

# Chiral Screening and Optimisation using Supercritical Fluid Chromatography and Design of Experiments

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## Introduction

- Due to the pharmacological activity and side effects of chiral enantiomers, the separation and quantification of enantiomers of racemic substances is key for the development and regulation of drug formulations.
- Unlike achiral separations, chiral separations can be harder to predict, so there is a need to screen and optimise a wide range of conditions and parameters to achieve the required resolution of enantiomers. Evaluating a wide design space can be a significant investment in time and cost.
- Compared with liquid chromatography, supercritical fluid chromatography (SFC) can offer a faster, more efficient and more sustainable alternative. Higher flow rates can be utilised to take advantage of the high diffusivity of supercritical fluids, reducing analysis time without sacrificing efficiency.
- Analytical Quality by Design (AQbD) can be used to vary multiple factors simultaneously using a different regression models. This structured approach provides more precise information in fewer experiments and evaluate interaction influences. A faster screening and optimisation process leads to reduced costs, resource usage and is more sustainable.

## Chiral Method Screening

### Chiral Stationary Phase

- Many column types exist, so set of columns are chosen to maximise a positive hit.
- Biggest impact on chiral selectivity.

### Organic Modifiers

- Screening solvents include methanol, ethanol, IPA and acetonitrile or mixtures.
- Added to supercritical CO<sub>2</sub> to influence selectivity and efficiency.

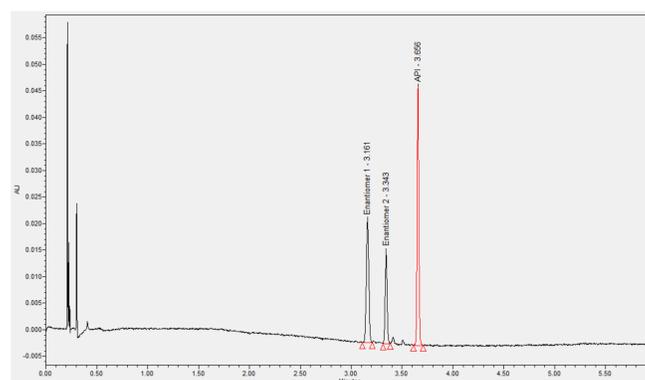


Figure 1: Chromatogram showing separation of enantiomers and API during a screening set.

### Mobile Phase Additives

- Acids, bases and salts are screened using generic values.
- Added to improve resolution and peak shape.

### Gradient

- Gradient injections used to quickly and systematically screen for acceptable conditions.
- SFC provides considerably shorter analysis times compared to traditional NP-LC to enable faster screening.

## Chiral Method Optimisation

### Parameters

- Following screening, quantitative parameters can be optimised.
- Many quantitative parameters require optimisation including additive concentration, mobile phase flow rate, system back pressure, column temperature and more.

### One Factor At a Time

- Evaluating one parameter at a time could lead to many injections and long analysis.
- This approach is time consuming and may miss the true optimum between a set of factors.

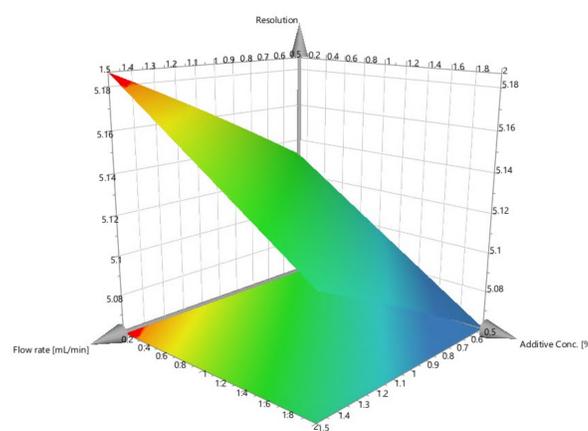


Figure 2: Example response surface plot showing change in X between two SFC parameters.

### Analytical Quality by Design

- The use of Modde Pro allows Resolian to design experiments that evaluate the entire design space efficiently with a reduced set of injections required.
- Methods can be evaluated for their robustness or optimality as required.

### Sustainability

- SFC significantly reduces the use of toxic and environmentally harmful solvents used in NP-LC such as hexane.
- Faster analysis leads to less solvent usage and reduced disposal requirements, and reduced energy consumption.

## Conclusion

- The properties of supercritical CO<sub>2</sub> provide a fast, efficient and sustainable chromatographic technique for chiral separations.
- The development of chiral methods requires evaluation of a wide range of parameters that can be difficult to predict for each separation.
- The use of a structured screening process coupled to a AQbD assisted optimisation procedure enables an accurate and robust development of chiral methods using a reduced set of experimental injections.