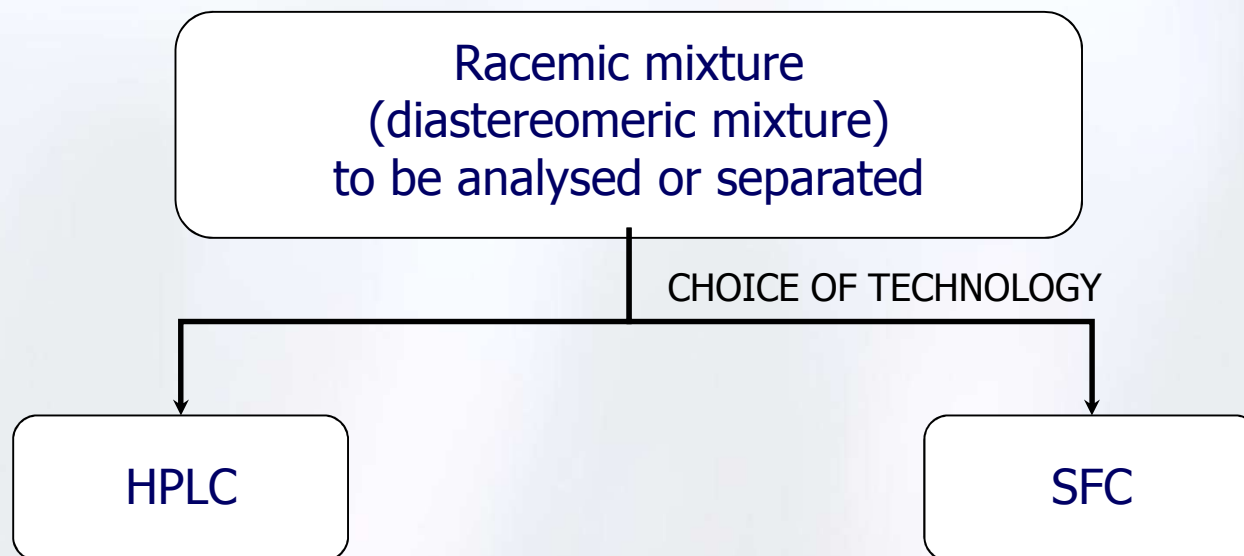


Supercritical Fluid Chromatography Screening Approach for Chiral Separations

T. Zhang, D. Colantuono, B. Freer, P. Franco

- Finding an approach for **Small and Medium Scale separations**
 - SFC or LC
 - SFC: which columns and mobile phases
 - Immobilised vs Coated Columns
 - Use of Additives
 - Troubleshooting in SFC
- CTE has an analytical method development & custom separation laboratory

This is what we do:



High success rates can be achieved by:

- Screening in LC or SFC with multiple columns (>7)
- Screening LC and SFC with a limited number of columns

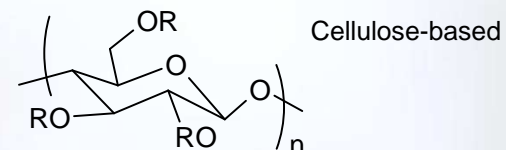
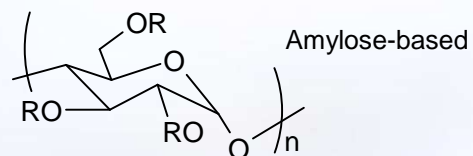
At Small Scale we use SFC and LC

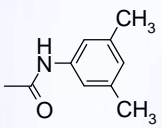
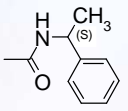
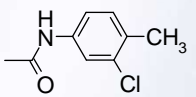
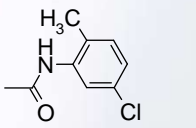
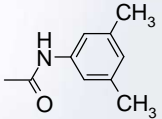
Fast primary screen - maximum hits

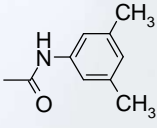
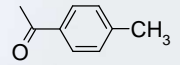
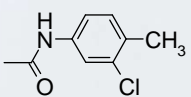
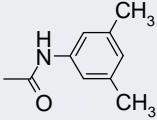
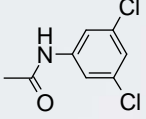
Secondary Screen - difficult compounds

Success rate at Small Scale

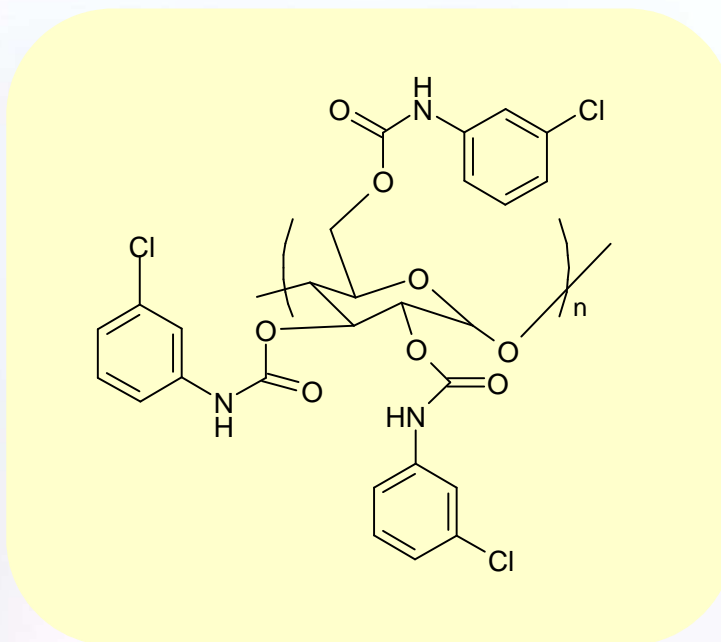
	Success rate 2008	Success rate 2009	Success rate 2008+2009
SFC+LC	99	99	99
SFC	86	81	83
LC	85	76	82



CSP	Nature	-R
CHIRALPAK AD-H	Coated	
CHIRALPAK AS-H	Coated	
CHIRALPAK AZ-H	Coated	
CHIRALPAK AY-H	Coated	
CHIRALPAK IA	Immobilised	

CSP	Nature	-R
CHIRALCEL OD-H	Coated	
CHIRALCEL OJ-H	Coated	
CHIRALCEL OZ-H	Coated	
CHIRALPAK IB	Immobilised	
CHIRALPAK IC	Immobilised	

CHIRALPAK ID



Based on
amylose *tris*-(3-chlorophenylcarbamate)

A new selector
for the immobilised series!!

Primary Screen

CHIRALPAK IA
CHIRALPAK IB
CHIRALPAK IC
CHIRALPAK ID

Mobile phase system

EtOH, MeOH, 2-PrOH
Acetonitrile

Dichloromethane
Ethyl acetate
THF
MtBE

Secondary Screen

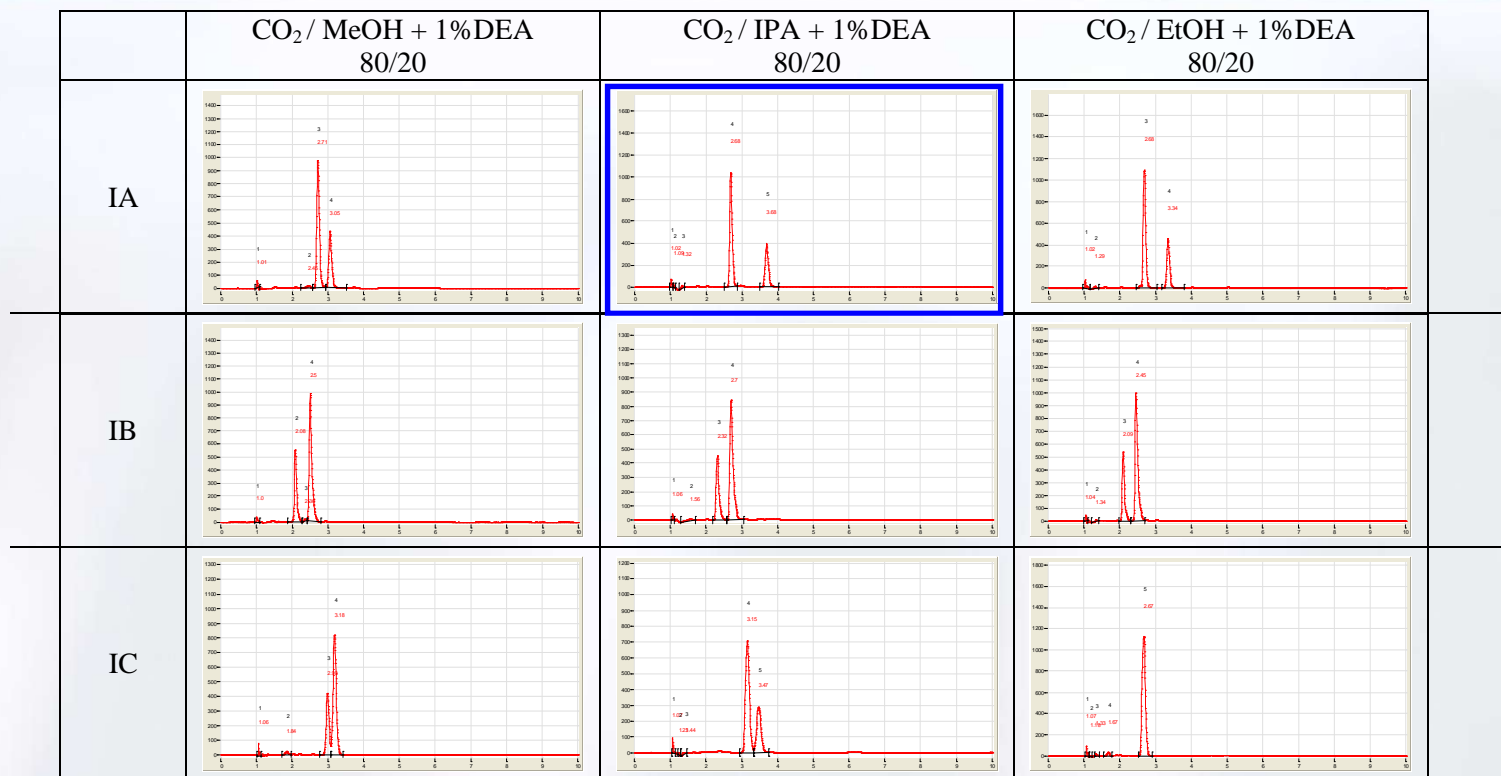
CHIRALPAK AD-H
CHIRALPAK AS-H
CHIRALPAK AY-H
CHIRALPAK AZ-H

CHIRALCEL OD-H
CHIRALCEL OJ-H
CHIRALCEL OZ-H

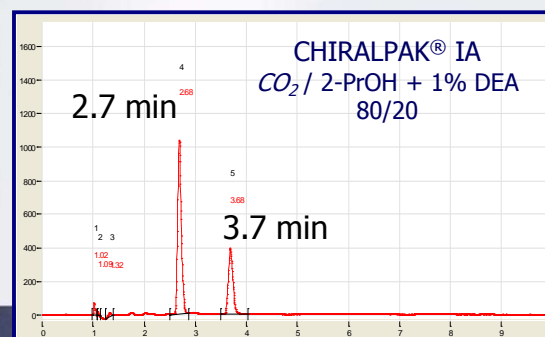
Co-solvents

EtOH, MeOH, 2-PrOH
Acetonitrile

- Samples are screened systematically in LC and SFC with a FAST screening
- Systematic screening (only 5 μ m)
 - LC on IA, IB, IC and ID (H/IPA, H/EtOH, H/THF, H/DCM, H/AcOEt, ACN and alcohol)
 - SFC on IA, IB, IC and ID (MeOH, IPA and EtOH)
- Coated phases are only screened if no efficient separation
- If separation on IA or IB present, but not enough a quick check on AD-H/OD-H
- QN/QD are also screened for acids if no efficient separation
- Short stability test of the sample (with racemate) in the MP
- Prep systems involved: 2 SP-LC and 2 SFC

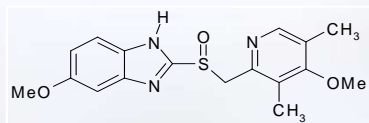


Also:
THF mixtures
MtBE mixtures
DCM mixtures
Ethyl acetate mixtures ...

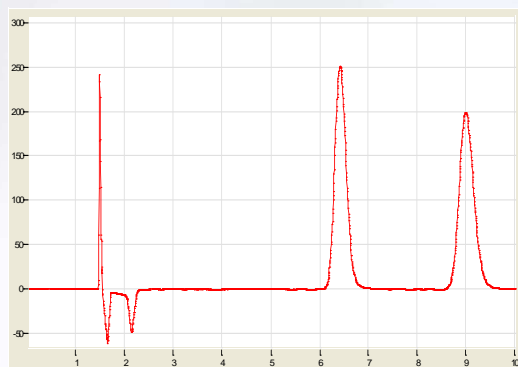


basic molecule

CHIRALPAK IC

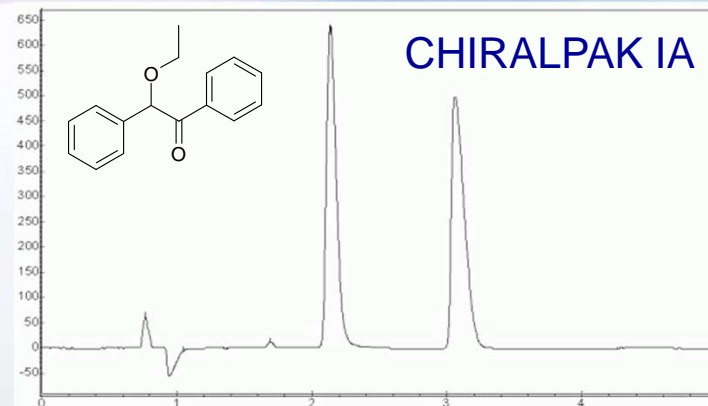


Omeprazole



CO₂ / THF (+1%DEA) 70/30

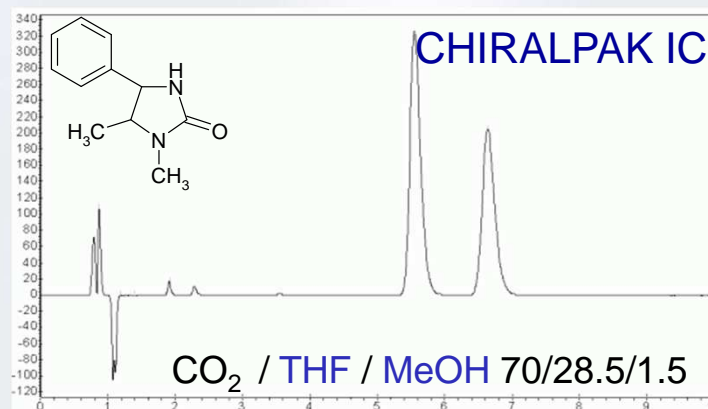
T=30°C
Flow rate: 3.0ml/min.



CHIRALPAK IA

CO₂ / ethyl acetate 80/20

T=35°C
Flow rate: 5.0ml/min.




CHIRALPAK IC

CO₂ / THF / MeOH 70/28.5/1.5


T=35°C
Flow rate: 4.0ml/min.

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Preparative chromatographic resolution of racemates using HPLC and SFC in a pharmaceutical discovery environment[☆]

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Supercritical fluid chromatography
Chiral stationary phase

ABSTRACT

The preparative chromatographic resolution of racemates has become a standard approach for the generation of enantiomers in pharmaceutical discovery laboratories. This paper will discuss the use of preparative HPLC and SFC to generate individual enantiomers for discovery activities. Analytical HPLC and SFC method development to rapidly screen chiral stationary phases and solvent combinations will be presented. The usefulness of preparative chromatographic resolution of racemates will be demonstrated through the presentation of numerous non-routine case studies from the laboratories at Amgen.

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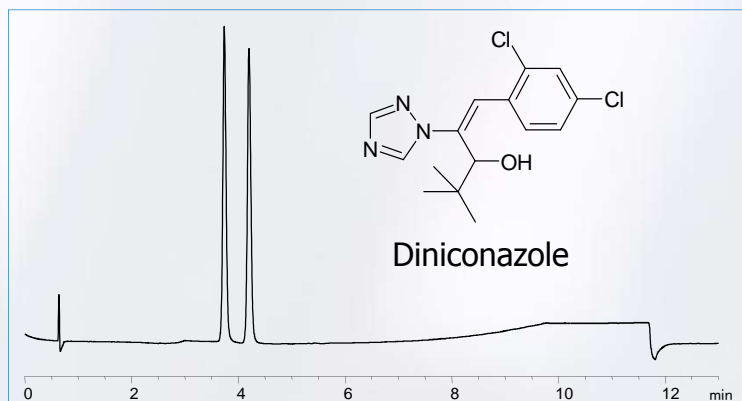
L. Miller et al., J. Chromatogr. B 875 (2008) 230-236

1. Combination of LC and SFC
2. Initial screening in gradient mode
3. Final isocratic method

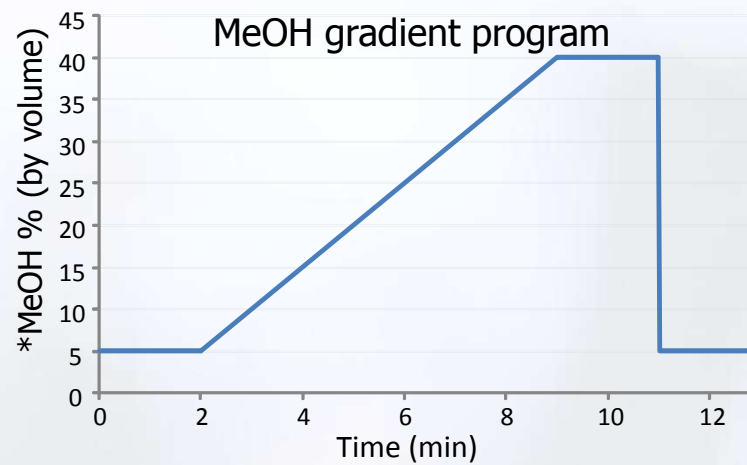
Literature example

CTE examples in SFC

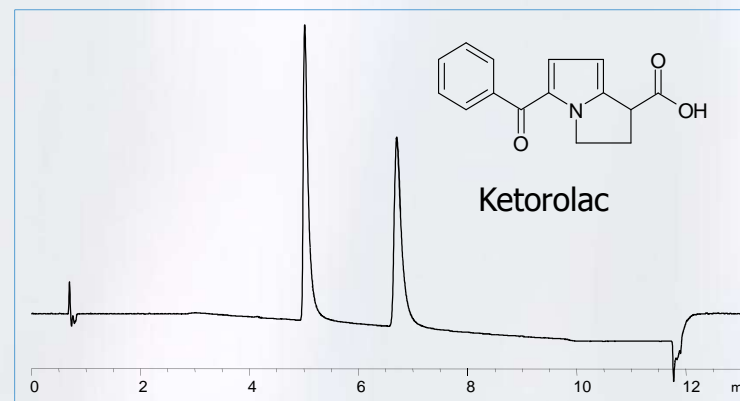
Back pressure: 150bar, Flow rate: 3.0ml/min,
Temperature: 35°C



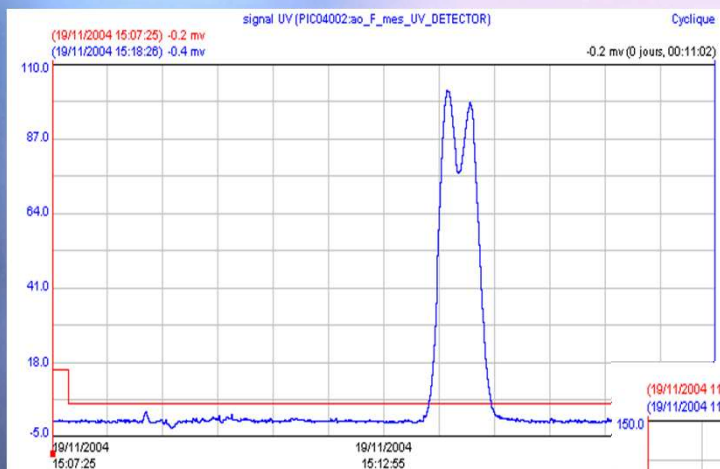
CHIRALPAK IA



* Containing 0.3% DEA for screening of basic compounds



CHIRALPAK ID

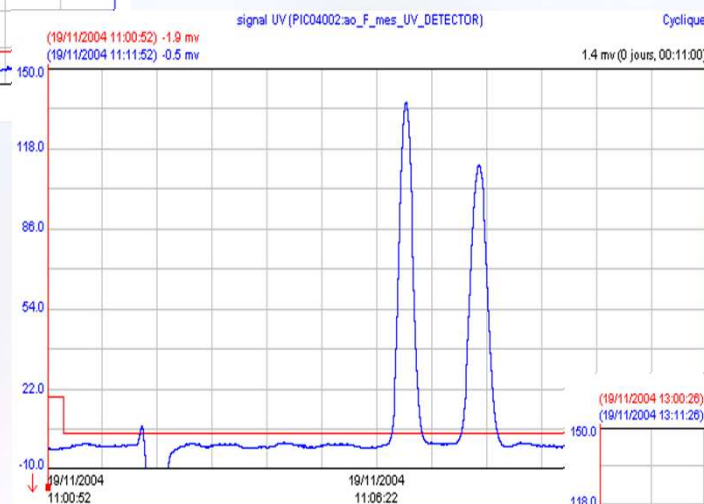


20% Isopropanol

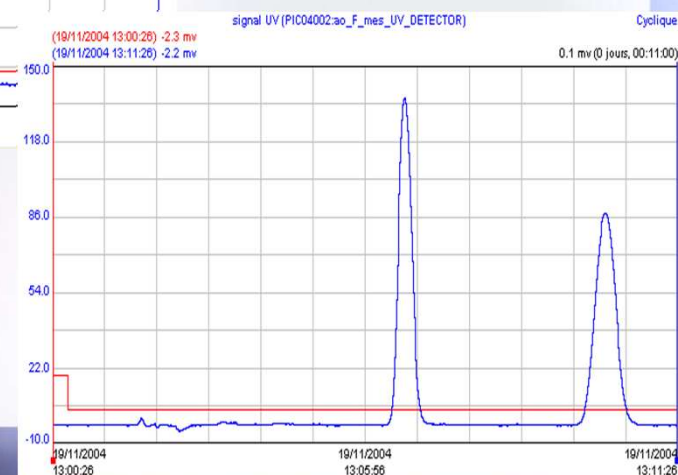
CHIRALPAK AD-H

(250 x 4.6 mm)
3 ml/min, 25°C
P outlet 150 bar

20% Isopropanol
+1% Diethylamine



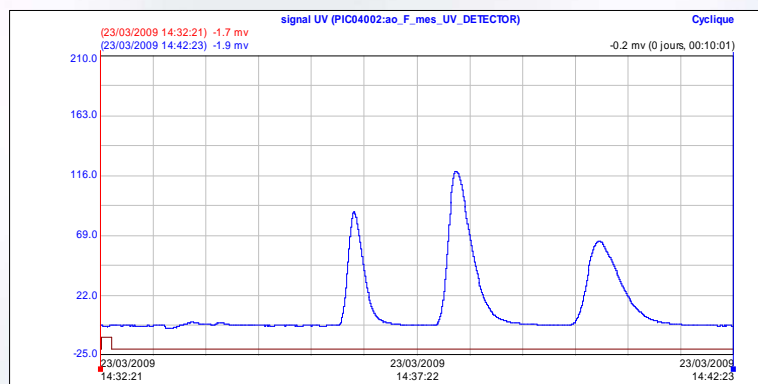
20% Isopropanol
+1% Butylamine



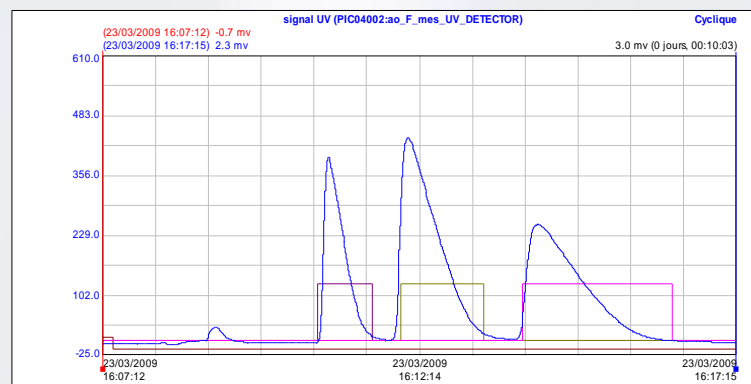
- Choice in the sample solvent dictated by sample solubility.
- Free choice of solvents in the mobile phase
- Greater options for chemical stability
- New selectivities
- **Use of DCM in sample solvent GREATLY improves solubility, loadability and SPEED**

- Column : **CHIRALPAK IC** 5 μm SFC – 25 x 3 cm
- Eluent : 70/30 CO_2 / EtOH
- Flow rate : 120 ml/min
- Temperature : 25°C
- P outlet : 150 bar
- Solubility in EtOH < 2 g/L → injection in EtOH/DCM 90/10

new solubility = 58 g/L

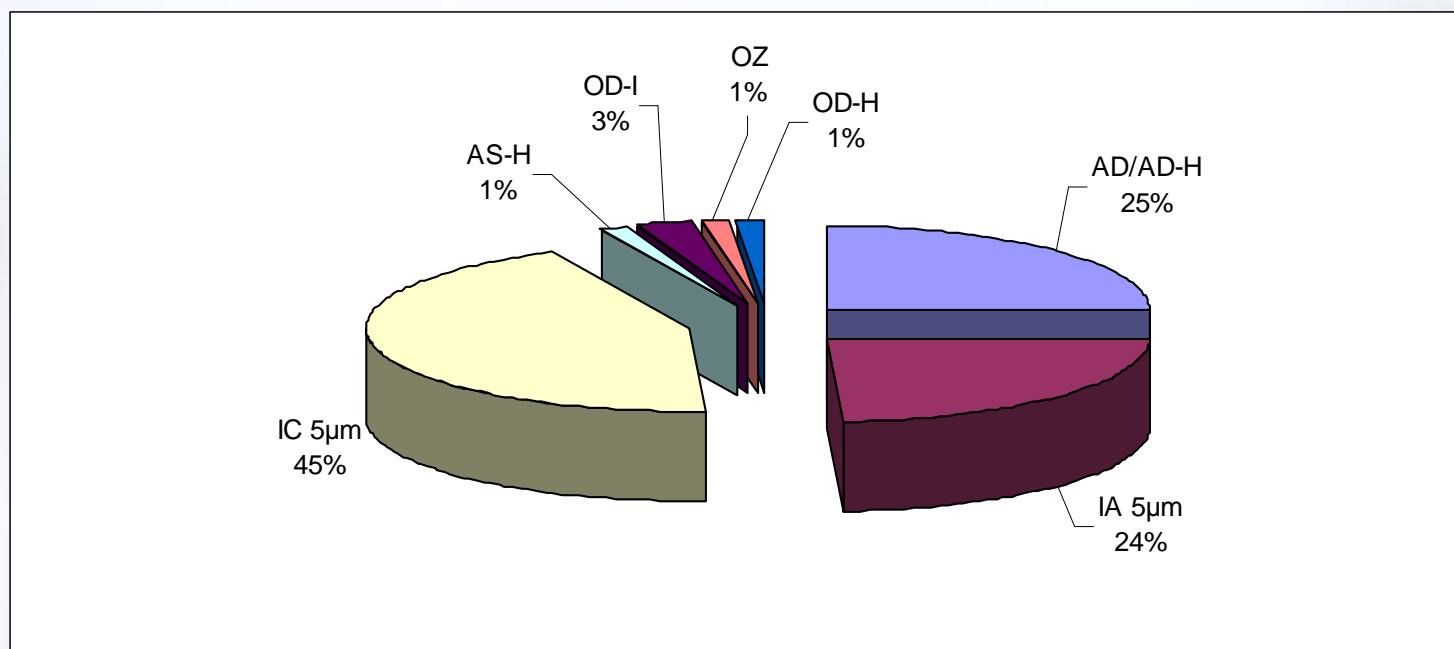


Analytical injection



Injection in EtOH/DCM 90/10 – 2ml - 116 mg

No perturbation of the separation



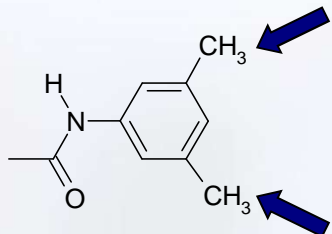
Screening with few CSPs
combining LC and SFC



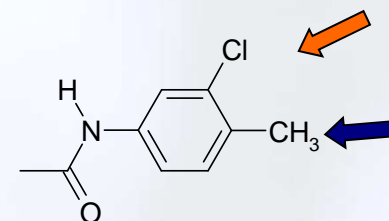
New chiral selectors

Primary and Secondary screen

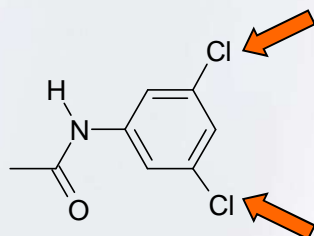
**CHIRALPAK IA
CHIRALPAK IB**



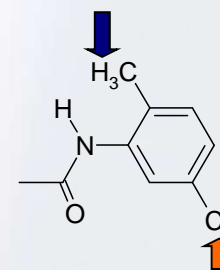
**CHIRALCEL OZ
CHIRALPAK AZ**



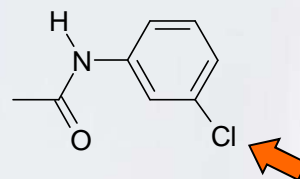
CHIRALPAK IC



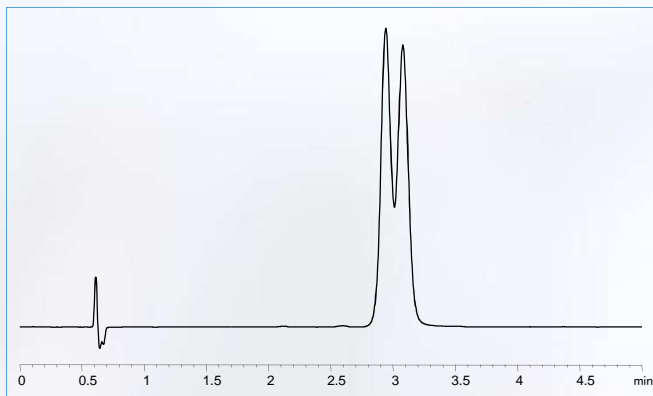
CHIRALPAK AY



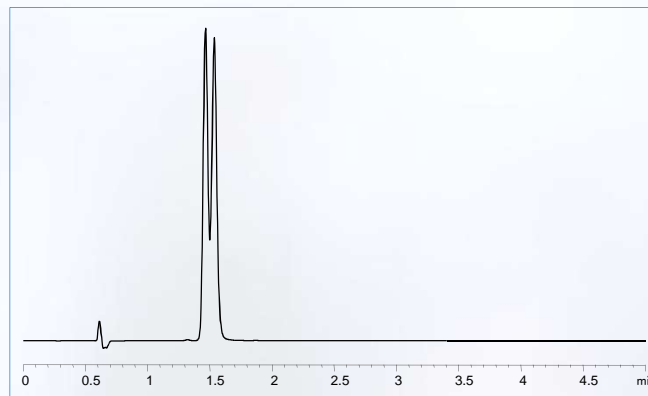
CHIRALPAK ID



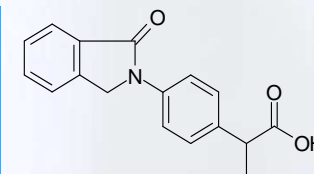
3.0 ml/min, BPr150bar, **30% MeOH** (+0.2% TFA) in CO₂



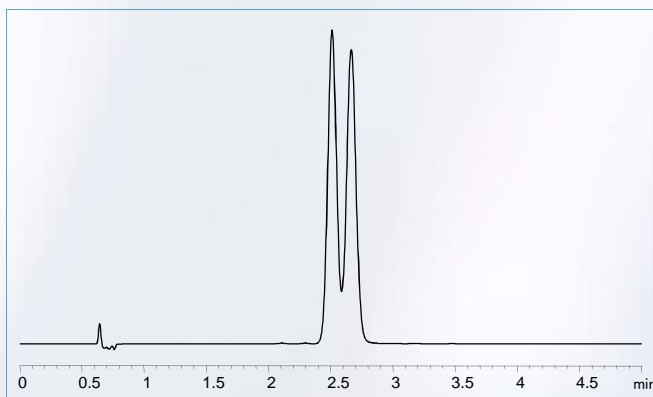
CHIRALPAK IA



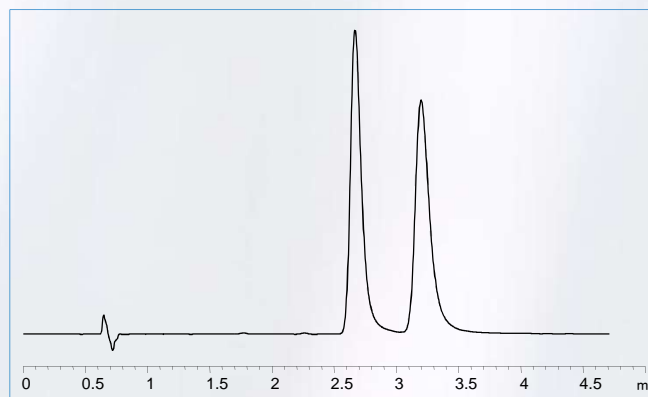
CHIRALPAK IB



Indoprofen



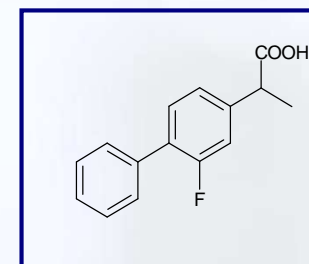
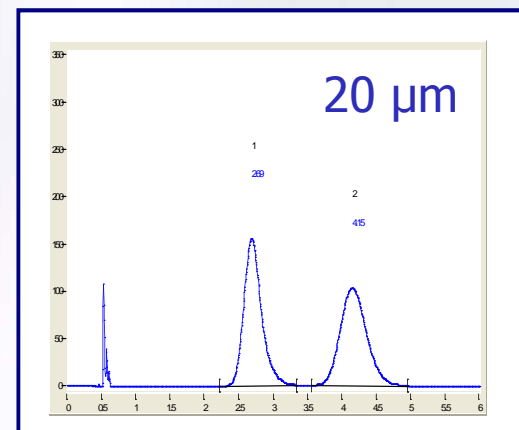
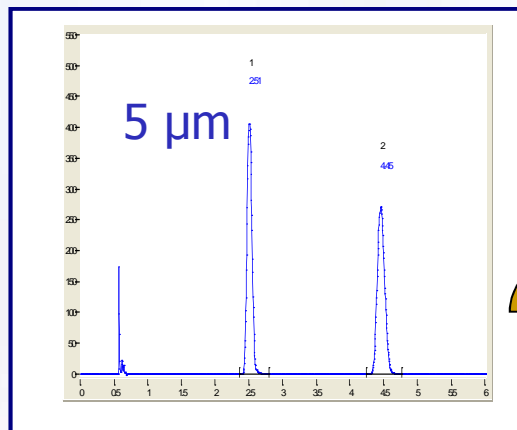
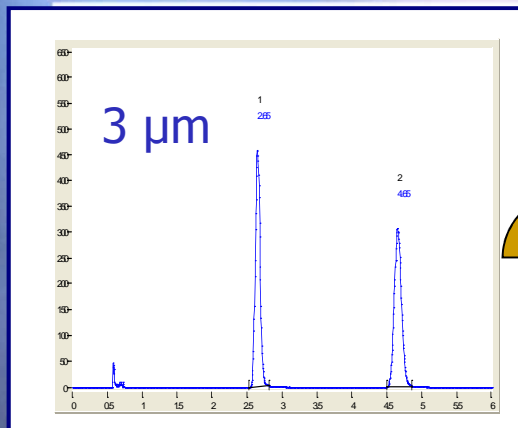
CHIRALPAK IC



CHIRALPAK ID



Moving to semipreparative
or
preparative scale



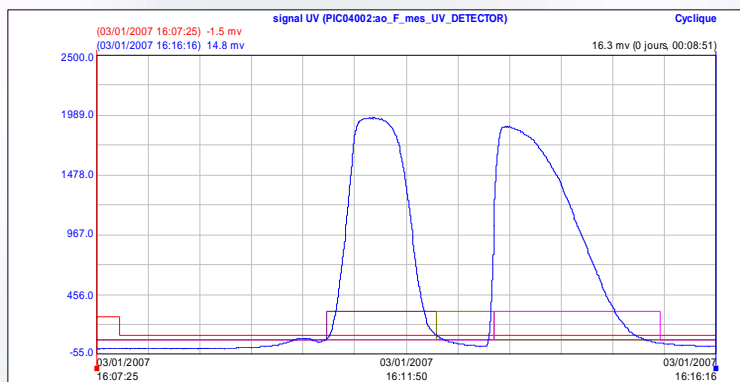
Flurbiprofen

CHIRALPAK® AD-type

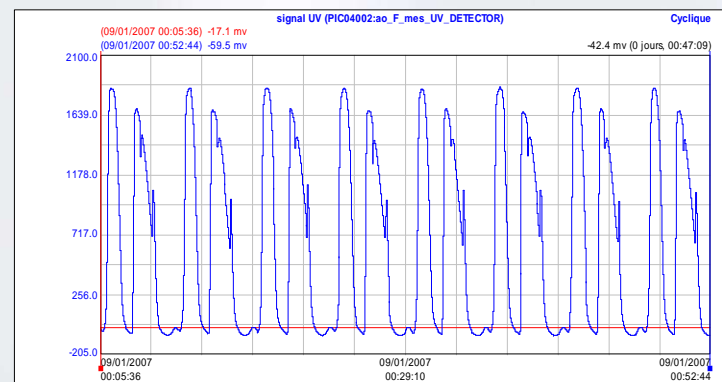
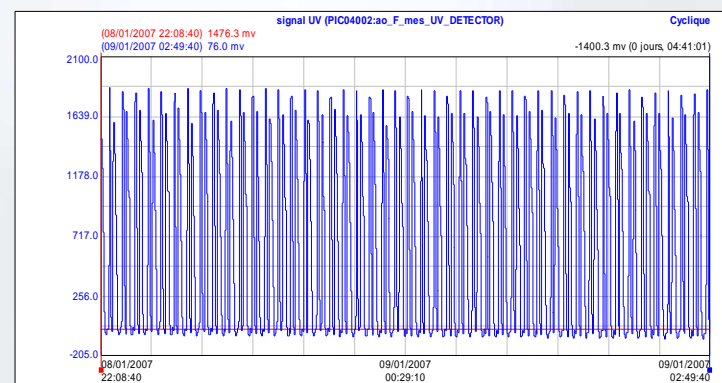
CO₂ / MeOH 90/10
3 ml/min, 30°C

Columns 150 x 4.6 mm

- Column : **CHIRALPAK AD-H SFC** – 25 x 3 cm
- Eluent : 80/20 CO₂/MeOH
- Flow rate : 120 ml/min
- Temperature : 25°C
- P outlet : 150 bar
- Loading : 200 mg

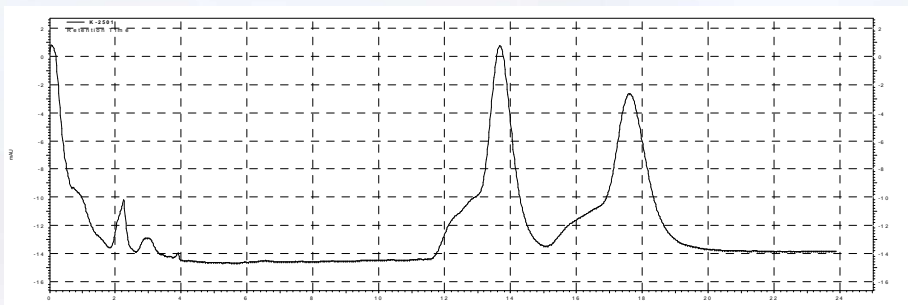


Injection 200 mg – run time 10 min

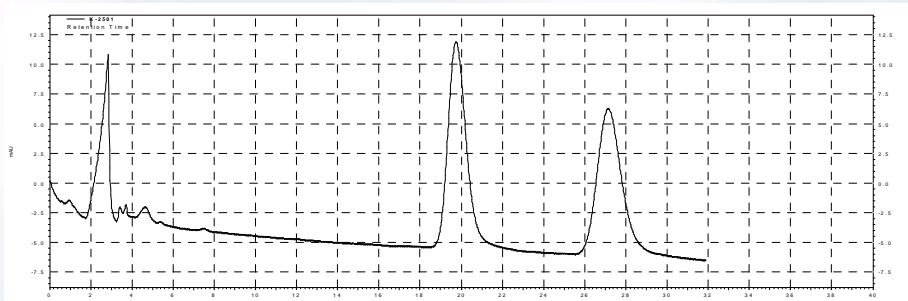


Stack Injections 200 mg every 6 min

First injection



Second injection - new inlet frit



Small and Medium Scale chromatography

by SFC

- SFC is a fast technique - so use a *fast* screen
- Primary screen in SFC alone will give you an 85% success rate overnight!
(99% by SFC and HPLC)
- Extending primary screen will give you 90% - 95%
- Secondary Screen will give you ca. 100%
- Using immobilised CSPs and DCM will make prep FASTER