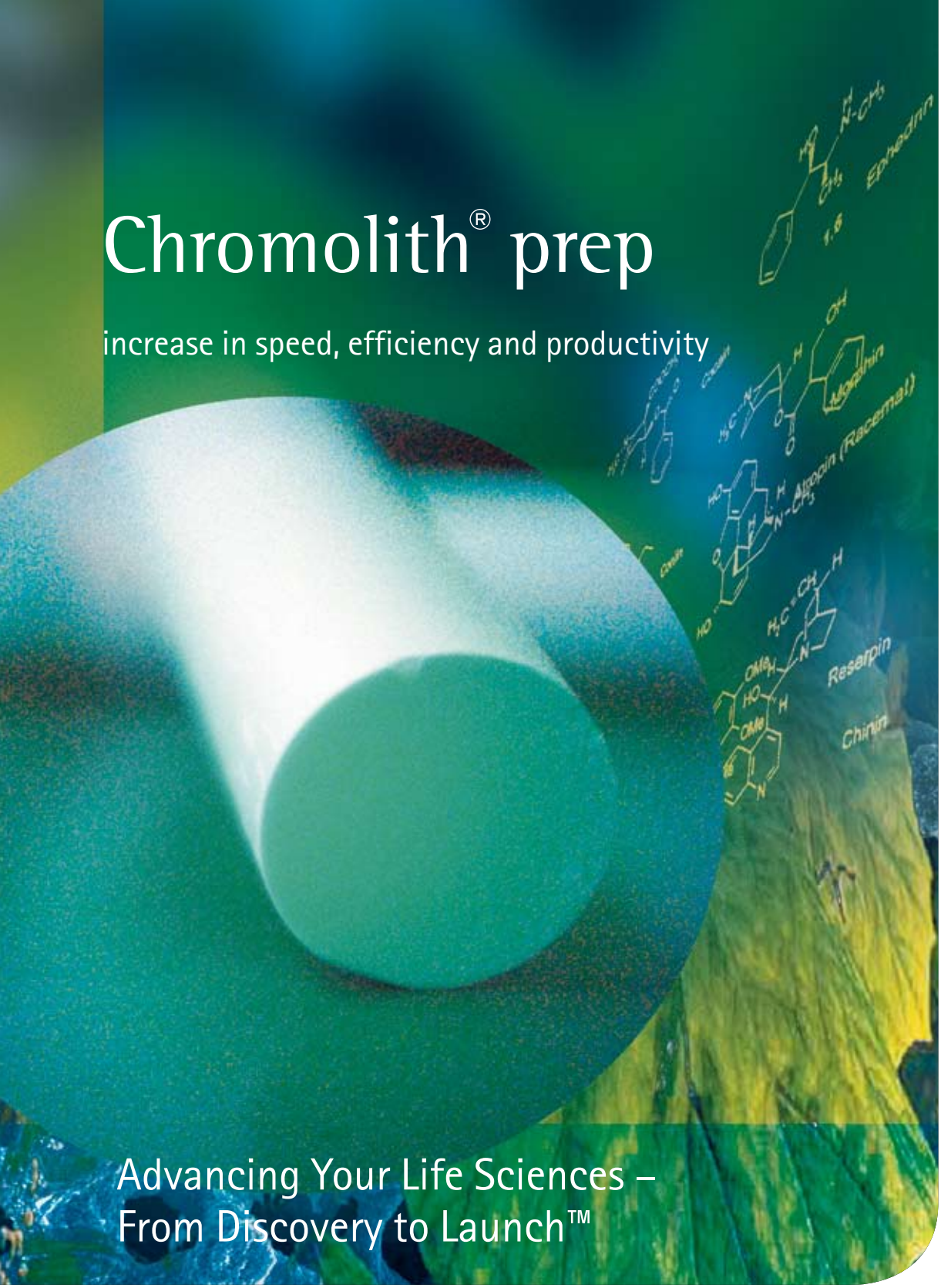


Chromolith® prep

increase in speed, efficiency and productivity

Advancing Your Life Sciences –
From Discovery to Launch™

MERCK

Chromolith® prep

increase in speed, efficiency and productivity

Chromolith® prep monolithic stationary phases are new ultra-pure silica phases that have special properties resulting from their bimodal pore structure. This structure is based on new "Sol-Gel" technology and consists of macropores and mesopores.

The mesopores, with an average diameter of 12 nm, form the fine porous structure of the column interior and create a very large surface area on which adsorption of the target compounds occurs.

The large macropores, with a pore average diameter ranging from 3 µm, form a dense network of pores and allows a high flow rate due to their low resistance factor. The resulting excellent accessibility of the mesopores (total porosity > 80 %) ensures fast adsorption and desorption kinetics due to the short diffusion length inside the pores. This results in dramatically reduced separation times and a large increase in productivity.

The monolith is cladded with a polymeric material (special PEEK) and can be connected directly to any HPLC system and used similar to a standard column.

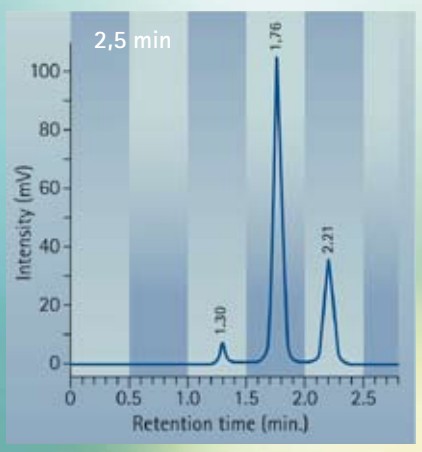
As a result of the monolithic structure of Chromolith® prep columns, inlet bed settling or bed splitting under high pressure have been eliminated. Column reliability, reproducibility and long life are ensured.

Applications with Chromolith® prep Si 100-25

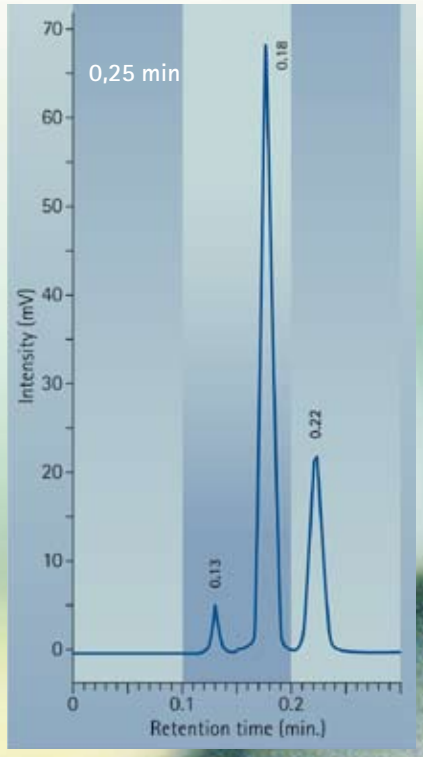
Separations at different flow rates

Sample: *Toluene, Dimethylphthalate, Dibutylphthalate*
Column : *Chromolith® prep Si 100-25*
Solvent: *n-heptane/dioxane (80/20, v/v)*

Flow rate 40 ml/min



Flow rate 390 ml/min



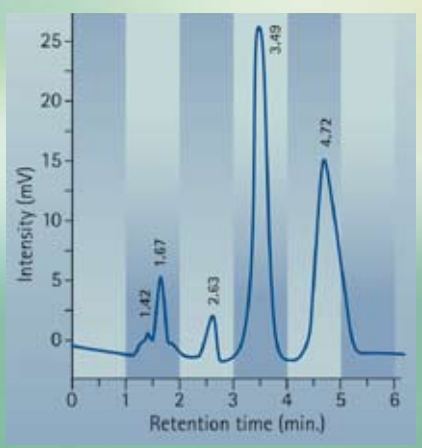
Chromolith® prep Si columns can be operated at flow rates of up to 400 ml/min and pressures of up to 100 bar. This is a tenfold increase of flow rate compared to equivalent size particulate packed columns.

Separation of γ- and δ-Tocopherol from sunflower oil at different flow rates

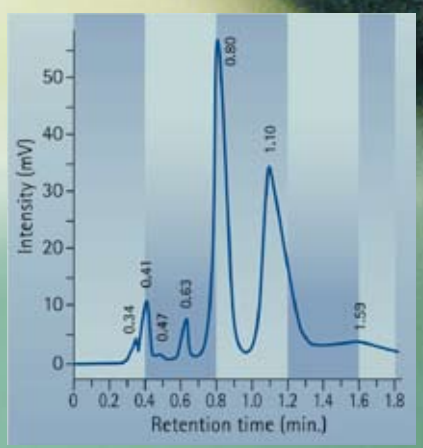
Sample: *Tocopherols*
Detecion: *UV 220 nm*
Mobile phase: *n-heptane/isopropanol (99.5/0.5, v/v)*
Column: *Chromolith® prep Si 100-25*

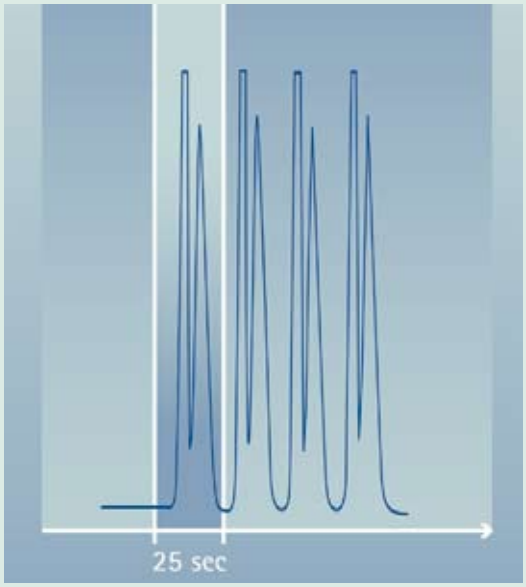
Both separations of Tocopherol Isomers show Chromolith® prep's high efficiency.

Flow rate 40 ml/min



Flow rate 160 ml/min





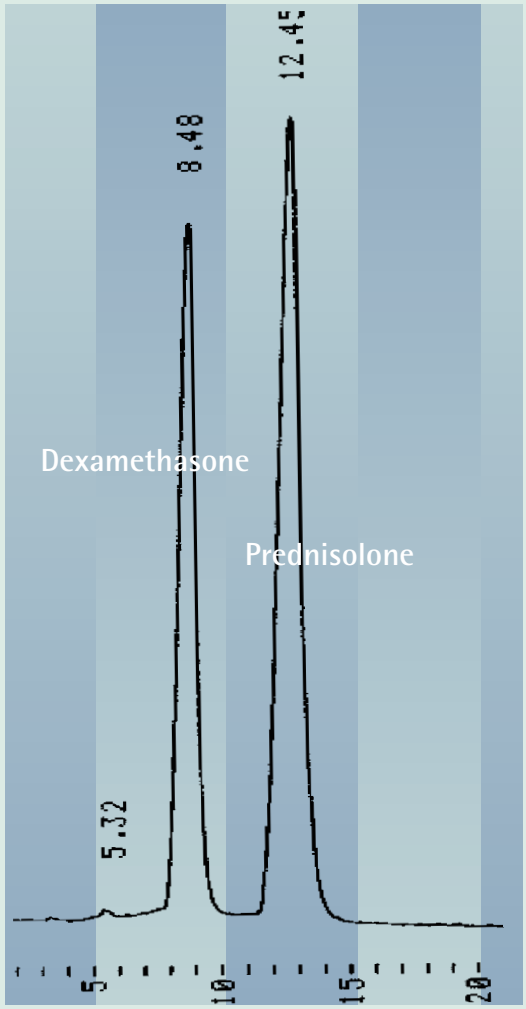
Separation of Diastereomers with a productivity of 861 g/d

Column: *Chromolith® prep Si 100-25*
Flow rate: *140 ml/min*
Injection: *249 mg*
Cycle Time: *25 sec.*
Sample: *Fluoro-dihydro-oxiranyl-benzopyran*
Productivity: *861 g/d*

$$\text{Productivity:} \quad \frac{\text{Injection amount [g]}}{\text{Time [d]} \cdot \text{Filling material [kg]}}$$

= 68294 g/(d · kg) --> 861 g/d

Regioisomers of Fluoro-dihydro-oxiranyl-benzopyran could be separated with Chromolith® prep Si at higher productivity (861 g/day) using a very short cycle times (25 sec.). The productivity can be increased further under increasing injection amount. Merck has performed the same separation with a particulate sorbent in 300 mm ID columns, but the separation took more than 60 mins per run.

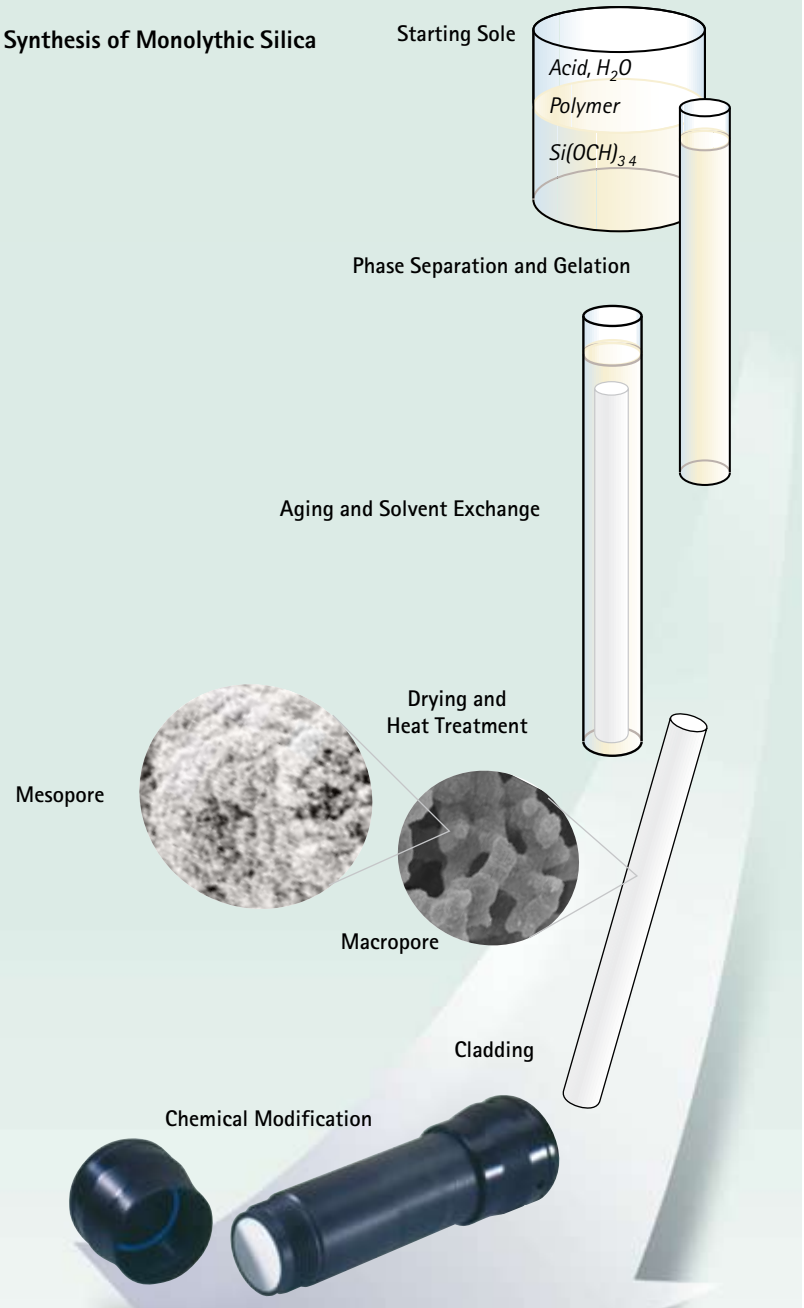


Separation of structurally closely related compounds: Dexamethason, Prednisolon

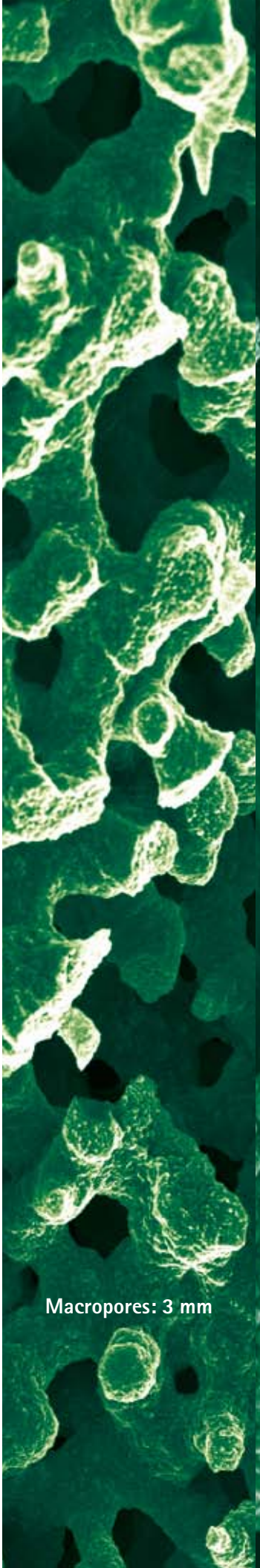
Stationary phase: *Chromolith® prep Si 100-25*
Mobile phase: *n-heptane/THF (85/15, v/v)*
Flow rate: *80 ml/min*
Detection: *UV 248 nm*
Sample: *4 ml (2 mg/ml)*

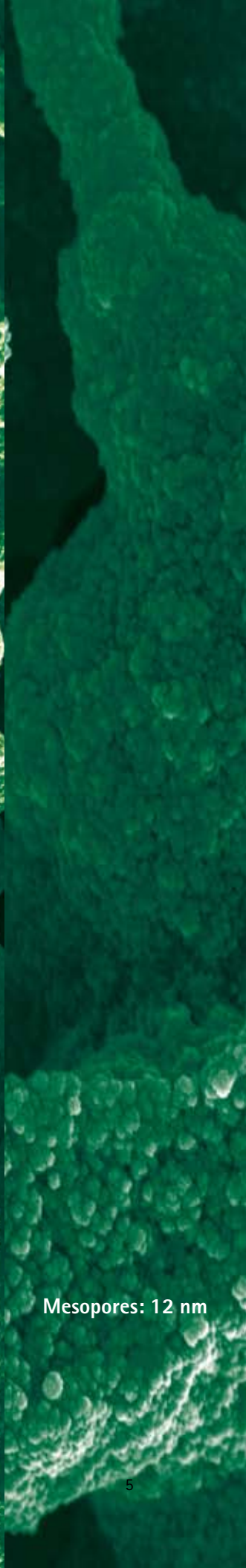
Phytosterines from plant or animal sources are the basic source for a variety of drug compounds with core steroid structures. They are chemically or enzymatically modified to yield a wide variety of structurally closely related compounds. Purification of such steroid compounds is often performed by process chromatography on silica sorbents. Monolithic stationary phases show good selectivities for such derivatives with only small differences in the steroid structure.

Synthesis of Monolythic Silica



HPLC





Mesopores and Macropores of Chromolith® prep

Preparative RP-Chromatography

Monolithic silica rod technology puts an end to the back pressure problem immediately! Compared to modern particulate columns filled with particles of a size 3.6 µm the Chromolith® prep RP 18e shows a significant reduction of back pressure at all flow rates (40 ml/min to 300 ml/min and even higher)! Only limited by the maximum back pressure tolerance of 100 bar (1450 psi).

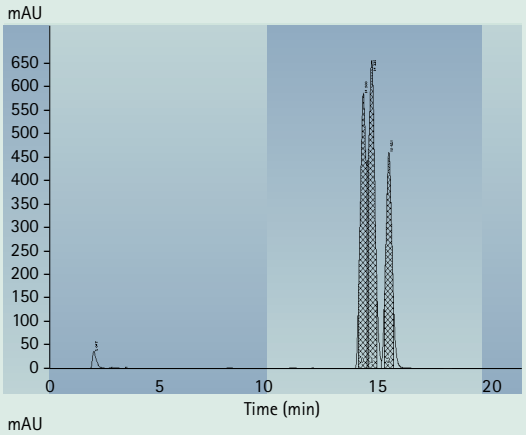
The selectivity of a Chromolith® prep RP 18e column is comparable to common RP 18e reversed phase columns. It provides you with an excellent tool to solve your separation problems regarding non-polar basic and acidic compounds as well as peptides.

In most cases your existing methods from using particulate columns can easily be transferred to Chromolith® prep. However for some applications it is worth optimizing the method to make use of the full potential of this enhanced technology. We would be happy to assist you – please find our contact details at the end of this information.

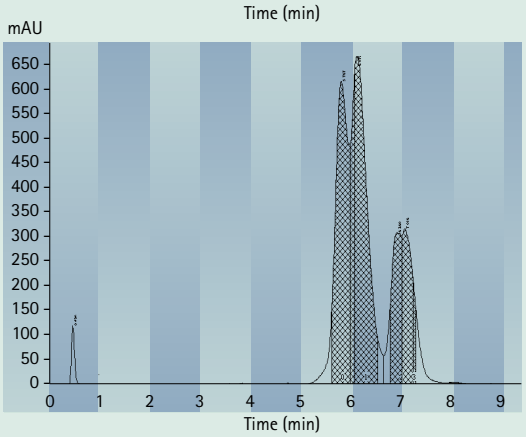
Chromolith® prep RP 18e 100-25 opens the door to high speed separation in preparative Chromatography

Comparison of Chromolith® prep RP 18e 100-25 with particulate material

Separation of Oxime-derivates

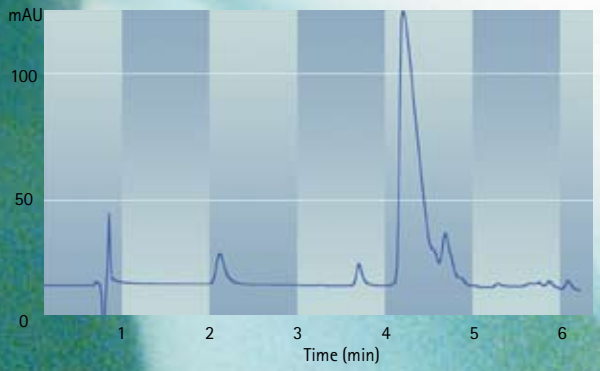


Column:	*Packing stand NW 50 (250-50) filled with Purospher RP 18e, 10 µm*
Sample:	*125 mg Oxime-derivates in 500 µl acetonitrile*
Flow rate:	*100 ml/min*
Detection:	*UV 210 nm*
Eluent A:	*water +0.05 % trifluoric acid*
Eluent B:	*acetonitrile +0.05 % trifluoric acid*
Gradient:	*linear in 30 min up to 100 % B*



Column:	*Chromolith® prep RP 18e 100-25*
Sample:	*125 mg Oxime-derivates in 500 µl acetonitrile*
Flow rate:	*100 ml/min*
Detection:	*UV 210 nm*
Eluent A:	*water +0.05 % trifluoric acid*
Eluent B:	*acetonitrile +0.05 % trifluoric acid*
Gradient:	*linear in 11 min up to 100 % B*

The comparison of Chromolith® prep RP18e with a particulate Purospher® RP18e-column (210-50 mm) shows that both separations have a similar resolution under the same chromatographic conditions, however Chromolith® prep RP18e has a better selectivity than Purospher® RP18e as exhibited by the resolution of the additional isomer peak at 7 minutes (see page 6).



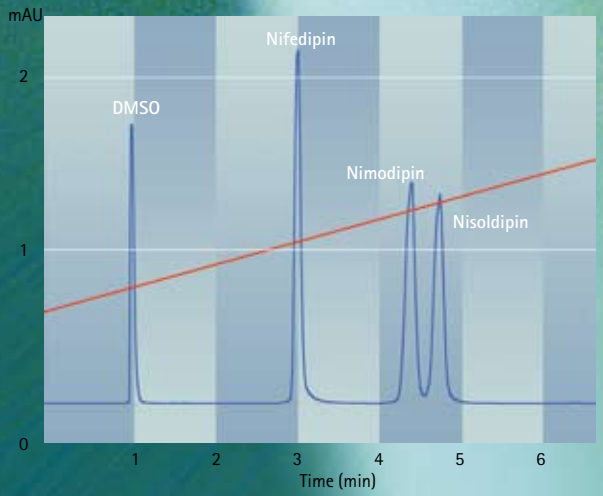
Separation of Hirudin (filtrate of crude extract)

Without any sample preparation the crude sample we injected straight on to the Chromolith® prep RP 18e. The separation took only 5 minutes. It was possible to isolate the desired product from the impurities.

Column:	*Chromolith® prep RP 18e 100-25*		
Sample:	*23 mg Hirudin (filtrate of crude extract) in 5 ml solution injected*		
Flow rate:	*60 ml/min*		
Detection:	*UV 254 nm*		
Eluent A:	*water + 0.1 % formic acid*		
Eluent B:	*acetonitrile 100 %*		
Gradient:	*Time (min.)*	*% A*	*% B*
	0	*90*	*10*
	10	*70*	*30*
	10,1	*90*	*10*

With monolithic silica rod technology it is possible to speed up your separation significantly!

Chromolith® prep RP18e shows a significant reduction of back pressure at a flow rate of 40 ml/min and even higher (up to 300 ml/min).



Separation of Dihydropyridines (Nifedipin, Nimodipin and Nisoldipin)

Column:	*Chromolith® prep RP 18e 100-25*		
Sample:	*90 mg mixture of Nifedipin, Nimodipin and Nisoldipin (1/1/1, v/v/v) in 450 µL DMSO injected*		
Flow rate:	*100 mL/min*		
Detection:	*UV 224 nm*		
Eluent A:	*water*		
Eluent B:	*acetonitrile*		
Gradient:	*Time (min.)*	*% A*	*% B*
	0	*80*	*20*
	8	*20*	*80*
	8,1	*80*	*2*

The formula for direct scale-up

An analytical separation can be simply transferred to semi-preparative and preparative 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. The maximum load on the column however, is dependent on the complexity of the separation problem and the nature of the sample.

If working in the linear or the non-linear mode the calculation of the flow rate or injection volume is made according to the equation.

$$\frac{X_{an}}{\pi r_{an}^2} = \frac{X_{pr}}{\pi r_{pr}^2} \cdot \frac{1}{c_L}$$

X_{an} = *flow rate in the analytical system*

X_{pr} = *flow rate in the preparative system* $\quad X_{pr} = X_{an} \cdot r_{pr}^2 \cdot c_L / r_{an}^2$

r_{an} = *radius of analytical column*

r_{pr} = *radius of preperative column*

c_L = *length of the preparative column to length of the analytical column*

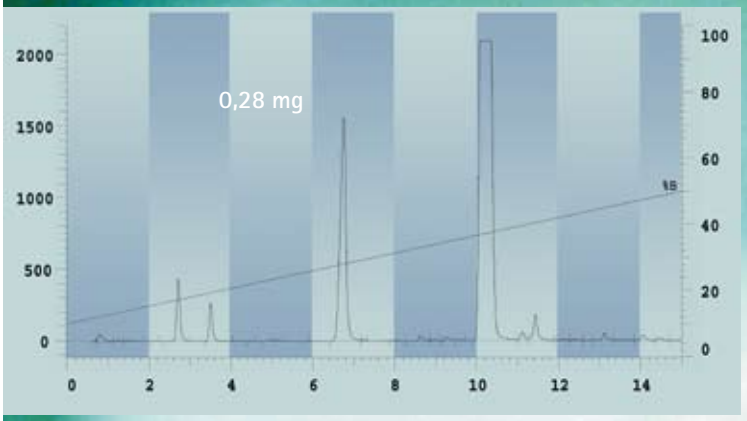
M = *substance mass* $\quad M_{pr} = M_{an} \cdot r_{pr}^2 \cdot c_L / r_{an}^2$

Column dimension length/diameter [mm]	Typical flow rate [ml/min]	Loading capacity [mg]	Loading volume [µl]
100 – 4.6	2	5	5 – 50
100 – 25	60	150 – 370	100 – 1500

Table 2:
Guide values of typical flow rates and loading capacity for the transfer from an analytical to a preparative column.

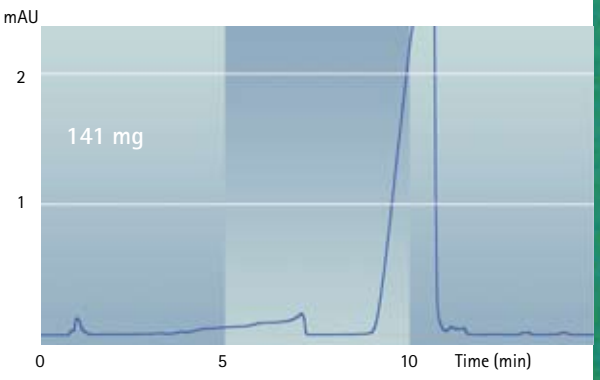
Analytical separation

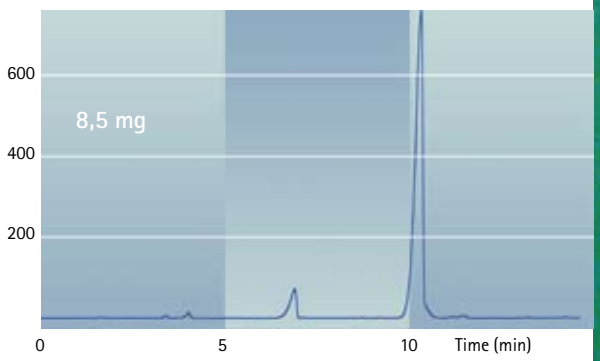
Column: *Chromolith® performance RP 18e 100-4.6*
Sample: *0,28 mg Heterocyclic racemate (EMD 53986) in 10 µl DMSO*
Flow rate: *2 ml/min*
Detection: *UV 254 nm*
Eluent A: *water + 0.1 % formic acid*
Eluent B: *acetonitrile*
Gradient: *linear gradient from 10 % B to 40 % in 14 min*



Preparative separation

Column: *Chromolith® RP 18e 100-25*
Sample: *8.46 mg or 141 mg Heterocyclic racemate (EMD 53986) in 300 µl DMSO*
Flow rate: *60 ml/min*
Detection: *UV 254 nm*
Eluent A: *water + 0.1 % formic acid*
Eluent B: *acetonitrile*
Gradient: *linear gradient from 10 % B to 40 % in 14 min*





Chromolith® prep
Ordering information

Packing material	Dimensions length x inner diameter [mm]	Cat.-No.	Package
Chromolith® prep Si	100 – 25	1.25251.0001	1 piece
Chromolith® prep RP 18e	100 – 25	1.25252.0001	1 piece
Filter-set for Chromolith® prep		1.25253.0001	10 pieces

For further information please call your local Merck agency or contact our headquarters:

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