

90% Zeit, Lösungsmittel und
Kosten einsparen

mit

„ultra“-schneller
und konventioneller
Chromatographie

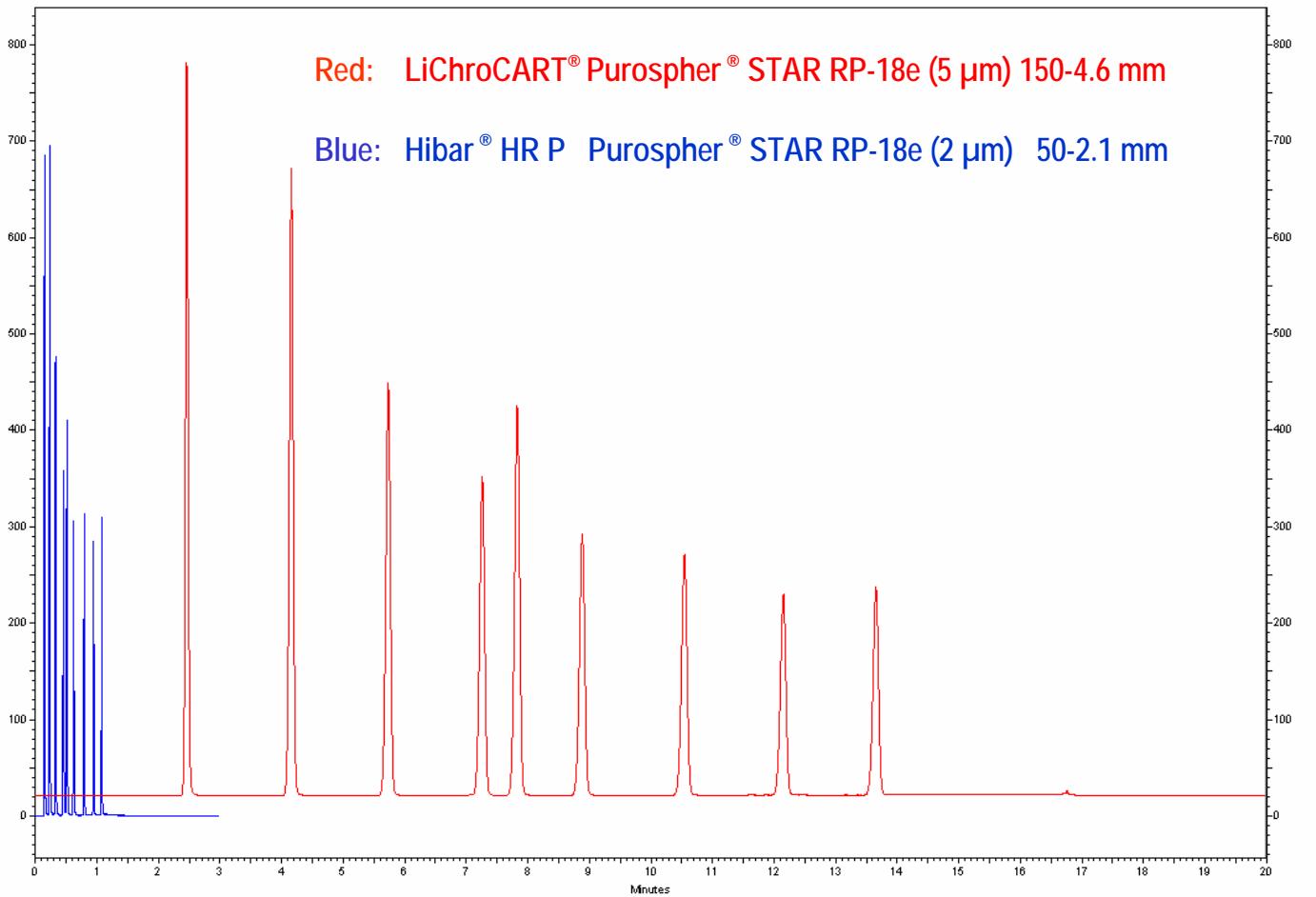
- Verwendung von Säulen mit Partikeln $\leq 2 \mu\text{m}$
- Verwendung von Chromolith[®]-Säulen
- Optimierung der Methoden mit ChromSword Auto[®]
- Anwendungsbeispiele



Applications

Alkylphenones

Time: 19,9883 Minutes - Amplitude: -- mAU



Sample: Alkylphenone standard

1. Acetanilide
2. Acetophenone
3. Propiophenone
4. Butyrophenone
5. Benzophenone
6. Valerophenone
7. Hexanophenone
8. Heptanophenone
9. Octanophenone

Column temperature: 40°C

Eluent A: Water

Eluent B: Acetonitrile

Gradient: 0 min 45% B , from 45 to 95 % B in 15 min, from 15.1 to 20 min reequilibration with 45 % B

Flow rate: 1.0 ml/min,

Pressure: 105 bar

UV: 247 nm

Injection volume: 10 µl

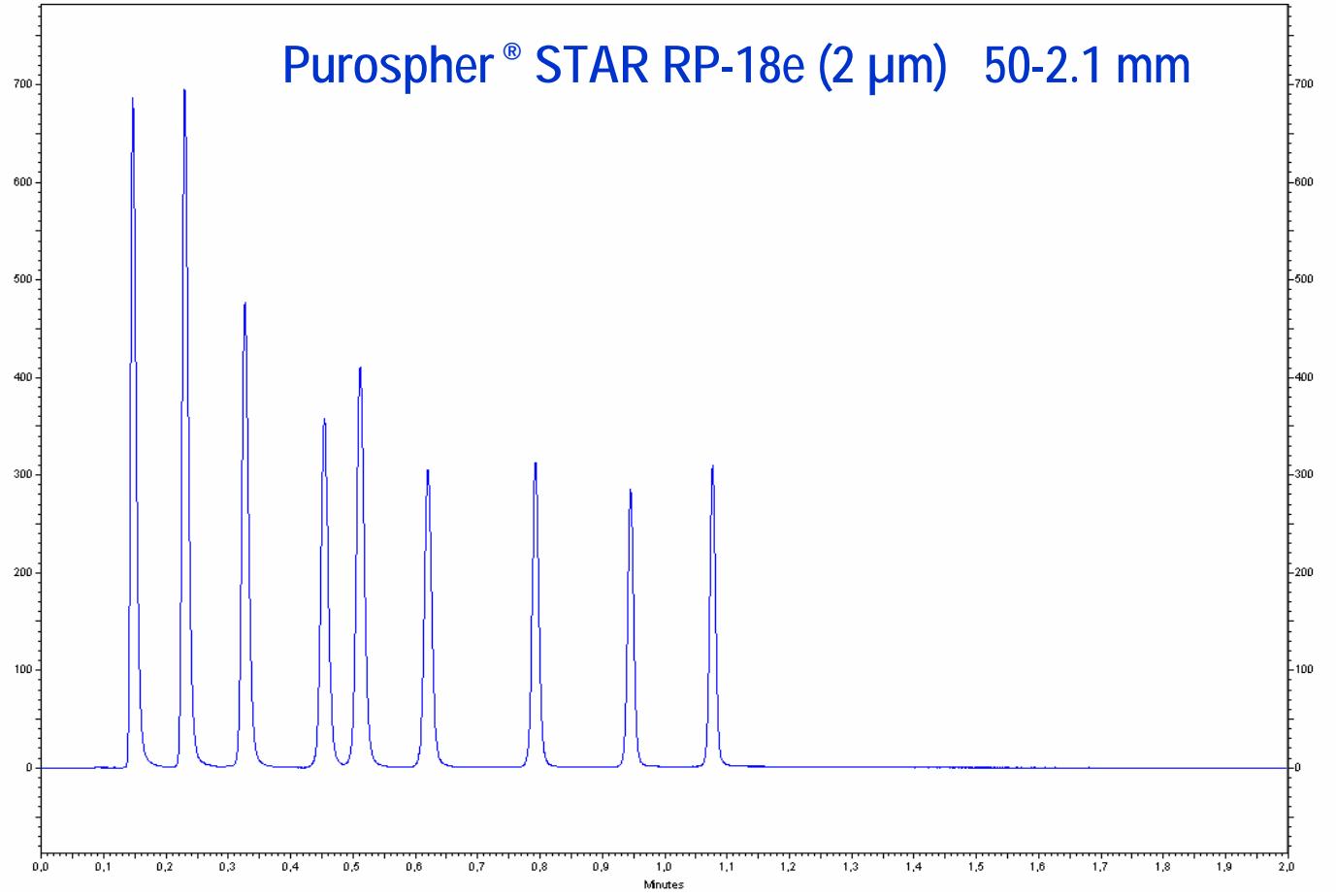


Applications

Alkylphenones

Time: 1.99863 Minutes - Amplitude: 0,0895 mAU

Purospher® STAR RP-18e (2 µm) 50-2.1 mm



Sample: Alkylphenone standard

1. Acetanilide
2. Acetophenone
3. Propiophenone
4. Butyrophenone
5. Benzophenone
6. Valerophenone
7. Hexanophenone
8. Heptanophenone
9. Octanophenone

Column temperature: 40°C

Eluent A: Water

Eluent B: Acetonitrile

Gradient: 0 min 55% B , from 55 to 100 % B in 0.8 min, from 0.9 to 2 min reequilibration with 55 % B

Flow rate: 1.1 ml/min,

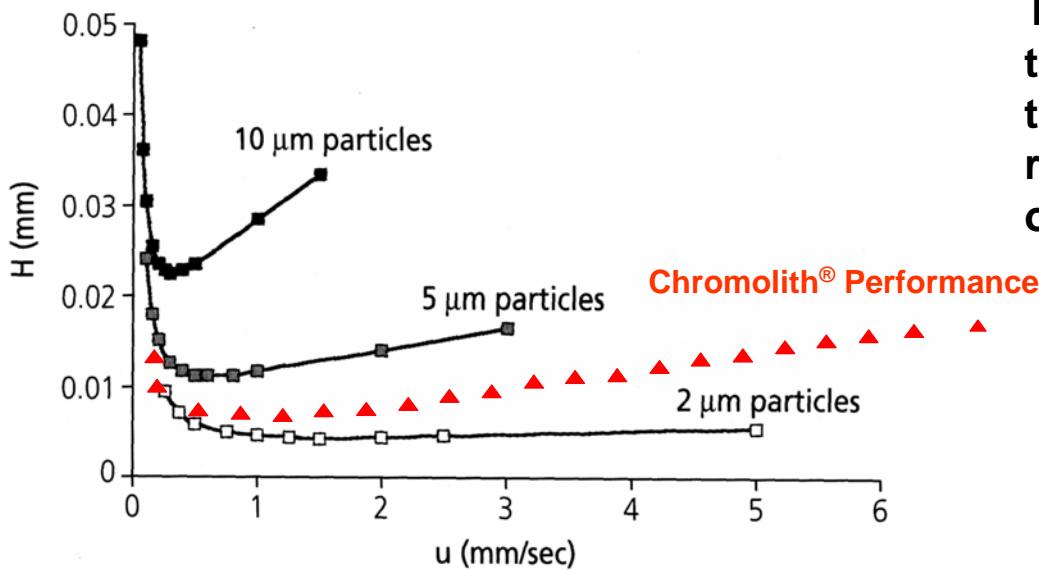
Pressure: 505 bar

UV: 247 nm

Injection volume: 1 µl

Fast Chromatography

Van Deemter Plot



H = Height Equivalent to a Theoretical Plate (HEPT)

u = Flow speed of the mobile phase

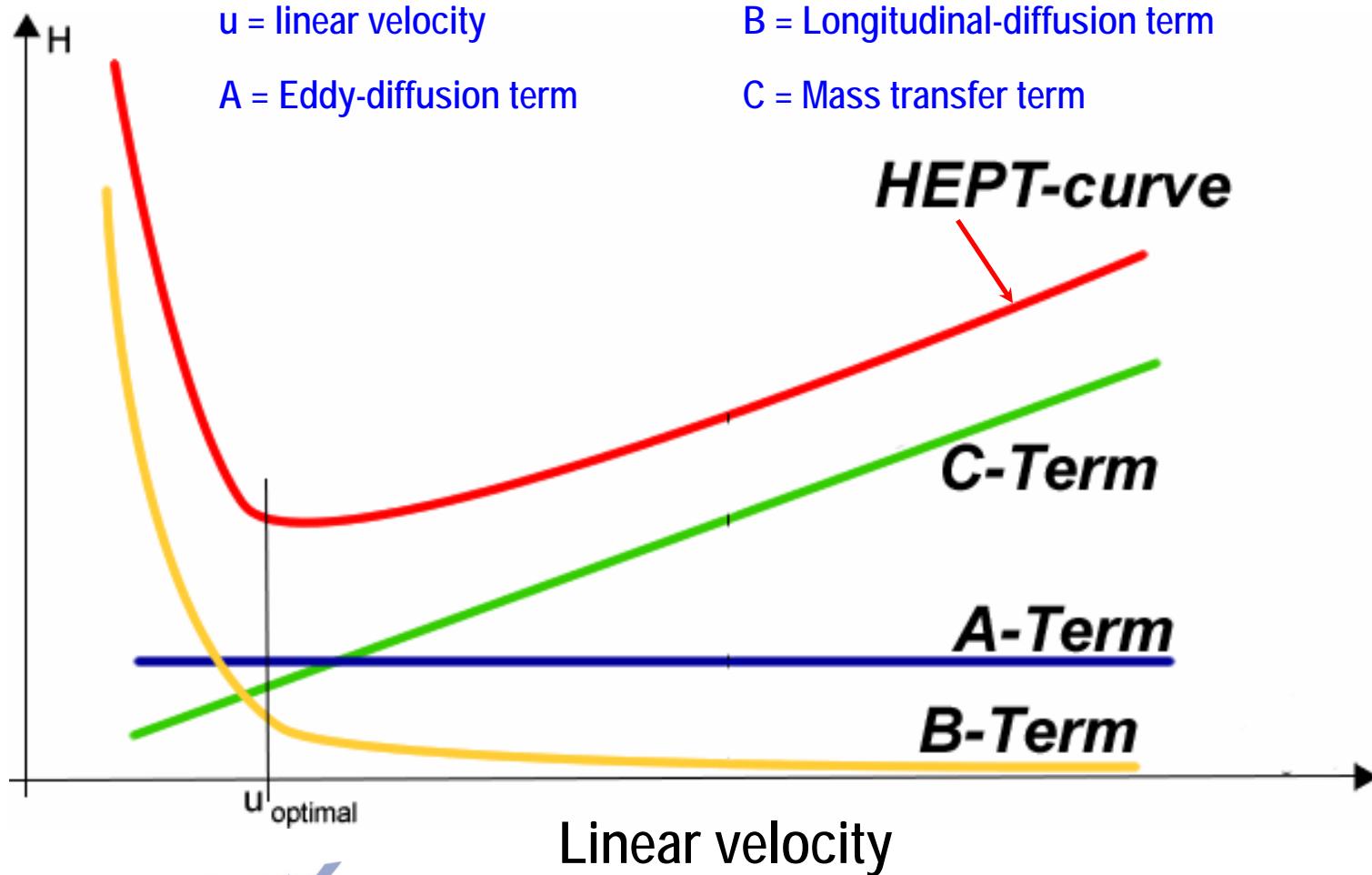
What shows this curve?

The smaller the particle diameter, the more it is possible to increase the flow rate while keeping the resolution performance (efficiency) of the column.



Van Deemter Curve

$$h = A + \frac{B}{u} + C \bullet u$$

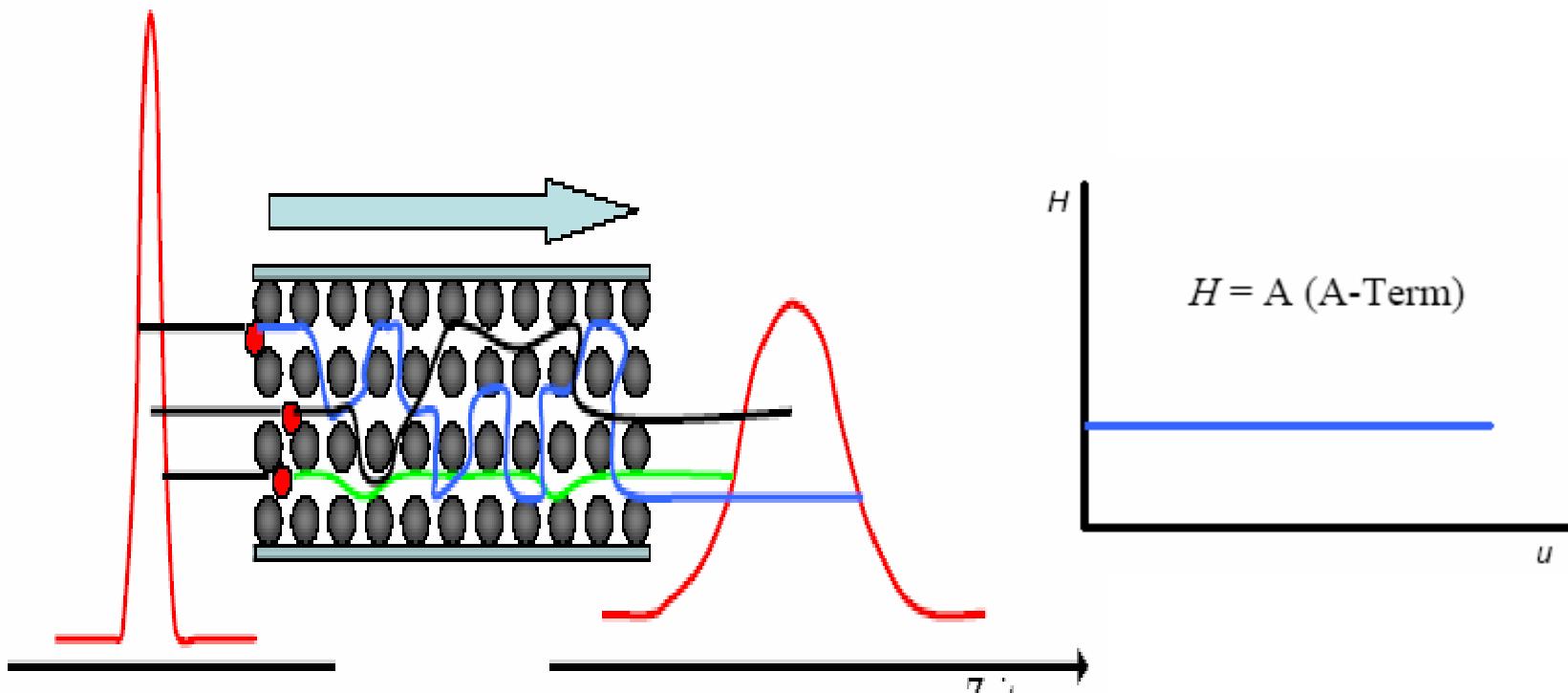


Contributions to the van Deemter Plot

Eddy-Diffusion term A :

$$A = 2 \cdot \lambda \cdot d_p$$

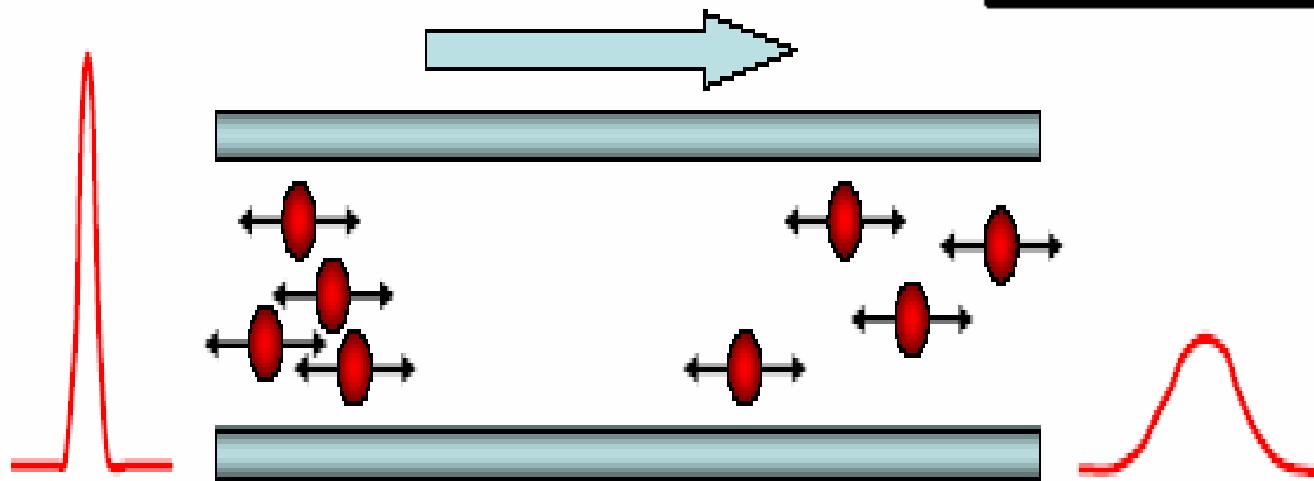
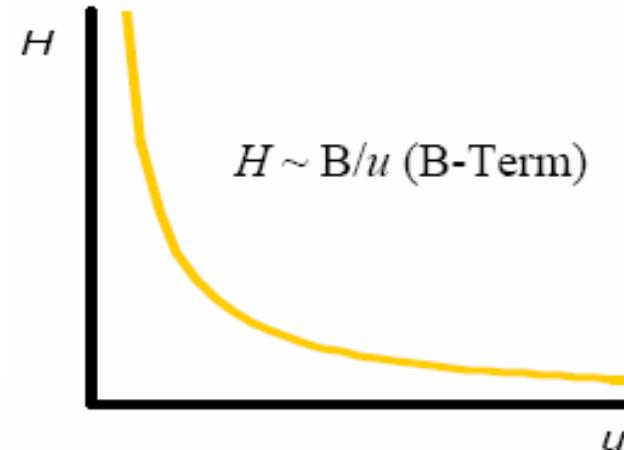
λ = factor of irregularities
 d_p = particle diameter



Contributions to the van Deemter Plot

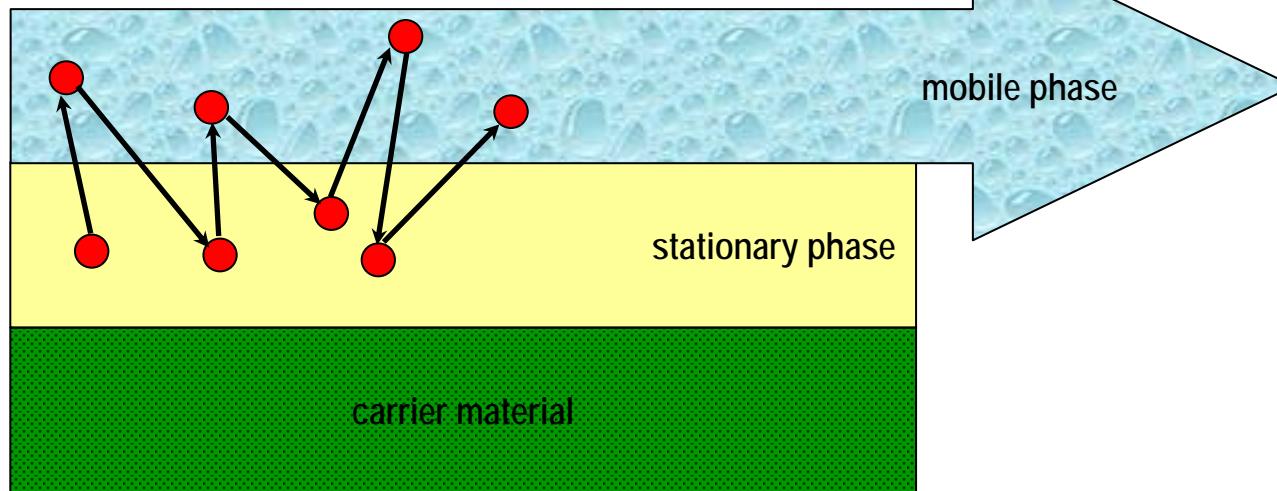
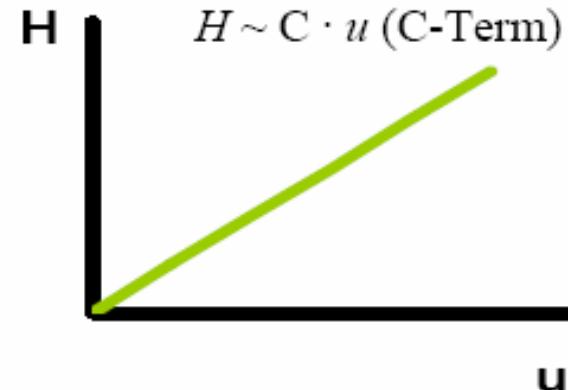
Longitudinal-Diffusion term B :

$$B = \gamma \frac{Dm}{u}$$



Contributions to the van Deemter Plot

Mass transfer term C





Ultra-Fast Chromatography

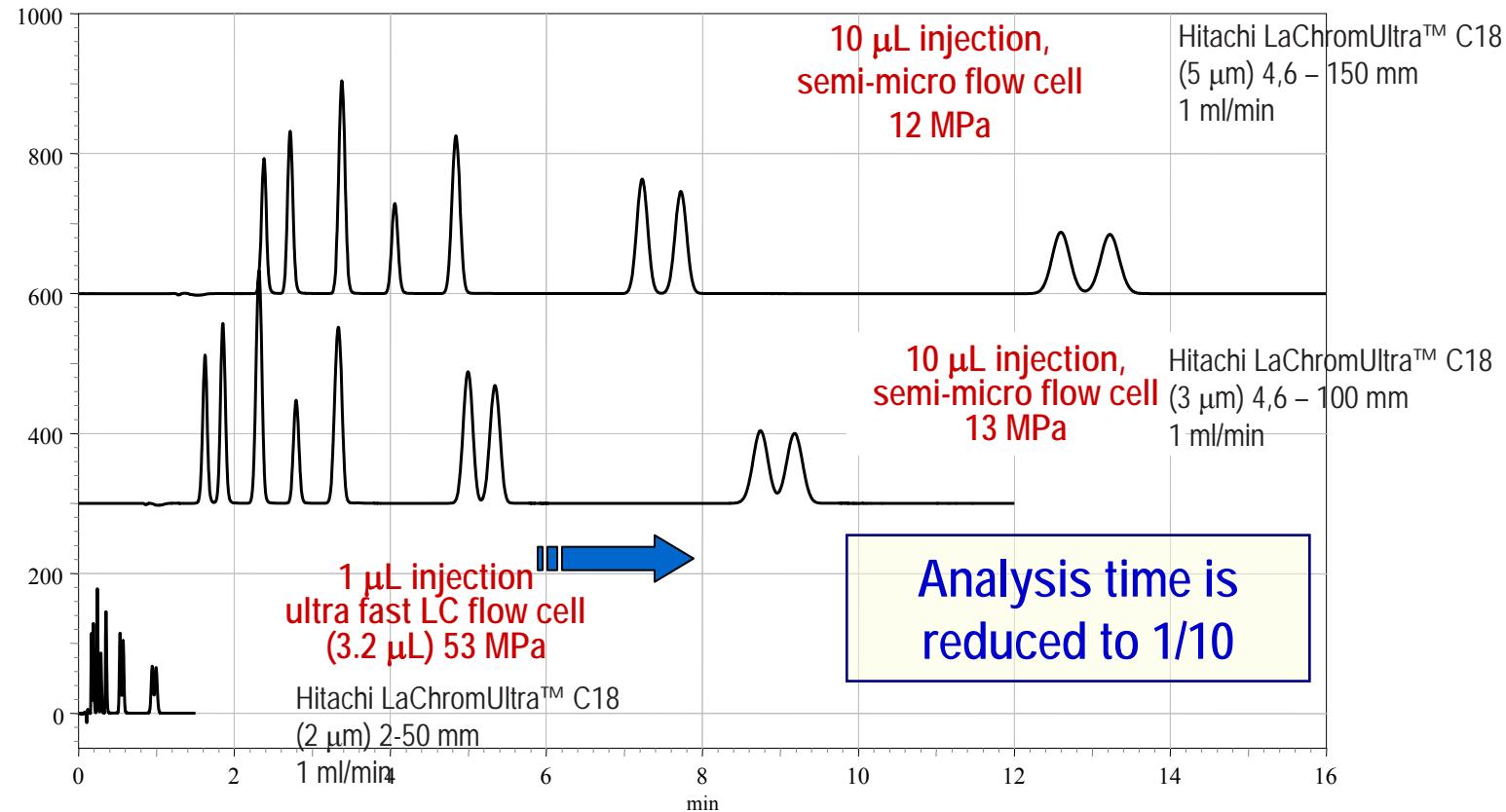
Application: Preservatives

Particle
size

5 μm

3 μm

2 μm





Requirements to the HPLC-System

Ultra-fast Chromatography with $\leq 2 \mu\text{m}$ Particles requires:

- High pressure resistance
- Minimised internal volumes (in order to avoid peak broadening)
- Precise and accurate gradient formation at high pressures
- Minimised sample carry-over
- Extremely fast detector reaction

The new VWR-Hitachi LaChrom Ultra HPLC System



Superior Resolution in shortest Time



Features and Benefits

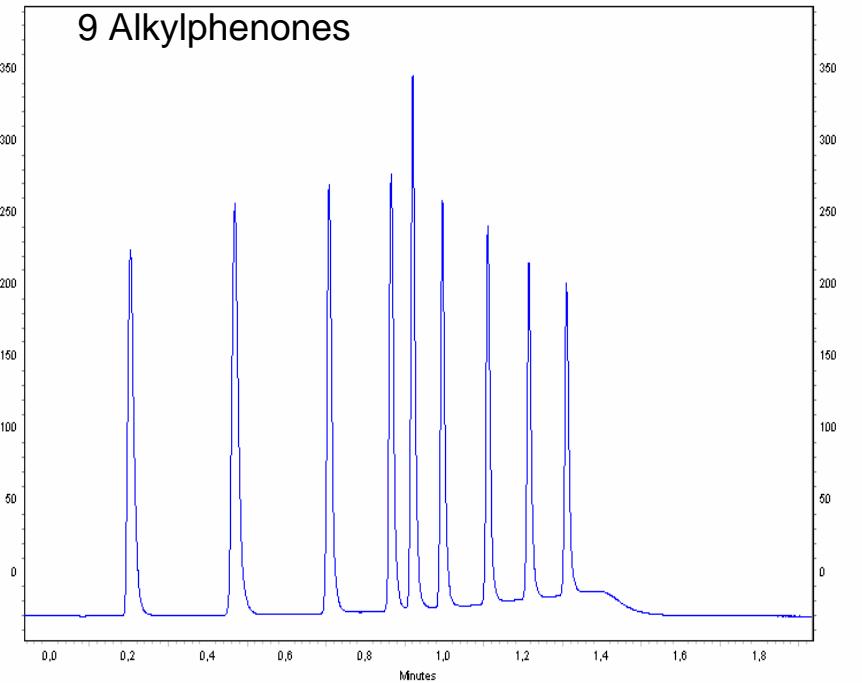
The System

- General purpose HPLC system with highest performance, which allows ultra fast LC with $\leq 2 \mu\text{m}$ particle columns, fast LC using Chromolith columns as well as all standard HPLC applications.
Max. pressure: 600 bar
Max. flow rate: 5 ml/min
- High-pressure gradient solvent delivery system with A-EPIC control function for elimination of pulsations and compensation of solvent compressibility and with extremely low gradient dwell volume to ensure sharpest gradient steps.
- Autosampler with further reduced carry-over (0.005%) and minimised internal volume
- High performance column ovens for temperature control and reduction of solvent viscosity
- Detectors (UV, DAD, FL) with fastest response time constants (10 ms) and shortest data collection intervals (10 ms corresponds to 100 Hz) for accurately recording shortest peaks.



Gradient Performance

Time: 1,9979 Minutes - Amplitude: -0,1925 mAU



Linear gradient 35% B to 95% B
in 1 minute

Re-equilibration in less than 1 minute



Retention Time + Injection Precision

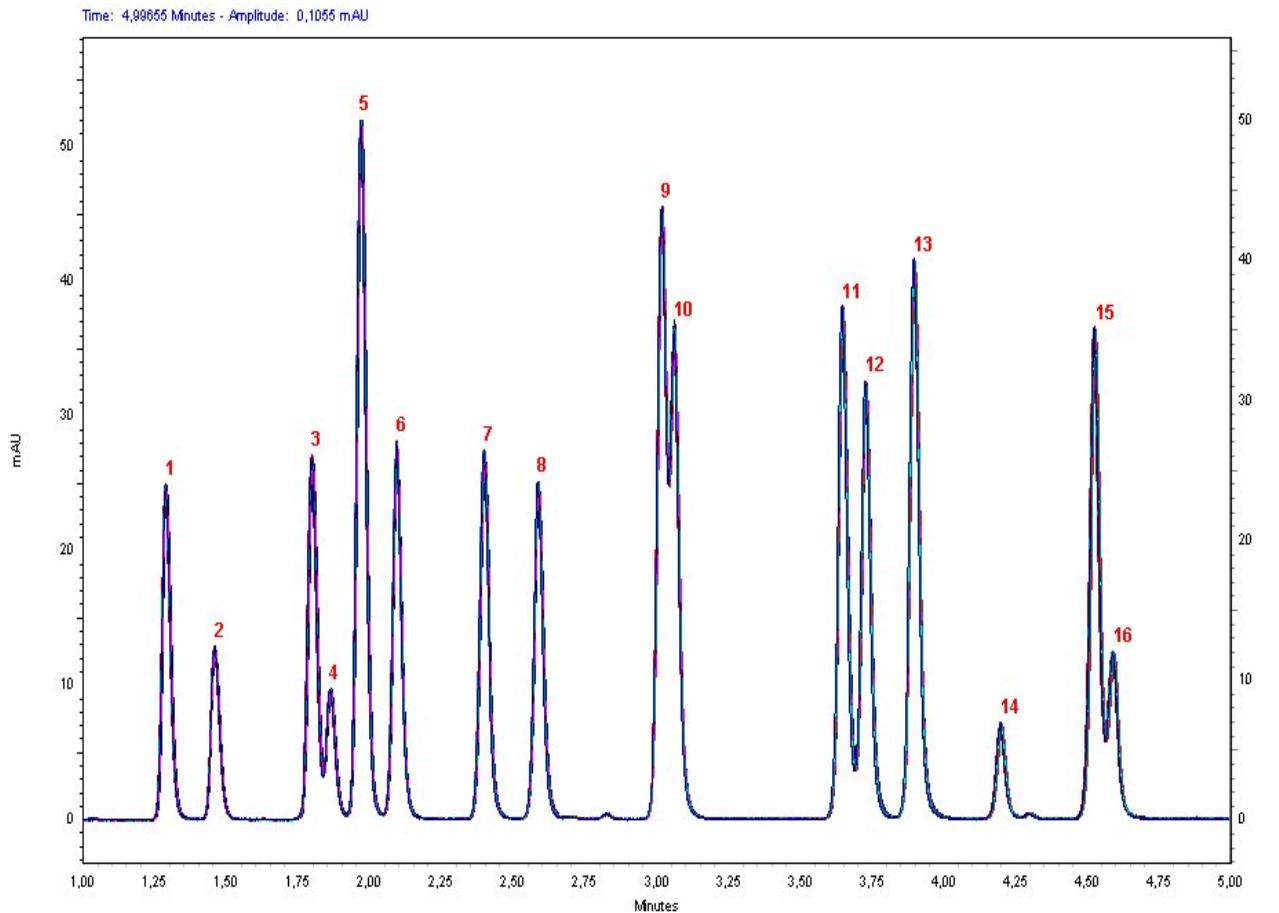
9 Alkyl phenones, inj.vol: 1 μL , n = 10 consecutive runs

Peak	Compound	RT (min)	RT RSD%	Area ($\mu\text{V}^*\text{s}$)	Area RSD%
1	Acetanilide	0.255	0.10	1,105,478	0.26
2	Acetophenone	0.520	0.09	1,366,703	0.41
3	Propiophenone	0.886	0.12	1,039,794	0.47
4	Butylophenone	1.048	0.13	912,533	0.30
5	Benzophenone	1.100	0.14	1,088,765	0.45
6	Valerophenone	1.160	0.13	806,033	0.22
7	Hexanophenone	1.264	0.12	760,224	0.44
8	Heptanophenone	1.365	0.08	711,477	0.62
9	Octanophenone	1.474	0.06	694,373	0.36

Result: The LaChromUltra system has a superior flow + gradient stability of the solvent delivery system + a high injection precision

Polyaromatic Hydrocarbons

Overlay of 2 series of 10 Standard Runs



Sample: NIST-16 PAH
Standard

- 1.Naphthalene, 2.Acenaphthylene,
- 3.Acenaphthene, 4.Fluorene,
- 5.Phenanthrene, 6.Anthracene,
- 7.Fluoranthene, 8.Pyrene,
- 9.Benz(a)anthracene, 10.Chrysene,
- 11.Benzo(b)fluoranthene, 12.
- Benzo(k)fluoranthene, 13.
- Benzo(a)pyrene,
- 14.Dibenz(a,h)anthracene,
- 15.Benzo(g,h,i,)perylene,
- 16.Indeno(1,2,3-cd)pyrene

Column: Agilent Zorbax Eclipse XDB C-18 (1.8 μ m), 100-2.1 mm

Column temperature: 30°C
Eluent A: LiChroSolv Water
Eluent B: LiChroSolv Acetonitrile

Gradient: from 70 to 97 % acetonitrile in 4.5 min
Flow rate: 0.5 ml/min,
injection volume: 2 μ l
UV: 254 nm

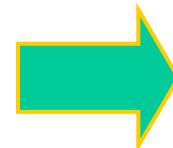
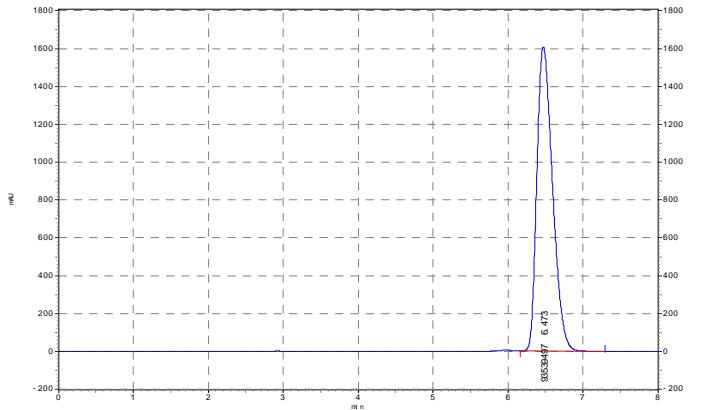


L-2200U Autosampler

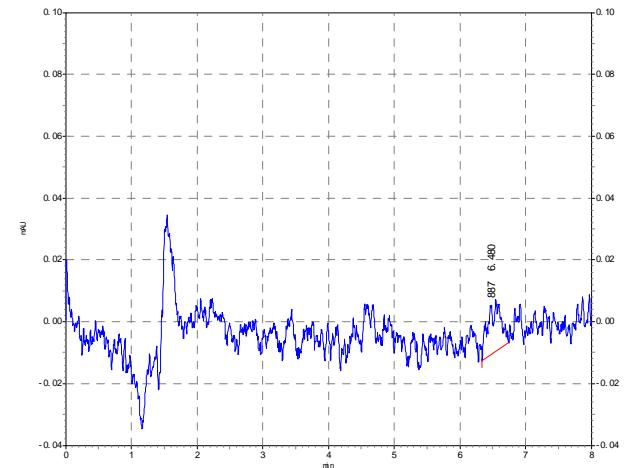
Sample carry-over

Sample data

Chlorhexidine 1000 ppm

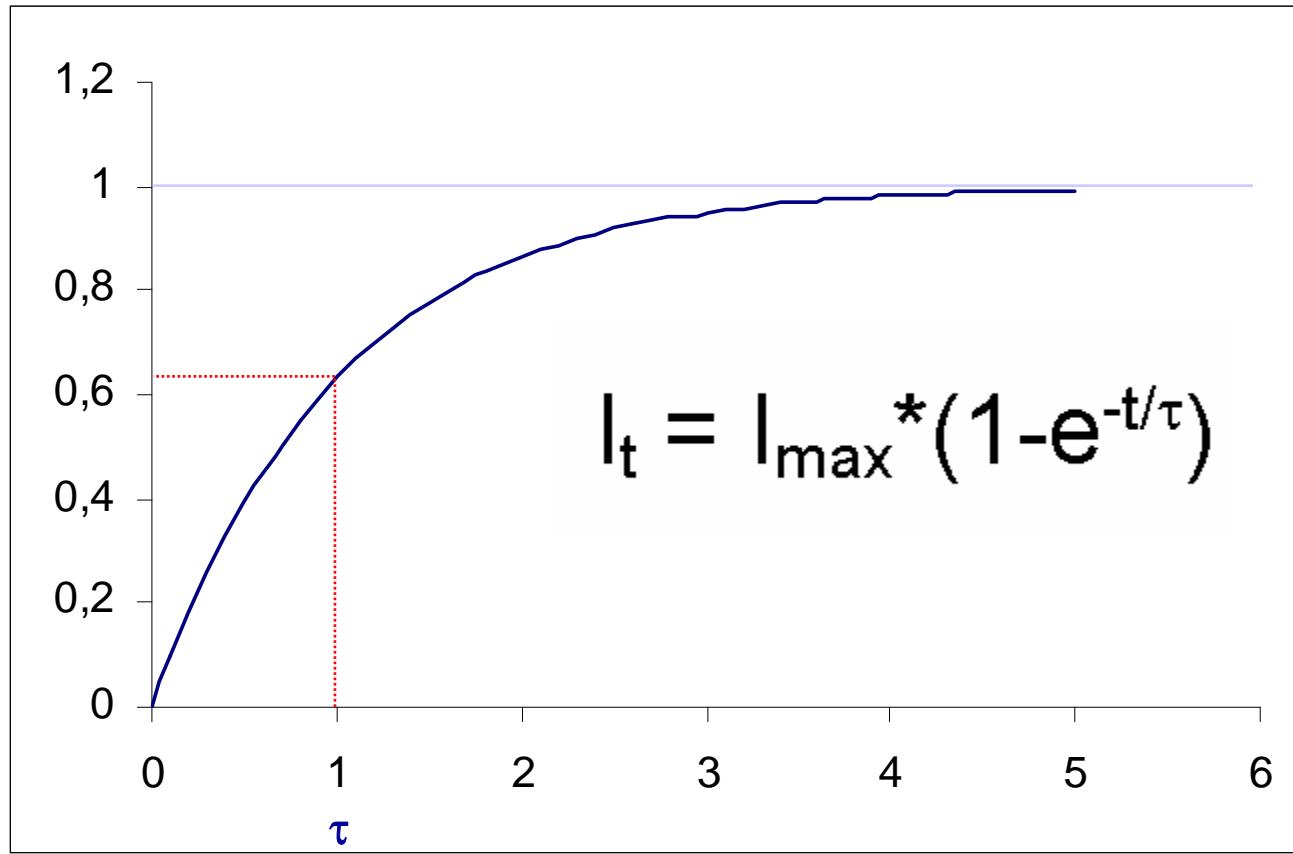


Blank data



Extremely low carry-over of
0.005% or less is realized

Response Time Constant

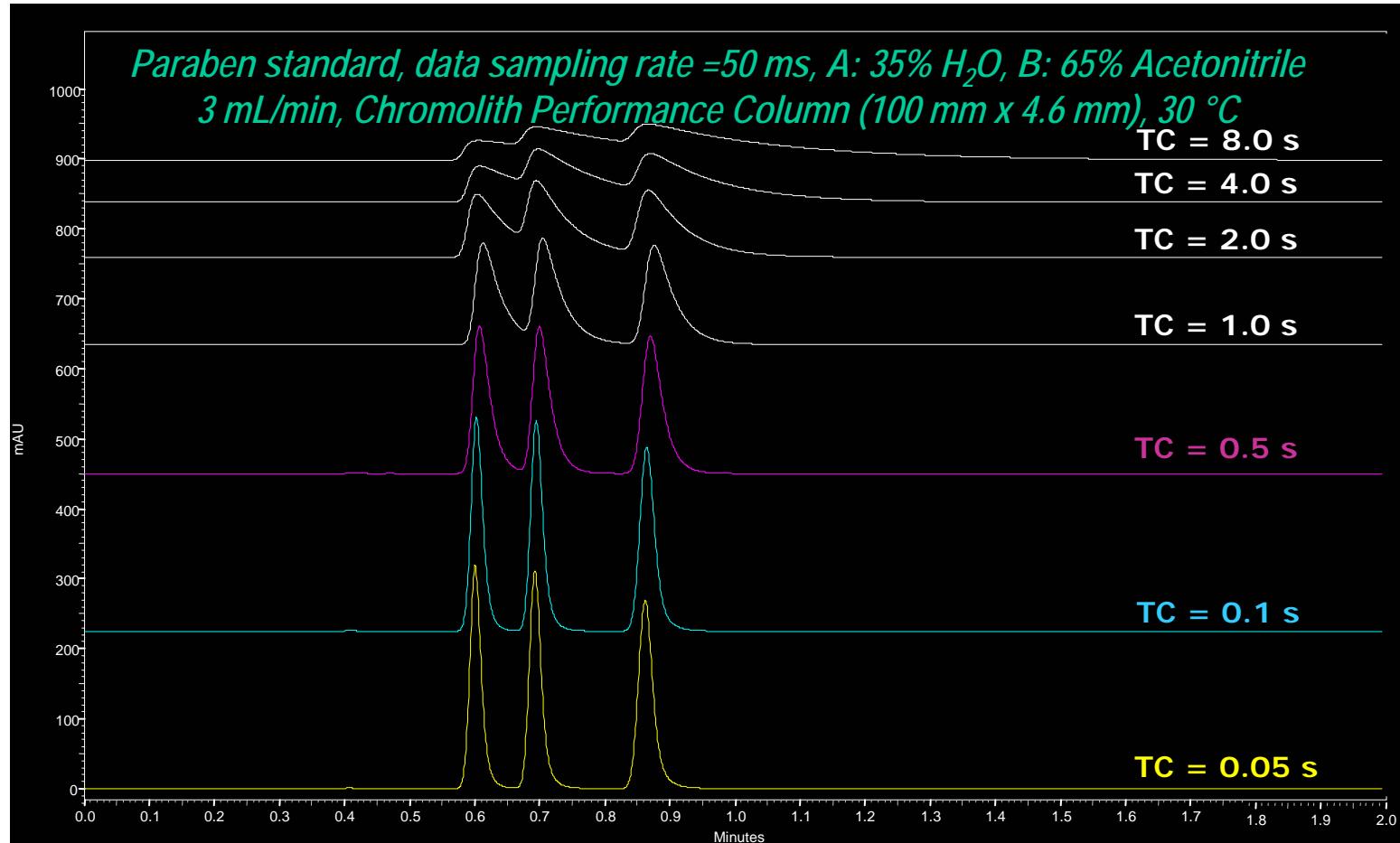


A detector reacts with a certain retardation on an incoming signal.

The response time constant τ is defined as the time where the detector outputs 63.2% ($= 1-e^{-1}$) of the real signal.

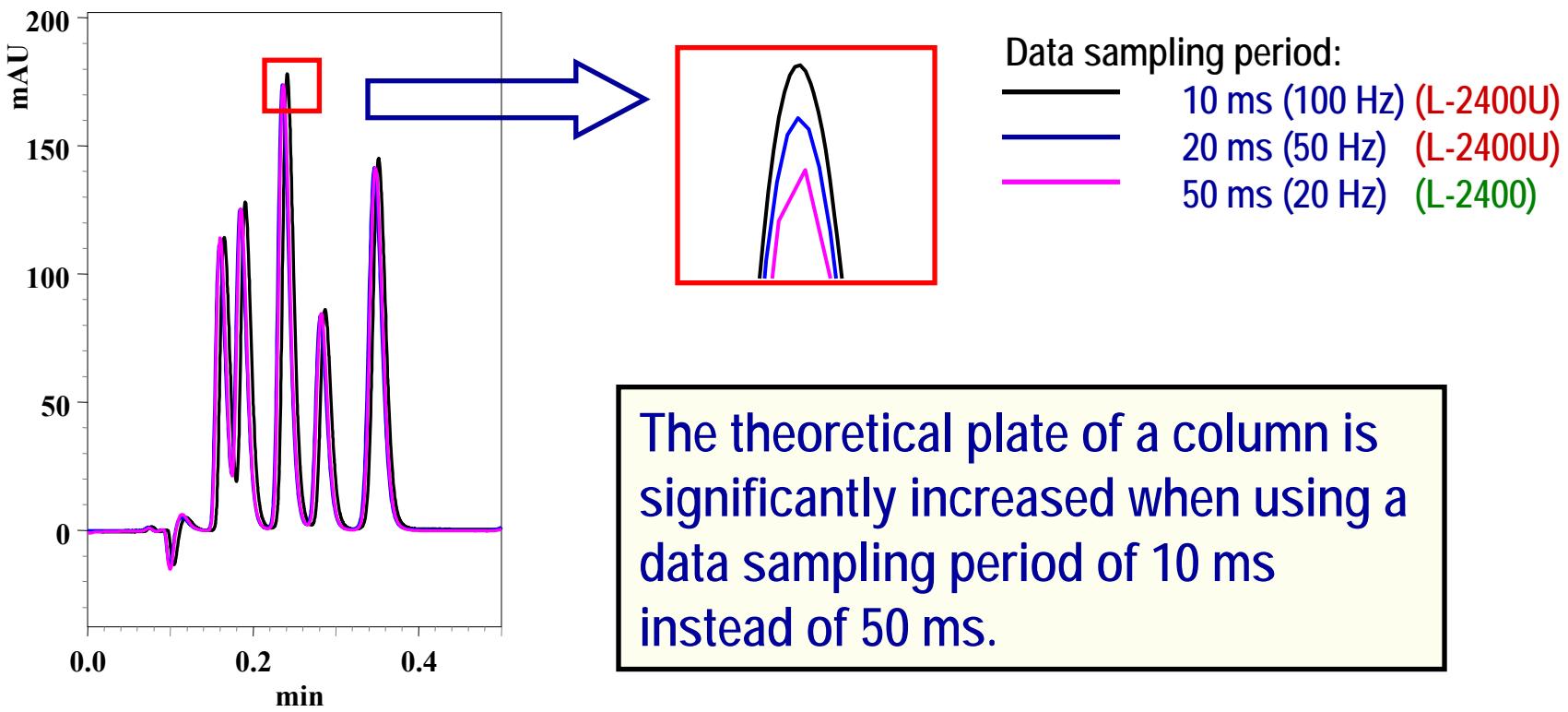
Modern detectors allow the selection of different response time constants.

Effect of different response times



Effect of different data sampling rates

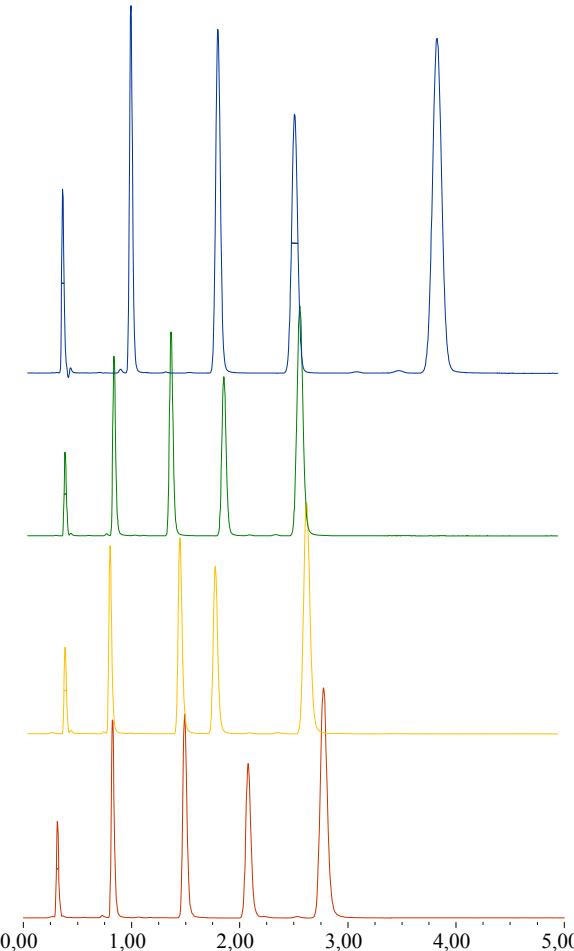
Only detectors with lowest response times and very high data sampling rates (low sampling periods) guarantee the best peak performance and separation.





Ultra-fast HPLC with particular columns

Which column to select?



Purospher STAR RP-18 endcapped, 2 μ m

174080 N/m

130 bar

Waters, 1.7 μ m

187620 N/m

225 bar

Restek, 1.9 μ m

172840 N/m

209 bar

Agilent, 1.8 μ m

173260 N/m

175 bar

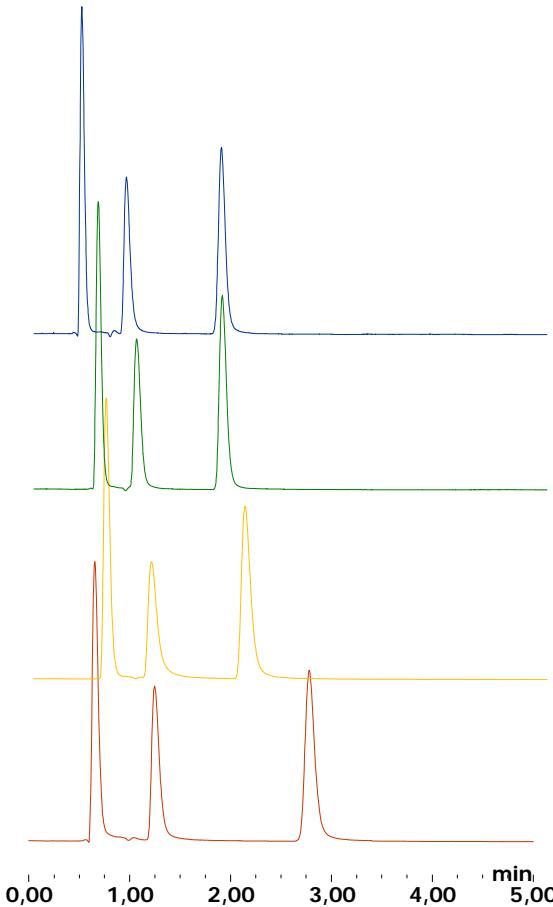
RP Test mixture

Mobile phase:	Acetonitril/Water 60/40
Detection:	UV 254nm Response fast
Temperature:	RT
Flow:	0,350 mL/min
Injection:	0,2 μ L
Sample:	Thioharnstoff 2,1 mg
	Biphenyl-2-ol 5,4 mg
	Progesteron 11,6 mg
	Hexanophenon 12,3 mg
	Anthracen 1,8 mg



Ultra-fast HPLC with particular columns

Which column to select?



Procainamide

Purospher STAR RP-18 endcapped, 2 μ m

Waters, 1.7 μ m

Restek, 1.9 μ m

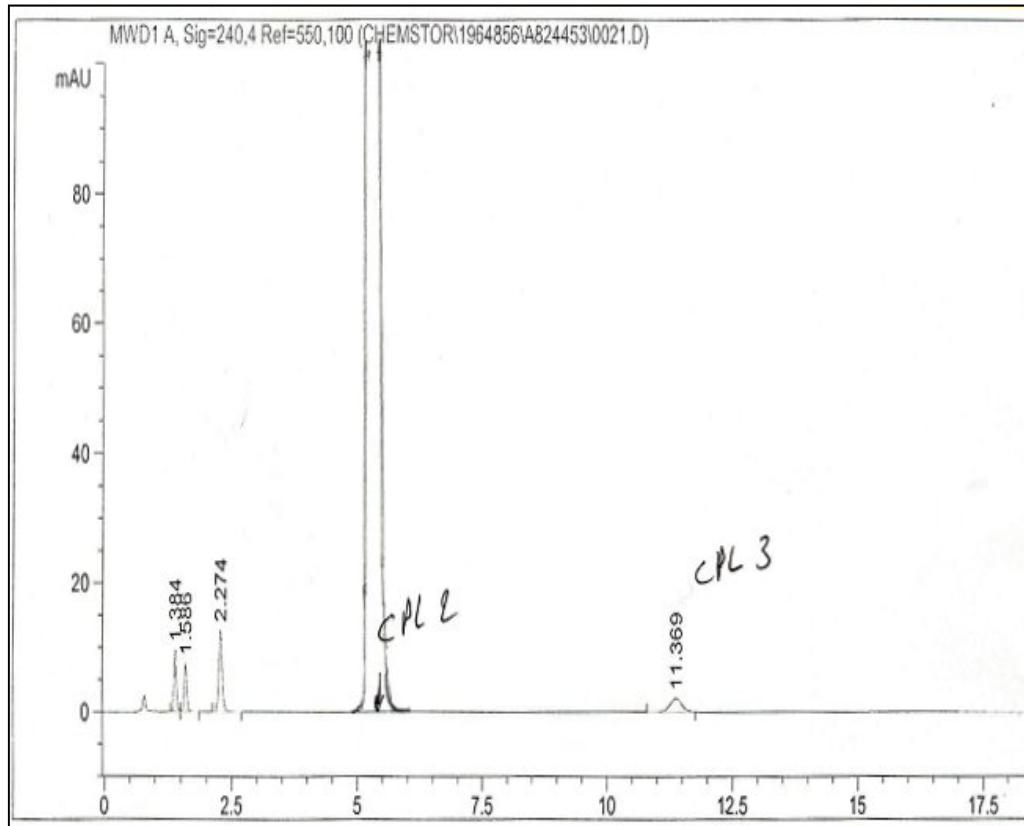
Agilent, 1.8 μ m

Mobile phase: Methanol/0,02M NaH₂PO₄ pH 7,6 30/70 v/v
Detection: UV254nm ,Res.0.1s
Temperature: RT
Flow: 0,350 mL/min
Injection: 0,2 μ L
Sample: Uracil, Procainamide, N-Acetyl-Procainamide



Customer Application

Pharmaceutical sample



Column: Xbridge shield RP-18
(2.5 μ m), 50-4.6

Eluent:

A: Phosphate buffer (1.77 g KH_2PO_4) + 3.08 g Hexan-sulfonic acid Na-salt per 1000 ml, adjusted with 85 % phosphoric acid to pH 2.5

B: LiChroSolv Acetonitrile 37%

Isocratic Run with 63 % A and 37 % B

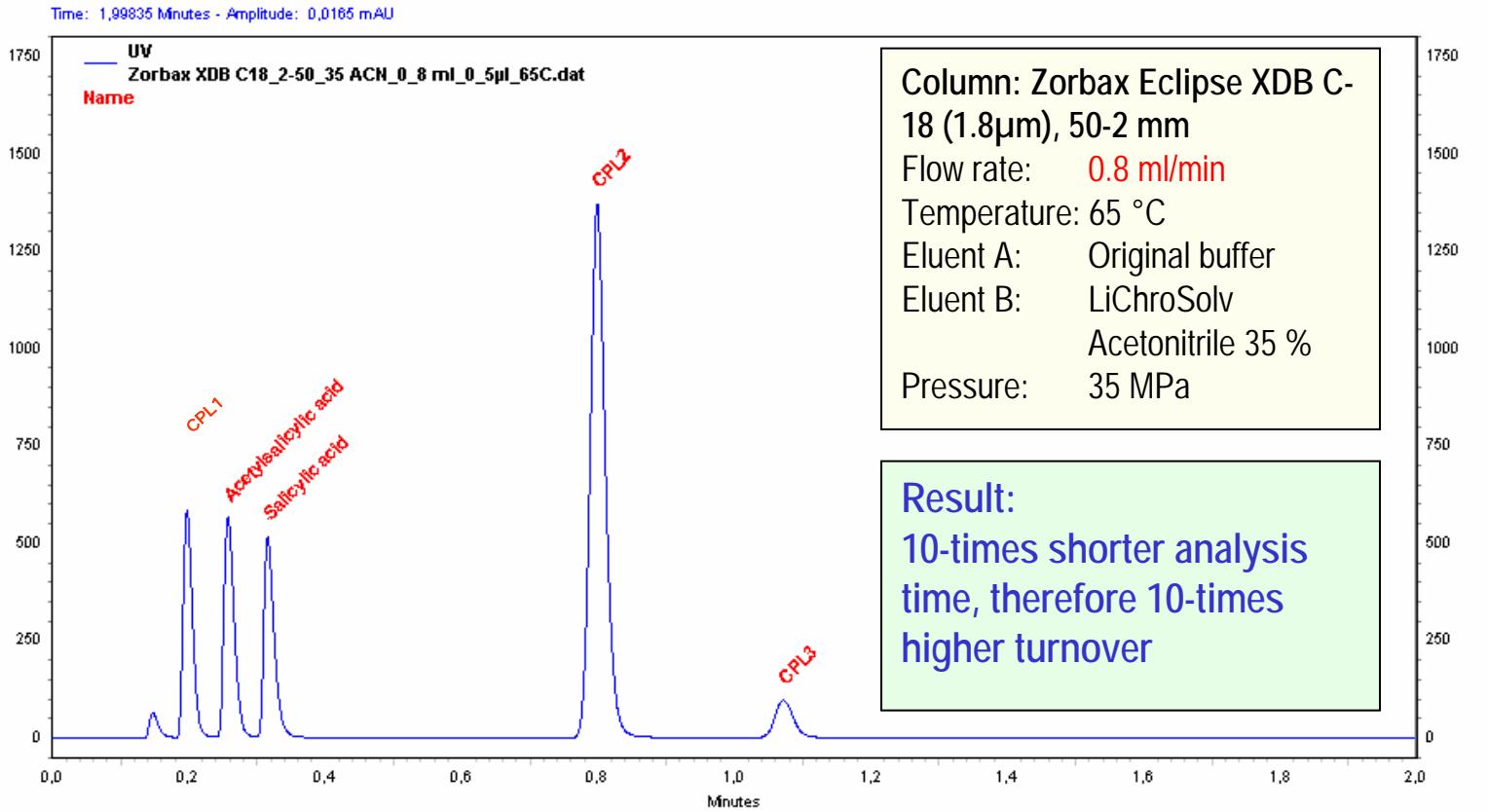
Flow rate: 1.0 ml/min

Temperature: 30 °C

Analysis time: 15 min

Customer Application

Pharmaceutical sample: Optimised



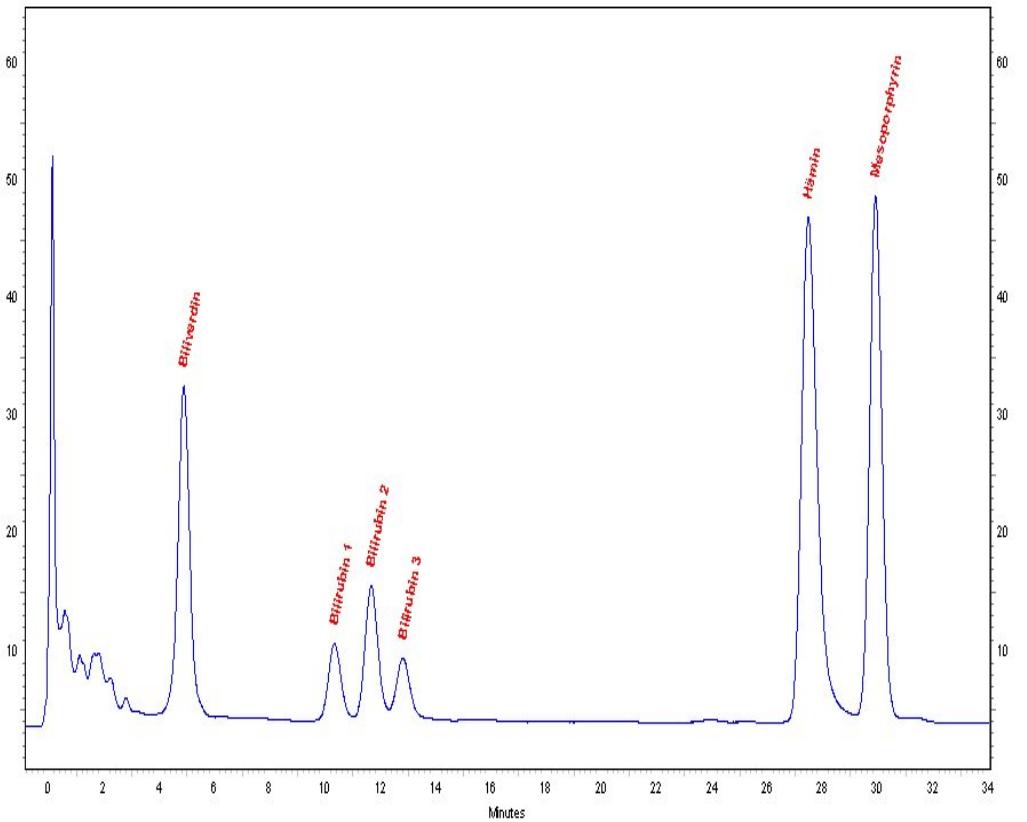
Method transfer to the Zorbax Eclipse column, 1,8 μ m, 50-2 mm



Customer Application

Hemins & Porphyrins Original

Time: 34.9703 Minutes - Amplitude: 0.2665 mAU

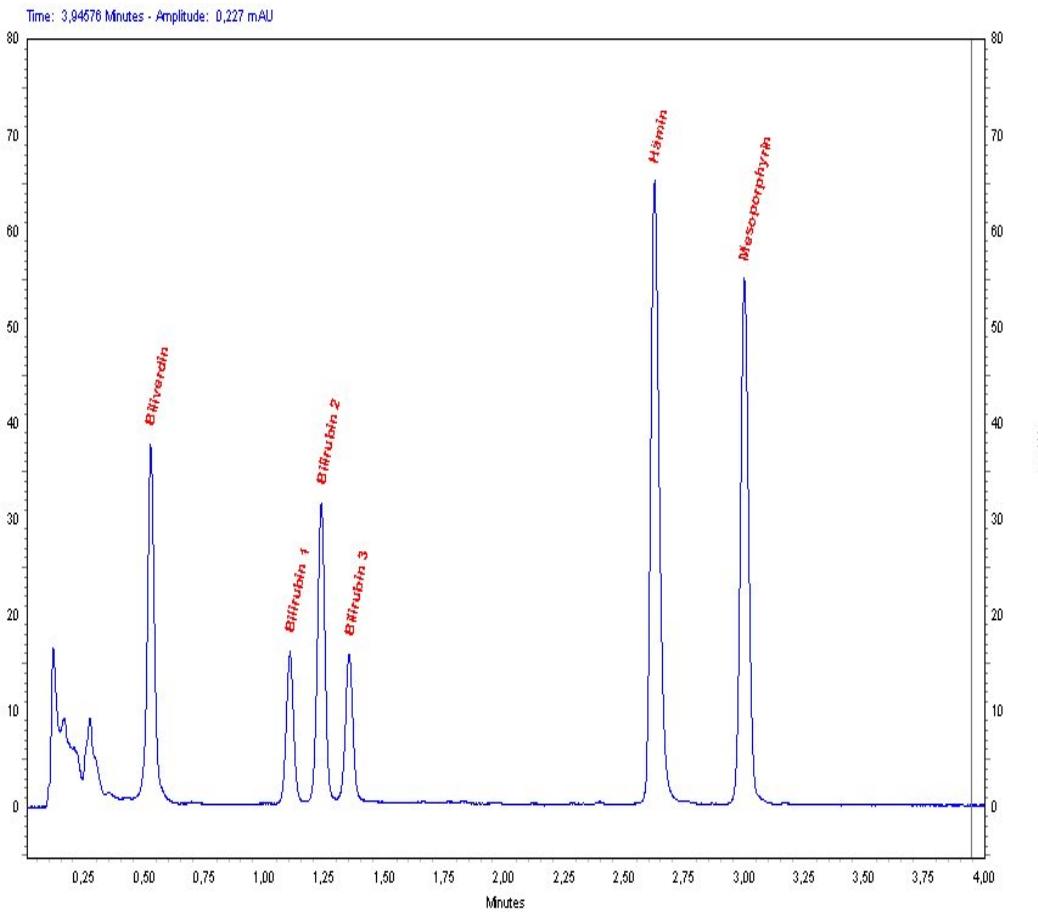


Column: Purospher, C-18 (3 μ m),
125-3 mm
Flow rate: 0.6 ml/min
Temperature: 40 °C
Eluent A: Ammonium acetate,
0,1 M, pH 5, 40%
Eluent B: LiChroSolv
Methanol 40 %
Gradient: to 100% Methanol
in 32 min
Run time: 30 min



Customer Application

Hemins & Porphyrins Optimised



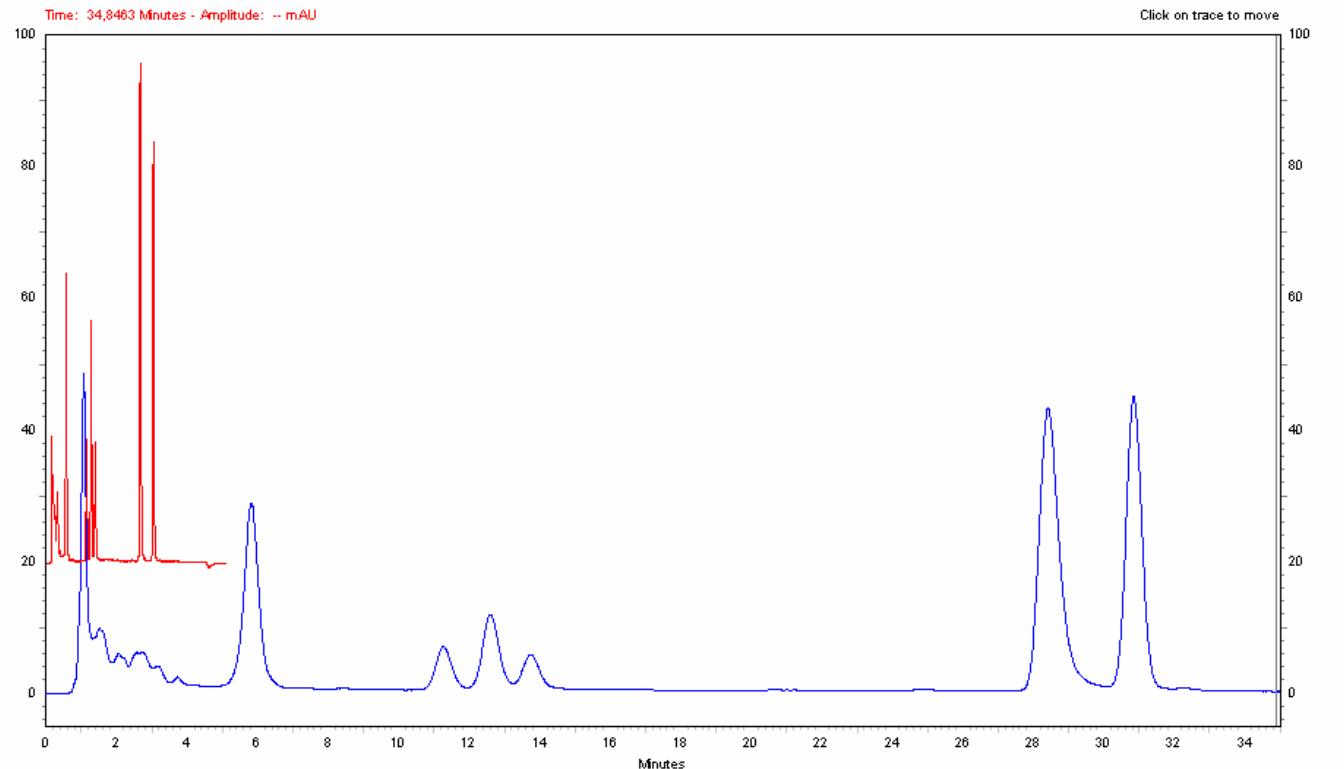
Column: Zorbax, C-18 (1,8 µm), 30-2 mm
Flow rate: 0.7 ml/min
Temperature: 40 °C
Eluent A: Ammonium acetate, 0,1 M, pH 5, 40%
Eluent B: LiChroSolv Methanol 40 %
Gradient: to 100% Methanol in 3,2 min
Run time: 3 min

Result:
10-time shorter run time, therefore about 10-times higher turnover possible



Customer Application

Hemins & Porphyrins Optimised



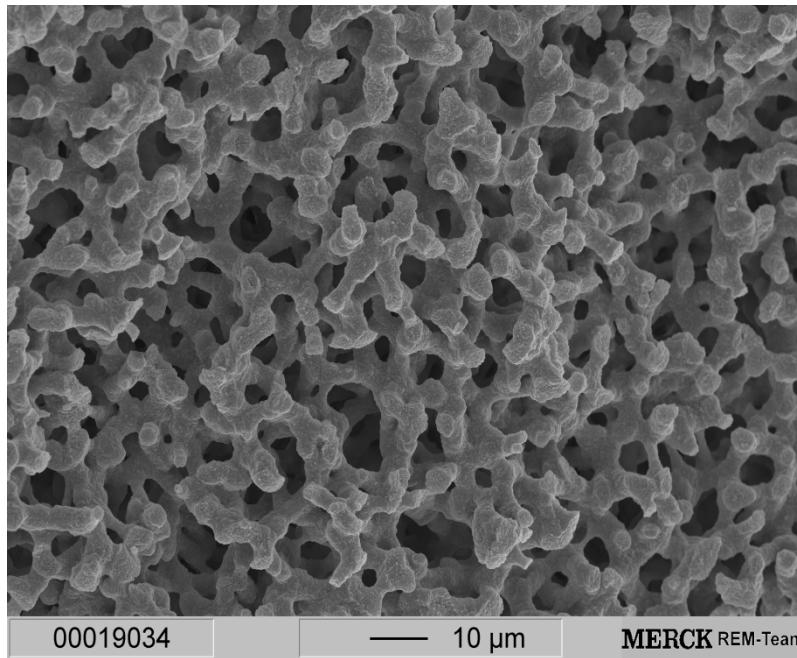
Result:

10-time shorter run time, therefore
10-times higher turnover possible

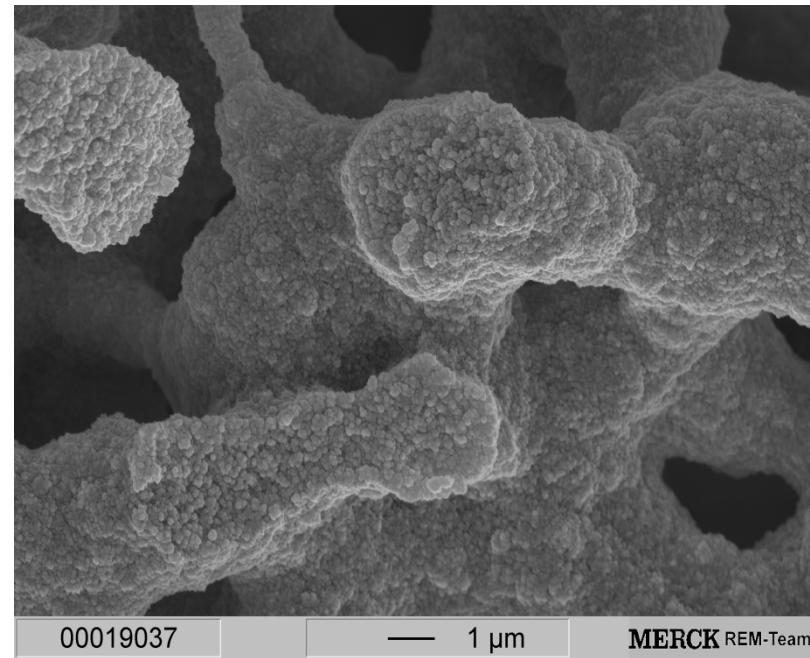


Chromolith Bimodal Pore Structure

SEM of a cross section of a monolithic silica rod



Macropores: 2 μm



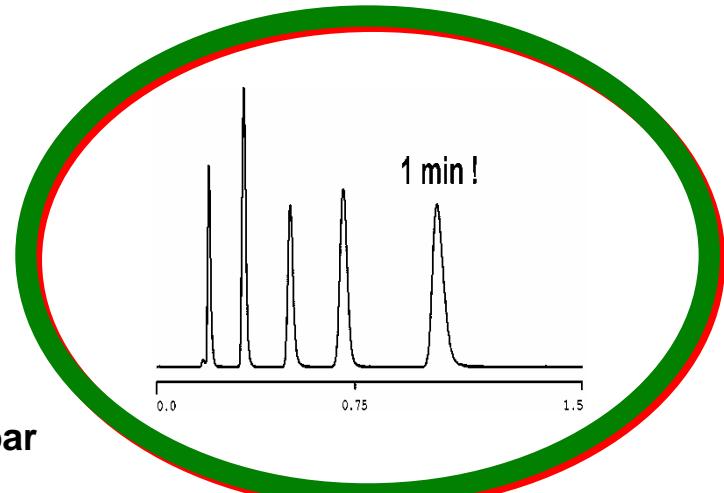
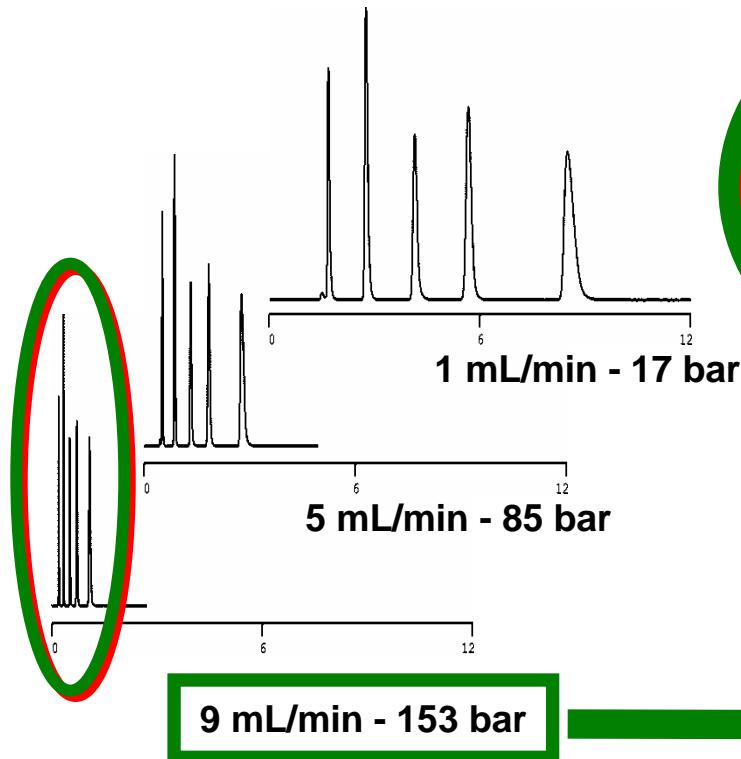
Mesopores: 130 Å

Total porosity 81%



Fast Chromatography

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

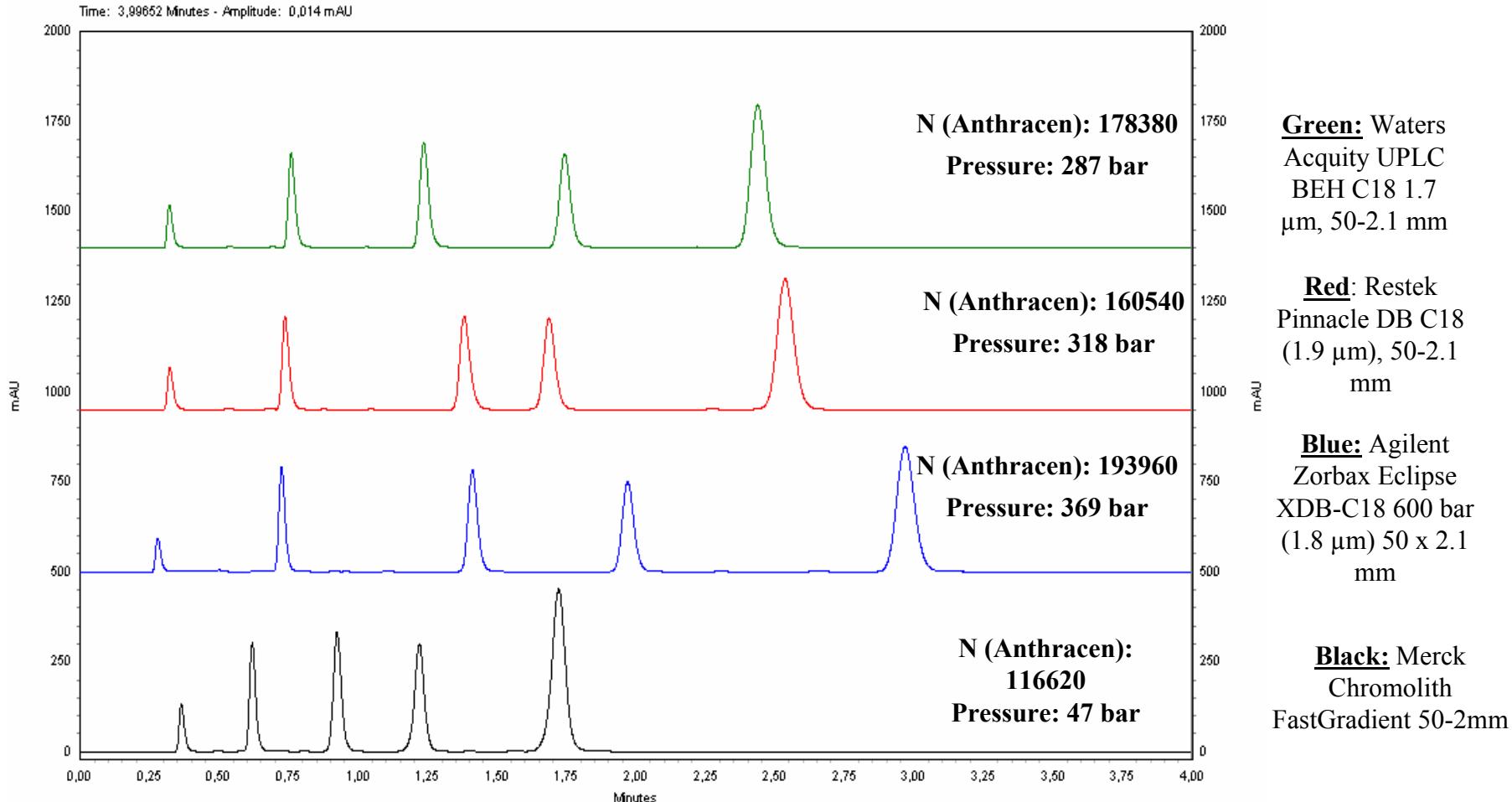


9 mL/min - 153 bar



Chromolith® HPLC Columns

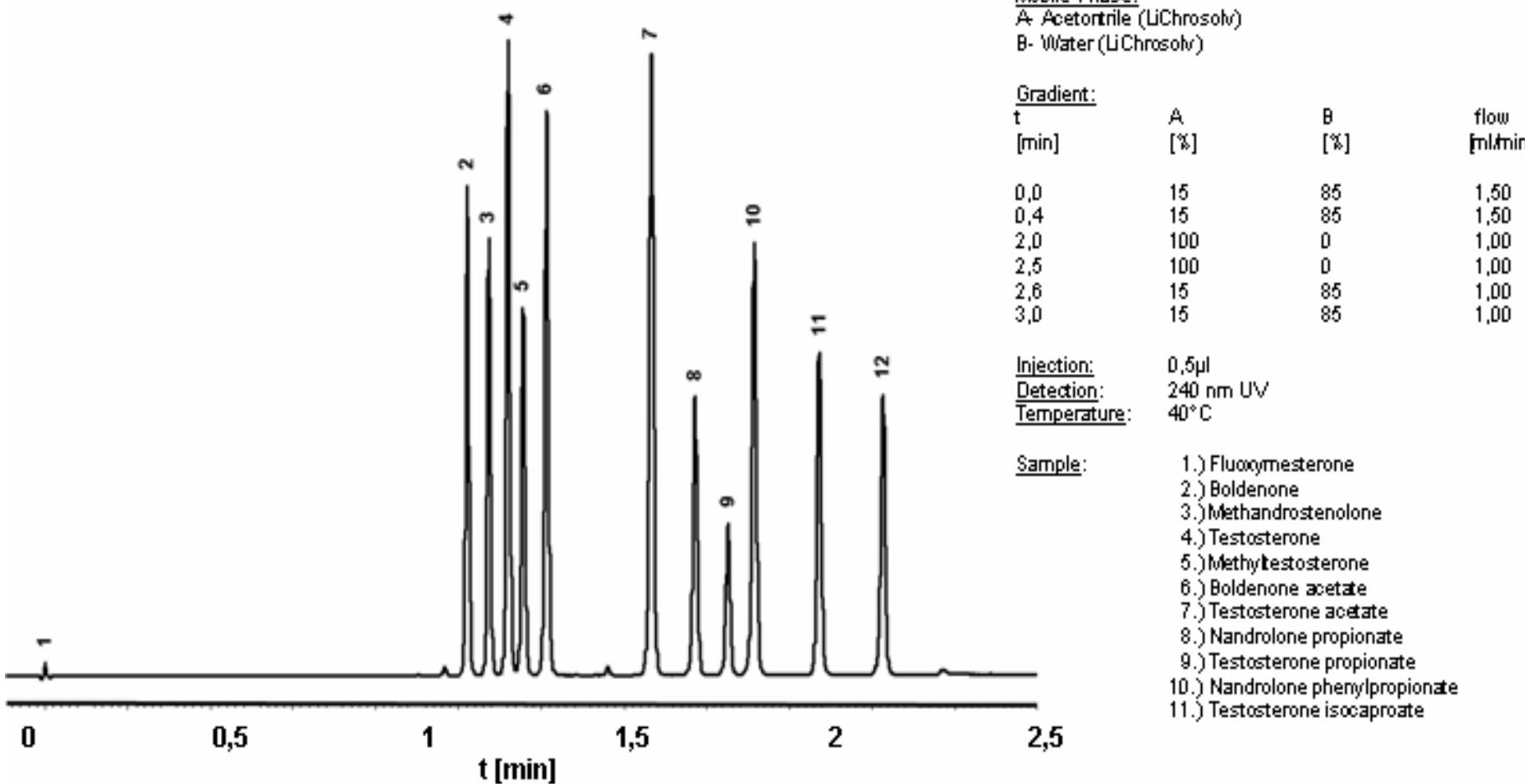
Comparison to sub 2µm particulate columns





Chromolith® FastGradient RP-18e

50 – 2mm



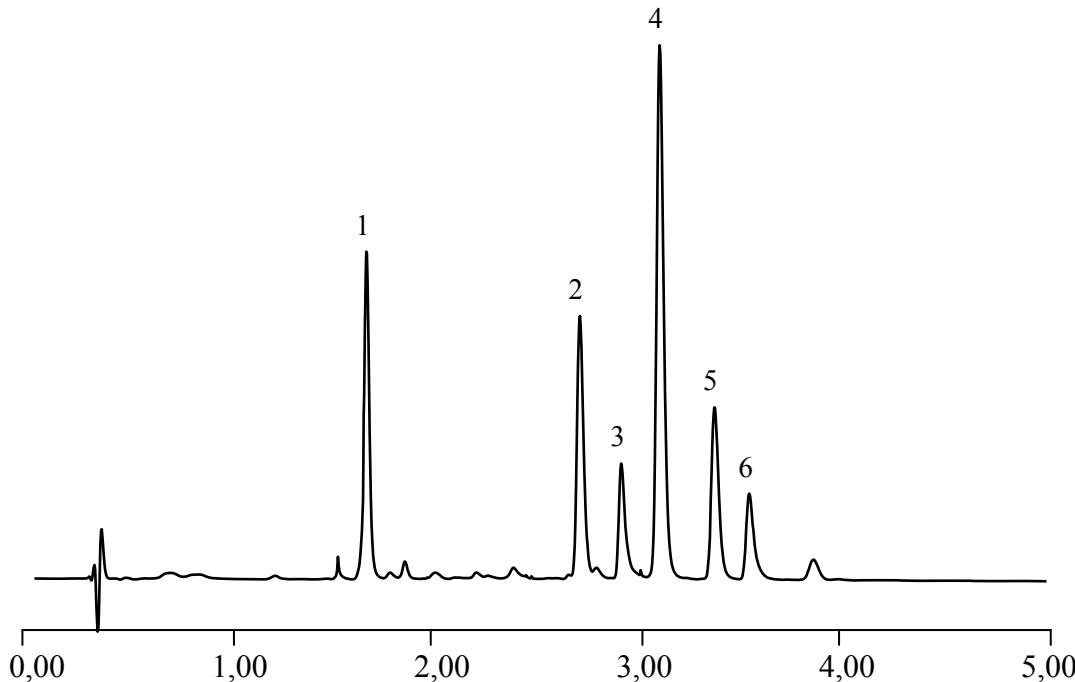


Chromolith® FastGradient RP-18e

50 – 2mm

Separation of Bio-Flavonoids

on a Micro or Ultra HPLC system



Mobile Phase:

A-0,1% TFA in H₂O
B- MeOH

Gradient:

t [min]	A [%]	B [%]	flow [ml/min]
0,0	85	15	0,50
2,5	50	50	0,50
5,0	50	50	0,50
5,1	85	15	0,50
8,5	85	15	0,50

Injection: 0,5µL

Detection: 220 nm UV

Temperature: ambient

Sample:

- 1.) Isoquercetin
- 2.) Troxerutin
- 3.) Naringin
- 4.) Morin
- 5.) Quercetin
- 6.) Trihydroxyethyluteolin



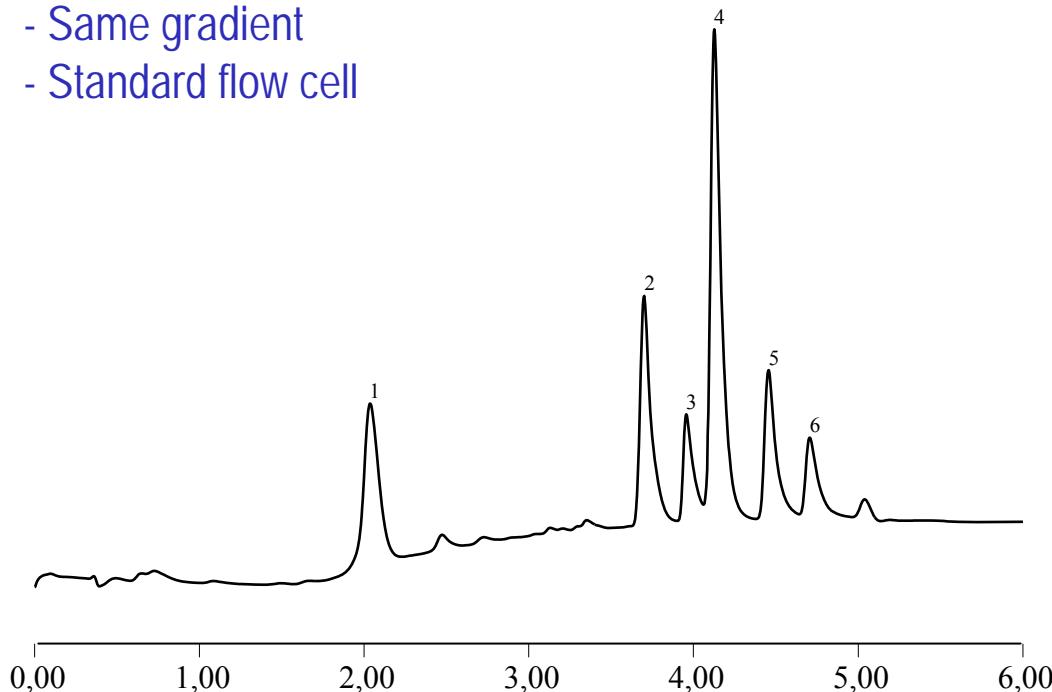
Chromolith® FastGradient RP-18e

50 – 2mm

Separation of Bio-Flavonoids

on a Conventional HPLC system

- Same gradient
- Standard flow cell



Mobile Phase:

A-0,1% TFA in H₂O
B- MeOH

Gradient:

t [min]	A [%]	B [%]	flow [ml/min]
0,0	85	15	0,50
2,5	50	50	0,50
5,0	50	50	0,50
5,1	85	15	0,50
8,5	85	15	0,50

Injection: 0,5µL

Detection: 220 nm UV

Temperature: ambient

Sample: 1.) Isoquercetin

2.) Troxerutin

3.) Naringin

4.) Morin

5.) Quercetin

6.) Trihydroxyethyluteolin



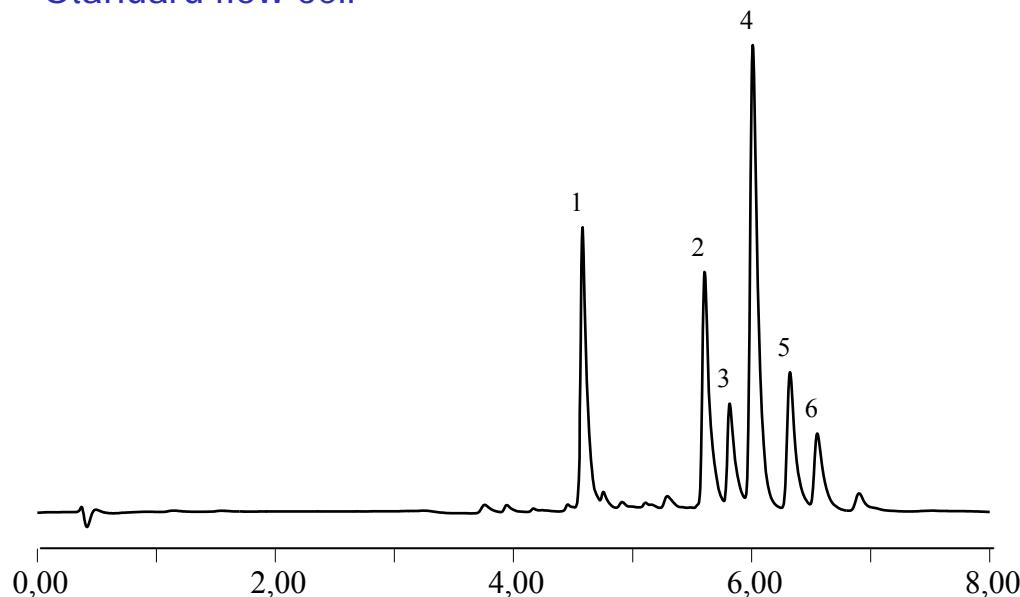
Chromolith® FastGradient RP-18e

50 – 2mm

Separation of Bio-Flavonoids

on a Conventional HPLC system

- Optimised gradient (due to different system dwell volume)
- Standard flow cell



Mobile Phase:

A-0,1% TFA in H₂O
B- MeOH

Gradient:

t [min]	A [%]	B [%]	flow [ml/min]
0,0	95	5	0,50
1,8	95	5	0,50
4,3	50	50	0,50
6,8	50	50	0,50
6,9	95	5	0,50
10	95	5	0,50

Injection: 0,5µL

Detection: 220 nm UV

Temperature: ambient

Sample: 1.) Isoquercetin

2.) Troxerutin

3.) Naringin

4.) Morin

5.) Quercetin

6.) Trihydroxyethyluteolin



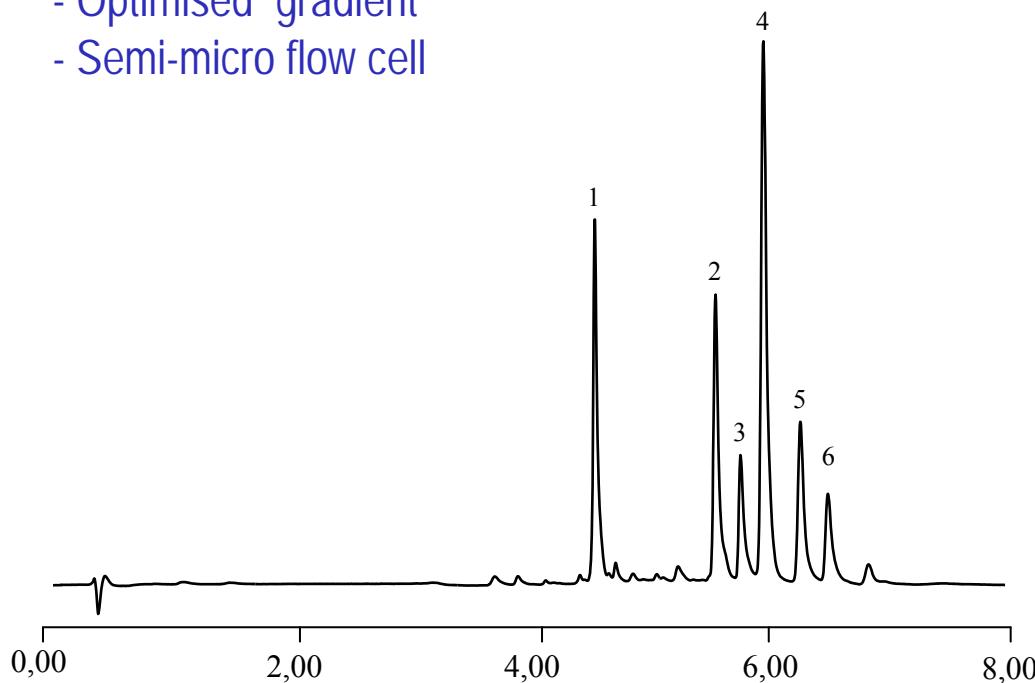
Chromolith® FastGradient RP-18e

50 – 2mm

Separation of Bio-Flavonoids

on a Conventional HPLC system

- Optimised gradient
- Semi-micro flow cell



Mobile Phase:

A-0,1% TFA in H₂O
B- MeOH

Gradient:

t [min]	A [%]	B [%]	flow [ml/min]
0,0	95	5	0,50
1,8	95	5	0,50
4,3	50	50	0,50
6,8	50	50	0,50
6,9	95	5	0,50
10	95	5	0,50

Injection: 0,5µL

Detection: 220 nm UV

Temperature: ambient

Sample: 1.) Isoquercetin

2.) Troxerutin

3.) Naringin

4.) Morin

5.) Quercetin

6.) Trihydroxyethyluteolin



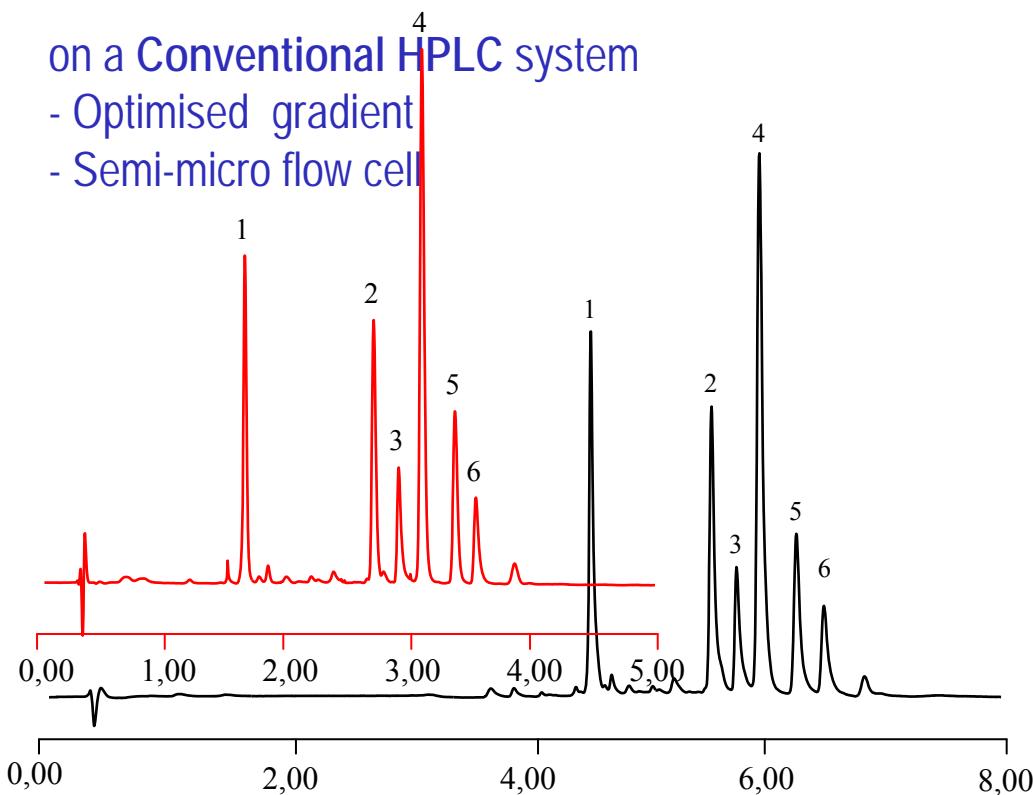
Chromolith® FastGradient RP-18e

50 – 2mm

Separation of Bio-Flavonoids

on a Conventional HPLC system

- Optimised gradient
- Semi-micro flow cell



Mobile Phase:

A-0,1% TFA in H₂O

B- MeOH

Gradient:

t [min]	A [%]	B [%]	flow [ml/min]
0,0	95	5	0,50
1,8	95	5	0,50
4,3	50	50	0,50
6,8	50	50	0,50
6,9	95	5	0,50
10	95	5	0,50

Injection: 0,5µL

Detection: 220 nm UV

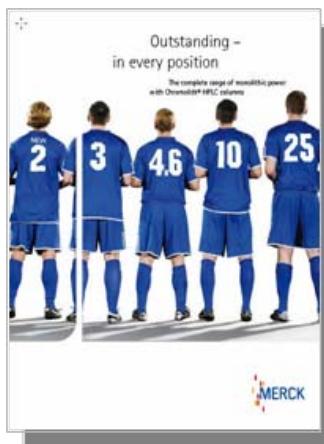
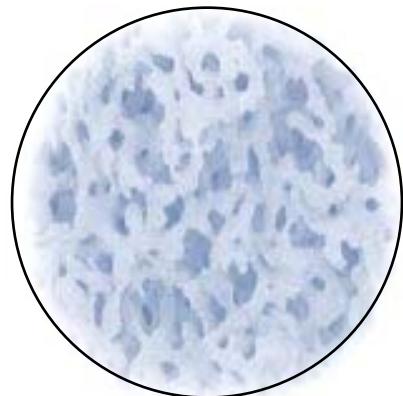
Temperature: ambient

Sample:

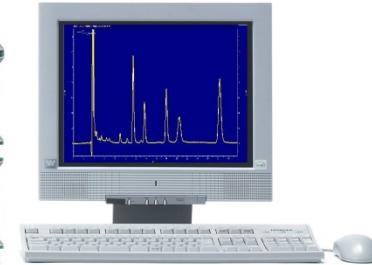
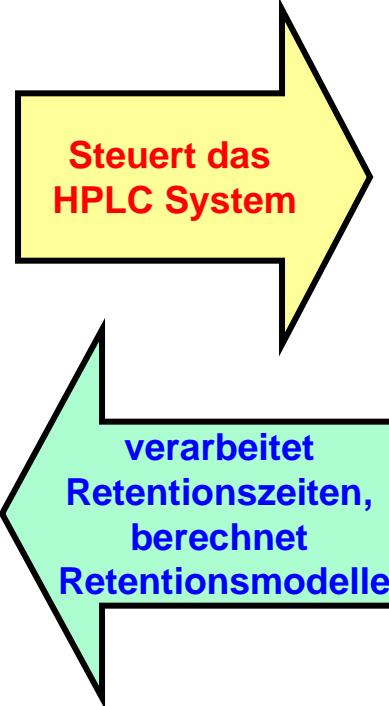
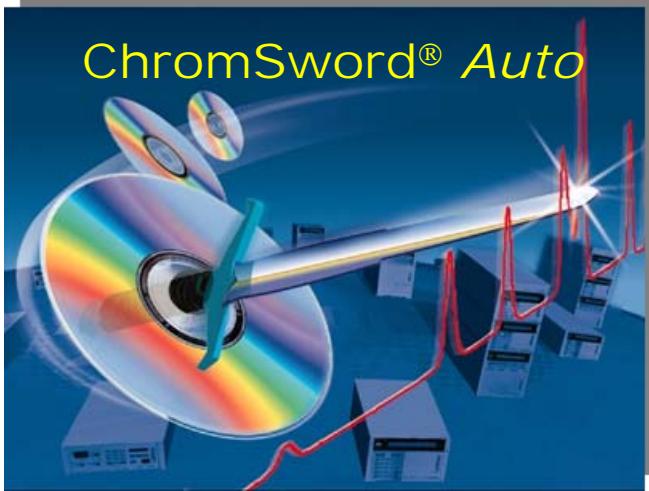
- 1.) Isoquercetin
- 2.) Troxerutin
- 3.) Naringin
- 4.) Morin
- 5.) Quercetin
- 6.) Trihydroxyethyluteolin



Chromolith® HPLC Columns



- Extremely low back-pressure
 - = Shorter analysis time / higher sample throughput
- Less sample preparation (filtration) required
 - = Ideal for real samples
- Extremely long column lifetime (rigid silica skeleton)
 - = Significant cost savings
- Coupling of columns possible
 - = Improved separations
- Use with every HPLC or UHPLC system
 - = fast separations for everyone



ChromSword Auto® Methodenentwicklungs- und Optimierungssoftware optimiert vollautomatisch Reversed-Phase-HPLC-Methoden. ChromSword Auto® steuert dabei den kompletten mathematischen und experimentellen Optimierungsprozess.

Die Software kann mit folgenden HPLC-Systemen kombiniert werden:

- VWR-Hitachi LaChrom, LaChrom Elite and LaChrom Ultra
- Agilent Systemen
- Waters Systemen

Zwei Optimierungsmodi stehen zur Auswahl:

1. Schnelle Optimierung/ schnelles Screening:

ChromSword® Auto führt mit einer Säulen-Lösungsmittel-Kombination einen Übersichtsgradienten aus und berechnet daraus einen optimalen linearen Gradient und einen optimalen Stufengradient.

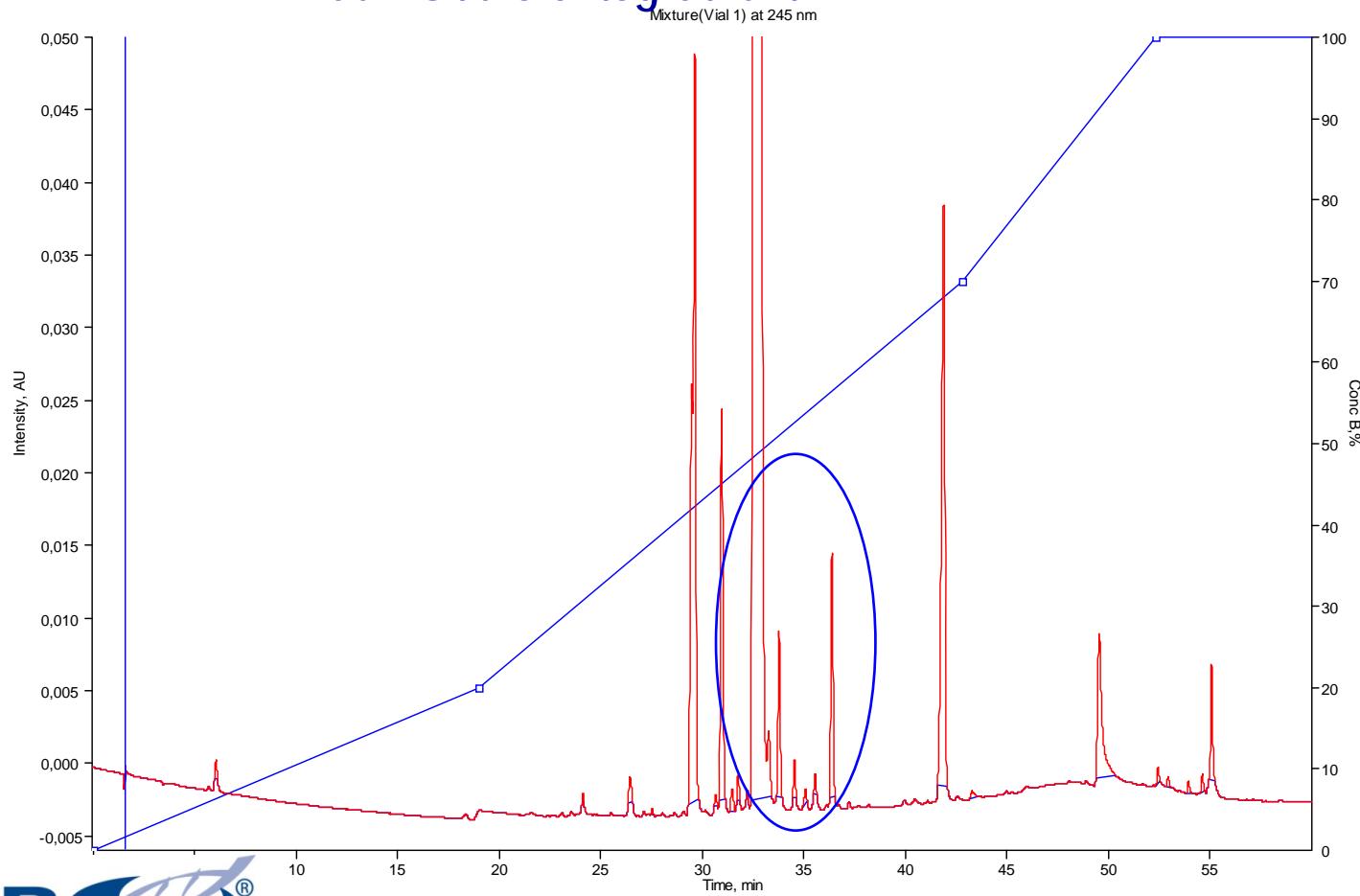
2. Fein-Optimierung

Im Verlauf der Feinoptimierung nimmt ChromSword® Auto weitere Retentionsdaten auf, um exakte Retentionsmodelle zu berechnen. Anschließend werden bis zu 10 Chromatogramme mit den berechneten optimalen Bedingungen aufgenommen.

ChromSword® Auto

Schnelle Optimierung/ Schnelles Screening: Methodenentwicklung in 3 Läufen

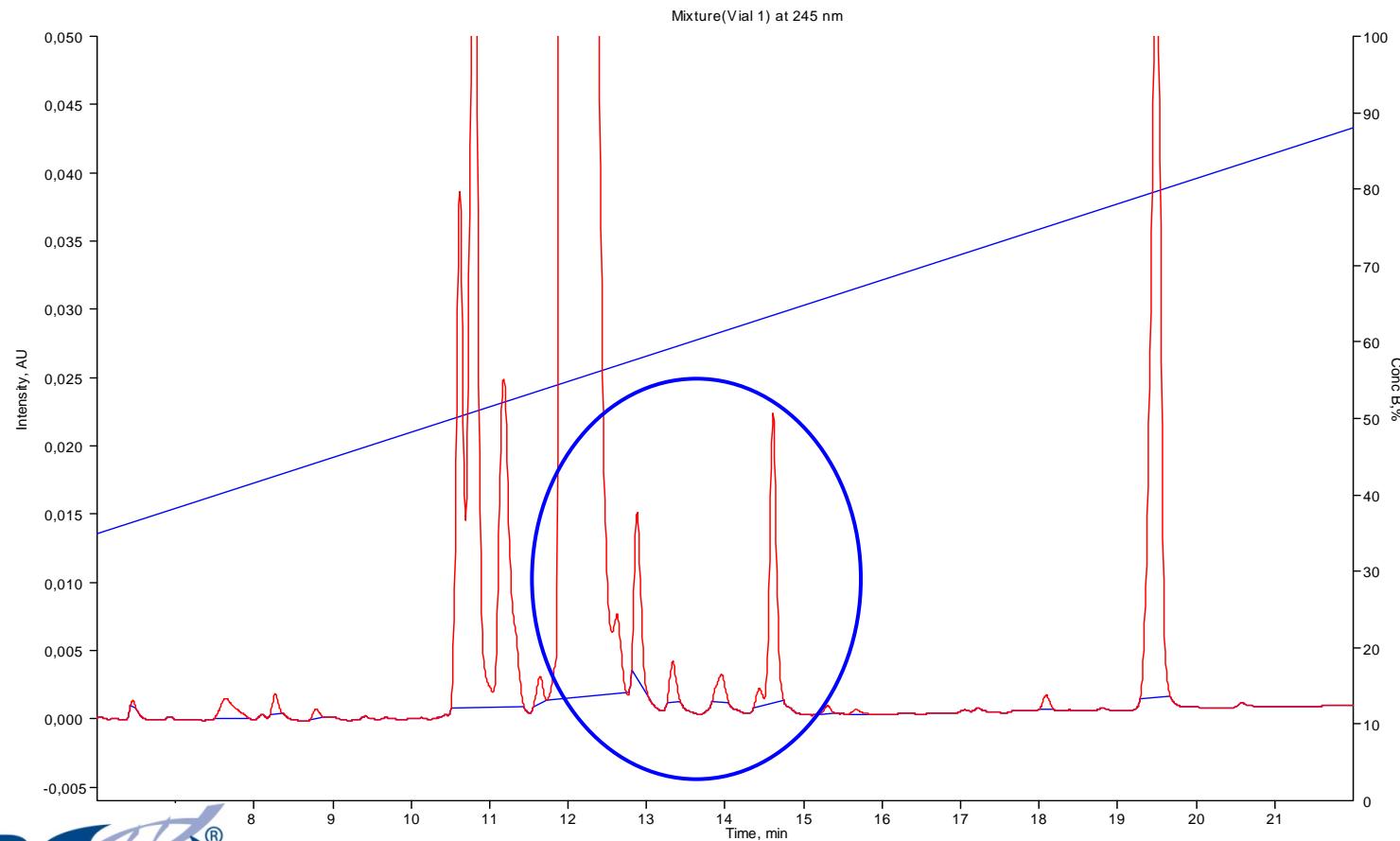
1. Lauf: Übersichtsgradient



ChromSword® Auto

Schnelle Optimierung/ Schnelles Screening: Methodenentwicklung in 3 Läufen

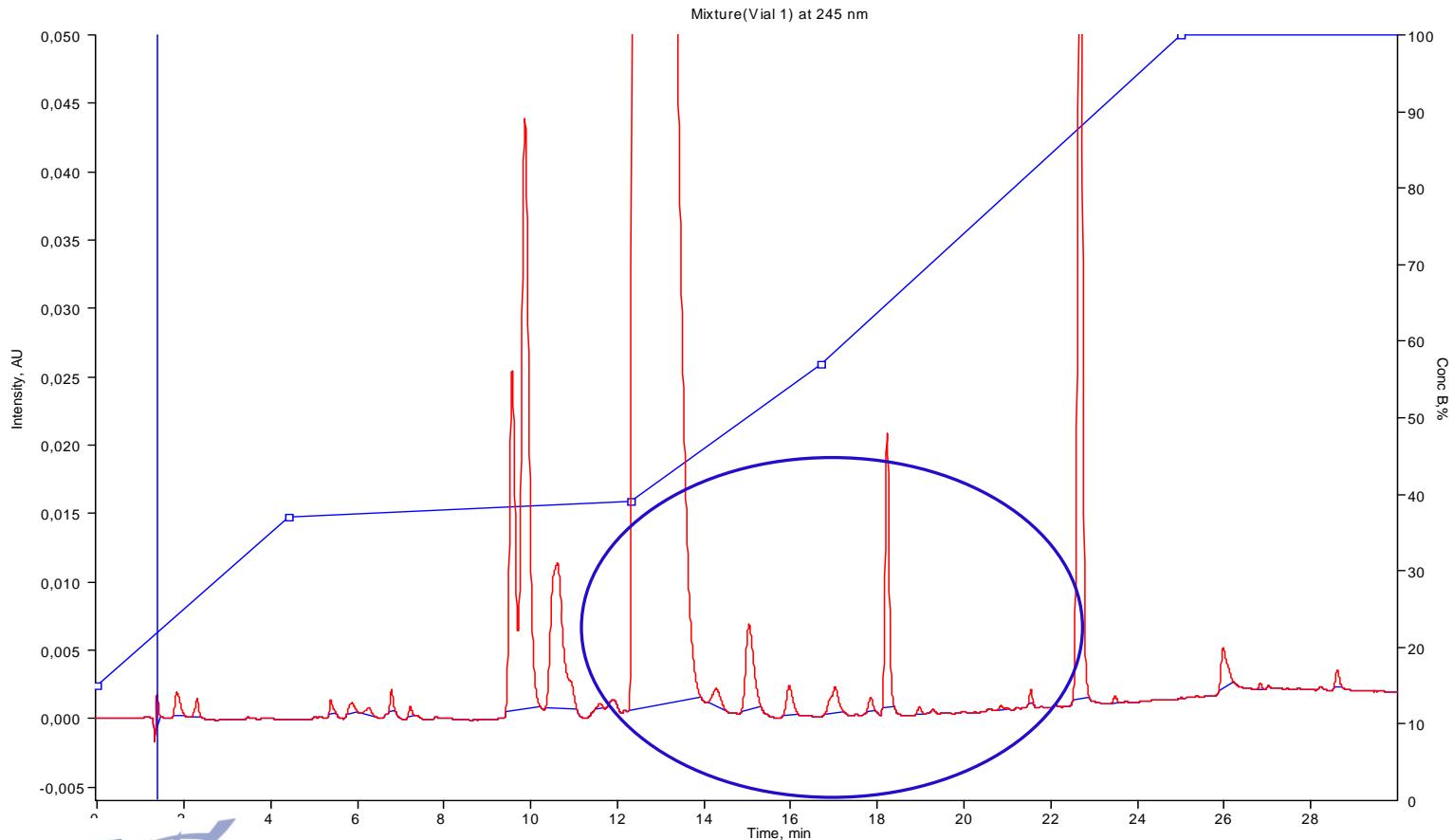
2. Lauf: optimierter linearer Gradient



ChromSword® Auto

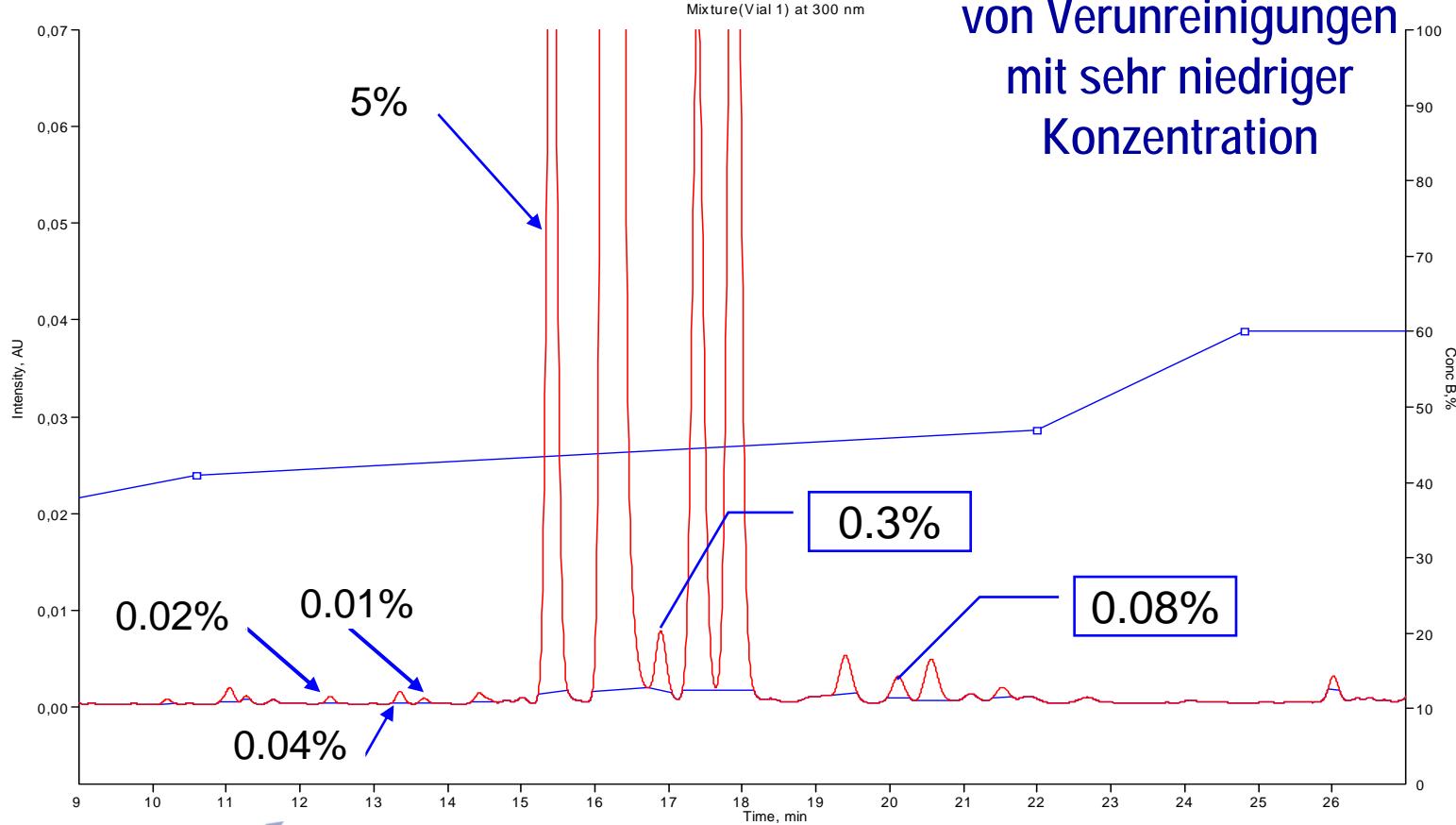
Schnelle Optimierung/ Schnelles Screening: Methodenentwicklung in 3 Läufen

3. Lauf: optimierter Stufen-Gradient



ChromSword® Auto

Fein-Optimierung einer pharmazeutischen Probe



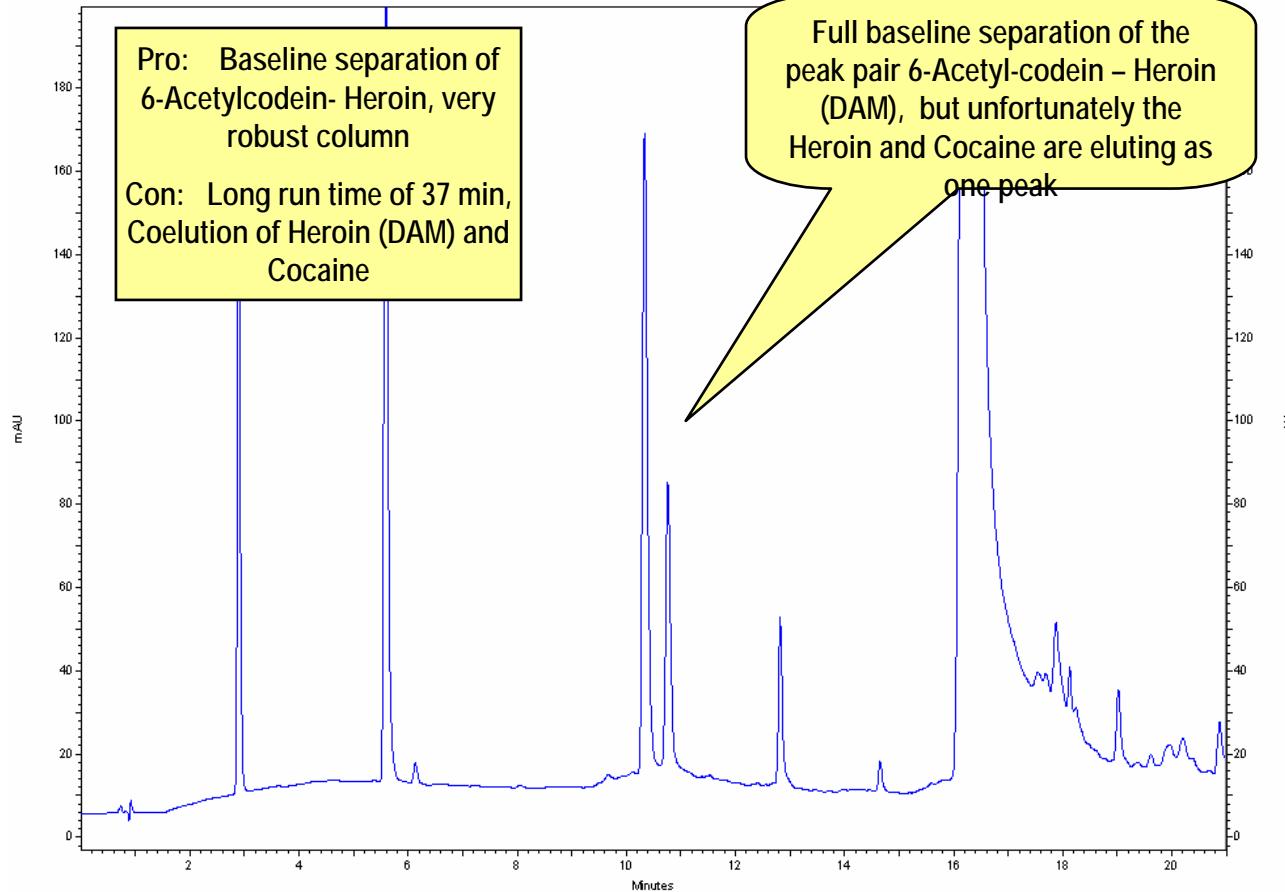
Trennoptimierung auch
von Verunreinigungen
mit sehr niedriger
Konzentration



Optimisation: Drugs of Abuse Standard mixture on LaChromUltra system

Column: Chromolith® Performance RP-18e, 100-4.6 mm

Time: 20,9837 Minutes - Amplitude: 18,704 mAU



Original customer's application

Sample: Standard mixture

1.Paracetamol, 2.Caffeine
3.6-Acetylcodeine, 4.Diacetylmorphine (DAM, Heroin),

Column: Chromolith® Performance RP-18e, 100-4.6 mm

Eluent A: Phosphate buffer,
pH 2.5 (2.4 g NaH₂PO₄ + 20 ml ACN ad 1000 ml > adjust with 85 % H₃PO₄ to pH 2.5)

Eluent B: Acetonitrile

Column temperature: 40 °C

Time	A	B	Flow
0	100	0	2.0
14	79	21	2.0
20	52	48	2.0
21	22	78	2.0
23	22	78	2.0
31	100	0	2.0
37	100	0	2.0

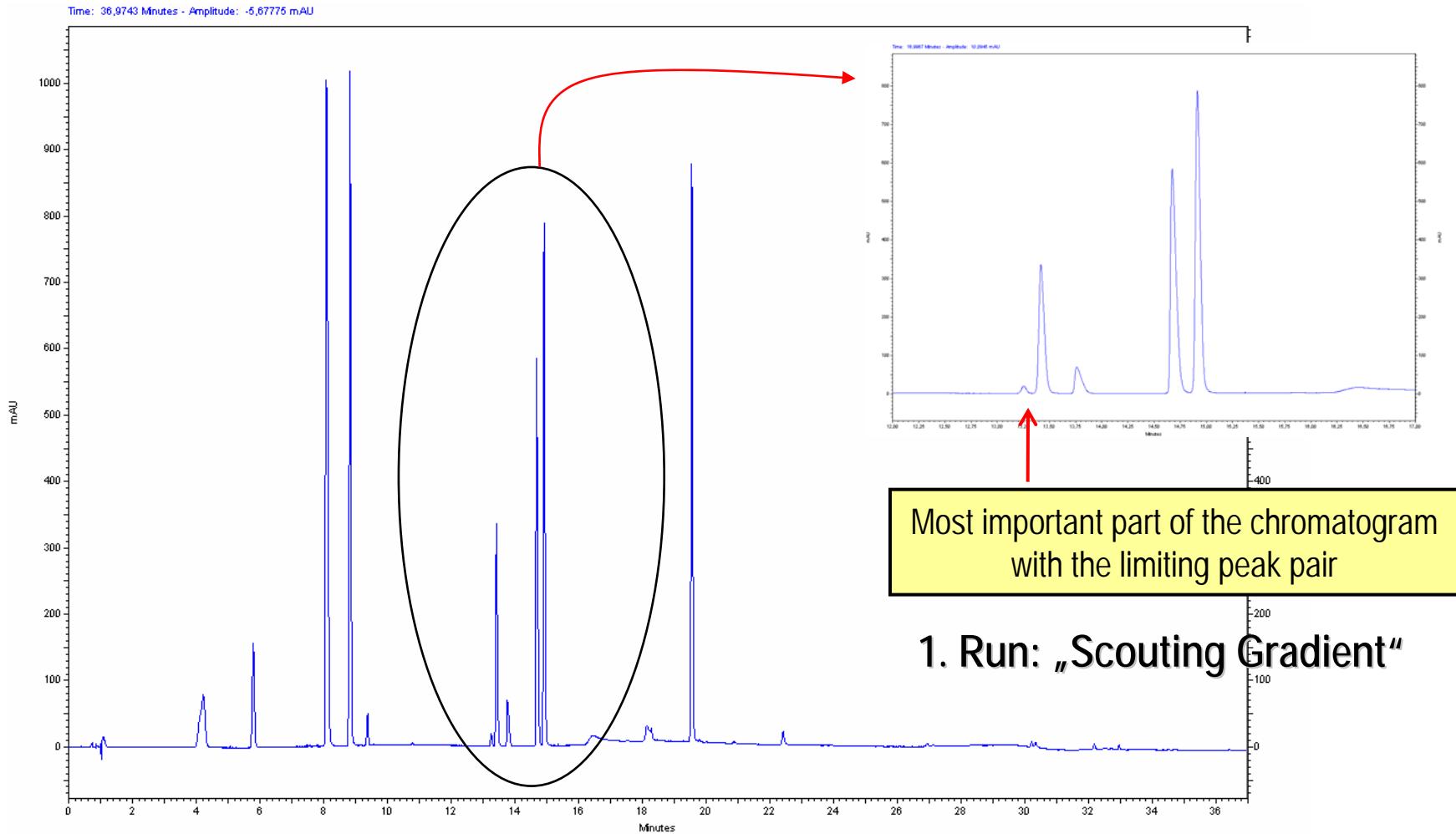
Flow rate: 2.0 ml/min,
UV: 210 nm

Pressure: 90 bar



ChromSword Auto® Optimisation: Drugs of Abuse Standard mixture of 11 illegal drugs

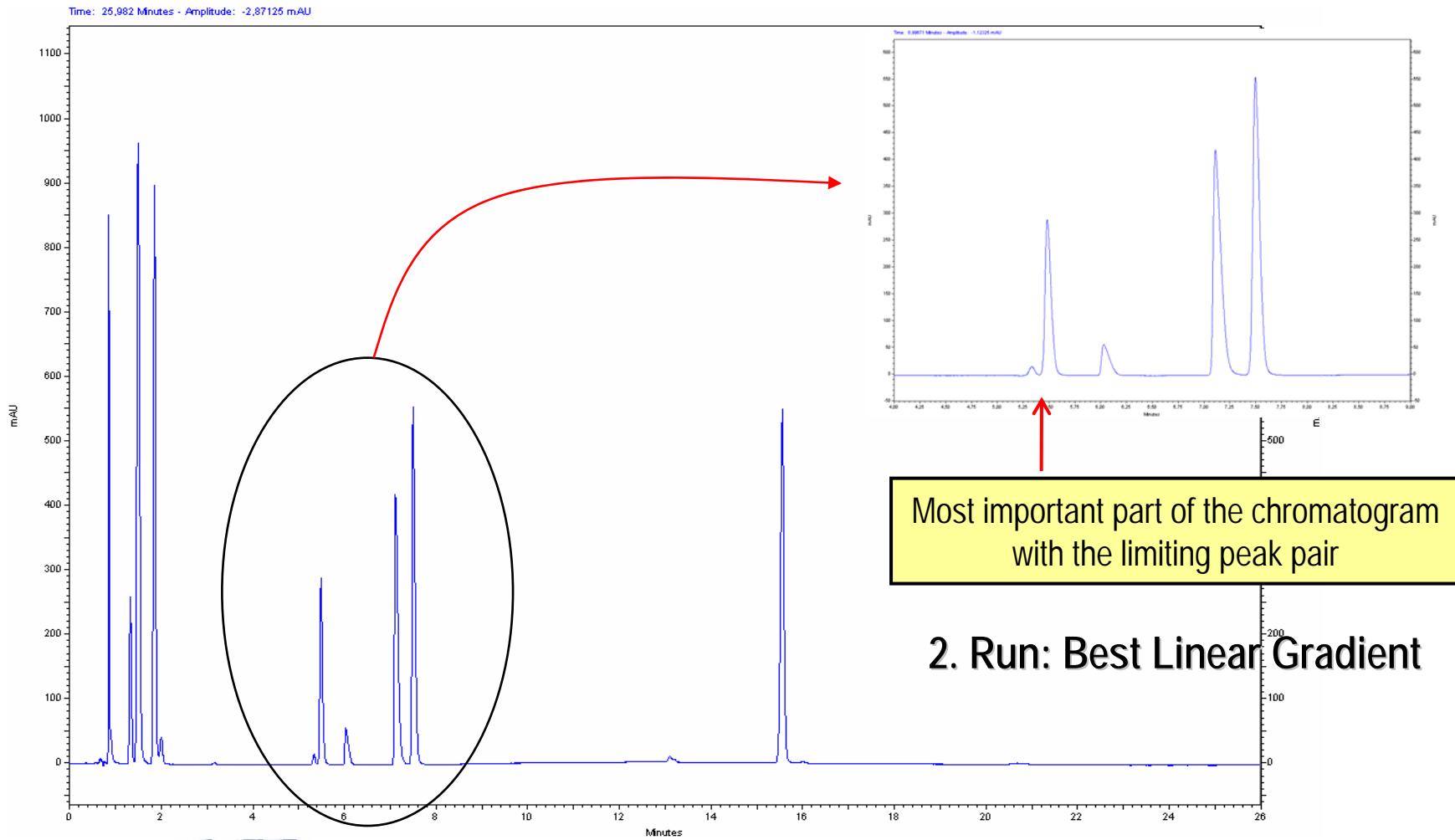
Column: Purospher® STAR RP-18e (3 µm), Hibar HR 150-2.1 mm





ChromSword Auto® Optimisation: Drugs of Abuse Standard mixture of 11 illegal drugs

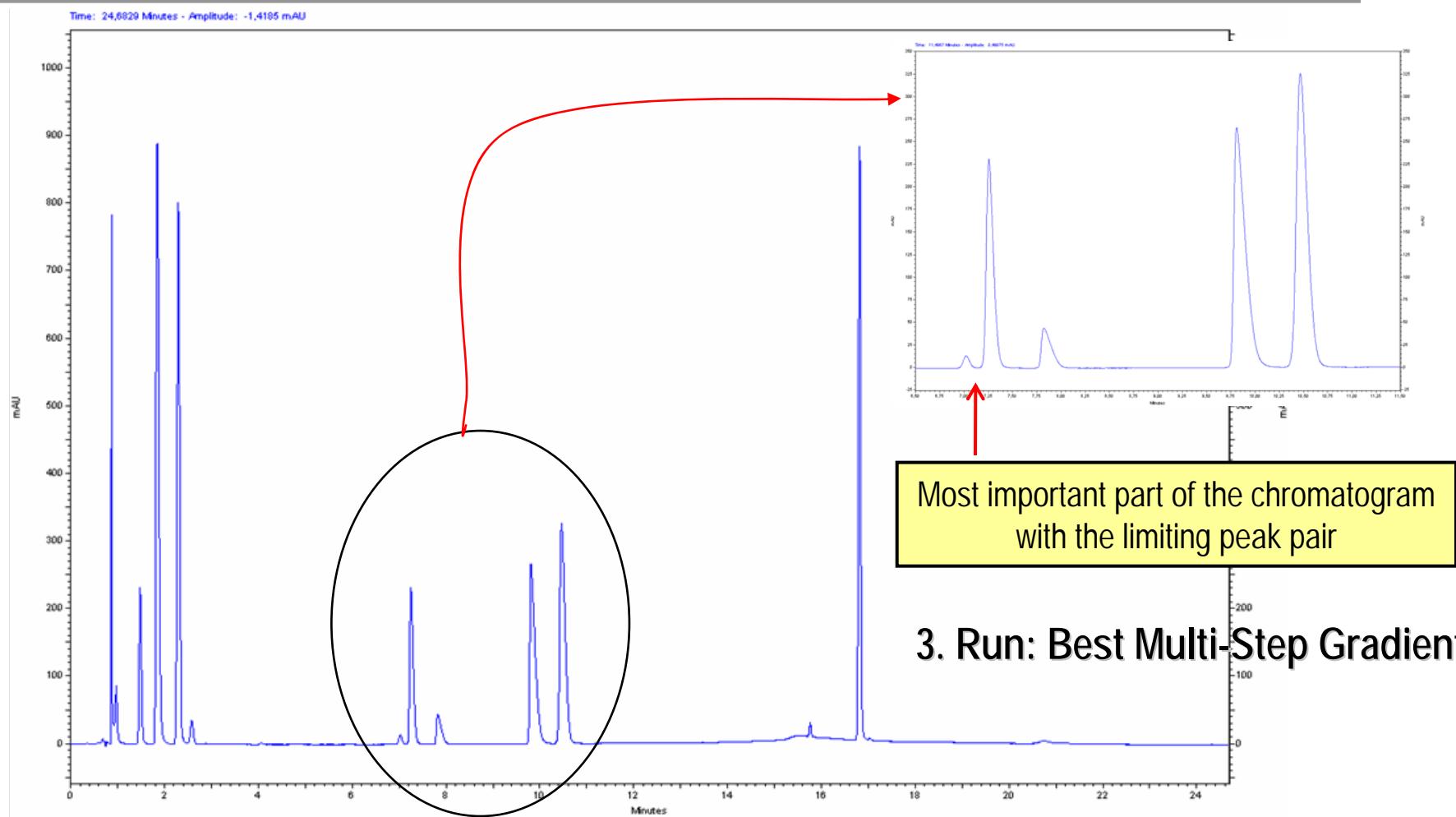
Column: Purospher® STAR RP-18e (3 µm), Hibar HR 150-2.1 mm





ChromSword Auto® Optimisation: Drugs of Abuse Standard mixture of 11 illegal drugs

Column: Purospher® STAR RP-18e (3 µm), Hibar HR 150-2.1 mm





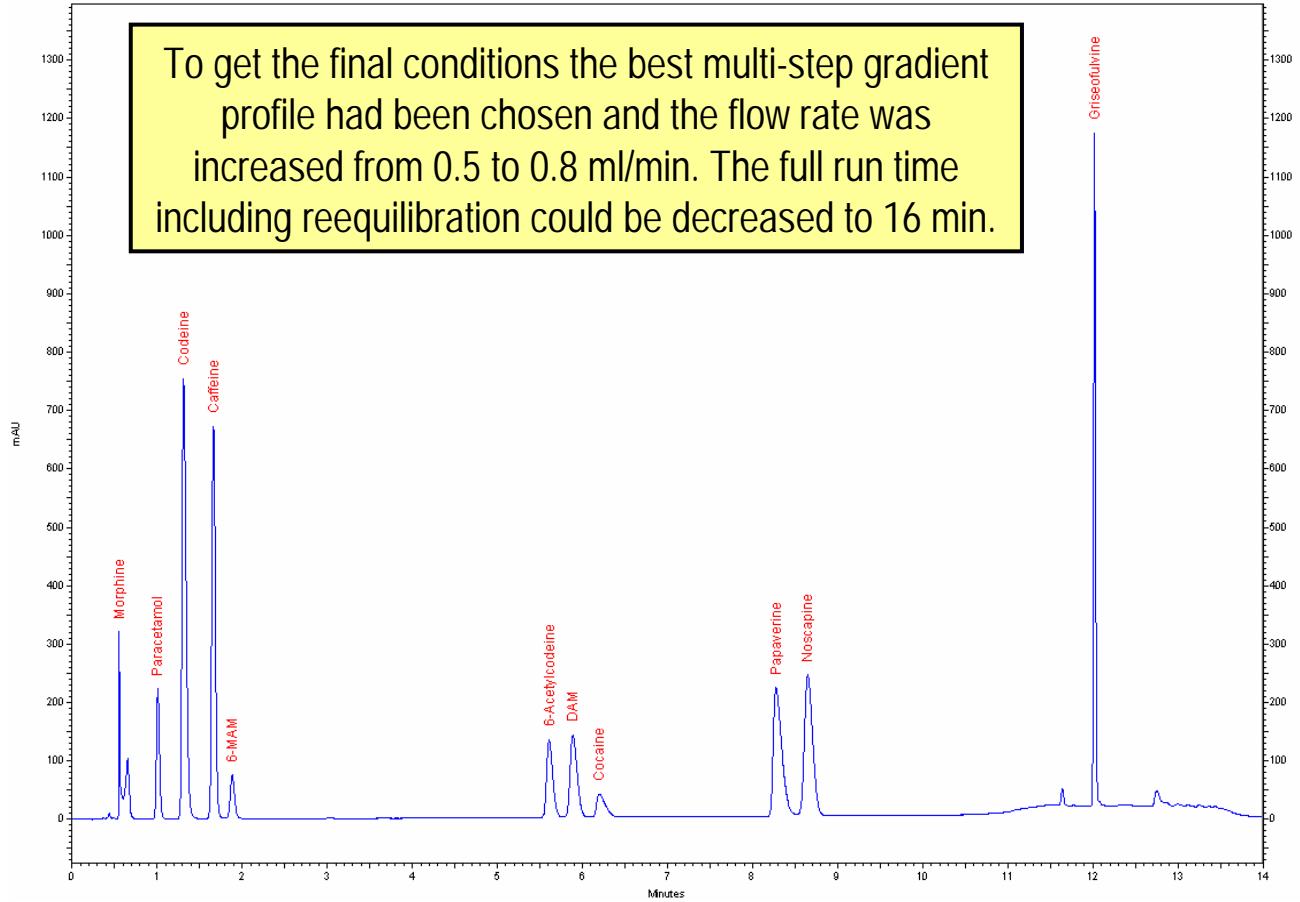
ChromSword Auto® Optimisation: Drugs of Abuse

Standard mixture of 11 illegal drugs

Column: Purospher® STAR RP-18e (3 µm), Hibar HR 150-2.1 mm

Time: 13,9903 Minutes · Amplitude: 3,9735 mAU

To get the final conditions the best multi-step gradient profile had been chosen and the flow rate was increased from 0.5 to 0.8 ml/min. The full run time including reequilibration could be decreased to 16 min.



Sample: Std-Mix of 11 illegal drugs

1.Morphine, 2.Paracetamol, 3.Codein,
4.Coffeine, 5.6-Mono-acetylmorphine
(MAM), 6.6-Acetyl-codeine,
7.Diacetylmorphine (DAM, Heroin),
8.Cocaine, 9.Papaverine,
10.Noscapine, 11.Griseofulvine

Column: Purospher®
STAR RP-18e (3 µm), Hibar
HR 150-2.1 mm

Column temperature: 40 °C
Eluent A: Phosphate buffer,
pH 2.5 (2.4 g NaH₂PO₄ + 20 ml
ACN ad 1000 ml water > adjust
with 85 % H₃PO₄ to pH 2.5)

Eluent B: Acetonitrile

Time	A	B	Flow
0	89	11	0.8
5.8	82	18	0.8
7	81.5	18.5	0.8
9.5	75	25	0.8
12	30	70	0.8
13	30	70	0.8
13.1	89	11	0.8
16	89	11	0.8

Flow rate: 0.8 ml/min,

UV: 210 nm

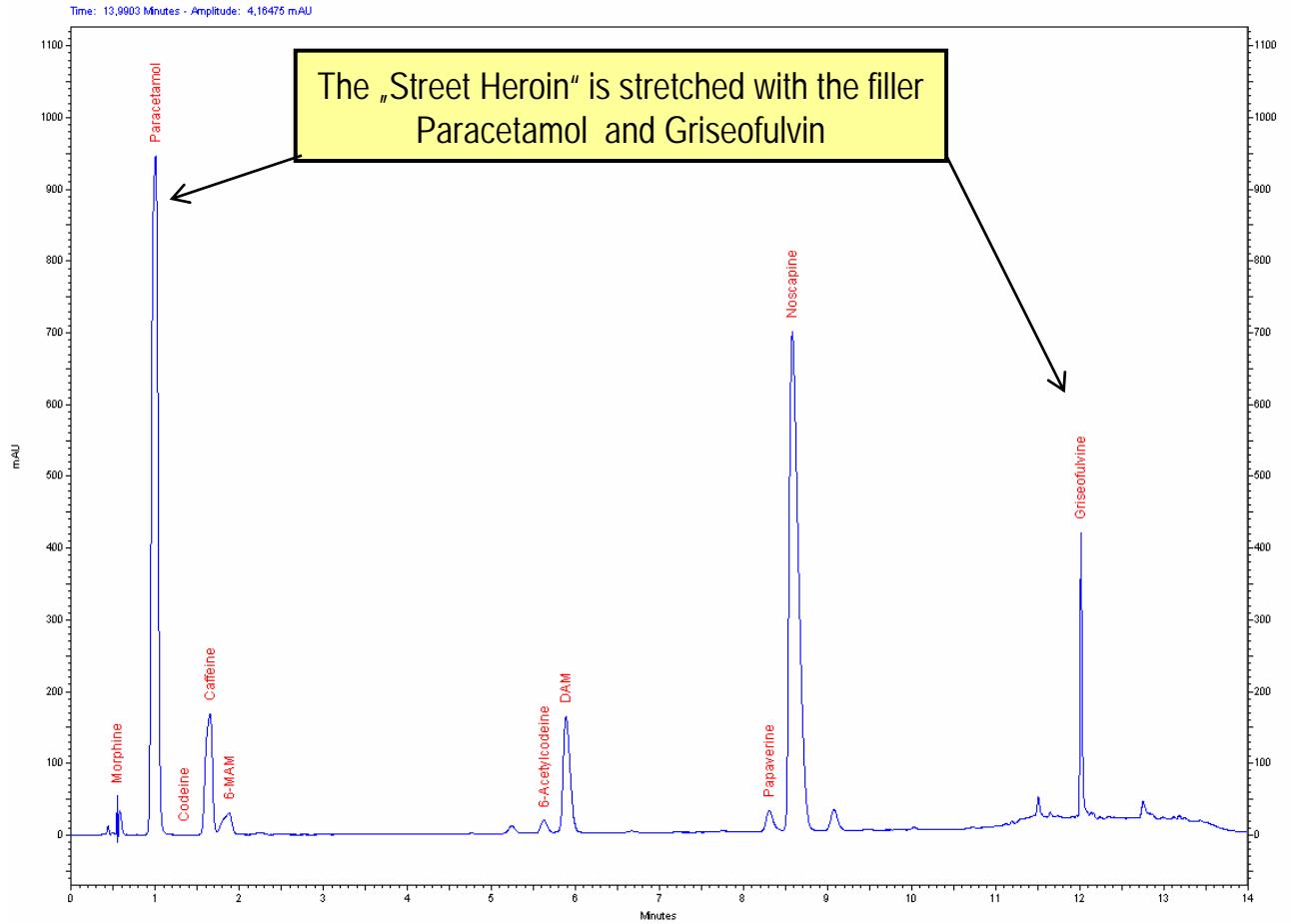
Pressure: 520 bar



ChromSword Auto® Optimisation: Drugs of Abuse

Real sample: „Street Heroin“

Column: Purospher® STAR RP-18e (3 µm), Hibar HR 150-2.1 mm



Sample: "Street Heroin"
1.Morphine, 2.Paracetamol, 3.Codein,
4.Coffeine, 5.6-Mono-acetylmorphine
(MAM), 6.6-Acetyl-codeine,
7.Diacetylmorphine (DAM, Heroin),
8.Cocaine, 9.Papaverine,
10.Noscapine, 11.Griseofulvine
Column: Purospher®
STAR RP-18e (3 µm), Hibar
HR 150-2.1 mm
Column temperature: 40 °C
Eluent A: Phosphate buffer,
pH 2.5 (2.4 g NaH₂PO₄ + 20 ml
ACN ad 1000 ml water > adjust
with 85 % H₃PO₄ to pH 2.5)
Eluent B: Acetonitrile

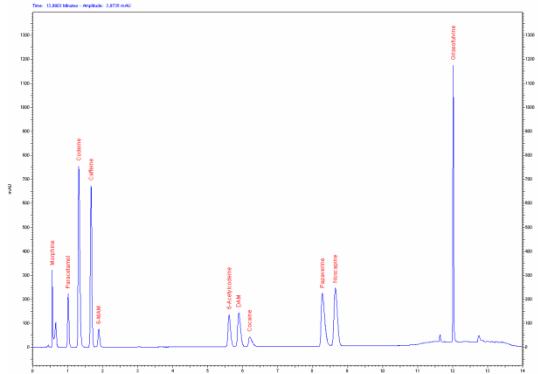
Time	A	B	Flow
0	89	11	0.8
5.8	82	18	0.8
7	81.5	18.5	0.8
9.5	75	25	0.8
12	30	70	0.8
13	30	70	0.8
13.1	89	11	0.8
16	89	11	0.8

Flow rate: 0.8 ml/min,
UV: 210 nm
Pressure: 520 bar



ChromSword Auto® Optimisation: Drugs of Abuse

Column: Purospher® STAR RP-18e (3 µm), Hibar HR 150-2.1 mm



Why a 3 µm column has been selected?

Purospher® STAR RP-18e Number of theoretical plates:

2 µm : 190 000 plates /m

50 mm column: 9 500 plates/m

3 µm : 150 000 plates /m

150 mm column: 22 500 plates/m

The 3 µm 15 mm column supplies a much better resolution at similar backpressure!

Sample: Std-Mix of 11 illegal drugs

1.Morphine, 2.Paracetamol, 3.Codein,
4.Caffeine, 5.6-Mono-acetylmorphine
(MAM), 6.6-Acetyl-codeine,
7.Diacetyl/morphine (DAM, Heroin),
8.Cocaine, 9.Papaverine,
10.Noscapine, 11.Griseofulvine

Column: Purospher®
STAR RP-18e (3 µm), Hibar
HR 150-2.1 mm

Column temperature: 40 °C

Eluent A: Phosphate buffer,
pH 2.5 (2.4 g NaH₂PO₄ + 20 ml
ACN ad 1000 ml water > adjust
with 85 % H₃PO₄ to pH 2.5)

Eluent B: Acetonitrile

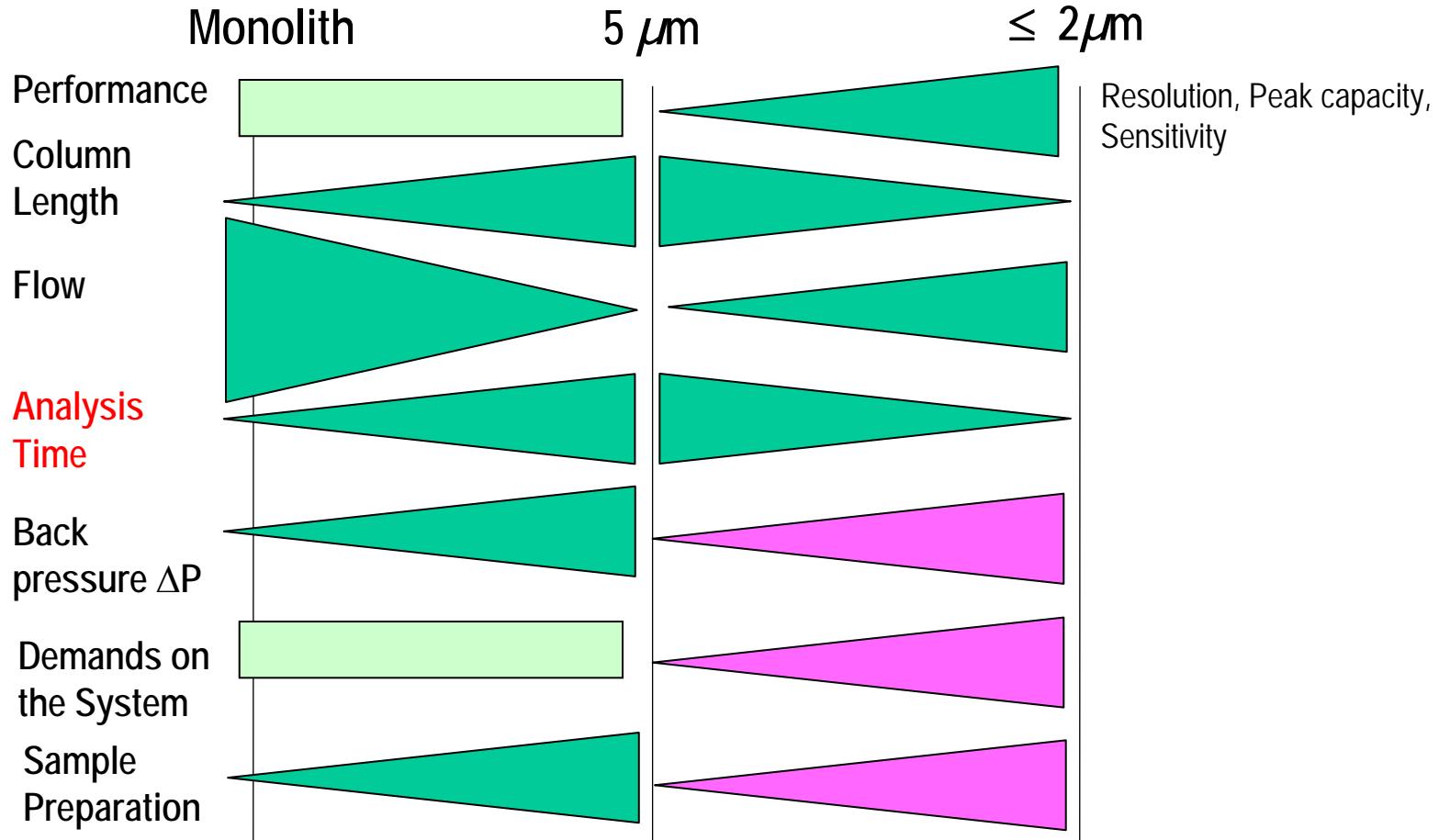
Time	A	B	Flow
0	89	11	0.8
5.8	82	18	0.8
7	81.5	18.5	0.8
9.5	75	25	0.8
12	30	70	0.8
13	30	70	0.8
13.1	89	11	0.8
16	89	11	0.8

Flow rate: 0.8 ml/min,
UV: 210 nm

Pressure: 520 bar

Conclusion

Chromolith vs. Particular Columns





Fazit

Bis zu 90% Zeit, Lösungsmittel und Kosten lassen sich einsparen durch „ultra“-schnelle Chromatographie

- mit partikulären Säulen $\leq 3 \mu\text{m}$ auf UHPLC-Systemen
- mit Chromolith[®] 50-2 mm Säulen
auf UHPLC- und konventionellen HPLC-Systemen
- Besondere Aufmerksamkeit sollte der Methoden-Optimierung geschenkt werden - ChromSword Auto[®] ist dafür ein einzigartiges Werkzeug