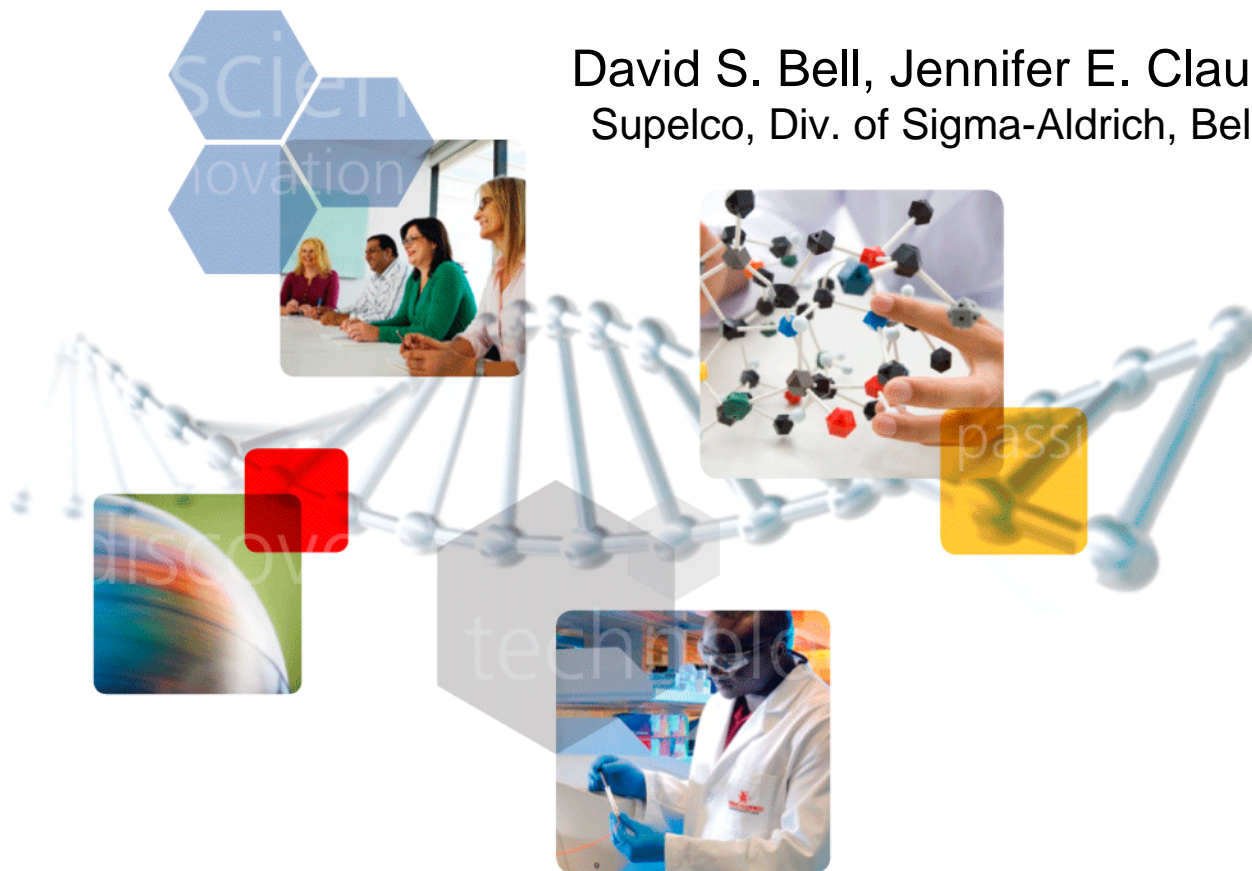


# Significant Improvements in Chiral Method Development using an LC-MS-Based Screening Approach

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T409117



## Introduction

Because retention mechanisms in chiral separations are highly complex and analyte specific, the approach of using 'experience' to achieve enantiomeric selectivity is not as effective as it is with typical achiral reversed-phase method development. Chiral screening, therefore, is the recommended procedure for method development in chiral separation.

LC-MS analysis may be used as a valuable tool in the rapid screening and investigation of the variables that contribute to retention and selectivity in chiral separations. Since LC-MS has the ability to separate in the mass/charge dimension, it should be possible to run a composite set of probes to assess the impact of operational parameters on enantiomeric selectivity for many analytes simultaneously.

CHIROBIOTIC™ stationary phases operate well in both reversed-phase and polar ionic modes (polar organic solvents containing low buffer concentrations), and are highly amenable to LC-MS. In this study, the LC-MS approach for a set of basic probes differing in  $pK_a$  values, hydrophobicity, and molecular weight is utilized to probe the impact of buffer (salt) type, buffer concentration, and acid/base ratio on retention and selectivity.

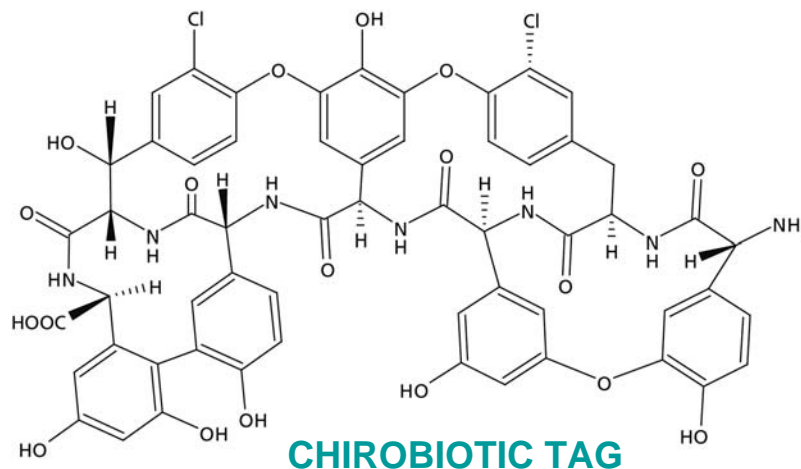
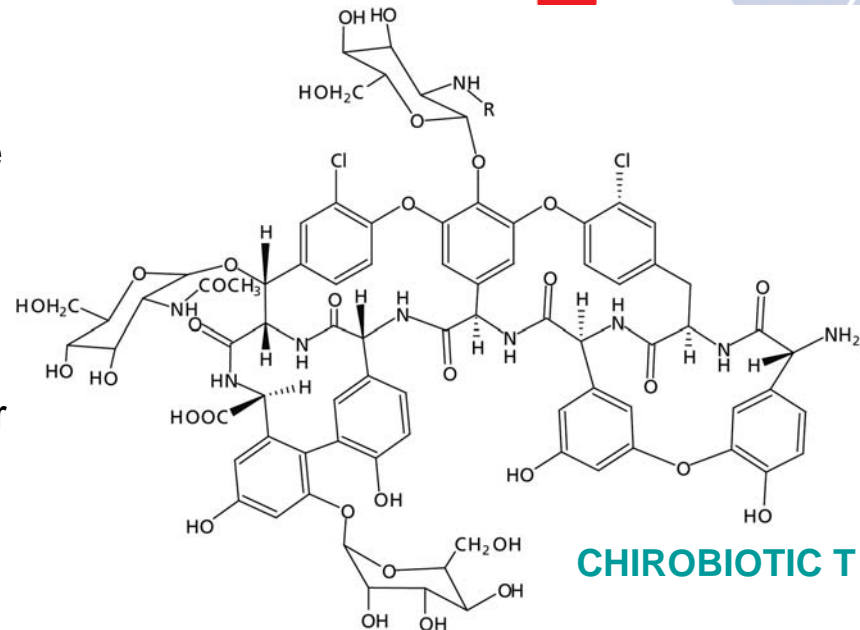


## Introduction (contd.)

Specifically, the CHIROBIOTIC T in polar-ionic mode will be examined, and the extracted ion current of  $\beta$ -blocker metoprolol will be used to monitor impact of the variables on selectivity and retention. In addition, the approach is utilized to screen several CHIROBIOTIC stationary phases to identify unique selectivity.

# Mobile Phase Types for CHIROBIOTIC CSPs

- **Polar Ionic Mode** – a *non-aqueous* mobile phase. Unique to CHIROBIOTICS: fast, perfect for prep, **MS** detection
  - for *ionizable* molecules – any acid or base
- **Reversed Phase** – **MS** compatible, ideal for manufacturing QC, bioanalysis
  - for *all types* of molecules
- **Polar Organic Mode** – Ideal for prep HPLC
  - for *neutral* molecules only
- **Normal Phase** –
  - about 15% of all applications





## Experimental

All analyses were done using the CHIROBIOTIC T on a Waters/Micromass single quadrupole instrument, interfaced to a Waters Alliance LC system using an electrospray ion source. All acquisitions were performed in positive ion mode with a scan range of  $m/z$  150-500.

## Impact of Buffer Type on Retention and Enantiomeric Selectivity

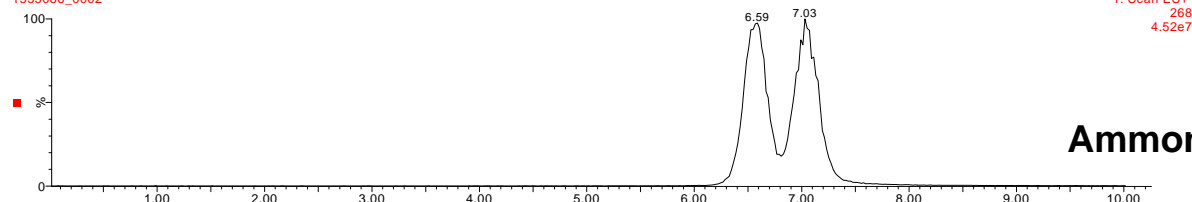
Retention and selectivity were monitored as a function of the buffer type. Refer to Figure 1 for results.

# Figure 1. Comparison of Various Buffers in the Chiral Separation of Metoprolol



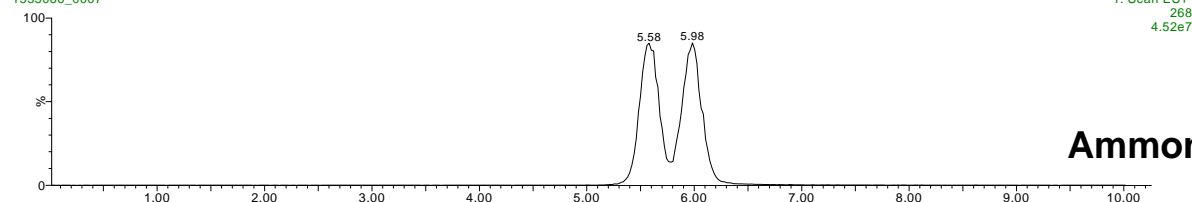
instrument: Waters/Micromass ZQ, Single Quadrupole, Waters Alliance 2690  
column: CHIROBIOTIC T, 15 cm x 4.6 mm, 5  $\mu$ m  
mobile phase: 0.1% wt/v ammonium acetate, ammonium formate and ammonium trifluoroacetate were prepared in methanol  
flow rate: 1 mL/min.  
temp.: 35° C  
det.: ESI, Positive Ion Mode, scan range m/z 150–500  
injection: 5  $\mu$ L

metoprolol only\_T\_0.1%AA in methanol  
1535066\_0002



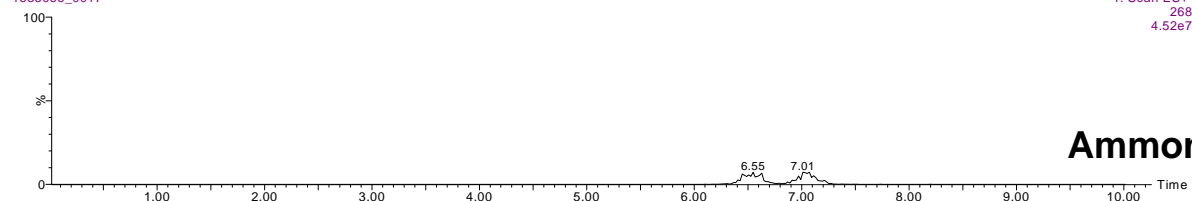
**Ammonium Acetate**

1535066\_0007




**Ammonium Formate**

1535066\_0017



**Ammonium TFA**

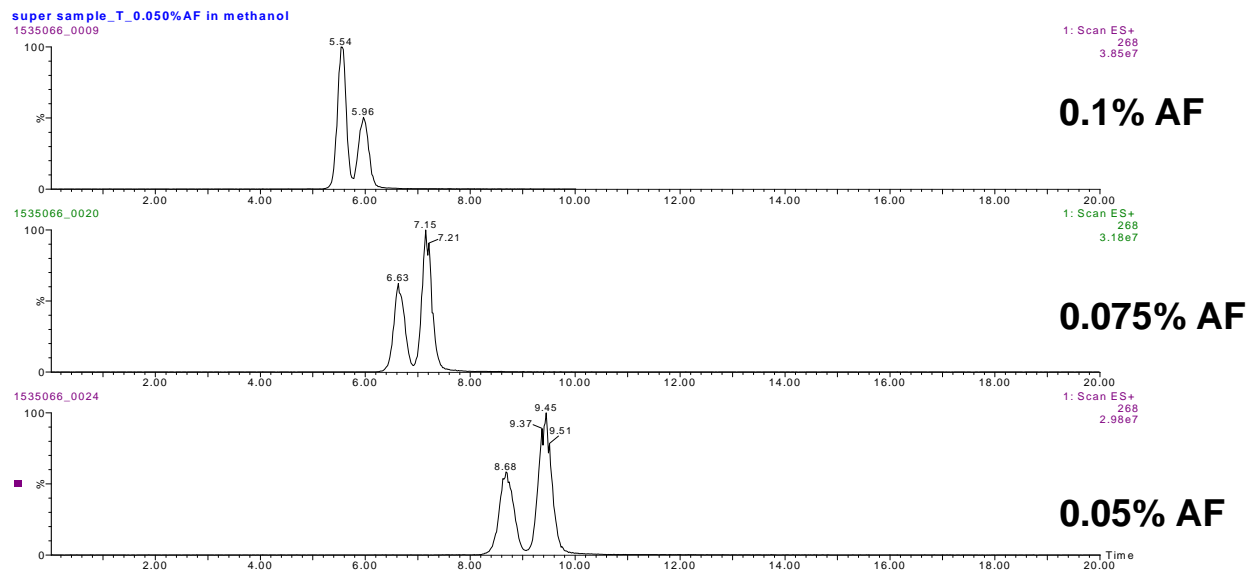
- 
- Retention using ammonium formate was shorter than that observed using ammonium acetate and ammonium TFA. The same general retention trend (with a few exceptions) was observed with the other compounds in the probe mix (not shown).
  - No selectivity was observed using one buffer that was not observed when using the other two buffers in any of the probe mix separations (not shown).
  - Selectivity for the  $\beta$ -blockers was slightly better when using ammonium TFA due to improved peak shape.
  - The anion has a major affect on response, a slight affect on retention, and no significant affect on selectivity.



# Impact of Buffer Concentration on Retention and Enantiomeric Selectivity

- The mixture was run using 0.1%, 0.075% and 0.05% ammonium formate (AF) in methanol.
- Retention and selectivity were monitored as a function of the buffer concentration (see Figure 2).

# Figure 2. The Chiral Separation of Metoprolol at Various Ammonium Formate Concentrations



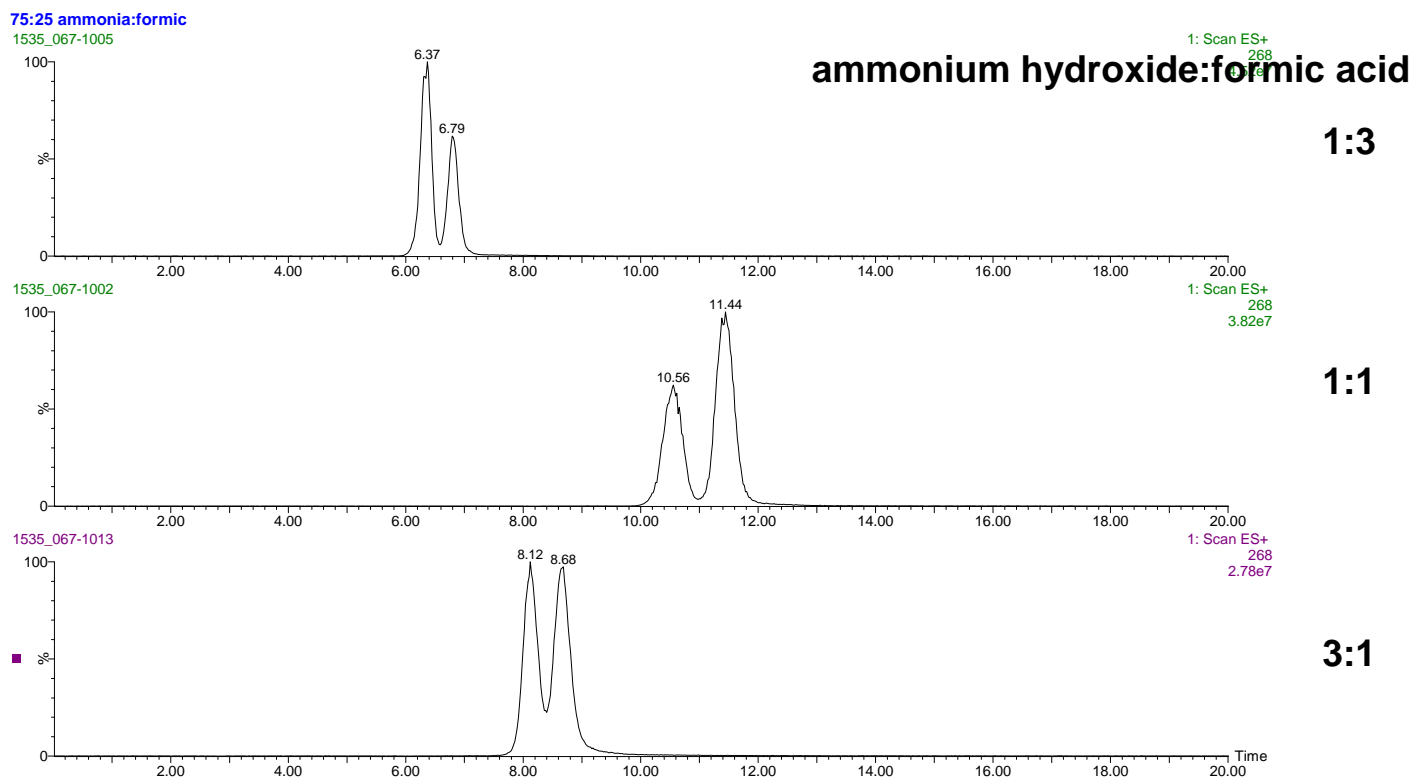
- In general, lowering buffer concentration increases base retention (inverse relationship). The dependence of retention on buffer concentrations implies a strong ion-exchange component toward retention.
- Lowering buffer concentration appears to aid overall in enantiomeric selectivity. The buffer strength appears to only enhance or reduce selectivity, not create or destroy it.
- Concentration has an affect on retention, but no major affect on selectivity or response.



## Impact of Buffer Component Ratio on Retention and Selectivity

- 13 mM ammonium hydroxide and 13 mM formic acid were independently prepared in methanol
- The complex sample was run using acid:base ratios of 3:1, 1:1 and 1:3
- Retention and enantiomeric selectivity were monitored (see Figure 3)

# Figure 3. Chiral Separation of Metoprolol at Different Acid:Base Ratios



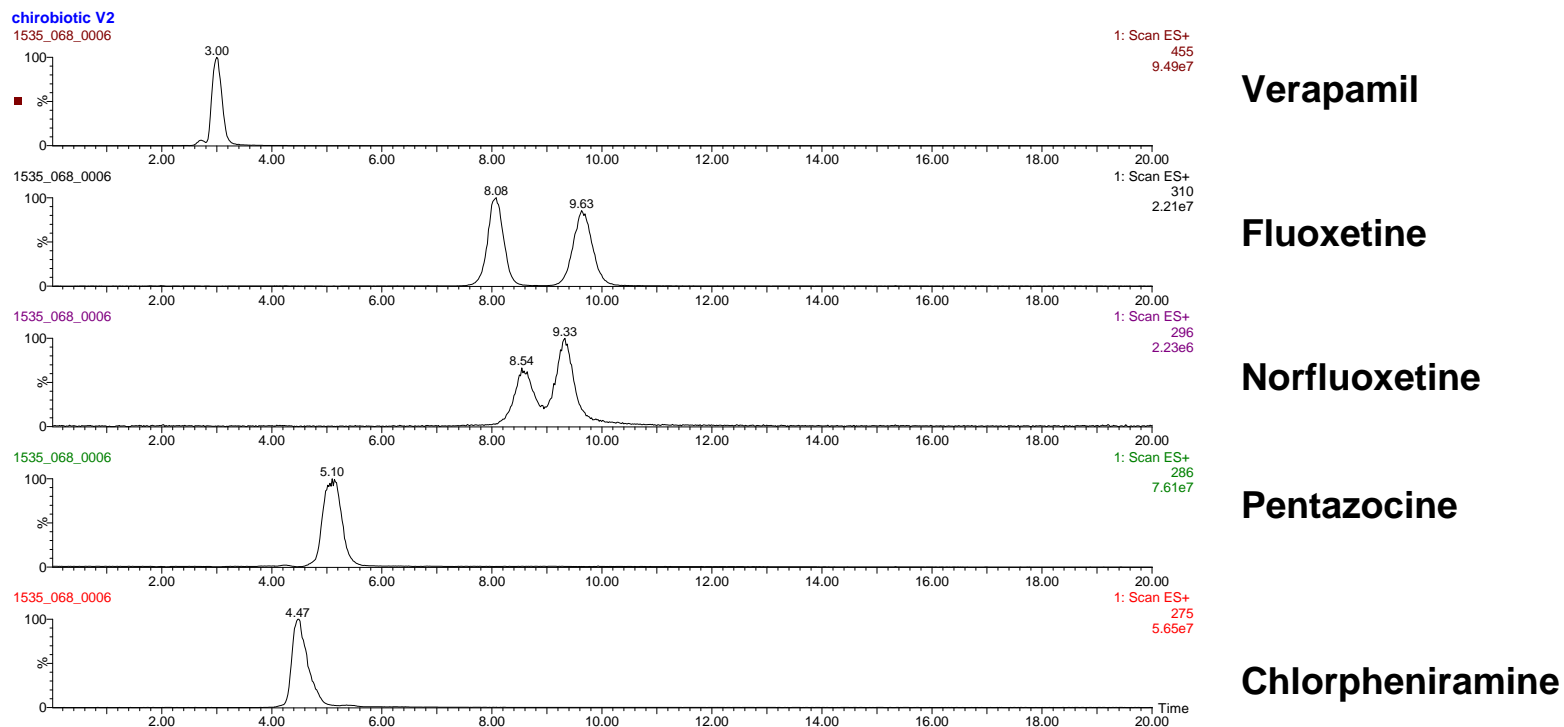
- In general, changing acid/base ratios shows more of an impact on selectivity than buffer concentration; however, to this point it is not shown to be dramatic.
- The change in retention shows that this is not a continuous function. The retention does not continue to decrease as one or the other buffer components changes.
- Changing the acid:base ratio creates a significant change in retention plus some change in selectivity.

## Impact of Stationary Phase Chemistry for Three CSPs on Retention and Selectivity

CHIROBIOTIC V2, TAG and R were run using the 1:1 mobile phase to assess the impact of stationary phase on the set of basic analytes.

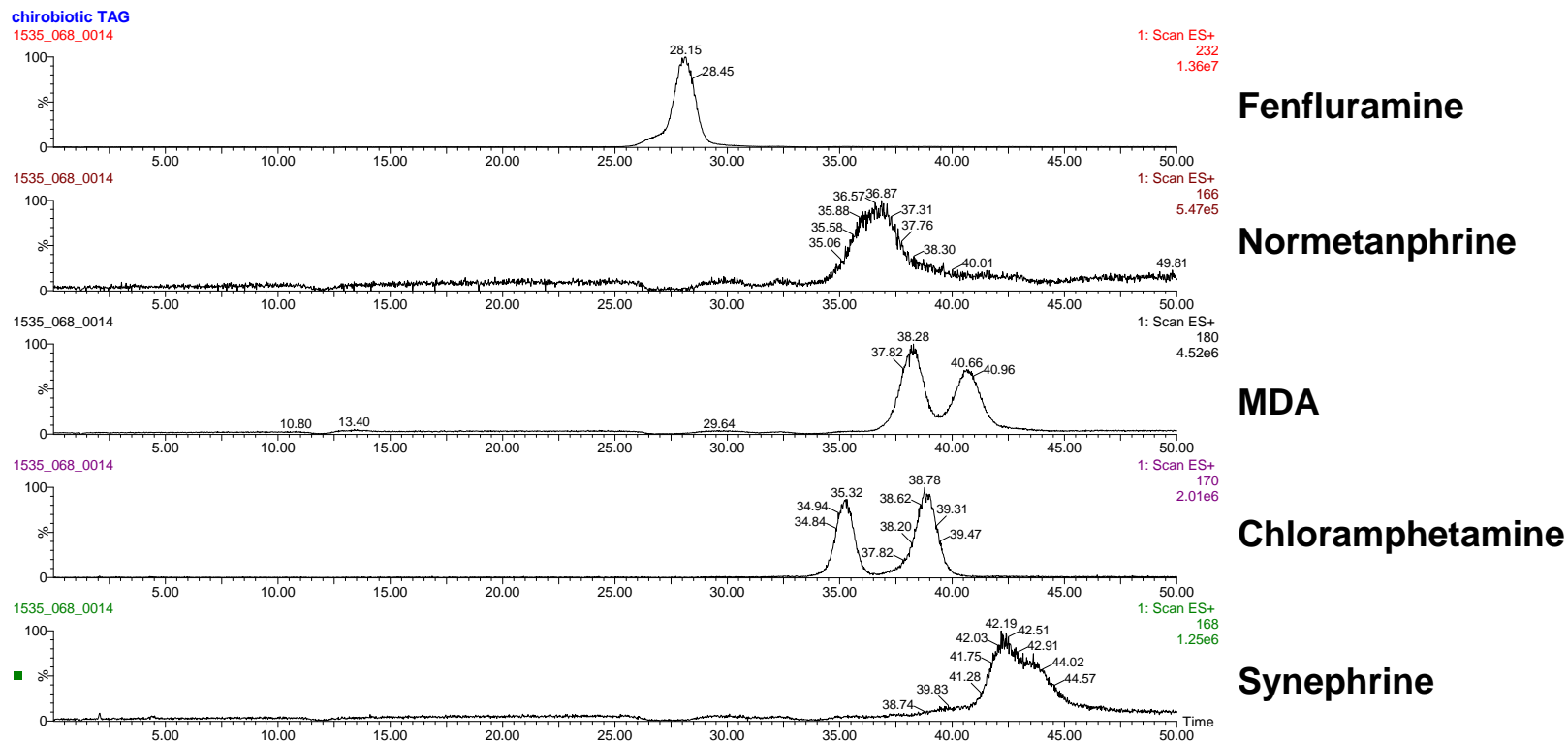
instrument: Waters/Micromass ZQ, Single Quadrupole, Waters Alliance 2690  
column: CHIROBIOTIC V2, TAG and R, 15 cm x 4.6 mm, 5  $\mu$ m  
mobile phase: ammonium formate in methanol (13 mM)  
flow rate: 1 mL/min.  
temp.: 35° C  
det.: ESI, Positive Ion Mode, scan range m/z 150–500  
injection: 5  $\mu$ L

# Figure 4. CHIROBIOTIC V2 Shows Selectivity Towards Fluoxetine and Norfluoxetine



- As seen in Figure 4, unique selectivity is shown between the V2 phase and the fluoxetine compounds in the probe mix.
- The TAG, T, and R showed no selectivity toward the fluoxetines.
- 13 • The beta blockers also showed selectivity on the V2 in polar-ionic mode.

# Figure 5. CHIROBIOTIC TAG Shows Selectivity Towards the Amphetamines



- Figure 5, shows selectivity of the amphetamines unique to the TAG in this mode. The TAG also showed selectivity toward the beta blockers.
- The TAG shows much greater retention for the basic compounds when compared to the other CHIROBIOTICS.

## Importance of Column Screening

- Changing the CSP stationary phase remains the most useful means of altering enantiomeric selectivity.
- As seen in Figures 4 and 5, each of the CHIROBIOTIC phases showed selectivity toward different analytes (except R, which is generally more applicable to acidic compounds).
- Other than very general trends, selectivity remains unpredictable, necessitating a column screening approach to method development.
- When CSPs are compatible, LC-MS can make this easier by allowing mixtures of different enantiomers to be screened simultaneously.

## Discussion and Conclusion

- The utility of LC-MS to study the impact of variables on retention and selectivity has been demonstrated.
- Variables such as buffer type, buffer concentration, and acid:base ratio were investigated in polar ionic mode.
- Selectivity was impacted the greatest by changing stationary phase, confirming the need for column screening as a first step in method development.
- Once selectivity has been observed on a CSP, the adjustment of buffer component ratios appears to have the greatest impact on enantiomeric resolution.
- Both the type of buffer salt and the concentration can be used to manipulate peak shape and retention, but have limited impact on selectivity.
- Further work is planned to rapidly investigate variables on Cyclodextrin and other CSPs using this batch LC-MS screening technique.

# Acknowledgements

J.T. Lee, Craig Aurand, and Dick Henry

## References

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2. Jensen, B. P., C. F. Sharp, et al. (2008). "Development and validation of a stereoselective liquid chromatography-tandem mass spectrometry assay for quantification of S- and R-metoprolol in human plasma." *Journal of Chromatography B* 865(1-2): 48-54.
3. Bell, D., C. Aurand, J. Claus, D. Schollenberger and J. Jones, Chiral LC-MS Analysis of Drug Substances (Beta-Blockers) from Plasma Using Macrocyclic Glycopeptide Chiral Stationary Phases, Pittcon 2009.