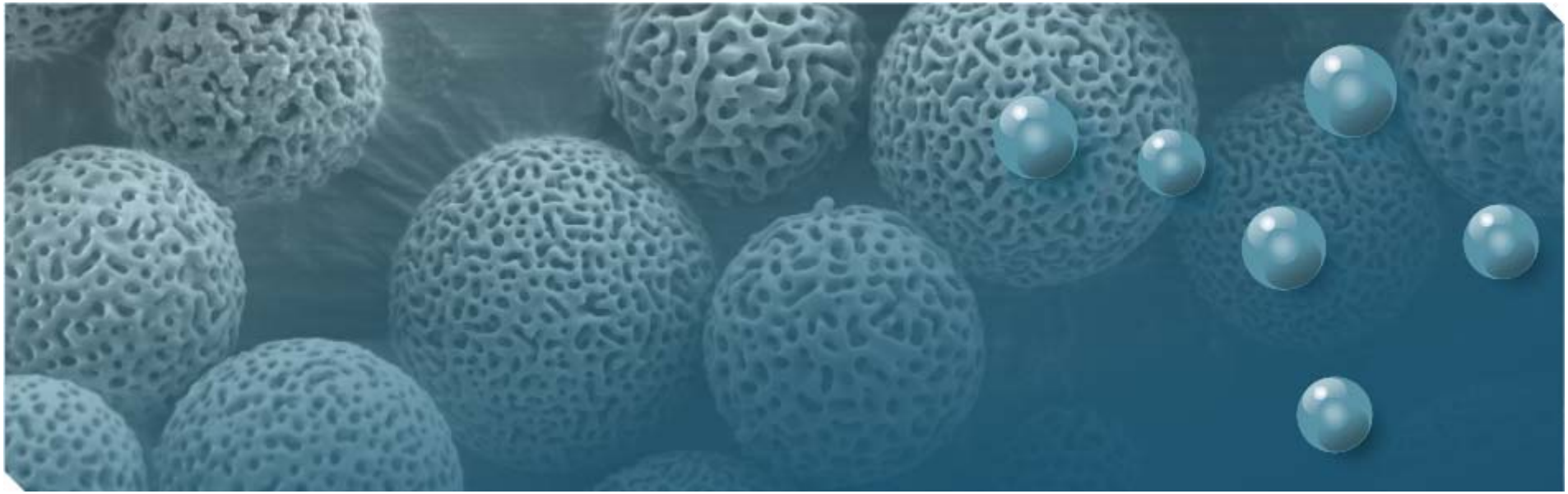
Kromasil®

AKZO NOBEL



New Kromasil Chiral Phases for HPLC and SFC

Eka Chemicals AB – Separation Products, SE-445 80 Bohus, Sweden

Website: www.kromasil.com

Kromasil CSP:s

- **Kromasil Chiral DMB:** The chiral monomer is O,O'-bis (3,5-dimethylbenzoyl)-N,N'-diallyl-*L*-tartar diamide. (1996)

  chemically stable, bonded co-polymer, tartaric acid, available in analytical columns and in bulk

- **Kromasil Chiral TBB:** The chiral monomer is O,O'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-*L*-tartar diamide. (1996)

  chemically stable, bonded co-polymer, tartaric acid, available in analytical columns and in bulk

- **Kromasil CelluCoat:** adsorbed polysaccharide (2006)

  available in analytical columns and in bulk

Unveiling Kromasil AmyCoat

On July 8 th the market for amylose-based chiral HPLC phases will never be the same again

Kromasil CSP:s

- **Kromasil Chiral DMB:** The chiral monomer is O,O'-bis (3,5-dimethylbenzoyl)-N,N'-diallyl-*L*-tartar diamide. (1996)

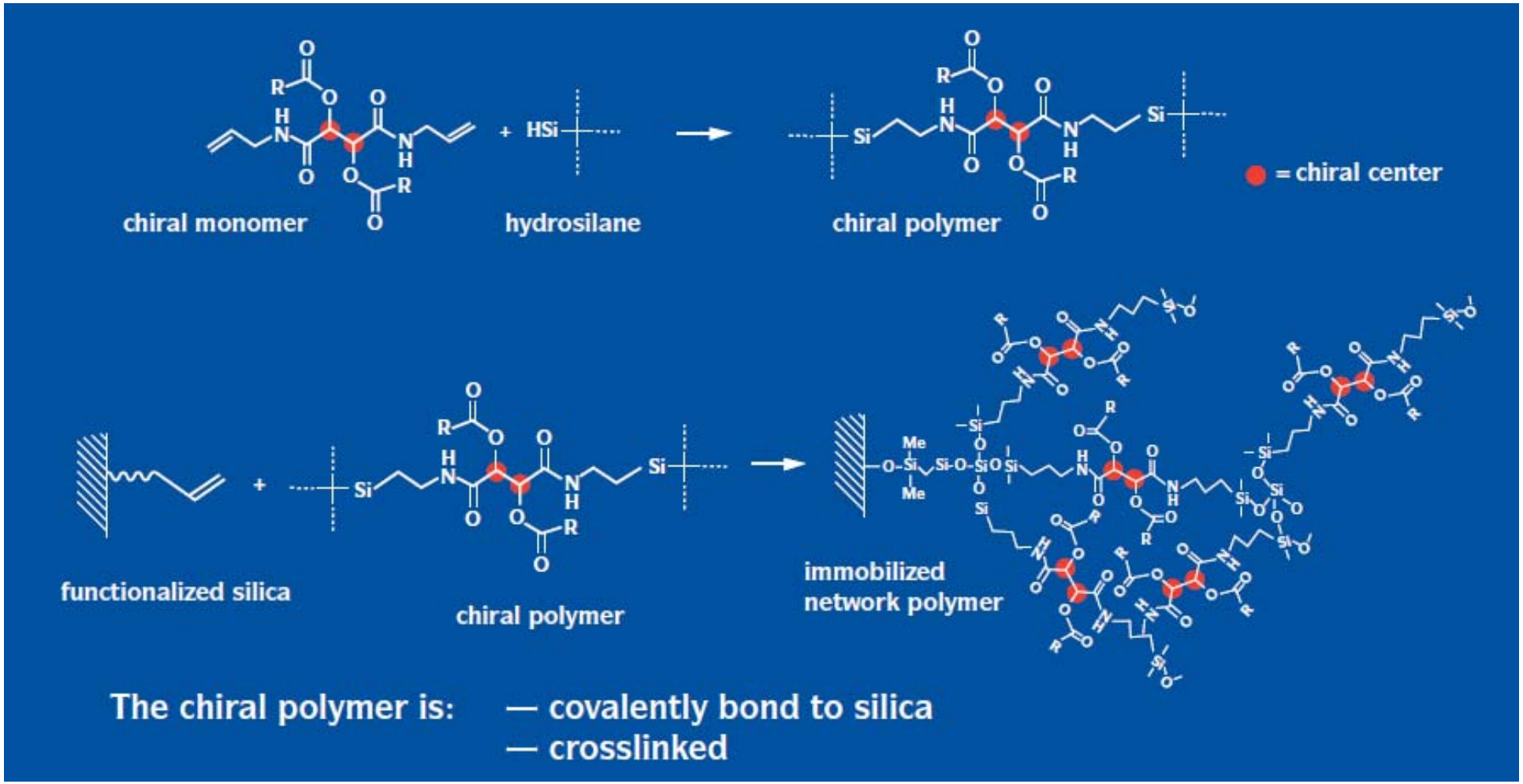
  chemically stable, bonded co-polymer, tartaric acid, available in analytical columns and in bulk
- **Kromasil Chiral TBB:** The chiral monomer is O,O'-bis (4-tert-butylbenzoyl)-N,N'-diallyl-*L*-tartar diamide. (1996)

  chemically stable, bonded co-polymer, tartaric acid, available in analytical columns and in bulk
- **Kromasil CelluCoat:** adsorbed polysaccharide (2006)

  available in analytical columns and in bulk
- **Kromasil AmyCoat:** adsorbed polysaccharide (2007)

  available in analytical columns and in bulk

Kromasil Chiral TBB/DMB



Outline of the Presentation

- Scope during development
- Features of the new chiral phases, Kromasil CelluCoat and Kromasil AmyCoat
- Development of new silica backbone
- The chiral selector
- Stability of the phases
- Fast analysis
- Scale up

Scope during development

- Extend the Kromasil CSP assortment with more general phases, polysaccharide-based

- Develop the polysaccharide phases so that they are more user-friendly (robust) without sacrificing the general selectivity

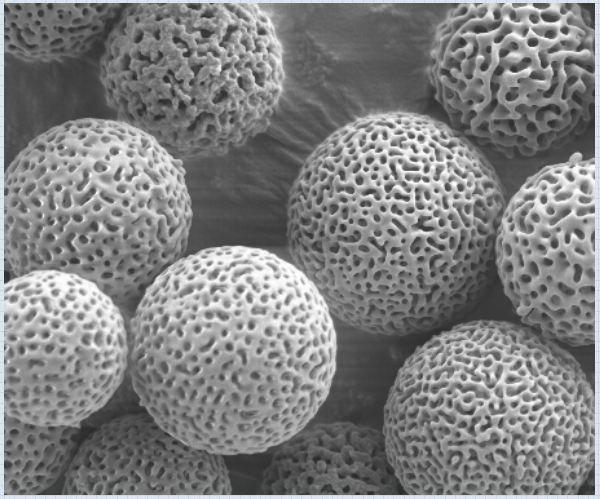
Features of Kromasil CelluCoat and AmyCoat

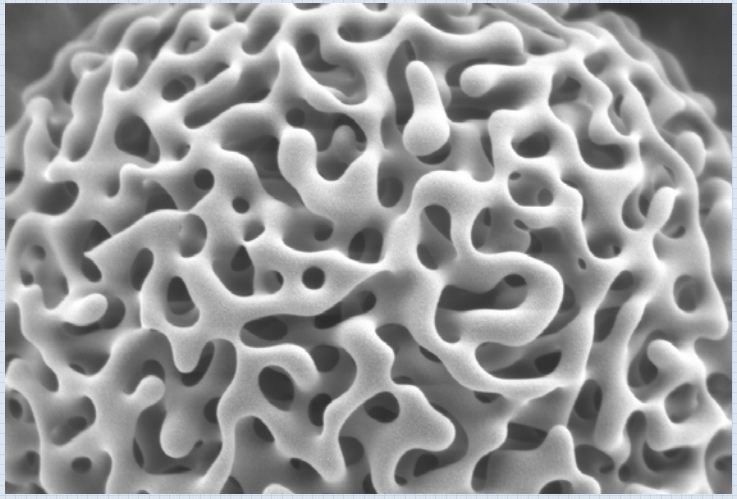
- High and broad selectivity
  - Coated cellulose and amylose based phases
- Fast analysis
  - 3 µm particles
- Mechanically and chemically stable
  - Tailor-made Super Wide Pore silica and a novel coating technology
- Fully back-integrated phase
  - Silica/Selector/Column

Development of a new silica matrix

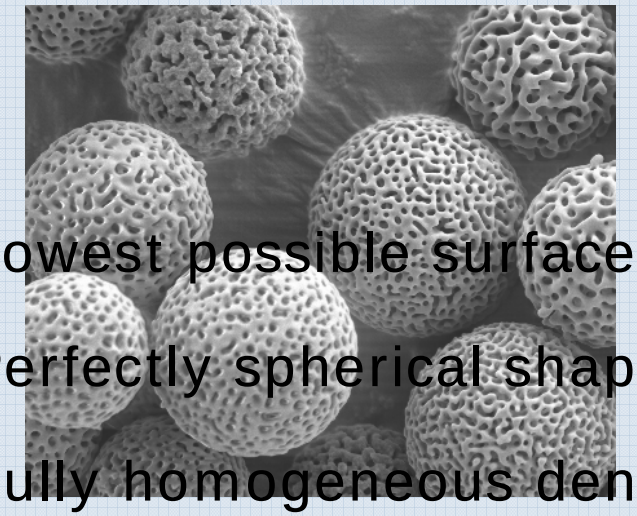
Super Wide Pore Silica designed to:

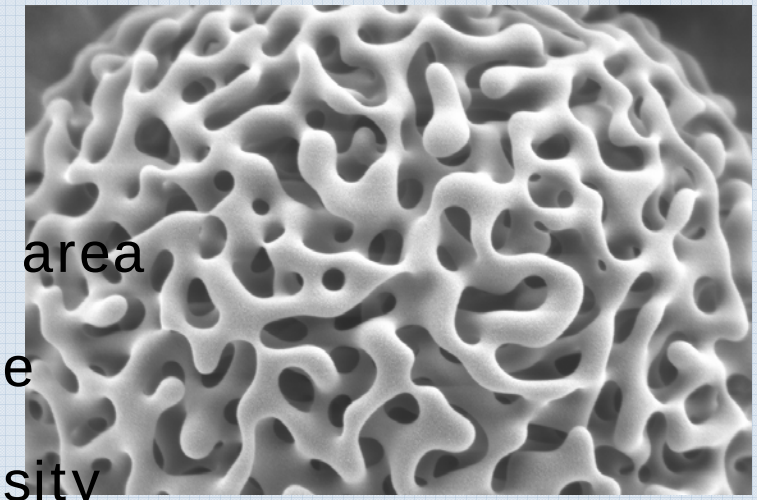
- Minimize achiral interactions
- Maintain high mechanical strength





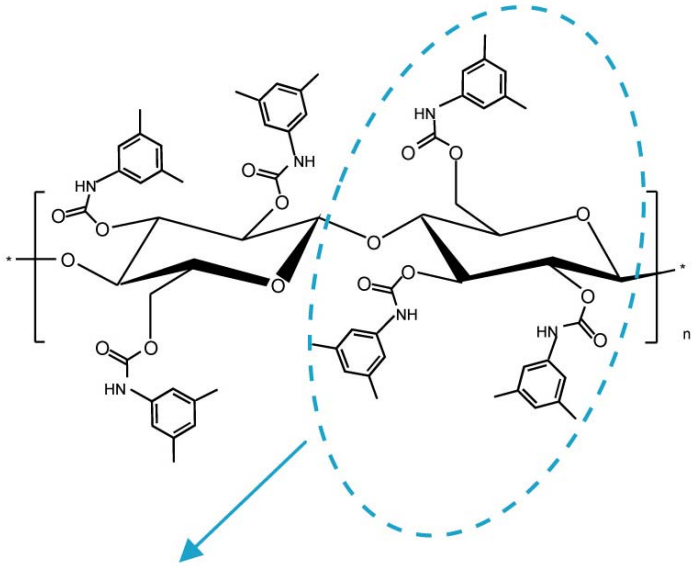
Development of a new silica matrix



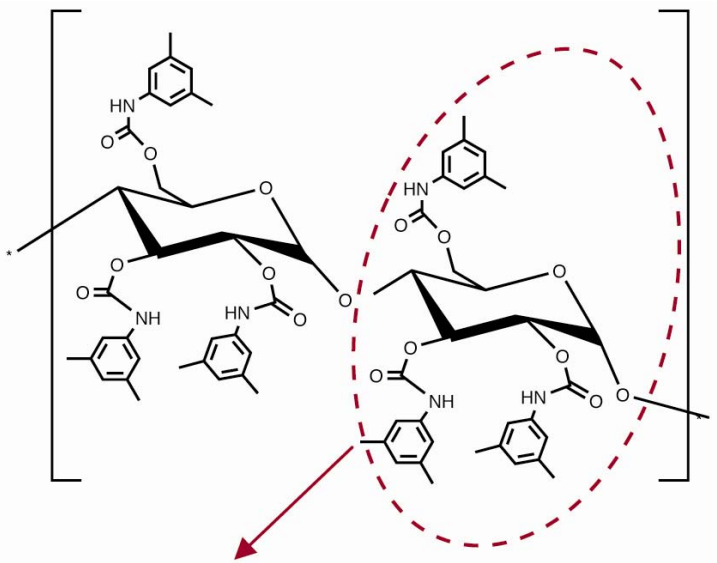


- Lowest possible surface area
- Perfectly spherical shape
- Fully homogeneous density

The Chiral Selectors



tris-(3,5-dimethylphenyl)carbamoyl cellulose



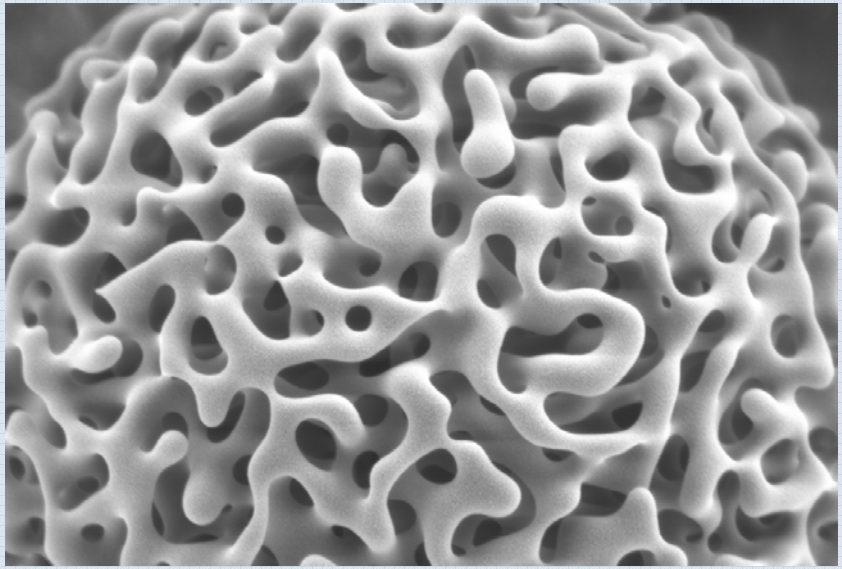
tris-(3,5-dimethylphenyl)carbamoyl amylose

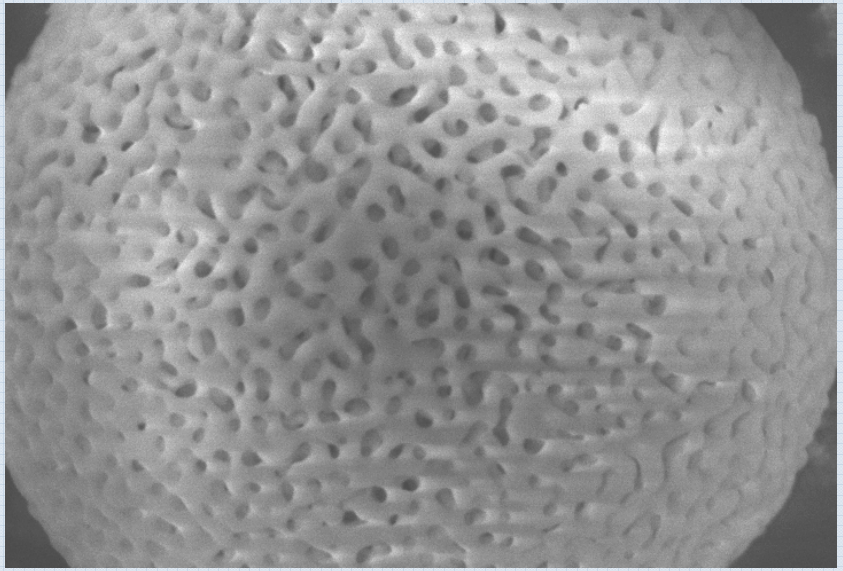
Provides high and broad chiral discrimination
A unique coating technology ensures maximal long term stability and resolution

The Chiral Selector

A novel coating technology ensures long term stability, maximal resolution and high loading capacity

- Homogenous distribution of the selector
- Optimal thickness of the selector
- Consistent result for all particle sizes
- Fully scalable coating method



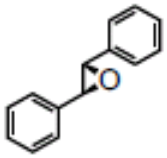


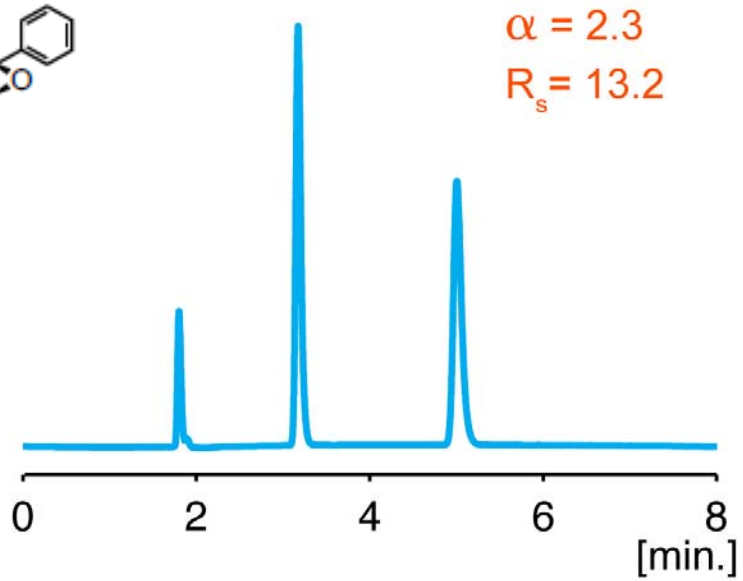
Selectivity and resolution

Racemate	AmyCoat		CelluCoat	
	α	R_s	α	R_s
trans-Stillbene oxide	3.1	18.0	2.3	13.2
Benzoin	1.3	6.5	1.5	8.6
TFAE	1.4	6.4	2.9	14.7
Hexobarbital	1.4	4.7	1.2	2.7
Carbinoxamine	1.5	6.6	1.4	3.8
Ambucetamide	1.4	4.8	4.7	11.5
Proglumide	2.6	10.1	1.8	4.6

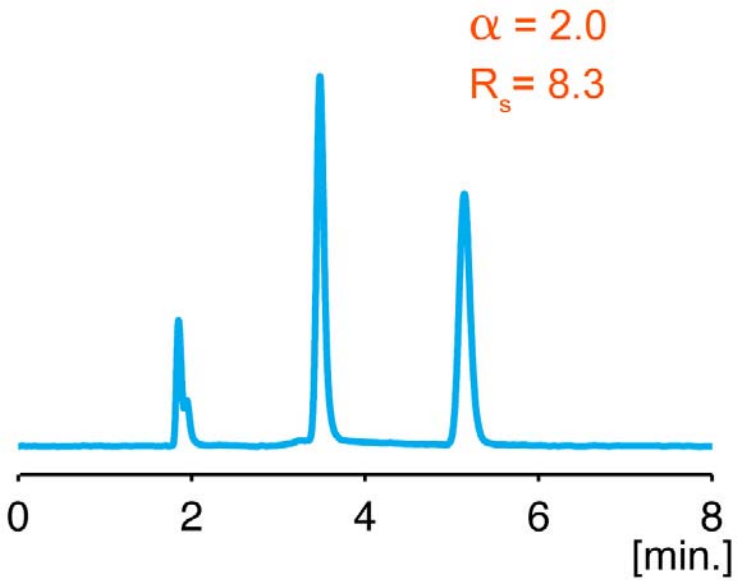
Expected selectivity – enhanced performance

Kromasil CelluCoat 3 µm





Chiralcel OD-H



Common conditions

Solute: trans-Stilbene oxide
Mobile phase: Heptane/2-Propanol (90/10)
Column size: 4.6 x 150 mm
Flow rate: 1 ml/min
Temperature: 25 °C
Detection: UV 229 nm

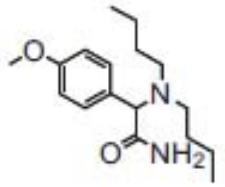
Expected selectivity – enhanced performance

	α		R_S	
	CelluCoat 3 µm	**Chiralcel OD-H***	**CelluCoat 3 µm**	**Chiralcel OD-H***
Trans-Stilbene oxide	2.3	2.0	13.2	8.3
Benzoin	1.5	1.5	8.6	5.7
TFAE	2.9	2.9	14.7	11
Tröger's base	1.4	1.4	3.7	2.7
Oxprenolol	5.6	5.5	18.1	15.1
Naproxen	1.2	1.2	2.9	2.2
Proglumide	2.0	2.0	7.6	3.2

*** (5 µm)**

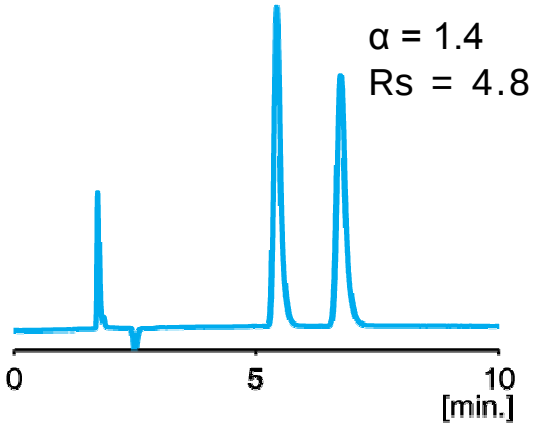
Expected selectivity – enhanced performance

Ambucetamide

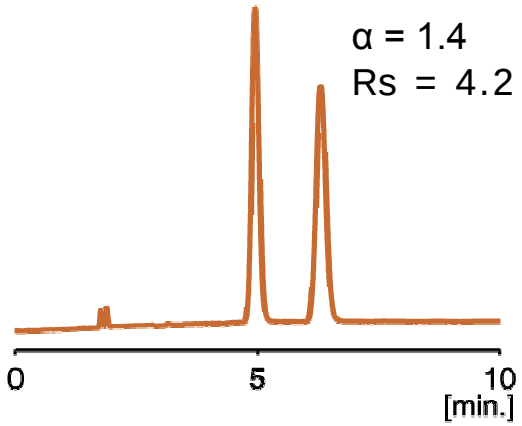


Flow rate: 1 mL/min
Detection: UV 229 nm

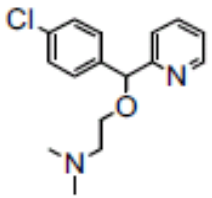
Kromasil 3-AmyCoat



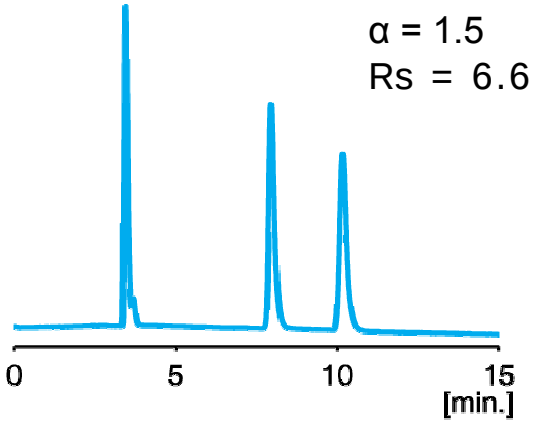
CHIRALPAK AD-H

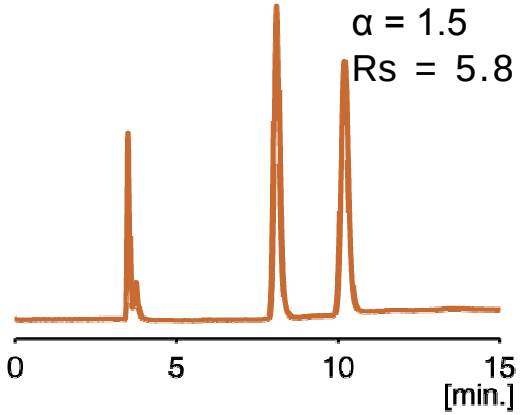


Carbinoxamine



Flow rate: 0.5 mL/min
Detection: UV 226 nm



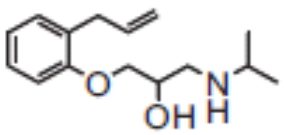


Column size: 4.6 x 150 mm, Mobile phase: Heptane/IPA/DEA (90/10/0.1), Temperature: 22 °C

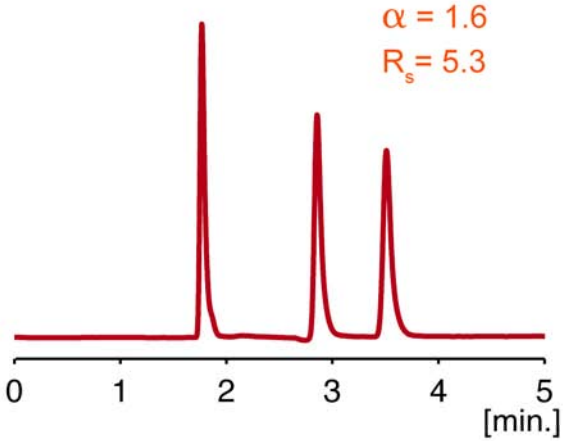
Selectivity and resolution – comparison 3 µm and 5 µm

	α		R_S	
	AmyCoat 3 µm	**AmyCoat 5 µm**	**AmyCoat 3 µm**	**AmyCoat 5 µm**
Alprenolol	1.6	1.7	5.3	4.4
Benzoin	1.3	1.3	6.5	4.4
Bucetin	1.8	1.7	8.2	5.8
Hexobarbital	1.4	1.4	4.7	3.2
Metoprolol	1.5	1.4	3.2	2.0
Oxamniquine	1.2	1.2	3.2	2.5
TFAE	1.4	1.4	6.4	4.2

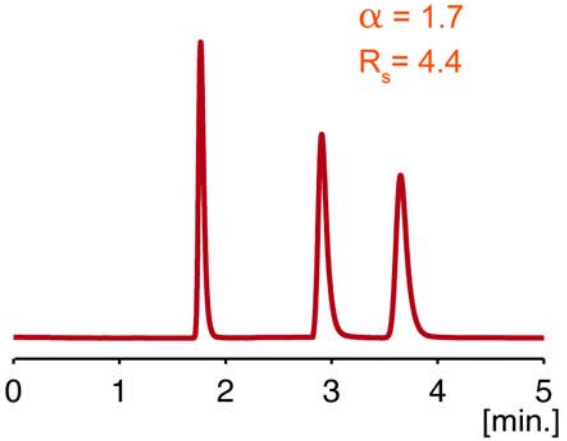
Selectivity and resolution – comparison 3 µm and 5 µm



Kromasil AmyCoat 3 µm



Kromasil AmyCoat 5 µm



Common conditions

Solute: Alprenolol
Mobile phase: Heptane/Ethanol/DEA (90/10/0.1)
Column size: 4.6 × 150 mm
Flow rate: 1 ml/min
Temperature: 25 °C
Detection: UV 220 nm

Selectivity and resolution – comparison 3 µm and 5 µm

Kromasil AmyCoat 5µm
4.6x150 mm

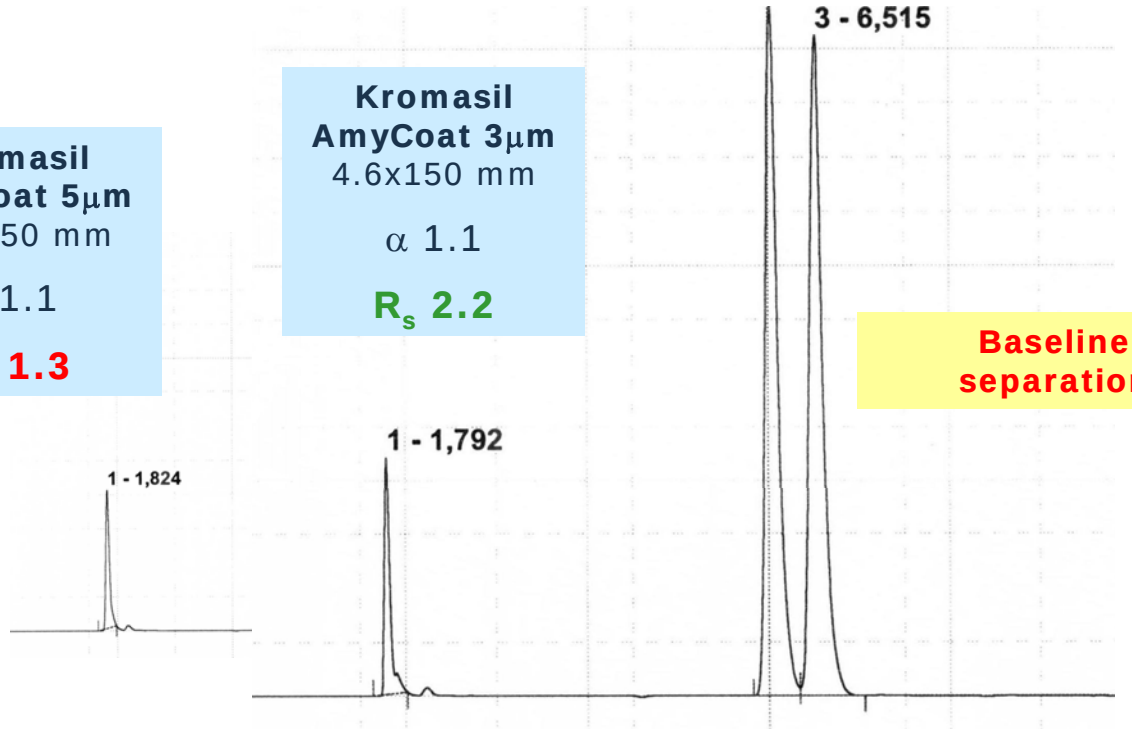
α 1.1

R_s 1.3

Kromasil AmyCoat 3µm
4.6x150 mm

α 1.1

R_s 2.2

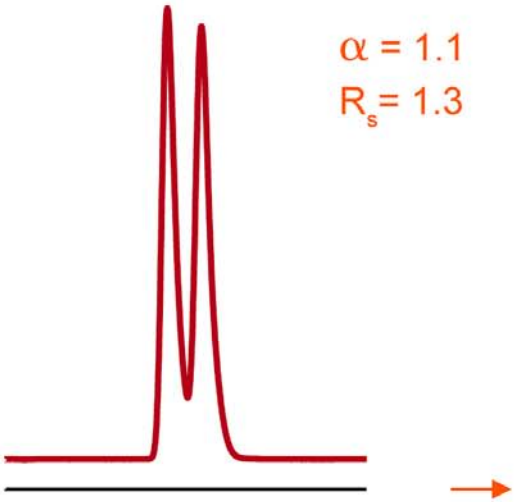


Baseline separation

Common conditions: trans-2-phenyl-1-cyclohexanol, heptane/isopropanol 95/5, 1 mL/min, UV@220 nm

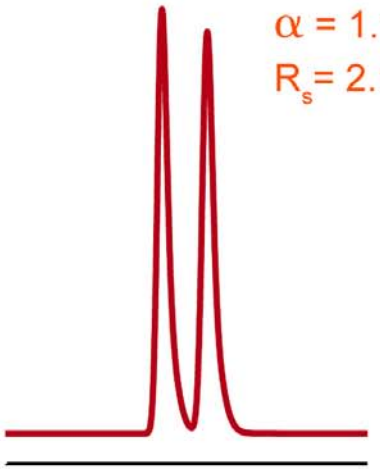
Selectivity and resolution – difficult separations

Particle size: **5** µm
Column length: 150 mm
Flow rate: 1 ml/min



$\alpha = 1.1$
$R_s = 1.3$

Particle size: **3** µm
Column length: 150 mm
Flow rate: 1 ml/min



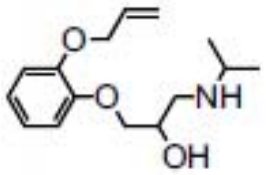
$\alpha = 1.1$
$R_s = 2.1$

Baseline separation using 3 µm particles

Common conditions
Solute: trans-2-Phenyl-1-cyclohexanol
Mobile phase: Heptane/2-Propanol (95/5)
Column diameter: 4.6 mm
Temperature: 25 °C
Detection: UV 220 nm

Selectivity and resolution –SFC

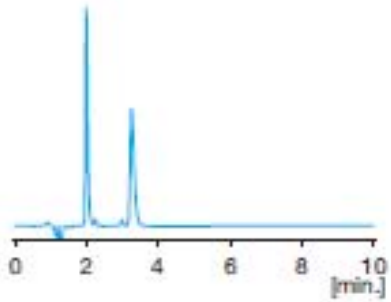
Oxprenolol



Conditions:

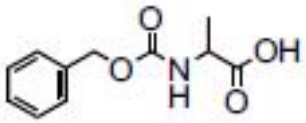
CelluCoat

Stationary phase:	Kromasil CelluCoat, 3 µm
Column size:	4.6 x 250 mm
Mobile phase:	CO_2/Methanol/IPEA (80/20/0.5)
Flow rate:	3 ml/min
Temperature:	30 °C
Outlet pressure:	100 bar
Detection:	UV 220 nm



k'_1	0.64
k'_2	1.68
N_1	27100
N_2	18700
α	2.6
R_S	8.8

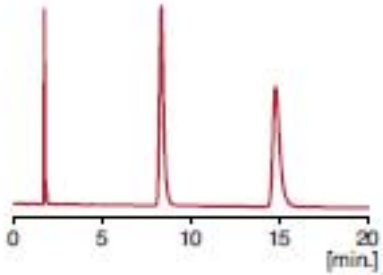
CBZ-Alanine



Conditions:

AmyCoat

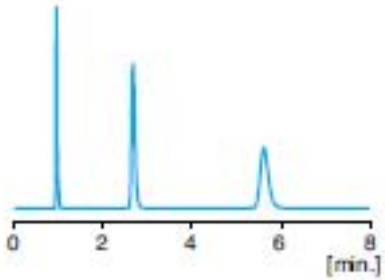
Stationary phase:	Kromasil AmyCoat, 3 µm
Column:	4.6 x 150 mm
Mobile phase:	Heptane/2-Propanol/TFA (90/10/0.1)
Flow rate:	1 ml/min
Temperature:	22 °C
Detection:	UV 215 nm



k'_1	3.78
k'_2	7.49
N_1	44800
N_2	50400
α	2.0
R_S	11.9

CelluCoat

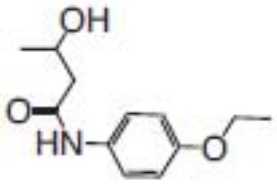
Stationary phase:	Kromasil CelluCoat, 3 µm
Column size:	4.6 x 150 mm
Mobile phase:	Heptane/2-Propanol/TFA (90/10/0.1)
Flow rate:	2 ml/min
Temperature:	22 °C
Detection:	UV 215 nm



k'_1	1.82
k'_2	4.93
N_1	40700
N_2	31200
α	2.7
R_S	12.7

Selectivity and resolution –SFC

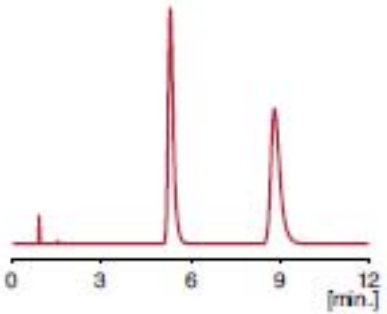
Bucetin



Conditions:

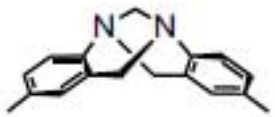
AmyCoat

Stationary phase:	*Kromasil AmyCoat, 3 µm*
Column:	*4.6 x 150 mm*
Mobile phase:	*Heptane/2-Propanol (90/10)*
Flow rate:	*2 ml/min*
Temperature:	*22 °C*
Detection:	*UV 254 nm*



k'_1	5.02
k'_2	9.01
N_1	30900
N_2	27700
α	1.8
R_S	8.2

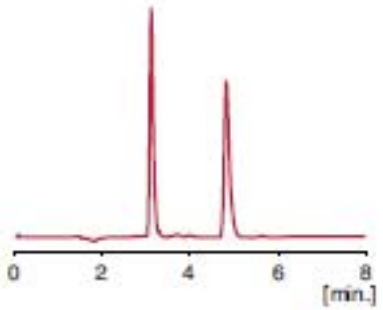
Tröger's Base



Conditions:

AmyCoat

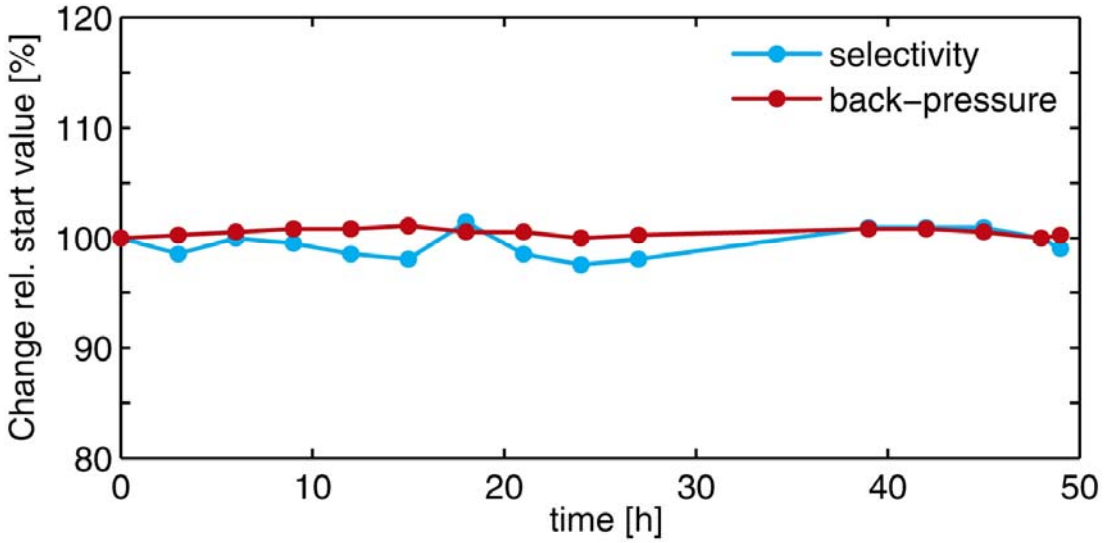
Stationary phase:	*Kromasil AmyCoat, 5 µm*
Column:	*4.6 x 250 mm*
Mobile phase:	*CO_2/Methanol (80/20)*
Flow rate:	*3 ml/min*
Temperature:	*40 °C*
Outlet pressure:	*150 bar*
Detection:	*UV 220 nm*



k'_1	1.38
k'_2	2.70
N_1	108200
N_2	109000
α	2.0
R_S	17.8

High flow rates – system stability

- The unique coating technology ensures maintained efficiency



Flow rate: 7 mL/min
Time: 49 h
Pressure: 310 bar
(~78000 col. volumes)

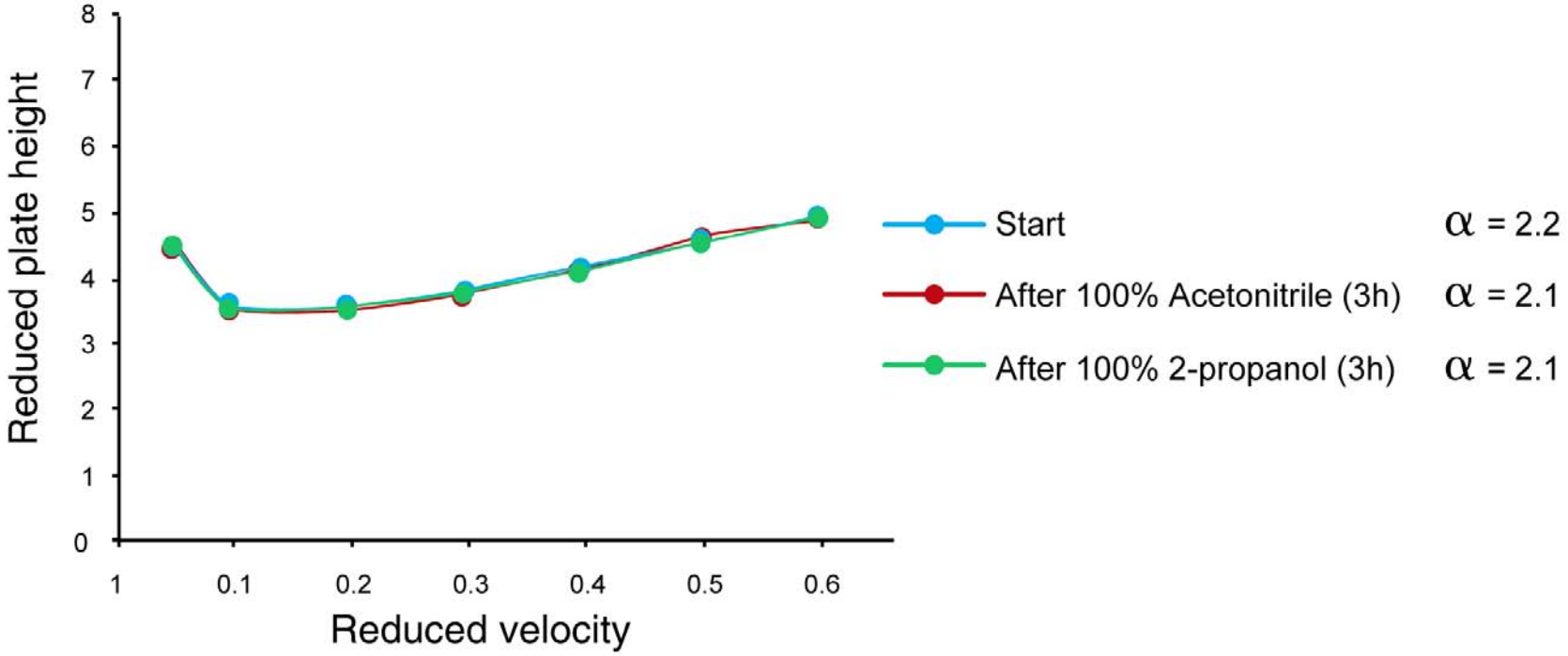
Conditions

Solute: trans-Stilbene oxide
Stationary phase: Kromasil CelluCoat, 3 µm
Column size: 4.6 x 50 mm
Mobile phase: Heptane/2-Propanol (90/10)
Flow rate: 7 ml/min
Temperature: 25 °C

* Kromasil CelluCoat can withstand flow rates equivalent to pressures of up to 400 bar—i.e. the approximate limit for most HPLC systems.

Maintained efficiency after extended use

- The unique coating technology ensures maintained efficiency – switching of solvents



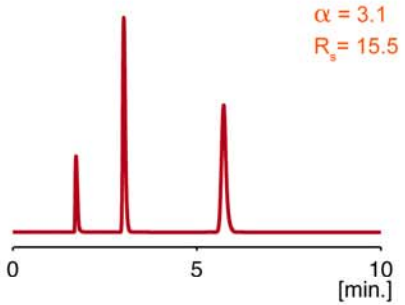
Common conditions

Solute: trans-Stilbene oxide
Stationary phase: Kromasil CelluCoat, 5 µm
Column size: 4.6 x 250 mm
Mobile phase: Heptane/2-Propanol (90/10)
Flow rates: 0.1-1.2 ml/min
Temperature: 25 °C

Maintained efficiency after extended use

Before

Solute: trans-Stilbene oxide

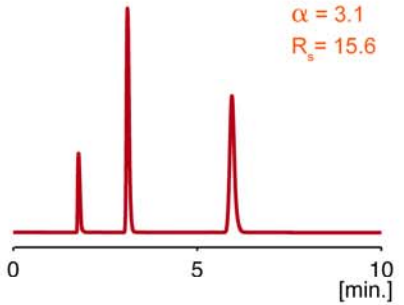


Test sequence

Mobile phase	Flow rate [ml/min]	Run time [h]	Total back-pressure [bar]
1. Heptane/2-Propanol (90/10)	4.3	2	345
2. 2-Propanol	0.7	2	260
3. Acetonitrile	1	2	58
4. Ethanol	1	2	188
5. Heptane/2-Propanol (90/10)	1	2	72

After

Solute: trans-Stilbene oxide

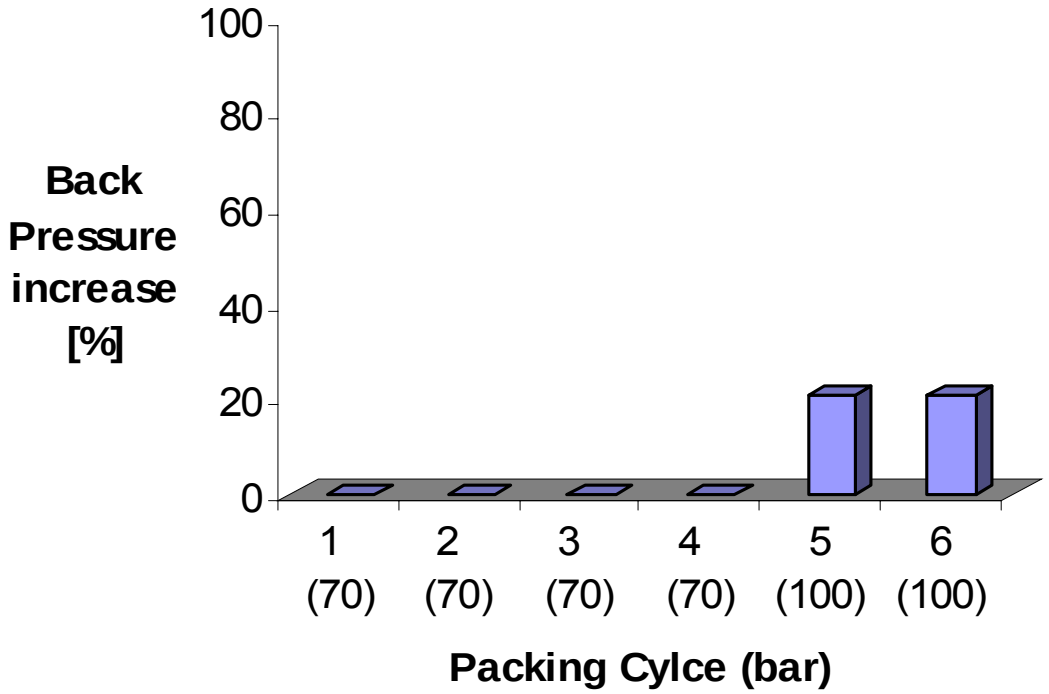


Common conditions
Stationary phase: Kromasil AmyCoat, 3 µm
Column size: 4.6 × 150 mm
Temperature: 25 °C

Mobile phase: Heptane/2-Propanol (90/10)
Flow rate: 1 ml/min
Detection: UV 229 nm

- Stable at high pressures and freedom to switch between solvents

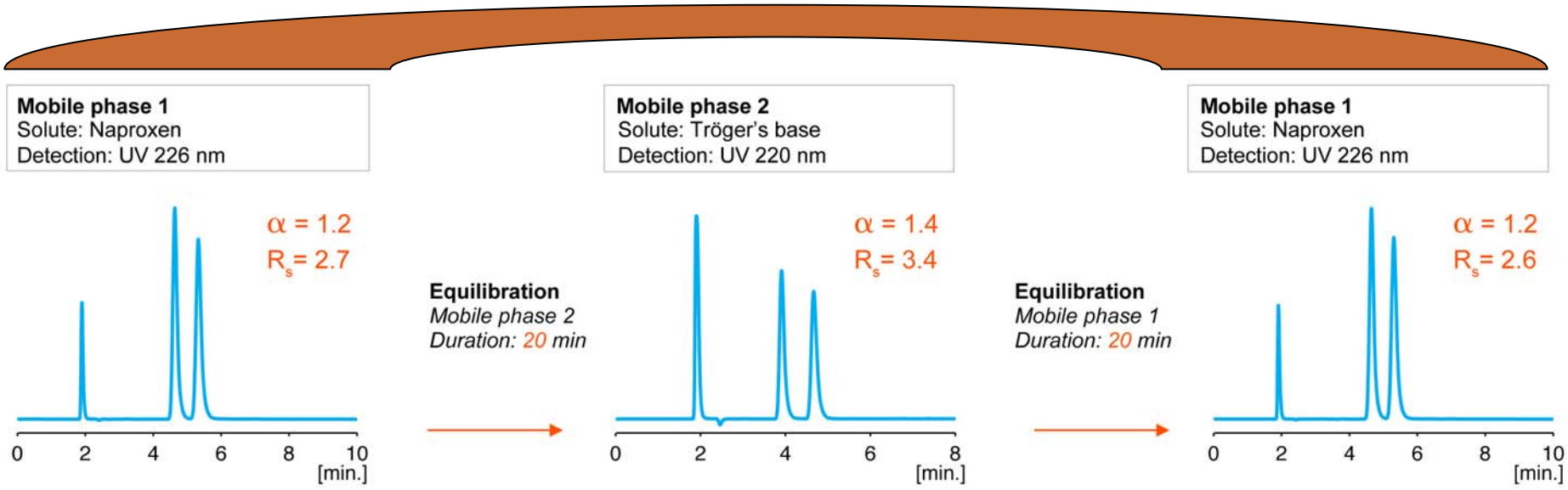
Mechanical strength – no deterioration



Back-pressure increase after repeated slurry-packing of Kromasil CelluCoat in a DAC column

Stability under acid-base switching – Kromasil CelluCoat

Identical results



Common conditions

Stationary phase: Kromasil CelluCoat, 3 µm
Column size: 4.6 x 150 mm

Mobile phase 1: Heptane/2-Propanol/TFA (90/10/0.1)
Mobile phase 2: Heptane/2-Propanol/DEA (90/10/0.1)

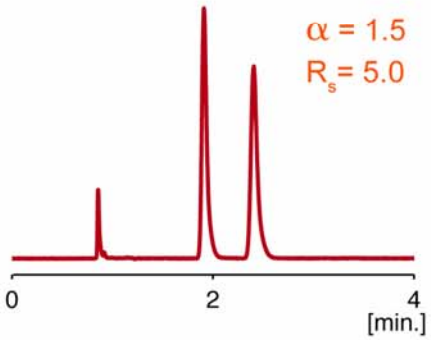
Flow rate: 1 ml/min
Temperature: 25 °C

- A specially designed silica and surface modification - no visible irreversible adsorption

Stability under acid-base switching – Kromasil AmyCoat

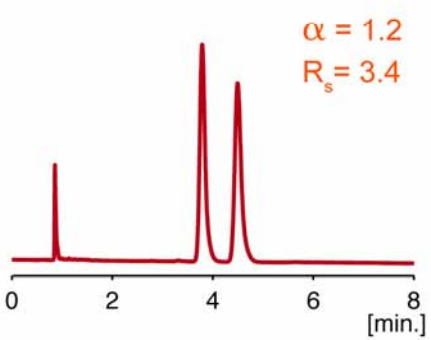
Identical results

Mobile phase 1
Solute: Carbinoxamine
Detection: UV 228 nm



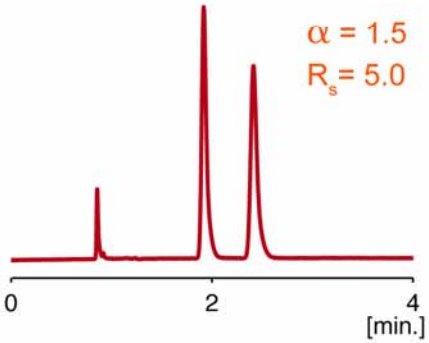
Equilibration
Mobile phase 2
Duration: 18 min

Mobile phase 2
Solute: Ketoprofen
Detection: UV 250 nm



Equilibration
Mobile phase 1
Duration: 12 min

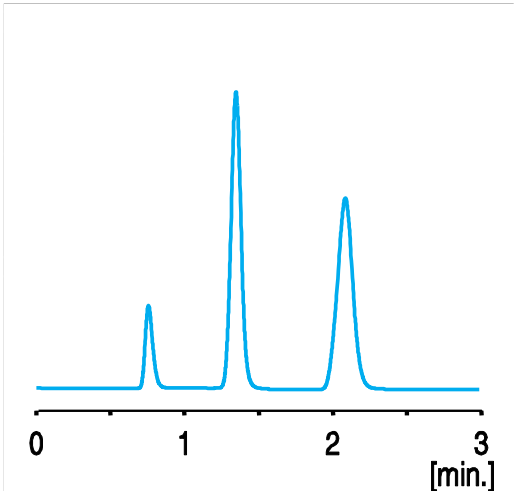
Mobile phase 1
Solute: Carbinoxamine
Detection: UV 228 nm



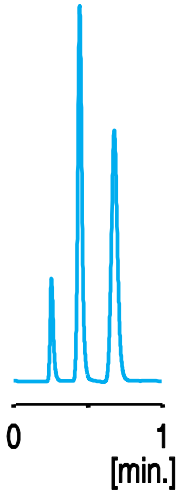
Common conditions
Stationary phase: Kromasil AmyCoat, 3 µm
Column size: 4.6 x 150 mm
Mobile phase 1: Heptane/2-Propanol/DEA (90/10/0.1)
Mobile phase 2: Heptane/2-Propanol/TFA (90/10/0.1)
Flow rate: 2 ml/min
Temperature: 25 °C

Kromasil CelluCoat™ 3 µm → High Speed Chromatography!

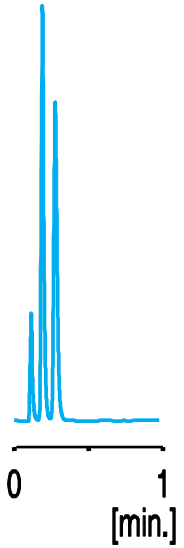
Column: Kromasil CelluCoat 3 µm 4.6 * 50 mm
trans-Stilbene Oxide in Heptane/IPA 90:10



1 mL/min



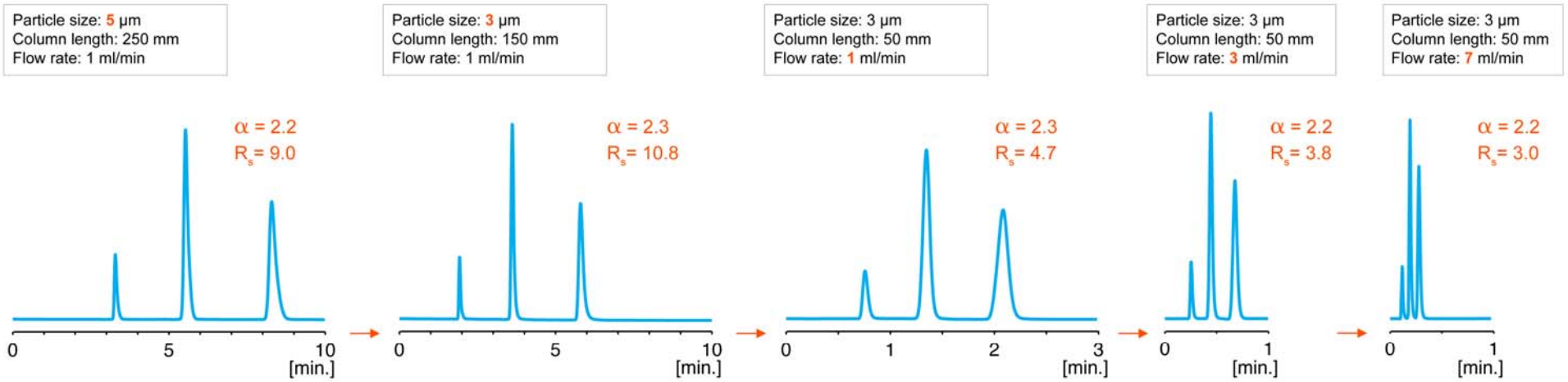
3 mL/min



7 mL/min

Baseline separation in 20 seconds!
Pressure: 310 bar
α = 2.2
R_s = 3.0

Kromasil CelluCoat™ 3 µm → High Speed Chromatography!

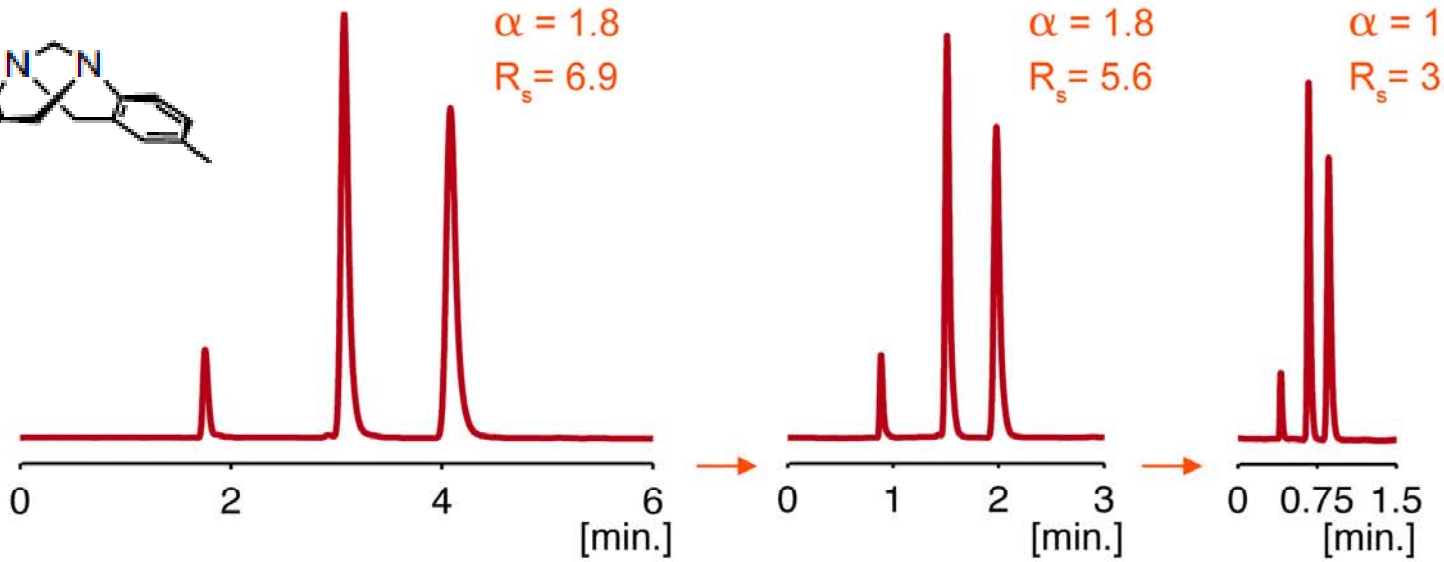


Common conditions
Solute: trans-Stilbene oxide
Mobile phase: Heptane/2-Propanol (90/10)
Column diameter: 4.6 mm
Temperature: 25 °C
Detection: UV 229 nm

Baseline separation in 20 seconds!
Reduction of analysis time >95 %
Pressure: 310 bar
α = 2.2
R_s = 3.0

Kromasil AmyCoat™ 3 µm → High Speed Chromatography!

Particle size: 3 µm Column length: 150 mm Flow rate: **1** ml/min	Particle size: 3 µm Column length: 150 mm Flow rate: **2** ml/min	Particle size: 3 µm Column length: 150 mm Flow rate: **4.5** ml/min



Common conditions

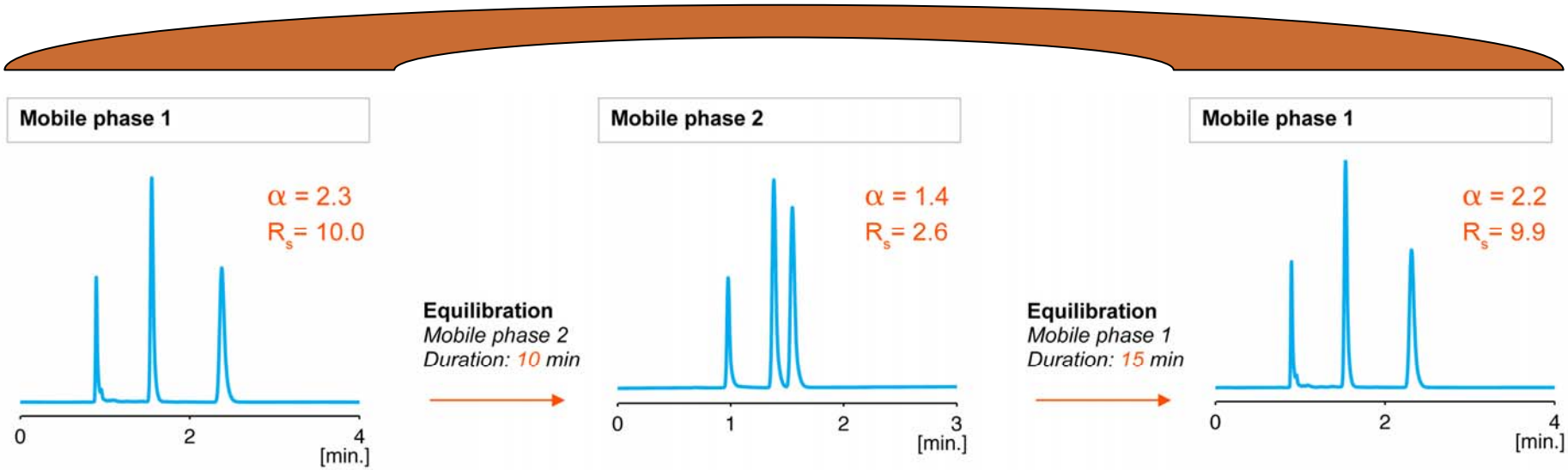
Solute: Tröger's base
Mobile phase: Heptane/2-Propanol/DEA (90/10/0.1)
Column diameter: 4.6 mm
Temperature: 25 °C
Detection: UV 220 nm

Kromasil CelluCoat™ and Amycoat™ 3 µm → High Speed Chromatography!

- Reduce the analysis time by 90% by exchanging a 250 mm column for a 50 mm column and increasing flow rate from 1 mL/min to 2 mL/min; increasing the flow rate to 7 mL/min will give you ultra-speed chromatography
- Increasing flow rate and decreasing column length results in reduced equilibration times
- Different Knox plots for 3 and 5 µm particles: most optimal to use 3 µm at elevated flows

Fast analysis – short equilibrium times
100% polar mode - Kromasil CelluCoat

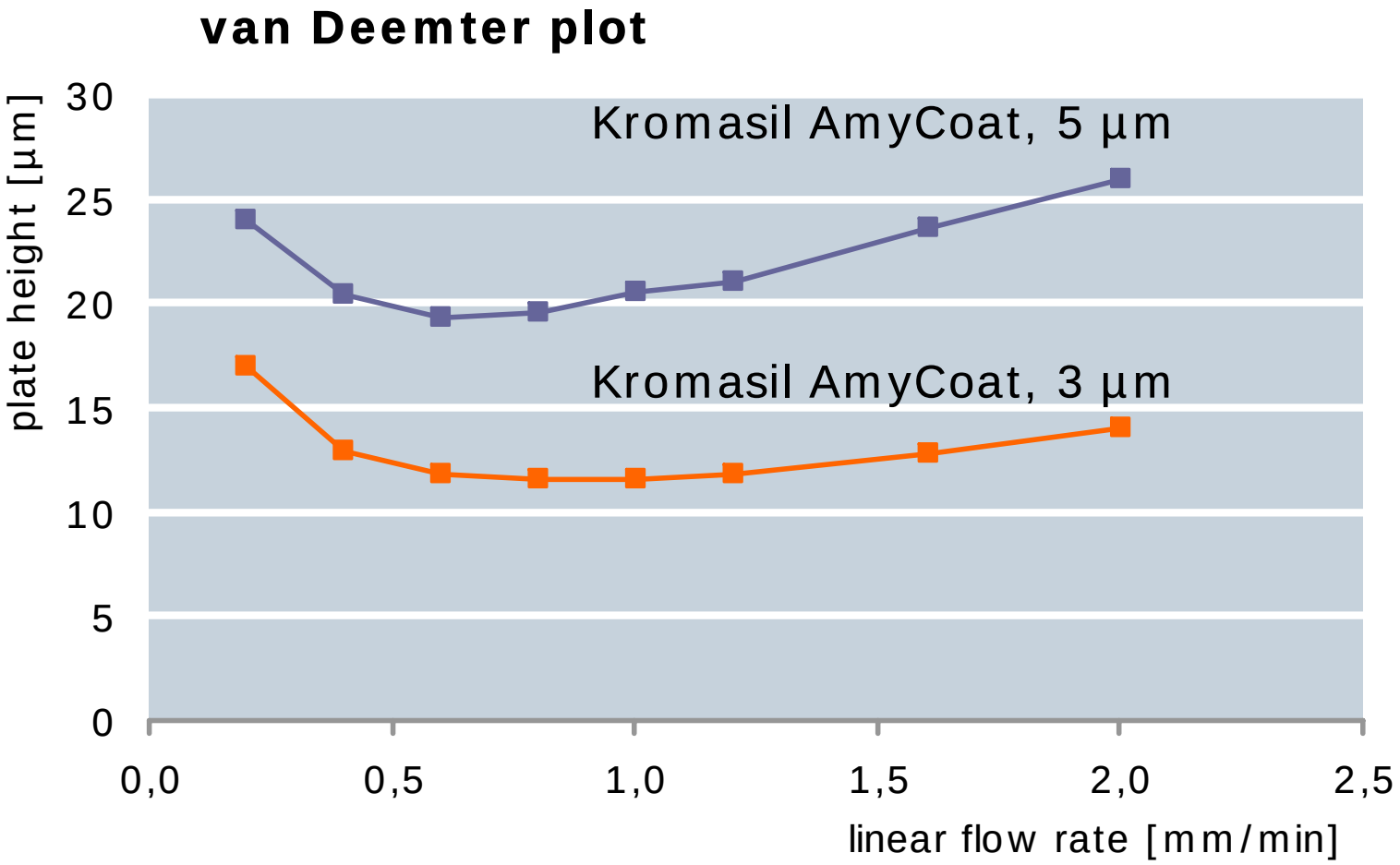
Identical results



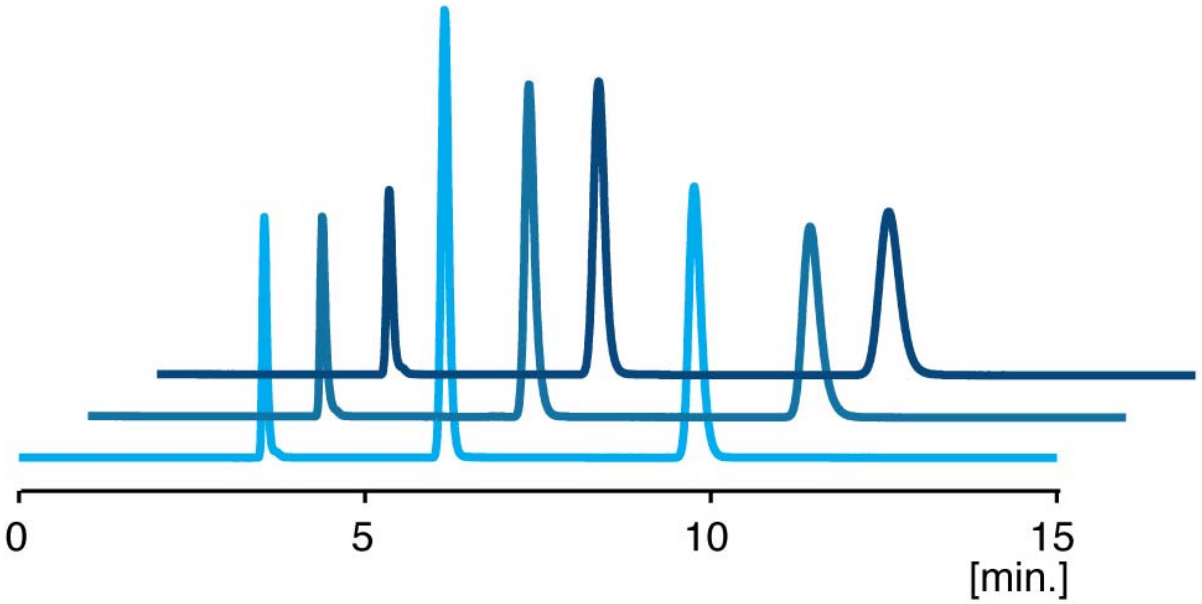
Common conditions

Solute: trans-Stilbene oxide
Detection: UV 229 nm
Stationary phase: Kromasil CelluCoat, 3 µm
Column size: 4.6 x 150 mm
Mobile phase 1: Heptane/2-Propanol (90/10)
Mobile phase 2: Ethanol
Flow rate: 2 ml/min
Temperature: 25 °C

Fast analysis – use the appropriate particle size



Scale-up – consistent results



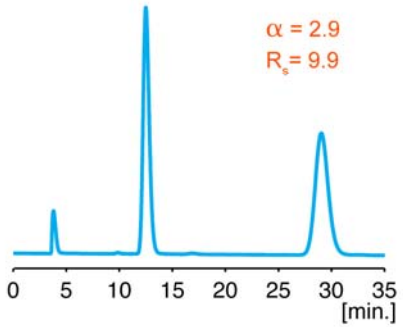
- Kromasil 10-CelluCoat
  α = 2.4 R_s = 10.0
- Kromasil 5-CelluCoat
  α = 2.4 R_s = 11.4
- Kromasil 3-CelluCoat
  α = 2.4 R_s = 13.9

Common conditions

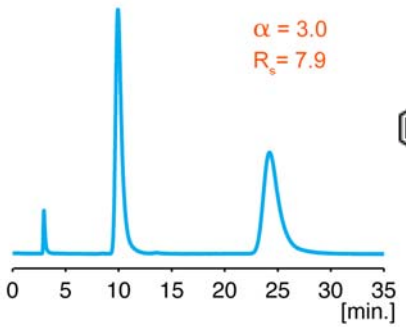
Column size: 4.6 × 150 mm
Mobile phase: Heptane/2-Propanol (90/10)
Flow rate: 0.5 ml/min
Temperature: 25 °C
Solute: trans-Stilbene oxide
Detection: UV 229 nm

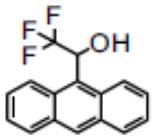
Scale-up – DAC packing

Conditions:
Column size: 4.6 × 150
Flow rate: 0.5 ml/min



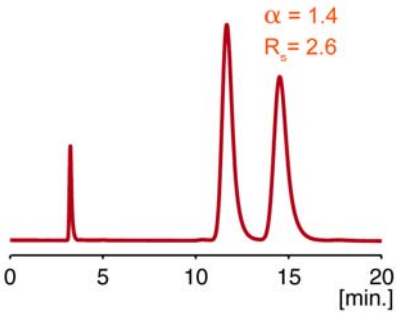
Conditions:
DAC system: NovaSep Pack-n-Sep, 50 mm i.d.
Bed length: 132 mm
Flow rate: 60 ml/min



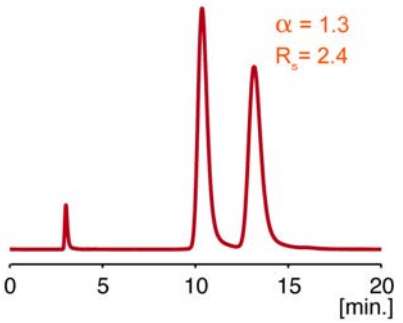


Common conditions
Stationary phase: Kromasil CelluCoat, 10 µm
Mobile phase: Heptane/2-Propanol (90/10)
Solute: Trifluoro-anthrylethanol
Detection: UV 254 nm
Temperature: 20 °C

Conditions:
Column size: 4.6 × 150 mm
Flow rate: 0.5 ml/min



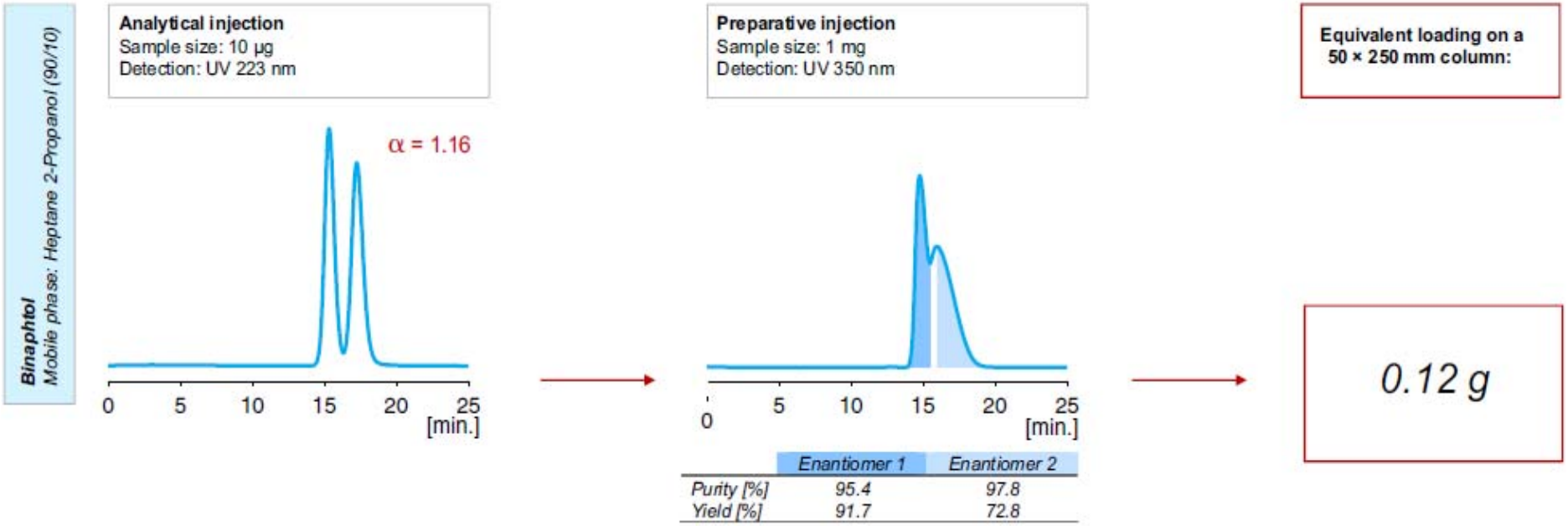
Conditions:
DAC system: NovaSep Pack-n-Sep, 50 mm i.d.
Bed length: 135 mm
Flow rate: 60 ml/min



Common conditions
Stationary phase: Kromasil AmyCoat, 10 µm
Mobile phase: Heptane/2-Propanol (90/10)
Solute: Trifluoro-anthrylethanol
Detection: UV 254 nm
Temperature: 20 °C

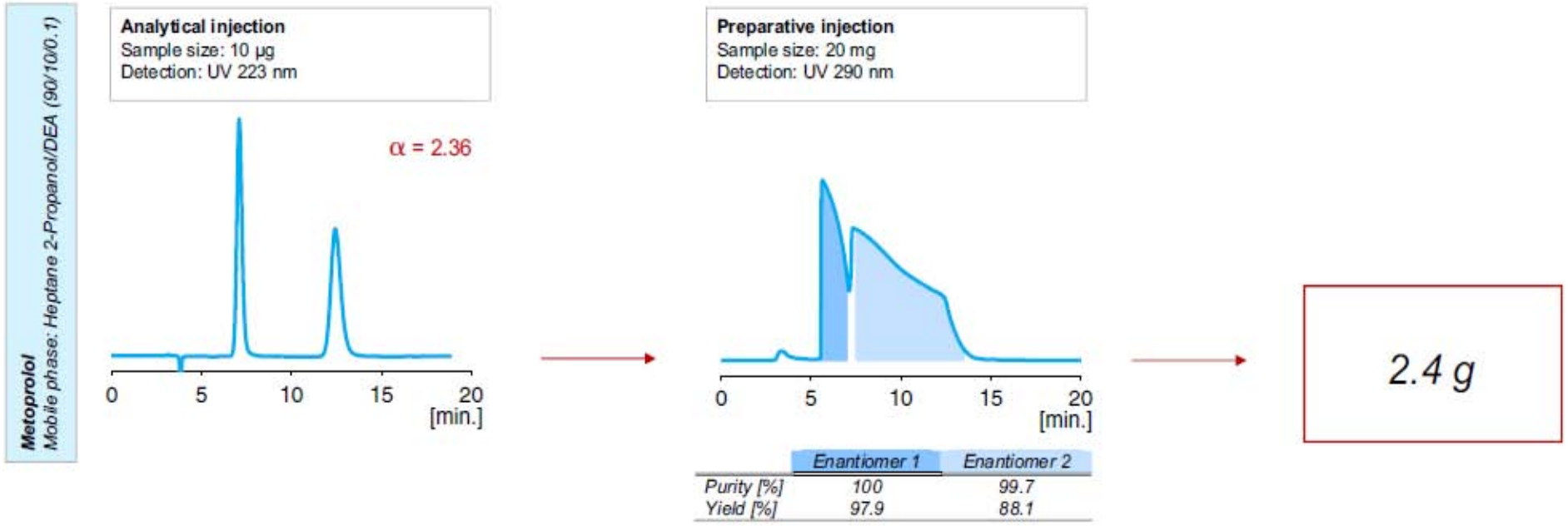
Scale-up – Overloaded injection on CelluCoat

- Preparative injection of Binaphtol

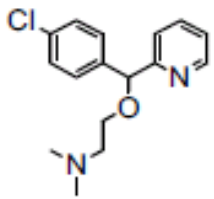


Scale-up- Overloaded injection on CelluCoat

- Preparative injection of Metoprolol

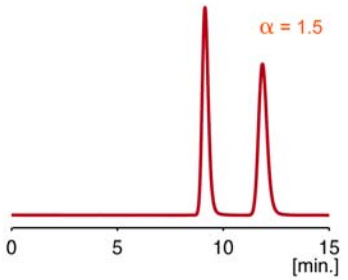


Scale-up- Overloaded injection on AmyCoat

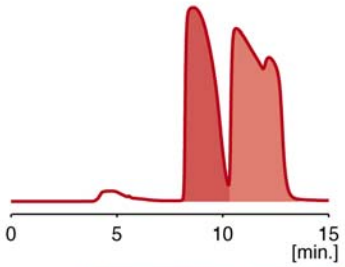


Carbinoxamine
Mobile phase: Heptane/2-Propanol/DEA (90/10/0.1)
Flow rate: 0.7 ml/min

Analytical injection
Sample size: 10 µg
Detection: UV 254 nm



Preparative injection
Sample size: 7.5 mg
Detection: UV 280 nm



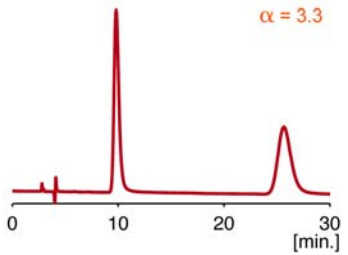
	Enantiomer 1	Enantiomer 2
Purity [%]	100	99.7
Yield [%]	99.7	100

Equivalent loading on a 50 × 250 mm column:

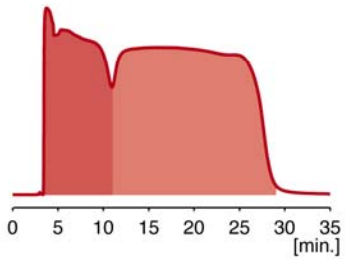
0.9 g

Warfarin
Mobile phase: Heptane/2-Propanol (85/15)
Flow rate: 1 ml/min

Analytical injection
Sample size: 8.5 µg
Detection: UV 230 nm



Preparative injection
Sample size: 17.1 mg
Detection: UV 325 nm



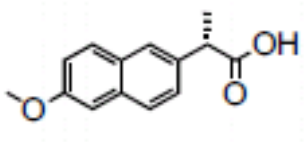
	Enantiomer 1	Enantiomer 2
Purity [%]	99.7	99.5
Yield [%]	99.4	99.7

2.0 g

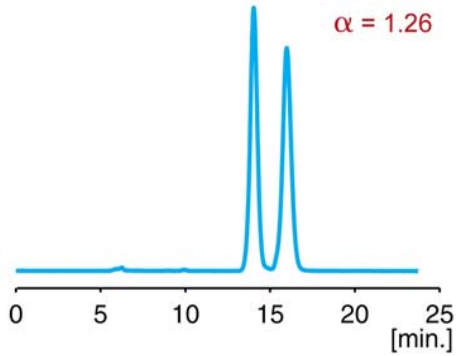
Common conditions
Stationary phase: Kromasil AmyCoat 10 µm
Column size: 4.6 x 250 mm
Temperature: 25 °C

Scale-up – Comparative example

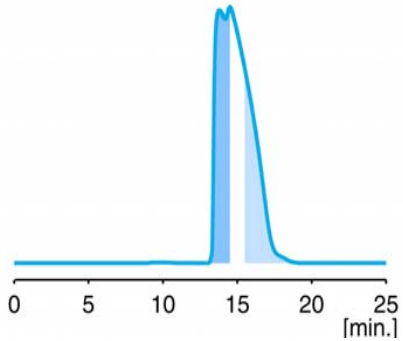
Kromasil CelluCoat 10 µm



Analytical injection: 20 µg

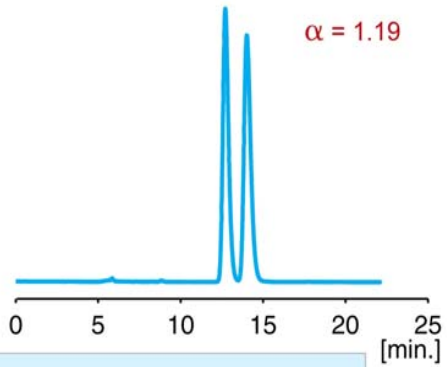


Preparative injection: 5 mg
Collected fractions size: 0.5 min

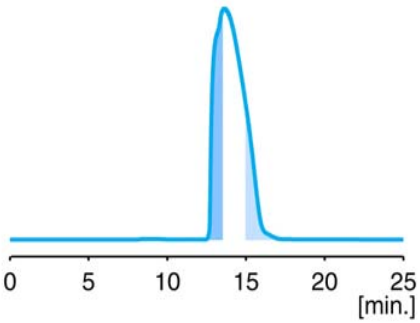


Chiralcel OD 10 µm

Analytical injection: 20 µg



Preparative injection: 5 mg
Collected fractions size: 0.5 min

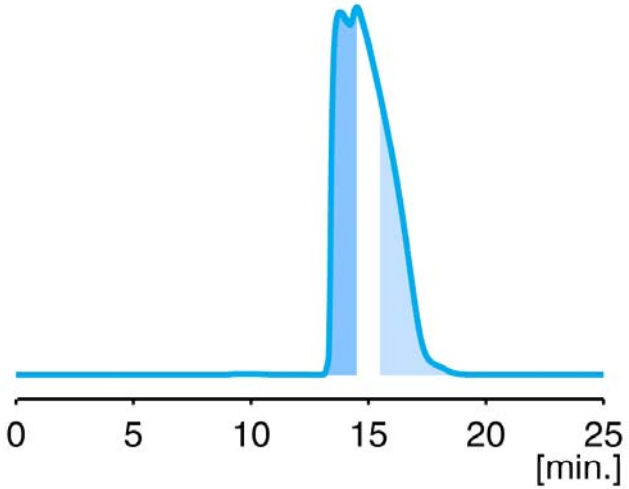


Common conditions
Solute: Naproxen
Mobile phase: Heptane/2-Propanol/TFA (90/10/0.1)
Column size: 4.6 x 250 mm
Temperature: 25 °C
Flow rate: 1 ml/min

Scale-up – Comparative example

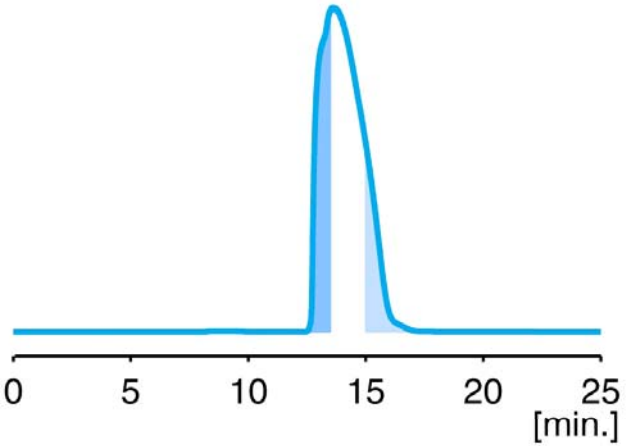
Kromasil CelluCoat 10 µm

Preparative injection: 5 mg
Collected fractions size: 0.5 min



Chiralcel OD 10 µm

Preparative injection: 5 mg
Collected fractions size: 0.5 min

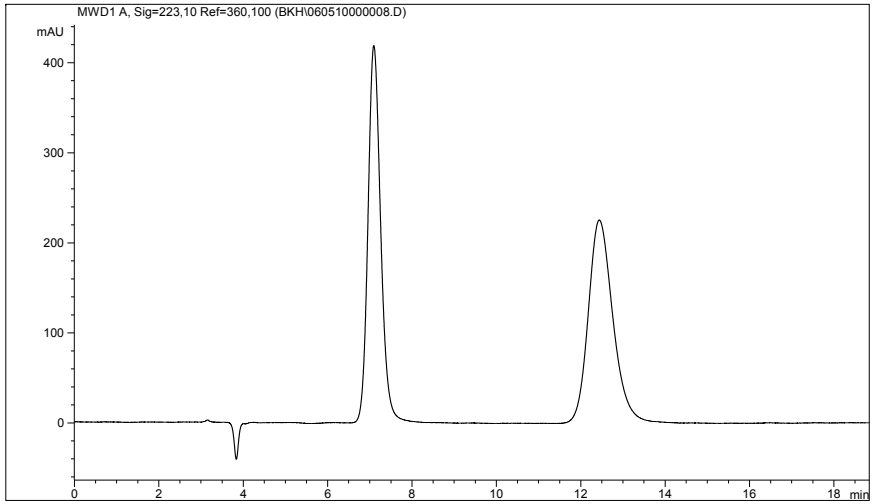


	Enantiomer 1		Enantiomer 2	
	Purity [%]	Yield [%]	Purity [%]	Yield [%]
Kromasil CelluCoat	91.2	73.3	94.4	50.1
Daicel Chiralcel OD	91.4	46.7	96.6	24.9

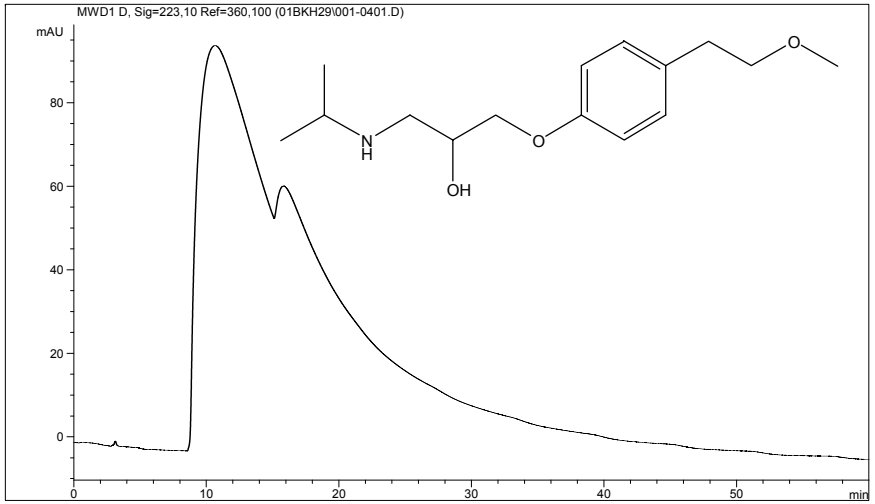
Scale up – With/without additive

- General facts
  - Frequently used in order to improve peak shape and hence increase the efficiency of the column
  - Analytical-prep differences in α
  - Complicates solvent recycling process
  - Stability problems, racemization, additives are accumulated during evaporation

Scale up – With/without additive



Heptane/2-propanol 90/10 0.1% DEA
Flow: 1mL/min, UV @ 223 nm
Kromasil-10-CelluCoat 4.6x250 mm



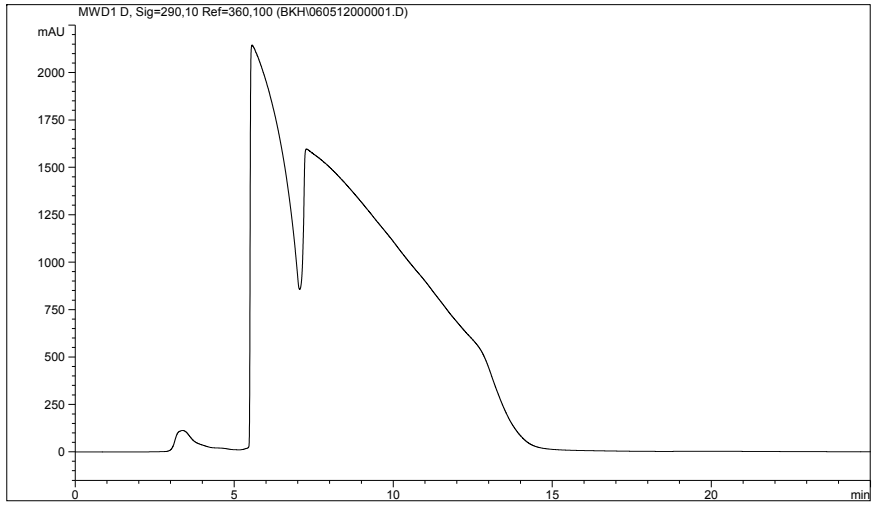
Heptane/2-propanol 90/10
Flow: 1 mL/min, UV @ 223 nm
Kromasil-5-CelluCoat 4.6x250 mm

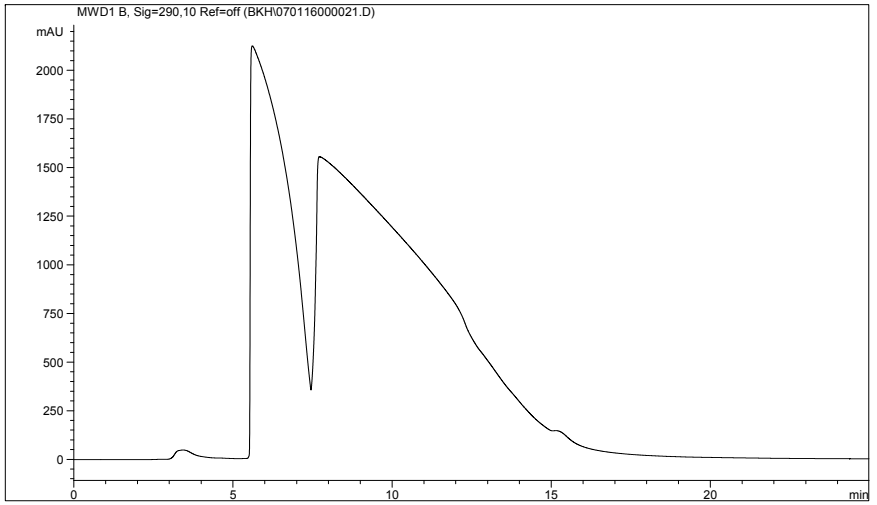
Conclusion:

Additive is needed for CelluCoat –

a disadvantage in prep/semi prep

Scale up – With/without additive

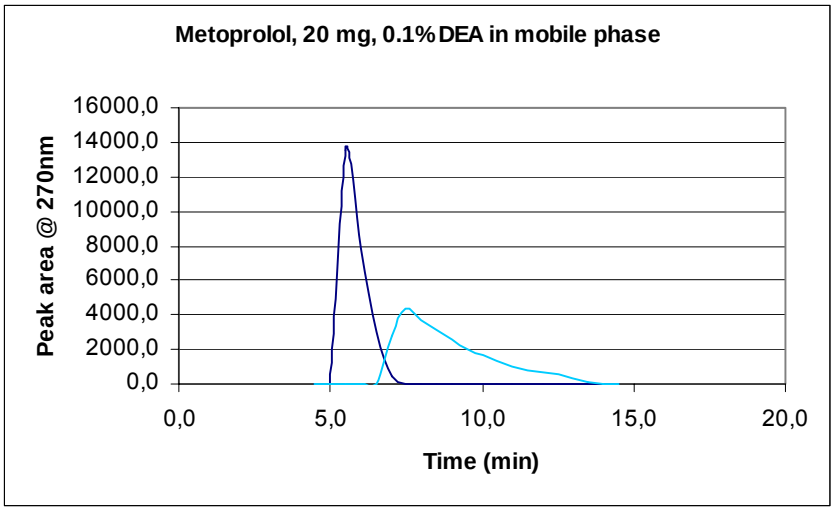


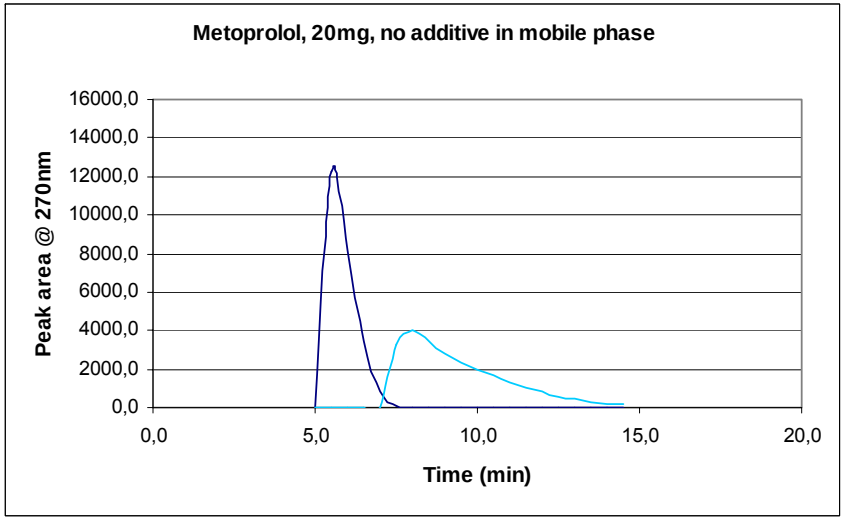


Preparative sample: Metoprolol free base 40 mg/mL
Mobile Phase: Heptane/2-propanol 90/10
Preparative load: 500 µl, 20 mg
Column: Kromasil-10-CelluCoat 4.6 x 250 mm
Mobile phase: Heptane/2-propanol 90/10, with and without 0.1% DEA as additive
Flow rate: 1.0 mL/min
Temperature: 25°C
Preparative detection: UV @ 290nm

Scale up – With/without additive

- Elution profile reconstructed from fraction analysis data





Pool	Purity	Recovery
1	100,00%	97,91%
2	99,73%	88,11%

Pool	Purity	Recovery
1	100,00%	99,49%
2	99,48%	97,42%

Scale up – With/without additive

RECOMMENDATION:

- Analytical injections: always use additive for basic compounds
- Preparative injections: large amounts ➔ self correction

Product assortment

Kromasil CelluCoat - Product code/part no.					
	4.6 x 50 mm	**4.6 x 150 mm**	**4.6 x 250mm**	**10 x 250 mm**	**21.1 x 250 mm**
3µm - Kromasil CelluCoat	KROMASIL 3-CelluCoat 4.6 x 50 mm	KROMASIL 3-CelluCoat 4.6 x 150 mm	KROMASIL 3-CelluCoat 4.6 x 250 mm	—	—
5µm - Kromasil CelluCoat	KROMASIL 5-CelluCoat 4.6 x 50 mm	KROMASIL 5-CelluCoat 4.6 x 150 mm	KROMASIL 5-CelluCoat 4.6 x 250 mm	KROMASIL-5-CelluCoat 10 x 250 mm	KROMASIL 5-CelluCoat 21.2 x 250 mm
10µm - Kromasil CelluCoat	—	KROMASIL 10-CelluCoat 4.6 x 150 mm	KROMASIL 10-CelluCoat 4.6 x 250 mm	KROMASIL-10-CelluCoat 10 x 250 mm	KROMASIL 10-CelluCoat 21.2 x 250 mm

Kromasil AmyCoat - Product code/part no.					
	4.6 x 50 mm	**4.6 x 150 mm**	**4.6 x 250mm**	**10 x 250 mm**	**21.1 x 250 mm**
3µm - Kromasil AmyCoat	KROMASIL 3-AmyCoat 4.6 x 50 mm	KROMASIL 3-AmyCoat 4.6 x 150 mm	KROMASIL 3-AmyCoat 4.6 x 250 mm	—	—
5µm - Kromasil AmyCoat	KROMASIL 5-AmyCoat 4.6 x 50 mm	KROMASIL 5-AmyCoat 4.6 x 150 mm	KROMASIL 5-AmyCoat 4.6 x 250 mm	KROMASIL-5-AmyCoat 10 x 250 mm	KROMASIL 5-AmyCoat 21.2 x 250 mm
10µm - Kromasil AmyCoat	—	KROMASIL 10-AmyCoat 4.6 x 150 mm	KROMASIL 10-AmyCoat 4.6 x 250 mm	KROMASIL-10-AmyCoat 10 x 250 mm	KROMASIL 10-AmyCoat 21.2 x 250 mm

- Other column dimensions available upon request
- 10 µm also available in bulk

Conclusions

What has been developed is:

- A new Super Wide Pore silica tailor-made for chiral chromatography
- A unique coating technology for perfectly homogeneous distribution of a polymer in porous materials
- A new coated cellulose-based chiral phase having improved properties
- A new coated amylose-based chiral phase having improved properties

Conclusions

Properties of the CSP:

- A chiral phase based on 3 µm particles for fast analysis (< 1 min)
- High loadability in prep applications
- High stability towards pressure and flow rate

Conclusions

- Kromasil can offer CSP:s for a wide range of applications both for analysis and purifications
- Two general phases, Kromasil AmyCoat and Kromasil CelluCoat
- Two specific phases, TBB and DMB, with very high chemical stability

Kromasil product assortment 2007

Kromasil 60 Å

Phases	Particle sizes, µm 3.5	5	7	10	13	16
SIL		■	■	■	■	■
CN		■		■		■
Diol		■		■		■

Kromasil 100 Å

Phases	Particle sizes, µm 3.5	5	7	10	13	16
SIL	■	■	■	■	■	■
C4	■	■	■	■	■	■
C8	■	■	■	■	■	■
C18	■	■	■	■	■	■
NH2	■	■	■	■	■	■
Phenyl		■		■		■

Kromasil 300 Å

Phases	Particle sizes, µm 5	10	16
SIL	■	■	■
C4	■	■	■
C8	■	■	■
C18	■	■	■

Kromasil Chiral

Phases	Particle sizes, µm 3	5	10	16
DMB			■	■
TBB			■	■
CelluCoat	■	■	■	
AmyCoat	■	■	■	

■ *Available as bulk or in pre-packed columns*

■ *Only available in pre-packed columns*

Availability Kromasil columns

Kromasil HPLC columns

- High pressure slurry-packed columns are with analytical performance
- Particle sizes available: from 3/3.5 to 16 µm
- Column diameters available: 2.1, 4.6, 10, 21.2 & 50 mm
- Column lengths available: 50, 100, 150 and 250 mm

For detailed availability, check our website: www.kromasil.com