



Extending the SFC applicability in the field of enantioselective separations

Eric Francotte and Georg Diehl

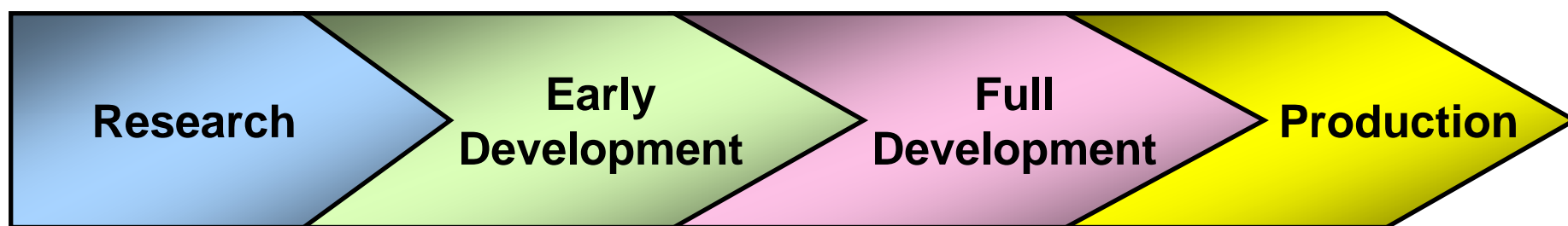
2nd International Conference on Packed-Column SFC, Zurich,
1-2. October 2008



Development Cycle of Chiral Drugs

About 70% of all new drugs under development are chiral !

Discovery → **Marketing**

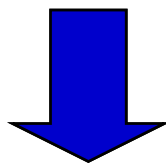


- **Synthesis**
- **Biological activity**
- **Chemistry**
- **Pharmacology**
- **Toxicology**
- **Formulation**
- **Clinical trials**
- **Quality control**

Requirements for Development of Chiral Drugs

What do we need today !

- *Rapid analysis of optical purity*
- *Rapid access to 5 mg -1 g of single stereoisomers for biological testing*
- *Rapid access to 2-50 g for lead optimization*
- *Rapid access to 100 g to 1 kg for lead optimization and toxicological studies*
- *Economically viable options to classical approaches of preparation of pure stereoisomers (including validated analytical methods)*
- *Competitive production tools for manufacturing of pure stereoisomers*

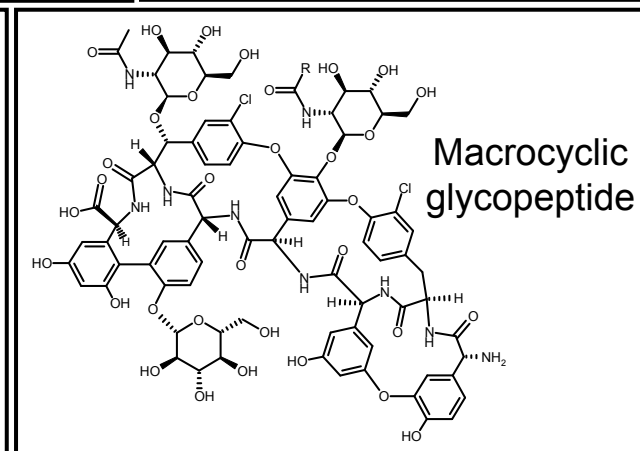
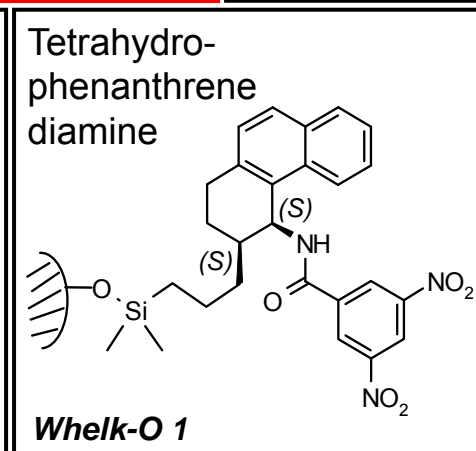
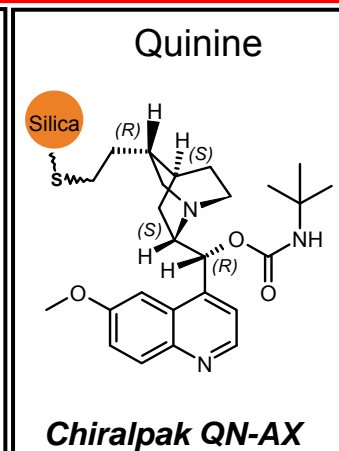
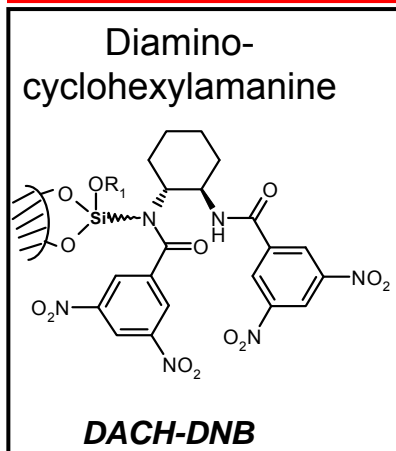
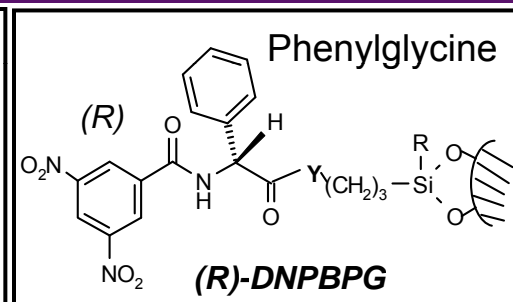
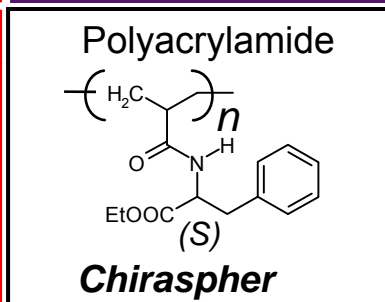
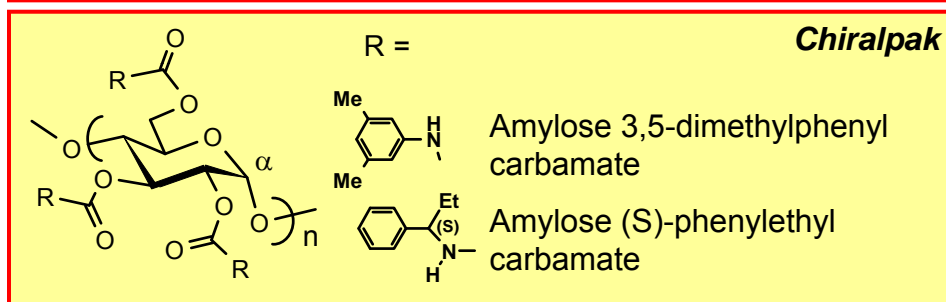
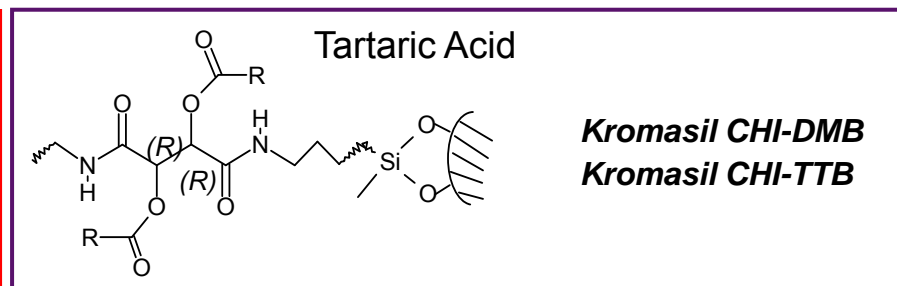
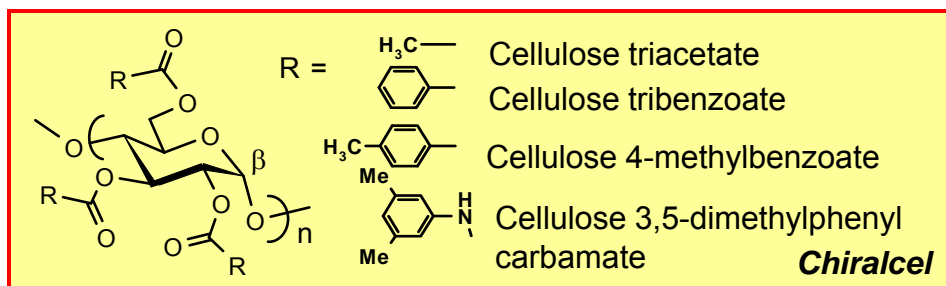


Chromatography can do it!

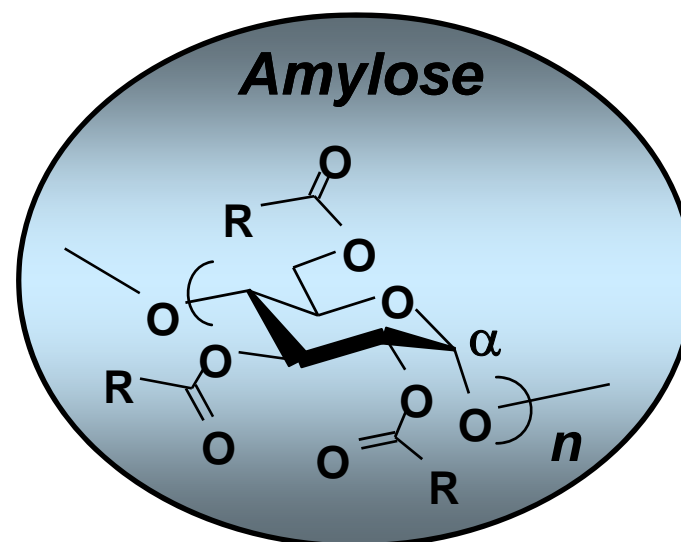
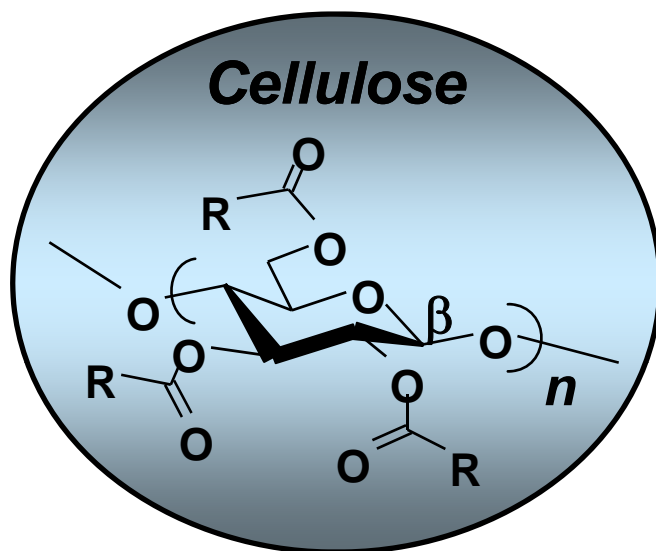
Enantioselective Chromatographic Techniques

- **Gas chromatography**
- **High Performance Liquid chromatography**
- **Supercritical fluid chromatography**
- **Electrophoresis**
- **Micellar electrokinetic chromatography**
- **Capillary electrochromatography**
- **Simulated moving bed chromatography**
- **Centrifugal partition chromatography (CCC, CPC)**
- **Membrane technology**

Most Used CSPs in HPLC and SFC



Cellulose- and Amylose-based CSPs

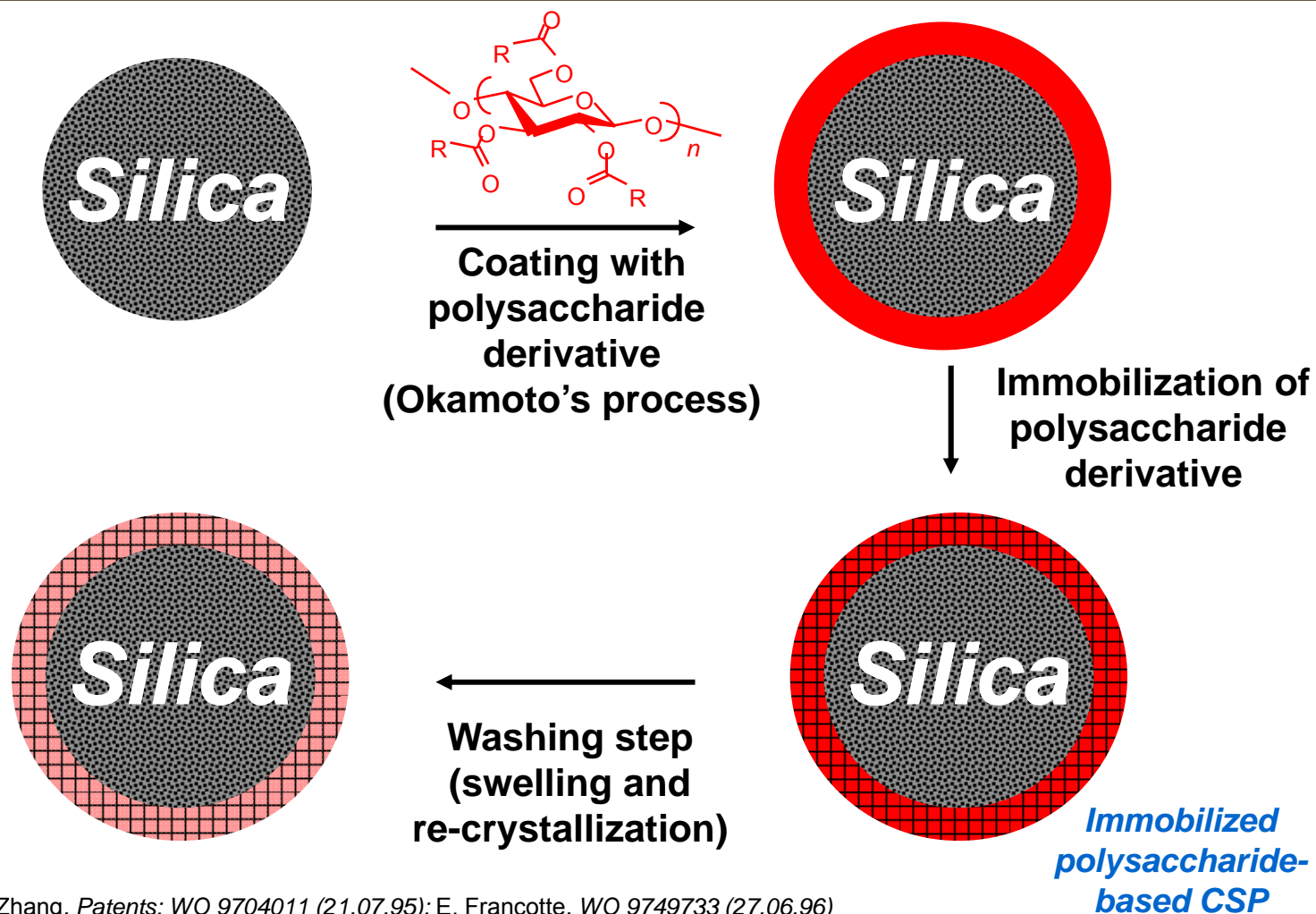


Can resolve ~ 90 % of all racemic compounds but show some limitations for preparative applications due to the high solubility of the chiral polymer selector in many organic solvents used for chromatographic applications



- limited possibility of improving the selectivity by varying the mobile phase
- limited (poor) solubility of the racemate (essential for preparative separations)
- limited possibility of adjusting the capacity factors k'

Immobilization Concept

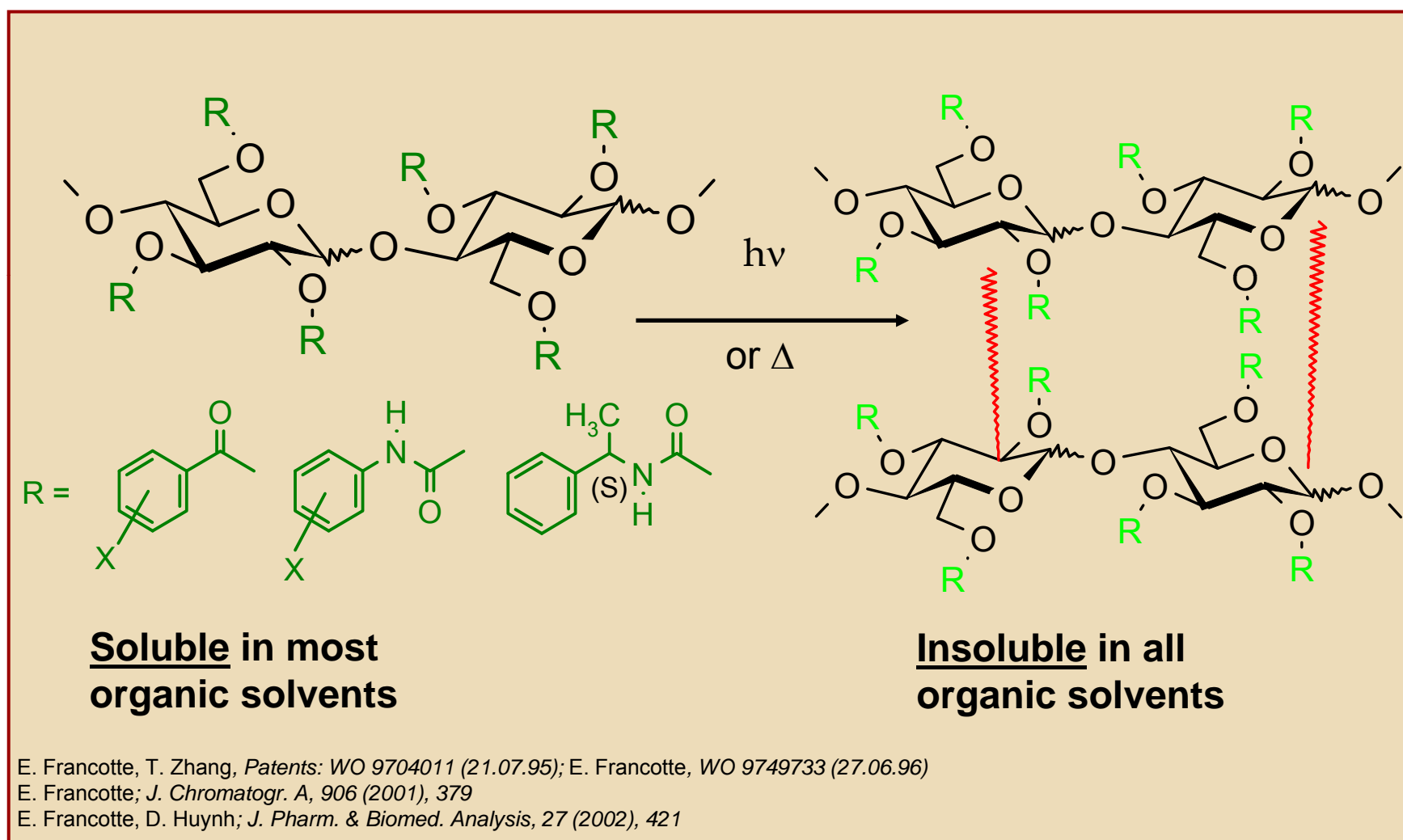


E. Francotte, T. Zhang, *Patents: WO 9704011 (21.07.95); E. Francotte, WO 9749733 (27.06.96)*

E. Francotte; *J. Chromatogr. A*, 906 (2001), 379

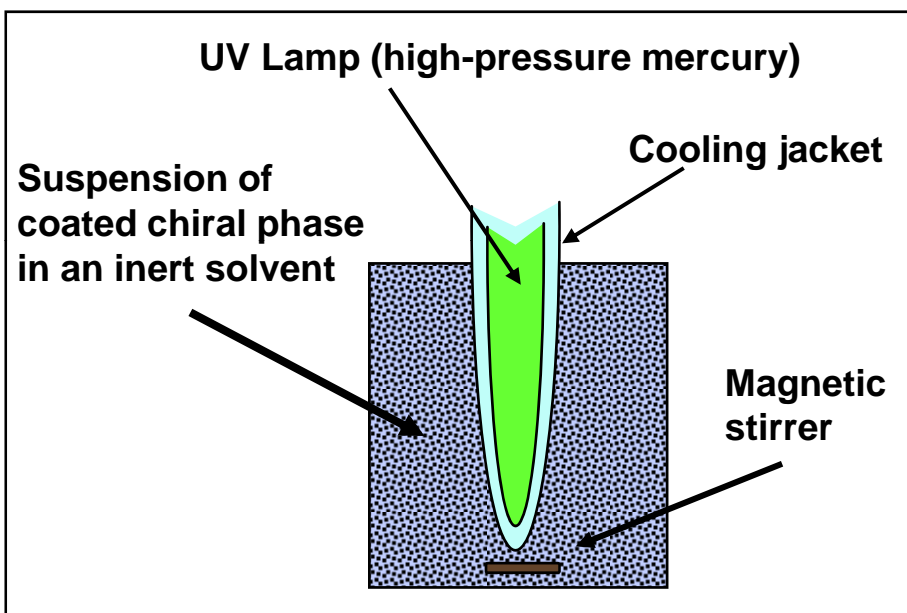
E. Francotte, D. Huynh; *J. Pharm. & Biomed. Analysis*, 27 (2002), 421

Immobilized Halogenophenylcarbamates of Cellulose



Immobilization Process

Photochemical ($h\nu$) process

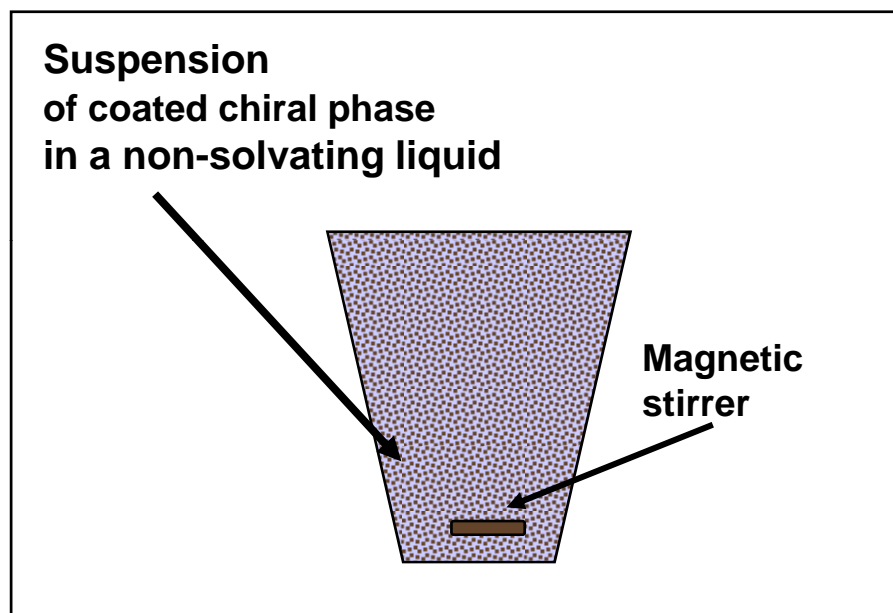


Radical source: high-pressure mercury

Suspension solvent: Acetonitrile
Methanol/water

Temperature: RT

Thermal process



Radical source: t-BPO CC(C)(C)OC(=O)OC(C)(C)C

Suspension solvent: Nonane
1,2-Dichlorobenzene

Temperature: 130° C

Immobilized Polysaccharide-CSPs

Chiral Stationary Phase

Cellulose 3,5-dimethylphenyl carbamate (OD)

Amylose 3,5-dimethylphenyl carbamate (AD)

Cellulose 4-methylphenyl carbamate (OG)

Cellulose 4-methylbenzoate (OJ)

Cellulose 3-Cl, 4-methylphenyl carbamate

Cellulose 4-chlorophenyl carbamate (OF)

Cellulose 3-methylbenzoate

Cellulose 3,5-dichlorophenyl carbamate

Amylose (S)-phenylethyl carbamate (AS)

Cellulose phenylcarbamate (OC)

Immobilization process

Peroxide in 1,2 dichlorobenzene

Peroxide in nonane, 130° C

Peroxide in nonane, 130° C

Peroxide in nonane, 130° C

UV in MeOH/water

UV in MeOH/water

UV in MeOH/water

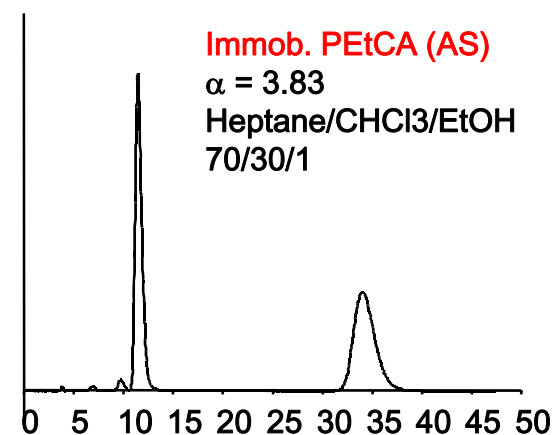
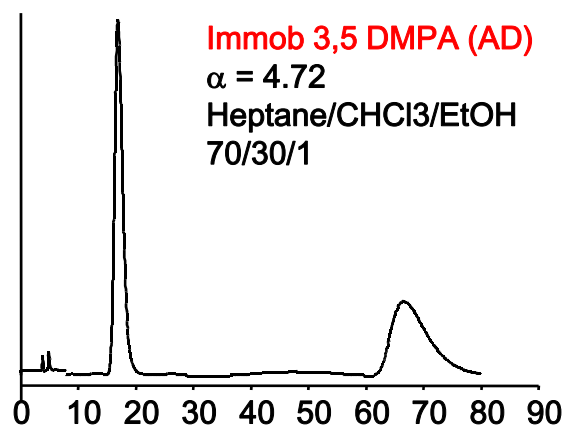
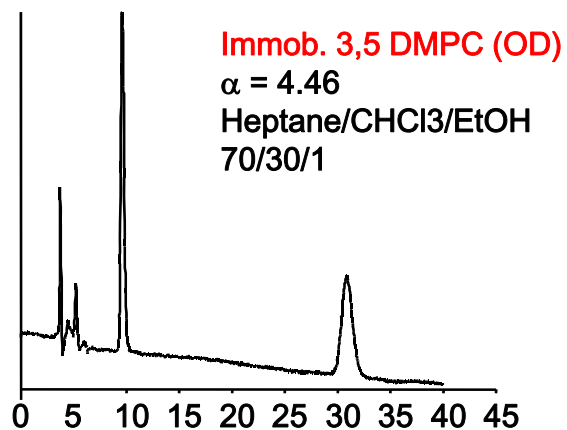
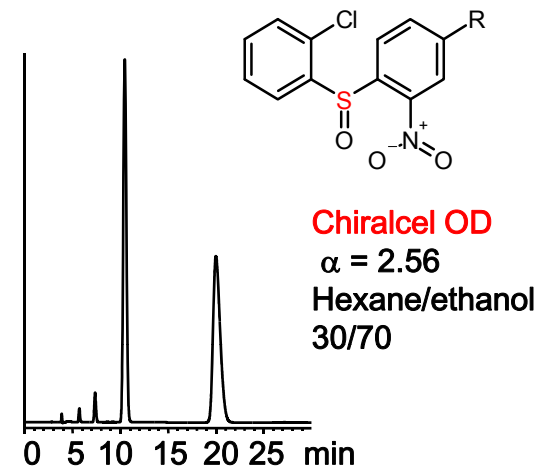
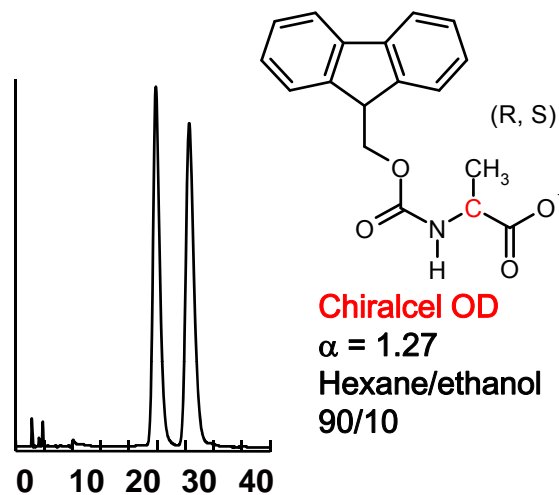
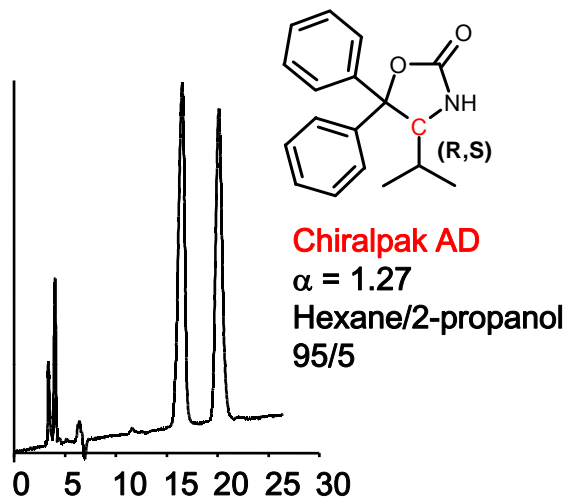
UV in acetonitrile

UV (+ thioxanthone) in MeOH/water

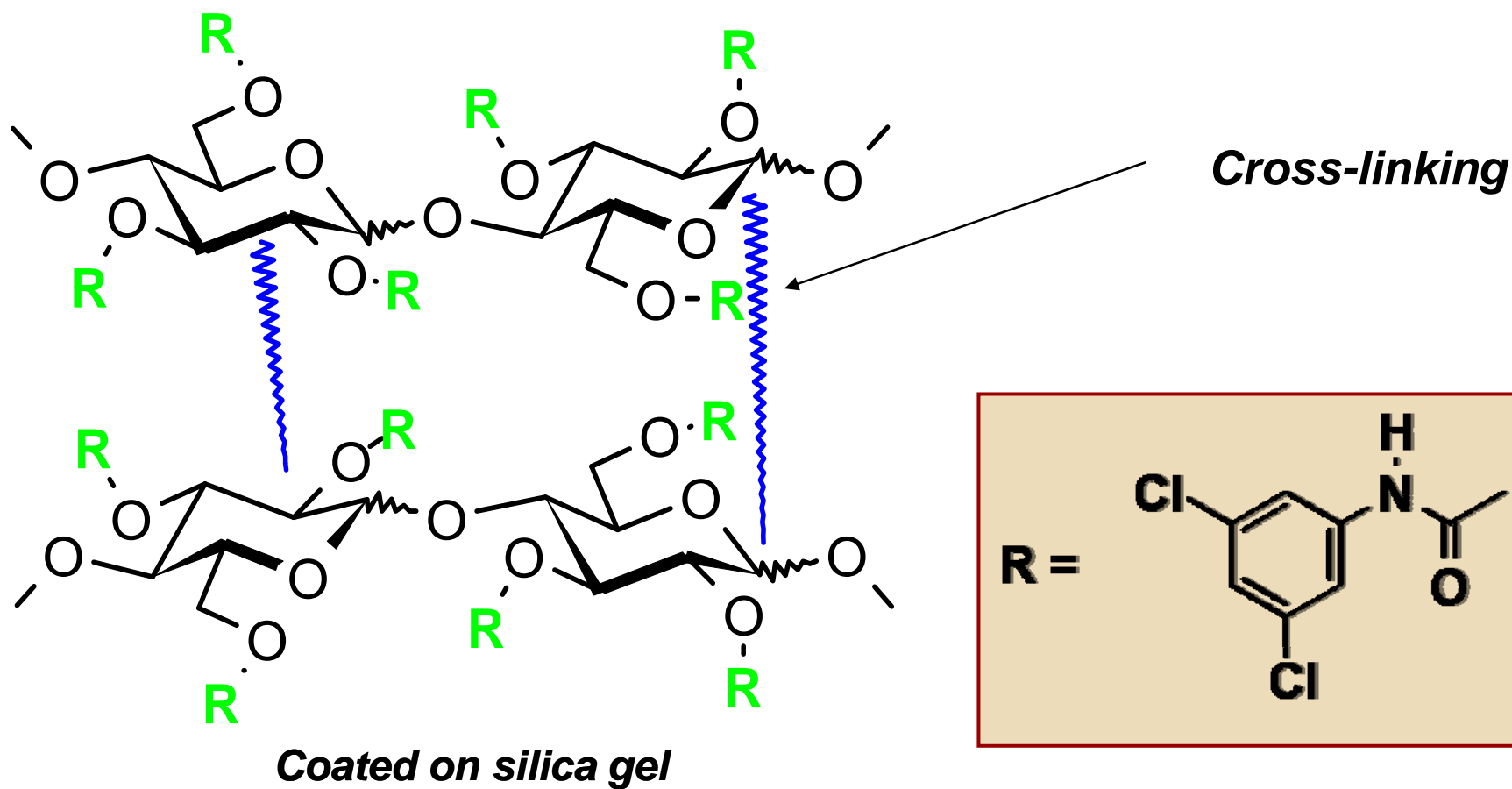
UV (+ thioxanthone) in MeOH/water

Patents: WO 9704011 (21.07.95); WO 9749733 (27.06.96)

Enhanced Enantioselectivity



Immobilized Halogenophenylcarbamates of Cellulose



E. Francotte, D. Huynh; J. Pharm. & Biomed. Analysis, 27 (2002), 421.

Supercritical fluid chromatography

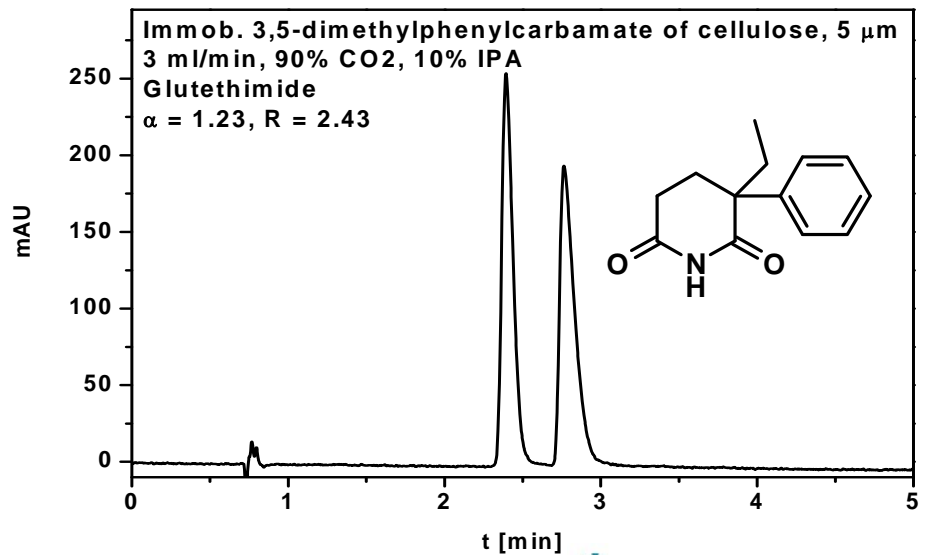
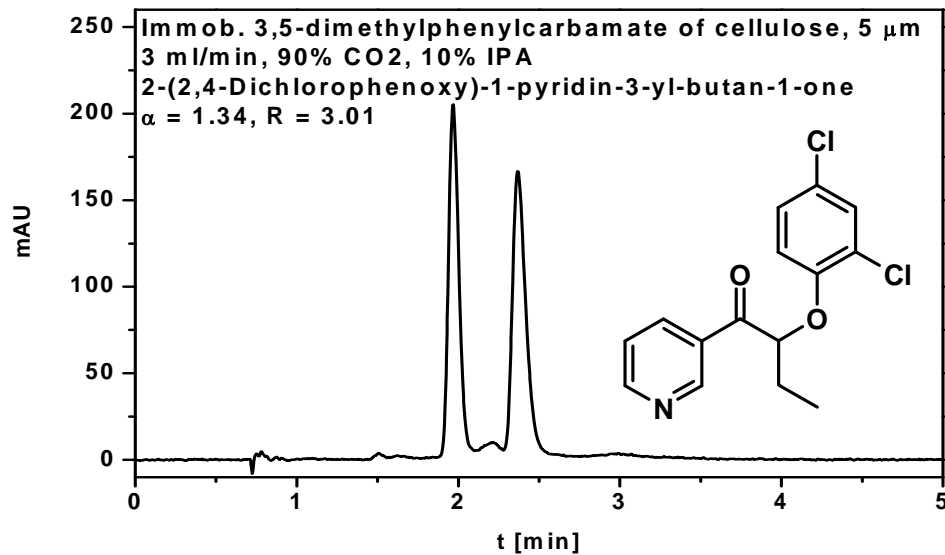
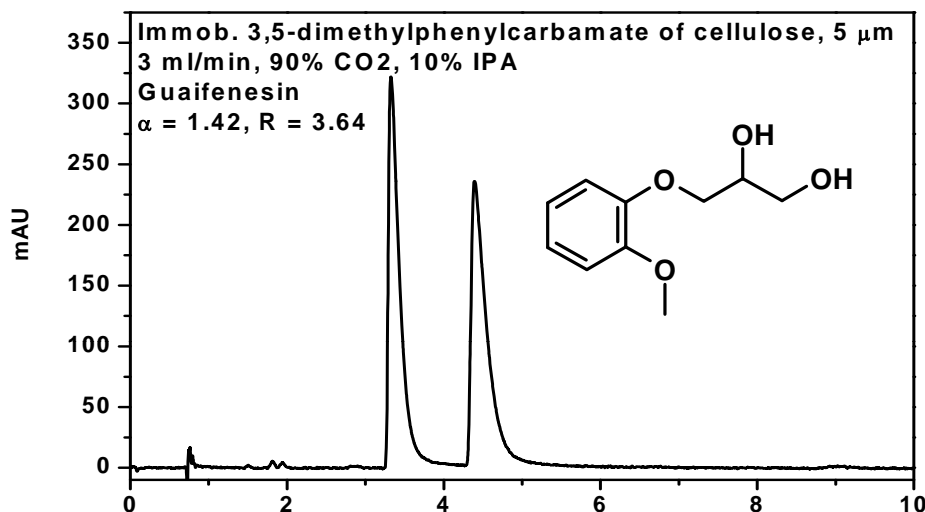
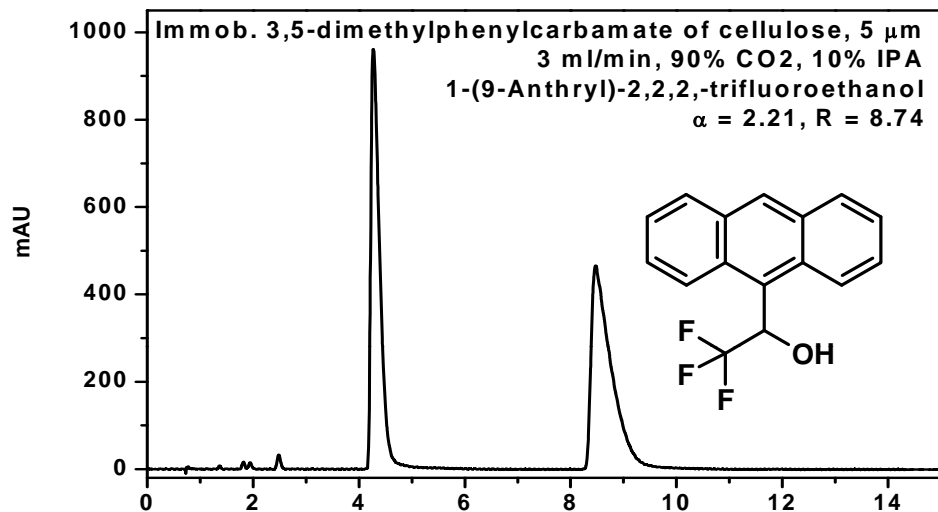
- Thar Prep SFC-200
Max flow rate CO₂:
200 g/min
- Thar Analytical/Semi-
Prep SFC
Mass spectrometry
coupling with Waters
Platform
- Mettler-Toledo/Berger
Analytical SFC



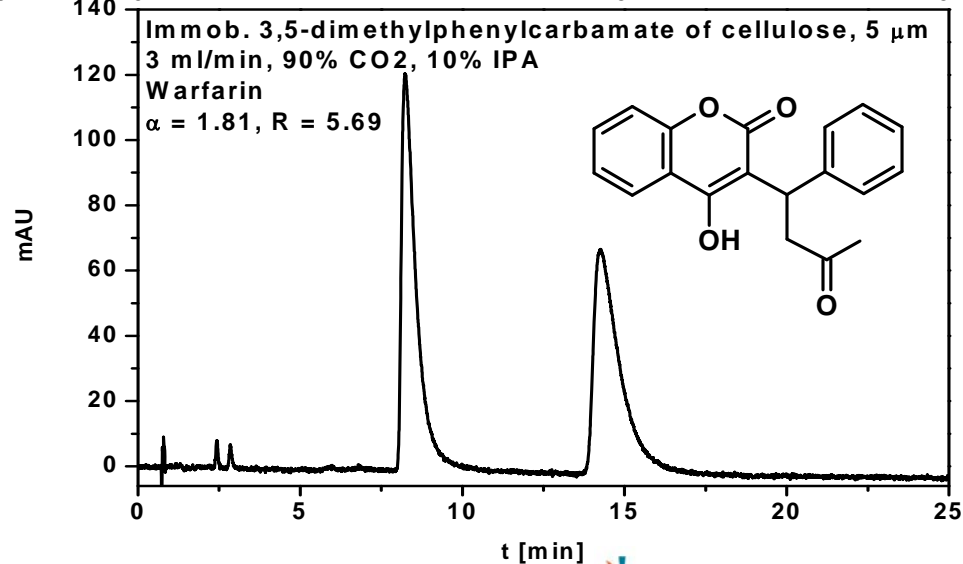
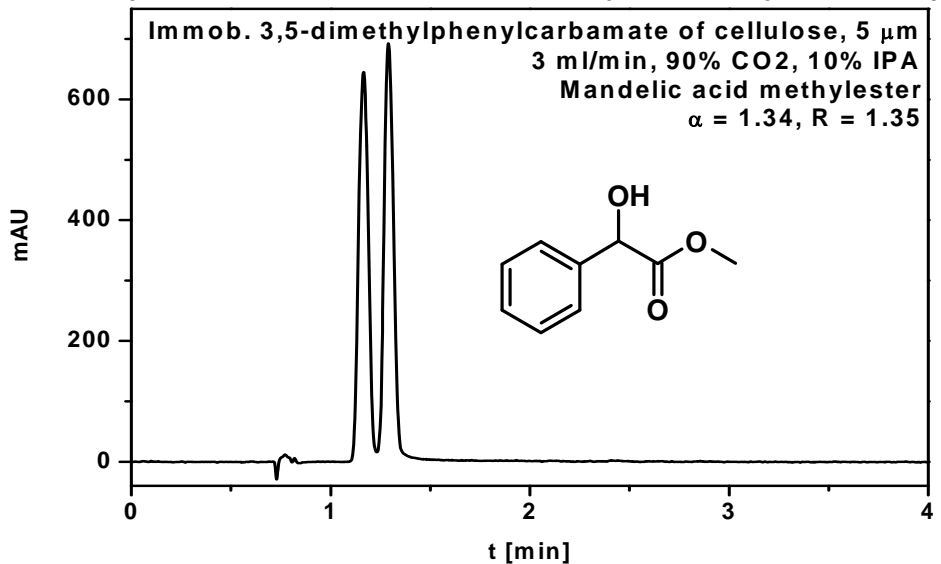
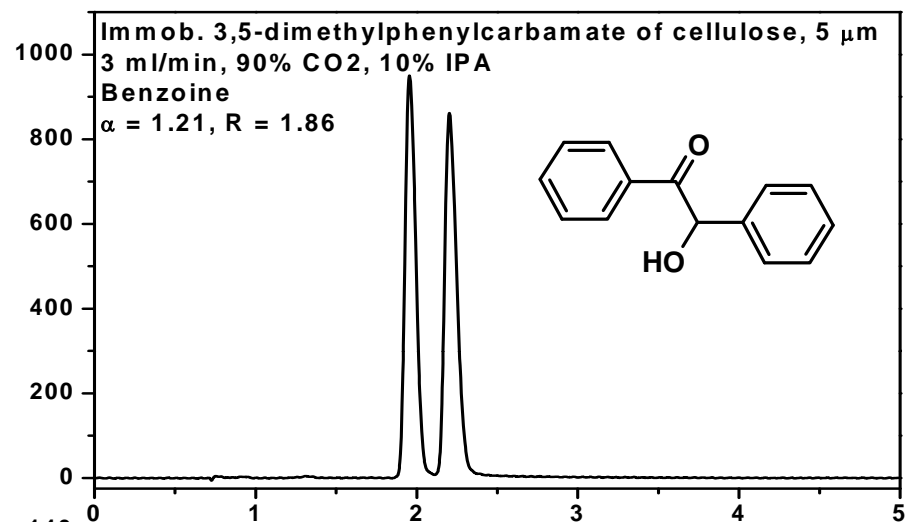
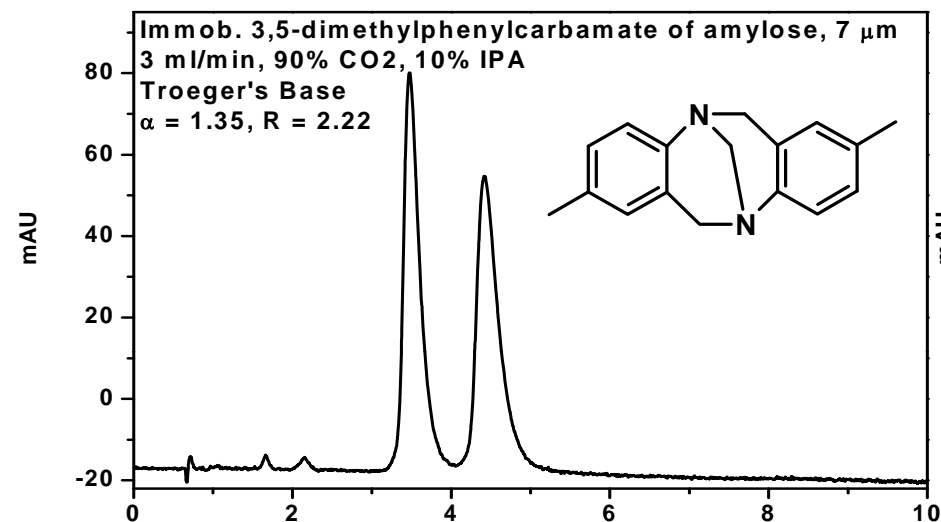
ANALYTICAL EXAMPLES

(all columns, 250 mm x 4.6 mm)

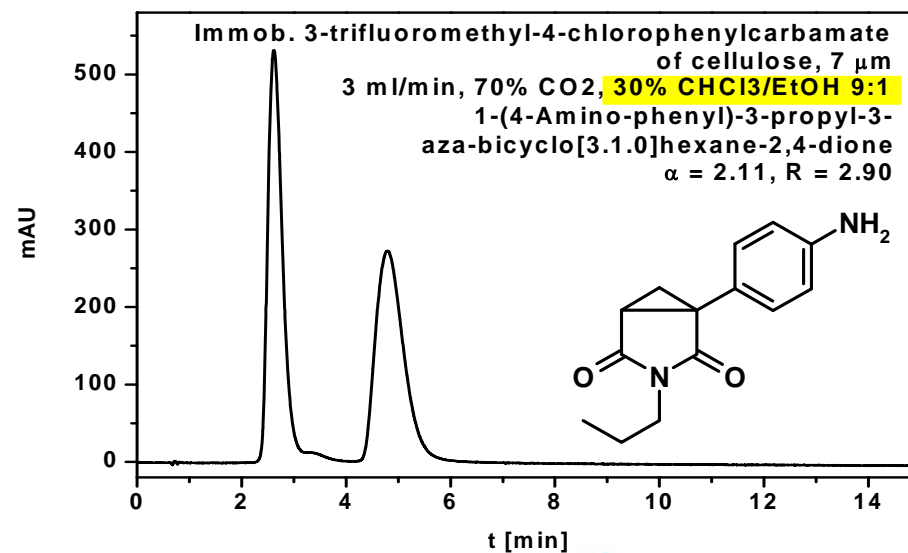
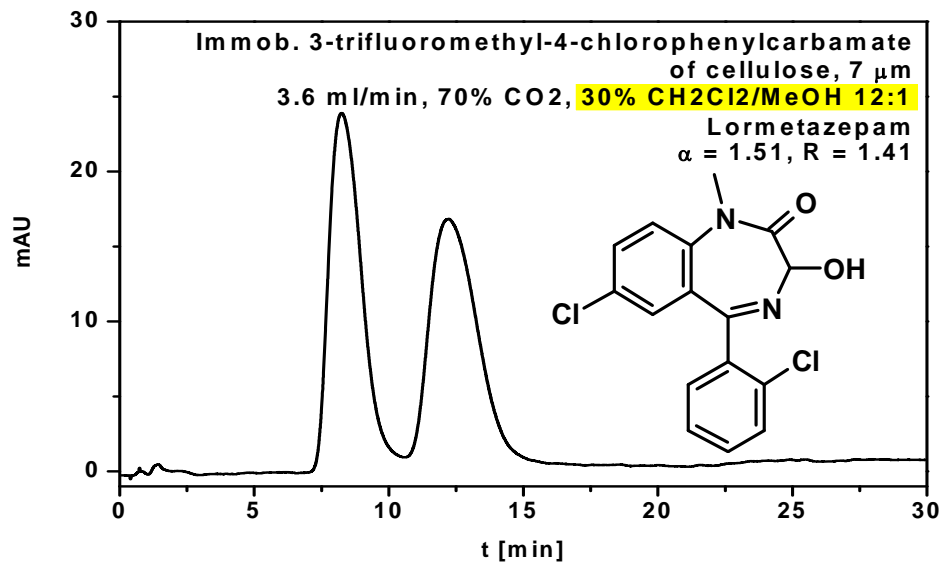
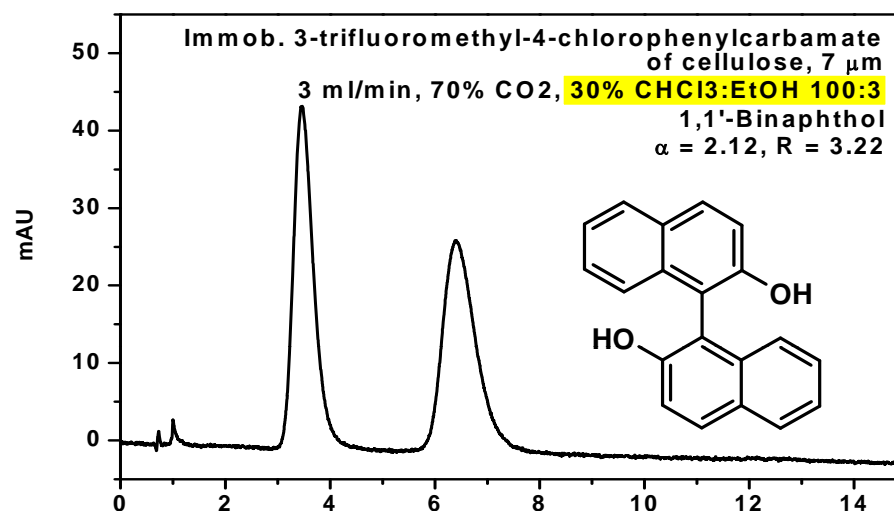
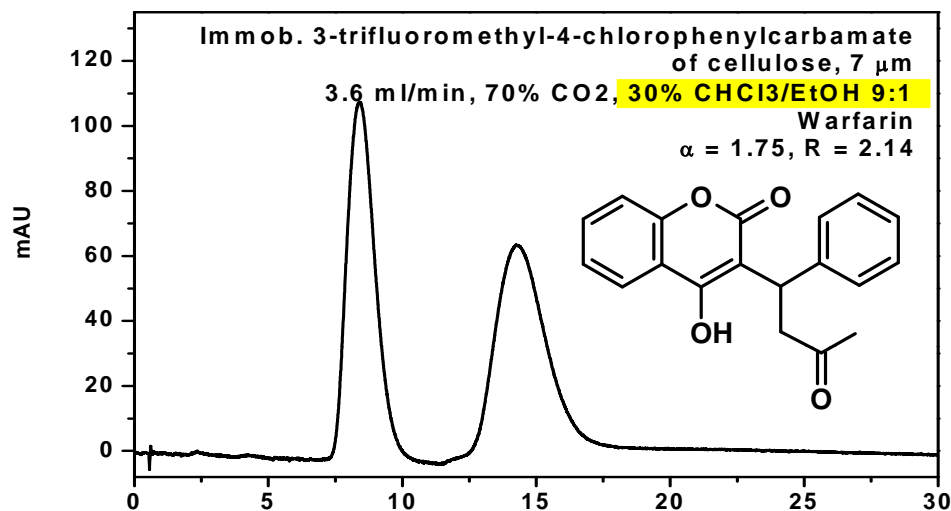
Analytical SFC on immobilized 3,5-dimethylphenylcarbamate of cellulose



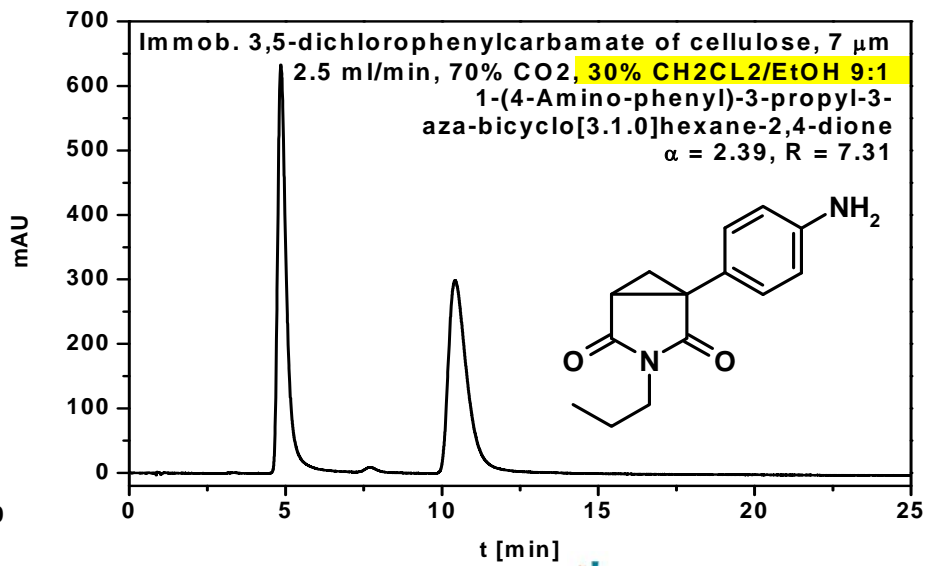
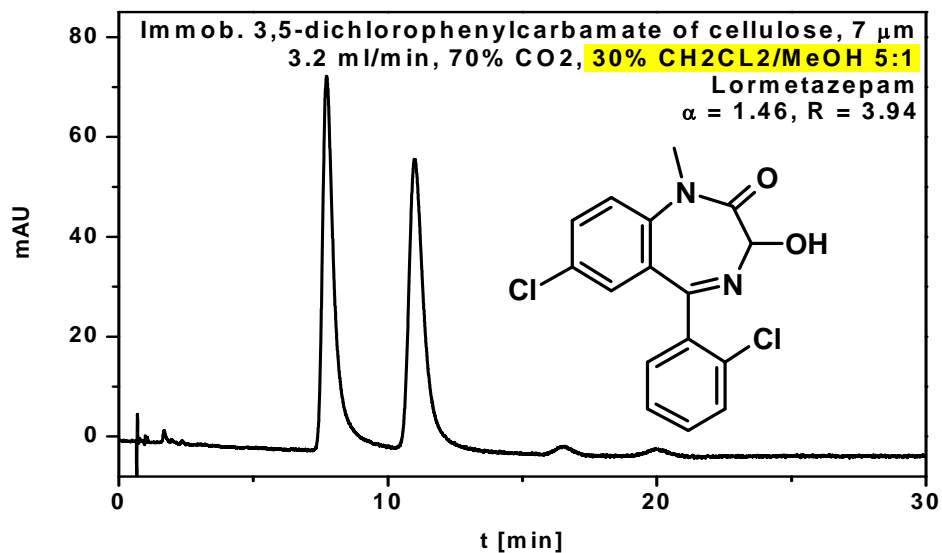
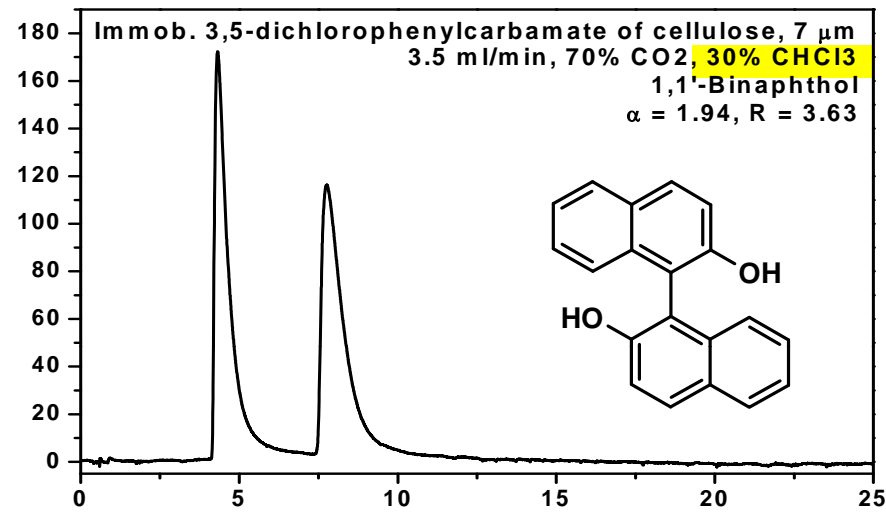
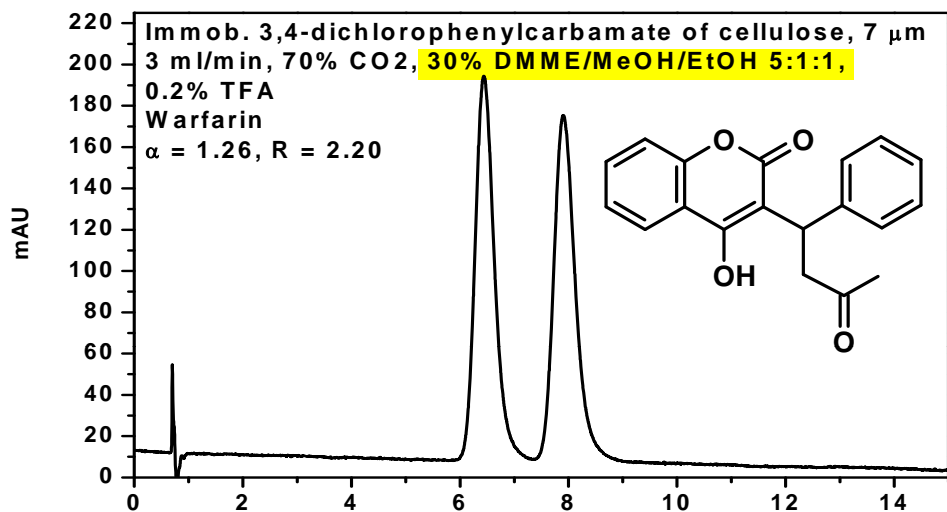
Analytical SFC on immobilized 3,5-dimethylphenylcarbamate of amylose



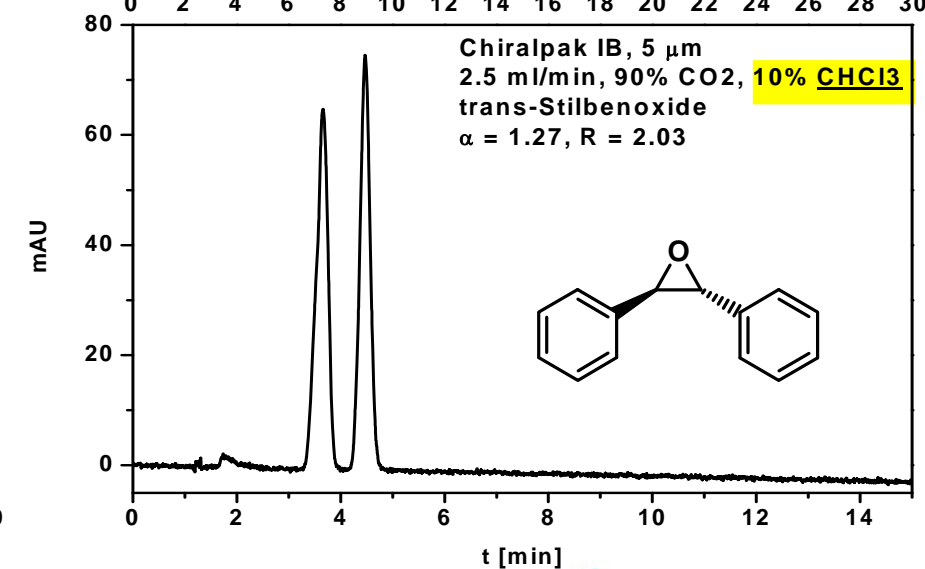
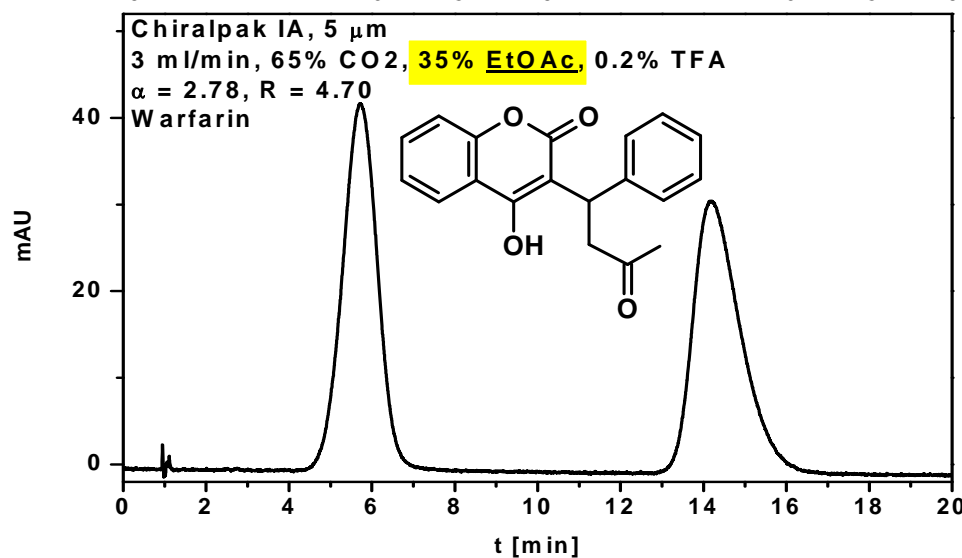
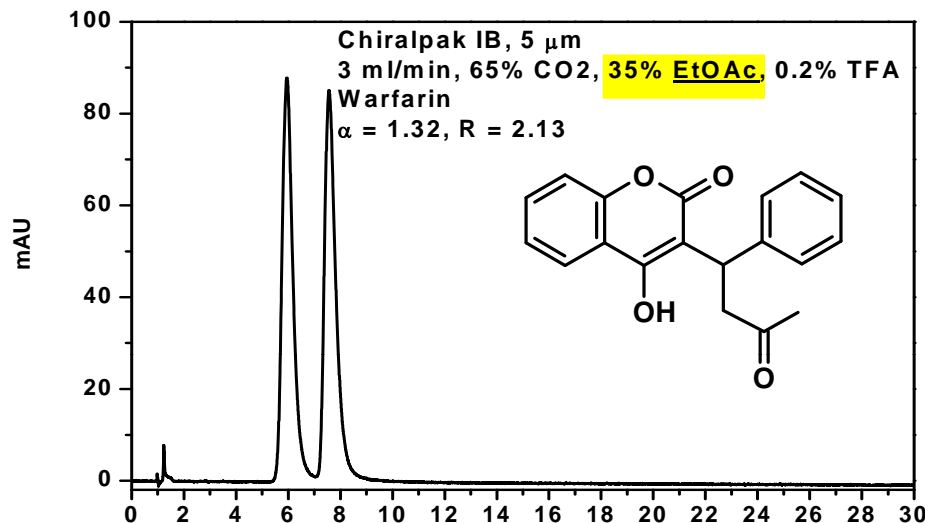
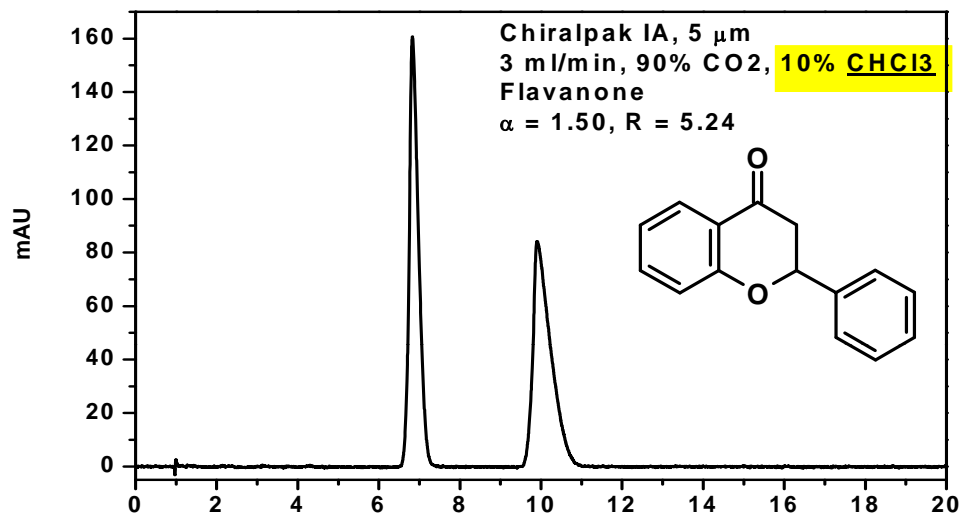
Analytical SFC on immobilized 3-trifluoromethyl-4-chlorophenylcarbamate of cellulose



Analytical SFC on immobilized 3,5-dichlorophenylcarbamate of cellulose



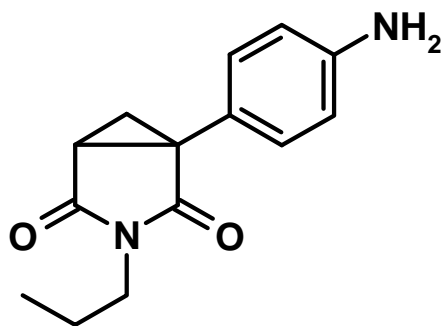
Analytical SFC on Commercial Chiralpak IA, IB



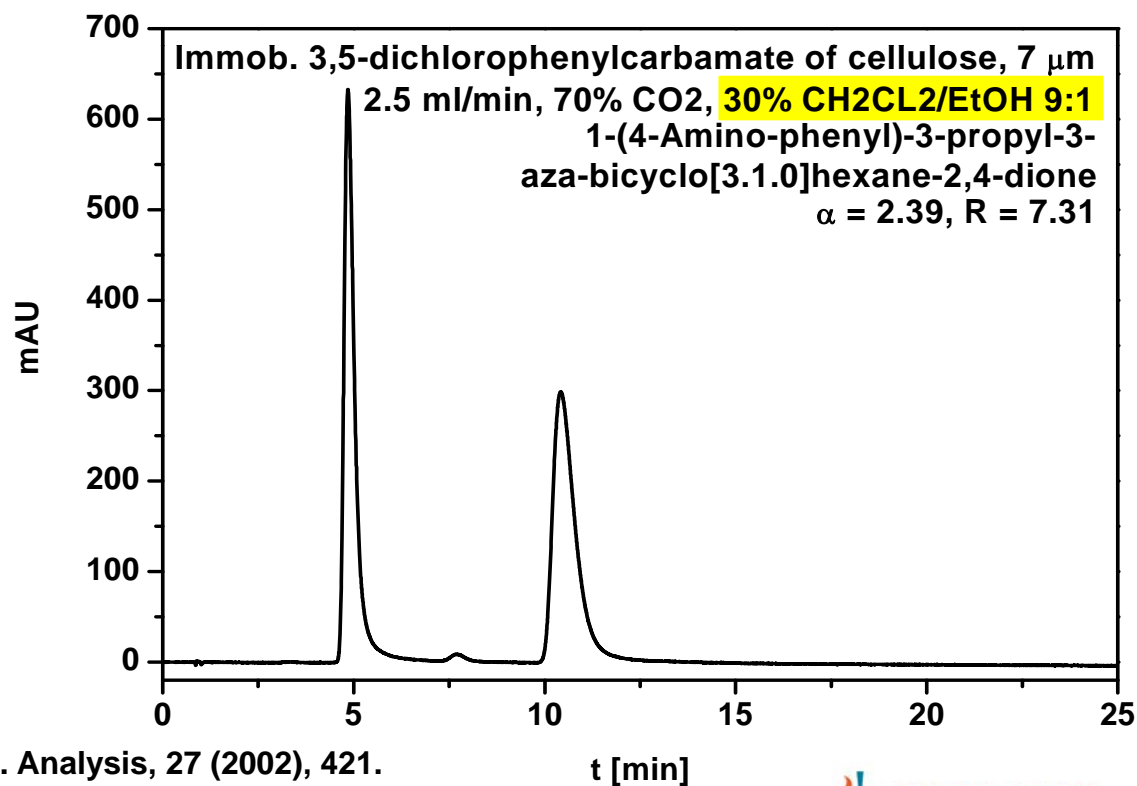
PREPARATIVE APPLICATIONS

Prep. Enantioseparation of Aromatase Inhibitor

- Low solubility in classical mobile phase, high solubility in chlorinated solvents:
n-heptane/IPA 90:10 \Rightarrow 3 mg/ml CH_2Cl_2 \Rightarrow >40 mg/ml
TBME \Rightarrow 17 mg/ml $\text{CHCl}_3/\text{MeOH}$ 9:1 \Rightarrow >40 mg/ml
THF \Rightarrow >40 mg/ml
- SFC separation on immobilized halogenophenylcarbamates of cellulose



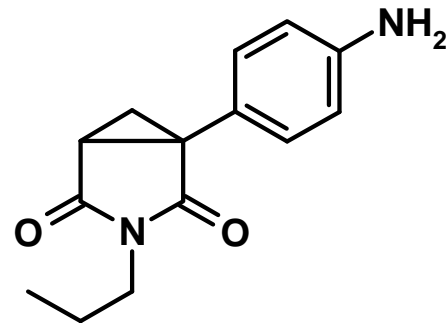
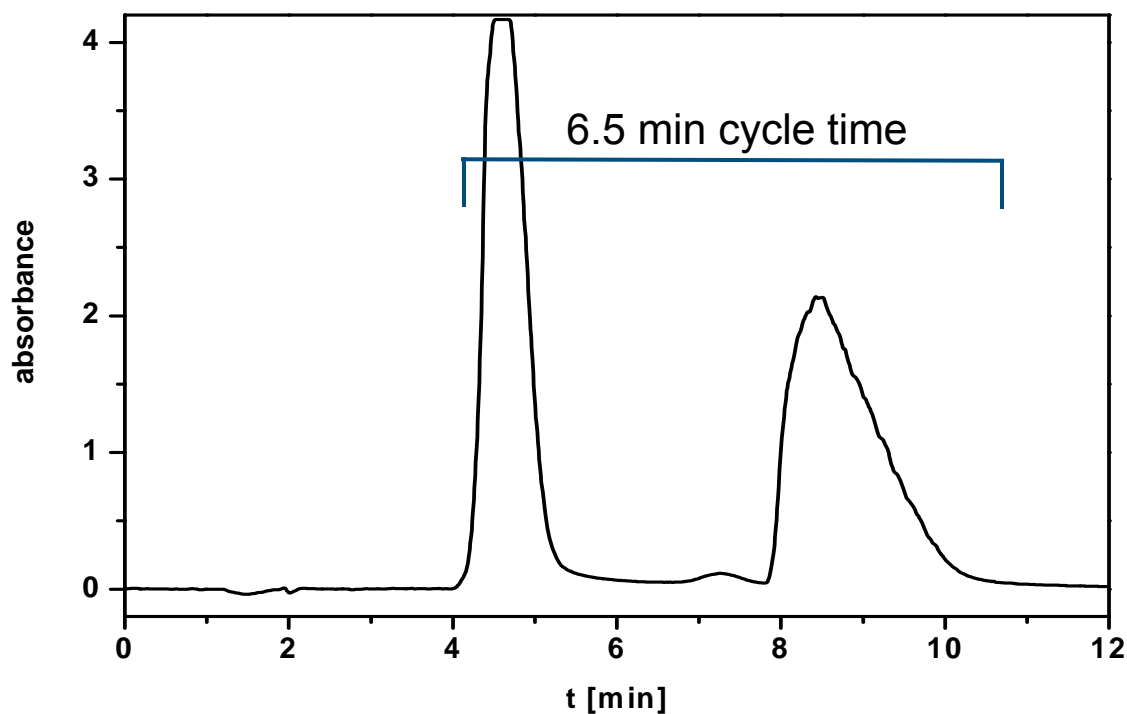
1-(4-Amino-phenyl)-3-propyl-
3-aza-bicyclo[3.1.0]hexane-2,4-dione



E. Francotte, D. Huynh; J. Pharm. & Biomed. Analysis, 27 (2002), 421.

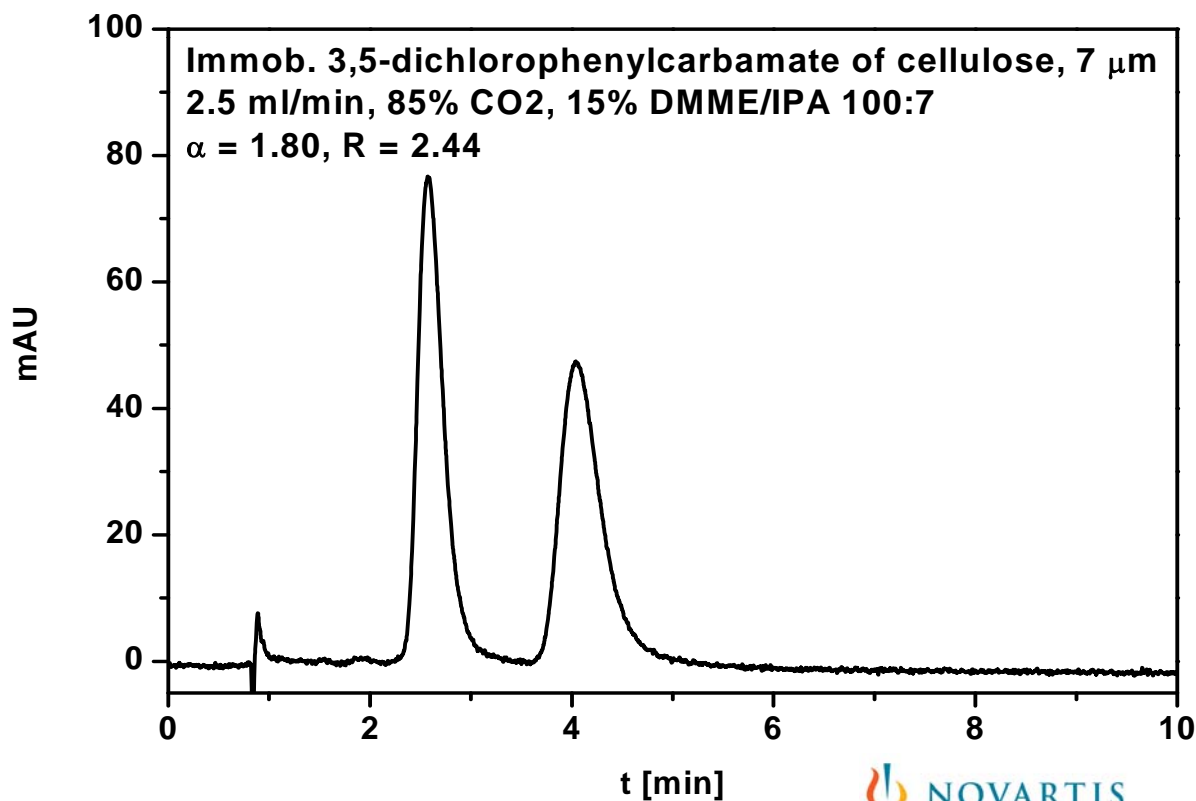
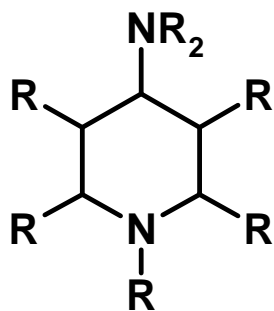
Prep. Enantioseparation of Aromatase Inhibitor

- Prep. SFC on proprietary immobilized CSP (OCI), 21.2 x 250 mm (7 μ m)
- High feed concentration in modifier solvents ($\text{CH}_2\text{Cl}_2/\text{EtOH}$ 8:2) \Rightarrow 70 mg/ml
- Column load: 140 mg, 590 mg of compound resolved by SFC
- Flow: 50 g/min, 70% CO_2 , 30% modifier; 150 bar, 40°C
- Productivity: 0.477 kg/kg CSP/24 h



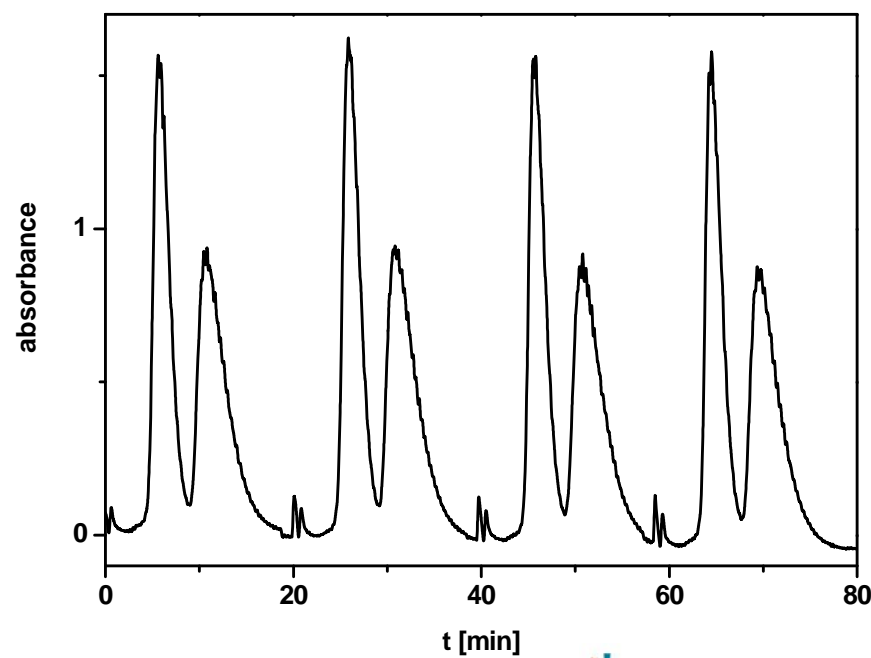
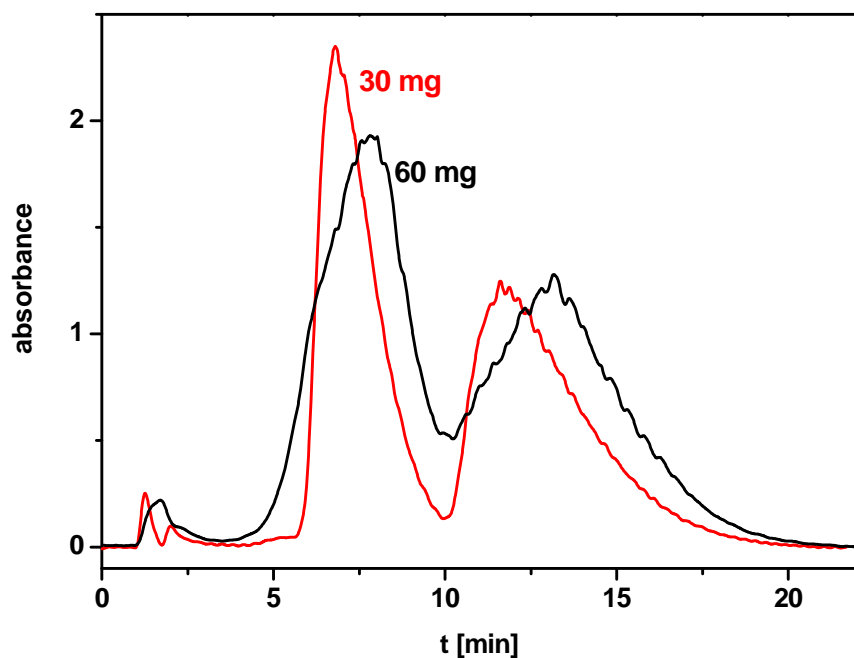
Research Project: Piperidine-Derivative

- Screening of classical CSPs (AD, OD, OJ, AS) => no separation
- Screening of six different polysaccharide-based immobilized phases => separation on immobilized 4-methylphenylcarbamate (corresponds to OG) and 3,5-dichlorophenylcarbamate of cellulose (OCI)
- Separation on OCI selected for further optimization on SFC



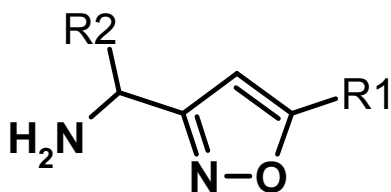
Prep. Separation of Piperidine-Derivative

- Prep. SFC on proprietary immobilized CSP (OCI)
- Compound very well soluble in modifier (DMME/IPA 100:7)
- Column load studied on 21.2 x 250 mm column (7 μm particles)
- Stacked injections for separation of 350 mg racemate
- Flow: 50 g/min, 90% CO_2 , 10% modifier; 150 bar, 40°C

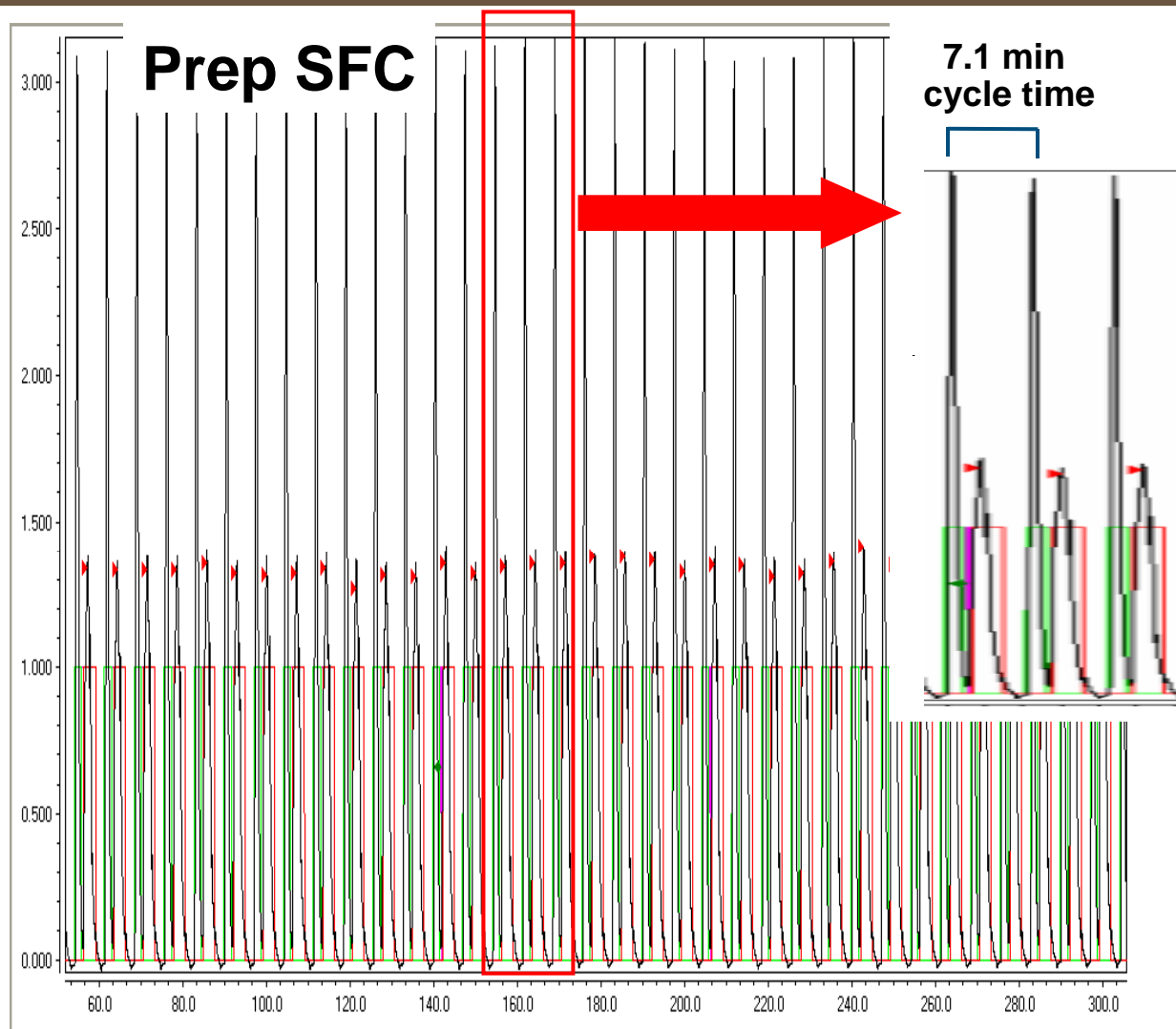


SFC Application on Immobilized 3,5-Dichlorophenyl Carbamate of Cellulose

- Request for chiral separation of 12 g rac. drug intermediate

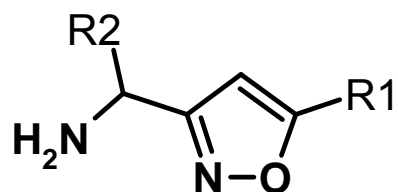


- Column: **Immobilized 3,5-dichlorophenyl carbamate of cellulose**, 7 μ m, 2.1 x 25 cm
- Mobile phase: CO₂/ethanol 85:15 + 0.15% isopropylamine
- Cycle time of 7.1 min
- 330 stacked injections (à 35 mg racemate) **in 39 hours overnight** (automated CO₂ supply!)



SFC Application on Immobilized 3,5-Dichlorophenyl Carbamate of Cellulose

Drug intermediate



Recovery:

90% (each enantiomer)

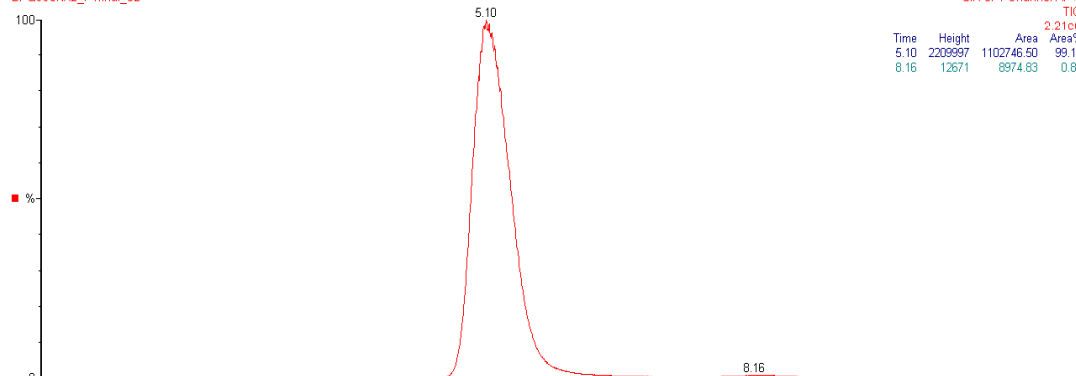
Optical purity:

Peak 1: 5.55 g, ee > 98.4%

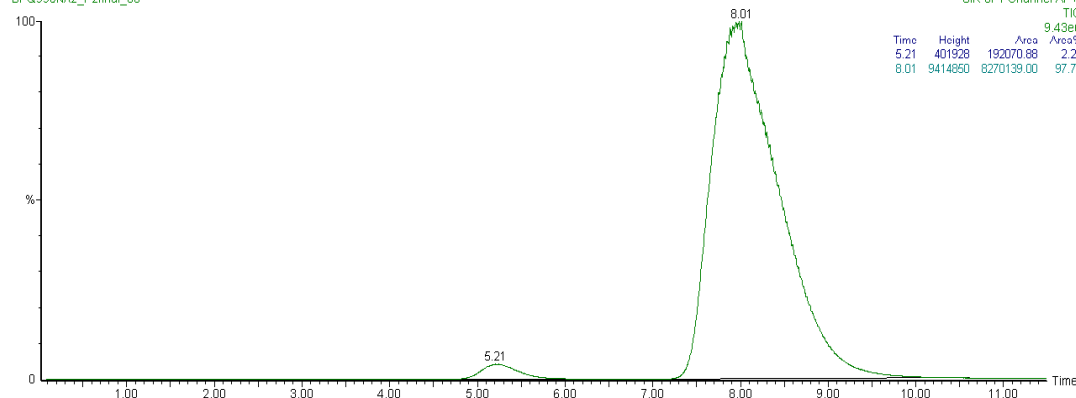
Peak 2: 5.23 g, ee > 95.4%

10% IPA+1%IPAm, OCI321-May-2007

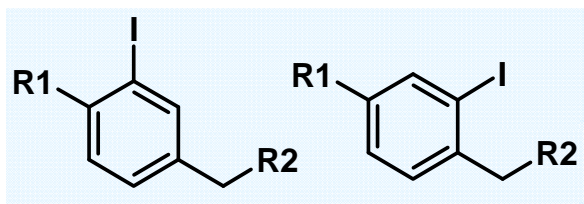
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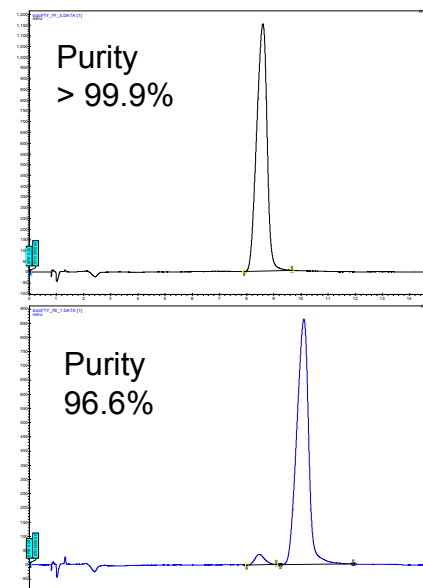
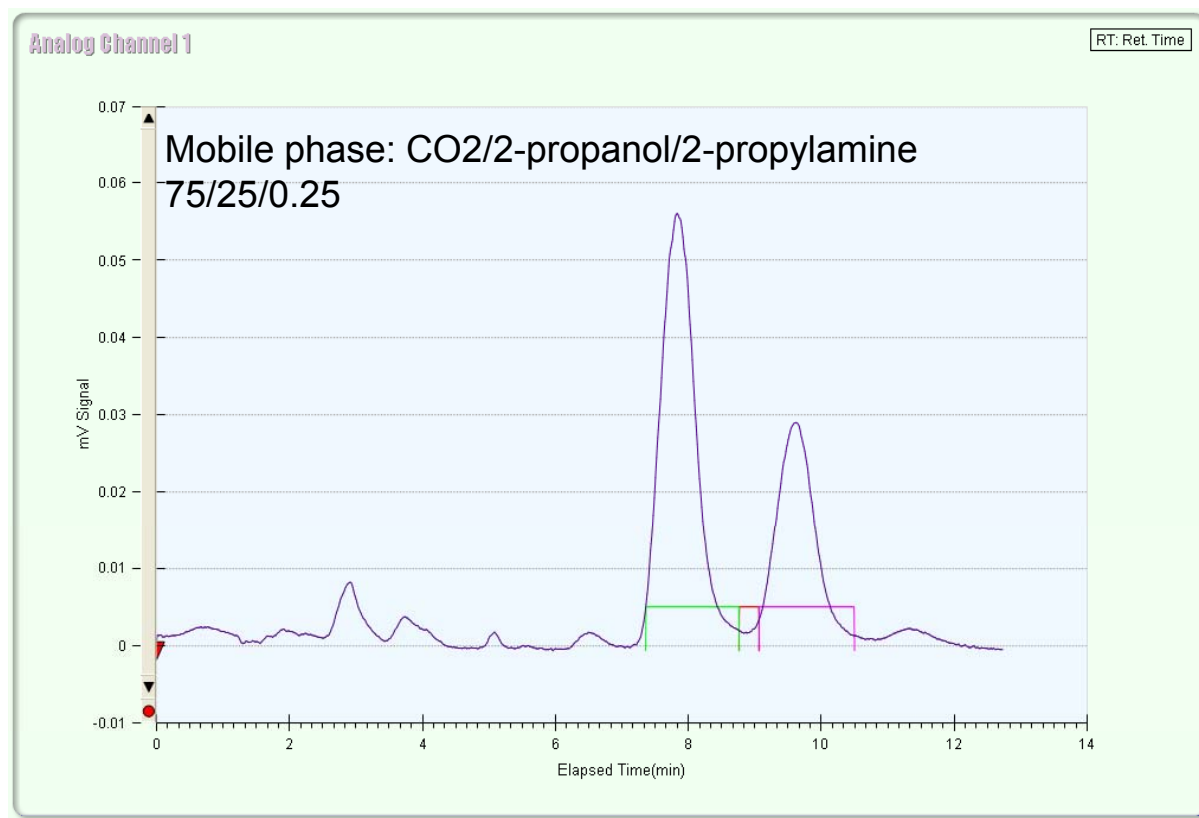
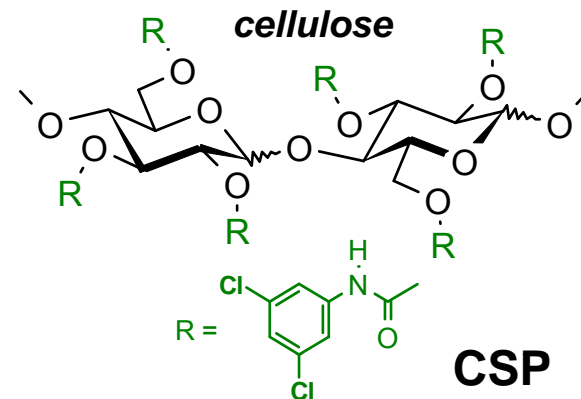
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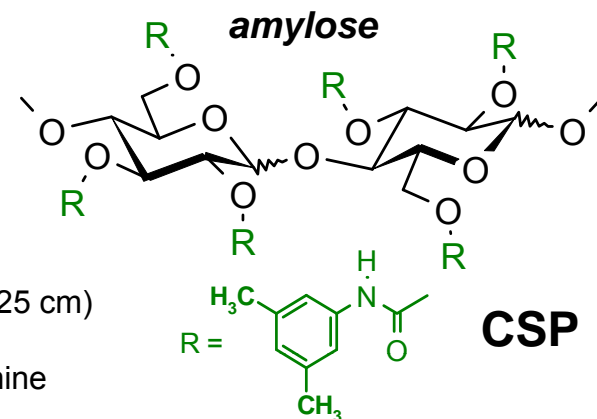
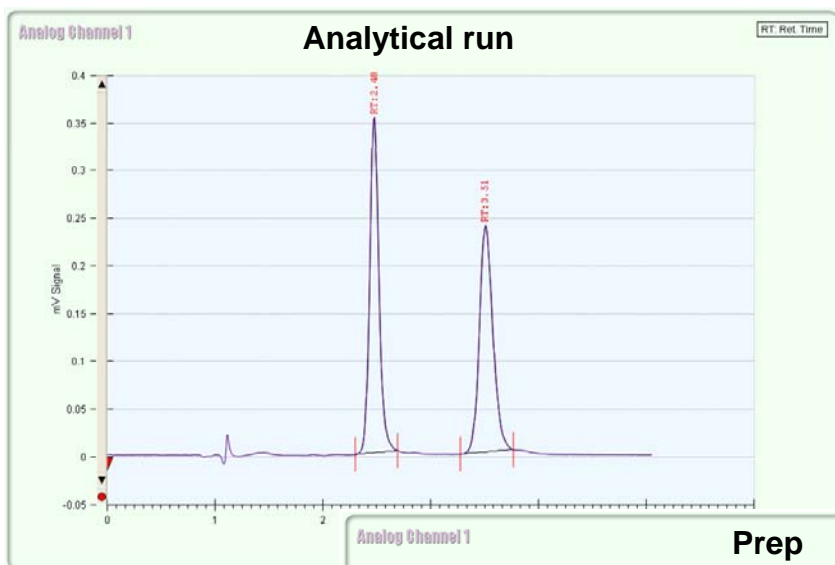
SFC Application on Immobilized 3,5-Dichlorophenyl Carbamate of Cellulose



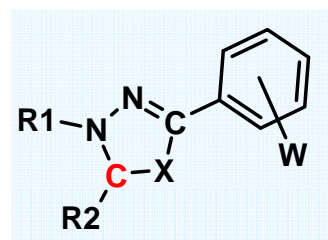
Separation of
Regioisomers
(~2/1)



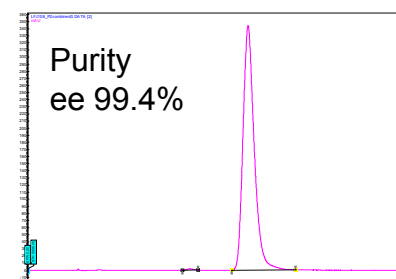
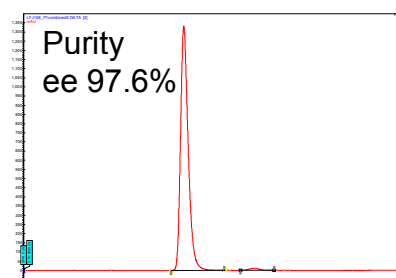
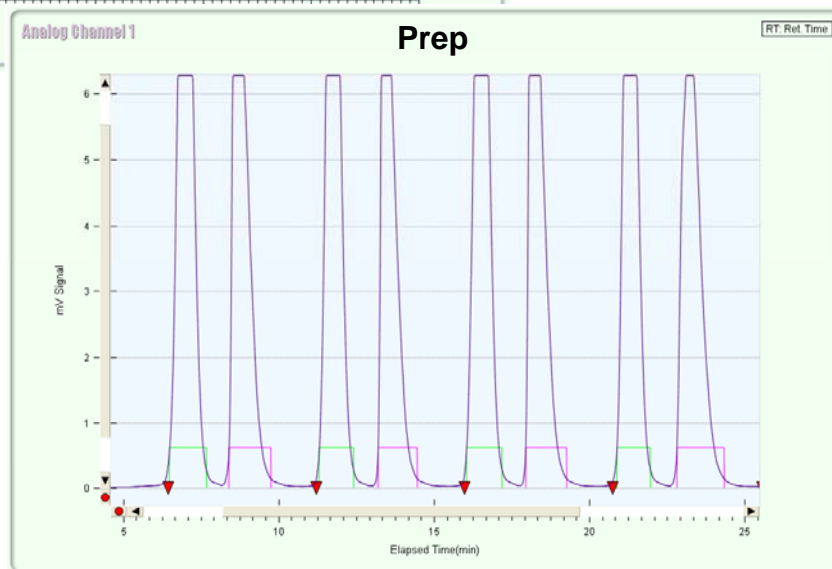
SFC Applications on Immobilized 3,5-Dimethylphenyl Carbamate of Amylose



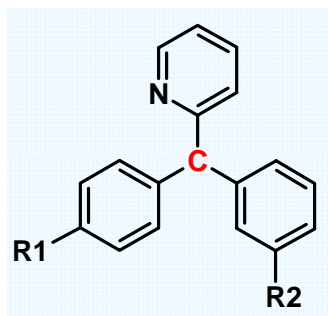
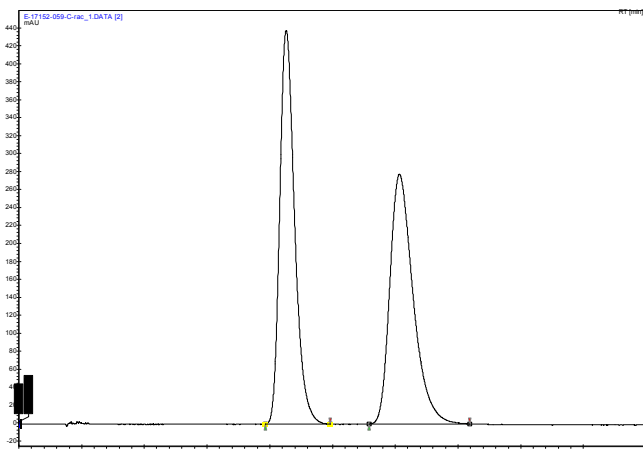
Column: Chiralpak IA (1 x 25 cm)
Mobile phase: CO₂/2-propanol/2-propylamine
 75/25/0.25
Cycle time: 4.9 min



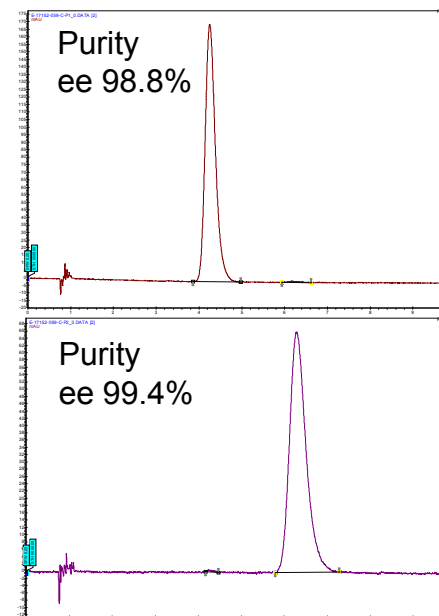
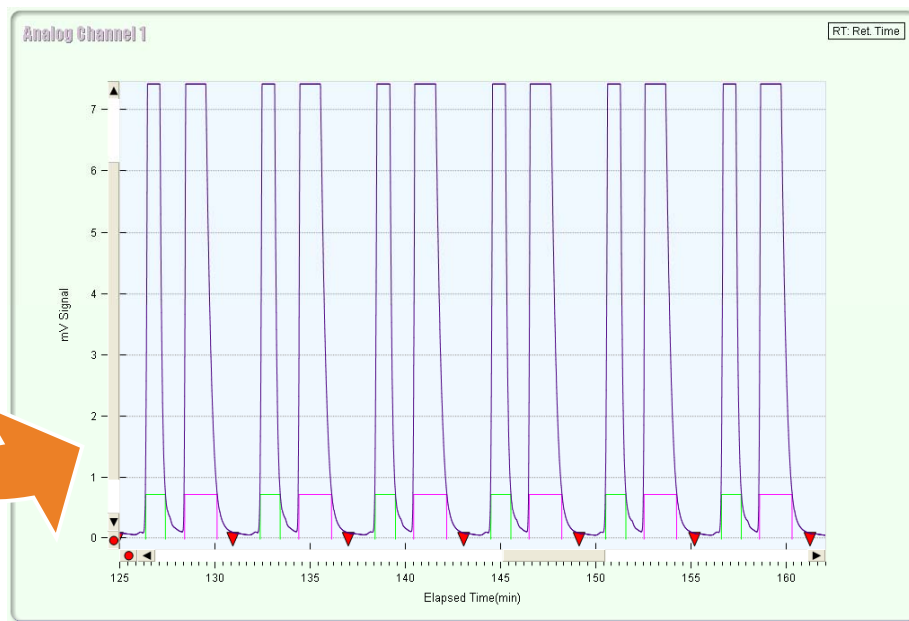
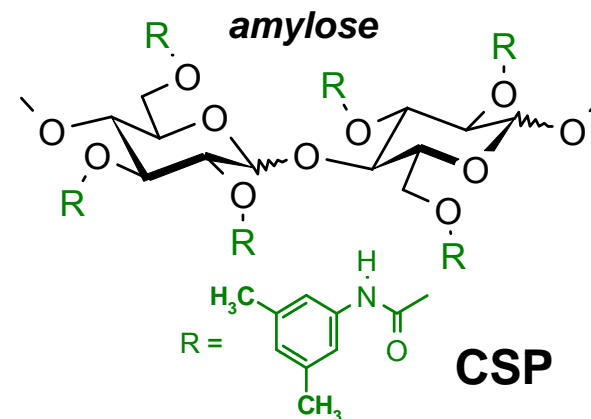
Separation of enantiomers (final drug)



SFC Applications on Immobilized 3,5-Dimethylphenyl Carbamate of Amylose



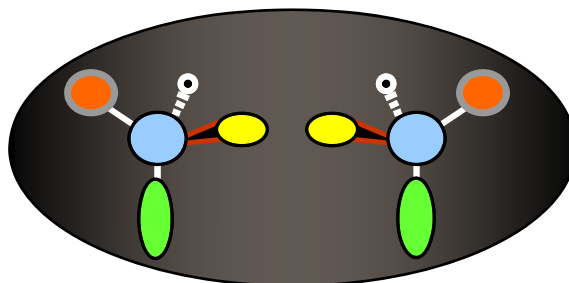
Column: Chiralpak IA (1 x 25 cm)
Mobile phase:
CO₂/ethanol/2-propylamine 70/30/0.06



Acknowledgements

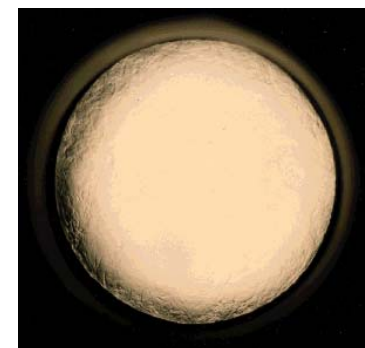
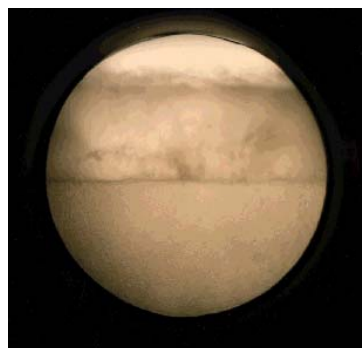
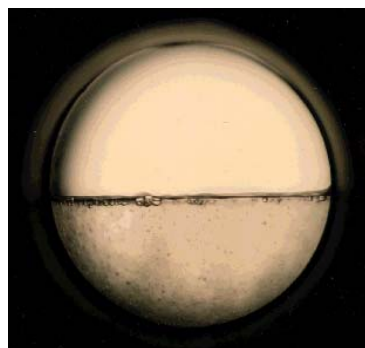
Chiral phases

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SFC

A. Meishammer
P. Richert



Pictures taken from <http://www.chem.leeds.ac.uk/People/CMR/>