

Fast Chiral Method Development combining Parallel Screening and Analytical SFC Systems

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Introduction

Enantiomer separation of drug candidates has gained in importance for pharmaceutical companies. The process of identifying the optimal conditions for the separation of racemic samples usually involves the screening of a given set of solvents on several columns packed with different chiral stationary phases (CSP's). This process is often time-consuming and is generally regarded as a bottleneck prior to enantiomer analysis. A way to speed up the chiral screening procedure is to improve the method development capacity by using the Sepiatec parallel supercritical fluid chromatography (SFC) system (Fig 1) in combination with the Agilent analytical SFC with the Aurora Fusion A5 Module (Fig 2).

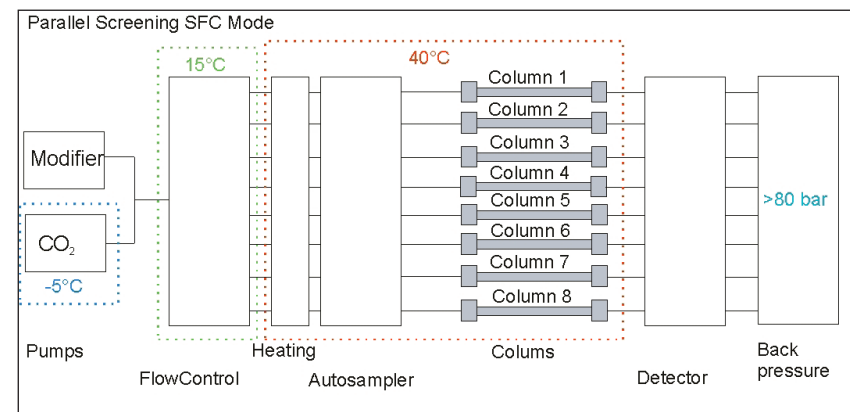
Goal

The goal was to separate two pairs of racemates in one method for two different samples. Sample 1: product 2-bromo-1-(4-nitrophenyl)ethanol and the epoxide side product (p-Nitrophenyl)oxirane (Fig 3). Sample 2: racemic precursor and racemic product (structures not published).

Experimental

Instrumentation

Fig 1: Schematic Sepiatec 8x parallel SFC



Parallel SFC Screening System (Fig 1):

- Sepmatix 8x Flow Control SFC
- Sepmatix 8x Auto Sampler, small version
- Sepmatix 8x Diode Array Detector SFC, analytical
- Sepmatix Back Pressure Regulator SFC
- Sepmatix Column Oven SFC
- Automatic Purge Valve for Sepmatix 8x Flow Control
- Sepmatix Solvent Selection Valve
- SFC-Pump, Knauer K180
- Modifier-Pump Knauer K1800

Columns

3 µm, 100 x 4.6 mm
 CHIRALPAK® AS-3, CHIRALPAK® IC-3

5 µm, 250 x 4.6 mm
 CHIRALPAK® IA, CHIRALPAK® IB, CHIRALPAK® IC CHIRALPAK® AS-H,
 CHIRALPAK® AD-H, CHIRALCEL® OJ-H, CHIRALPAK® AY-H, CHIRALCEL® OZ-H

Solvents

For the generic gradient method:
 Methanol (MeOH) / ethanol (EtOH) / isopropanol (IPA) / diethylamine (DEA) 100 / 100 / 100 / 0.1

For the detailed isocratic screening:

MeOH / DEA 100 / 0.1
 EtOH / DEA 100 / 0.1
 IPA / DEA 100 / 0.1

Fig 3: Structures of Sample 1

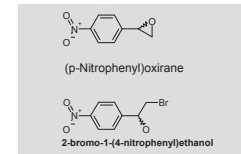
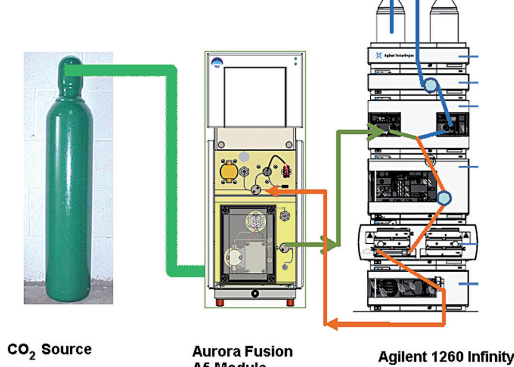


Fig 2: Schematic Agilent Analytical SFC with the Aurora Fusion A5 Module



Analytical SFC System (Fig 2):

Agilent 1260 Infinity Analytical SFC System consists of:
 1260 Binary SFC pump
 1260 DAD
 1260 SFC Autosampler
 1260 Thermostatted Column Compartment
 Aurora Fusion A5 SFC Module

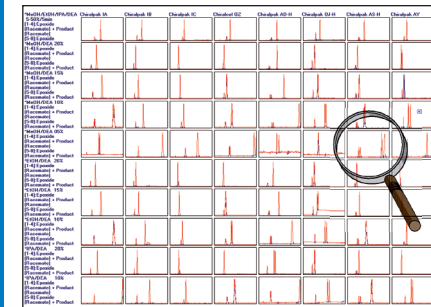
Working Concept

The column setup of the parallel SFC Sepmatix screening system used the following 8 CSPs: CHIRALPAK® IA, CHIRALPAK® IB, CHIRALPAK® IC CHIRALPAK® AS-H, CHIRALPAK® AD-H, CHIRALPAK® AY-H, CHIRALCEL® OJ-H and CHIRALCEL® OZ-H. All column dimensions were 250 x 4.6 mm, 5 µm.

The first screening step was performed by using a generic method with a 15 min run time (5 min equilibration and 10 min process time), in which a gradient of 5 – 40 % over 5 minutes was applied using a modifier composed of MeOH / EtOH / IPA / DEA (100 / 100 / 100 / 0.1). Flow rate was 3 mL / min, oven temperature was 40° C and the back pressure was set at 150 bar [1].

This initial gradient run is useful to ensure that all sample components are eluting and to determine which modifier strength is necessary for the method optimization screening runs. Often, separation is observed during this initial run because of the presence of all three common alcohols in the modifier.

Fig 4: Sepmatix Screening Wizard Chromatograms Overview Sample 1



0 min

Screening Overview

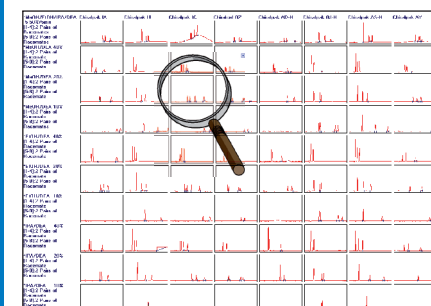
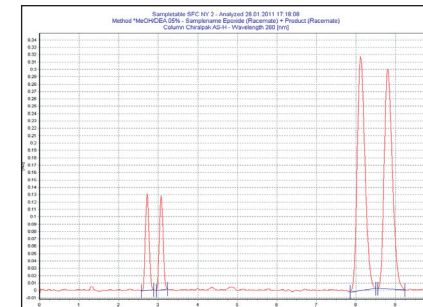


Fig 8: Sepmatix Screening Wizard Chromatograms Overview Sample 2

Fig 5: Zoom of Screening Hit Sample 1



150 min

Zoom of Scening Hit

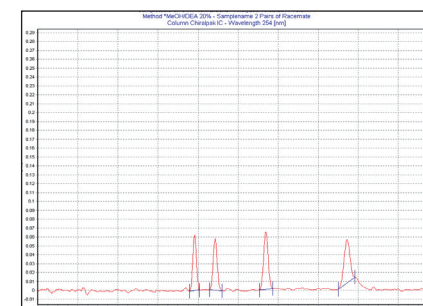
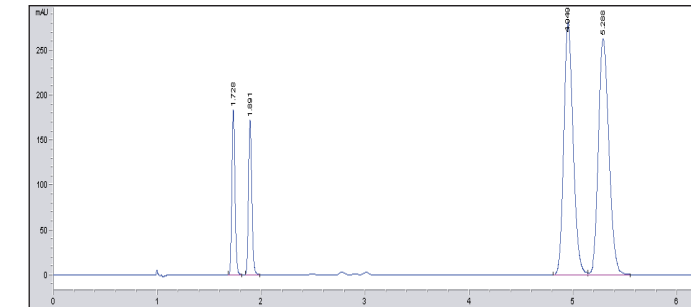


Fig 9: Zoom of Screening Hit Sample 2

Fig 6: Transferred Conditions to Analytical System Sample 1



Transferred Conditions to Analytical System

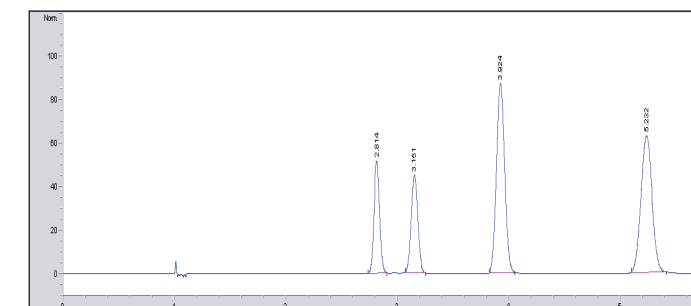
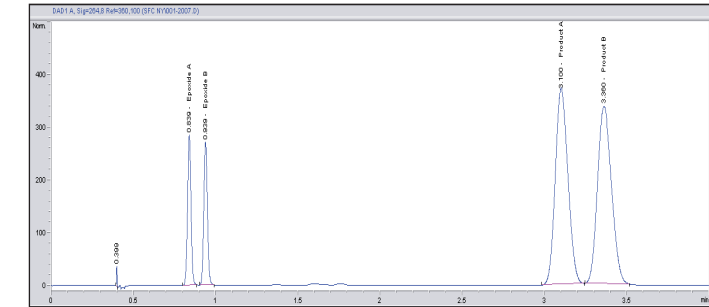


Fig 10: Transferred Conditions to Analytical System Sample 2

Fig 7: Optimized and Final Established Conditions Sample 1



Optimized and Final Established Conditions

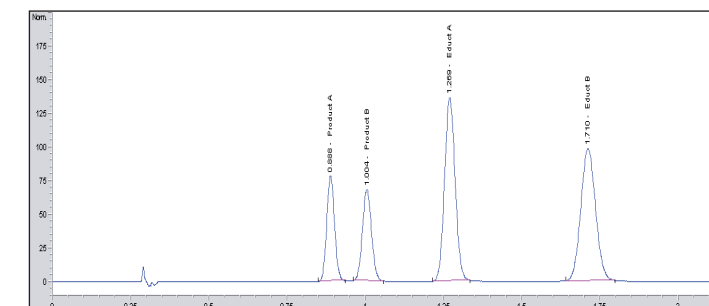


Fig 11: Optimized and Final Established Conditions Sample 2

Result and Discussion

Sample 1:

During the screening process, isocratic methods were used to find the most efficient alcohol and the concentration required to achieve the best resolution. The best separation for sample 1 was achieved by using 5 % of MeOH / DEA (100 / 0.1) with 95% supercritical CO₂ on a CHIRALPAK® AS-H column (Fig 4 / 5).

In order to establish a faster chiral method with the analytical system, two more optimization runs were done by using 3 µm particles CHIRALPAK® AS-3 (100 x 4.6 mm) and 4 % MeOH as the modifier with a run time of 4 min (Fig 7). The DEA additive was excluded in the final conditions because of possible chemical reactions with the analytes.

Sample 2:

From the screening process, MeOH was found to be the preferred alcohol. The best resolution was achieved by using 20 % of MeOH / DEA (100 / 0.1) with 80 % supercritical CO₂ on a CHIRALPAK® IC column (Fig 8 / 9).

After the conditions were transferred to the analytical system, three more optimization runs were done by using 3 µm particles CHIRALPAK® IC-3 (100 x 4.6 mm). In addition, the flow rate was increased to 4 ml / min, and the MeOH was set at 25 % with a run time of 2.3 min (Fig 11). The DEA additive was excluded in the final conditions, because it did not appear to make a difference on resolution and peak shape.

Conclusion

The above described process was applied to both samples, and each method was established in less than 4 hours. Therefore, the analytical development capacity regarding enantiomer separation was significantly enhanced by combining the above-mentioned systems, and work time was efficiently reduced.

When conditions were transferred from the screening parallel system to the analytical system, the same selectivity often remained, as these examples confirmed (Fig 5 / 6 & 9 / 10). That makes it easy to establish an analytical method without further time-consuming tests. In addition, the analytical system was less occupied by screening work and available for incoming routine samples.

References

[1] W. W. Barnhart, S. Thomas, Z. Hua, and K. H. Gahm. A tiered approach to rapid chiral method development for supercritical fluid chromatography in drug discovery. ISCD 2009