Beyond Column Screening: Chiral Method Development for Small Molecules Utilizing Design-of-Experiments Principles

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Background

With over 50% of the 19 FDA-approved small molecules in 2022 containing stereocenters [(1)], determination of enantiomeric purity in drug substance remains an essential part of the analytical control strategy. In response to this, most of the focus in both the literature and from chiral column manufacturers has been towards novel column chemistries [(2,3)] and screening strategies[(4)]. However, only screening diverse column chemistries often does not provide the specificity required to resolve, and therefore accurately quantitate, the undesired enantiomer from not only the active pharmaceutical ingredient (API), but also from other stereoisomers, process impurities, and/or degradation products that may be present.

Presented here is a robust chiral method development strategy utilizing design of experiments (DoE) principles to optimize chiral separations. This DoE approach allows for a better understanding of which LC parameters have significant impact on method performance, while also ensuring that the condition selected provides accurate and robust quantitation of the enantiomer. Using a more traditional one-variable-at-a-time (OVAT) approach to acquire the same amount of information can take > 200 experiments for 5 LC variables. However, using DoE, the same wealth of information can be acquired in ~ 20 experiments, which represents a significant savings of both time and resources. In addition, incorporating DoE principles at the outset of chiral method development provides a foundation for the integration of analytical quality by design (AQbD) and lifecycle management approaches described in the ICH Q14 (Step 2) guideline.

Methods

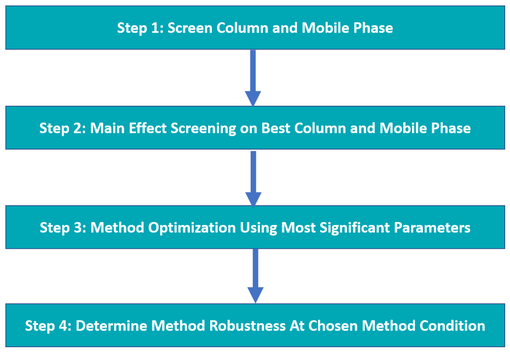

Figure 1. Schematic of the workflow for a robust chiral method development strategy utilizing DoE principles.

All chromatograms were acquired on an Agilent 1290 Infinity II (Agilent Technologies, CA, USA) UHPLC system with a photodiode detector. Empower CDS (Waters, MA, USA) was used for all chromatographic analysis. JMP Pro 16 (SAS, NC, USA) was used to analyze main effect screening chromatographic data. Fusion QbD (S-Matrix, CA, USA) was used to analyze data for method optimization and determine method robustness. Generally, main effect screening is analyzed in JMP utilizing a fractional factorial design such as Burman-Plackett or Resolution IV. Either a central-composite or 3-level full factorial design is utilized for optimization, with second order polynomial utilized for fitting. A 2-level full factorial design is utilized for method robustness study with a linear model utilized for fitting.

Samples contain an API, the enantiomer of that API, and a structurally similar process impurity (PI). The enantiomer and PI are present at ~ 0.5% by weight of the API.

Screening of column and mobile phase is done using OVAT. Initial screen consisted of 14 unique stationary phases, 2 mobile phase additive types, and 2 organic eluents.

Column Screening Result

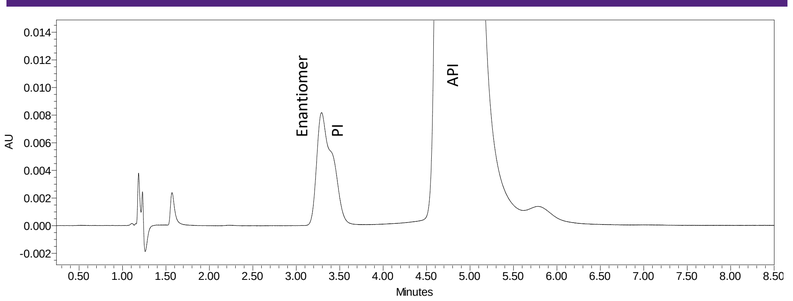

Figure 2. Example chromatogram at the conclusion of a typical column/MP screening step where resolution of the API and the enantiomer is achieved but partial co-elution with the PI prevents accurate quantitation of the enantiomer. Column: Daicel IC-U, 2.1 x 100 mm, 1.9 µm; flow rate:0.4 mL/min; Mobile phase: 20 mM ammonium Bicarbonate in 30/70 (v/v) Acetonitrile/pH 9.0 Water; column temperature: 20 ˚C

Effect Screening and Optimization

Statistical Analysis of Main Effect Screening:

A fractional factorial design of five LC variables was generated in JMP to determine the main effects for the resolution of the Enantiomer/PI and the PI/API. pH and column temperature ranges were limited for column stability purposes. LogWorth of each variable was calculated. Higher logWorth equates to increased influence.

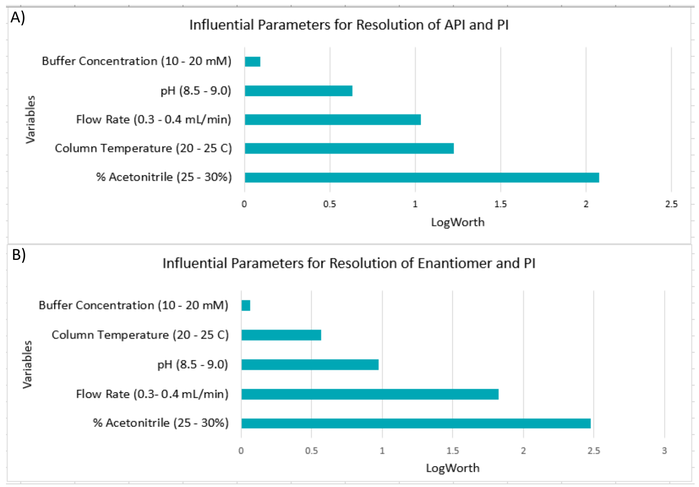

Figure 3. Pareto plot of A) the resolution between the API and PI
B) the resolution between the enantiomer and PI

pH was not included as a variable for the optimization due to the limitations of the column, the buffering range, and the pKa of the analytes. Instead, the pH was controlled at 8.5 during optimization.

Method Optimization

A central composite design with the three most significant variables (% Acetonitrile, Column Temperature, Flow Rate) was generated in Fusion QbD. A 2nd order quadratic fitting was used to build the resolution map.

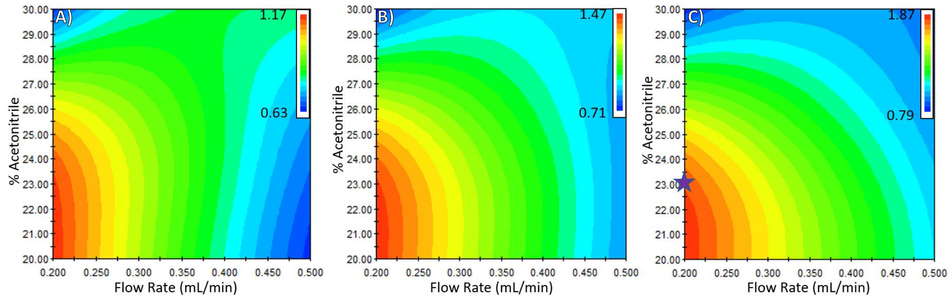

Figure 4. Resolution map of the API, PI, and the enantiomer plotting % acetonitrile of the mobile phase vs mobile phase flow rate with the column compartment temperature set at A) 15 ˚C B) 20 ˚C and C) 25 ˚C. The purple star indicates the chosen nominal method condition

The resolution maps indicate that the optimal condition for resolution is to set the LC method at lower flow rate, lower % acetonitrile, and higher column temperature. A decision was made to operate at a flow rate of 0.20 mL/min, 23% acetonitrile, column temperature of 25 ˚C to balance the resolution of peaks and the method run time. The resulting chromogram, Figure 5, shows a notable improvement in resolution between the enantiomer and PI compared to the initial chromatogram in Figure 2.

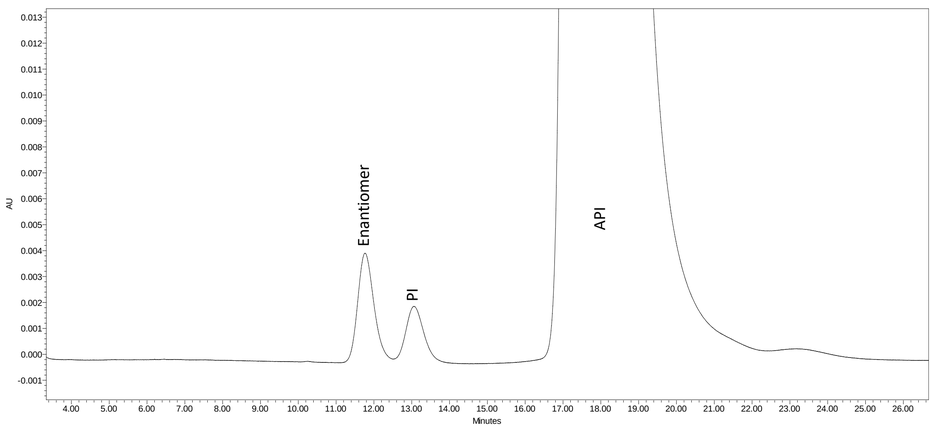

Figure 5. A chromatogram after the optimization step where sufficient resolution is seen between all peaks of interest. The USP resolution between the enantiomer and PI was 1.7. Column: Daicel IC-U, 2.1 x 100 mm, 1.9 µm; flow rate: 0.20 mL/min; Mobile phase: 20 mM ammonium Bicarbonate in 23/77 (v/v) Acetonitrile/pH 8.5 Water ; column temperature 25 ˚C

Robustness Determination

A 2-level full factorial design (5 variables) with a center point at the selected condition shown in Figure 5 was generated in Fusion QbD to determine the robustness of the method. A linear model of the resolution between the enantiomer and PI as well as the % area of the enantiomer relative to the API was built. Trellis plots of the LC conditions were generated from the model to determine the robustness of the method and to guide setting proper resolution criteria.

The initial trellis plot demonstrated that the method met robustness criteria with a RS of ≥ 1.5 between the enantiomer and PI, and with % area of enantiomer relative to the API within ± 10% of target (plots not shown). For demonstrative purposes, the criteria for robustness were set to RS of ≥ 1.6 between the enantiomer and PI, and to % area of enantiomer relative to API within ± 5% of target. The trellis plot demonstrates that by selecting a system suitability criteria for RS of ≥ 1.6, improved accuracy (quantitation) of the enantiomer is expected.

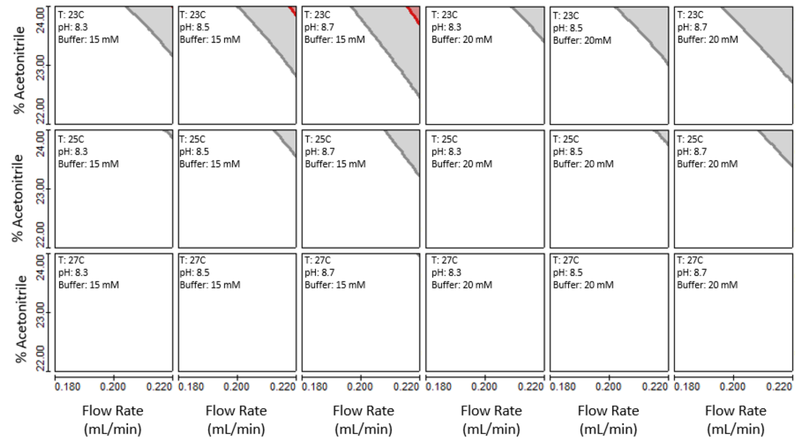

Figure 6. Trellis plot of LC conditions where each plot has % acetonitrile on the Y-axis, and flow-rate on the X-axis. Gray shaded region represents conditions where resolution of the enantiomer and PI is below 1.6. The red shaded region is where the % area of the enantiomer is not within ± 5% of target. 25 mM buffer concentration was also evaluated (plots not shown) and the model showed similar results to 20 mM buffer concentration.

Conclusion

Shown here is a chiral method development strategy based on DoE principles and statistical analysis. This approach yields a chiral method that not only meets the separation requirements but is also robust. Trellis plots can also be used to set appropriate system suitability criteria to align with the desired accuracy for the enantiomer. This chiral method development approach has been successfully applied to multiple programs with different chemical moieties, typically taking ~ 1-2 weeks to develop an optimized method.

Acknowledgement

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