Impact of Reversed-Phase Chiral Chromatography on the LC-MS Analysis of Drugs in Biological Fluids

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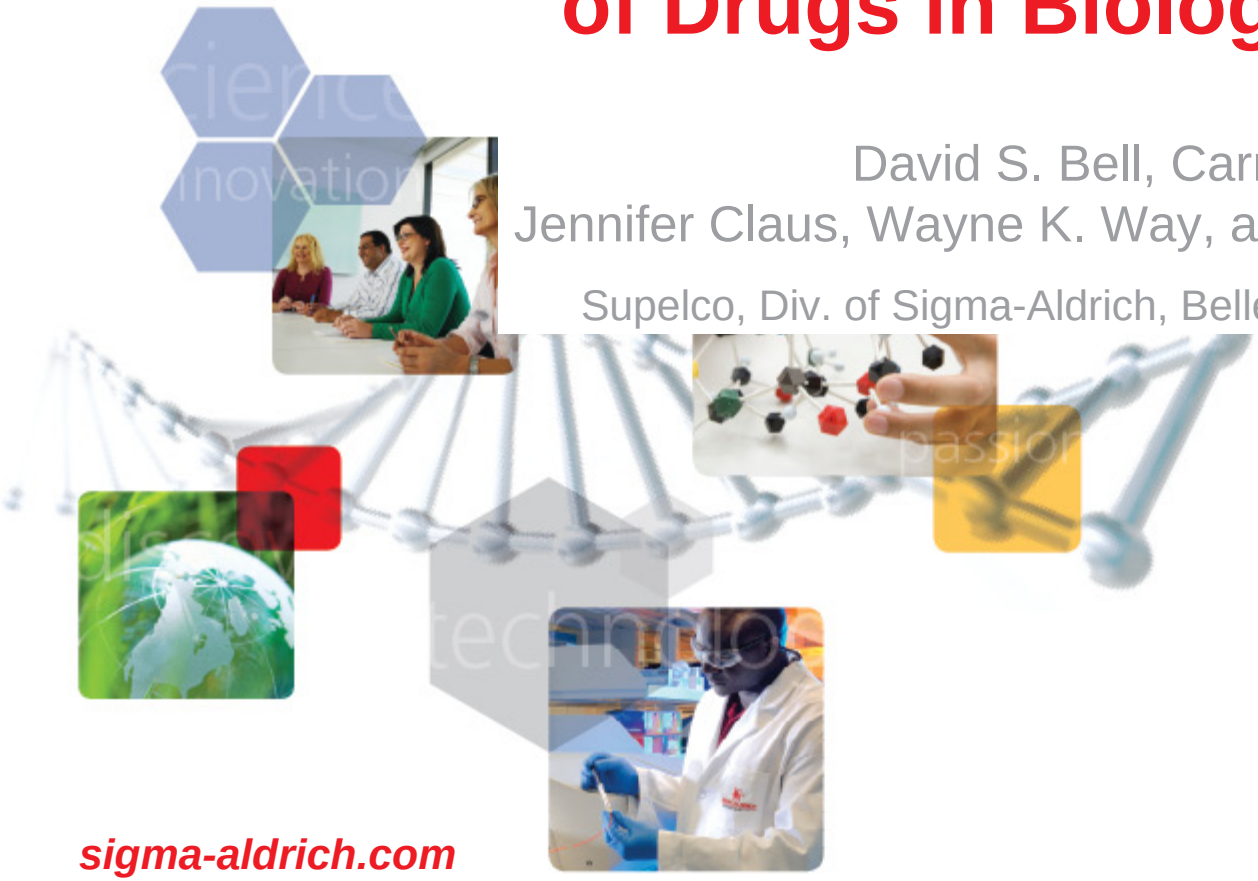

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Abstract

Macrocyclic glycopeptide and cyclodextrin-based chiral stationary phases (CSPs) often provide enantiomeric selectivity using polar organic and aqueous-organic mobile phases. As these mobile phases are highly amenable to mass spectrometry (MS) sources, separations intended for LC-MS detection may be improved over traditional chiral separations utilizing normal phase solvents.

In this study, chiral methods for several drugs and metabolites were developed utilizing both reversed-phase and normal phase conditions. The methods were then applied to LC-MS analysis in various biological fluids and the results were compared and contrasted. It is demonstrated that, when possible, chiral methods operating in reversed-phase mode, can greatly improve sensitivity in LC-MS.

Introduction

- Upon attempting to achieve enantiomeric selectivity of chiral analytes, scientists tend to focus their initial efforts on the examination of cellulosic and amylosic stationary phases using normal phase solvents and organic modifiers.
- Although these normal phase chromatographic systems tend to produce selectivity over a broad range of analytes, the use of normal phase solvents and organic modifiers is often unfavorable to mass spectrometry (MS), limiting analyte detection to much less sensitive methods.
- Since macrocyclic glycopeptide and cyclodextrin-based CSPs produce enantiomeric selectivity of chiral analytes in polar organic and aqueous-organic mobile phases, chromatographic systems containing these phases are highly amenable to LC-MS detection.
- A case study focuses on the chiral nonsteroidal anti-inflammatory drug (NSAID) ketorolac (1), and illustrates, in detail, the process of converting its chiral separation method from UV to MS detection. Another study describing the LC-MS analysis of β-blockers in plasma is then highlighted.

Experimental and Results

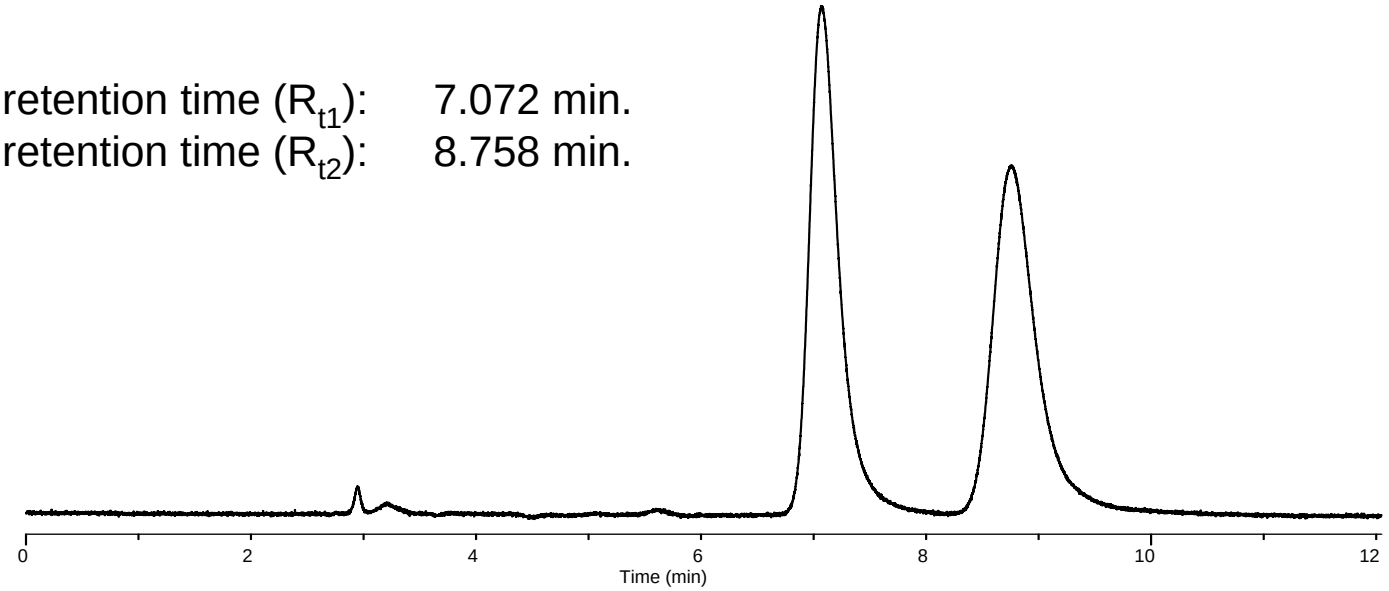
Racemic ketorolac was tested through a chiral screening protocol employing 12 columns and 6 mobile phases. Results showed that ketorolac separated enantiomerically under normal phase conditions on the Kromasil® AmyCoat™ as well as under reversed-phase conditions on the Astec CHIROBIOTIC® TAG. The methods were then made compatible to LC-MS analysis, and the results were compared and contrasted.

The normal phase separation of the ketorolac enantiomers is illustrated in Figure 1. The chromatographic system employs the organic solvent heptane and 2-propanol with the organic modifiers triethylamine (TEA) and trifluoroacetic acid (TFA) on an amylosic stationary phase, and utilizes UV detection at 254 nm.

Figure 1. The Chiral Separation of (+/-)-Ketorolac Under Traditional Normal Phase Conditions

column:	Kromasil AmyCoat, 25 cm x 4.6 mm I.D., 5 µm particles
mobile phase:	80:20, heptane:2-propanol (w/0.1% TEA and 0.1% TFA)
flow rate:	1.0 mL/min.
temp.:	25 ºC
det.:	UV at 254 nm
injection:	10 µL
sample:	0.2 mg/mL in 80:20, heptane:2-propanol

Peak 1 retention time (R_{t1}): 7.072 min.
Peak 2 retention time (R_{t2}): 8.758 min.

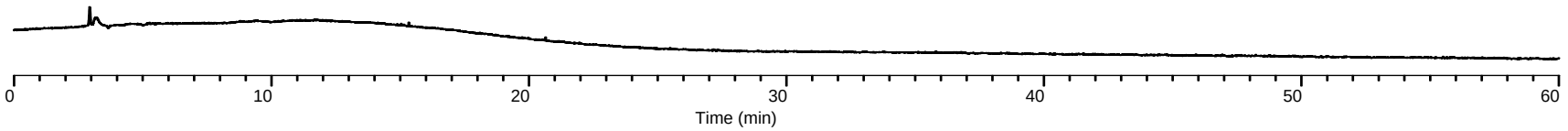


While normal phase solvents are not particularly compatible with MS, the organic modifiers TEA and TFA tend to cause strong ion suppression in MS, limiting the detection of analytes. Thus, these additives are highly unfavorable for use in MS. To make this chromatographic system more amenable to MS, an experiment to remove these additives from the normal phase mobile phase was performed.

Figure 2 shows the chromatographic results attained upon removal of mobile phase additives TEA and TFA. Note that removal of the organic modifiers caused ketorolac to remain retained on the column, leading one to believe that one or both of the modifiers was essential for the elution of ketorolac. Therefore, the normal phase conditions could not be directly converted to MS compatible conditions.

Figure 2. Injection of (+/-)-Ketorolac Under Normal Phase Conditions Without Organic Modifiers

column:	Kromasil AmyCoat, 25 cm x 4.6 mm I.D, 5 µm particles
mobile phase:	80:20, heptane:2-propanol
flow rate:	1.0 mL/min.
temp.:	25 ºC
det.:	UV at 254 nm
injection:	10 µL
sample:	0.2 mg/mL in 80:20, heptane:2-propanol

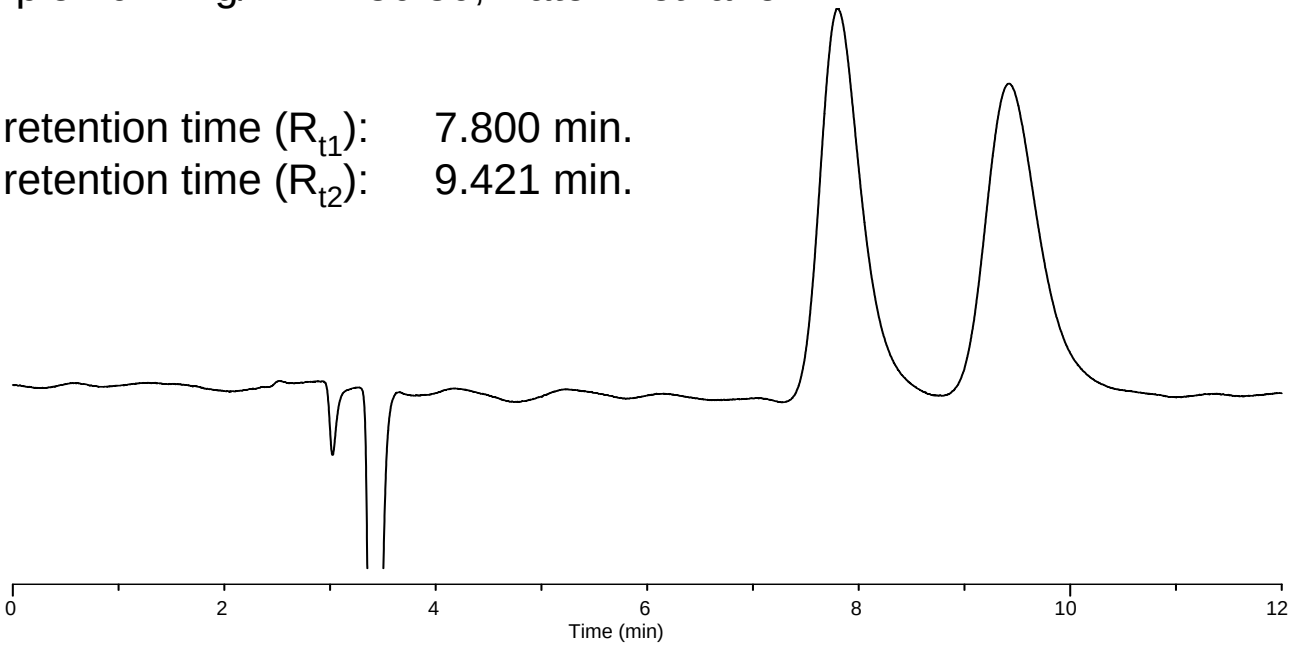


Resulting from the chiral screen mentioned previously, separation of the ketorolac enantiomers was also achieved on an Astec CHIROBIOTIC TAG, a macrocyclic glycopeptide, in reversed-phase mode utilizing UV detection at 220 nm. The separation is illustrated in Figure 3.

Figure 3. The Chiral Separation of (+/-)-Ketorolac Under Reversed-Phase Conditions

column:	CHIROBIOTIC TAG, 25 cm x 4.6 mm I.D., 5 µm particles
mobile phase:	50:50, 20 mM ammonium acetate (pH 4.0):methanol
flow rate:	1.0 mL/min.
temp.:	25 ºC
det.:	UV at 220 nm
injection:	10 µL
sample:	0.2 mg/mL in 50:50, water:methanol

Peak 1 retention time (R_{t1}): 7.800 min.
Peak 2 retention time (R_{t2}): 9.421 min.



Since the solvents and additives used in the reversed-phase chiral separation of ketorolac on the CHIROBIOTIC TAG are MS compatible, the method was directly transferred to an LC-MS system for analysis. It was, however, observed that ketorolac produced a poor MS response in both +ESI, -ESI, +APCI modes under the aforementioned mobile phase conditions. In order to improve the response, the ammonium acetate buffer was replaced with 0.1% formic acid (1). The formic acid mobile phase additive showed a significant improvement over the ammonium acetate buffer for detection of the chiral enantiomers in +ESI mode. Please refer to Figure 4.

Figure 4. The Chiral Separation of (+/-)-Ketorolac Under Reversed-Phase Conditions and Detection by MS

column:	CHIROBIOTIC TAG, 15 cm x 4.6 mm I.D., 5 µm particles
mobile phase:	50:50, 0.1% formic acid in water:methanol
flow rate:	0.5 mL/min.
temp.:	ambient
det.:	Waters/Micromass ZQ, Single Quadrupole, Waters Alliance 2690, ESI (+), SIR m/z 256
injection:	2 µL
sample:	10 µg/mL in 50:50, water:methanol

Peak 1 retention time (R_{t1}): 18.30 min.
Peak 2 retention time (R_{t2}): 20.75 min.

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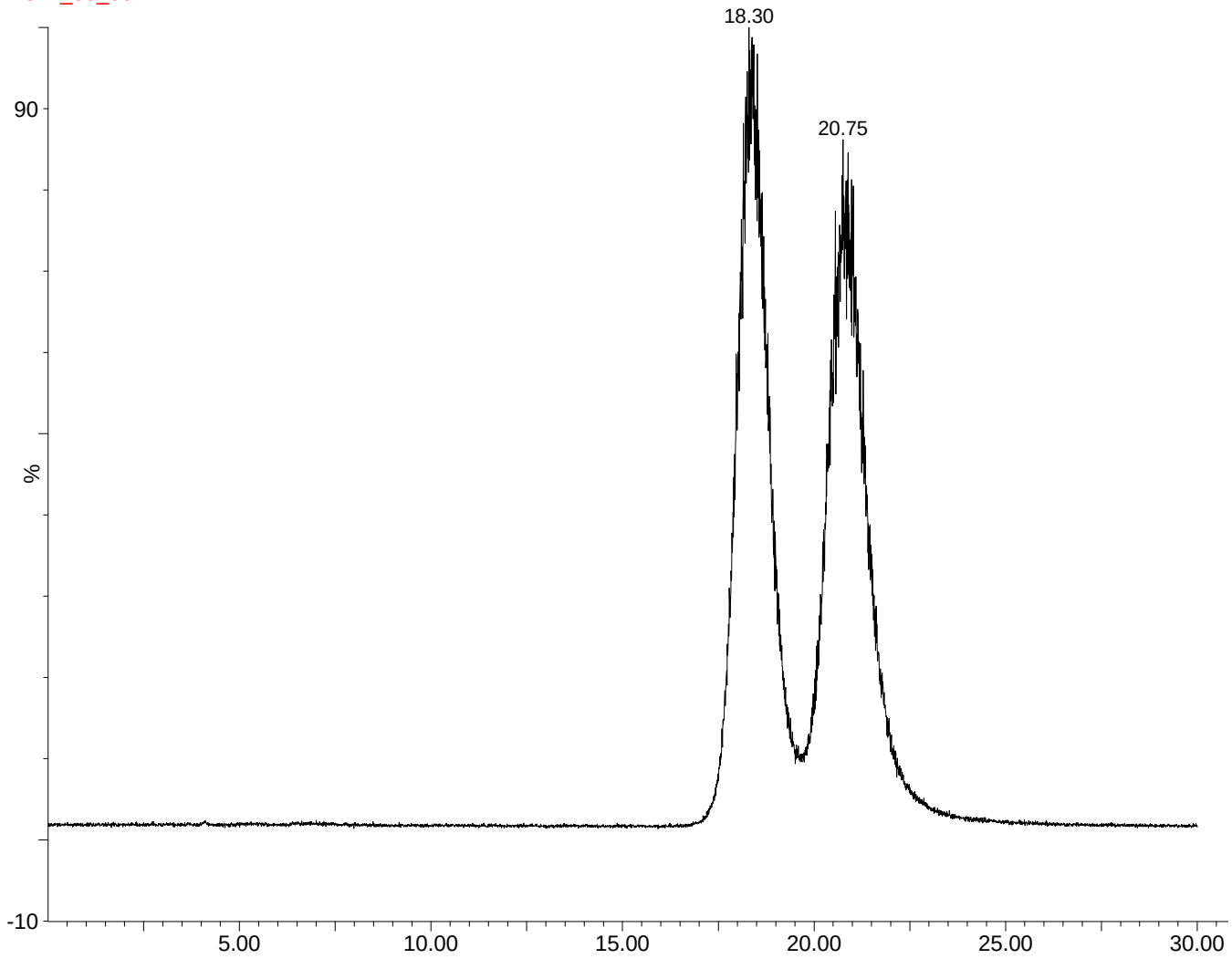


The following experiment illustrates the use of LC-MS to for the analysis of various β-blockers extracted from rat plasma. The HybridSPE approach for sample preparation was chosen for extraction of the chiral analytes alprenolol, metoprolol, clenbuterol, pindolol, and salbutomol (2).

Sample Prep HybridSPE: To 200 µL spiked rat plasma, add 600 µL of 1% formic acid acetonitrile, vortex to precipitate proteins then centrifuge at 15000 for 2 min. Collect 400 µL of supernate and pass through HybridSPE 96-well plate using 10 mm Hg vacuum for 4 minutes. Collect filtrate and analyze directly (2).

column:	CHIROBIOTIC T, 25 cm x 2.1 mm I.D., 5 µm particles
mobile phase:	15 mM ammonium formate in methanol
flow rate:	0.3 mL/min.
temp.:	25 ºC
det.:	6210 TOF, Agilent 1200RR HPLC, ESI (+), 50-2000 m/z profile scan
injection:	1 µL
sample:	1 µg/mL of each β-blocker standard

The extracted ion currents for the β-blockers from rat plasma is shown in Figure 5. The described conditions produced excellent selectivity and MS response of the respective β-blocker enantiomers (2).

Figure 5. Composite Extracted Ion Currents (2)

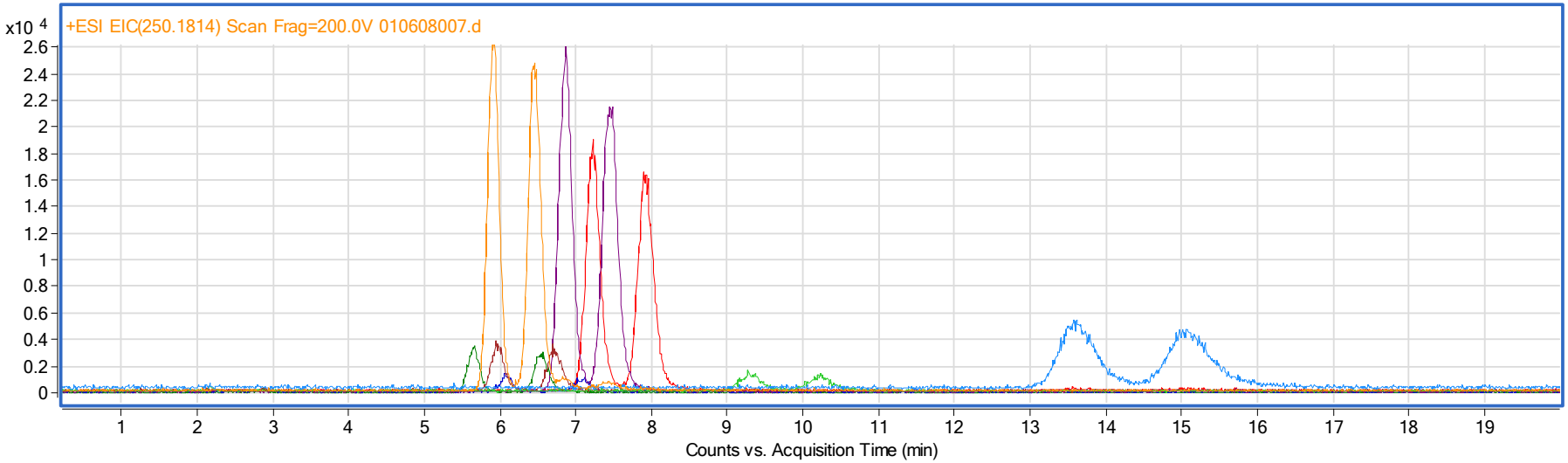
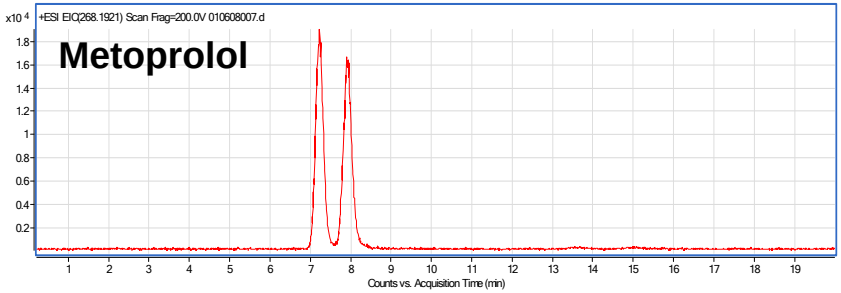
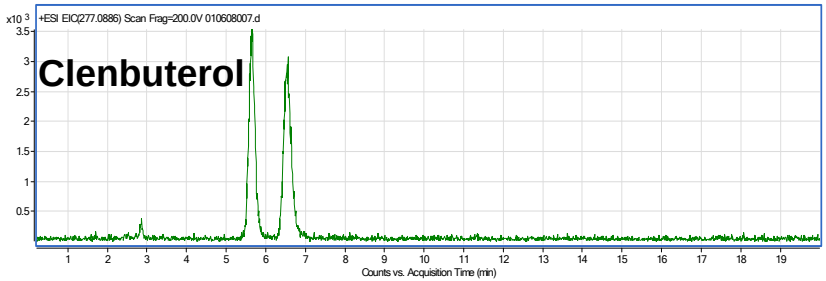
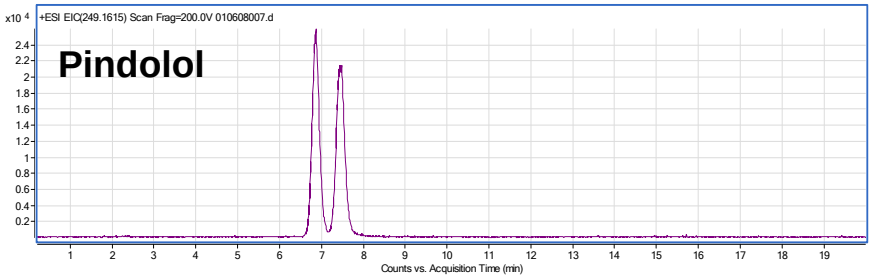


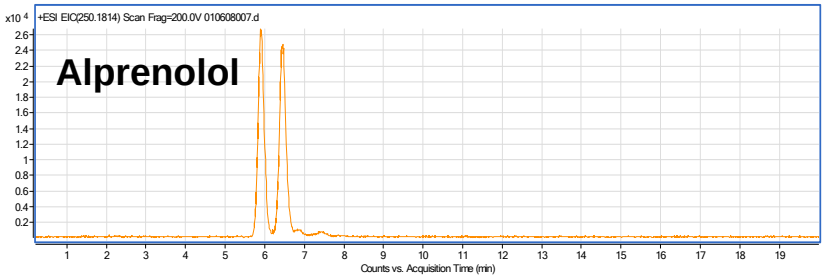
Figure 6 illustrates the extracted ion current for all five of the β-blockers in the mixture (2).

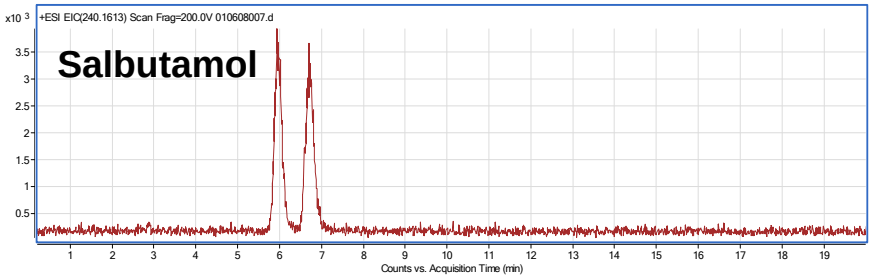
Figure 6. Extracted Ion Currents of β-blockers (2)











Conclusions

- Although chiral separations achieved in normal phase mode often seem favorable, conversion of this methodology to LC-MS tends to be arduous and often unsuccessful. While normal phase solvents are, at best, somewhat compatible with MS, commonly used organic modifiers, such as TEA and TFA, tend to cause strong ion suppression in MS. Removal of these additives is often detrimental to the chiral separation.
- Enantiomeric separations utilizing macrocyclic glycopeptide CSPs in reversed-phase mobile phases are compatible with mass spectrometry (MS) sources, and can be typically converted from LC-UV to LC-MS systems with few adjustments. MS response can often be enhanced by changing buffers and mobile phase additives, ionization mode, flow rate, sample concentration, injection size, or column dimensions. Unlike that observed in normal phase mode, these adjustments for optimized detection produce minimal impact on selectivity in normal-phase mode.
- The demonstrated separation of the β-blocker enantiomers on the CHIROBIOTIC T from extracted rat plasma employs methodology that may be utilized in clinical, PK and/or ADME/Tox type chiral LC-MS analyses.

References

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