Strategies for Increasing Throughput of Chiral Separations by Supercritical Fluid Chromatography

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The chirality of a drug can potentially have a large impact on its biological activity, metabolism and toxicity. Obtaining optically pure compounds has become increasingly important in the discovery of therapeutic compounds since one enantiomer can have positive therapeutic properties while the other can display non-therapeutic or negative biological activity. Due to the often difficult and limiting nature of achiral synthesis, chiral chromatography is typically applied to access stereochemically pure compounds. The application of Supercritical Fluid Chromatography (SFC) to chiral separations has proven very effective in recent years due to its many advantages over chiral HPLC, such as shorter retention times, higher efficiencies per unit time, and the reduction of organic solvent waste. At Abbvie, the Analytical and Purification Sciences (APS) group has provided a chiral preparative SFC service since 2000 [1, 2], which has steadily grown to impact over 30 projects and over 200 samples per year. Recently, the APS group at AbbVie has developed strategies to meet the growing need and variability of chiral separations within Drug Discovery, such as developing a streamlined scale-up approach, applying structure similarity software to minimise method development, and capitalising on the powerful stacked injection capability of SFC to provide a 3-5 day turnaround for chiral separations [3].

Impact and Discussion

At AbbVie, SFC is the technique of choice for all chiral separations supporting Discovery. SFC provides higher diffusivity of analytes in the mobile phase and can handle faster flow rates, resulting in higher efficiency over HPLC. In addition, the use of CO_2 and alcohol modifier, such as MeOH, provide SFC with a green advantage, since shorter run times with only a small percentage of organic solvent in the mobile phase results in less organic solvent consumed and waste generated. SFC also permits rapid drydown of wet fractions without the use of aqueous solvents in the mobile phase. For chiral separations, where samples are generally less complex than typical medicinal chemistry reverse-phase purifications and where gram-scale separations are often requested, these advantages make SFC the preferred choice in the purification laboratory. At AbbVie, our chiral separation capabilities have evolved to handle chiral separations in high throughput by implementing a variety of SFC instrumentation to meet the demand for chiral separations at the milligram to 10s of grams scale, developing a custom column-screening analytical SFC to screen 20 columns in a single login, and by employing spectral data handling software from ACD/Labs to do structure similarity searches to reduce chiral stationary phase (CSP) screening for similar analogues.

Analytical method development is performed within 24 hours of receiving the sample with reports emailed to the client and also linked directly to their ELN. Three analytical Aurora/Agilent SFC instruments are employed to meet the screening and method development needs of the chiral separation service. To effectively screen up to 20 unique CSPs a login wizard was developed using Agilent macros to control column-switching valves and build a sequence table *(Figure 1)*.

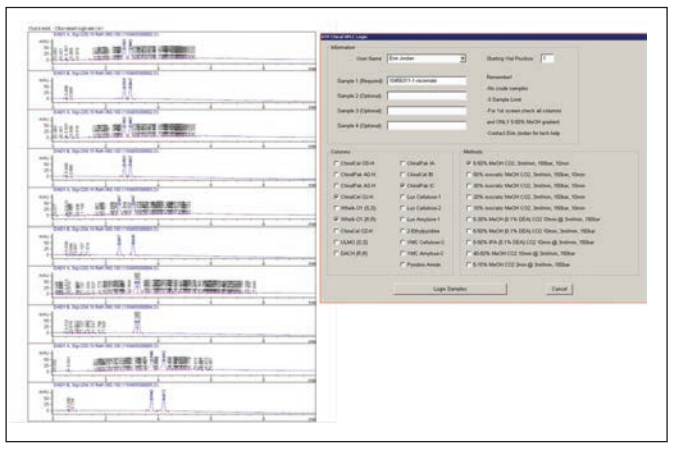

Figure 1. Custom Aurora/Agilent Column Screening Macro. The macro is able to screen up to 20 unique CSPs against 10 columns. The runs are typically 8-10 minutes in length and all data is emailed and archived to electronic laboratory notebooks.

Furthermore, an additional custom Aurora/Agilent analytical column screening system is being provided as an open-access service to medicinal chemists to determine the feasibility of chiral separation or to monitor the chiral purity of their reactions without having to submit to the APS purification queue.

Preparative SFC in the APS group is performed on the JASCO 2088 preparative SFC, THAR/Waters 80 preparative SFC and THAR/Waters 350 preparative SFC systems. In order to achieve the maximum throughput for chiral separations, analytical hits are rapidly scaled from an analytical 5-50% MeOH hit to an isocratic preparative run based on the retention time of the analyte on the analytical gradient method *(Figure 2)*. In addition, very little time is dedicated to preparative method development. Typically, only 2-3 'test' injections are executed to optimise the mobile phase and loading conditions, and often non-optimal methods with small mixed fractions or low loading are preferred for samples less than 5g, since the efficiency of running in stacked injection mode and resubmitting mixed material typically results in higher throughput, compared to the time required to for more extensive method development.

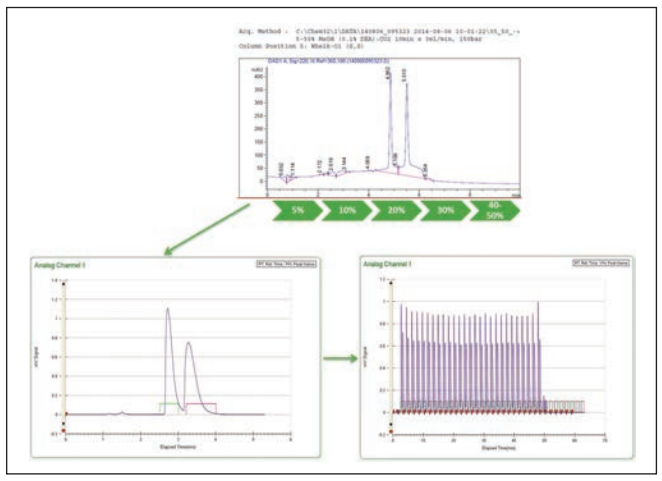

Figure 2. Rapid Scale Up from Analytical Hit to Preparative SFC in Stacked Injection Mode. Method development and analytical screening is streamlined through companion software provided by ACD/Labs to display a series of associated chromatograms with structures, peak data, and annotations, and to perform structure similarity searches.

Conclusion

The APS chiral separation service employs SFC technology to resolve racemic or diastereomeric mixtures to support Drug Discovery on a milligram to tens of grams scale with a 3-5 business day turnaround. Relying on the chromatographic advantages of SFC instrumentation to efficiently screen and separate chiral samples on a preparative scale with high recovery and chiral purity has been a valuable asset to the service. Rapid scale up from analytical screening methods can result in large time savings rather than extending analytical method development and integrated software shortens screen time for structurally similar racemates.

References

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(2) Searle, P. A.; Glass, K. A.; Hochlowski, J. E. J Comb Chem 2004, 6, 175.

(3) Jordan, E. E.; Searle, P. A. Poster Presentation. SFC International Basel, Switzerland, 2014.