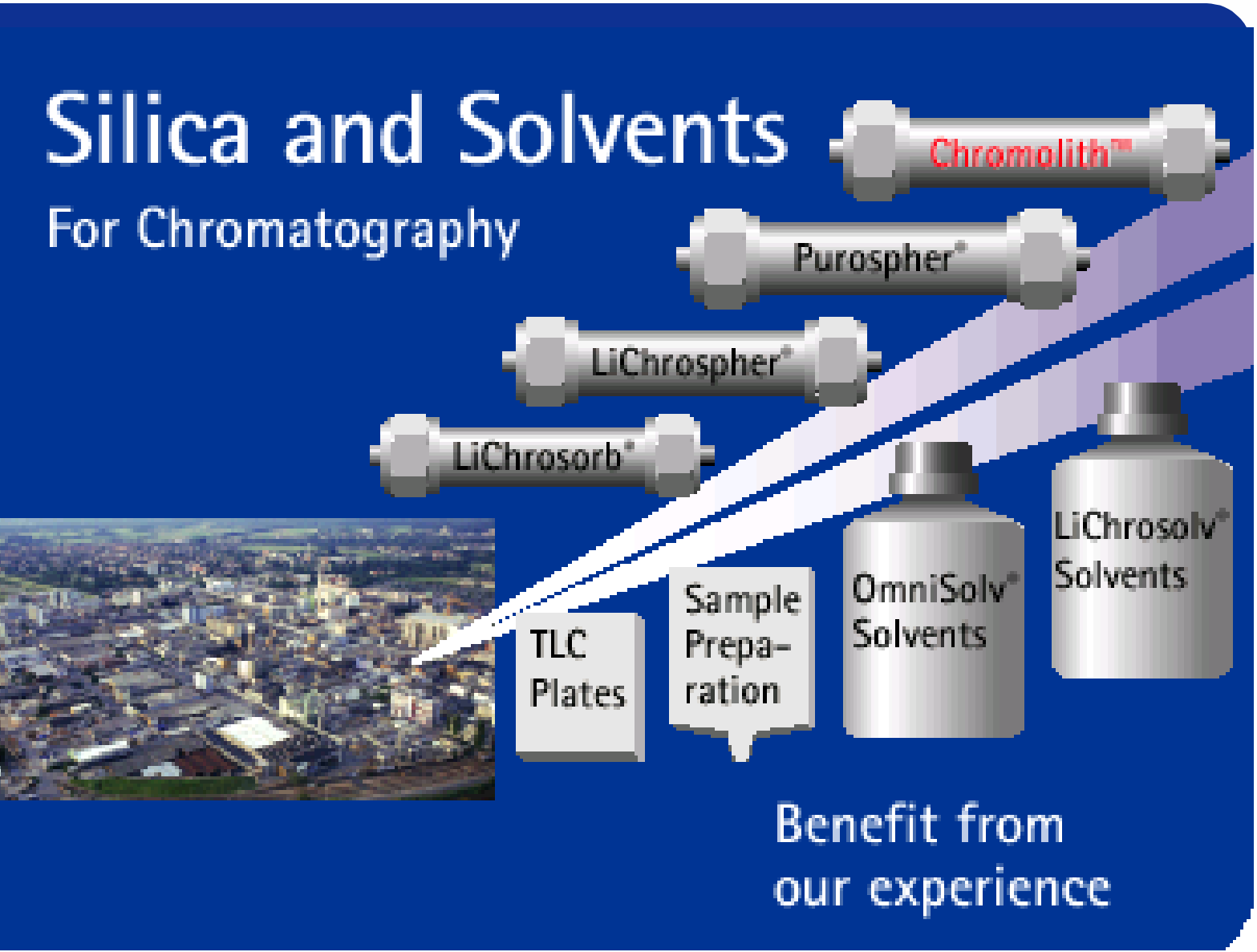
Monolithic silica columns: benefits and applications of Chromolith® HPLC columns

Dr. Rod McIlwrick

Merck KGaA, Darmstadt

Merck Solvents & Chromatography

100 Years of Chromatography at Merck – *Experience drives innovation*



Merck‘s HPLC Stationary Phases

First generation (1970’s): LiChrosorb®
- the first high performance HPLC columns; irregular particles; 1st RP-18 phase

Second generation (1980’s): LiChrospher®, Superspher®
- 4 and 5 µm spherical particles with optimised selectivity

Third generation (1990’s): Purospher®
-Metal impurities < 5 ppm. Spherical particles
-Excellent peak symmetry

Fourth generation (2000’s): Purospher® STAR
-3 µm and 5 µm spherical particles
-Extended stability: pH range 1.5 to 10.5

Chromolith® HPLC Columns

Discover the New Chrom Age!

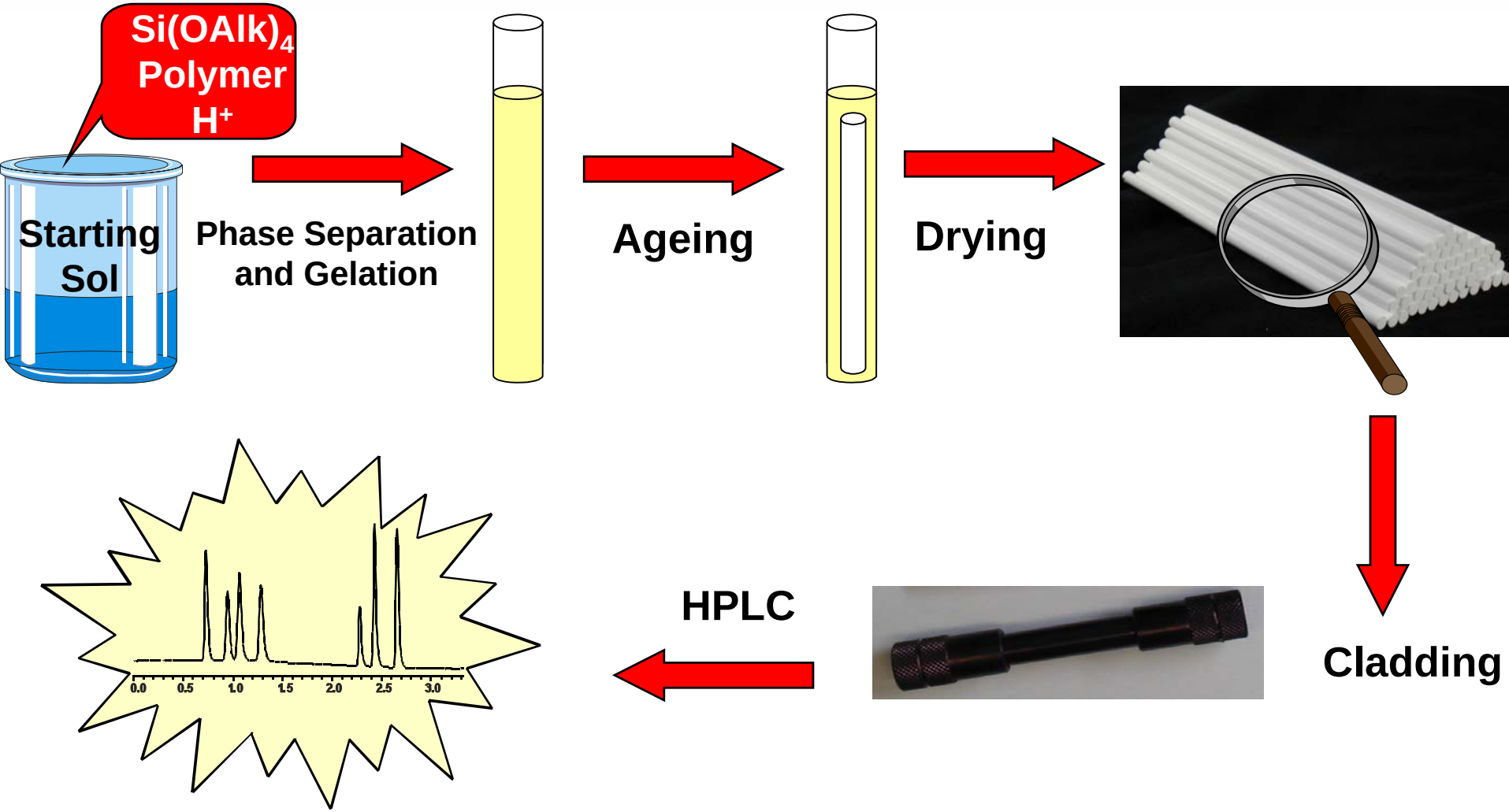
Chromolith® HPLC Columns

1.51463.0001 Chromolith Flash RP-18e (25 x 4.6 mm)
1.51450.0001 Chromolith SpeedROD RP-18e (50 x 4.6 mm)
1.02129.0001 Chromolith Performance RP-18e (100 x 4.6 mm)

Chromolith® HPLC Columns

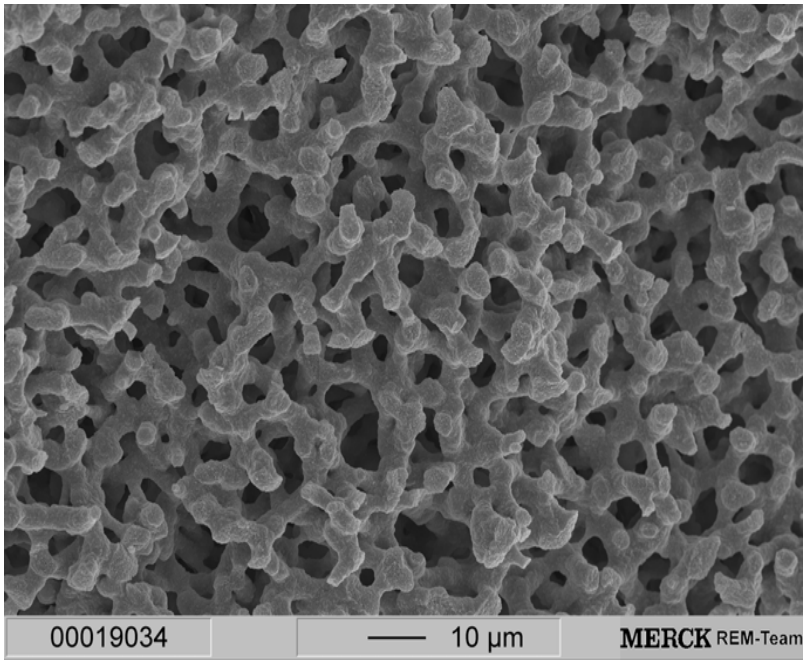
Column cladding material is PEEK (poly-ether-ether-ketone)
- stable with all standard HPLC solvents
- limitations: 5% DMSO, 5% CH_2Cl_2, 50% THF

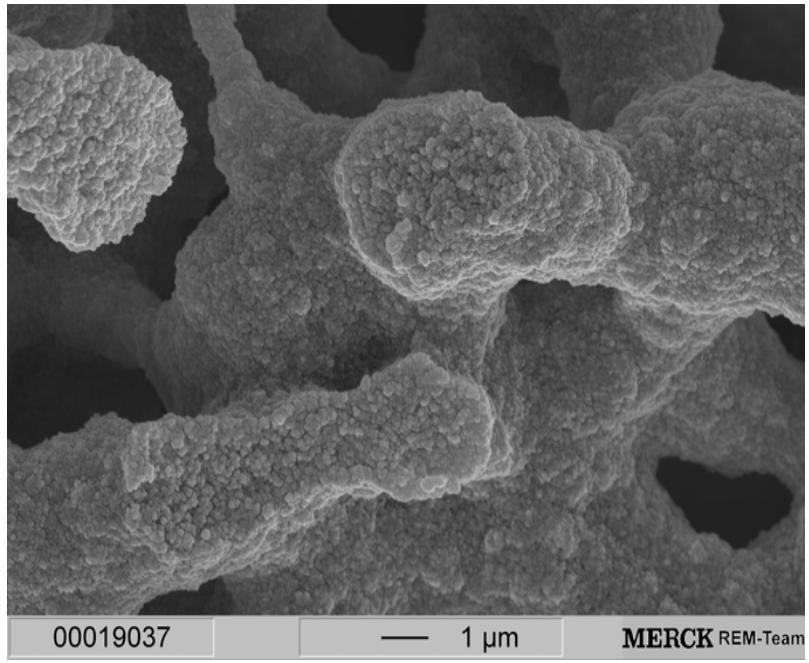
Preparation



The Bimodal Pore Structure

SEM of a cross section of a monolithic silica rod





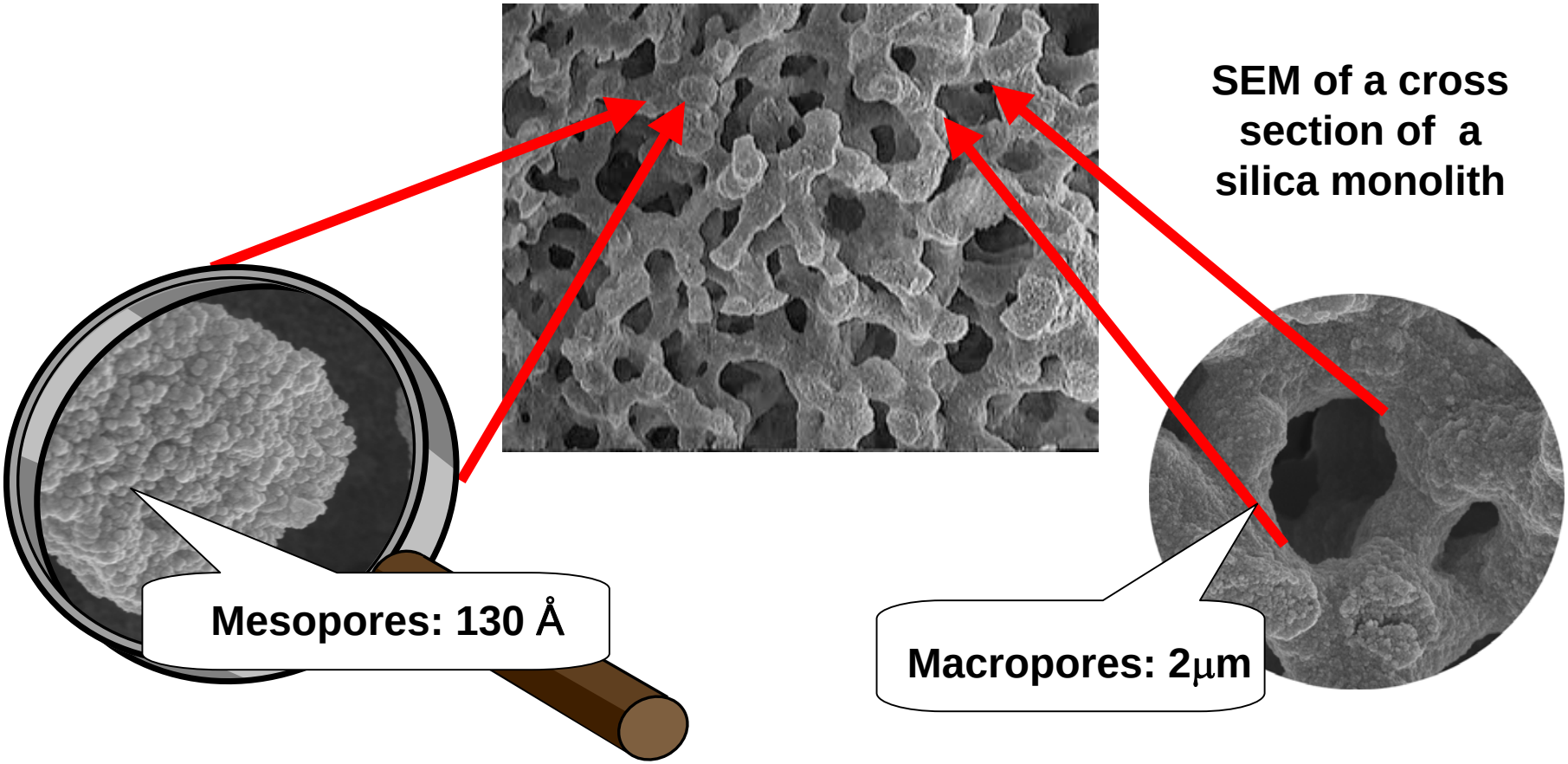
Macropores: 2 µm

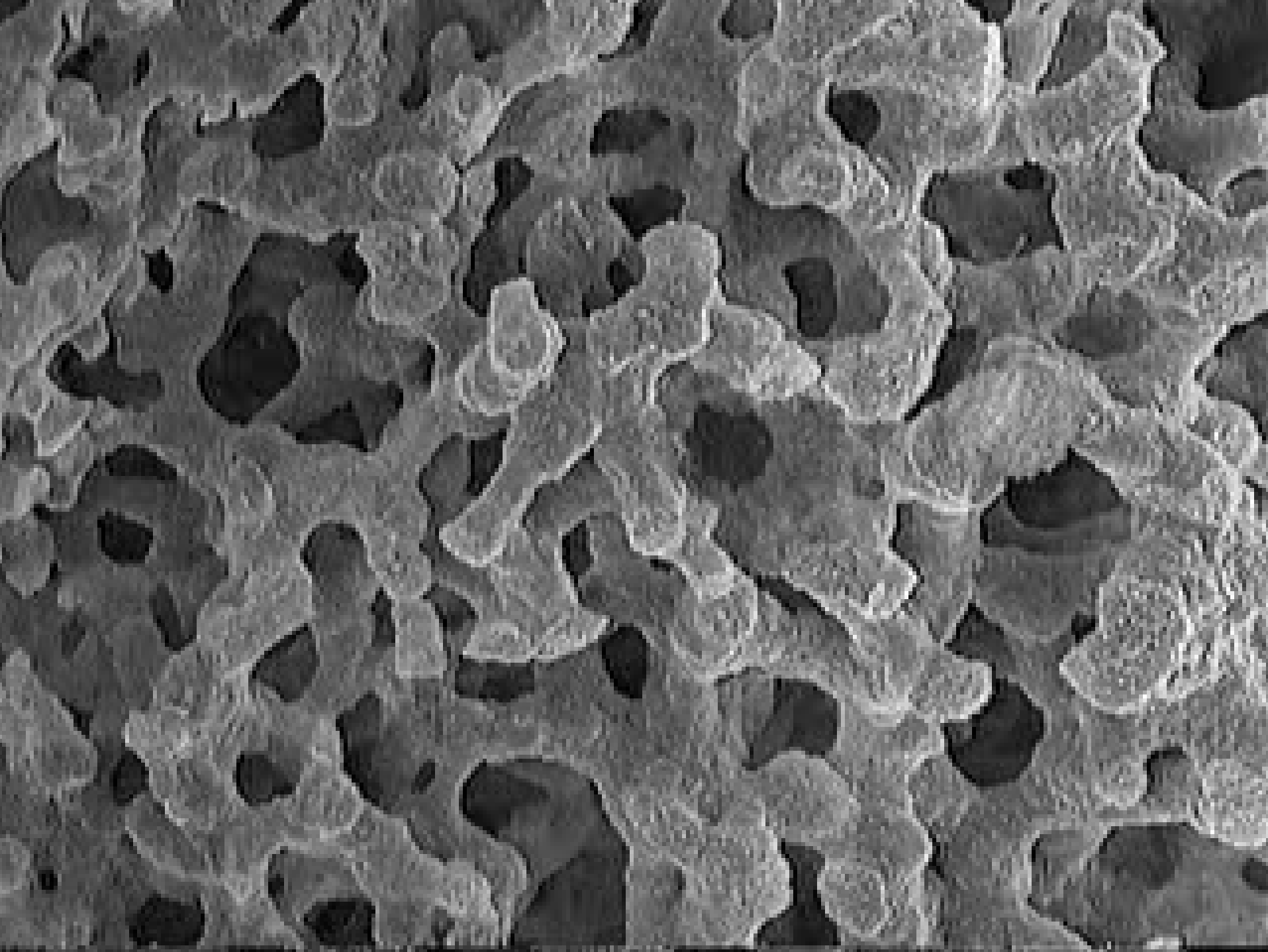
Mesopores: 130 Å

Total porosity 81%

Feature of monolithic silica columns

Defined bimodal pore structure





Chromolith® Specification

- Silica type: High purity
- Particle size: Monolithic shape
- Macropore size: 2 µm
- Mesopore size: 130 Å (13 nm)
- Surface area: 300 m^2/g
- Pore volume: 1 ml/g
- Surface modification: RP-18 endcapped
- Carbon content: 18 %
- Surface coverage: 3.6 $mmol/m^2$
- pH range 2 – 7.5
- Total porosity: >81%

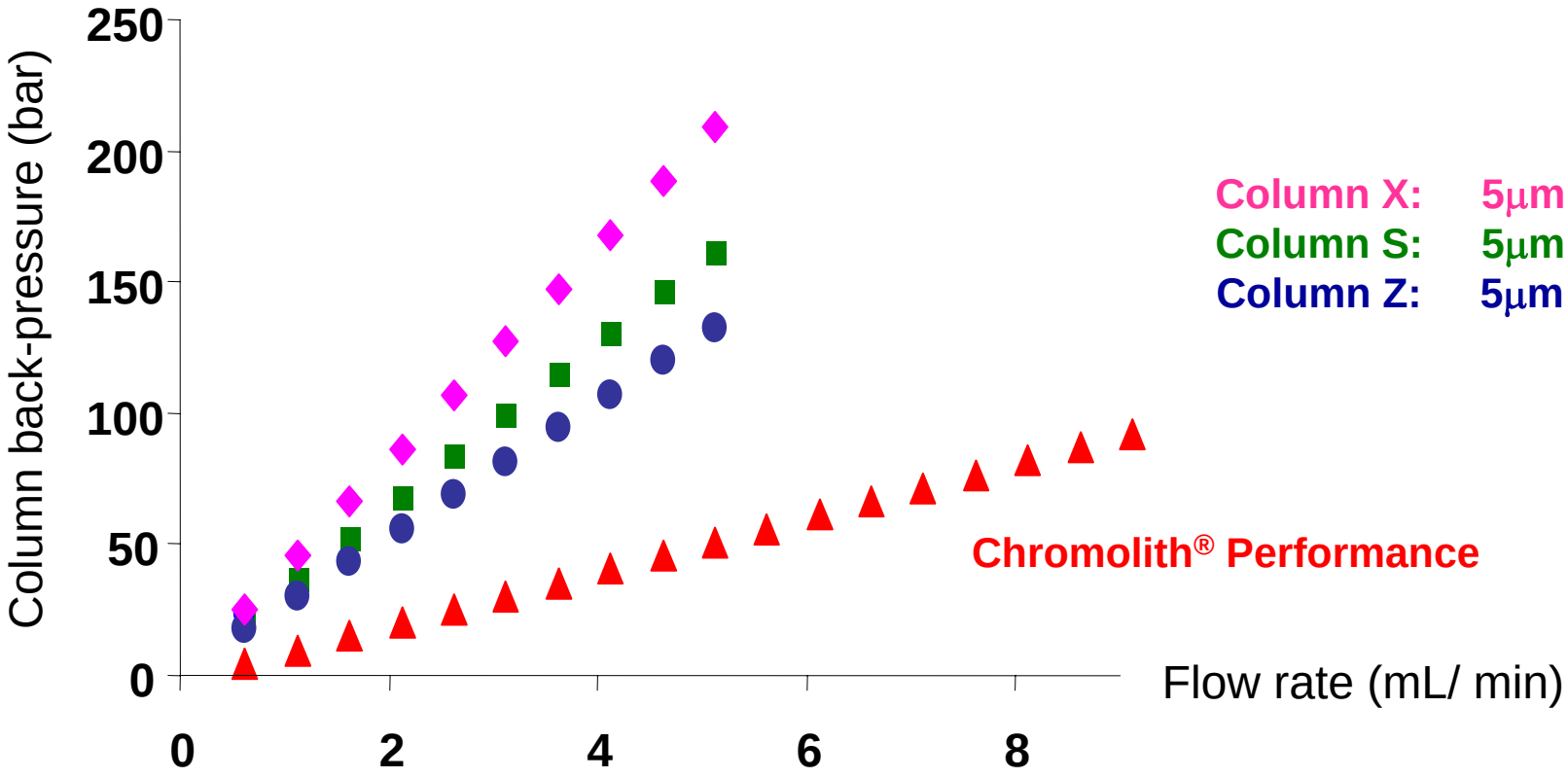
Purospher® STAR

Specification

Sorbent:	High-purity, silica gel
Metal content:	Na, Ca, Mg, Al: 1 ppm; Fe: 3 ppm
Particle size:	3 µm and 5 µm
Pore diameter:	120 Å (12 nm)
Specific surface:	330 m^2/g
Pore volume:	1.1 ml/g
Surface modification:	C-18 endcapped
Carbon load:	17%
Coverage of the surface	3 $\mu mol/m^2$
Performance	5µm: >85,000; 3µm:>130.000 N/m
pH range:	1.5 - 10.5

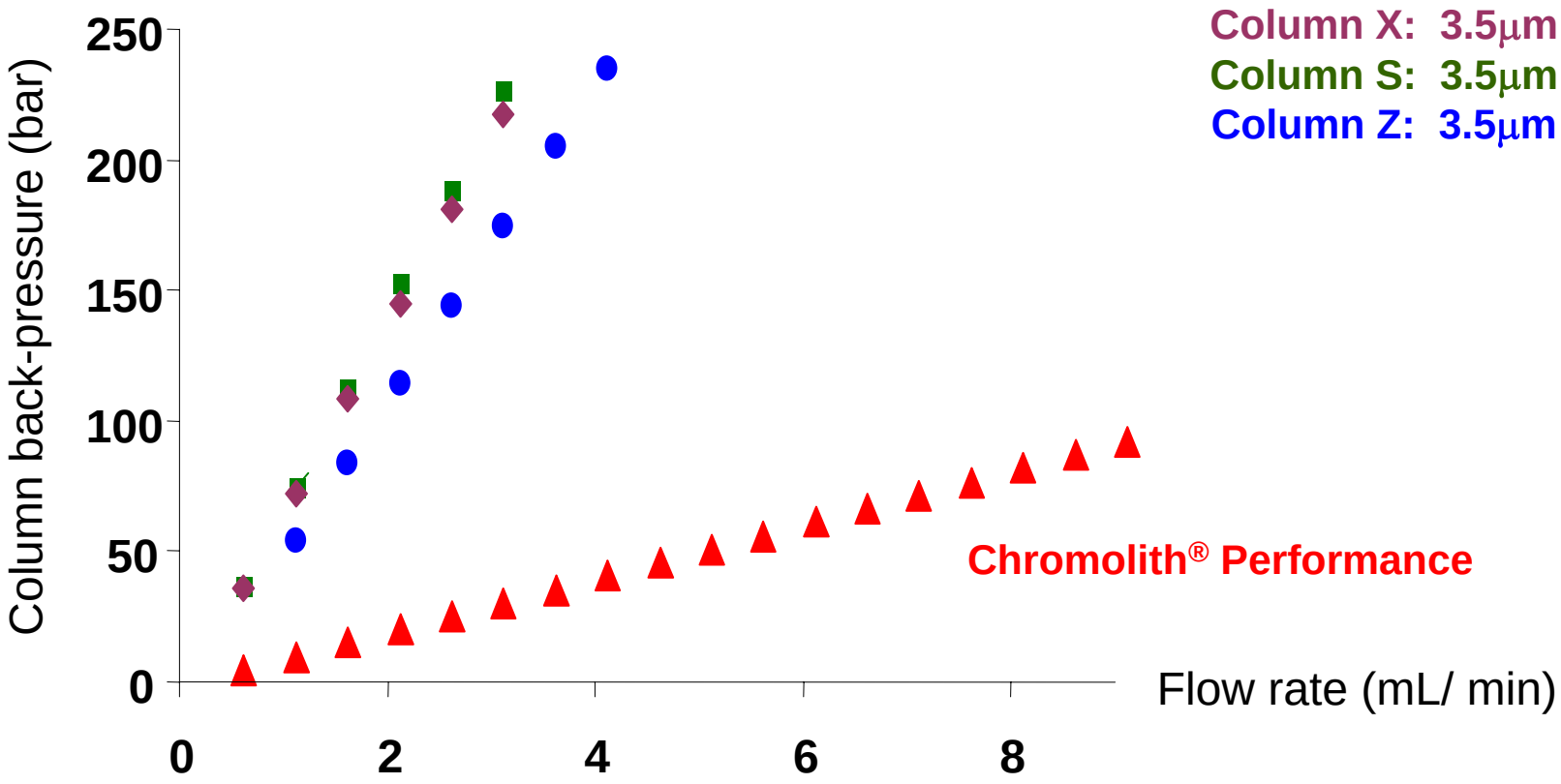
Column back-pressure – 5µm columns

p/u curves for Chromolith® and particulate HPLC columns



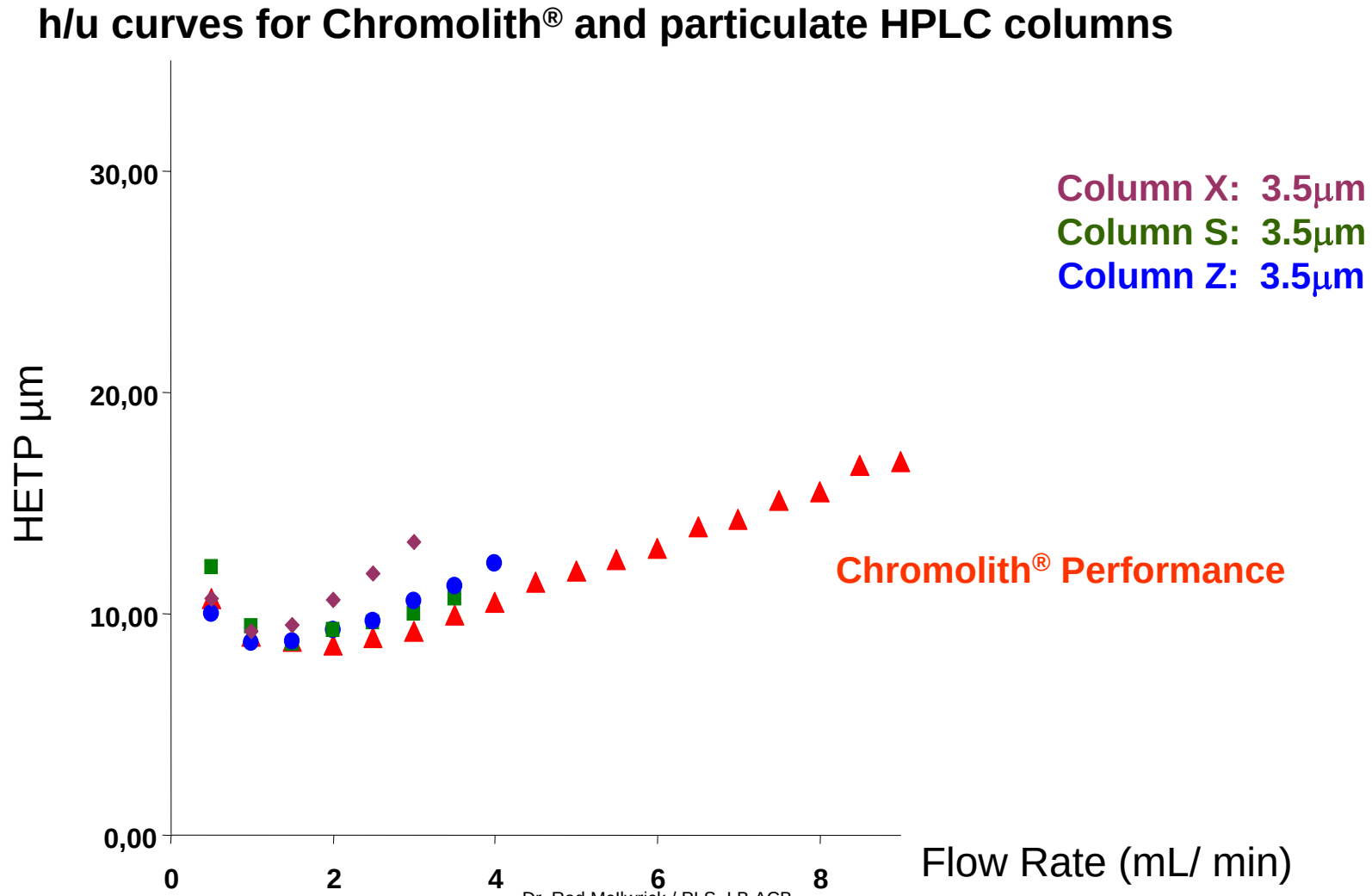
Column back-pressure – 3.5µm columns

p/u curves for Chromolith® and particulate HPLC columns



Column performance – 3.5µm

Van Deemter Plot

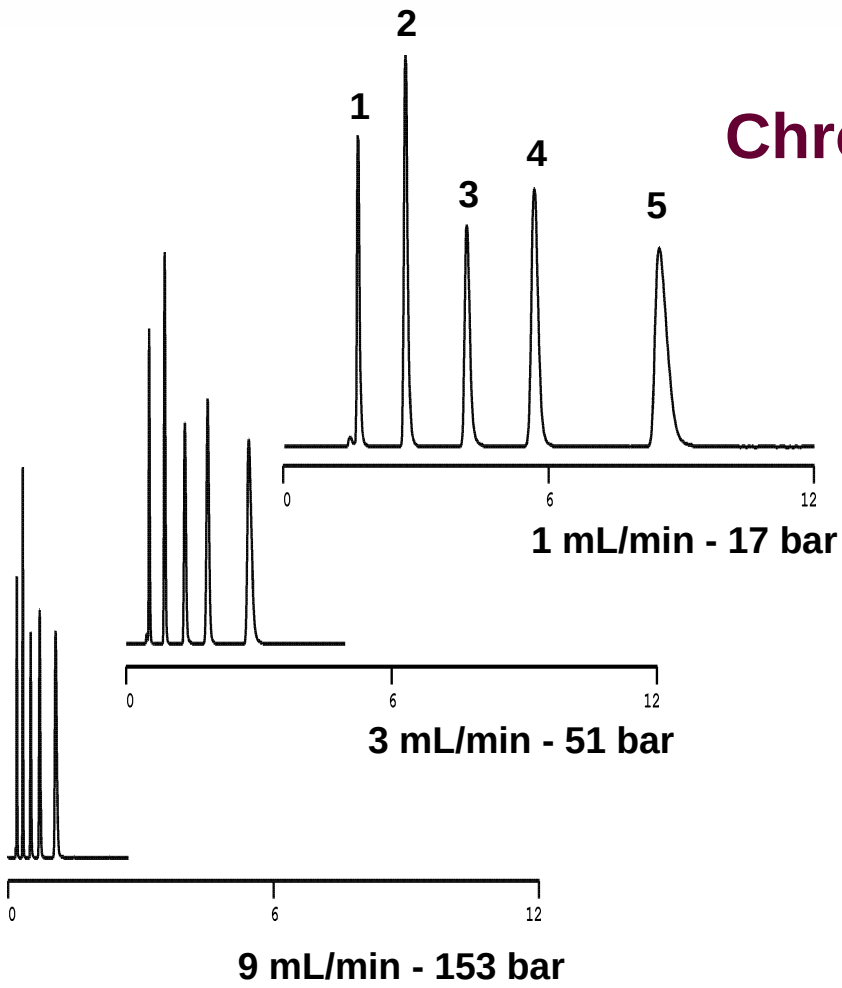


Chromolith® HPLC Columns

- unique properties and benefits (1)

1. **Columns back-pressure much lower**

Speed and Quality in Practice



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

5 ß-Blocker

Mobile	Acetonitrile/ 0.1% TFA in water
Phase	(20(80; v/v)
Flow rate	1-9 ml/min
Detection	222 nm
Sample	1. Atenolol 2. Pindolol 3. Metoprolol 4. Celiprolol 5. Bisoprolol

A Rapid and Low-Cost Method for Quantification of Reduced Iso-α-Acids in Brewing

Alexis Bolívar, Mónica Gasparri,[1] and Carsten Zufall, *Corporative Quality, Innovation and Development Department, Cervecería Polar C. A., Caracas, Venezuela*

ABSTRACT

J. Am. Soc. Brew. Chem. 64(1):39-46, 2006

A rapid HPLC method for routine wort and beer analysis during production has been developed. The method is based on the separation of the reduced iso-α-acids such as dihydro-, tetrahydro-, and hexahydro-iso-α-acids. This is done by means of a new column technology and direct injection of the sample. The new method is significantly faster and more economical than are the existing methods, obtaining 86% savings in time and 60% savings in cost. The method has been validated and implemented in our five laboratories with excellent results in repeatability and reproducibility.

Keywords: Beer bitterness, Dihydro-iso-α-acids, Hexahydro-iso-α-acids, Light-stable beer, Tetrahydro-iso-α-acids, Validation method

RESUMEN

Un Método Rápido y de Bajo Costo para Cuantificar los Iso-α-Ácidos Reducidos en la Elaboración de Cerveza

Initially, a standard HPLC method including a sample pretreatment (1) by manual solid-phase extraction (SPE) and analysis by a C-18 reverse phase column had been used with a resulting relative standard deviation (RSD) of 1.6%. This article describes a rapid HPLC method (9) for routine analysis using a new HPLC column, Chromolith (Darmstatdt, Germany. It permits direct analysis of wort and beer without presample preparation. The 60 min of sample preparation and HPLC run required previously could be reduced to just eight minutes. The new method leads to the quantitative and qualitative analysis of the different reduced iso-α-acids while also correlates to their varying relative bitterness intensity (5) or organoleptic bitterness units.

The improved method with an RSD of 0.9% has been validated and implemented in all five of our laboratories. To fulfill the validation requirements, we have tested specificity/selectivity, linearity and range, accuracy, precision, sensitivity, and repeatability and reproducibility.

EXPERIMENTAL

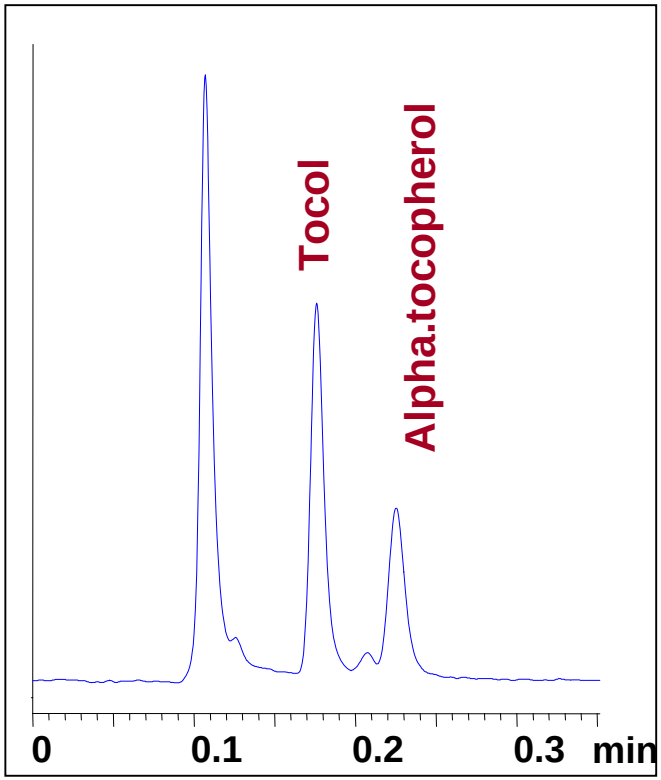
Chromolith® HPLC Columns

- unique properties and benefits (1)

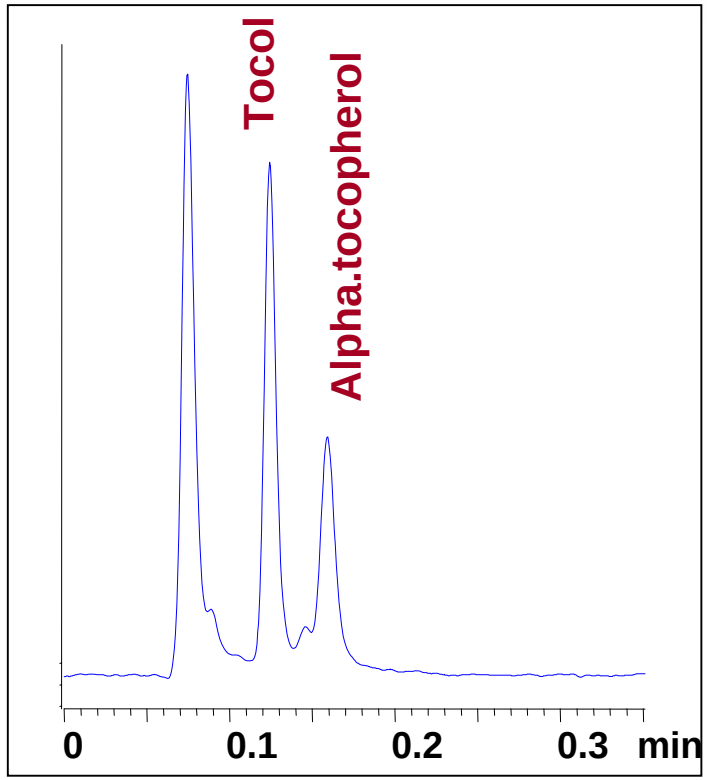
1. **Columns back-pressure much lower**

   = faster analysis and higher sample throughput

Tocopherols



Chromolith Speed ROD
7 ml/min



Chromolith Speed ROD
10 ml/min

By courtesy of Dr. Thomas E Gundersen
AS Vitas, Norway

High throughput analysis with monolithic silica columns

N. Barbarin, B.D. Mawhinney, R. Black, J. Henion
J. Chromatogr. B, 2003, 783, 73-83

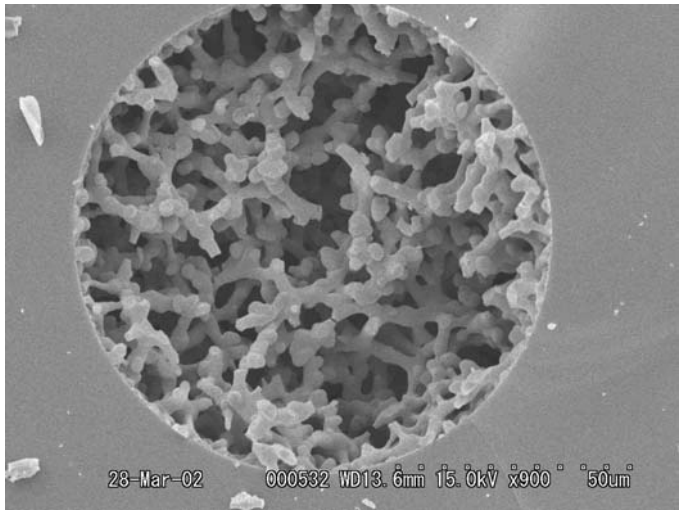
“Determination of methylphenidate (central nervous stimulant) and its metabolite ritalinic acid in rat plasma with LC/MS”

Chromatographic conditions:

Column:	**Chromolith® Flash RP-18e, 25 x 4.6mm**
flow rate:	**3.5ml/ min**
analysis time:	**15 s**

768 protein precipitated samples analysed in 3h 45min !!!!!

Monolithic Silica Capillaries



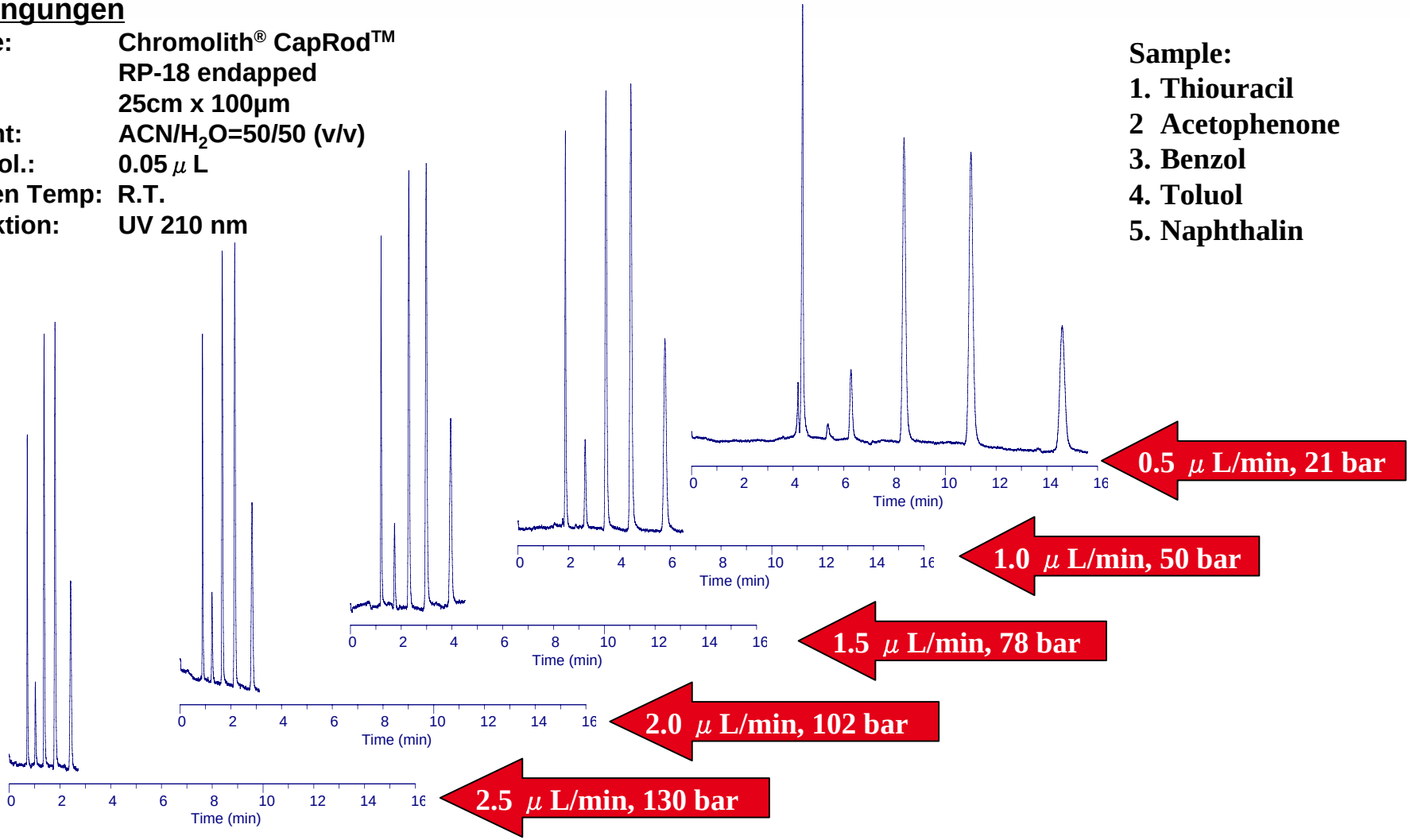
Chromolith® CapROD RP 18e, 150 x 100µm

Fast analysis

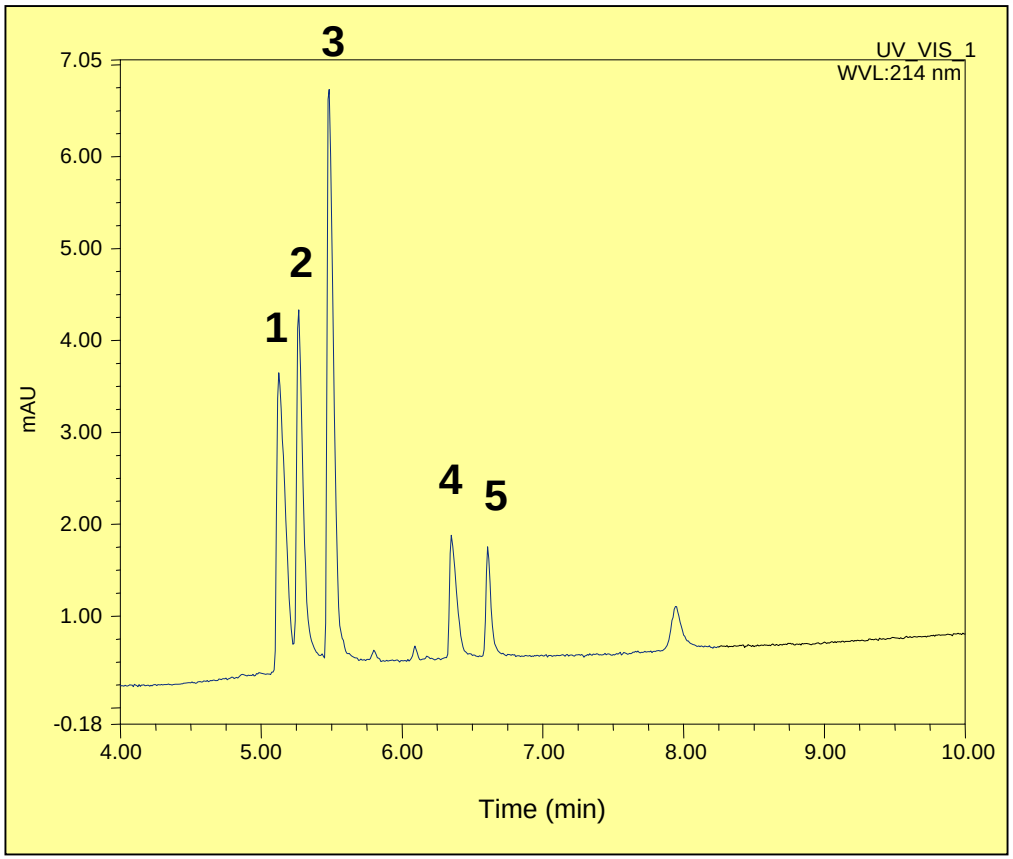
Bedingungen

Säule:	**Chromolith® CapRod™ RP-18 endapped 25cm x 100µm**
Eluent:	**ACN/H_2O=50/50 (v/v)**
Inj. Vol.:	**0.05 µ L**
Säulen Temp:	**R.T.**
Detektion:	**UV 210 nm**

Sample:
1. **Thiouracil**
2. **Acetophenone**
3. **Benzol**
4. **Toluol**
5. **Naphthalin**



Separation of Peptides



System: UltiMate Nano LC (LC Packings)

Chromolith™ CapRod™

RP-18e 150cm x 100µm

U = 2.5µl/min

Solvent A: 2% ACN / 0,05% **TFA**

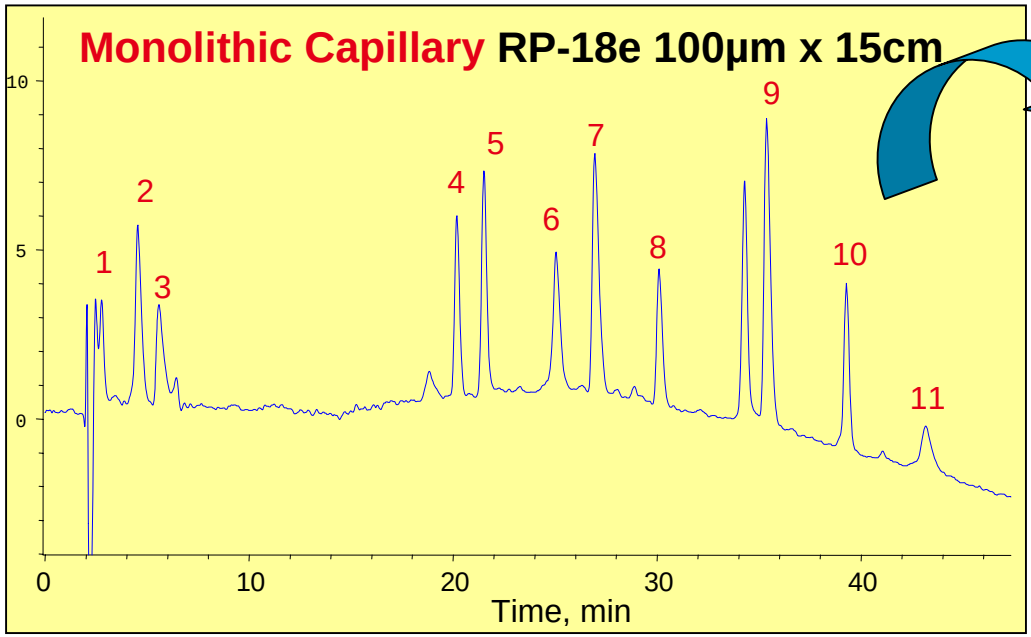
Solvent B: 50% ACN /0,04% **TFA**

Gradient: 0-50 % B in 7.5 min with 0.5 min wash step and 7 min equilibration.
Total run time: 15 minutes.

Sample: 1 ng Peptide Standard

Peptides (Peak Nr)	ret. Time (min)	PWHH (sec)
1. bradykinin frag 1-5	5.2	4.7
2. vasopressin [Arg8]	5.3	3.6
3. methionin enkephalin	5.5	2.6
4. leucin enkephalin	6.4	3.2
5. oxytocin	6.6	2.5

nanoLC/MS of a digested Cytochrome C



Mass spectrometry analysis of a digested protein (cytochrome c) using an Esquire 3000plus (Bruker).

U = 1,5µl/min
Solvent A: 2% ACN / 0,1% **FA**
Solvent B: 80% ACN /0,08% **FA**
Gradient: 0% to 45% B in 45 min
0,5 pmol tryp. digested cytochrome c

ESI MS mediated Sequence Analysis of digested cytochrome c

Peak	*m/z*	*Sequence*
1	not identified	
2	677,37	YIPGTK
3	633,38	IFVQK
4	1583,76	KTGQAPGFSYTDANK
5	1455,66	TGQAPGFSYTDANK
6	1167,61	TGPNLHGLFGR
7	778,44	MIFAGIK
8	963,53	EDLIAYLK
9	1632,81	IFVQKCAQCHTVEK
10	2137,04	GITWGEETLMEYLENPKK
11	2008,95	GITWGEETLMEYLENPK

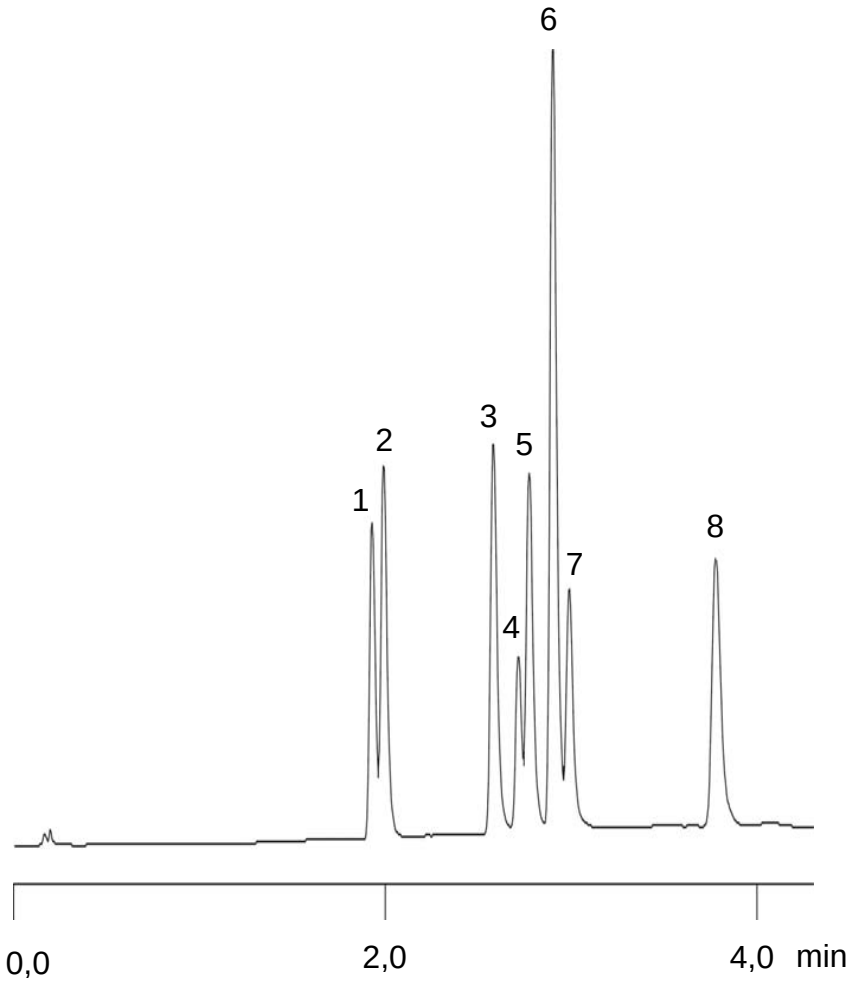
Chromolith® HPLC Columns

- unique properties and benefits (2)

1. **Columns back-pressure much lower**
   = faster analysis and higher sample throughput

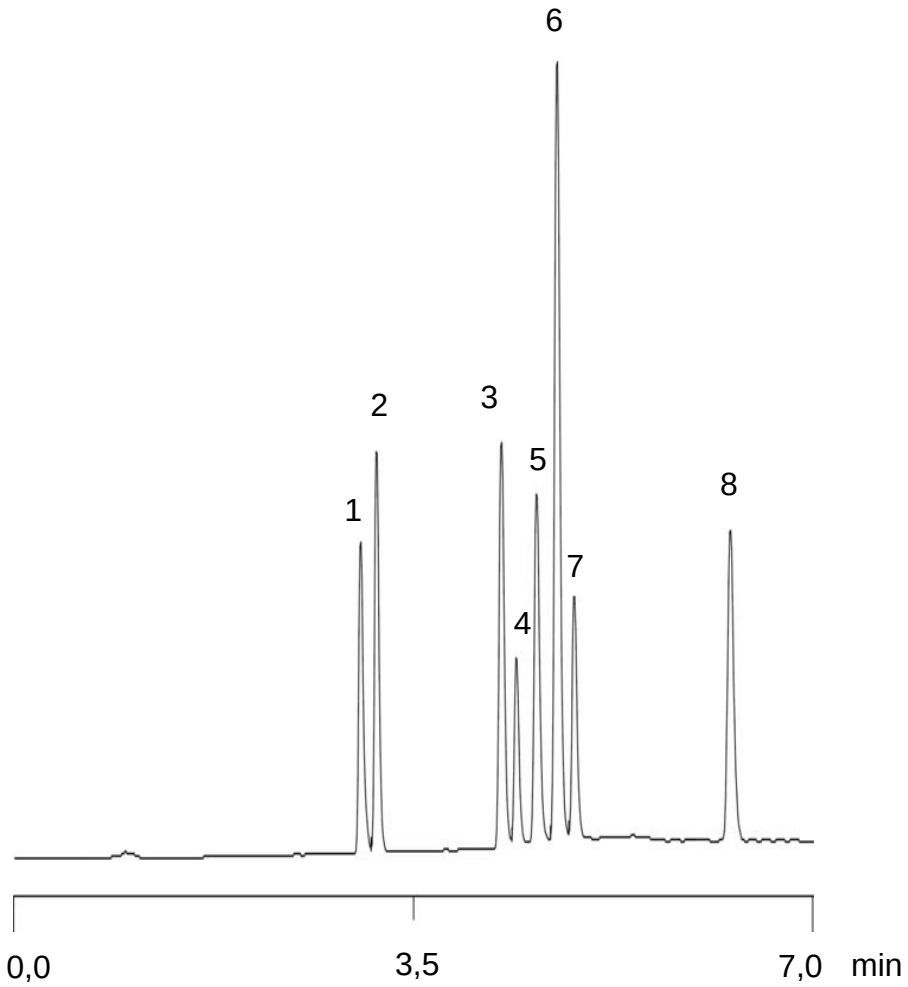
2. **Length of column not pressure limited**

Separation of 8 Steroids



Column	**Chromolith SpeedROD RP-18e 50-4.6mm**		
Mobile phase	A: Acetonitrile B: Water		
Gradient	Time/min	%A	%B
	0,0	10	90
	3,0	50	50
	4,5	50	50
Flow rate	4 mL/min		
Detection	UV 220 nm		
Temp.	ambient		
Inj.Volume	10 µL		
Sample	1. Prednisolone 2. Cortisone 3. Nortestosterone 4. Estradiol 5. Testosterone 6. Corticosterone 7. Estrone 8. Progesterone		

Separation of 8 Steroids



Column	**2 columns of Chromolith Performance RP-18e 100-4.6mm**		
Mobile phase	A: Acetonitrile B: Water		
Gradient	Time/min	%A	%B
	0,0	20	80
	7,0	90	10
Flow rate	3 mL/min		
Detection	UV 220 nm		
Temp.	ambient		
Inj.Volume	10 µL		
Sample	1. Prednisolone 2. Cortisone 3. Nortestosterone 4. Estradiol 5. Testosterone 6. Corticosterone 7. Estrone 8. Progesterone		

Chromolith® HPLC Columns

- unique properties and benefits (2)

1. **Columns back-pressure much lower**
   = faster analysis and higher sample throughput

2. **Length of column not pressure limited**
   = better peak resolution

Chromolith® HPLC Products

1.51467.0001
Chromolith Column Coupler

Longer columns

Column	Length (mm)	Back pressure (bar)	Plate Number N (Anthracene)
Chromolith Performance 1x	100	30	10.000
Chromolith Performance 2x	200	60	19.000
Chromolith Performance 3x	300	90	27.000
Chromolith Performance 4x	400	120	35.000
Chromolith Performance 5x	**500**	**150**	**41.000**
Particulate 5 µm Competitor X	250	320	16.000
Particulate 5 µm Competitor Z	250	210	17.500
Particulate 5 µm Competitor L	250	220	18.500
Particulate 3.5 µm Competitor X	150	330	11.500
Particulate 3.5 µm Competitor Z	150	260	14.000
Particulate 3.5 µm Competitor L	150	400	19.000

[column diameter: 4.6 mm, mobile phase: acetonitrile/water (60/40; v/v), flow: 3 mL/min, temperature: 25°C, injection: 10 µLl anthracene (10 µg/mL)]

Coupling of Chromolith® - columns

Chromolith® Performance, 100-4.6mm

1x

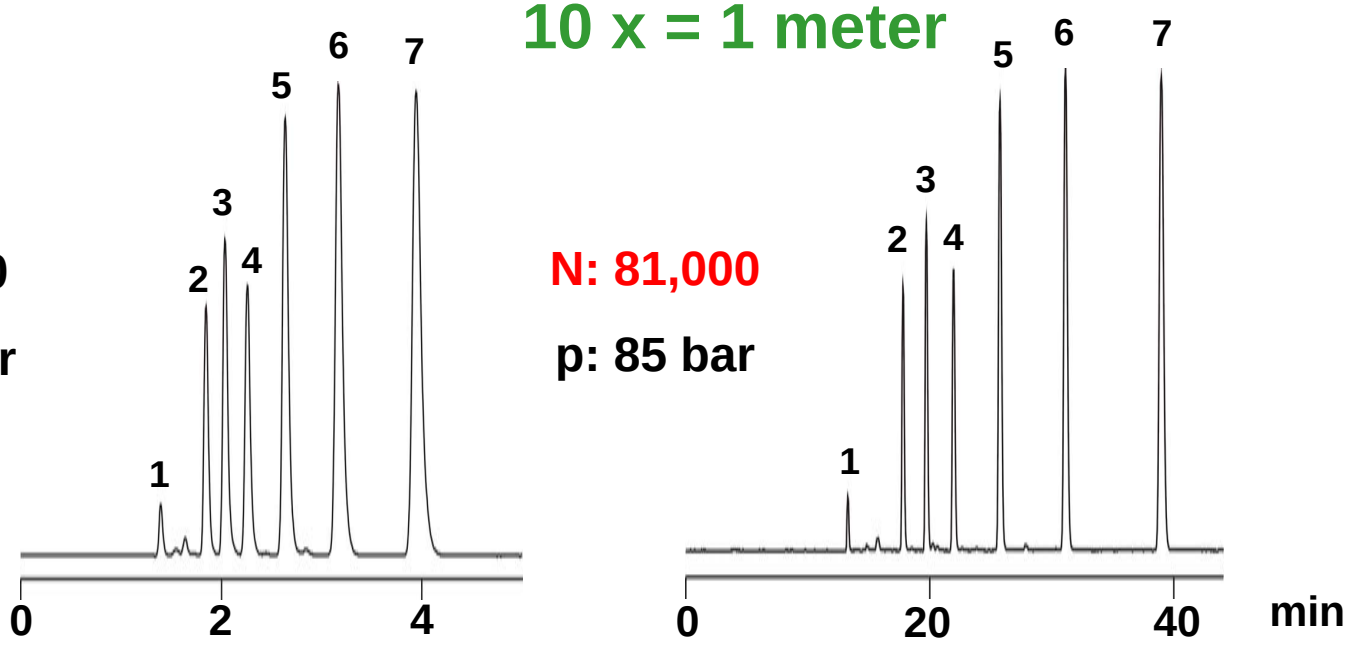
N: 9,500

p: 11 bar

10 x = 1 meter

N: 81,000

p: 85 bar



ACN/ water (80/ 20; v/v), 1mL/ min

1. thiourea, 2. benzene, 3. toluene, 4. ethyl-, 5. propyl-, 6. butyl-, 7. pentylbenzene

Chromolith® HPLC Columns

- unique properties and benefits (3)

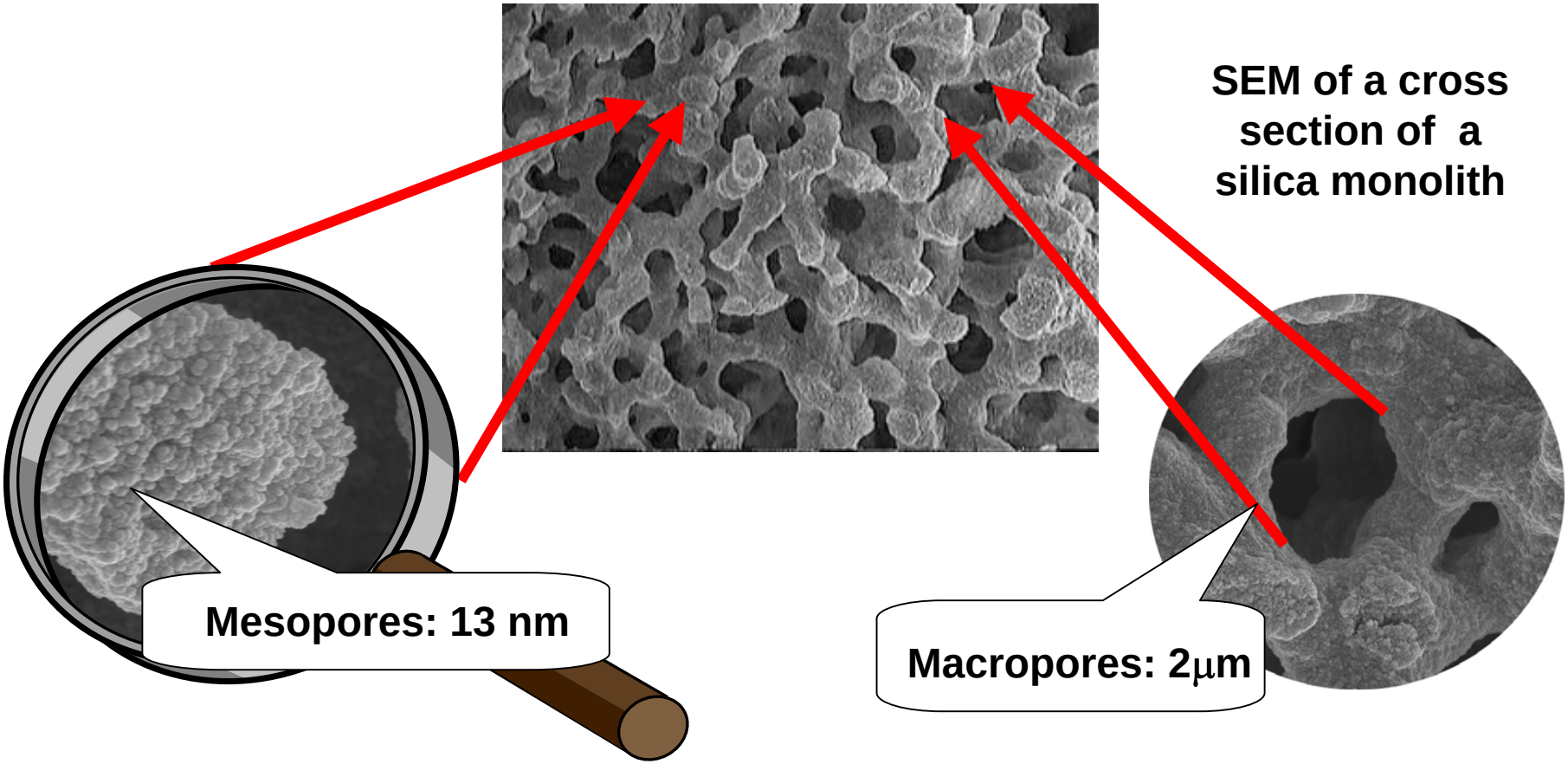
1. **Columns back-pressure much lower**
   = faster analysis and higher sample throughput

2. **Length of column not pressure limited**
   = better peak resolution

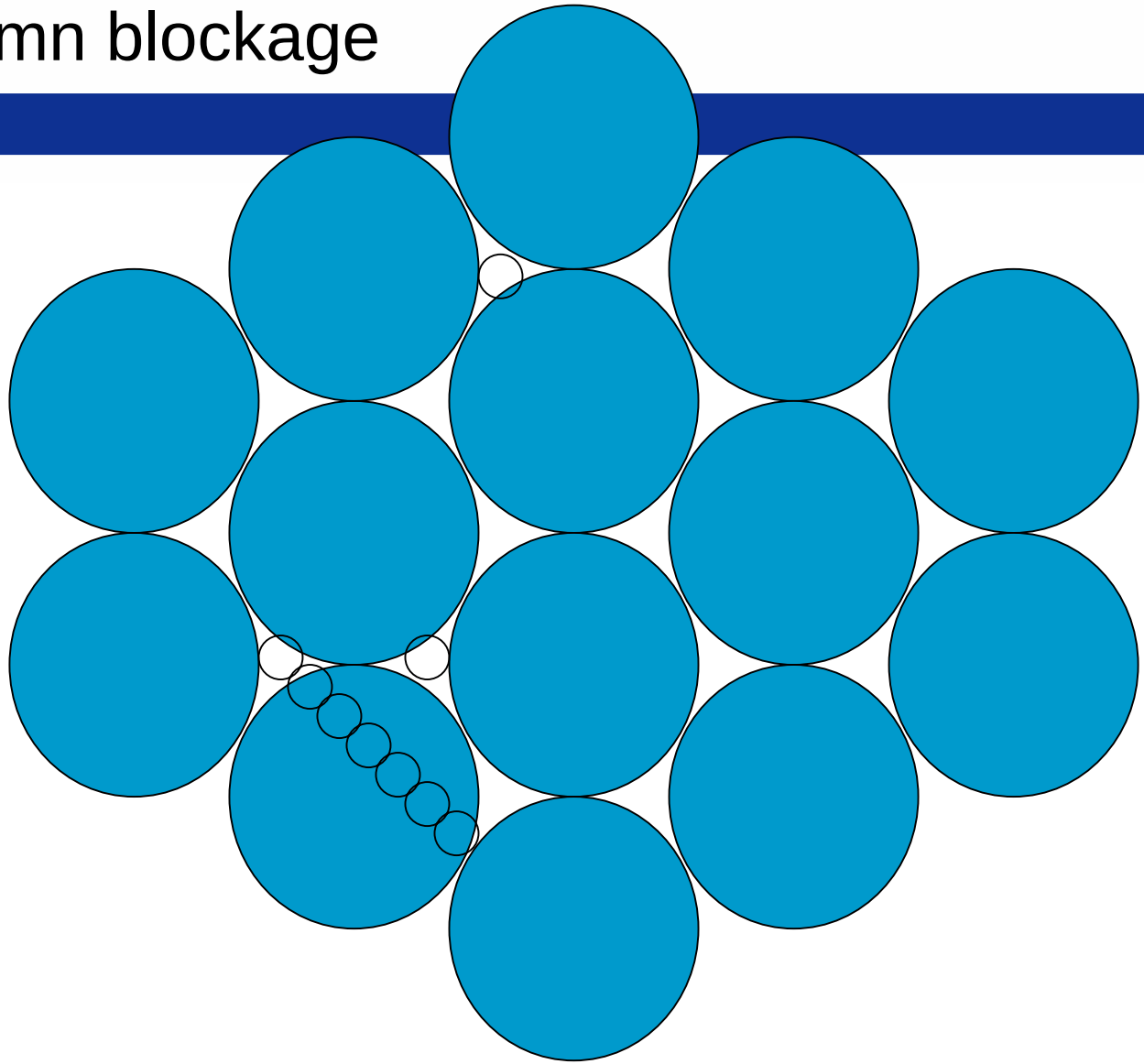
3. **Strong porous monolithic silica**

Feature of monolithic silica columns

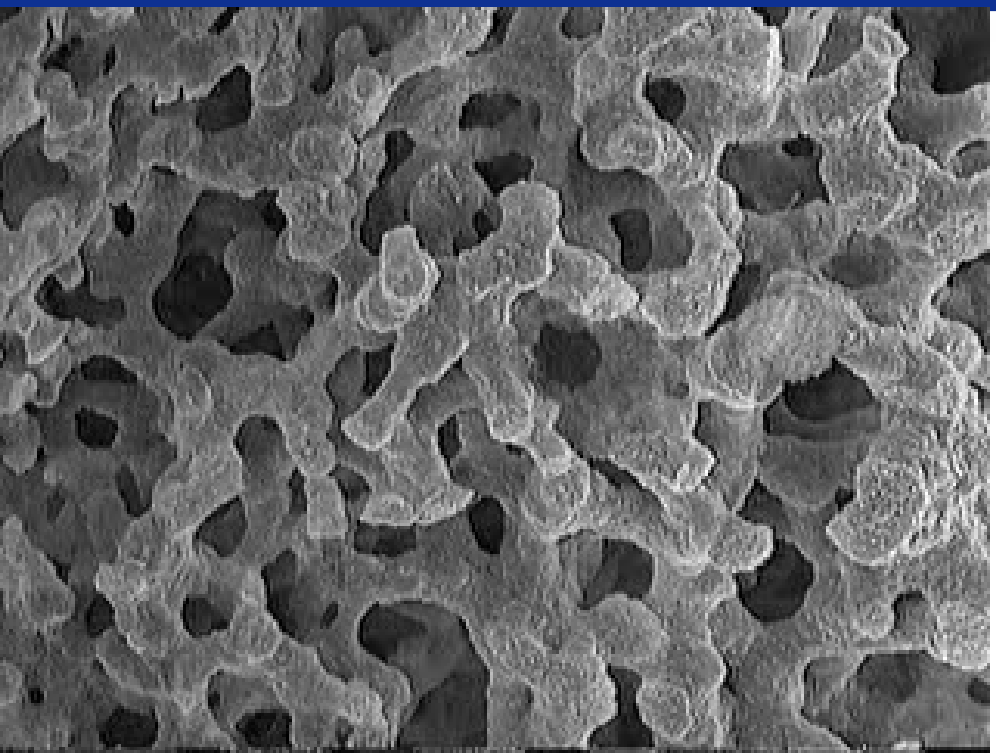
Defined bimodal pore structure

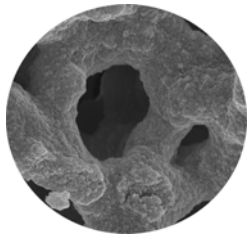


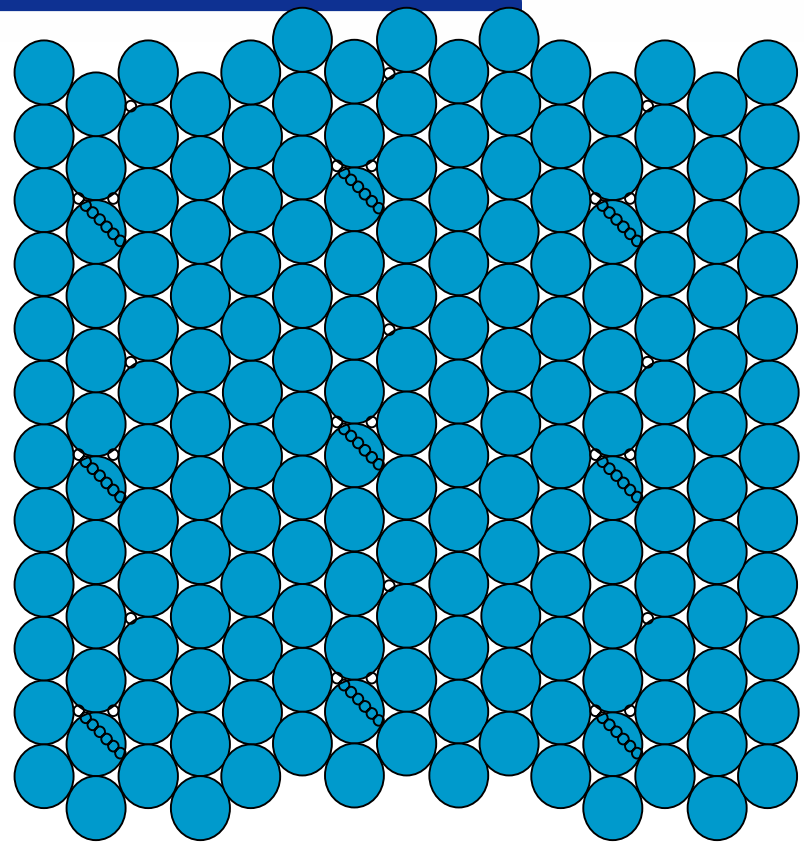
Sub 2µm / 6 < 0,3µm means high risk of column blockage



Chromolith® HPLC columns are robust







High throughput analysis with monolithic silica columns

N. Barbarin, B.D. Mawhinney, R. Black, J. Henion
J. Chromatogr. B, 2003, 783, 73-83

“Determination of methylphenidate (central nervous stimulant) and its metabolite ritalinic acid in rat plasma with LC/MS”

Chromatographic conditions:

Column:	**Chromolith® Flash RP-18e, 25 x 4.6mm**
flow rate:	**3.5ml/ min**
analysis time:	**15 s**

768 protein precipitated samples analysed in 3h 45min !!!!!

Chromolith® HPLC Products

- Guard Columns

Chromolith® HPLC Columns

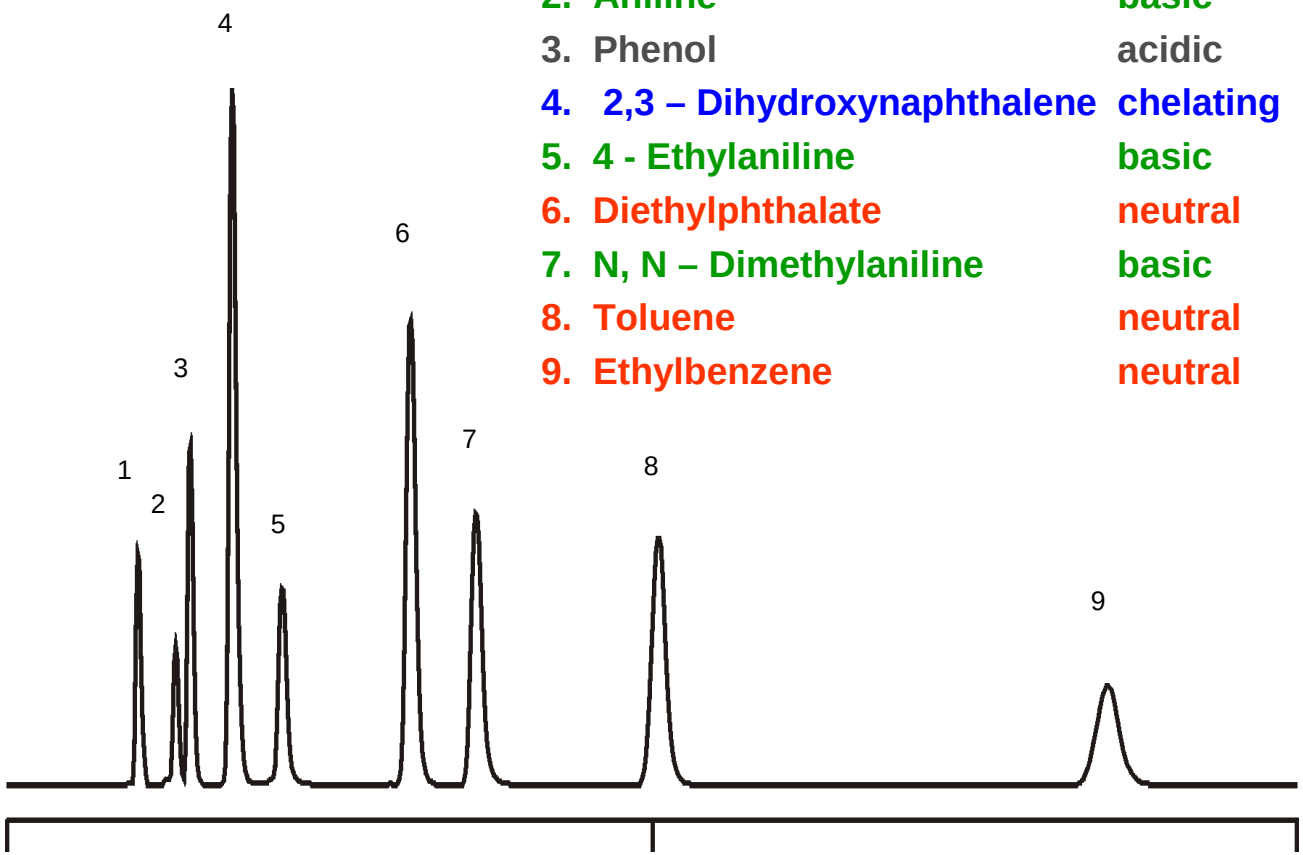
- unique properties and benefits (3)

1. **Columns back-pressure much lower**
   = faster analysis and higher sample throughput

2. **Length of column not pressure limited**
   = better peak resolution

3. **Strong highly porous monolithic silica**
   = longer column lifetime

Selectivity (1)



1. Thiourea t_0
2. Aniline basic
3. Phenol acidic
4. 2,3 – Dihydroxynaphthalene chelating
5. 4 - Ethylaniline basic
6. Diethylphthalate neutral
7. N, N – Dimethylaniline basic
8. Toluene neutral
9. Ethylbenzene neutral

Chromatographic Conditions:

Column:

Chromolith Performance

RP18e

4.6 mm x 100 mm

Mobile Phase:

Methanol/Water

55/45 (v/v)

Flow rate:

1.0 mL/min

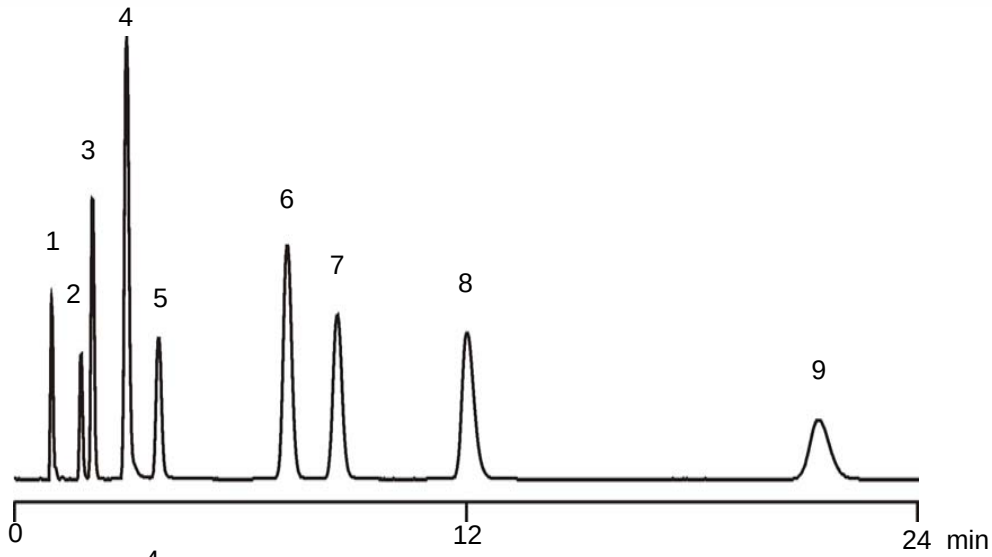
Temperature:

ambient

Detection:

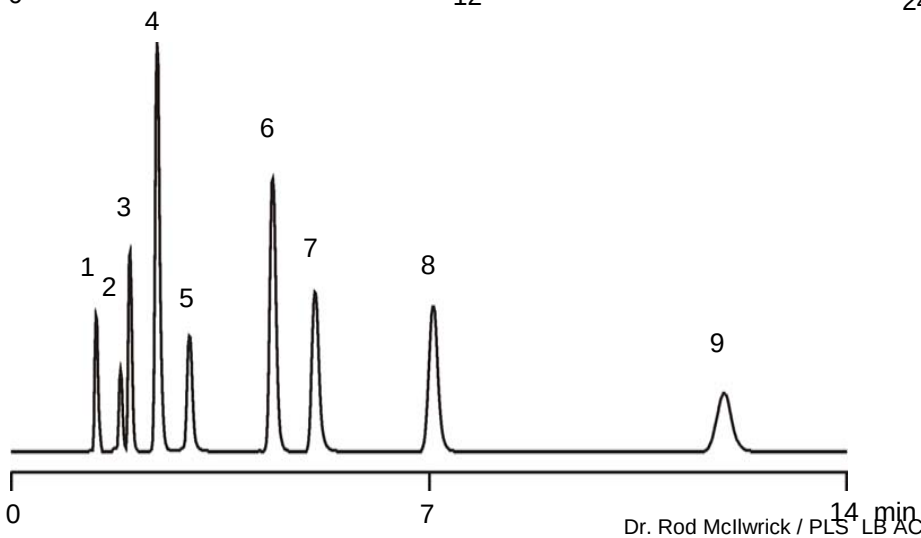
UV 254nm

Selectivity (2)



Purospher RP-18e, 5 µm

125 x 4mm



Chromolith Performance RP-18e

100 x 4.6mm

USP includes monolithic silica HPLC columns in the L1 category

In the US Pharmacopoeia, Volume 29 Supplement 1, April 2006 – the USP has officially added monolithic silica HPLC columns to the list of HPLC columns in General Chapter 621.

Chromolith® Performance, SpeedROD and Flash HPLC RP-18 columns from Merck become listed in **USP 29 as L1** columns.

Potential cost saving with Chromolith HPLC columns

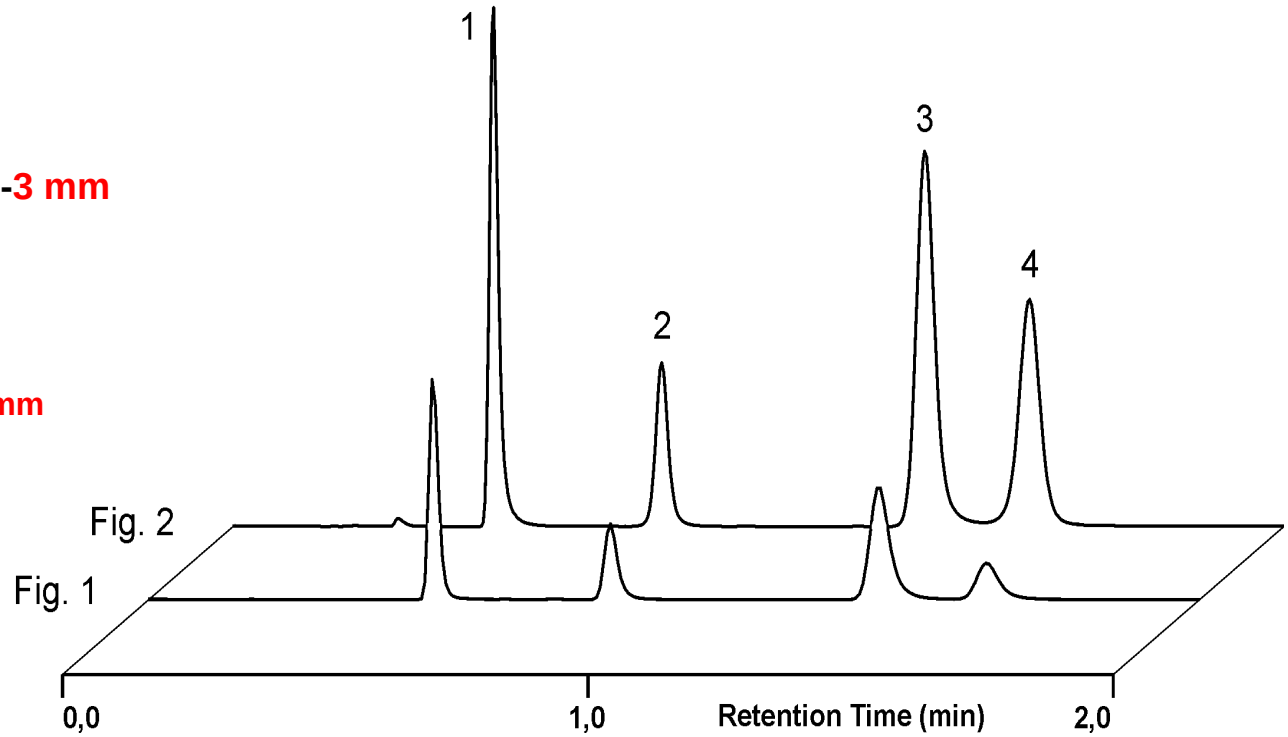
1 hour HPLC lab time in USA typically	costs	$100	per hour
Revalidating one hplc method requires 3 weeks lab time and	costs	$12,000	per revalidation
New faster Chromolith HPLC method cuts analysis time by 50% saving 4 hours per day	saves	$400	per day
Run the new faster Chromolith method for 30 days	total savings	$12,000	after 30 days, revalidation has paid for itself
After running the new faster Chromolith method for one year	total savings	$80,000	assuming only 200 days

Chromolith® 100-3 mm HPLC columns

- higher sensitivity at lower flow rate

Fig. 2
Chromolith® RP-18e 100-3 mm
Flow rate: 1.7 mL/min
Pressure: 100 bar

Fig. 1
Chromolith® RP-18e 100-**4,6 mm**
Flow rate: 4.0 mL/min
Pressure: 137 bar



Chromatographic conditions:
Acetonitrile/ Water 60/ 40, UV 254 nm (2.4 µL detector cell), ambient temperature, 1 µL injection
Sample: 1) Biphenyl-4,4'-ol, 2) Biphenyl-2,2'-ol, 3) Biphenyl-4-ol, 4) Biphenyl-2-ol

Chromolith® 100-3 mm HPLC columns

Practical benefits:

- Very fast results with optimum column efficiency between flow rates 0.5 and 2 mL/min

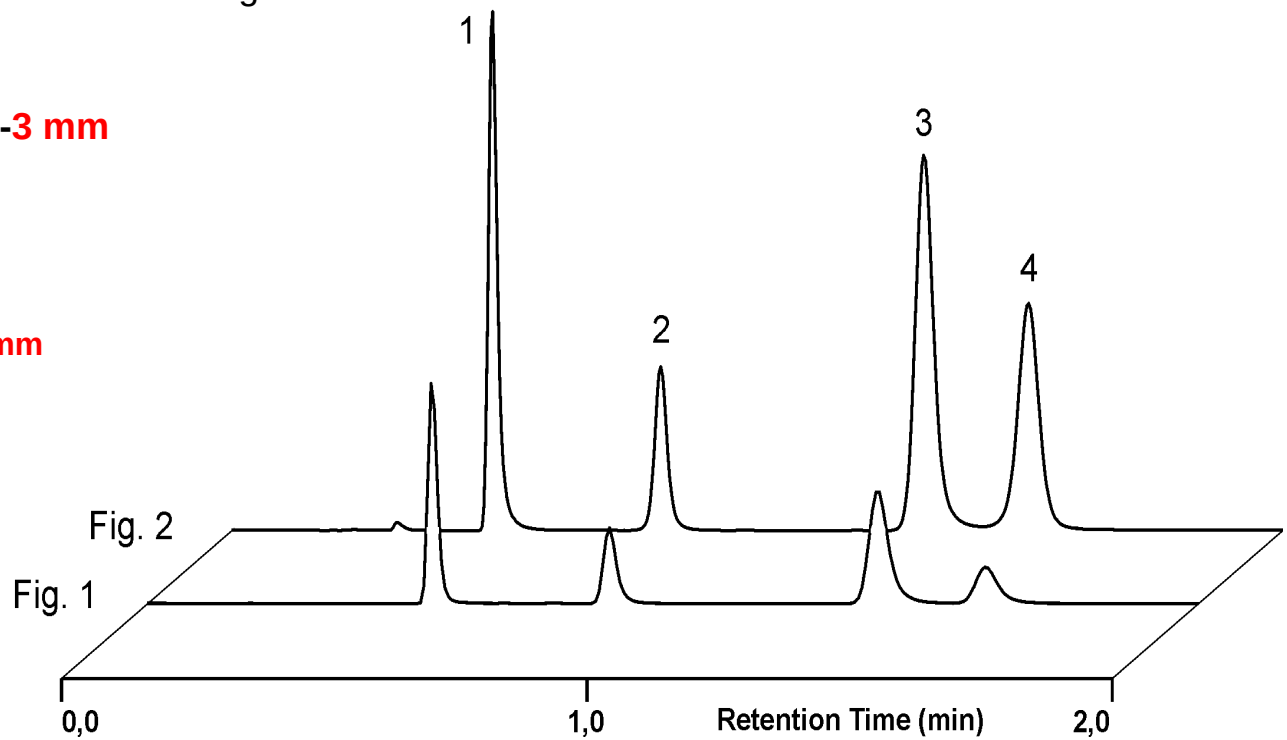
Chromolith® 100-3 mm HPLC columns

- higher sensitivity at lower flow rate

With Chromolith® 3 mm columns, detection sensitivity is markedly improved, the flow rate is reduced and the quality of the separation is unchanged

Fig. 2
Chromolith® RP-18e 100-3 mm
Flow rate: 1.7 mL/min
Pressure: 100 bar

Fig. 1
Chromolith® RP-18e 100-**4,6 mm**
Flow rate: 4.0 mL/min
Pressure: 137 bar



Chromatographic conditions:
Acetonitrile/ Water 60/ 40, UV 254 nm (2.4 µL detector cell), ambient temperature, 1 µL injection
Sample: 1) Biphenyl-4,4'-ol, 2) Biphenyl-2,2'-ol, 3) Biphenyl-4-ol, 4) Biphenyl-2-ol

Chromolith® 100-3 mm HPLC columns

Practical benefits:

- Very fast results with optimum column efficiency between flow rates 0.5 and 2 mL/min
- Increased detection sensitivity, particularly in LC/MS
- Cost saving for solvents typically > $1000 per annum, when compared to 4.6 mm columns

Chromolith® 100-3 mm HPLC columns

- increased peak resolution

Fig. 1
1 column
Chromolith® RP-18e 100-3mm
Flow: 2,0 mL/min
Back-pressure: 92 bar

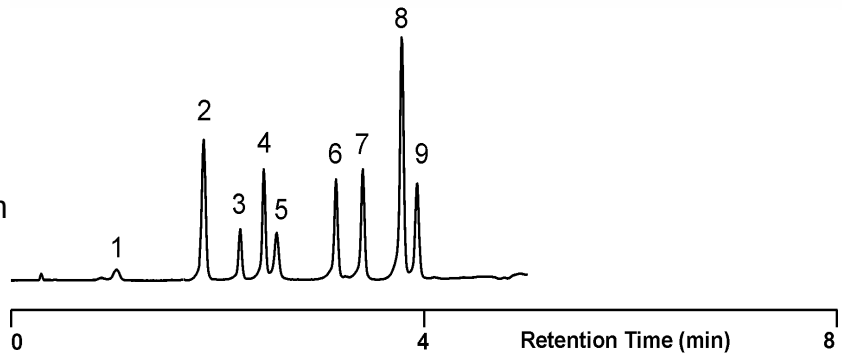
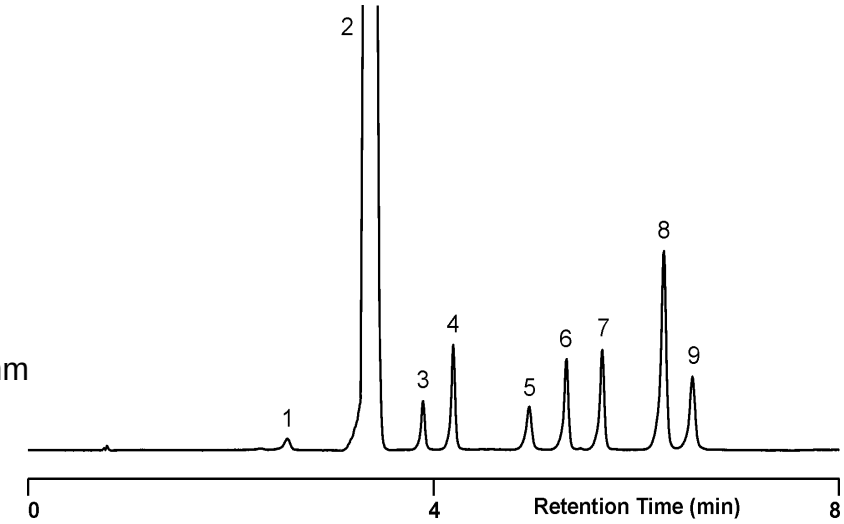


Fig. 2
2 columns
Chromolith® RP-18e 100-3 mm
Flow: 1,5 mL/min
Back-pressure: 140 bar



Chromolith® 3mm columns can be easily coupled to give very high peak resolution

Chromatographic conditions:
Acetonitrile/ Puffer pH 1,8 (Gradient), UV 254 nm, 30 °C, 1 µL Injection standards mixture, Peak 2 - Levothyroxin, peaks 1 and 3 to 9 possible impurities

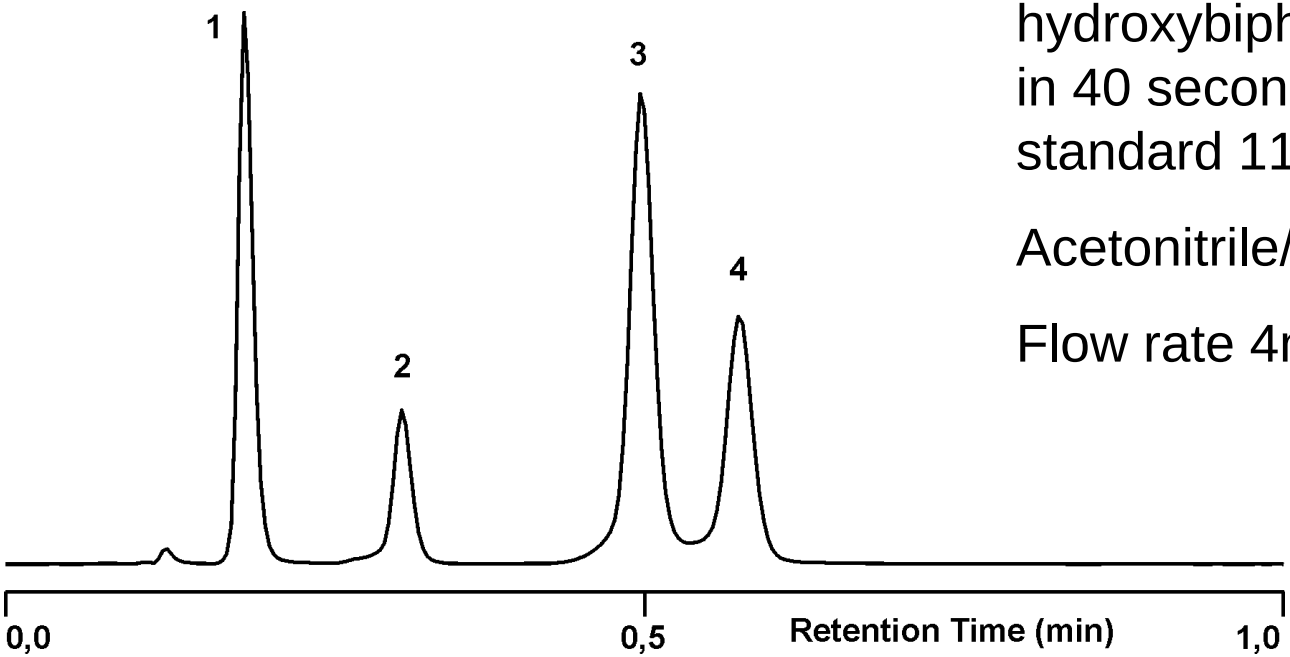
Benefits at a glance

Chromolith® 3 mm, like Chromolith® 4,6 mm, is

- Ideal for increasing the number of samples run per instrument per day - cost saving up to $400 per day
- Ideal for use with all standard HPLC instruments
- and has longer column lifetime and reduced risk of column failure - thanks to robust monolithic silica

Ultra fast LC

Separation of hydroxybiphenyls



Separation of hydroxybiphenyls in 40 seconds using a standard 11.3µL detector cell

Acetonitrile/water 40/60

Flow rate 4mL/min (183 bar)

Ultra fast gradient LC

Separation of 8 sulphonamides standards

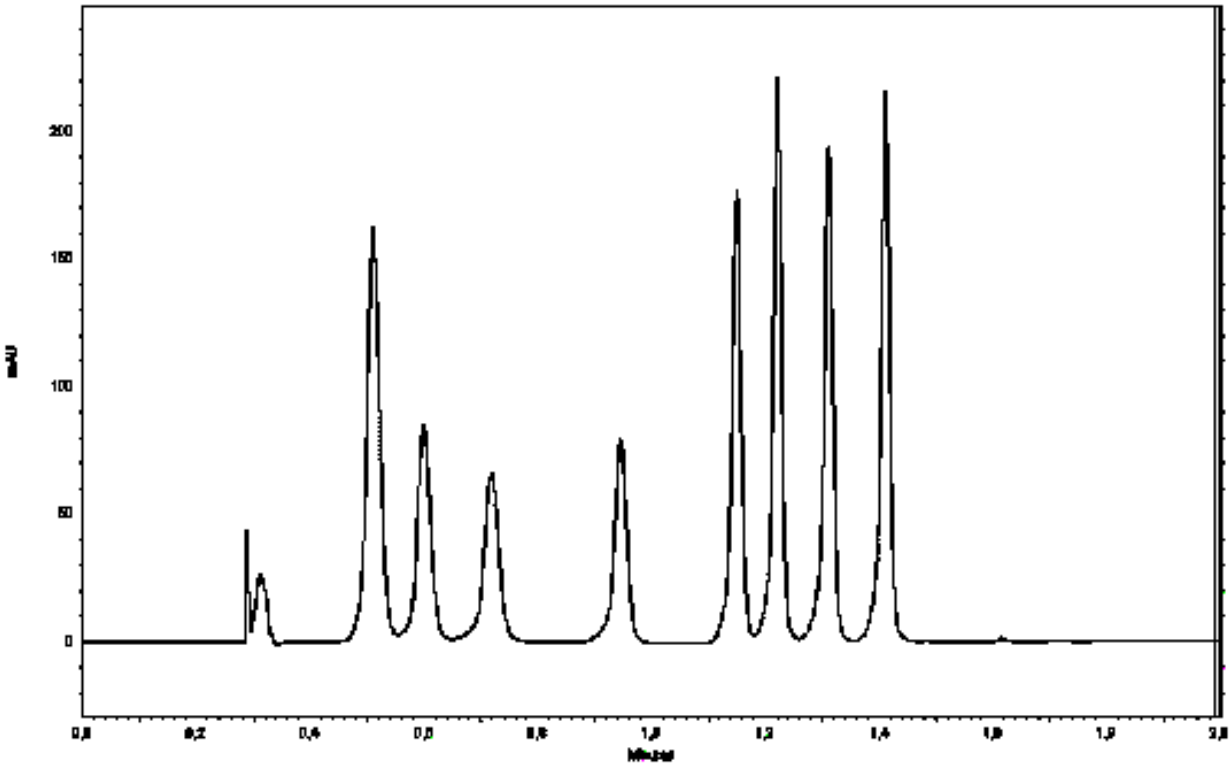


Figure 1: VWR-Hitachi LaChrom Elite® Application with 3 mm Chromolith® Performance RP-18e column.

Peaks: 1. sulfacetamide, 2. sulfamerazine, 3. sulfamethazine, 4. sulfamethoxypyridazine, 5. sulfachloropyridazine, 6. sulfadoxine, 7. sulfadimethoxine, 8. sulfaisoxazole

Standard 11.3µL detector cell
Sample 5µL

Acetonitrile/ Water 0.1% TFA
pH 2.8 (Gradient), 45°C

Flow rate 2mL/min
104 bar pressure

Analysis time < 1.5 minutes

Institute of Pharmaceutical Chemistry

Technical University Braunschweig

- „Evaluation of monolithic C18 columns for the analysis of Pilocarpine and degradation products“

  – S.El Deeb, U.Schepers and **H.Wätzig**
  - poster presentation, DPHG Tagung, Mainz, October 2005

- Comparison of Superspher® 100 RP-18 endcapped (Merck) and Chromolith® Perfomance RP-18 endcapped for the analysis of Pilocarpine and degradation products

- Pilocarpine is a widely used miotic agent for the treatment of glaucoma

9. „Monolithic columns have been shown as an excellent alternative to conventional silica based columns“

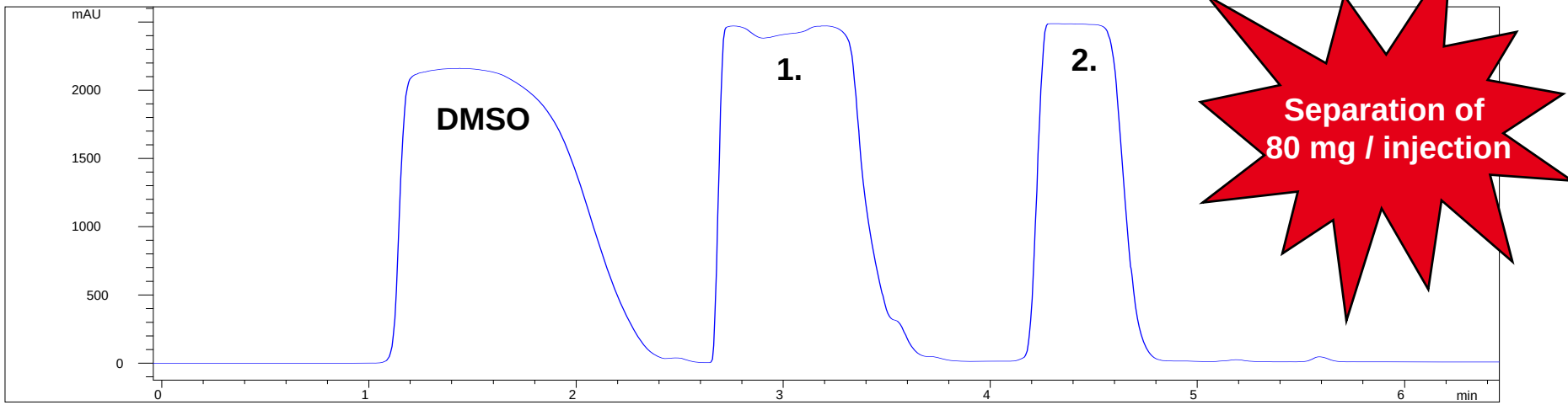
10. „This new trend may be highly important in quality control of drugs, it may be applied to a large number of samples in a short time, thus being a practical choice for routine quality control“

Chromolith® SemiPrep RP-18 endcapped 100-10mm – Capacity test

Chromatographic conditions

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

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



Chromolith® HPLC Columns
- additional information on Internet

Chromolith website

www.chromolith.com

www.chrombook.merck.de (also CD)

www.chromatography.merck.de

Summary and conclusion

Thank you