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
1) **Preparative chiral chromatography**

   - Features
   - Potential

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

   - Sample
   - Chromatographic method
   - Chromatographic system
   - Product recovery

3) **Conclusions**
Preparative chiral chromatography

Easily scalable from the analytical study

Analytical injection

Loading study
Preparative chiral chromatography

Different to achiral chromatography

Uses Chiral Stationary Phases (CSP)

Batch and Continuous Systems (SMB)

Isolation of two components, not purification from a crude

Can separate racemates, diastereomers and atropisomers

Yield is ca. 90%

Specification is ≥ 98% e.e.
Preparative chiral chromatography

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
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Potential causes of insolubility:
• Nature of the sample
• Presence of impurities

Potential associated problems:
• Frit blockage, with or without associate higher pressure
• Perturbation 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 – $\alpha$ = 2.1

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

Rs = 3.5 – $\alpha$ = 4.5

Two improvements:

• Larger resolution
• Lower $n$-heptane content, which may enhance solubility
Sample solubility

The importance of filtration

Peak distortion 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.
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 distortion 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)...

Preparative Chiral HPLC: Sample solubility

Preparative Chiral HPLC: Sample solubility
Injecting in a solvent different from mobile phase

CHIRALPAK IC
(250 x 30 mm)
CO\textsubscript{2}/EtOH 70/30
120 ml/min, 25°C

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

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

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

CHIRALPAK IC
ACN / DCM
90/10

FULLY STABLE CONDITIONS

1st eluted enantiomer after 12 h at 55°C
DEGRADATION AND RACEMISATION

Compound with glutarimide moiety

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

A case study

Starting conditions

100% ACN
Run >20 min
Rs=5.0 – α=3.5

Target
Run time < 9 min

ACN / EtOAc 40/60
Rs=2.9 – α=2.6

ACN / THF 90/10
Rs=2.8 – α=2.4

ACN / MeOH 90/10
Rs=3.2 – α=2.8

CHIRALPAK IC – 20 µm

Final conditions should consider method robustness and solvent recovery
Optimising conditions in method development

Suitability of chromatographic method

CHIRALPAK IA

$n$-heptane / THF 60/40

Racemate

Target enantiomer

CHIRALPAK IC

$n$-heptane / THF 60/40

Target enantiomer eluted first

Optimising conditions in method development
- 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°C
- Eluent Temperature: 30°C
• 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
- Column: **CHIRALPAK IA** 5 µm
- Eluent: n-Heptane / DCM
- Flow rate: 20 ml/min
- Temperature: 25°C

Transfer on the LC preparative column

+1% EtOH in the mobile phase
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 Hickey AB, S-421 83 Mölndal, Sweden

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together with:


Influence of water in the separation of metoprolol analogues
Method optimisation

The use of additives

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

20% Isopropanol

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

20% Isopropanol
+1% Butylamine
(in co-solvent)
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
Essential parameter either in batch or continuous chromatography.

Retention times $t_{r1}$ and $t_{r2}$ (TSO)

Efficiency values $N_1$ and $N_2$ (TSO)

Need of well packed columns and homogenous sets, with clean frits.
The operation in pure ethyl acetate produced electrostatic energy!!

It was necessary to add additional earth contact points in the mini-SMB system.
Consequences of the use of solvent mixtures with high alkane content
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
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.
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
PREPARATIVE CHIRAL SEPARATIONS

... more details

Our Vendor seminar today at 12:15 h