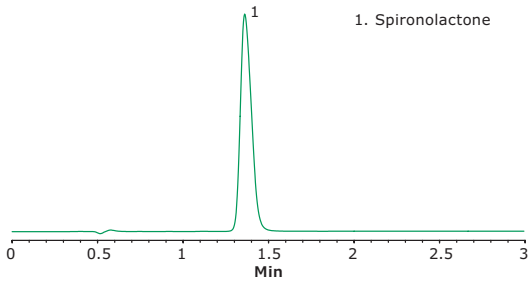
Spironolactone on Chromolith® HighResolution RP-18 endcapped USP38-NF33

Chromatographic conditions

Columns:	A) Chromolith® HighResolution RP-18 endcapped, 150 x 4.6 mm (1.52023.0001) B) Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001) C) Chromolith® HighResolution RP-18 endcapped, 50 x 4.6 mm (1.52021.0001)
Mobile phase:	[A] Water; [B] Methanol; (40:60, A:B)
Flow rate:	1.5 mL/min
Column temp.:	ambient
Pressure:	A) 1595 psi (110 bar) B) 1175 psi (81 bar) C) 856 psi (59 bar)
Injection volume:	A) 20 µL B) 13 µL C) 7 µL
Detector:	230 nm cell: 11 µL
Sample:	0.5 mg/mL (500 ppm) of Spironolactone RS in diluent. (diluent: acetonitrile/water 1:1 (v/v))



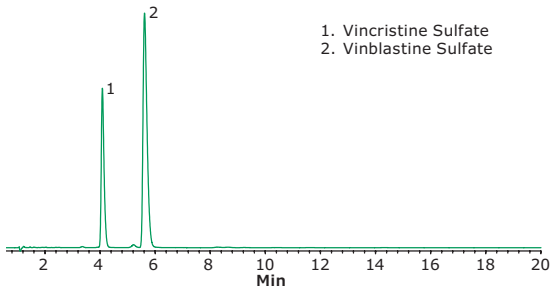




Vinblastine Sulfate on Chromolith® HighResolution RP-18 endcapped USP38-NF33

Chromatographic conditions

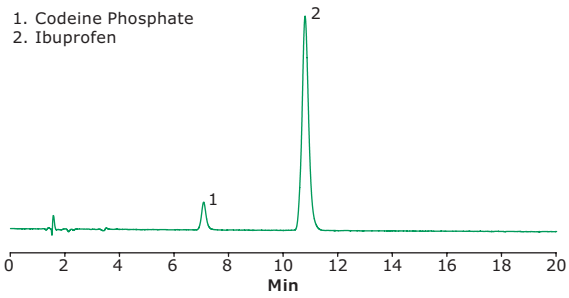
Column:	Chromolith® HighResolution RP-18 endcapped, 150 x 4.6 mm (1.52023.0001)
Mobile phase:	[A] Dimethylamine and water 14/986 (v/v). Adjusted with phosphoric acid to a pH of 7.5; [B] Acetonitrile and Methanol 20/80 (v/v); (38:62, A:B)
Flow rate:	2.0 mL/min
Column temp.:	ambient
Pressure:	2030 psi (140 bar)
Injection volume:	20 µL
Detector:	262 nm; cell: 11 µL
Sample preparation:	Transfer USP Vincristine Sulfate RS (or Assay RS) to a suitable volumetric flask, and dissolve in the Standard solution.
Sample:	0.4 mg/mL each of Vincristine sulfate and Vinblastine Sulfate in water (Diluent: water)



Ibuprofen & Codeine Phosphate Tablet on Chromolith® HighResolution RP18e

Chromatographic conditions

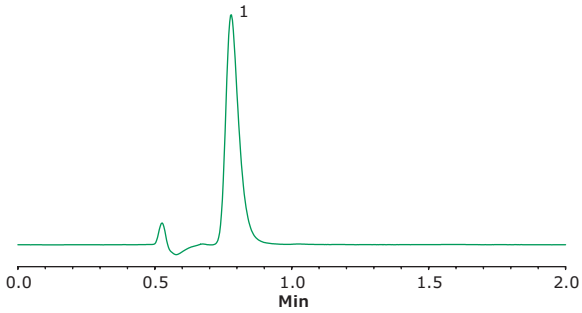
Column:	Chromolith® HighResolution RP18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 2.24 g of Potassium dihydrogen phosphate and 5.4 g of Sodium octane sulfonate in 1000 mL water containing 7 mL triethylamine, adjust pH to 7.2 with ortho-phosphoric acid; [B] methanol; (70:30, A:B)
Flow rate:	1.0 mL/min
Column temp.:	40 °C
Injection volume:	10 µL
Detector:	214 nm cell: 10 µL
Sample preparation:	Weigh 20 tablets & crush. Transfer equivalent to one tablet weight to a 100 mL volumetric flask. Dissolve in 70 mL methanol & sonicate for 20 min. Dilute up to the mark with methanol. Pipette out 5 mL of the above solution & dilute to 50 mL with mobile phase.



Albuterol Tablets - Assay on Chromolith® HighResolution RP-18 endcapped

Chromatographic conditions

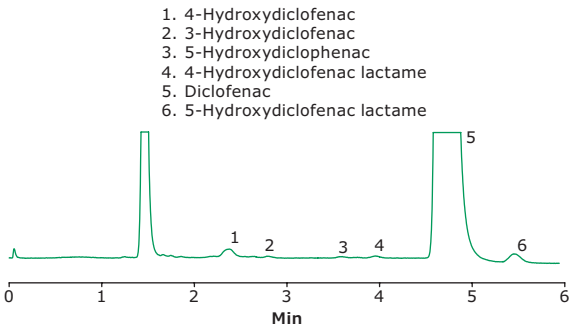
Column:	Chromolith® HighResolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 10 mL/L of glacial acetic acid in water; [B] 1.13 g of Sodium 1-hexanesulfate in 1200 mL water, add 12 mL of glacial acetic acid; (36:64,:A:B)
Flow rate:	1.5 mL/min
Column temp.:	ambient
Pressure:	1553 psi (108 bar)
Injection volume:	17 µL
Detector:	276 nm cell: 11 µL
Sample:	Albuterol RS 30 µg/mL in diluent (30 µg/mL of Albuterol Sulfate RS in Diluent from Standard stock solution)
Sample preparation:	120 µg/mL of Albuterol Sulfate RS prepared as follows. Transfer USP Albuterol Sulfate RS to a suitable volumetric flask and add a volume of Solution A corresponding to 60% of the flask volume. Sonicate for 5 minutes and dilute with Methanol to volume.



Diclofenac and Metabolites on Chromolith® High Resolution RP-18e

Chromatographic conditions

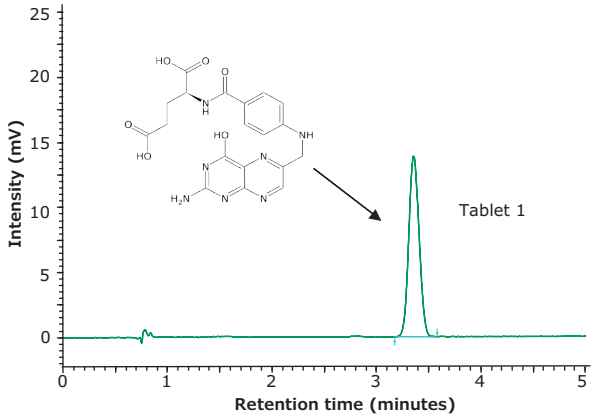
Column:	Chromolith® High Resolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] Acetonitrile; [B] 10 mM Sodium phosphate buffer, pH 3.5; (50:50, A:B)
Flow rate:	1.0 mL/min
Column temp.:	ambient
Injection volume:	5 µL
Detector:	280 nm cell: 11 µL
Sample:	Diclofenac 0.1 mg/mL
Sample preparation:	Diclofenac was dissolved in 10 mM Sodium phosphate buffer, pH 3.5, and stirred overnight at 40 °C. Then, Acetonitrile was added to get a mixture of acetonitrile/buffer 50/50 v/v



USP Dissolution Testing Method (HPLC) for Folic Acid Tablets using a Monolith Column and UV detection

Chromatographic conditions

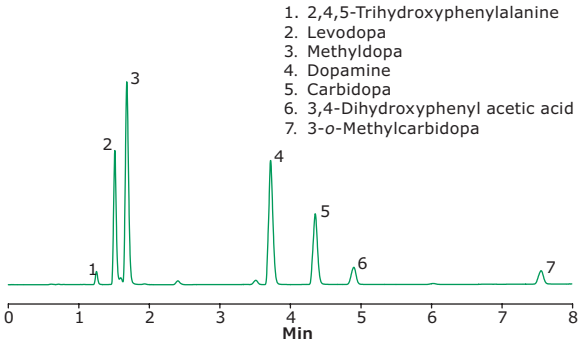
Column:	Chromolith® HighResolution RP-18e 100 x 4.6 mm (1.52022.0001)
Mobile phase:	Transfer 35.1 g of sodium perchlorate and 1.40 g of monobasic potassium phosphate to a 1.0 L volumetric flask. Add 7.0 mL of 1 N potassium hydroxide and 40 mL of methanol, dilute with water to volume and mix. Adjust with 1 N potassium hydroxide or phosphoric acid to a pH of 7.2.
Flow rate:	2.0 mL/min
Temperature:	Ambient
Injection:	10 µL
Detection:	UV, 254 nm
Cell:	20 µL
Sample preparation:	10 ppm in diluent
Pressure drop:	130 Bar (1885 psi)



Carbidopa on Chromolith® HighResolution RP-18e

Chromatographic conditions

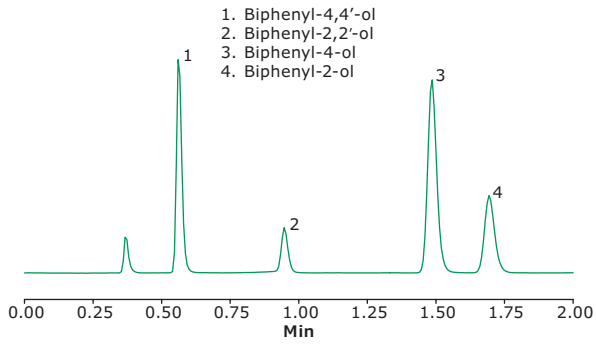
Column:	Chromolith® High Resolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] Methanol; [B] Water + 0.1% (v/v) TFA
Gradient:	100% B held for 0.01 min; to 80% B in 0.9 min; held at 80% A for 9 min
Flow rate:	2.5 mL/min
Column temp.:	ambient
Injection volume:	1 µL
Detector:	UV, 282 nm
Sample:	2,4,5-Trihydroxyphenylalanine 4 µg/mL, Levodopa 402 µg/mL, Methyldopa 384 µg/mL, Dopamine 217 µg/mL, Carbidopa 232 µg/mL, 3,4-Dihydroxyphenyl acetic acid 4 µg/mL, 3-o-Methylcarbidopa 4 µg/mL



Biphenyles on Chromolith® High Resolution RP-18e

Chromatographic conditions

Column:	Chromolith® High Resolution RP-18 endcapped, 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] Acetonitrile; [B] Water; (40:60, A:B)
Flow rate:	4.0 mL/min
Column temp.:	ambient
Injection volume:	5 µL
Detector:	254 nm cell: 11 µL
Sample:	Biphenyl-4,4'-ol 1.5 mg/10 mL, Biphenyl-2,2'-ol 7.0 mg/10 mL, Biphenyl-4-ol 1 mg/10 mL, Biphenyl-2-ol 1 mg/10 mL



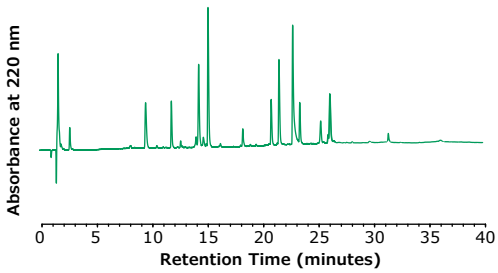
Biopharmaceuticals

Considered by many to be the next frontier for breakthrough medicines, biopharmaceuticals are drug molecules that are proteinaceous in nature. These drugs can exist in the form of peptides, proteins, antibodies, and virus-like particles, among many other forms. These molecules are much larger than typical, small molecule drugs, and, due to this size, can lead to complex mixtures of structurally similar variants. To combat this separation challenge, Chromolith® WP 300 columns were developed. These columns have 300 Å mesopores capable of allowing large biomolecules to enter and elicit separation. In addition, the large macropores allow for high flow rates to be utilized without a drop in efficiency.

Tryptic Digest Analysis on Chromolith® WP 300 RP-18

Chromatographic conditions

Column:	Chromolith® WP 300 RP-18 2 mm I.D. (1.52370.0001)
Mobile phase:	[A] Water (0.1% (v/v) TFA); [B] Acetonitrile (0.08% (v/v) TFA)
Gradient:	0 min 5% B 45 min 50% B
Detection:	UV, 214 nm MS
Temperature:	30 °C
Injection volume:	0.2 µL
Flow rate:	190 µL/min
Sample:	Cytochrome C Equine Heart
Sample preparation:	SOLu-Trypsin Rapid Digestion Kit 4 mg Cytochrome C, Equine Heart, was added in a PCR vial and dissolved in 320 µL Rapid Trypsin Digestion Buffer. 80 µL SOLu-Trypsin was added to the solution and the sample was incubated at 60 °C for 1 hour in a Thermomixer. The digestion was quenched by adding 12 µL of hydrochloric acid 32%



Resulting peptide sequence	Peptide length	Peptide mass [Da]	Identified
K	1	146,189	Y
GK	2	203,241	Y
GGK	3	260,293	Y
HK	2	283,330	Y
TER	3	404,423	N
ATNE	4	433,418	Y
GITWK	5	603,719	Y
IFVQK	5	633,789	Y
MGDVEK	6	677,771	Y
MIFAGIK	7	779,008	Y
EDLIAYLK	8	964,126	Y
CAQCHTVEK	9	1018,171	N
TGPNLHGLFGR	11	1168,321	Y
TGQAPGFTYTDANK	14	1.470,558	Y*
EETLMEYLENPK	12	1.495,664	Y*

Data from ExPASy Calculator (https://web.expasy.org/peptide_cutter/)
* Double ionized

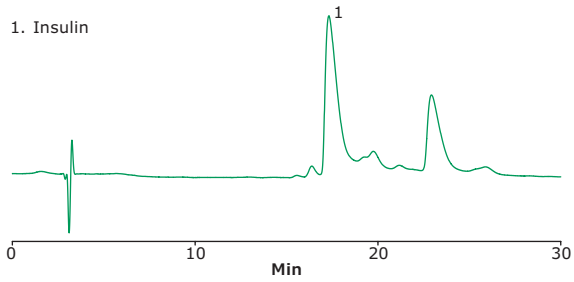
Cleaving of Cytochrome c from equine heart with trypsin is expected to lead to 15 different peptides.

LC-MS with a Single Quadrupole ACQUITY QDA Detector (Waters) leads to a sequence coverage of 87%. Note: maximum m/z ratio is about 1250.

Insulin on Chromolith® Performance 100-2 mm

Chromatographic conditions

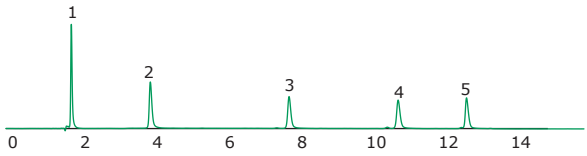
Column:	Chromolith® Performance 100 x 2 mm (1.52006.0001)
Mobile phase:	[A] Acetonitrile; [B] Water + 0.1% TFA v/v; (30:70, A:B)
Flow rate:	95 µL/min
Column temp.:	ambient
Pressure:	1581 psi (109 bar)
Injection volume:	0.4 µL
Detector:	215 nm cell: 1.4 µL
Sample preparation:	9 mg Insulin are dissolved in 9 mL 0.01 M Hydrochloric acid and put into the vacuum dryer at 50 °C for 4 hours. Then, it was stored for 96 hours at 25 °C.
Sample:	Insulin, 1 mg/mL in 10 mM Hydrochloric acid



Peptides on Chromolith® WP 300 RP-18

Chromatographic conditions

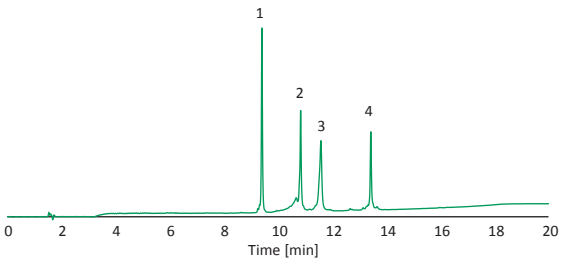
Column:	Chromolith® WP 300 RP-18 100 x 4.6 mm (1.52270.0001)
Mobile phase:	[A] Acetonitrile + 0.1% (v/v) TFA; [B] Water + 0.1% (v/v) TFA
Gradient:	90% A held for 1 min; to 80% A in 10 min; held at 80% A for 5 min
Flow rate:	1.0 mL/min
Column temp.:	60 °C
Injection volume:	1.0 µL
Detector:	UV, 220 nm
Sample:	HPLC peptide standard mixture (1. Gly-Tyr, 2. Val-Tyr-Val, 3. Met enkephalin, 4. Leu enkephalin, 5. Angiotensin II)



Proteins on Chromolith® WP 300 RP-8

Chromatographic conditions

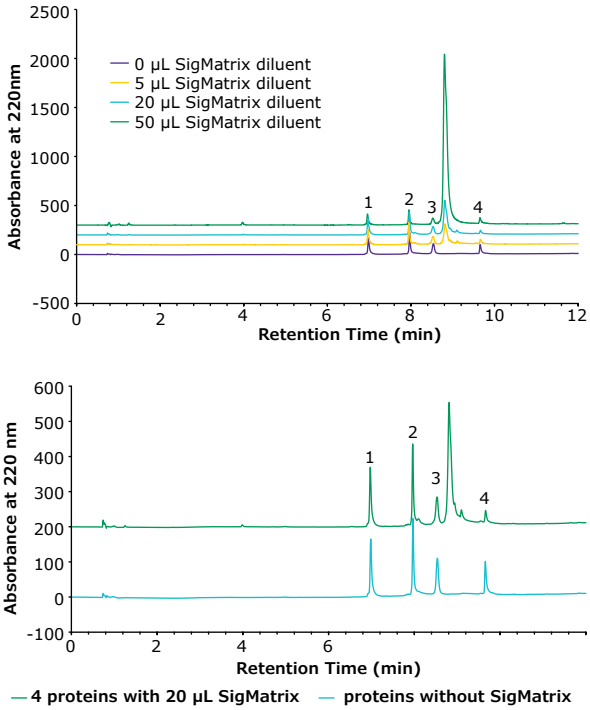
Column:	Chromolith® WP 300 RP-8 100 x 4.6 mm (1.52265.0001)
Mobile phase:	[A] Acetonitrile + 0.1% (v/v) TFA; [B] Water + 0.1% (v/v) TFA
Gradient:	90% A held for 1 min; to 80% A in 10 min; held at 80% A for 5 min
Flow rate:	1.0 mL/min
Column temp.:	60 °C
Injection volume:	1.0 µL
Detector:	UV, 220 nm
Sample:	HPLC protein standard mixture (1. Ribonuclease, 2. Cytochrome c, 3. holo-transferrin, 4. Apo myoglobin)



Proteins in SigMatrix Serum Diluent on Chromolith® WP 300 RP-18

Chromatographic conditions

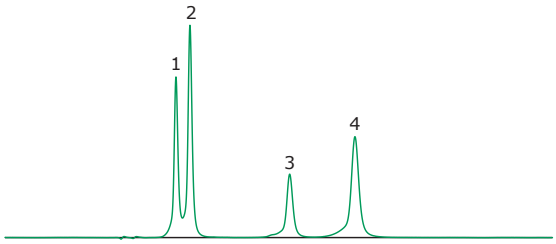
Column:	Chromolith® WP 300 RP-18 100 x 2 mm (1.52370.0001)
Mobile phase:	[A] Acetonitrile + 0.08% TFA; [B] Water + 0.1% TFA
Gradient:	4% B to 60% B in 10 min
Flow rate:	0.38 mL/min
Column temp.:	30 °C
Injection volume:	0.5 µL
Detector:	220 nm
Sample:	HPLC Protein Mix 1. Ribonuclease, 2. Cytochrome c, 3. holo-Transferrin, 4. apomyoglobin 6% SigMatrix Serum diluent 0 µL / 5 µL / 20 µL / 50 µL of SigMatrix Serum diluent were added to 100 µL Protein Mix



Nucleosides on Chromolith® WP 300 Epoxy immobilized with Cysteine

Chromatographic conditions

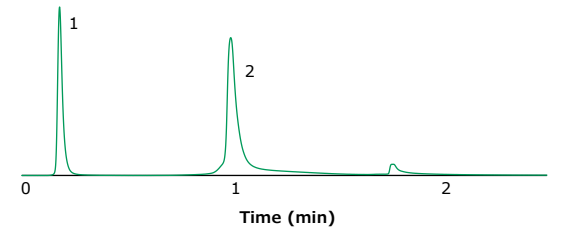
Column:	Chromolith® WP 300 Epoxy 100 x 4.6 mm (1.52250.0001)
Immobilization:	5.01 g Cysteine dissolved in 25 mL, 50 mM sodium phosphate + 1.9 M ammonium sulfate pH 8.0; Immobilization for 24 hours at 0.2 mL/min
Mobile phase:	[A] Acetonitrile [B] 5 mM Ammonium acetate A/B 90/10
Flow rate:	1.0 mL/min
Column temp.:	23 °C
Injection volume:	1.0 µL
Detector:	254 nm
Sample:	1. Adenosine 2. Uridine 3. Cytidine 4. Guanidine in water/acetontrile 10/90

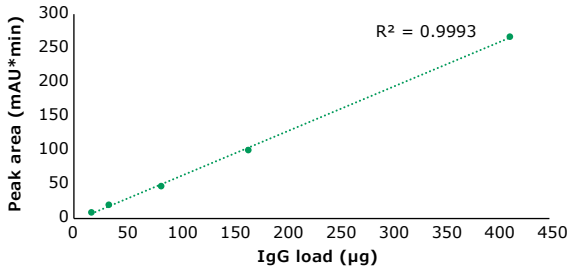


IgG on Chromolith® WP 300 Epoxy immobilized with Protein G

Chromatographic conditions

Column:	Chromolith® WP 300 Epoxy 100 x 4.6 mm (1.52250.0001)
Immobilization:	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
Mobile phase:	[A] 100 mM sodium phosphate, pH 7.4 [B] 100 mM sodium phosphate, pH 2.5
Gradient:	100% A 0.0 min; to 100% B in 0.5 min; held at 100% B for 0.1 min; 100% A in 0.6 min
Flow rate:	2.0 mL/min
Column temp.:	25 °C
Injection volume:	10 µL 1 µL, 2 µL, 5 µL, 10 µL, 25 µL (for linearity)
Detector:	280 nm
Sample:	1. BSA 2. IgG (Gammanorm) – 16.5 mg/mL

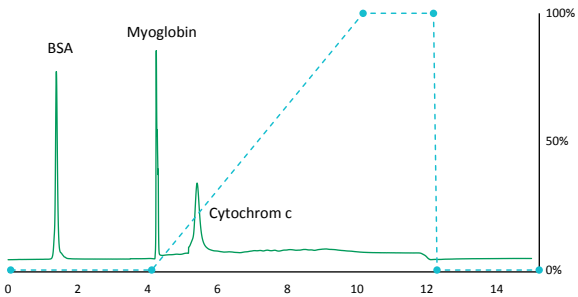




Proteins on Chromolith® WP 300 Epoxy immobilized with Iminodiacetic Acid

Chromatographic conditions

Column:	Chromolith® WP 300 Epoxy 100 x 4.6 mm (1.52250.0001)
Immobilization:	1 g imidodiacetic acid dissolved in 25 mL, 50 mM Disodium hydrogen phosphate + 1.9 M Ammonium sulfate, pH 8.0; Immobilization for 72 hours at 0.2 mL/min
Mobile phase:	[A] 20 mM sodium phosphate + 100 mM sodium chloride, pH 7.0 [B] A + 200 mM imidazole
Gradient:	100% A 0.0 min; held at 100% A for 4 min; to 100% B in 6 min; held at 100% B for 2 min; 100% A in 0.1 min; held at 100% A for 2 min
Flow rate:	1.0 mL/min
Column temp.:	25 °C
Injection:	20 µL
Detector:	280 nm
Sample:	1. BSA, 0.97 mg/mL in water 2. Myoglobin, 0.76 mg/mL in water 3. Cytochrome c, 0.81 mg/mL in water



Cosmetics

Cosmetic samples, such as Creams or Lipsticks, are very matrix-rich and therefore challenging in HPLC analysis. Due to their high matrix-tolerance, Chromolith® columns provide several advantages over particle packed columns and make them the preferred choice for Cosmetic sample analysis. Chromolith® columns require less sample preparation and allows for a simplified analytical workflow which results in significant time and cost savings.

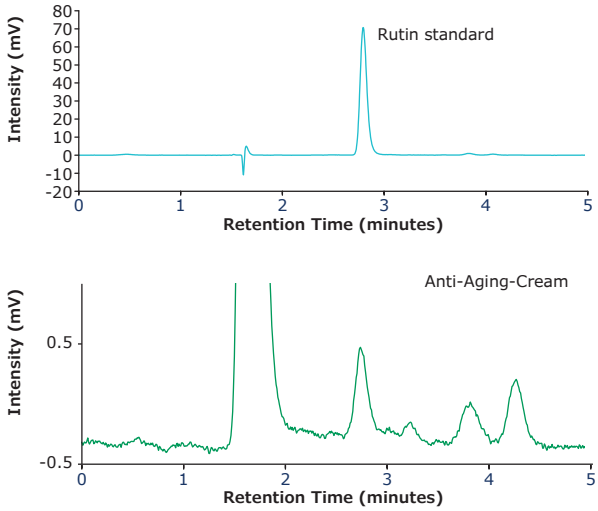
Rutin in Anti-Aging Skin Cream Formulations using Chromolith® HighResolution RP-18 endcapped Column and UV Detection

Rutin is believed to possess antioxidant properties, and/or possess anti-aging effects on human dermal fibroblasts and human skin. Like other bioflavonoids, Rutin is investigated for its biological activity and often formulated as skin cream, i. e. semi-solid emulsions of oil and water, products.

HPLC analysis of skin cream formulations can profit from use of monolithic columns over particle-packed columns due to higher tolerance against matrix heavy samples.

Chromatographic conditions

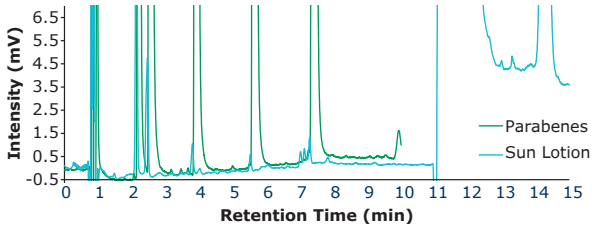
Column:	Chromolith® HighResolution RP-18e 100 x 4.6 mm (1.52022.0001)		
Injection volume:	1 µL		
Flow rate:	1 mL/min		
Temperature:	25 °C		
Pressure drop:	56 - 85 bar (812-1233 psi)		
Detection:	Dionex™ Ultimate 3000 VWD-3400, 5 Hz, Response Time 0.1 s, UV, 220 nm		
Mobile phase:	[A] 10 mM Phosphate buffer, pH 3.0; [B] Methanol		
Gradient:	**Time (min)**	**% A**	**% B**
	0	55	45
	5	55	45
	10	10	90
	10.1	55	45
	15	45	45
Diluent:	Ethanol		
Standard solution (282 µg/mL):	Rutin was accurately weighed and diluted to volume with diluent.		
Sample preparation (cream):	50 mg Day-Cream were weighed into a 25 mL volumetric flask and topped off with ethanol. The cream suspension was placed into an ultrasonic bath for 20 minutes. The sample solution was filtered through a 0.45 µm PTFE filter directly into the HPLC vial prior to analysis and placed in the autosampler.		



Parabenes in Sun Lotion on Chromolith® HighResolution RP-18e

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18e 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 20 mM Potassium phosphate, pH 4.5 [B] Acetonitrile
Gradient:	80% A 0 min; to 60% A in 6 min; held at 60% A for 4 min; to 10% A in 5 min
Flow rate:	2.0 mL/min
Column temp.:	25 °C
Injection volume:	10 µL
Detector:	230 nm
Sample preparation:	50 mg Sun Lotion were weight into a 25 mL volumetric flask and diluted to mark with ethanol. The suspension was transferred into an ultra sonic bath for 20 min. The solution was filtered through a 0.45 µm filter directly into the vial.
Sample:	Phenoxyethanol 4.16 mg/mL, Methylparaben 4.24 mg/mL, Ethylparaben 4.32 mg/mL, Propylparaben 4.24 mg/mL, Butylparaben 4.64 mg/mL in acetontrile/water 40/60

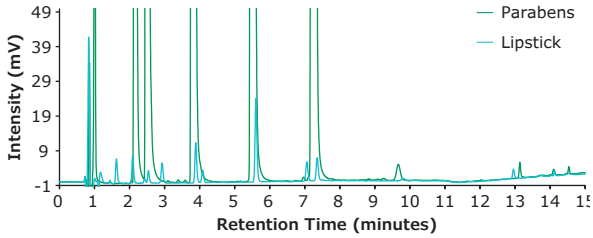


Compound	Asymmetry	Retention Time (min)	Area mAU*min	S/N
t0 void volume		0.8		
Phenoxyethanol	0.90	2.1	0.833	139.70
Methylparaben	0.81	2.5	0.402	57.50
Ethylparaben	1.04	3.8	0.095	10.40
Propylparaben	1.02	5.5	0.045	5.80
Butylparaben	1.07	7.3	0.061	2.80

Parabenes in Lipstick on Chromolith® HighResolution RP-18e

Chromatographic conditions

Column:	Chromolith® HighResolution RP-18e 100 x 4.6 mm (1.52022.0001)
Mobile phase:	[A] 20 mM Phosphate buffer, pH 4.5; [B] Acetonitrile
Gradient:	80% A 0 min; to 60% A in 6 min; held at 60% A for 4 min; to 10% A in 5 min
Flow rate:	2.0 mL/min
Column temp.:	25 °C
Injection volume:	10 µL
Detector:	230 nm
Sample preparation:	250 mg of lipstick were weighed into a 25 mL volumetric flask and dissolved in 2.5 mL THF by stirring in a water bath (70 °C). Then, 15 mL hot methanol was added, and the waxes dropped out. After cooling down, the volumetric flask was topped off with Methanol. The combined solution was filtered through a 0.45 µm filter directly into the vial.
Sample:	Phenoxyethanol 4.16 mg/mL, Methylparaben 4.24 mg/mL, Ethylparaben 4.32 mg/mL, Propylparaben 4.24 mg/mL, Butylparaben 4.64 mg/mL in acetontrile/water 40/60



Compound	Retention Time (min)	Plates	Area mAU*min	Asymmetry
t0 void volume	0.7			
Propylparaben	5.6	49796	1.549	1.2

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Ordering Information

Chromolith® Guard Columns, Holder and Coupler

Chromolith® Guard column Holder

Chromolith® HPLC guard cartridges are extremely easy to use. The guard cartridges 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 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 2 mm and 4.6 mm I.D. cartridges and holders for 10 and 25 mm I.D. cartridges.

a) b) c) d)

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)	2, 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

Chromolith® Guard cartidge Holder

for dimension	Type	Material	Item No.
5 x 2 and 3	a	PEEK	1.52004.0001
5 x 2	b	Bioinert	1.52355.0001
5 x 4.6	b	Bioinert	1.52255.0001
10 x 4.6	b	Bioinert	1.52256.0001
5 x 4.6	c	SST	1.52032.0001
10 x 4.6	c	SST	1.52033.0001
10 x 10	d	PEEK/SST	1.52037.0001

Chromolith® Column Coupler
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.

Ordering Information

for 25 mm I.D. columns	1.25259.0001

Chromolith® columns for small molecule separations

Column dimension										
Length (mm)	I.D. (mm)	RP-18e	HR RP-18e	RP-8e	HR RP-8e	Phenyl	CN	Diol	NH_2	Si
Chromolith® HPLC Column [1 unit]										
25	x 4.6	1.51463.0001	1.52020.0001			1.52056.0001	1.52046.0001	1.53170.0001	1.52026.0001	
25	x 3	1.52003.0001								
25	x 2	1.52014.0001	1.52320.0001							
50	x 4.6	1.51450.0001	1.52021.0001			1.52057.0001	1.52047.0001	1.53171.0001	1.52027.0001	
50	x 3	1.52002.0001								
50	x 2	1.52007.0001	1.52321.0001							
100	x 4.6	1.02129.0001	1.52022.0001	1.51468.0001	1.52064.0001	1.52058.0001	1.52048.0001	1.53172.0001	1.52028.0001	1.51465.0001
100	x 3	1.52001.0001								
100	x 2	1.52006.0001	1.52322.0001							
150	x 4.6		1.52023.0001							
100	x 10	1.52016.0001								1.52015.0001
100	x 25	1.25252.0001								1.25251.0001
Validation Kits [3 Chromolith® HPLC cartidges from 3 different sorbent batches]										
50	x 2	1.52062.0001								
100	x 4.6	1.51466.0001	1.52019.0001							
100	x 3	1.52063.0001								
100	x 10	1.52036.0001								1.52035.0001
Chromolith® Guard cartidges [3 units]										
5	x 4.6	1.51451.0001	1.52025.0001	1.52013.0001		1.52059.0001	1.52050.0001	1.53175.0001	1.52030.0001	1.52011.0001
10	x 4.6	1.51452.0001								
5	x 3	1.52005.0001								
5	x 2	1.52009.0001	1.52325.0001							
10	x 10	1.52036.0001								
Chromolith® Guard cartidge Set [1 starter kit with holder and 3 guard cartridges]										
5	x 2	1.52008.0001								
5	x 3	1.52004.0001								

Chromolith® CapRod® Capillary Columns

Column dimension					
Length (mm)	I.D. (mm)	HR RP-18e	RP-8e	HR RP-8e
Chromolith®CapRod® HPLC capillary columns [1 unit]				
50	x 0.1 Trap		1.50426.0001	
50	x 0.2 Trap		1.50409.0001	
150	x 0.05		1.50403.0001	
150	x 0.1	1.50404.0001	1.50402.0001	1.50400.0001
150	x 0.2	1.50407.0001	1.50405.0001	
300	x 0.1		1.50424.0001	

Validation kits

The success of an HPLC method depends strongly on the consistent quality of the stationary phase. Long-term reproducibility is a key factor in achieving reliable results. Supelco® validation kits consist of three HPLC columns, packed with three different sorbent lots to confirm the reliability of HPLC methods and their robustness.

Chromolith® WP 300 for Biomolecule Separation

Column dimension						
Length (mm)	I.D. (mm)	RP-18	RP-8	RP-4	Protein A	Epoxy
Chromolith® WP 300 HPLC Column [1 unit]						
25	x 4.6				1.52258.0001	1.52252.0001
25	x 2				1.52358.0001	1.52352.0001
50	x 4.6	1.52271.0001	1.52266.0001	1.52261.0001		1.52251.0001
50	x 2	1.52371.0001				1.52351.0001
100	x 4.6	1.52270.0001	1.52265.0001	1.52260.0001		1.52250.0001
100	x 2	1.52370.0001				1.52350.0001
Chromolith® Guard cartidges [3 units]						
5	x 4.6	1.52273.0001	1.52268.0001	1.52263.0001		1.52254.0001
5	x 2	1.52372.0001				1.52353.0001
10	x 4.6	1.52272.0001	1.52267.0001	1.52262.0001		1.52253.0001

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