

Non Chiral Separation ? Think Chiral ! (And Vice Versa)



**Wim Dermaut, Hilde Vanbaelen, Albert Van Loon,
Peter Goeminne, Jef Proost, Luc Van Der Steen**

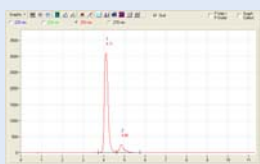
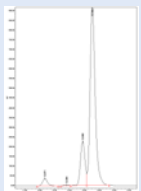
Johnson and Johnson Pharmaceutical Research and Development, API Small Molecule Development, Beerse, Belgium



Introduction

Two different compounds needed to be purified by preparative chromatography. In the first case, an elimination impurity had to be removed, but resolution on common RP HPLC was poor. Screening on chiral phases gave good results on Whelk-O 1, so this phase was used in the final preparative SFC separation. In the second case, two diastereomers needed to be separated. Here, chiral stationary phases did not show the selectivity needed, but sufficient separation was obtained on a cyano phase. Some issues with the solvent plug from the injection solution disturbing the elution profile were observed in this second case.

Case 1 : Non chiral compound Analytical

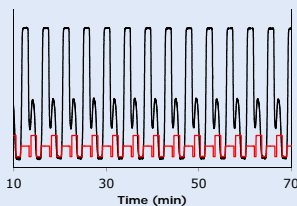


- HPLC, RP C18 (Hypersil BDS 5 μ)
- Grad 0.5% Amm. Acetate - Methanol
- Long runtime, poor selectivity $\alpha = 1.01$
- Not suitable for prep scale
- SFC Whelk-O 1, 10 μ
- Isocratic CO₂/Methanol + 0.2% iPrAm (70/30)
- Moderate runtime, better selectivity $\alpha = 1.18$
- Possible for use at prep scale

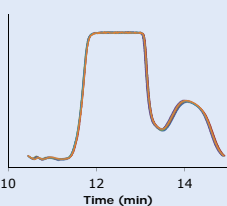
➔ Chiral phase gives better resolution than non-chiral RP, although compound is not chiral.

Case 1 : Non chiral compound Preparative

Semi-Prep (2 cm i.d.)

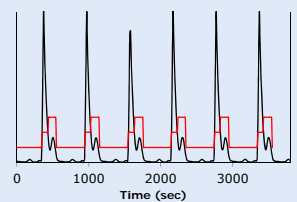


Stacked injections with fraction collection indicated

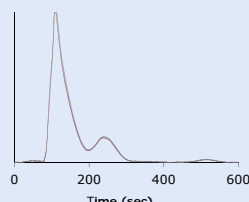


Overlay of 5 injections, showing excellent reproducibility

Prep (7.65 cm i.d.)



Stacked injections with fraction collection indicated. Longer runtime than in semi-prep, 1 extra critical and late eluting impurity needed to be removed.

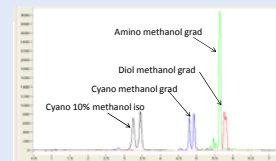
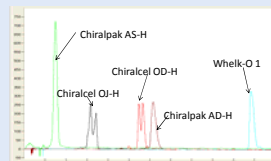


Overlay of 4 injections, showing excellent reproducibility

	Total flow	% MeOH	Runtime	mg/inj	g/hour	g/kg CSP/h	Quality
Semi-Prep (2cm i.d.)	40 ml/min	30	265 sec	40	0.54	11.0	97.5%
Prep (7.65cm i.d.)	600 g/min	30	600 sec	750	4.5	6.7	99%

➔ Product was purified rapidly and with high quality. Prep scale process can be further optimized.

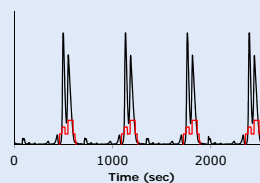
Case 2 : Diastereomeric compound Analytical



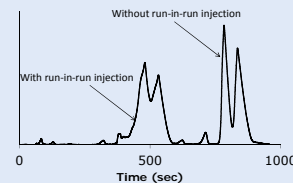
- Analytical chiral SFC screening, methanol + 0.2% iPrAm gradient, 4 ml/min, 150 bar
- No selectivity on the amylose and Pirle type phases tested
- Some selectivity on the cellulose phases, not sufficient for SFC prep work
- Analytical NP SFC screening, methanol + 0.2% iPrAm gradient, 4 ml/min, 150 bar
- Sufficient selectivity on Kromasil Cyano 5 μ
- Limited selectivity on the amino and diol modified phases

➔ Kromasil cyano gives better resolution than chiral phases, although compounds are diastereomers.

Case 2 : Diastereomeric compound Preparative



Four injections on the 7.65 cm cyano column. Flowrate 800 gr/min, 2% methanol (+0.2% iPrAm). Fraction collection indicated on graph.



Effect of run-in-run injection. Because of the low amount of cosolvent, the solvent plug of the injection will disturb the elution profile when run-in-run injection is performed. Shown on the graph is the effect of the injection of a dichloromethane solution, when injecting a methanol solution the effect is even more pronounced.

	Total flow	% MeOH	Runtime	mg/inj	g/hour	g/kg CSP/h	Quality
Prep (7.65cm i.d.)	800 g/min	2	950 sec	500	1.9	2.9	95-98%

➔ Product could be purified with SFC on cyano phase. Long runtimes were needed to obtain sufficient separation. Non optimal process, but product (18 grams) was purified within an acceptable time.

Instrumentation



Hybrid SFC system PIC-solution
Analytical : 9 chiral and 9 NP columns
Selection of 6 diff cosolvents
UV-detection (4 wavelengths simult.)
Flowrate : 0 - 5 ml/min
Semi-prep : 1 column, typical 1-2 cm i.d.
Flowrate : 5 - 40 ml/min
Fraction collection : 4 + waste



Prep SFC system Novasep (Supersep 50)
Flowrate : 0-1000 g/min CO₂
0-400 ml/min cosolv
UV-detection (single wavelength)
Fraction collection : 5 + waste
Columns : 5 x 7.65 cm fixed
1 x 5 cm DAC
1 x 8 cm DAC
CO₂ recycling
Loop injection (typical 20 ml)