# Improved Analytical Chiral Separations for Amino Acid Control Strategy

Bryan Overholser, Daniel Pazo, Marc Jacob
**AMPAC Analytical**

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## Summary

In this work, we present the development and implementation of 2 analytical chiral separations methods used for the control strategy in the large-scale manufacturing of a proprietary pharmaceutical. In the first part, we highlight the chiral separation optimization of a fully protected amino acid intermediate 3 without significant chromophores using HPLC and CAD. In the second part, we describe the development of a chiral separation for the free amino acid final product using a Zwitter ion HPLC column and UV as detection mode.

## Original methods

The customer provided two methods for the process.

**Method 1:** Intermediate 3 - CHIRALPAK AD-H 4.6 x 150 mm column (Daicel, Japan) and 90:10 hexane/IPA + 0.1% diethylamine as the mobile phase in isocratic conditions.

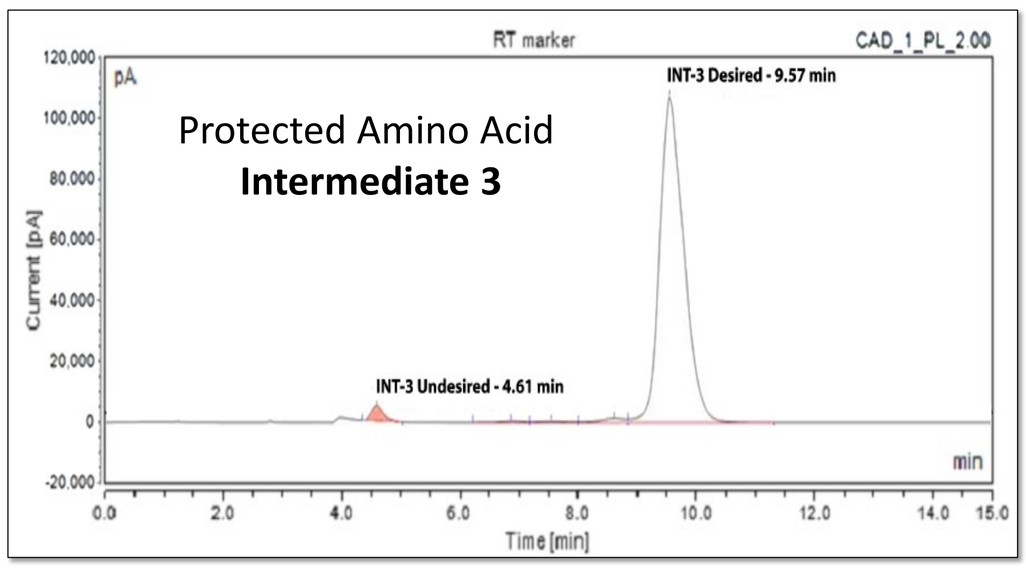
**Method 2:** API - 2 Infinity Lab Poroshell 120 Chiral-T columns in series with a phosphate/TEA buffered mobile phase.

## Intermediate 3 - Method Optimization

The method for the chiral analysis of “intermediate 3” was implemented at AMPAC Analytical, however wide peaks, baseline noise, riders and peaks eluting in close vicinity of the peaks of interest prompted method development to improve the conditions.

**Optimization** of column temperature, flow rate, and injection volume with detection via CAD resulted in a more robust method.

**Validation** of the method was performed under isocratic normal phase conditions using a CHIRALPAK AD-H 4.6 x 150 mm column (Daicel, Japan) and 90:10 hexane/IPA + 0.1% diethylamine as the mobile phase. The method was successfully validated for specificity, repeatability linearity and quantitation limit.

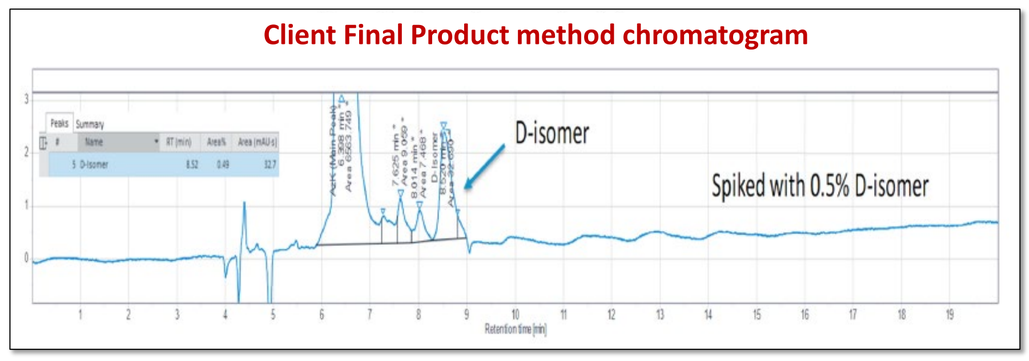


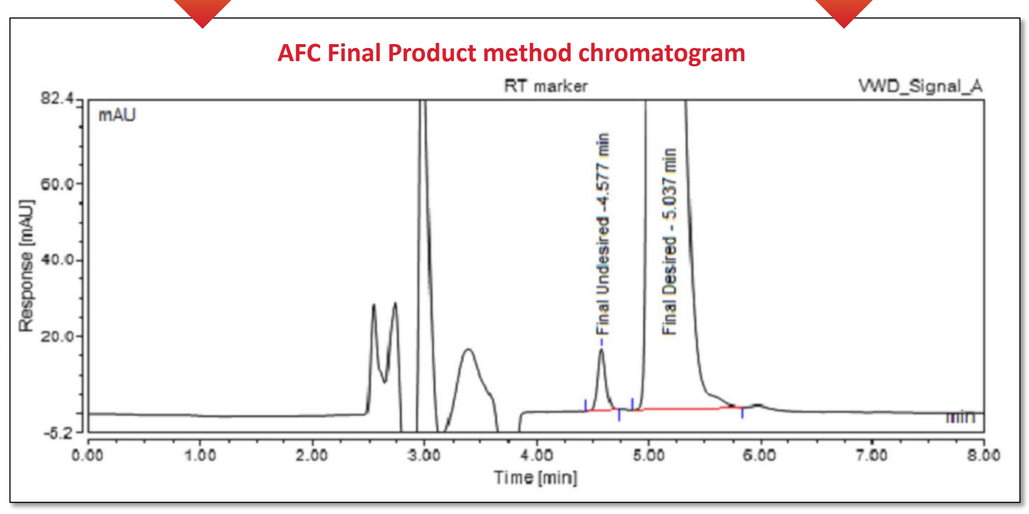

## Final API Product - Method Development

The CHIRALPAK Zwix(-) zwitter ion column was evaluated since the final product contains a free amino acid functionality.

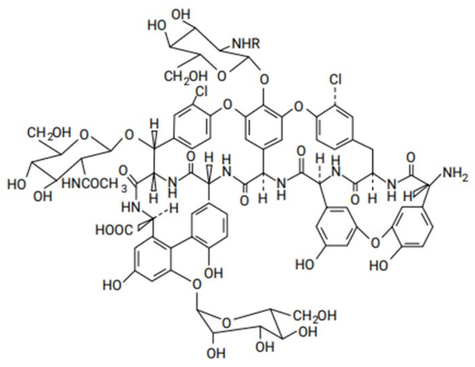
Adding DEA and FA to the mobile phase helps maintain the enantiomers as zwitterions, enhancing the column affinity, providing good peak shape and superior selectivity as well as shorter run times. After further **optimization**, the separation was successfully **validated** under isocratic reverse phase conditions using a mobile phase composed of Water:ACN:MeOH with FA and DEA as modifiers.

The new method provides a cleaner baseline, no co-eluting peaks or interference as well as sharper peaks, with the run-time cut from 20 minutes to 8 minutes. The final USP resolution between the two enantiomers was 2.2.

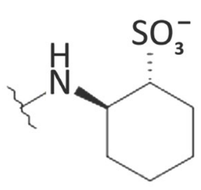


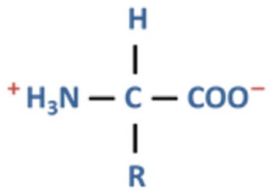




InfinityLab Poroshell 120 Chiral-T (Teicoplanin)



Zwix (-) Stationary Phase






## Conclusion

While the intermediate method provided by the customer was successfully validated after minimal optimization, their final product method needed significant improvement. This was achieved by AMPAC Analytical after identifying that the compound was a free amino acid, and therefore, amenable to zwitterionic separation conditions. Transitioning to the CHIRALPAK Zwix(-) column using a reverse phase isocratic method resulted in a more robust method with improved peak shape, selectivity and a shorter run time.

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