

Retention Mechanisms in Chiral Chromatography: LC-MS Analysis using Macrocyclic Glycopeptide and Cyclodextrin Chiral Stationary Phases



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T409045

Introduction

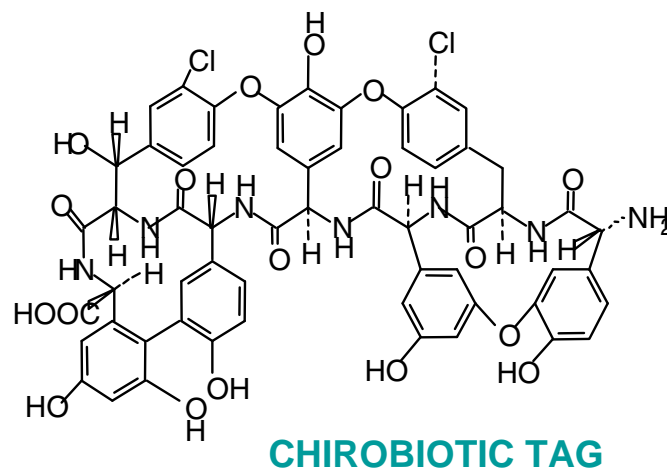
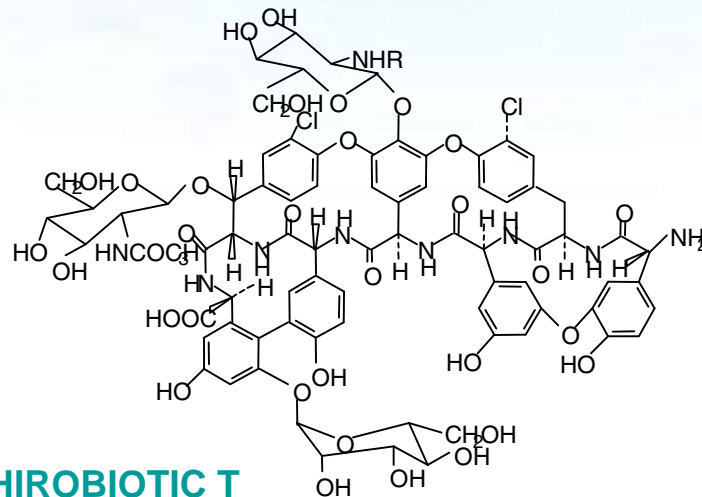
- Retention mechanisms in chiral separations are highly complex and analyte specific, therefore many columns and conditions must be screened to ensure that optimum conditions are found.
- To more quickly investigate the many variables that contribute to retention and selectivity in chiral separations, the use of a composite set of probes would be beneficial.
- 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.

Focus on CHIROBIOTIC CSPs

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

Instrument: Waters/Micromass ZQ, Single
Quadrupole, Waters Alliance 2690

Column: **Chirobiotic T**, 150 cm x 4.6 mm, 5 μ m

Temperature: 35° C

Flow Rate: 1 mL/min

Mobile Phase: 0.1%, w/w ammonium acetate in
methanol (**Polar Ionic Mode**)

Detection: ESI, Positive Ion Mode, scan range m/z
150 – 500 (Total Ion or Extracted Ion
Current)

Inj. Vol.: 5 μ L

Composite Test Probe- Basic Analytes

synephrine, m/z 168

chloramphetamine, m/z 170

methylenedioxyamphetamine (MDA) m/z 180

normetanphrine, m/z 184 (base peak m/z 166 $-H_2O$)

fenfluramine, m/z 232

bupropion, m/z 240

midodrine, m/z 255

propranolol, m/z 260

metoprolol, m/z 268

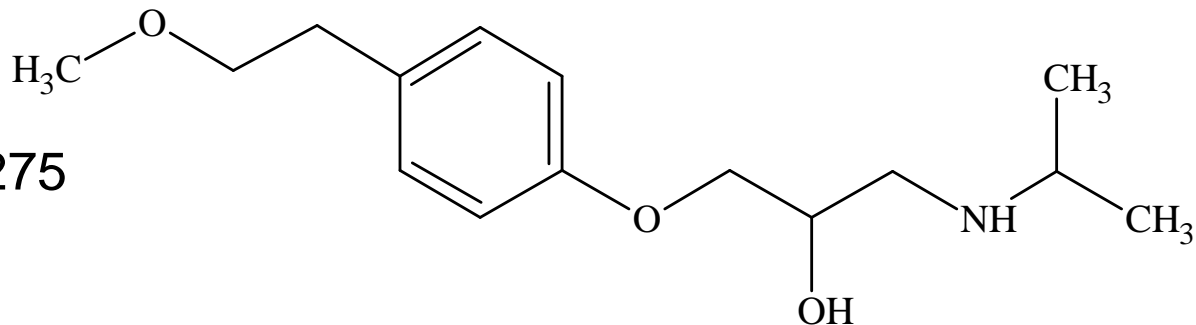
chlorpheniramine, m/z 275

pentazocine, m/z 286

norfluoxetine, m/z 296

fluoxetine, m/z 310

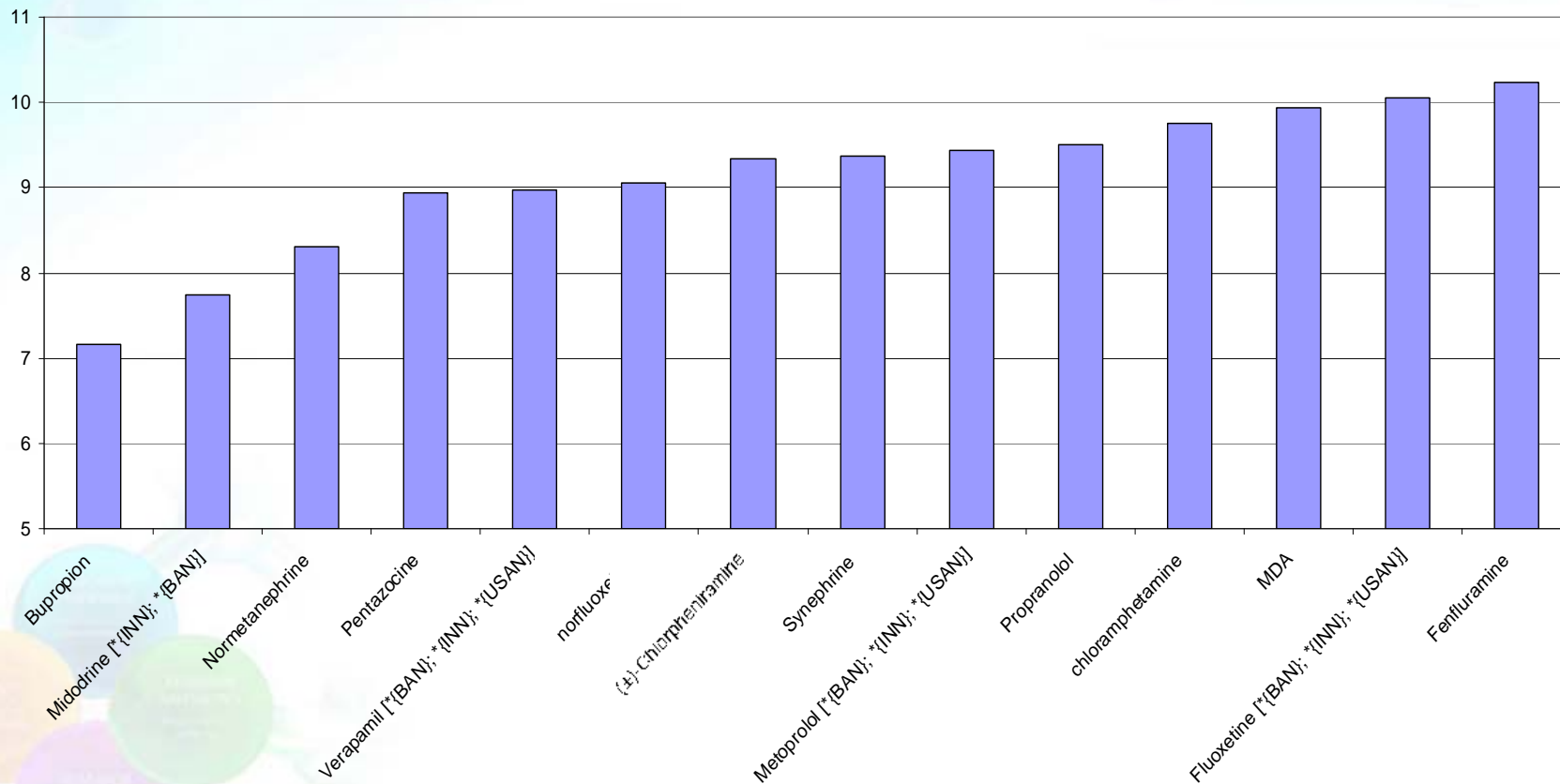
verapamil, m/z 455



Metoprolol

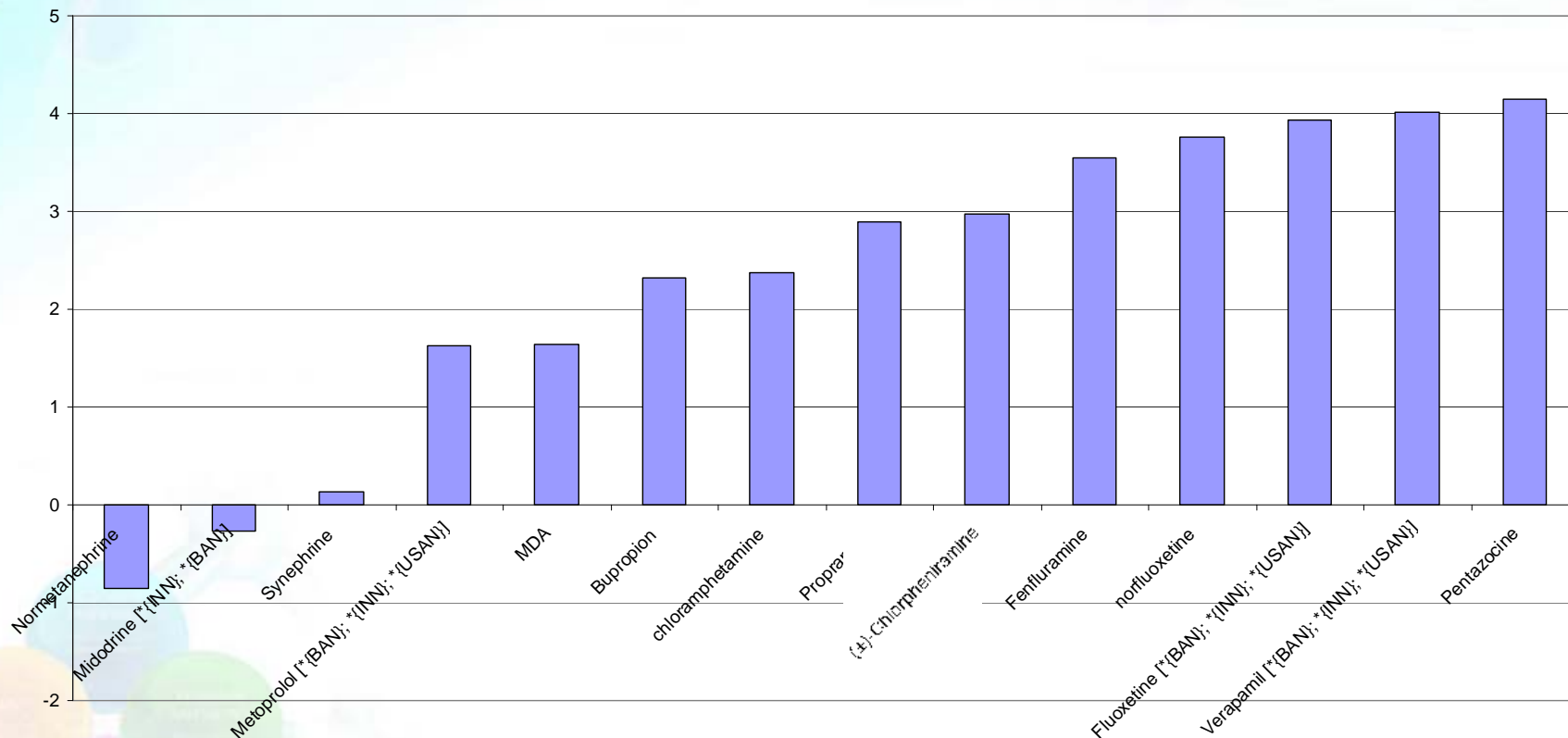
pK_a Values for Probe Analytes

Variation in pKa Values



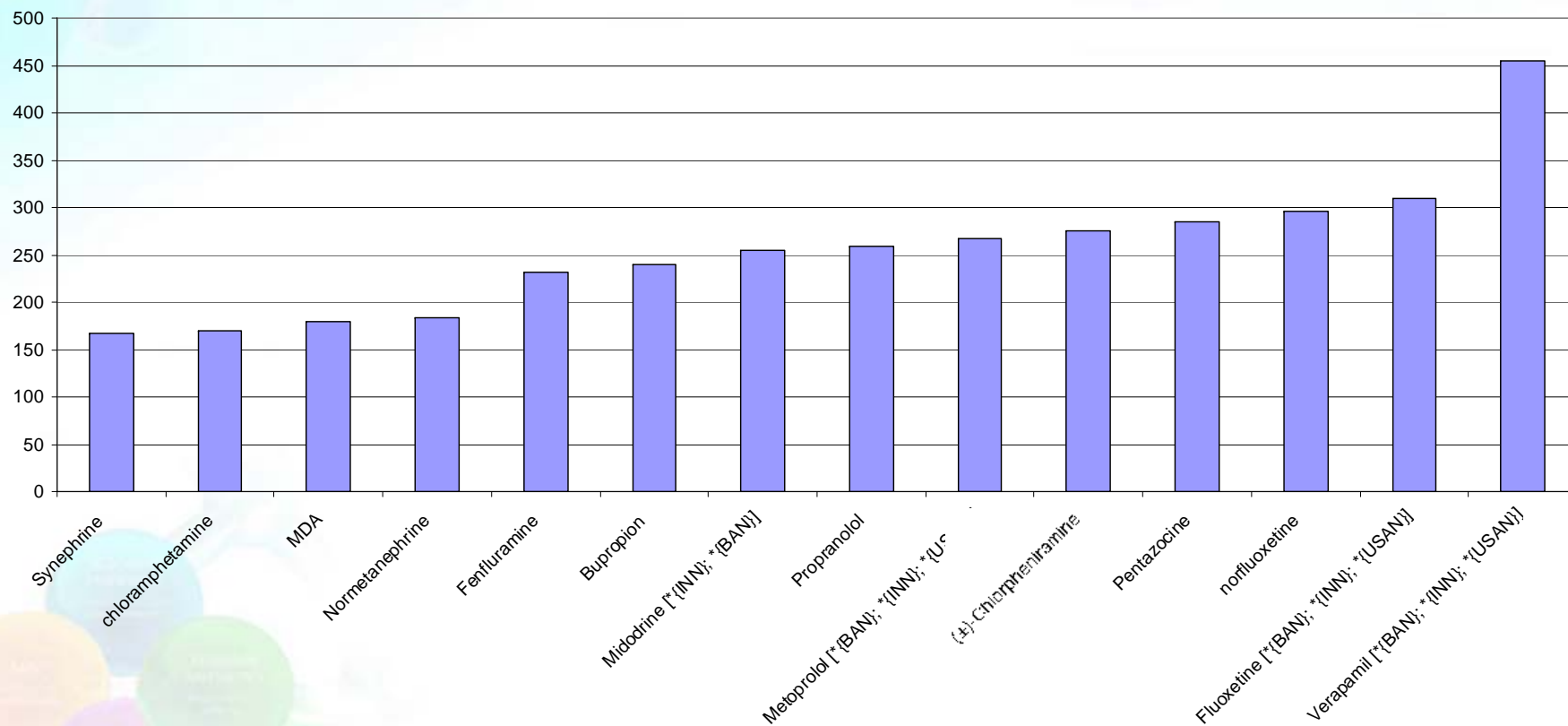
Log P Values for Probe Analytes

Variation in Log P Values



Molecular Weights for Probe Analytes

Variation in Molecular Weight



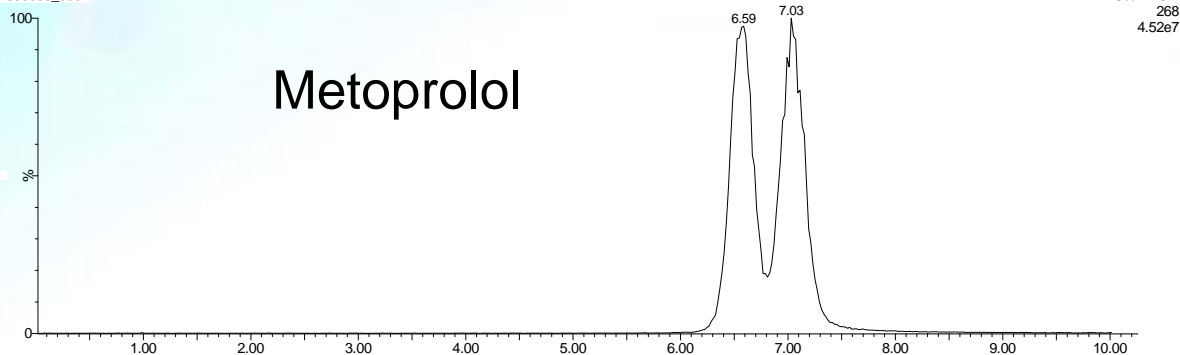
Validation of LC-MS Approach

- Chirobiotic T has been shown to be effective for the separation of β -blocker enantiomers (1-2).
- In this study, the β -blocker metoprolol was first injected alone and then in the presence of the 13 additional compounds; significant peak overlap was observed.
- The success of the approach was confirmed and then utilized to investigate the impact of buffer type, concentration and acid/base ratio on retention and selectivity in Polar Ionic Mode.

1. 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.
2. 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, Poster Tuesday PM.

Comparison of Metoprolol Alone and in Probe Mix

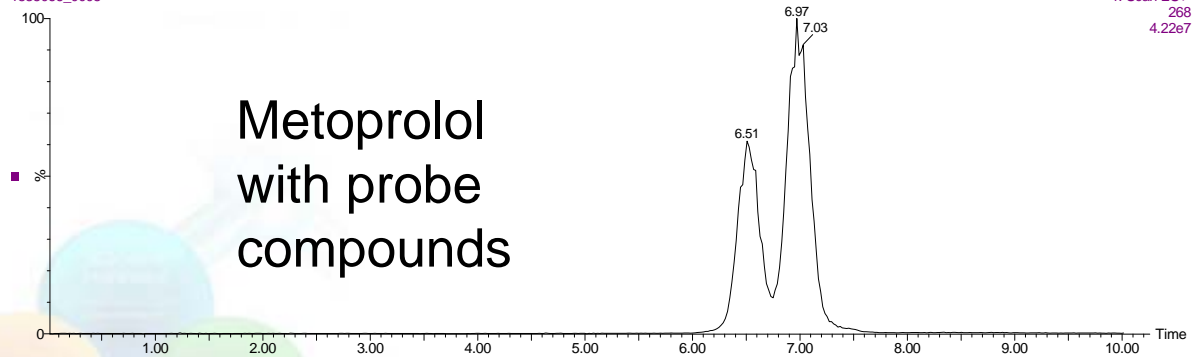
super sample_T_0.1%AA in methanol
1535066_0002



1: Scan ES+
268
4.52e7

Metoprolol

1535066_0005



1: Scan ES+
268
4.22e7

Metoprolol
with probe
compounds

Note a slight variation in enantiomer response due to ion-suppression by coeluting peaks; however, retention and selectivity is not compromised

Chirobiotic T, 0.1% NH₄Ac in Methanol (Polar Ionic Mode), ESI+ (Extracted Ion Current).

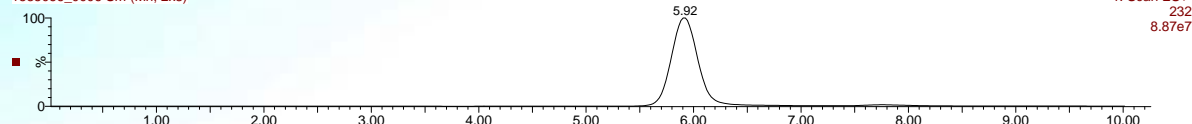
Probe Mix using 0.1% Ammonium Acetate in Methanol

Set 1:

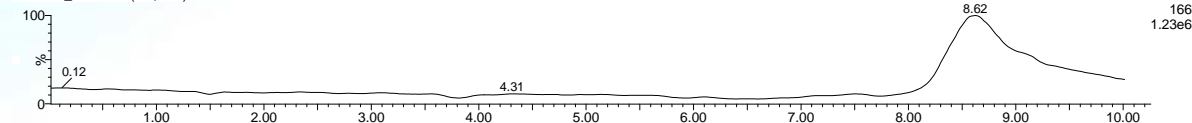
Extracted Ion Current (XIC)

super sample_T_0.1%AA in methanol

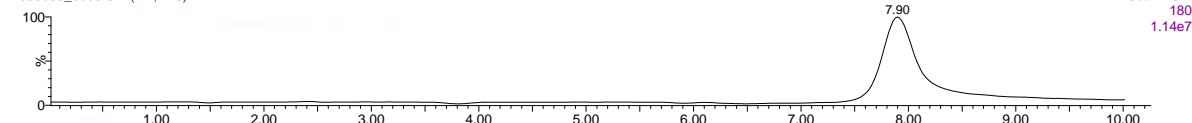
1535066_0006 Sm (Mn, 2x3)



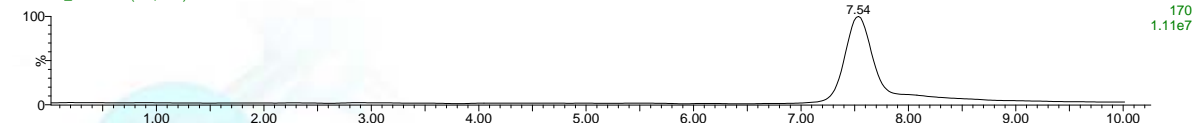
1535066_0006 Sm (Mn, 2x3)



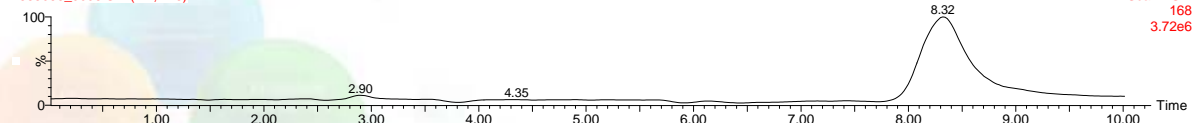
1535066_0005 Sm (Mn, 2x3)



1535066_0006 Sm (Mn, 2x3)



1535066_0006 Sm (Mn, 2x3)



Fenfluramine

Normetanphrine

MDA

Chloramphetamine

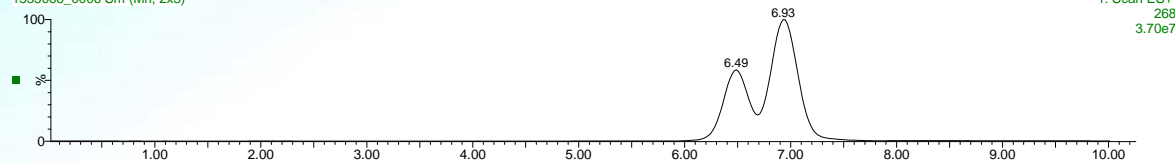
Synephrine

Probe Mix using 0.1% Ammonium Acetate in Methanol

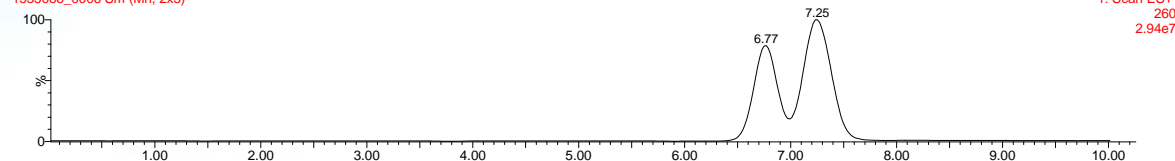
Set 2:

super sample_T_0.1%AA in methanol

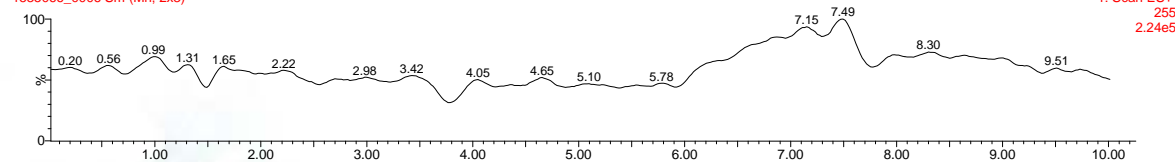
1535066_0006 Sm (Mn, 2x3)



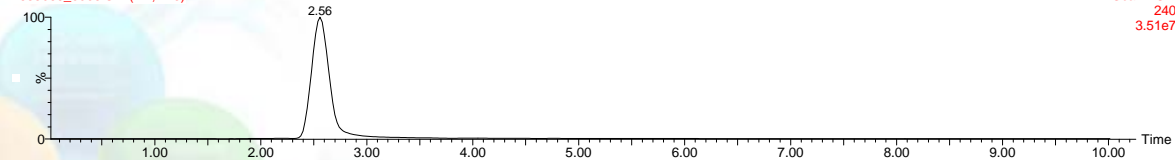
1535066_0006 Sm (Mn, 2x3)



1535066_0006 Sm (Mn, 2x3)



1535066_0006 Sm (Mn, 2x3)



Metoprolol

Propranolol

Midodrine¹

Bupropion

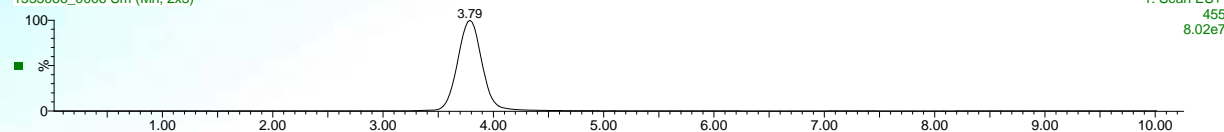
1. Midodrine was not observed under these conditions.

Probe Mix using 0.1% Ammonium Acetate in Methanol

Set 3:

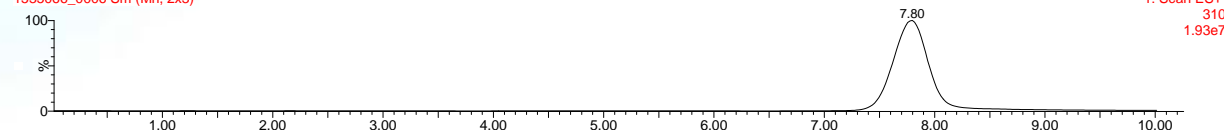
super sample_T 0.1%AA in methanol

1535066_0006 Sm (Mn, 2x3)



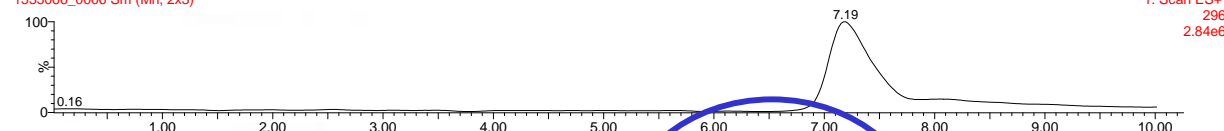
1: Scan ES+
455
8.02e7

1535066_0006 Sm (Mn, 2x3)



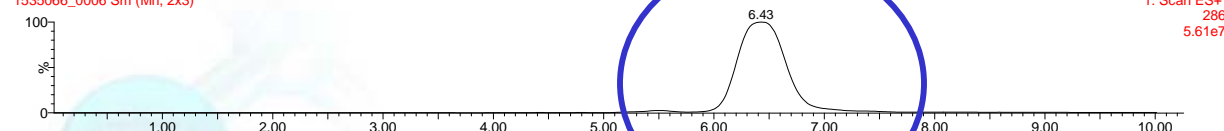
1: Scan ES+
310
1.93e7

1535066_0006 Sm (Mn, 2x3)



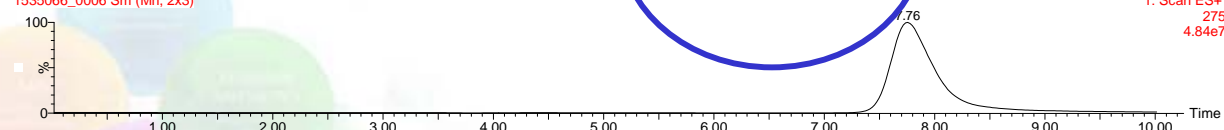
1: Scan ES+
296
2.84e6

1535066_0006 Sm (Mn, 2x3)



1: Scan ES+
286
5.61e7

1535066_0006 Sm (Mn, 2x3)



1: Scan ES+
275
4.84e7

Verapamil

Fluoxetine

Norfluoxetine

Pentazocine

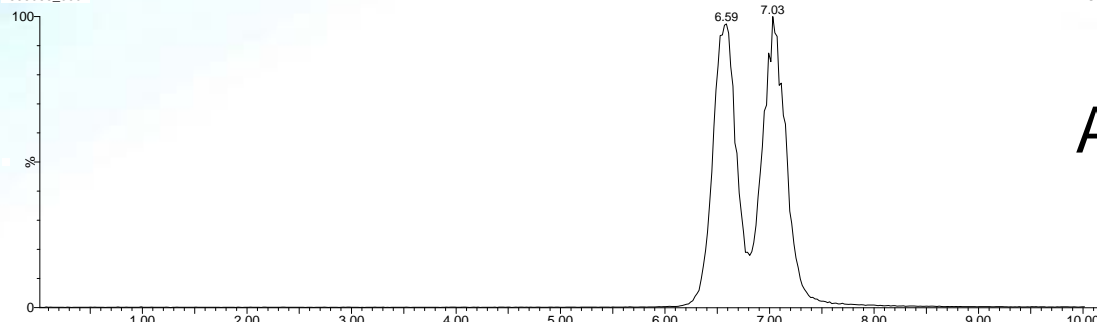
Chlorpheniramine

Chiral Retention Mechanisms: Impact of Buffer Type on Retention and Enantiomeric Selectivity

- 0.1% wt/v ammonium acetate, ammonium formate and ammonium trifluoroacetate were prepared in methanol.
- Retention and selectivity were monitored as a function of the buffer type.
- The complex probe mixture was run using multiple injections to confirm system equilibration.

Comparison of Ammonium Formate with Ammonium Acetate – Metoprolol in Probe Mix

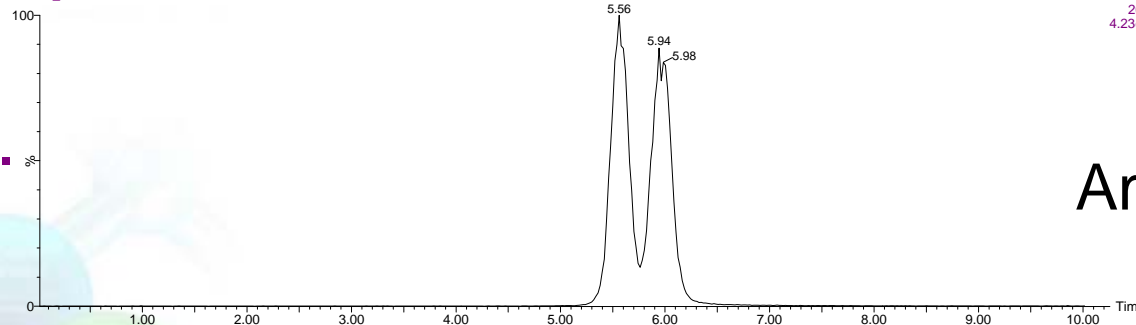
metoprolol only_T_0.1%AF in methanol
1535066_0002



1: Scan ES+
268
4.52e7

Ammonium Acetate

1535066_0008



1: Scan ES+
268
4.23e7

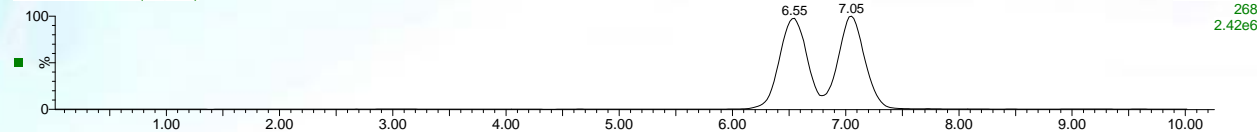
Ammonium Formate

Chirobiotic T (Polar Ionic Mode), ESI+ (Extracted Ion Mode).

Slow Equilibration with Ammonium TFA

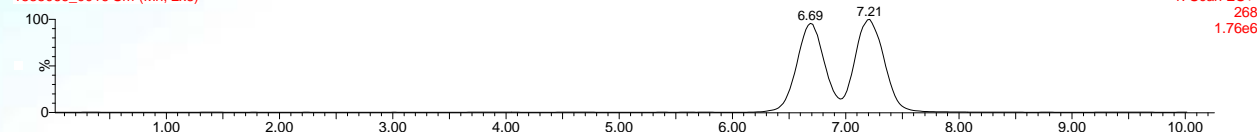
metoprolol_T 0.1%ATFA in methanol

1535066_0017 Sm (Mn, 2x3)



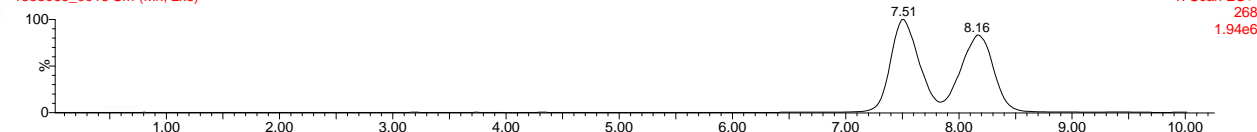
~100 min

1535066_0016 Sm (Mn, 2x3)



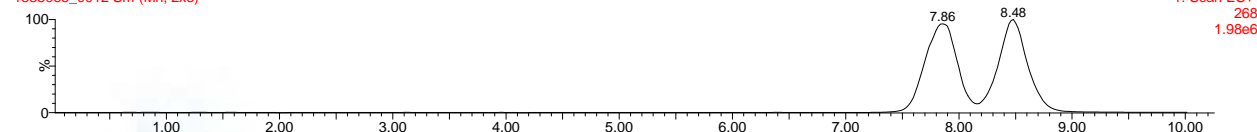
~90 min

1535066_0013 Sm (Mn, 2x3)



~40 min

1535066_0012 Sm (Mn, 2x3)



~30 min

1535066_0011 Sm (Mn, 2x3)

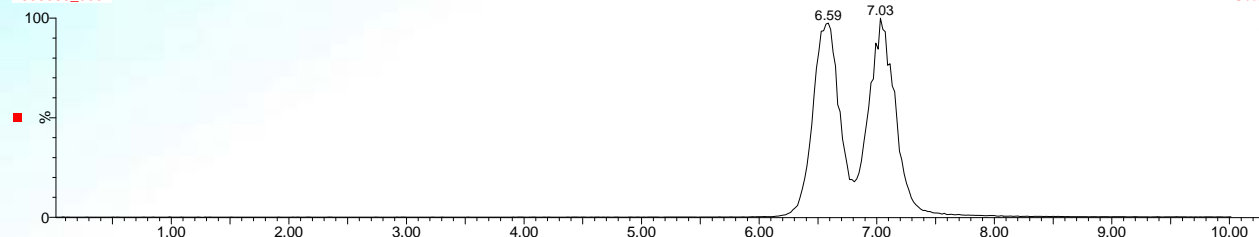


~20 min

Ion-Suppression using Ammonium TFA

Anion has a major effect on response, a slight effect on retention, and no significant effect on selectivity.

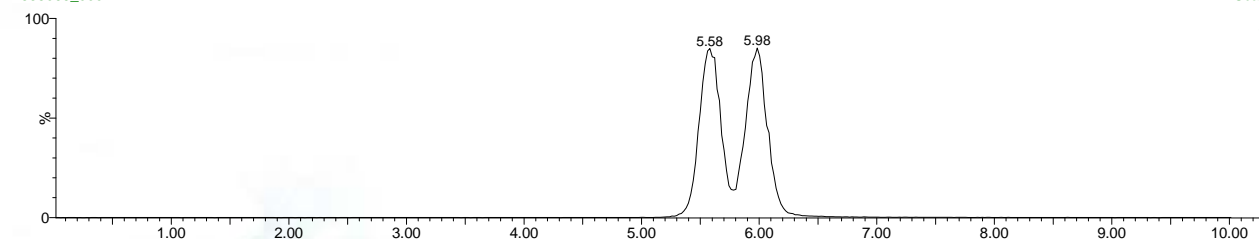
metoprolol only_T_0.1%AA in methanol
1535066_0002



1: Scan ES+
268
4.52e7

Ammonium Acetate

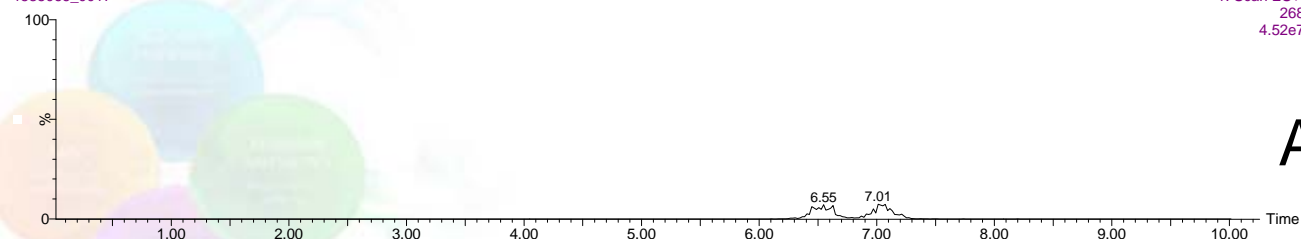
1535066_0007



1: Scan ES+
268
4.52e7

Ammonium Formate

1535066_0017



1: Scan ES+
268
4.52e7

Ammonium TFA

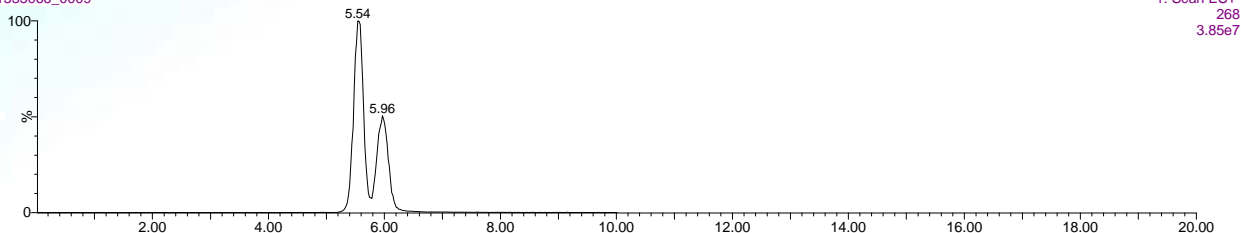
Chiral Retention Mechanisms: Impact of Buffer Concentration on Retention and Enantiomeric Selectivity

- The complex probe 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.

Impact of Buffer Concentration on Metoprolol Retention and Selectivity

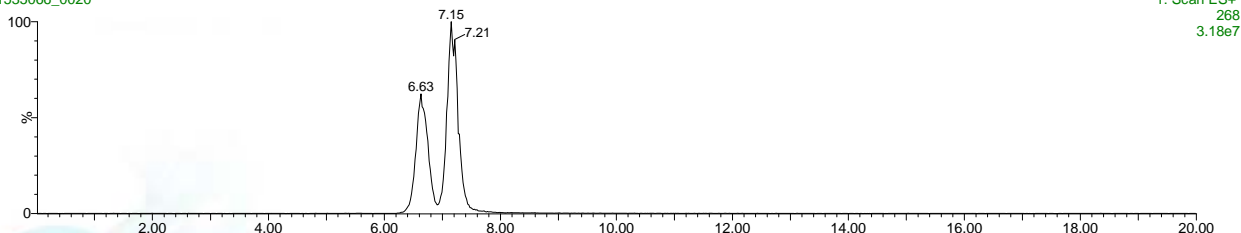
Concentration has an effect on retention, but no major effect on selectivity or response.

super sample_T_0.050%AF in methanol
1535066_0009



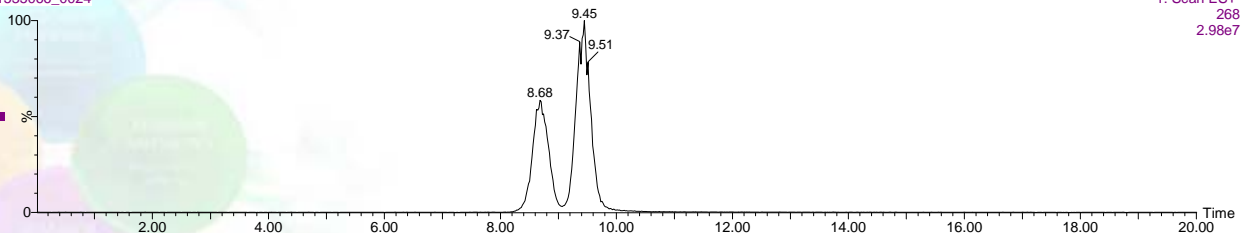
0.1% AF

1535066_0020



0.075% AF

1535066_0024

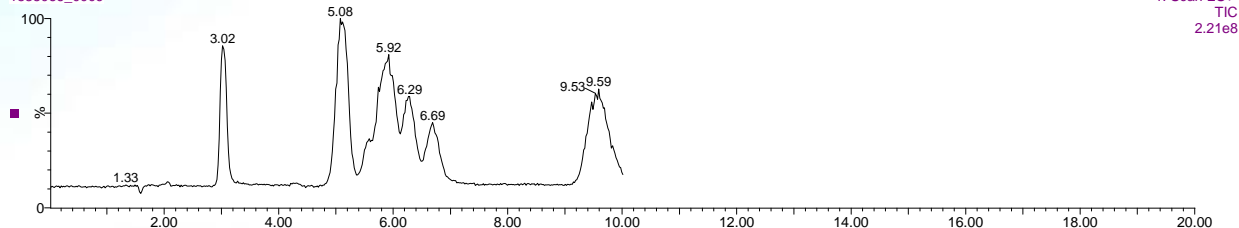


0.05% AF

Impact of Buffer Concentration on Retention and Selectivity for Complex Probe Mix

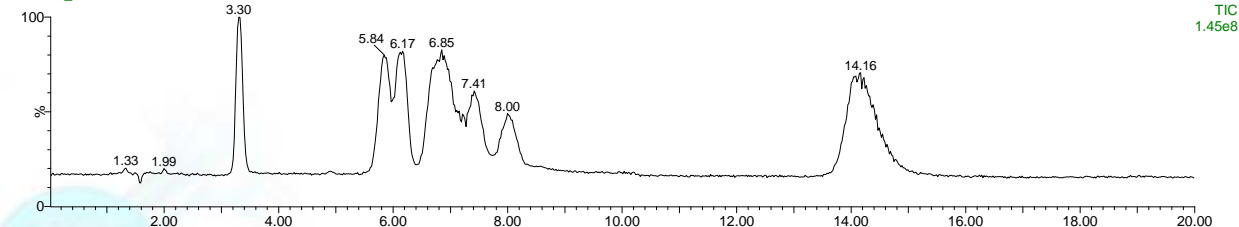
Concentration of ammonium formate has an effect on retention, but no major effect on selectivity or response.

super sample_T_0.1%AF in methanol
1535066_0009



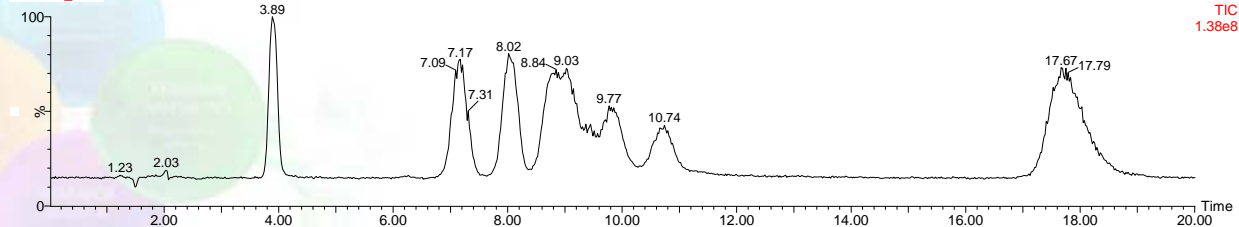
0.1% AF

1535066_0020



0.075% AF

1535066_0024



0.05% AF

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
- Runs were repeated to ensure equilibration

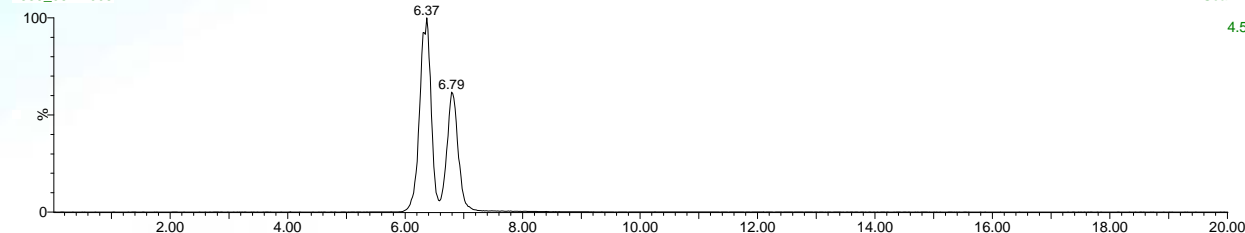
Impact of Buffer Component Ratio on Metoprolol Retention and Selectivity

Ratio creates a significant change in retention plus some change in selectivity.

Ratio is base:acid

75:25 ammonia:formic
1535_067-1005

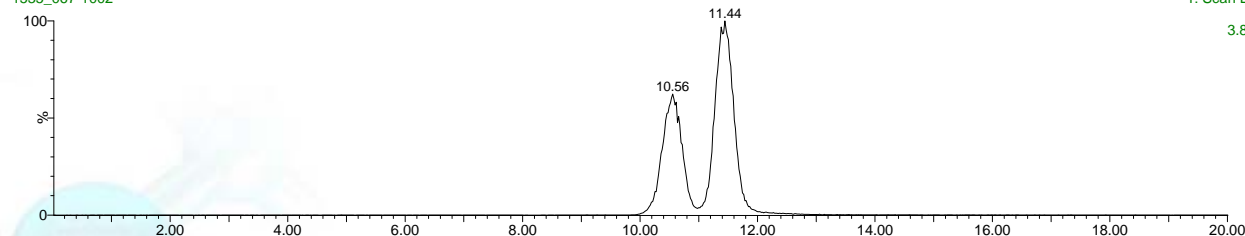
1: Scan ES+
268
4.52e7



1:3

1535_067-1002

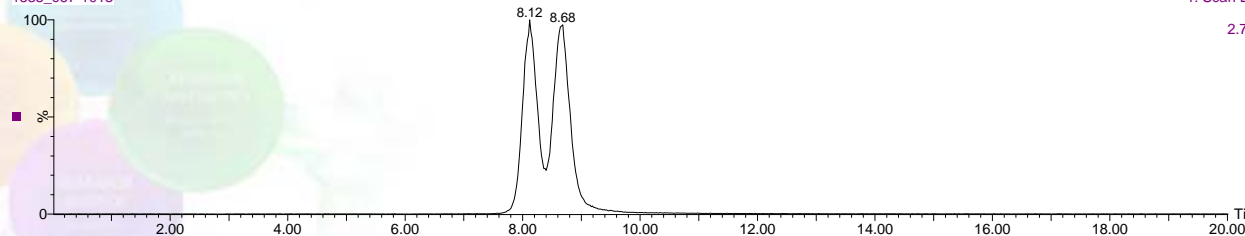
1: Scan ES+
268
3.82e7



1:1

1535_067-1013

1: Scan ES+
268
2.78e7



3:1

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, 150 x 4.6 mm

Temperature: 35° C

Flow Rate: 1 mL/min

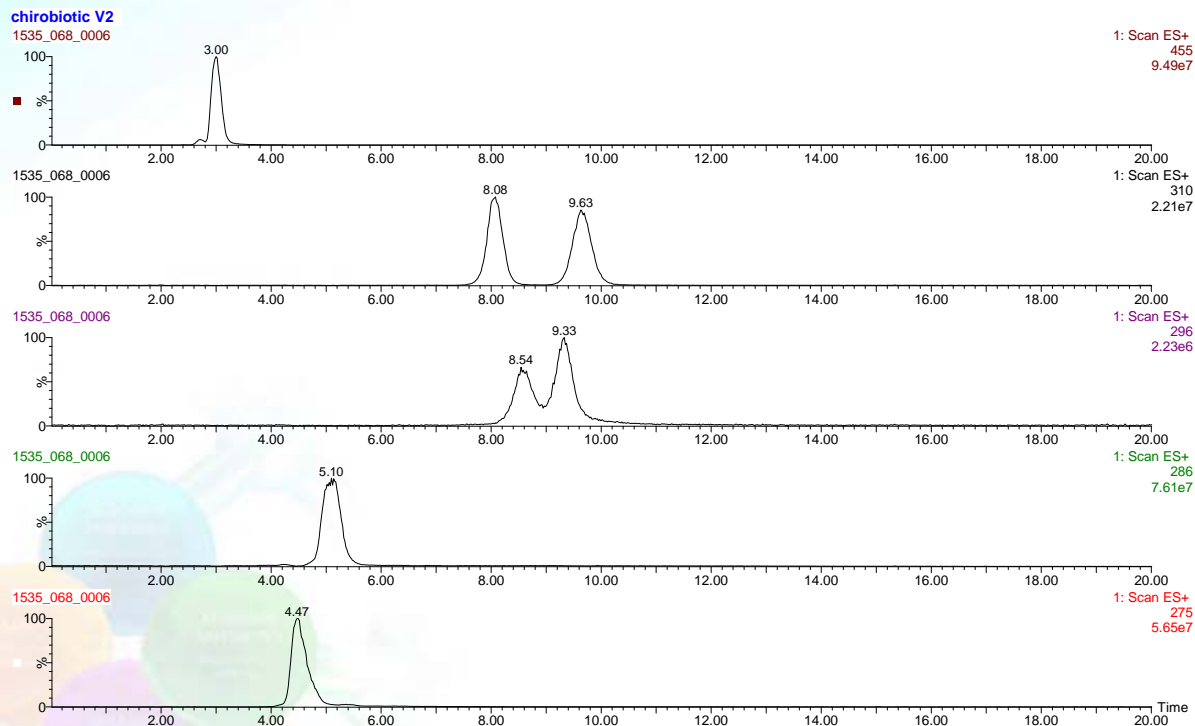
Mobile Phase: Ammonium formate in methanol (13 mM)

Detection: ESI, Positive Ion Mode, scan range m/z
150 – 500 (Total Ion or Extracted Ion
Current)

Inj. Vol.: 5 µL

Chirobiotic V2 Shows Selectivity Towards Fluoxetine and Norfluoxetine

Unique selectivity between V2 phase and certain solutes shows up in complex probe mix.



Verapamil

Fluoxetine

Norfluoxetine

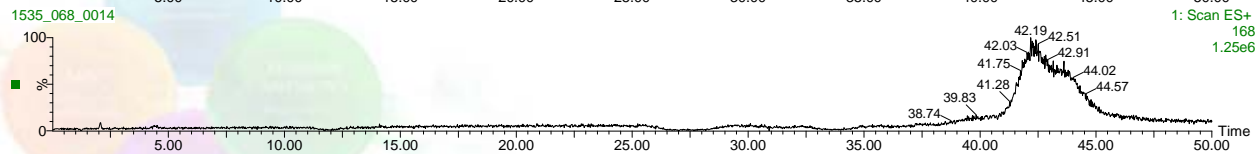
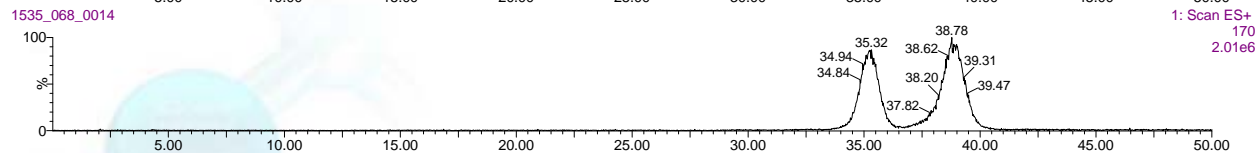
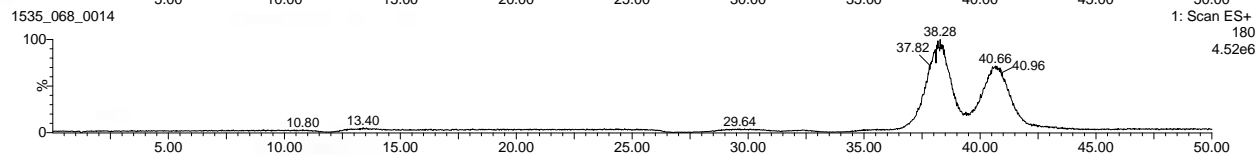
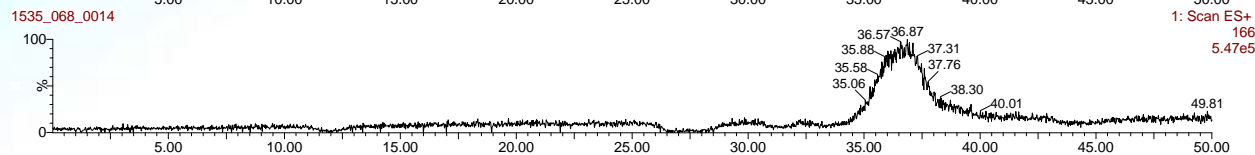
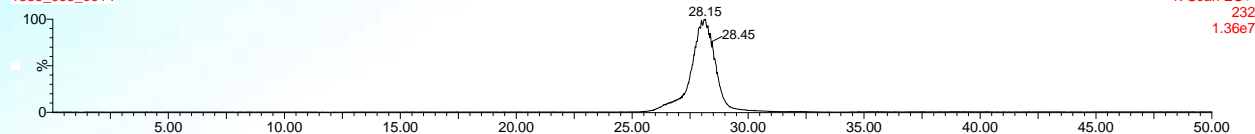
Pentazocine

Chlorpheniramine

Chirobiotic TAG Shows Selectivity Towards the Amphetamines

Unique selectivity between TAG phase and certain solutes shows up in complex probe mix.

chirobiotic TAG
1535_068_0014



Fenfluramine

Normetanphrine

MDA

Chloramphetamine

Cynephine

Column Screening

- Changing the CSP stationary phase is still the most useful means of altering enantiomeric selectivity.
- 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.

Conclusions

- The utility of LC-MS to study the impact of variables on retention and selectivity simultaneously for a large sample set in chiral separations has been demonstrated.
- Variables such as buffer type, buffer concentration, acid/base ratio and column phase chemistry were investigated in polar ionic mode.
- Equilibration problems were observed with ammonium TFA in methanol— further work is planned to investigate this phenomenon.
- Selectivity was impacted the greatest by changing stationary phase, confirming the need for column screening as a first step in method development

Conclusions, Contd.

- 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
- Batch screening shows excellent potential for speeding up the column selection process.
- Further work is planned to rapidly investigate variables on Cyclodextrin and other CSPs using this batch LC-MS screening technique.

Acknowledgements

- The assistance of the Supelco Applications Lab and Chiral Screening Lab is greatly appreciated.