The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Chromolith® HPLC columns
Race through separations with revolutionary technology
Tired of congestion?

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

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

### Plates per pressure (N/bar)

<table>
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<tr>
<th>Core shell a 2.7 µm</th>
<th>Core shell b 2.6 µm</th>
<th>Porous particles 3 µm</th>
<th>Porous particles 5 µm</th>
<th>Core shell 5 µm</th>
<th>Chromolith® HighResolution</th>
<th>Chromolith® Performance</th>
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All columns are C18e modified, 100-4.6 mm. Sample: anthracene, eluted isocratically using acetonitrile/water (60/40) at 2 mL/min flow rate. Injection volume: 5 µL, detection at 254 nm UV. All analyses performed at room temperature.

Speed up with Chromolith® columns

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

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

 SigmaAldrich.com/chromolith

The thin skeleton ensures fast mass transfer, while the large channels allow unobstructed flow through the column. SEM image: Cross section of a silica monolith. Total porosity > 80%.

A column packed with tiny particles is relatively easy to block since the space between particles is directly proportional to particle size (approx. one sixth of particle diameter). The smaller space leads to higher back pressure and blockage. SEM image: Silica particles.
## Why choose a Chromolith® column?

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

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

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

- **Most commonly used column**
- **Column with some successful separation cases**

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

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### Loadability
- +

### Sensitivity
+ -

### Solvent saving
+ -

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### Chromolith® columns are available in various lengths:

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Chromolith® HPLC columns
Revolutionary monolithic silica replaces particles

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

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

SEM image: Cross section of a silica monolith. Total porosity > 80%.

**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.
Characterization of Chromolith® HPLC columns

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

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

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

Analysis speed

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

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.

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

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

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

Chromolith® Performance RP-18 100-4.6 mm

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

High separation efficiency

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

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

For complex separations, it is still necessary to use long columns in order to provide the separation efficiency required for resolution of all compounds of interest. Chromolith® HPLC columns can be connected in series to produce a column with high plate count at low back pressure. (Please see: Chromolith® column coupler). With particulate columns, further column length is prevented by excessive back pressure.

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

Long-term stability

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

Column robustness

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

Chromolith® Performance RP-18e

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

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

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

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

**Typical column efficiency using the Chromolith® column coupler**

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

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

**Application of Chromolith® column coupler**

**81,000 plates at 85 bar pressure**

<table>
<thead>
<tr>
<th>Column</th>
<th>10 columns of Chromolith® Performance RP-18 endcapped 100-4.6 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>80/20 Acetonitrile / water</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>UV 254 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>ambient</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 μL</td>
</tr>
</tbody>
</table>

Sample:
1. Thiourea
2. Benzene
3. Toluene
4. Ethylbenzene
5. Propylbenzene
6. Butylbenzene
7. Pentylbenezene
Column coupling: 11 steroids

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

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

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

Full flexibility with mobile phases

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

Doping drug separation using ACN and MeOH

Chromolith® Performance RP-18e 100-2mm

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fluoxymesterone, Boldenone, Methandrostenolone, Testosterone, Methyltestosterone, Boldenone Acetate, Testosterone Acetate, Nandrolone Propionate, Testosterone Propionate, Nandrolone Phenylpropionate, Testosterone Isocaproate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>A - ACN or MeOH, B - water</td>
</tr>
<tr>
<td>Injection</td>
<td>0.2 µL, Detection: UV 240 nm</td>
</tr>
</tbody>
</table>

---

![Graph showing viscosity change with percent organic solvents](image)

![Graph showing separation of doping drugs using ACN and MeOH](image)
Chromolith®
Capillary columns
0.05 mm / 0.1 mm / 0.2 mm i.d.
Monolithic capillary columns have become increasingly important in the separation of biomolecules, especially in combination with mass spectrometry. In contrast to particulate columns, monolithic capillaries do not require frits, and have a much lower tendency to clog. This allows higher flow rates, improving the speed and quality of biomolecule characterization. To answer the growing interest in micro and nano-HPLC, we offer a wide range of outstanding monolithic silica capillaries with a variety of internal diameters, bonded phases, pore structures, and lengths.

**Why choose capillary Chromolith® columns?**

<table>
<thead>
<tr>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher flow rates than particle-packed capillary columns at low pressure</td>
</tr>
<tr>
<td>Long column lifetime</td>
</tr>
<tr>
<td>Robust and easy handling</td>
</tr>
<tr>
<td>Flow rates from 0.1–200 μL/min ensure ideal compatibility with LC/MS systems, with both ESI and APCI interfaces</td>
</tr>
</tbody>
</table>
**Chromolith® CapRod®**

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

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

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

---

**Recommended use and flow rate ranges**

<table>
<thead>
<tr>
<th>Recommended use</th>
<th>RP-18e 150 x 0.05 mm</th>
<th>RP-8e 150 x 0.1 mm</th>
<th>RP-18e 50 x 0.1 mm Trap</th>
<th>RP-18e 150 x 0.1 mm</th>
<th>RP-18e 300 x 0.1 mm</th>
<th>RP-18e 150 x 0.1 mm HR</th>
<th>RP-18e 50 x 0.2 mm Trap</th>
<th>RP-18e 150 x 0.2 mm</th>
<th>RP-18e 150 x 0.2 mm HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separation of small molecules</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>– of peptides</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>– of proteins</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Micro ESI</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Nano ESI</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>High Resolution</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
<td>●</td>
</tr>
<tr>
<td>Flow rates (μL/min)</td>
<td>0.2 – 0.8</td>
<td>0.4 – 3</td>
<td>1 – 10</td>
<td>0.4 – 3</td>
<td>0.2 – 1.5</td>
<td>0.1 – 0.4</td>
<td>10 – 50</td>
<td>5 – 20</td>
<td>0.5 – 2</td>
</tr>
<tr>
<td>Max back pressure (bar)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>218</td>
<td>218</td>
<td>218</td>
<td>218</td>
</tr>
</tbody>
</table>
Separation example: Sudan dyes

**Chromolith® CapRod® RP-18e 150-0.1 mm**

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

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

Separation example: Sudan dyes and capsaicinoids in hot chili sauce

**Chromolith® CapRod® RP-18e 150-0.1 mm**

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Compound</th>
<th>Retention Time (min)</th>
<th>Monoisotopic mass (g/mol)</th>
<th>(M+H)+ (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Nordihydrocapsaicin</td>
<td>4.05</td>
<td>293.2</td>
<td>294.11</td>
</tr>
<tr>
<td>2.</td>
<td>Capsaicin</td>
<td>4.23</td>
<td>305.2</td>
<td>306.18</td>
</tr>
<tr>
<td>3.</td>
<td>Dihydrocapsaicin</td>
<td>4.9</td>
<td>307.21</td>
<td>308.19</td>
</tr>
<tr>
<td>4.</td>
<td>Homodihydrocapsaicin</td>
<td>5.73</td>
<td>321.23</td>
<td>322.11</td>
</tr>
<tr>
<td>5.</td>
<td>Para red</td>
<td>7.02</td>
<td>293.08</td>
<td>294.05</td>
</tr>
<tr>
<td>6.</td>
<td>Sudan I</td>
<td>7.55</td>
<td>248.09</td>
<td>249.05</td>
</tr>
<tr>
<td>7.</td>
<td>Sudan II</td>
<td>9.33</td>
<td>276.13</td>
<td>277.1</td>
</tr>
<tr>
<td>8.</td>
<td>Sudan III</td>
<td>10.74</td>
<td>352.13</td>
<td>353.11</td>
</tr>
<tr>
<td>9.</td>
<td>Sudan IV</td>
<td>12.11</td>
<td>380.16</td>
<td>381.21</td>
</tr>
</tbody>
</table>
Chromolith® Analytical columns, 130/150Å mesopores

2 mm / 3 mm / 4.6 mm i.d.
Standard HPLC columns with 3 or 5 μm silica particles often suffer from high back pressure. Hence, they are limited in length, and have a lower number of theoretical plates.

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

Why choose analytical Chromolith® columns?

- Fast, high-performance results
- Substantially longer column lifetime
- High resistance to column blockage
- Cost savings from higher sample throughput and column durability
- Compatible with all low dead volume LC instruments (UHPLC, UPLC, HPLC)
- Possibility of flow gradients
- Increased column performance by column coupling
The chemical basis of Chromolith® RP-18 endcapped columns—from starting materials to surface modifications—is the same as high-end particulate columns. Thus, their selectivity is comparable to high-quality C18 endcapped reversed-phase packed columns. This allows the use of standard methods when developing new protocols. The columns are based on high-purity silica, hence they minimize the negative effect of trace metals. Furthermore, they are chemically modified with n-alkyl chains that possess a high ligand density, and are fully endcapped to reduce the effect of unmodified silanol groups.

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

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

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

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

**Chromolith® Performance RP-18e 100-4.6 mm**

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

**Chromolith® Performance RP-18e 100-2 mm**

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

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

Chromolith® FastGradient RP-18e 50-2 mm

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

Separation example: Proteomics

Chromolith® Performance RP-18e 100-2 mm

- **Column**: Chromolith® Performance RP-18 endcapped 100-2 mm
- **Mobile phase**:
  - A: 95 % H₂O/5 % ACN/0.1 % TFA (v/v/v)
  - B: 5 % H₂O/95 % ACN/0.085 % TFA (v/v/v)
- **Gradient**:
  - from 5 % B to 50 % B in 20 min
- **Flow rate**: 0.3 mL/min
- **Detection**: UV 214 nm
- **Sample**: 1 µL BSA digest (1 mg/mL)
**Chromolith® RP-18 endcapped 3 mm i.d. columns: Fast and solvent saving separations at lower flow rates**

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

**Chromolith® Performance RP-18e 100-4.6 mm**

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

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

**Chromolith® Performance RP-18e 100-3 mm**

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

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

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

The same separation on a Chromolith® 3 mm i.d. column demonstrates improved sensitivity at just 1.7 mL/min. This equates to solvent savings of 57%.
Chromolith® HighResolution RP-18 endcapped

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

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

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

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

**Chromolith® HighResolution RP-18 endcapped**

**Comparison: Chromolith® and Chromolith® HighResolution**

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

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

**Higher efficiency, symmetrical peaks**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile / water 60/40</td>
</tr>
<tr>
<td>Flow rate</td>
<td>2 mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>UV 254 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>ambient</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 μL</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
</tr>
<tr>
<td>1. Urea</td>
<td></td>
</tr>
<tr>
<td>2. Biphenyl-2-ol</td>
<td></td>
</tr>
<tr>
<td>3. Progesterone</td>
<td></td>
</tr>
<tr>
<td>4. Hexanophenon</td>
<td></td>
</tr>
<tr>
<td>5. Anthracene</td>
<td></td>
</tr>
</tbody>
</table>

**Chromolith® Performance RP-18e 100-4.6 mm**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/m</td>
<td>101.300</td>
</tr>
<tr>
<td>T&lt;sub&gt;UFP&lt;/sub&gt;</td>
<td>1.49</td>
</tr>
<tr>
<td>Pressure</td>
<td>21 bar</td>
</tr>
</tbody>
</table>

**Chromolith® HR RP-18e 100-4.6 mm**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/m</td>
<td>165.900</td>
</tr>
<tr>
<td>T&lt;sub&gt;UFP&lt;/sub&gt;</td>
<td>1.25</td>
</tr>
<tr>
<td>Pressure</td>
<td>71 bar</td>
</tr>
</tbody>
</table>
**Improved peak shape for basic compounds**

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

---

**Chromolith® HighResolution RP-18e 100-4.6 mm**

<table>
<thead>
<tr>
<th>Column</th>
<th>Chromolith® HighResolution RP-18 endcapped 100-4.6 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>A: ACN</td>
</tr>
<tr>
<td></td>
<td>B: 20 mM NaH₂PO₄ buffer pH 7.6</td>
</tr>
<tr>
<td>Gradient</td>
<td>0 min 20% A</td>
</tr>
<tr>
<td></td>
<td>0.5 min 45% A</td>
</tr>
<tr>
<td>Flow rate</td>
<td>2 mL/min</td>
</tr>
<tr>
<td>Column pressure</td>
<td>63 – 69 bar</td>
</tr>
<tr>
<td>Detection</td>
<td>UV 254 nm</td>
</tr>
<tr>
<td>Vol. detector cell</td>
<td>16 µL</td>
</tr>
<tr>
<td>Temperature</td>
<td>ambient</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>1. Caffeine</td>
</tr>
<tr>
<td></td>
<td>2. Aniline</td>
</tr>
<tr>
<td></td>
<td>3. N-Methylaniline</td>
</tr>
<tr>
<td></td>
<td>4. 2-Ethylaniline</td>
</tr>
<tr>
<td></td>
<td>5. 4-Nitranisole</td>
</tr>
<tr>
<td></td>
<td>6. N,N-Dimethylaniline</td>
</tr>
</tbody>
</table>

---

**Excellnt batch-to-batch reproducibility**

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

---

**Chromolith® HighResolution RP-18e 100-4.6 mm**

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

---

![Graph of Chromolith® HighResolution RP-18e 100-4.6 mm](image1.png)

![Graph of Chromolith® HighResolution RP-18e 100-4.6 mm](image2.png)
The ideal alternative to sub-3 μm particulate columns

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

Chromolith® HR Silica monolith

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

Core-shell, 2.6 μm particles

<table>
<thead>
<tr>
<th>Column</th>
<th>Core-shell RP-18 endcapped 50-4.6 mm, 2.6 μm particles</th>
</tr>
</thead>
</table>
| Mobile phase | A: Acetonitrile  
B: 20 mM Phosphate buffer pH 4.5 |
<table>
<thead>
<tr>
<th>Gradient</th>
<th>Time/min</th>
<th>% A</th>
<th>% B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>20</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>40</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pressure</td>
<td>100 bar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Detection</td>
<td>UV 230 nm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>22°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection volume</td>
<td>2 μL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Sample | 1. Ascorbic acid  
2. 4-Hydroxybenzoic acid  
3. Benzoic acid  
4. Sorbic acid  
5. Methyl 4-hydroxybenzoate  
6. Ethyl 4-hydroxybenzoate  
7. Propyl 4-hydroxybenzoate |
Chromolith® & Chromolith® HR RP-8 endcapped

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

Separation examples

**Chromolith® Performance RP-8e 100-4.6 mm**

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

**Chromolith® HighResolution RP-8e 100-4.6 mm**

<table>
<thead>
<tr>
<th>Column</th>
<th>Chromolith® HighResolution RP-8 endcapped 100x4.6 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>ACN/ Water 25/75 + 0.04% TFA Isocratic</td>
</tr>
<tr>
<td>Pressure</td>
<td>49 bar</td>
</tr>
<tr>
<td>Detection</td>
<td>UV 220 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>ambient</td>
</tr>
<tr>
<td>Injection volume</td>
<td>0.5 μL</td>
</tr>
<tr>
<td>Flow</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Sample</td>
<td>Gly-Tyr, Val-Tyr-Val, Met enkephalin, Leu enkephalin, Angiotensin II 0.5 mg/mL each</td>
</tr>
</tbody>
</table>
Due to their π-π interactions, Chromolith® Phenyl HPLC columns offer greater selectivity towards aromatic ring-containing compounds than standard alkyl phases. These columns are ideal for the separation of aromatic compounds, flavonoids, fatty acids, PAH, preservatives, purines and pyrimidines.

**Separation examples**

**Chromolith® Phenyl 100-4.6 mm**

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

**Chromolith® Phenyl 100-4.6 mm**

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

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

Separation example: Five estrogens

**Chromolith® CN 100-4.6 mm**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Chromolith® CN 100-4.6 mm</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Methanol/0.1% TFA 30/70 v/v</td>
</tr>
<tr>
<td>Flow rate</td>
<td>2.0 mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>220 nm</td>
</tr>
<tr>
<td>Cell volume</td>
<td>11 μL</td>
</tr>
<tr>
<td>Temperature</td>
<td>ambient</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 μL</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
</tr>
<tr>
<td>1. Estriol</td>
<td>9.4 mg/mL</td>
</tr>
<tr>
<td>2. Estradiol</td>
<td>8.7 mg/mL</td>
</tr>
<tr>
<td>3. Testosterone</td>
<td>11.6 mg/mL</td>
</tr>
<tr>
<td>4. Ethynylestradiol</td>
<td>7.9 mg/mL</td>
</tr>
<tr>
<td>5. Estrone</td>
<td>13.3 mg/mL</td>
</tr>
<tr>
<td>Solved in</td>
<td>100 mL ACN/H2O 5/5</td>
</tr>
</tbody>
</table>

**Separation example: Anisole**

**Chromolith® Diol 100-4.6 mm**

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

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

Separation examples

**Chromolith® Performance Si 100-4.6 mm**

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

**Chromolith® Performance Si 100-4.6 mm**

<table>
<thead>
<tr>
<th>Column</th>
<th>Chromolith® Performance Si 100-4.6 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>n-Heptane / Dioxane 95/5 v/v</td>
</tr>
<tr>
<td>Flow rate</td>
<td>2 mL/min</td>
</tr>
<tr>
<td>Pressure</td>
<td>14 bar</td>
</tr>
<tr>
<td>Detection</td>
<td>254 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>ambient</td>
</tr>
<tr>
<td>Injection volume</td>
<td>10 μL</td>
</tr>
<tr>
<td>Sample</td>
<td></td>
</tr>
<tr>
<td>1. Toluene</td>
<td>0.16 mg/mL</td>
</tr>
<tr>
<td>2. Nitrobenzene</td>
<td>0.02 mg/mL</td>
</tr>
<tr>
<td>3. 2,3-Dimethylanthraquinone</td>
<td>0.02 mg/mL</td>
</tr>
<tr>
<td>4. 2-Nitroacetanilide</td>
<td>0.10 mg/mL</td>
</tr>
</tbody>
</table>
Chromolith® aminopropyl-modified columns possess medium polarity, between those of bare (normal-phase) silica and reversed-phase silica. Consequently, they display hydrophilic as well as hydrophobic properties, and can be used under both reversed-phase and normal-phase conditions. However, retention is weaker than on silica or RP supports. In acidic solutions, the NH₂ groups are protonated (\(\text{NH}_3^+\)) and display the characteristics of a weak anion exchanger. Hence, the columns can also be used as ion exchangers.

Chromolith® NH₂ columns offer high matrix tolerance and analysis speed, as well as an extended lifetime within the pH range of 2.5 to 7.5. These columns are suitable for the separation of anions, organic acids, and carbohydrates (mono and disaccharides, such as fructose, glucose, sucrose, maltose and lactose).

### Separation example

**Chromolith® Performance NH₂ 100-4.6 mm**

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

-20 0 25 50 75 100 125 150 175
0 2 4 6 8 10
Retention time (min)

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

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

Accurate analysis of proteins, antibodies and large peptides requires columns with good permeability, along with better mass transfer and selectivity. In order for size-exclusion not to influence the separation, the pore size should be approximately ten-times larger than the molecule being analyzed. Chromolith® columns have shown great potential and superiority in comparison to standard silica. In contrast to conventional packed-particle columns, wide pore (300 Å) monolithic silica columns are made of a single continuous bed of high purity porous silica that is then bonded with C18, C8, C4 and Protein A depending on the use of the column.

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

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

Why choose Chromolith® WP 300 columns

- Completely bioinert column hardware
- High biorecovery
- Special selectivities for biomolecules
- Very low column back pressure
- High-speed separation possible
- Substantially longer column lifetime
- High resistance to column blockage
- Cost savings from higher sample throughput and column durability
- Possibility of flow gradients
Analytical columns for bioapplications 4.6 mm i.d.

**Column Stability & Reproducibility**

Chromolith® WP columns were burdened with 15,000 CV of water and acetonitrile containing 0.1% TFA. The chromatographic change in separation behavior is shown below.

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

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>A 0.1% TFA in water</th>
<th>B 0.1% TFA in acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection</td>
<td>220 nm</td>
<td></td>
</tr>
<tr>
<td>Peak Identification</td>
<td>Ribonuclease</td>
<td>Cytochrome C</td>
</tr>
<tr>
<td></td>
<td>Holo-Transferrin</td>
<td>Apomyoglobin</td>
</tr>
<tr>
<td>Gradient</td>
<td>1 min 4% B; 10 min 4 – 60% B; 5 min 60% B</td>
<td></td>
</tr>
</tbody>
</table>

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

**Separation conditions**

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

**Peak No.** | **RSD Ret. Time**
---|---
1 | 0.66%
2 | 0.62%
3 | 0.58%
4 | 0.48%
Fast chromatography with low column back pressure columns

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

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

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

| Mobile phase | A 0.1% TFA in water
|              | B 0.1% TFA in acetonitrile |
| Detection   | 220 nm |
| Peak Identification | 1. Gly-Tyr
|                  | 2. Val-Tyr-Val
|                  | 3. Met enkephalin
|                  | 4. Leu enkephalin
|                  | 5. Angiotensin II |
| Gradient     | 1 min (0.5/0.25 min) 10% B
|              | 10 min (5/2.5 min) 10 – 20% B
|              | 5 min (2.5/1.25 min) 20% B |

Column back pressure at different flow rates. Comparison of a Chromolith® Widepore column vs. equivalent classical particulate HPLC columns.
Chromolith® WP 300 RP-18, RP-8 and RP-4: reversed-phase HPLC columns for bioapplications

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>A 0.1% TFA in water</th>
<th>B 0.1% TFA in acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>2.2 ml/min</td>
<td></td>
</tr>
<tr>
<td>Detection</td>
<td>220 nm</td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>5 mg/mL Cetuximab®</td>
<td></td>
</tr>
<tr>
<td>Gradient</td>
<td>0.1 min 4% B</td>
<td>4.9 min 4 – 60% B</td>
</tr>
<tr>
<td></td>
<td>2 min 60% B</td>
<td></td>
</tr>
</tbody>
</table>
Chromolith® WP 300 Protein A – Fast monoclonal antibody quantitation

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

Separation of monoclonal antibodies

| Eluent A | 100mM sodium phosphate pH7.4 |
| Eluent B | 100mM sodium phosphate pH2.5 |
| Flow rate | 2.0 mL/min |
| Detection | 280 nm |
| Temperature | 25°C |
| Injection volume | 10µl |

<table>
<thead>
<tr>
<th>Gradient</th>
<th>Time</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0.26</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1.25</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1.26</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.50</td>
<td>100</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Linearity

![Linearity graph](image)

$R^2 = 0.9991$
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

<table>
<thead>
<tr>
<th>Flow rate</th>
<th>Unbound area</th>
<th>IgG area</th>
<th>Pressure (bar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,0 mL/min</td>
<td>39%</td>
<td>61%</td>
<td>3</td>
</tr>
<tr>
<td>2,0 mL/min</td>
<td>39%</td>
<td>61%</td>
<td>6</td>
</tr>
<tr>
<td>3,0 mL/min</td>
<td>39%</td>
<td>61%</td>
<td>10</td>
</tr>
<tr>
<td>4,0 mL/min</td>
<td>39%</td>
<td>61%</td>
<td>13</td>
</tr>
<tr>
<td>5,0 mL/min</td>
<td>39%</td>
<td>61%</td>
<td>21</td>
</tr>
</tbody>
</table>

Stability test

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

More than 53,000 column volumes of 100 mM sodium phosphate buffer including 10,000 pH shifts were applied to the column

<table>
<thead>
<tr>
<th>Stability test</th>
<th>2.0 mL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ret. time IgG</td>
<td>RSD &lt; 0.5%</td>
</tr>
<tr>
<td>Peak area IgG</td>
<td>RSD &lt; 1.1%</td>
</tr>
</tbody>
</table>

Buffer A 100 mM sodium phosphate pH7.4
Buffer B 100 mM sodium phosphate pH2.5

Gradient

<table>
<thead>
<tr>
<th>Time</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>0.05</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>0.06</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1.10</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1.15</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2.00</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Column separation reproducibility and life time

Reproducibility

Chromatographic overlay of 50 injections

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

<table>
<thead>
<tr>
<th>Batch</th>
<th>Retention Time (Min)</th>
<th>Peak Width (Min)</th>
<th>Peak Symmetry</th>
<th>Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.952</td>
<td>0.034</td>
<td>1.56</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>0.957</td>
<td>0.033</td>
<td>1.39</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>0.953</td>
<td>0.031</td>
<td>1.47</td>
<td>10</td>
</tr>
</tbody>
</table>

**Chromolith® WP 300 Epoxy**

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

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

**Preparing the column**

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

**Immobilization via epoxide functions**

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

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

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

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

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

- Connect the column to an HPLC pump (stand-alone-system is recommended) and equilibrate the column with 30 CV of 50mM sodium phosphate + 1.9M ammonium sulfate pH8.0 using a flow rate of 2.0mL/min. The column end is connected directly to the waste. The equilibration step is performed at room temperature.
Dissolve the desired amount (1–10 mg/ml) of ligand in 25 ml 50mM sodium phosphate + 1.9M ammonium sulfate pH 8.0 buffer and recheck the pH value of the ligand solution. Subsequently, connect the solution to the HPLC pump.

Connect the column end also to the ligand solution and immobilize the ligand to the column and circulate for a maximum of 24 hours at a flow rate of 0.2 mL/min at room temperature.

Quench the remaining epoxide groups with 1M glycine for 2 hours at a flow rate of 1.0 mL/min at room temperature. The column end is directly connected to the waste.

Finally, equilibrate the column with your working solvent.

Immobilization via Schiff base mechanism

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

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

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

Connect the column to an HPLC pump (stand-alone-system is best) and hydrolyze the epoxide functional groups to diols with 70 CV of 2% sulfuric acid using a flow rate of 2.0 mL/min at room temperature. The column end is connected directly to the waste.

Wash the column with at least 15 CV deionized water at a flow rate of 2.0 mL/min. The column end is still connected to the waste.

The arisen diol groups are oxidized by 100 CV 100mM sodium periodate in water/methanol 4:1 (v/v) to carbonyls at 2.0 mL/min and room temperature. The column end is still connected to the waste.

Again, wash the column with at least 15 CV deionized water at a flow rate of 2.0 mL/min. The column end is still connected to the waste.

Dissolve the desired amount (1–10 mg/mL) of ligand and 5mM sodium cyanoborohydride in 25 mL 50 mM sodium phosphate + 1.9 M ammonium sulfate pH 8.0 buffer and connect the solution to the HPLC pump.

Connect the column end also to the ligand solution to immobilize the ligand to the column by circulating for a maximum of 24 hours at a flow rate of 0.2 mL/min and room temperature.

Reduce the remaining carbonyl groups with 20mM sodium cyanoborohydride, dissolved in 50mM sodium phosphate pH 3.0, for 60 CV at 2.0 mL/min and room temperature. The column end is directly connected to the waste.

Finally, equilibrate the column with your working solvent.

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

Use as HPLC column

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

Chromolith® WP Epoxy columns can be used with all commonly used HPLC grade organic solvents, with the following restrictions. The mobile phase should NOT contain more than 50% Tetrahydrofuran (THF), 5% Chlorinated solvent (e.g. Dichloromethane) or 5% Dimethylsulfoxide (DMSO). However pure DMSO can be used as solvent for samples. Buffers, organic modifiers and ion pair reagents present no problems as long as the appropriate pH range is not exceeded. Nevertheless, be careful not to expose the column to conditions which could cause denaturation of your ligand.
Do not exceed the pH range from 1.5 to 7.5 with Chromolith® Widepore columns during analysis. Higher pH will dissolve the silica, creating voids in the column. Lower pH’s can eventually strip away some of the bonded phase. These defects will cause changes in retention times and a loss of resolution.

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

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

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

Example immobilization

**Immobilization of penicillin acylase**

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

![Immobilization Diagram](image)

---

**Immobilization of penicillin acylase – Enzymatic bioreactor**

<table>
<thead>
<tr>
<th>Gradient</th>
<th>Time</th>
<th>Valve</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
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<td>20</td>
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</tr>
<tr>
<td>9.5</td>
<td>2</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>9.6</td>
<td>2</td>
<td>0</td>
<td>80</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>0</td>
<td>80</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>

---

![Enzymatic Bioreactor Diagram](image)
**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 concanavalin A**
- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-4.6 mm column
- 50 mg concanavalin A from Jack bean dissolved in 25 mL 50mM Na₂HPO₄, 1mM CaCl₂ + 1,9M (NH₄)₂SO₄ pH 8.0
- Immobilization for 4 hours at 0.2 mL/min
- Quenching of remaining epoxide functions with glycine

**Immobilization of protein G – Affinity chromatography**

| Eluent A | 100 mM sodium phosphate pH7.4 |
| Eluent B | 100 mM sodium phosphate pH2.5 |
| Flow rate | 2.0 mL/min |
| Detection | 280 nm |
| Temperature | 25°C |
| Injection volume | 10 µL |

<table>
<thead>
<tr>
<th>Gradient</th>
<th>Time</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**Immobilization of concanavalin A – Affinity chromatography**

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

<table>
<thead>
<tr>
<th>Gradient</th>
<th>Time</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.25</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>0</td>
<td>100</td>
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<tr>
<td></td>
<td>3.6</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

**Measurements**

- 

\[ R^2 = 0.99935 \]

- IgG load (µg)

- Time (min)

- Peak area (mAU x min)

- Ribonuclease

- HRP

- Time (min)
**Immobilization of iminodiacetic acid**

- According to Epoxy method
- Chromolith® WP 300 Epoxy 100-4.6 mm column
- 1 g iminodiacetic acid dissolved in 25 mL 50 mM Na$_2$HPO$_4$ + 1,9M (NH$_4$)$_2$SO$_4$ pH 8,0
- Immobilization for 72 hours at 0.2 ml/min
- No quenching of remaining epoxide functions
- Column was flushed with CuSO$_4$ solution before separation

**Immobilization of iminodiacetic acid—Affinity chromatography**

<table>
<thead>
<tr>
<th>Eluent A</th>
<th>20mM sodium phosphate + 100mM sodium chloride pH7,0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent B</td>
<td>Eluent A + 200mM imidazole</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>280 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

![Graph of elution profile](image)
Chromolith® Preparative columns
10 mm / 25 mm i.d.
Offering faster sample throughput at lower pressure, Chromolith® Prep and SemiPrep HPLC columns are ideal for direct scale-up from analytical to prep and semi-prep. The excellent accessibility of the mesopores (total porosity > 80 %), and the short diffusion length inside the pores ensure fast adsorption and desorption kinetics. This leads to faster separations and higher productivity. The monolithic structure of Chromolith® SemiPrep and Prep columns also eliminates inlet bed settling or bed splitting under high pressure. Column reliability, reproducibility and extended lifetime are assured.

**Why choose preparative Chromolith® columns**

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

Optimum separation at flow rates exceeding 40 mL/min

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

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

Separation of a standard mixture

Chromolith® SemiPrep C18e, 100-10 mm

<table>
<thead>
<tr>
<th>Mobile phase</th>
<th>Acetonitrile/water 60/40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rate</td>
<td>2 mL/min</td>
</tr>
<tr>
<td>Detection</td>
<td>UV 254 nm</td>
</tr>
<tr>
<td>Temperature</td>
<td>ambient</td>
</tr>
<tr>
<td>Injection volume</td>
<td>5 µL</td>
</tr>
<tr>
<td>Sample</td>
<td>1. Thiourea</td>
</tr>
<tr>
<td></td>
<td>2. Progesterone</td>
</tr>
<tr>
<td></td>
<td>3. Anthracene</td>
</tr>
</tbody>
</table>

Acetonitrile/water 60/40, Data for anthracene (3rd peak)
Preparative HPLC involves much higher sample volumes than analytical chromatography. Consequently, greater sample throughput and separation speed are essential for optimal productivity. These criteria are best fulfilled by Chromolith® Prep columns. The combination of macro and mesopores maximizes separation efficiency and flow rate, while minimizing resistance.

**The formula for direct scale-up**

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

\[
\frac{X_{an}}{\pi r_{an}^2 \cdot c_l} = \frac{X_{pr}}{\pi r_{pr}^2} \cdot 1
\]

- \(X_{an}\): Flow rate in the analytical system
- \(X_{pr}\): Flow rate in the preparative system
- \(r_{an}\): Radius of analytical column
- \(r_{pr}\): Radius of preparative column
- \(c_l\): Length of the preparative column to length of the analytical column

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

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

**Analytical separation**

**Chromolith® Performance RP-18e 100–4.6 mm**

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

**Chromolith® Prep RP-18e 100-25 mm**

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

**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**

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

**Preparative separation**

**Chromolith® Prep RP-18e 100-25 mm**

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

**Chromolith® HPLC columns**
Separation of diastereomers with a productivity of 861 g/d

Chromolith® Prep Si 100-25 mm

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

Chromolith®

HPLC guard cartridges and kits

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

Guard cartridges

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

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

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

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

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

PEEK = Poly Ether Ether Ketone, SS = Stainless Steel
As seen in the examples, guard columns have very little negative effects on the separation. These may include a slight shift in elution time, or a minimal loss in efficiency.

### Separation examples with and without a pre-column

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

<table>
<thead>
<tr>
<th>Column</th>
<th>Chromolith® Performance RP-18e 100-2 mm with pre-column Chromolith® RP-18e 5-2 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile / water 60/40</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.38 mL/min</td>
</tr>
<tr>
<td>Pressure</td>
<td>20 bar</td>
</tr>
<tr>
<td>Detection</td>
<td>UV 254 nm</td>
</tr>
<tr>
<td>Anthracene</td>
<td>N/m 113540, TUSP 1.14, K’-value 3.79</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Column</th>
<th>Chromolith® Performance RP-18e 100-2 mm without pre-column</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile / water 60/40</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.38 mL/min</td>
</tr>
<tr>
<td>Pressure</td>
<td>20 bar</td>
</tr>
<tr>
<td>Detection</td>
<td>UV 254 nm</td>
</tr>
<tr>
<td>Anthracene</td>
<td>N/m 115460, TUSP 1.07, K’-value 3.90</td>
</tr>
</tbody>
</table>
## Ordering information

### Capillary columns

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* Guard column type examples and detailed information please find on page 47.
### Chromolith® HPLC columns

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

### Chromolith® WP 300 columns

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* Guard column type examples and detailed information please find on page 47.
## Semi-preparative and preparative columns

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<td>10 mm</td>
<td>d*</td>
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* Guard column type examples and detailed information please find on page 34.