CHIRAL TECHNOLOGIES EUROPE
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PREPARATIVE CHIRAL CHROMATOGRAPHY

What can go wrong and how to solve it

M. Schaeffer, T. Zhang, D. Robin, J.M. Heym, D. Colantuono, J. Lee, S. Khattabi, P. Franco

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Summary

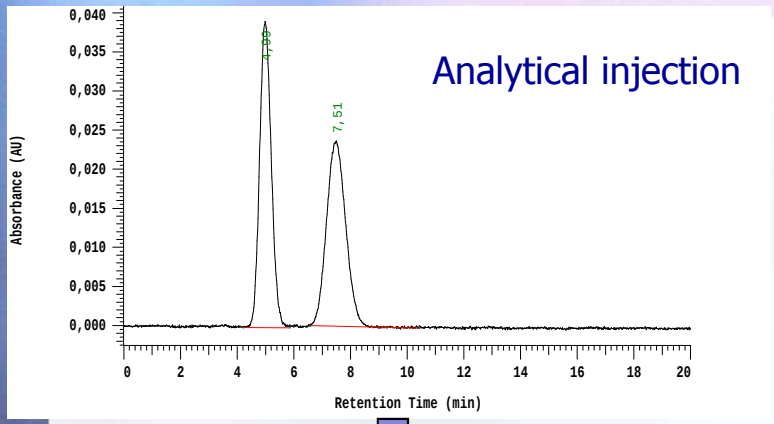
1) Preparative chiral chromatography

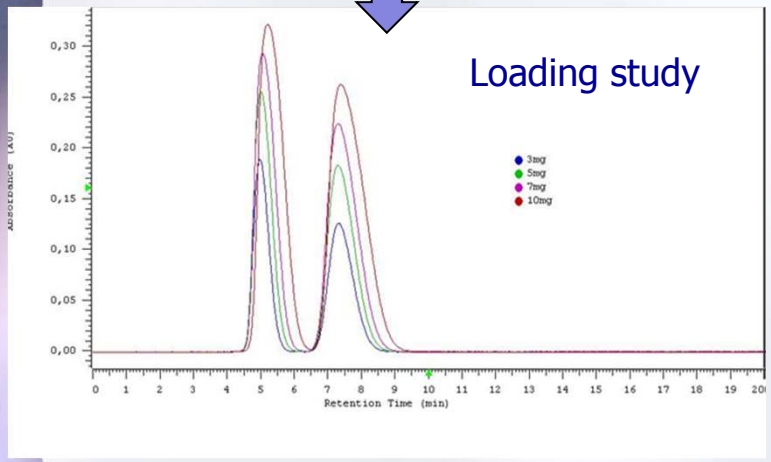
Features
Potential

2) What can go wrong? How to solve it?

Sample
Chromatographic method
Chromatographic system
Product recovery

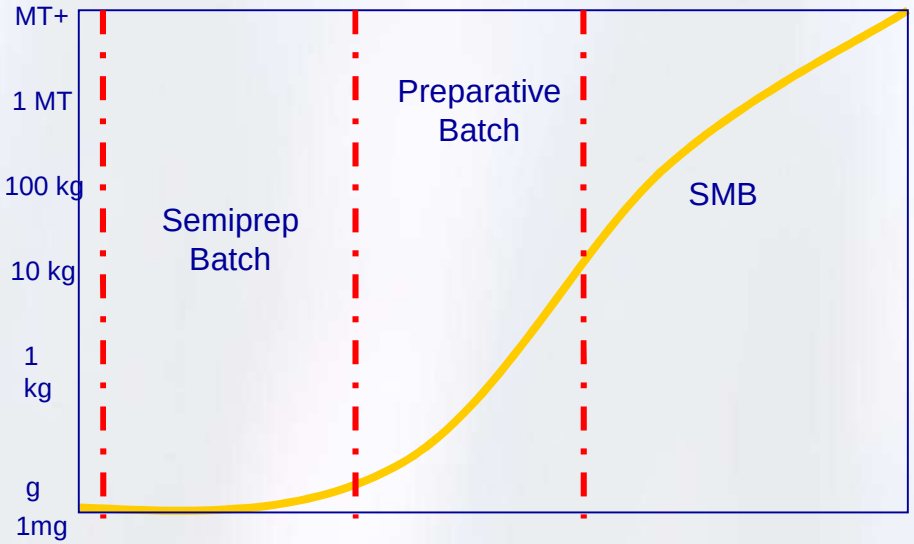
3) Conclusions





Easily scalable

from the analytical study



Preparative chiral chromatography

Different to achiral chromatography

Uses Chiral Stationary Phases (CSP)

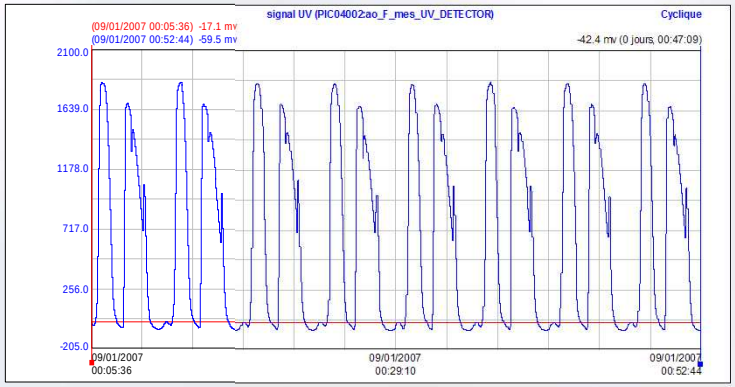
Isolation of two components, not purification from a crude

Can separate racemates, diastereomers and atropisomers

Batch and Continuous Systems (SMB)

Yield is ca. 90%

Specification is ≥ 98% e.e.

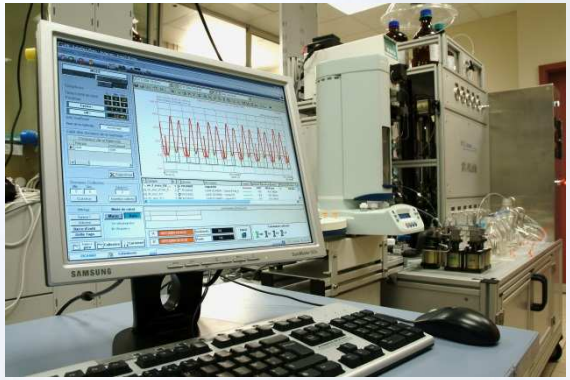


Several systems and modes possible

Batch LC

SMB

Batch SFC



To be chosen based on recognition and scale

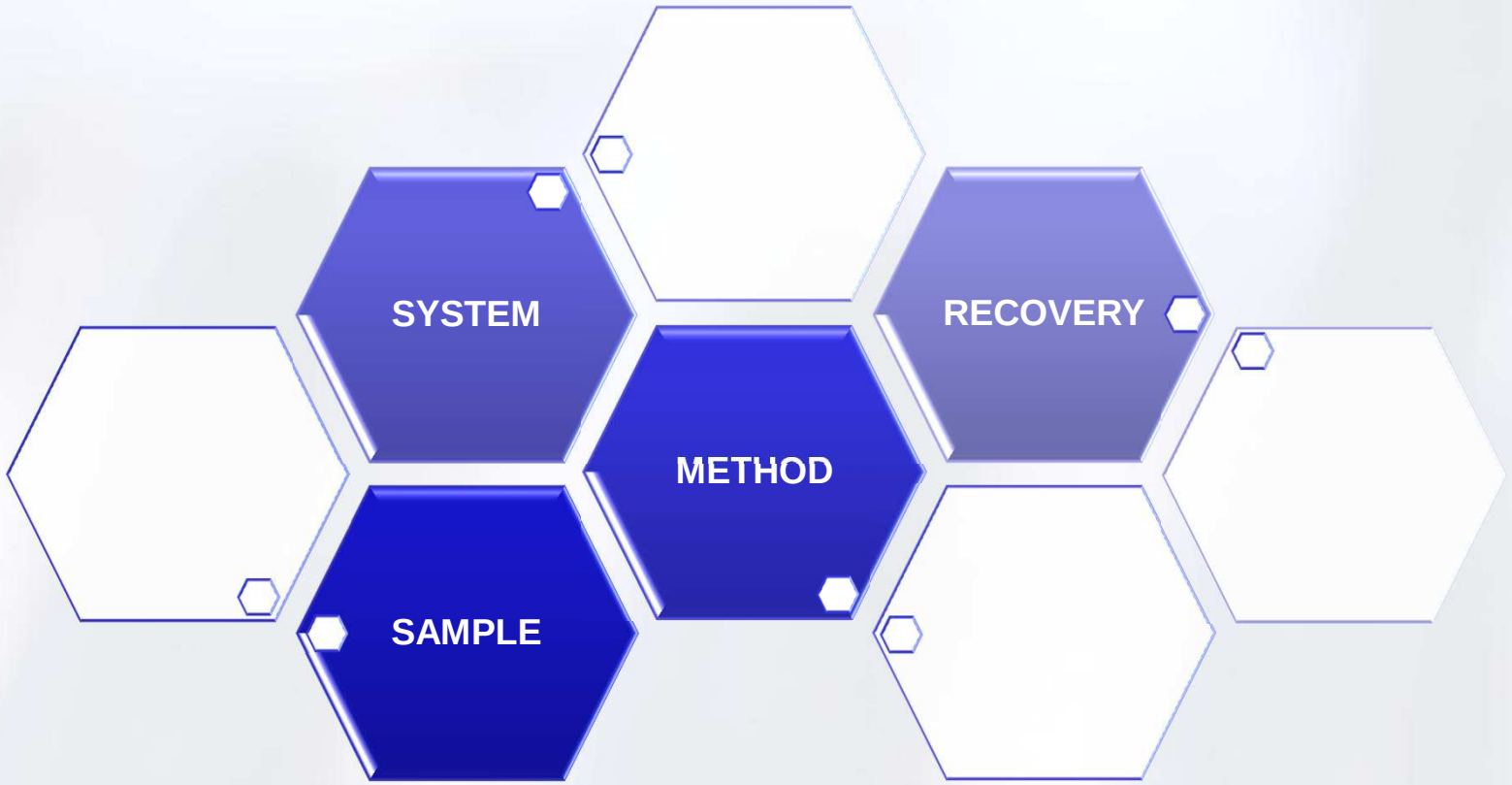
What can go wrong?

&

How to solve it?

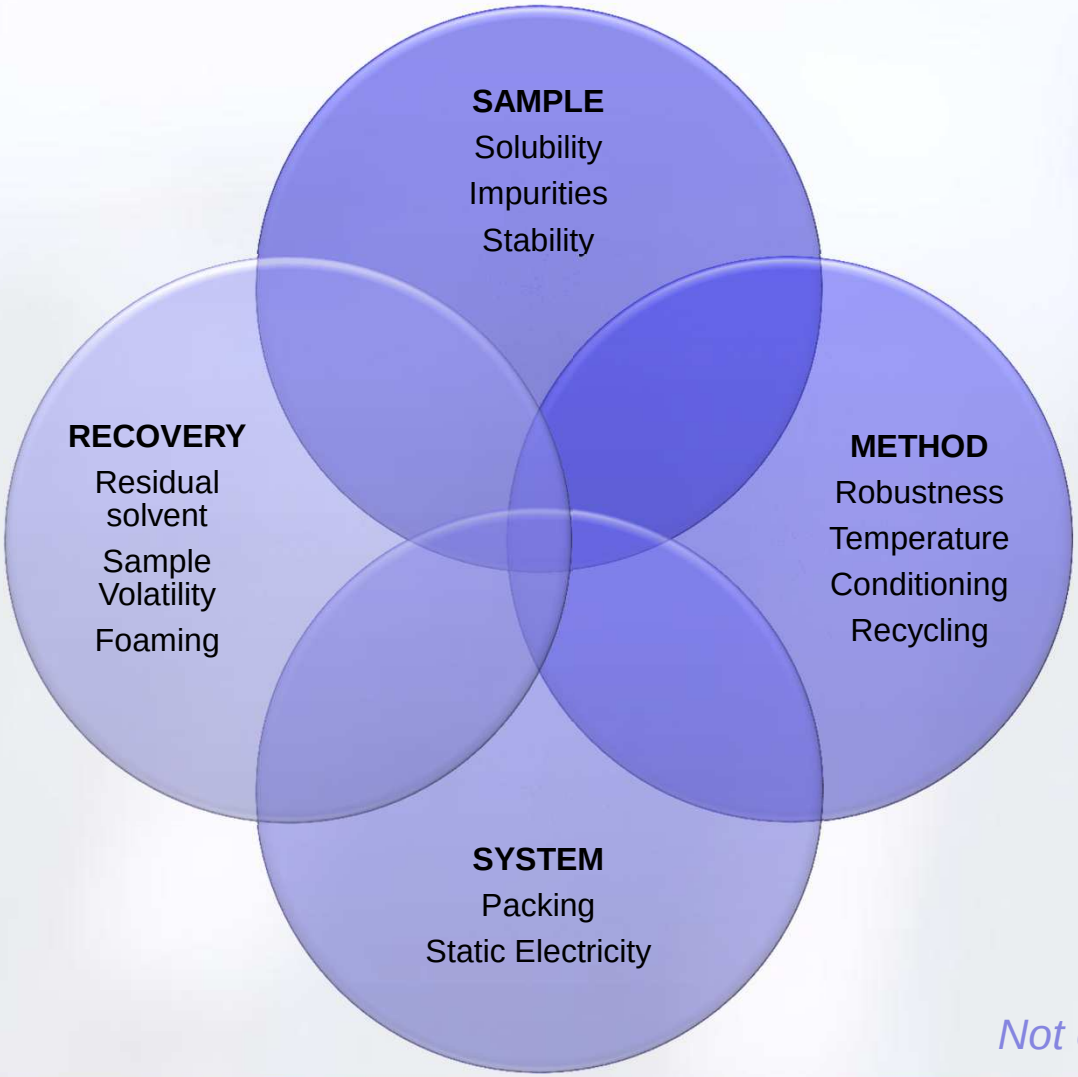
What can go wrong?

... Observing the different elements...

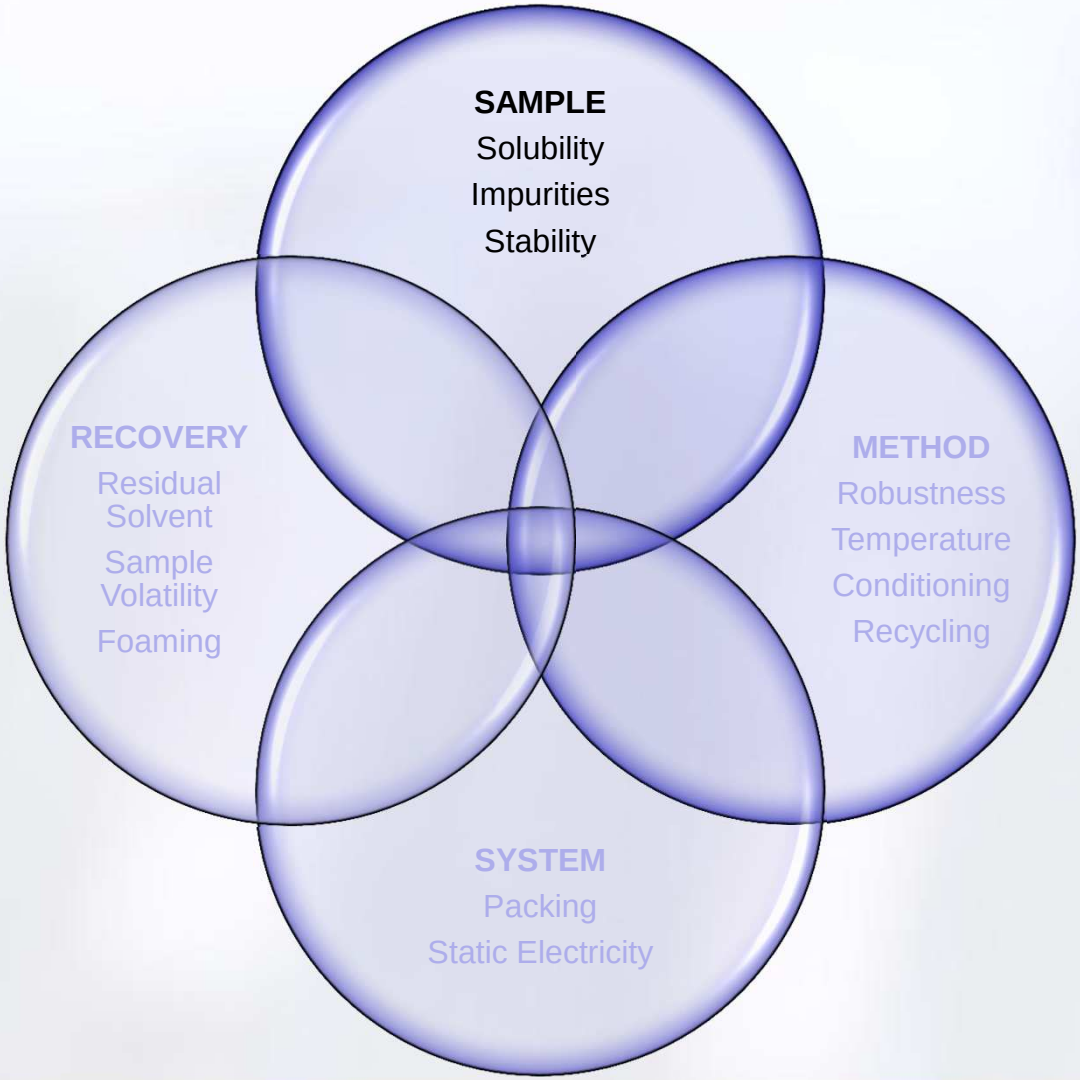


... to find the potential solution.

What can go wrong?
Some examples



Not exhaustive list


SAMPLE
Solubility
Impurities
Stability
RECOVERY
Residual Solvent
Sample Volatility
Foaming
METHOD
Robustness
Temperature
Conditioning
Recycling
SYSTEM
Packing
Static Electricity

Sample solubility

Potential causes of insolubility:

- Nature of the sample
- Presence of impurities

Potential associated problems:

- Frit blockage, with or without associate higher pressure
- Perturbance of the separation and method instability
- Precipitation of the sample in the chromatographic system or tanks
- Need for a very large sample volume to be injected

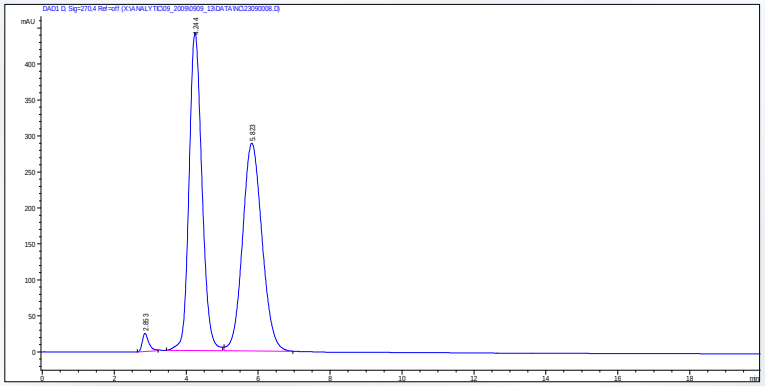
Potential ways to solve the problem:

- Look for alternative mobile phase combinations
- Apply thorough filtration of the sample or even preliminary chromatographic step
  - Choose a different molecule in the synthetic route or a more soluble derivative
- Inject sample in solvent different to mobile phase
- Thermostat the feed solution
- Try selective precipitation/crystallisation of selected components prior to chromatography

Optimising conditions in method development

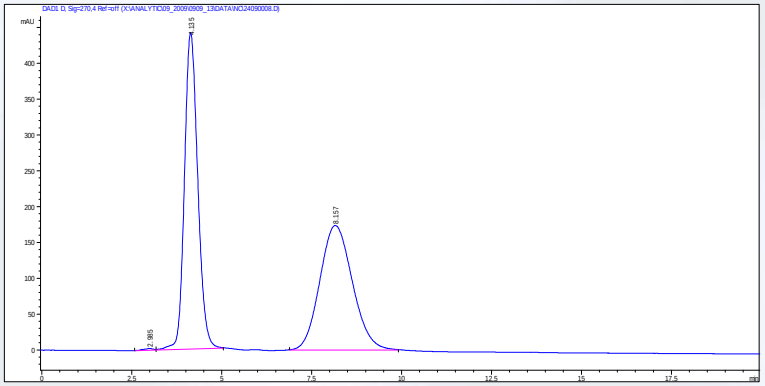
Our best investment!!

CHIRALPAK IC – 20 µm



***n*-heptane / THF 60/40**

Rs = 2.0 – α = 2.1



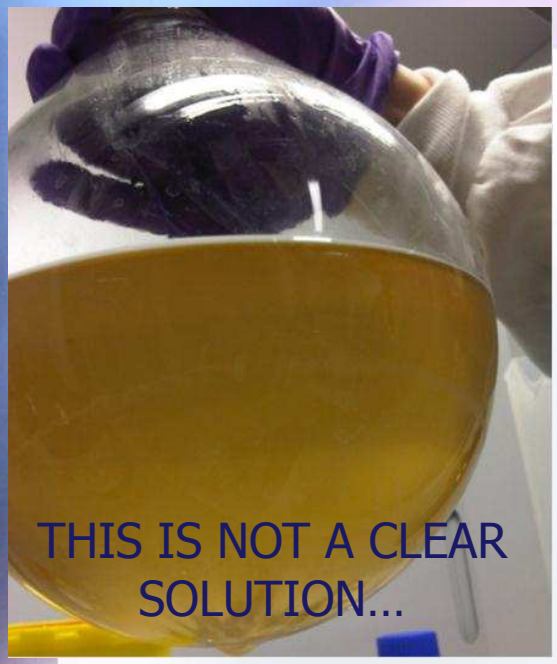
***n*-heptane / methyl-THF 40/60**

Rs = 3.5 – α = 4.5

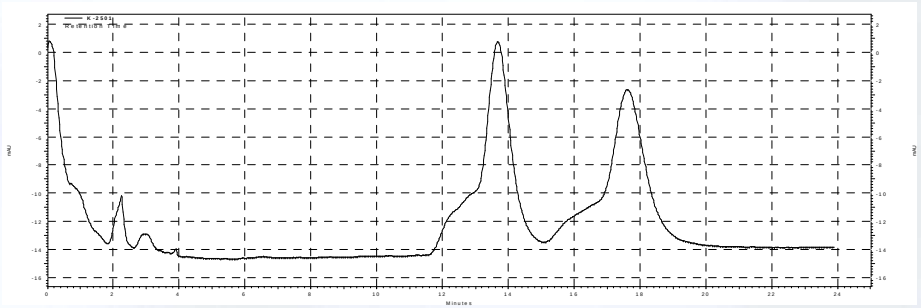
Two improvements:

- Larger resolution
- Lower *n*-heptane content, which may enhance solubility

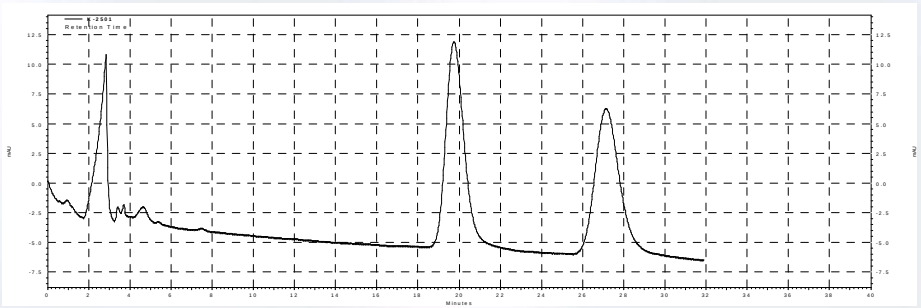
The importance of filtration



Peak distorsion in SFC due to dirty frits

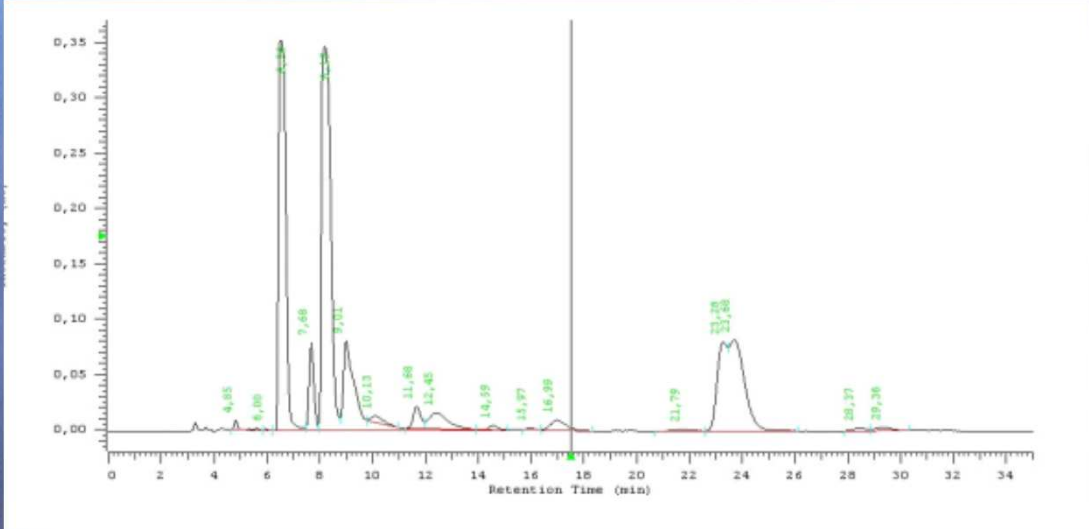


New injection after frit replacement



… and leads to dirty frits

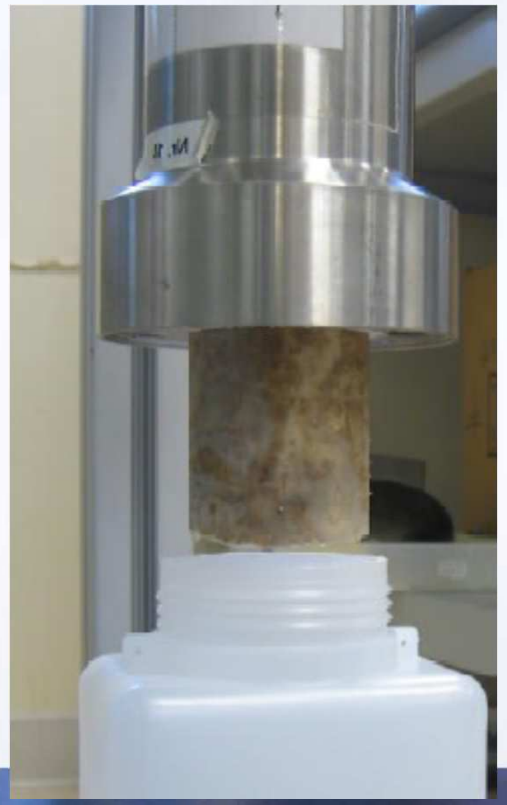
Presence of impurities in the sample



It is possible to work in the presence of impurities, but they may get absorbed in the CSP

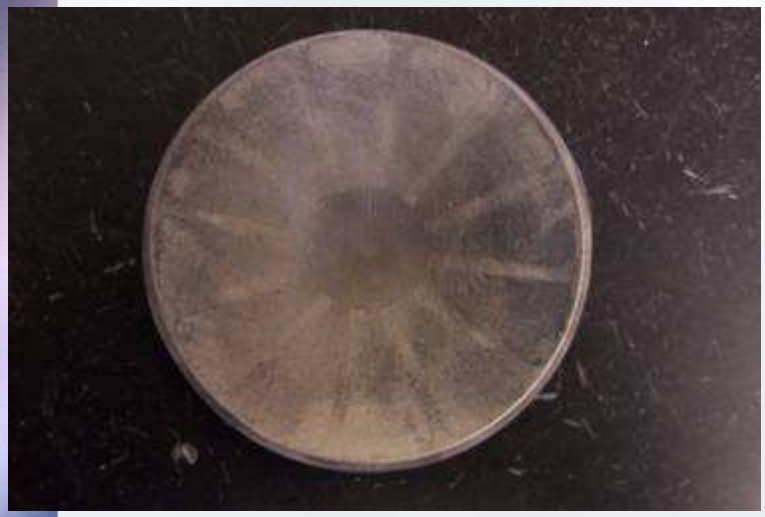
Stack injections will be more difficult

Backflush or washing steps with stronger solvents are possible



Troubleshooting Contaminants

Blocked Frits: 5 cm SFC columns



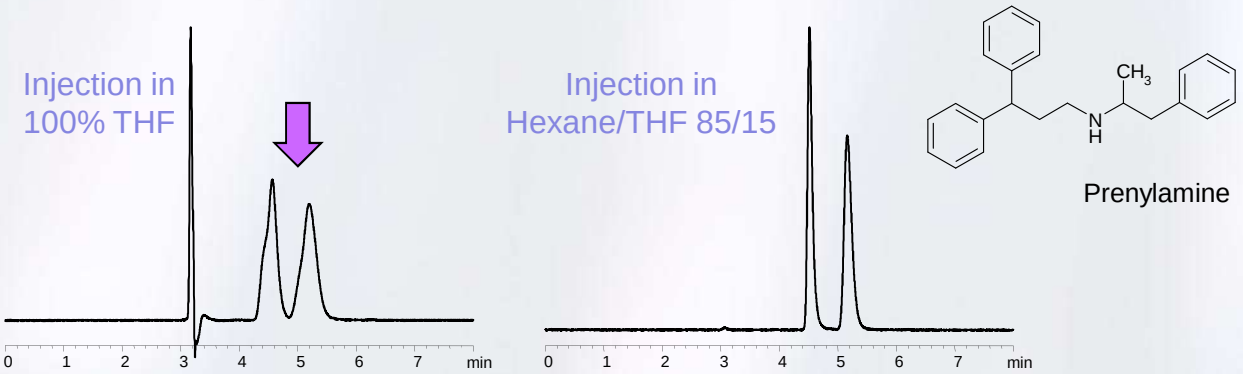
When the inlet frit is blocked, there is a high pressure differential across it; it may deform…

Injecting in a solvent different from mobile phase

There are several risks associated to such a practice:

- Strong peak distorsion due to sample solvent
- Changes in the separation due to solvent front
- Higher risks of sample precipitation due to different composition
- Difficulties for solvent recycling
- Process instability with continuous systems

CHIRALPAK IA
Hexane/THF/DEA 85/15/0.1



Ideally injection should be done in mobile phase (or close composition)…

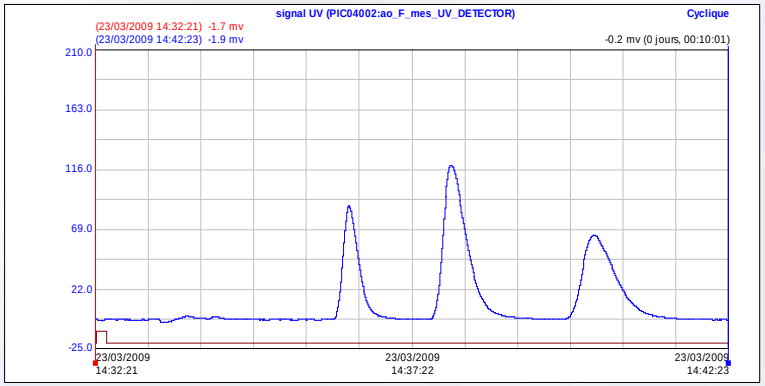
Sample solubility

Injecting in a solvent different from mobile phase

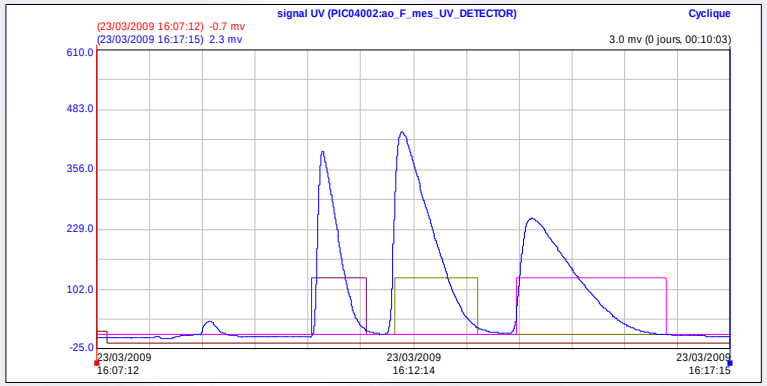
CHIRALPAK IC
(250 x 30 mm)
CO_2/EtOH 70/30
120 ml/min, 25°C

DCM is less polar than alcohols or THF
(not compatible with all columns)

Solubility in EtOH < 2 g/L
Solubility in EtOH/DCM 90/10 = 58 g/L



Analytical injection



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

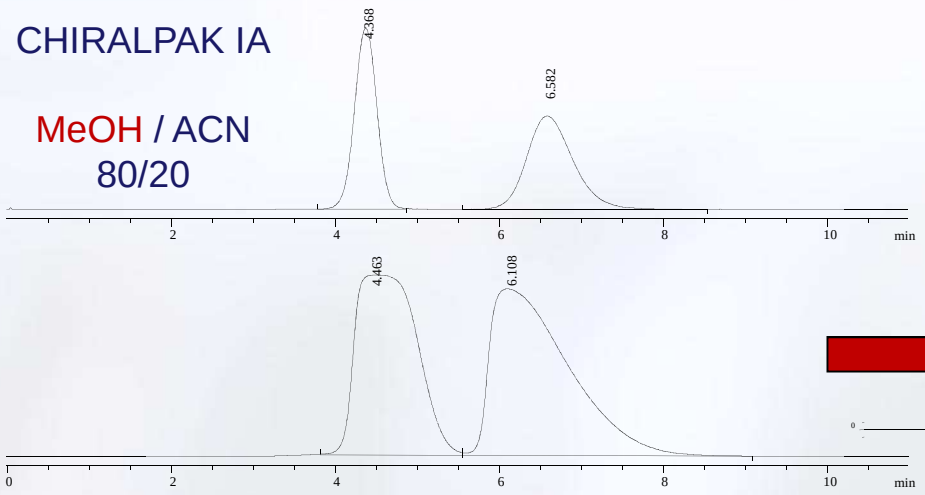
No perturbation of the separation

Compound stability

A case study

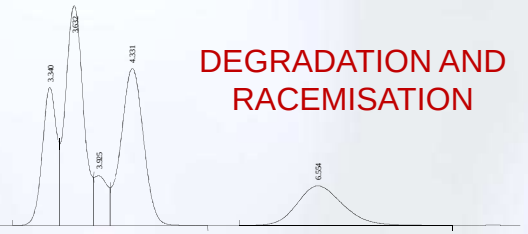
CHIRALPAK IA

MeOH / ACN
80/20



1st eluted enantiomer
after 12 h at 55°C

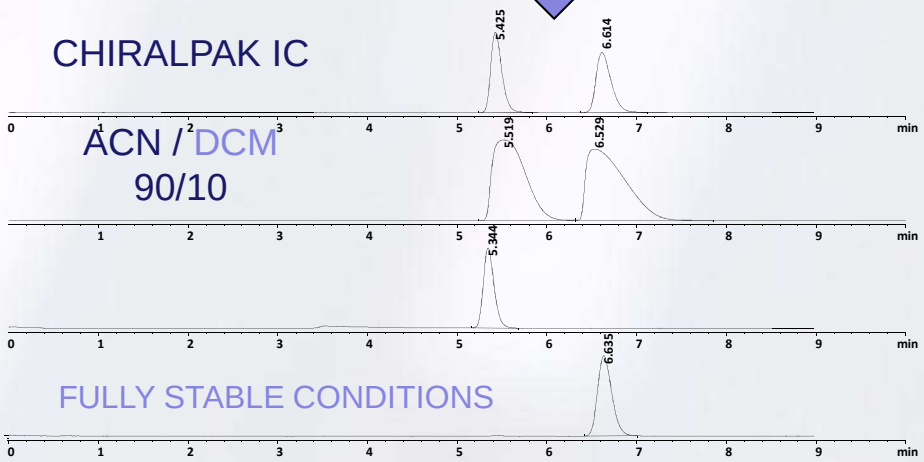
DEGRADATION AND
RACEMISATION



Compound with glutarimide moiety

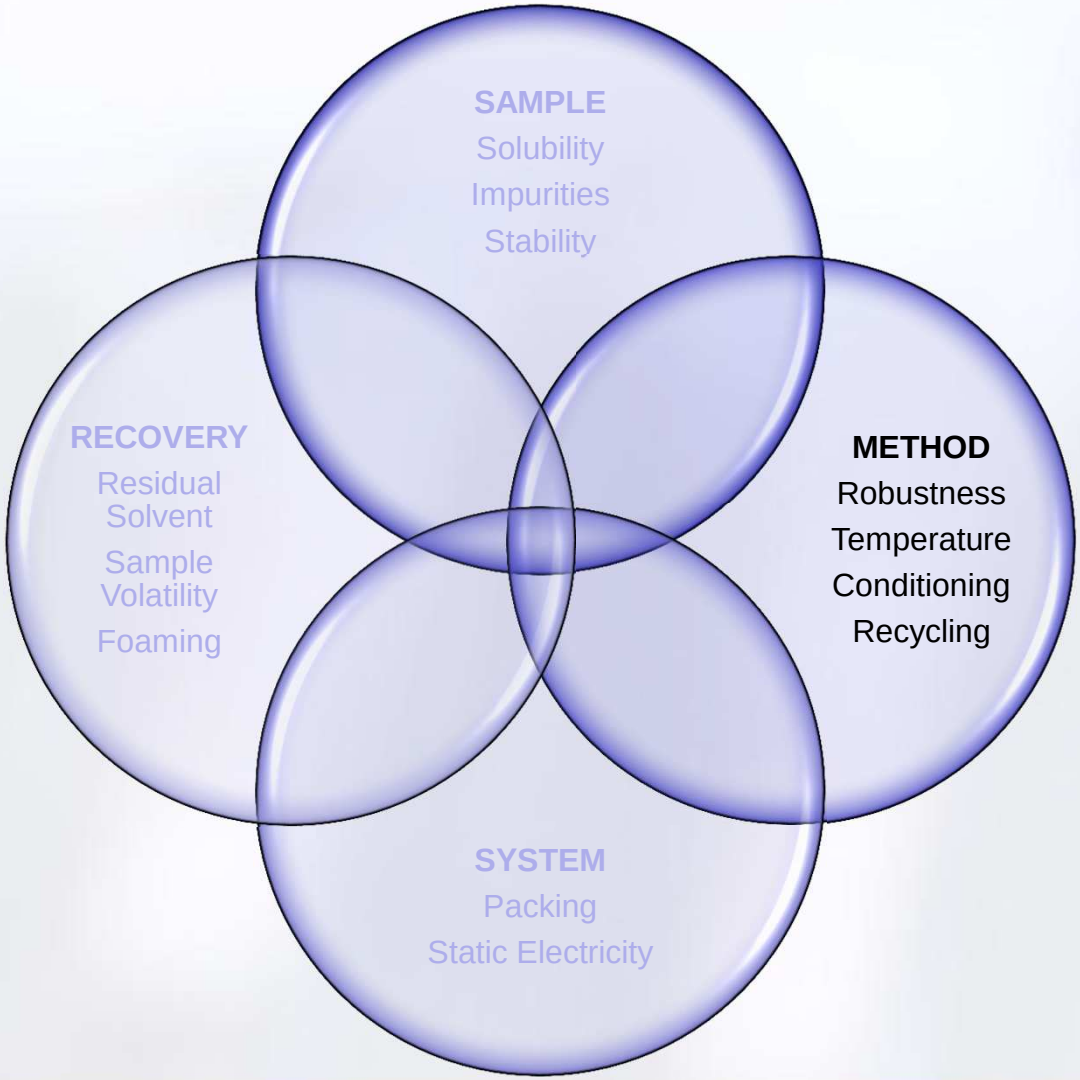
CHIRALPAK IC

ACN / DCM
90/10



FULLY STABLE CONDITIONS

In certain cases, degradation and/or racemisation can be controlled with lower evaporation temperature


SAMPLE
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Sample Volatility
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Robustness
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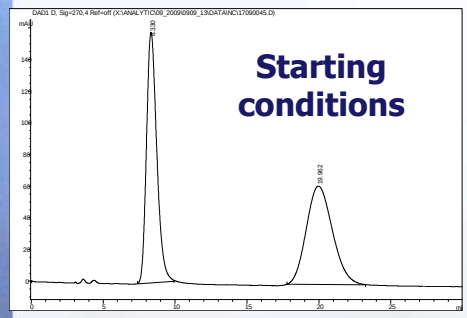
Recycling
SYSTEM
Packing
Static Electricity

Robustness of chromatographic method

A case study

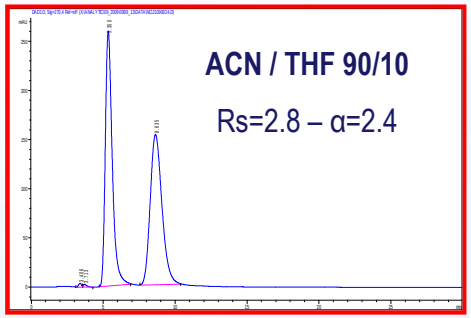


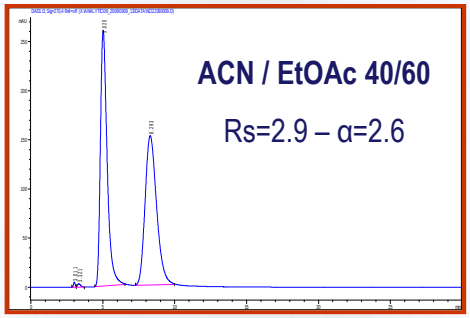
100% ACN

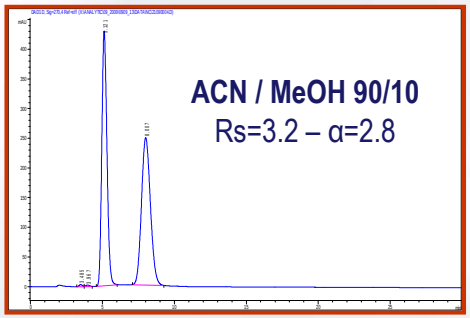
Run >20 min

Rs=5.0 – α=3.5

Target
Run time < 9 min



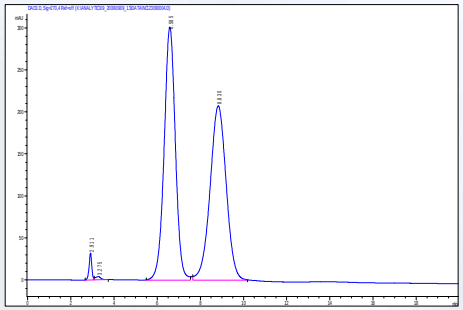
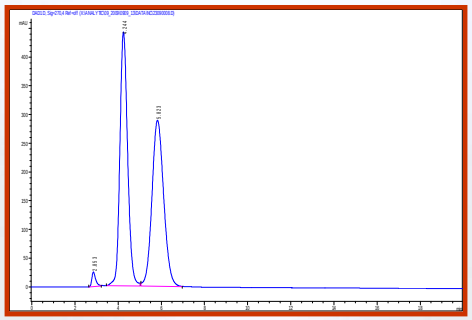
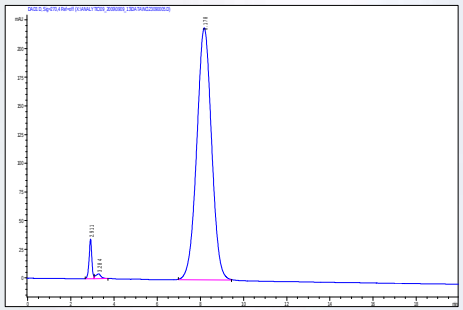
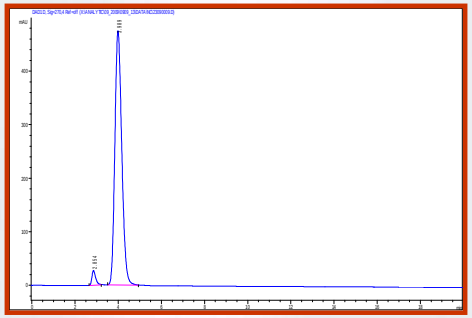




CHIRALPAK IC – 20 µm

Final conditions should consider method robustness and solvent recovery

Optimising conditions in method development

	CHIRALPAK IA	**CHIRALPAK IC**
	***n*-heptane / THF 60/40**	***n*-heptane / THF 60/40**
Racemate		
Target enantiomer		

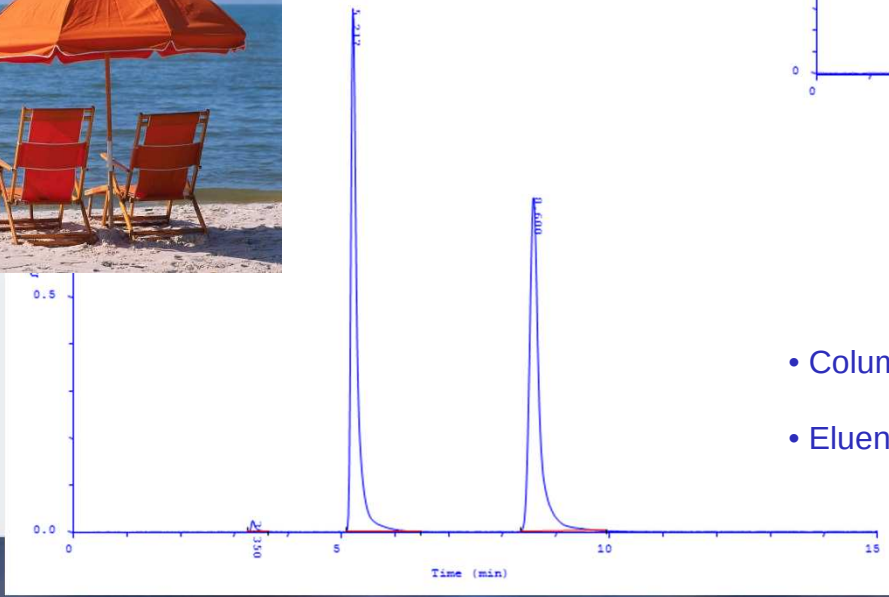
Target enantiomer

eluted first

Temperature effects

- Column : **CHIRALPAK IA** 5 µm LC – 250 x 50 mm
- Eluent : n-Heptane / 2-PrOH 90/10
- Flow rate : 120 ml/min
- Trans-stilbene oxide injections

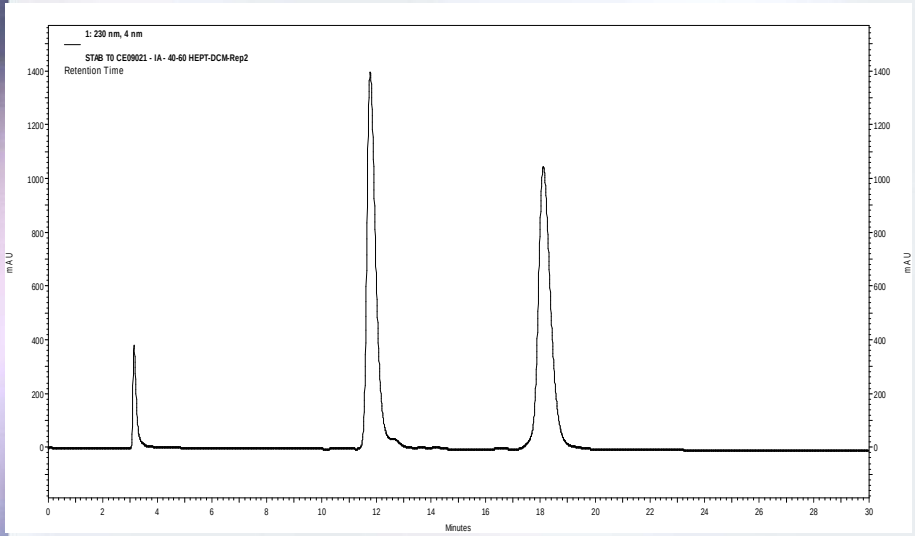
- Column temperature : ambient
- Solvent coming from external storage: in winter!!



- Column Temperature : 30℃
- Eluent Temperature : 30℃

CSP conditioning

- Column : **CHIRALPAK IA** 5 µm
- Eluent : n-Heptane / DCM
- Flow rate : 1 ml/min
- Temperature : 25°C

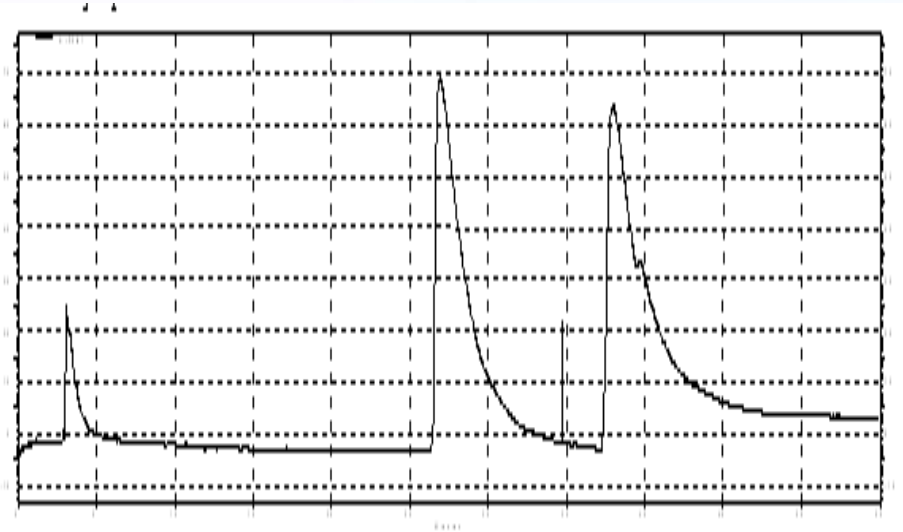


Initial separation found after the screening on the analytical column

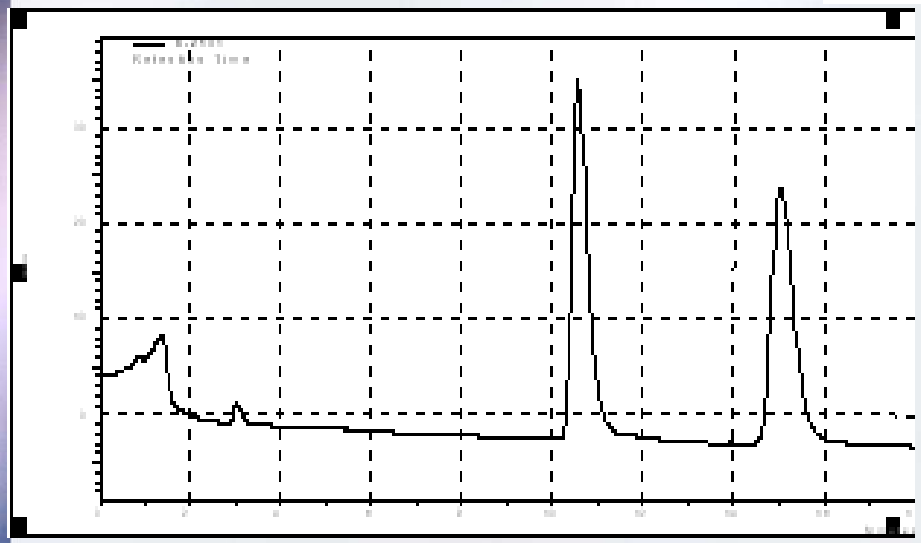
Column used for standard screening with different solvents including alcohols

CSP conditioning

- Column : **CHIRALPAK IA** 5 µm
- Eluent : n-Heptane / DCM
- Flow rate : 20 ml/min
- Temperature : 25℃



Transfer on the LC preparative column



+1% EtOH in the mobile phase

Influence of water in certain separations

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Journal of Chromatography A, 839 (1999) 23–39

JOURNAL OF CHROMATOGRAPHY A

Direct high-performance liquid chromatographic separations of metoprolol analogues on a Chiralcel OD column using chemometrics

S. Svensson, J. Vessman, A. Karlsson*
Analytical Chemistry, Astra Hässle AB, S-431 83 Mölndal, Sweden

Received 25 November 1998; received in revised form 26 January 1999; accepted 27 January 1999

S. Svensson et al., *J. Chromatogr. A* 839 (1999) 23

together with:

K. Balmér et al., *J. Chromatogr. A* 592 (1992) 331

Influence of water in the separation of metoprolol analogues

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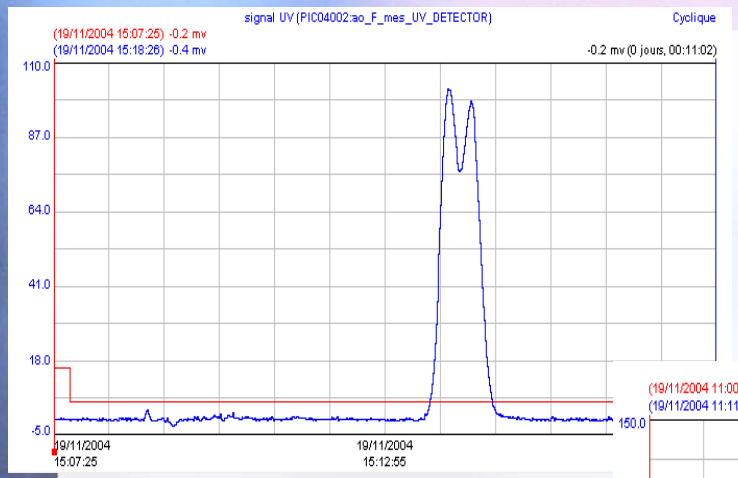
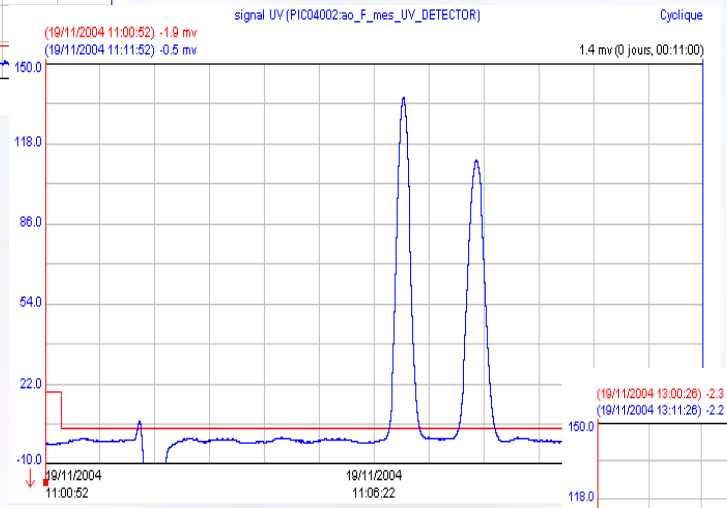
Method optimisation
The use of additives



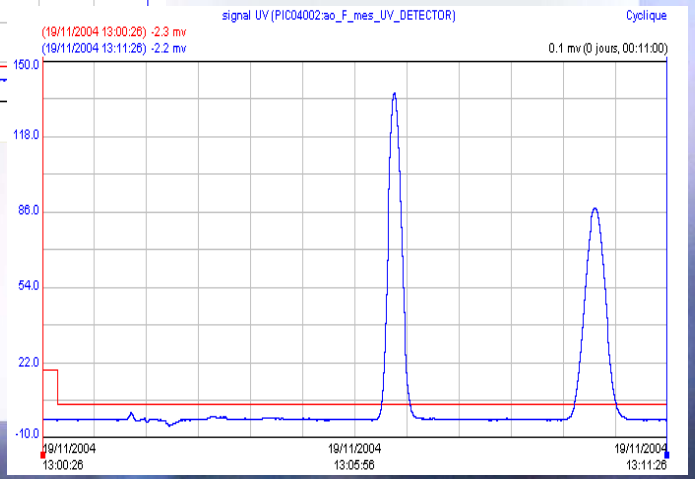
20% Isopropanol

CHIRALPAK AD-H

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



20% Isopropanol
+1% Diethylamine
(in co-solvent)



20% Isopropanol
+1% Butylamine
(in co-solvent)

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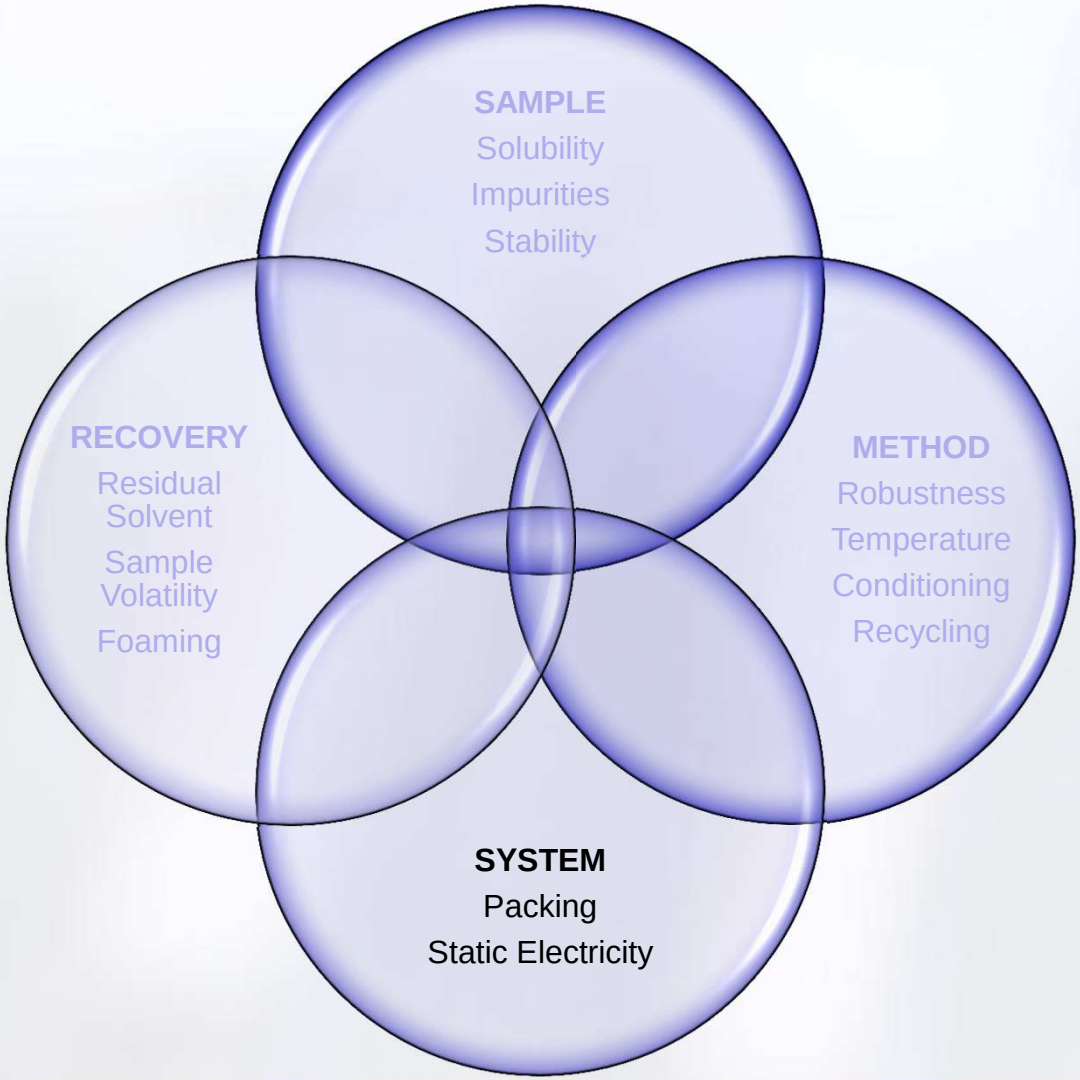
Mobile phase recycling

Some thoughts:

- Solvent recycling will get more challenging when increasing number of components (i.e. heptane/ethanol/methanol)
- Having mobile phases with relatively different boiling components (i.e. DCM and heptane)
- Working close to the azeotrope composition, when possible, can help
- Product carryover should be controlled in the recycled solvent


SAMPLE
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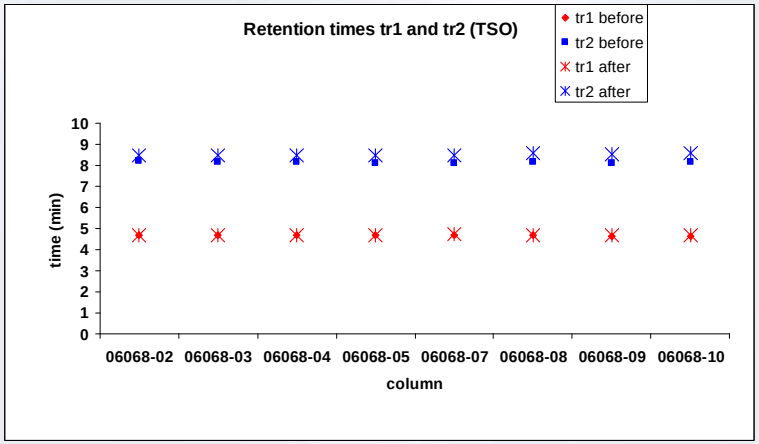
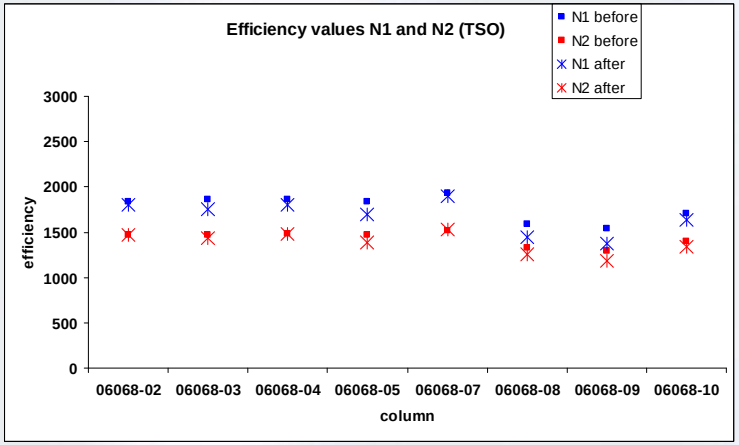
Column packing

Essential parameter
either in batch
or continuous chromatography





Need of well packed columns and homogenous sets, with clean frits

Separation of glutethimide on CHIRALPAK IA

Mini-SMB study in 100% ethyl acetate

The operation in pure ethyl acetate produced electrostatic energy!!

It was necessary to add additional earth contact points in the mini-SMB system

Electrostatic energy

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Journal of Chromatography A, 1094 (2005) 165–168

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Stability problems of polyether ether ketone and ethylene-tetrafluoroethylene copolymer tubing in simulated moving bed operation

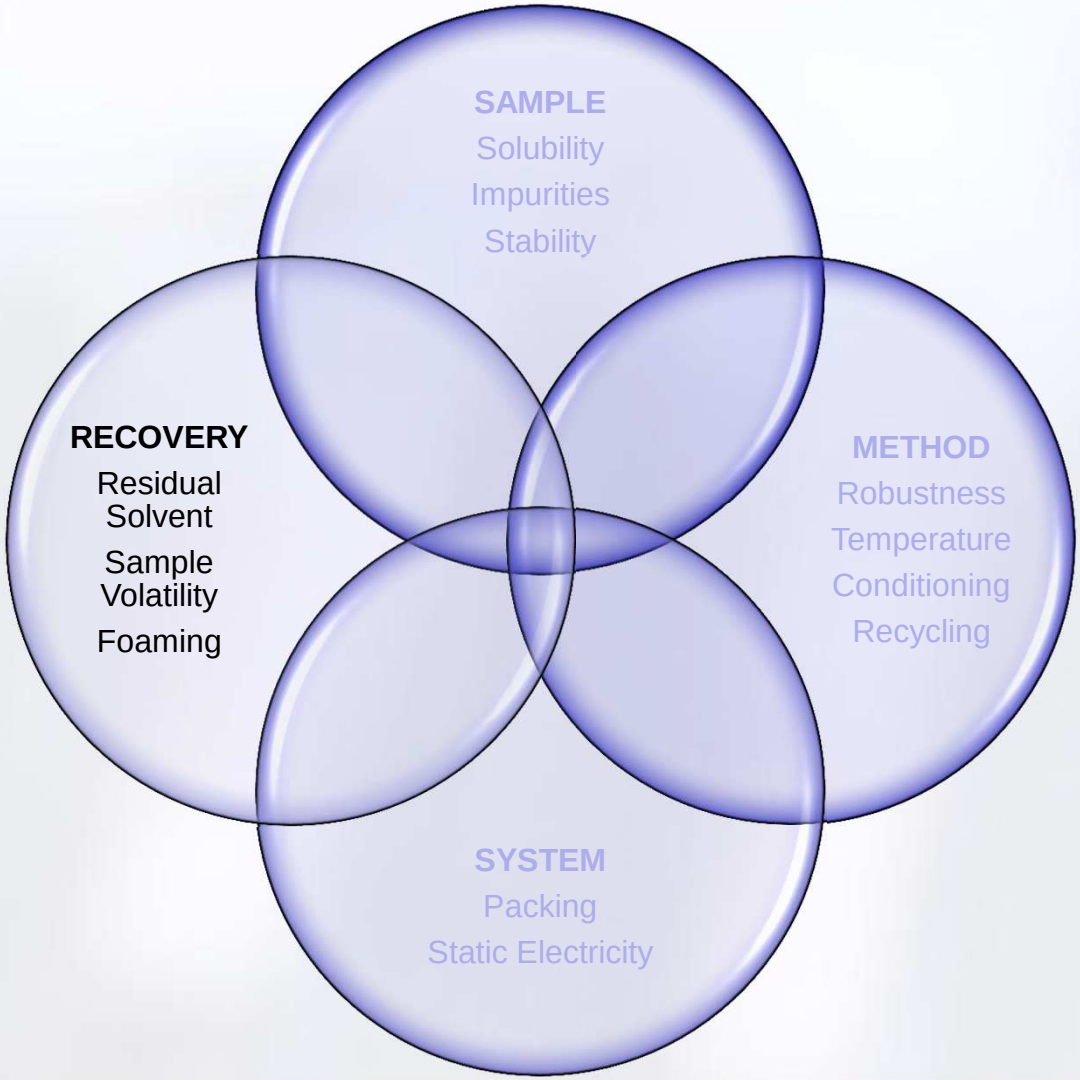
Larry Miller [a,1], Markus Juza [b,*]

[a] *Pfizer Inc, 4901 Searle Parkway, Skokie, IL, USA*
[b] *CarboGen AG, Schachenallee 29, CH-5001 Aarau, Switzerland*

Received 24 March 2005; received in revised form 12 August 2005; accepted 23 August 2005
Available online 19 September 2005

L. Miller and M. Juza, *J. Chromatogr. A* 1094 (2005) 165-168

Consequences of the use of solvent mixtures with high alkane content


SAMPLE
Solubility
Impurities
Stability
RECOVERY
Residual Solvent
Sample Volatility
Foaming
METHOD
Robustness
Temperature
Conditioning
Recycling
SYSTEM
Packing
Static Electricity

Product recovery

Residual solvent removal:
- Exhaustive drying, without compromising stability
- Azeotropic distillation with a different solvent
  - (i.e. THF removal with MeOH, ethyl acetate with acetone)

Removal of solvent stabiliser:
- The case of BHT in stabilised THF

Sample volatility:
- Adjustment of evaporation temperature and vacuum
- Choice of suitable chromatographic solvent, if possible
- Avoid complete evaporation and ship in selected solvent

Foam formation:
- Product to be recuperated from evaporator more frequently
- Frequent replacement of evaporator filter cartridge

Preparative chiral chromatography

Don’t take me wrong!!!

Preparative chromatography is a very reliable technique.

A number of industrial processes demonstrate this statement.

However, we are working very often in a developmental environment…

… at this point, we have limited information about the molecules and processes.

In all cases, our best investment would be having a proper method development.

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Conclusions

We will always have better chances with a solid basis

CSP

- Selectivity
- Loadability
- Stability in the operating conditions
- Availability (analytical and bulk)
- Batch-to-batch reproducibility

EQUIPMENT

- Fit for purpose
- Properly maintained

METHOD

- Loadability
- Solubility in mobile phase
- Viscosity of mobile phase
- Temperature
- Stability in operating conditions
- No interference with sample impurities
- Solvent recycling
- Repeatability

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PREPARATIVE CHIRAL SEPARATIONS

… more details

Our Vendor seminar today at 12:15 h