

# **Chiral LC-MS Analysis of $\beta$ -Blockers from Plasma using Macrocyclic Glycopeptide Chiral Stationary Phases**

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## Abstract

The analysis of enantiomers in a clinical setting requires rapid and sensitive methodology. The tool of choice is often liquid chromatography coupled with mass spectrometry (LC-MS). The separation of chiral drug compounds within biological samples is often problematic using traditional amylose and cellulose-based chiral stationary phases (CSPs), as the mobile phases utilized to provide separation are often not amenable to LC-MS. Macrocyclic glycoside and cyclodextrin-based CSPs often provide enantiomeric selectivity using polar organic solvents or aqueous-organic mixtures as mobile phases. These mobile phases are readily amenable to LC-MS sources and, when coupled to the mass spectrometer, can provide the speed and sensitivity required for clinical analyses.

## Abstract (contd.)

In this study, enantiomeric separation for several  $\beta$ -blockers was investigated using MS-compatible mobile phases on several macrocyclic glycoside CSPs. Applicability of the methodology toward clinical analyses is demonstrated using the analysis of selected  $\beta$ -blockers from rat plasma.

## Introduction

- Chiral analysis in drug development has become increasingly important over the past decade.
- The utilization of liquid chromatography coupled to mass spectrometry has also become prevalent.
- Traditional chiral separations have mostly been accomplished using cellulose/amylose stationary phases and normal-phase chromatography.
- Although possible with some LC-MS sources, normal-phase solvents are often detrimental to ionization in these tandem systems.
- Macrocyclic glycopeptide-based chiral stationary phases (CSPs) operate best in polar organic solvents and aqueous-organic solvents – systems highly compatible with LC-MS.

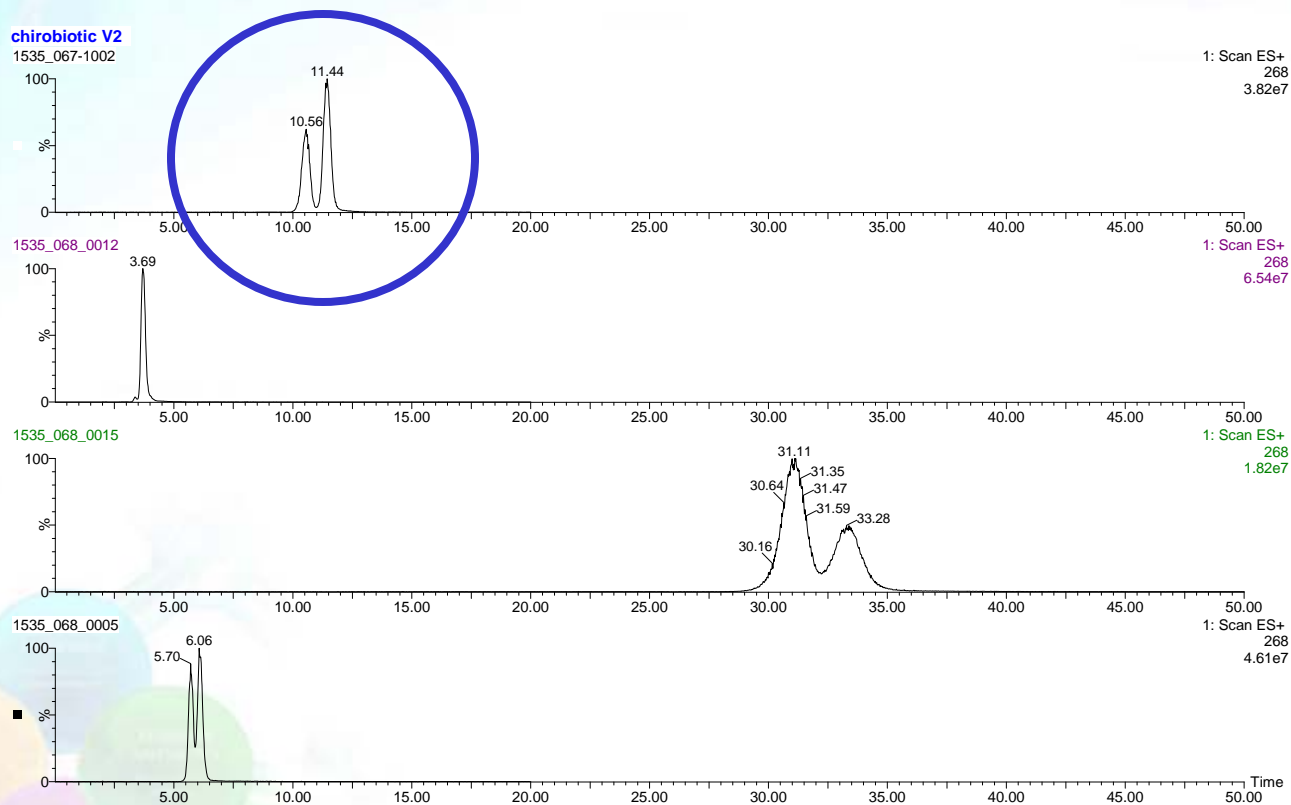
## Introduction (contd.)

- The use of these CSPs may provide improved chiral LC-MS analysis in realms such as clinical, pharmacokinetics and ADME/tox where complex samples are typically analyzed.
- In this study, several macrocyclic glycopeptide CSPs were screened for chiral selectivity toward a selected set of  $\beta$ -blockers.
- The subsequent chromatographic conditions were then adapted to LC-MS.
- Methods were then optimized for bioanalytical testing and sample prep techniques were evaluated.
- Rat plasma, spiked with  $\beta$ -blockers, were prepared using standard protein precipitation along with a novel HybridSPE™ approach and analyzed using the optimized method.

## Introduction (contd.)

- Initial screening of the macrocyclic glycopeptide CSPs revealed the Chirobiotic™ T (teicoplanin) to be the most suitable for the widest variety of  $\beta$ -blockers – See Figure 1.
- Chirobiotic T was therefore utilized for further method development.

# Figure 1a. Comparison of Several Macrocyclic Glycopeptide Columns for Metoprolol Enantiomeric Resolution



Chirobiotic T

Chirobiotic R

Chirobiotic TAG

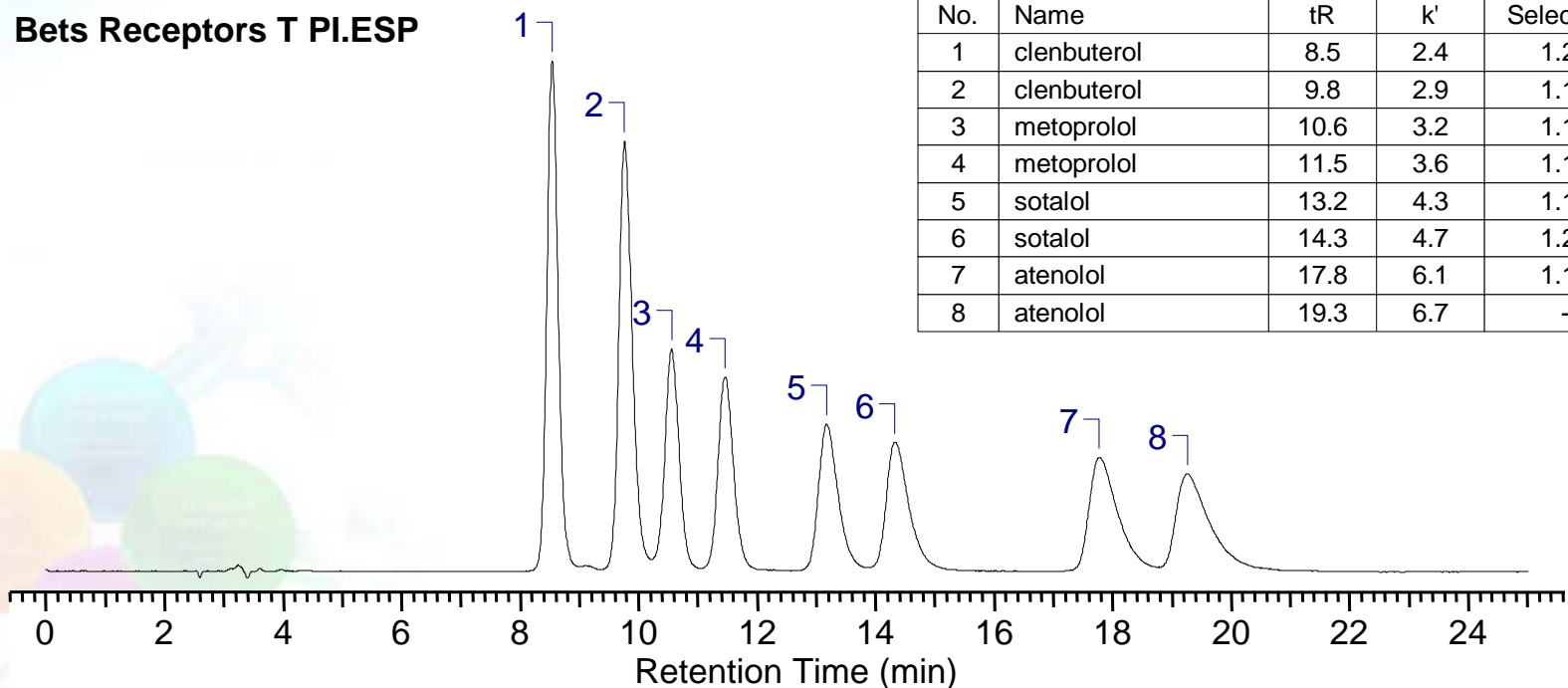
Chirobiotic V2

# Figure 1b. $\beta$ -Blocker Separation on Chirobiotic T

column: Chirobiotic T, 25 cm X 4.6 mm I.D., 5  $\mu$ m particles  
mobile phase: 15 mM ammonium formate; methanol  
flow rate: 1 mL/min.  
temp.: 25  $^{\circ}$ C  
det.: UV (220 nm)  
injection: 3  $\mu$ L

Bets Receptors T PI.ESP

No.	Name	tR	k'	Selectivity
1	clenbuterol	8.5	2.4	1.20
2	clenbuterol	9.8	2.9	1.11
3	metoprolol	10.6	3.2	1.11
4	metoprolol	11.5	3.6	1.19
5	sotalol	13.2	4.3	1.11
6	sotalol	14.3	4.7	1.29
7	atenolol	17.8	6.1	1.10
8	atenolol	19.3	6.7	-



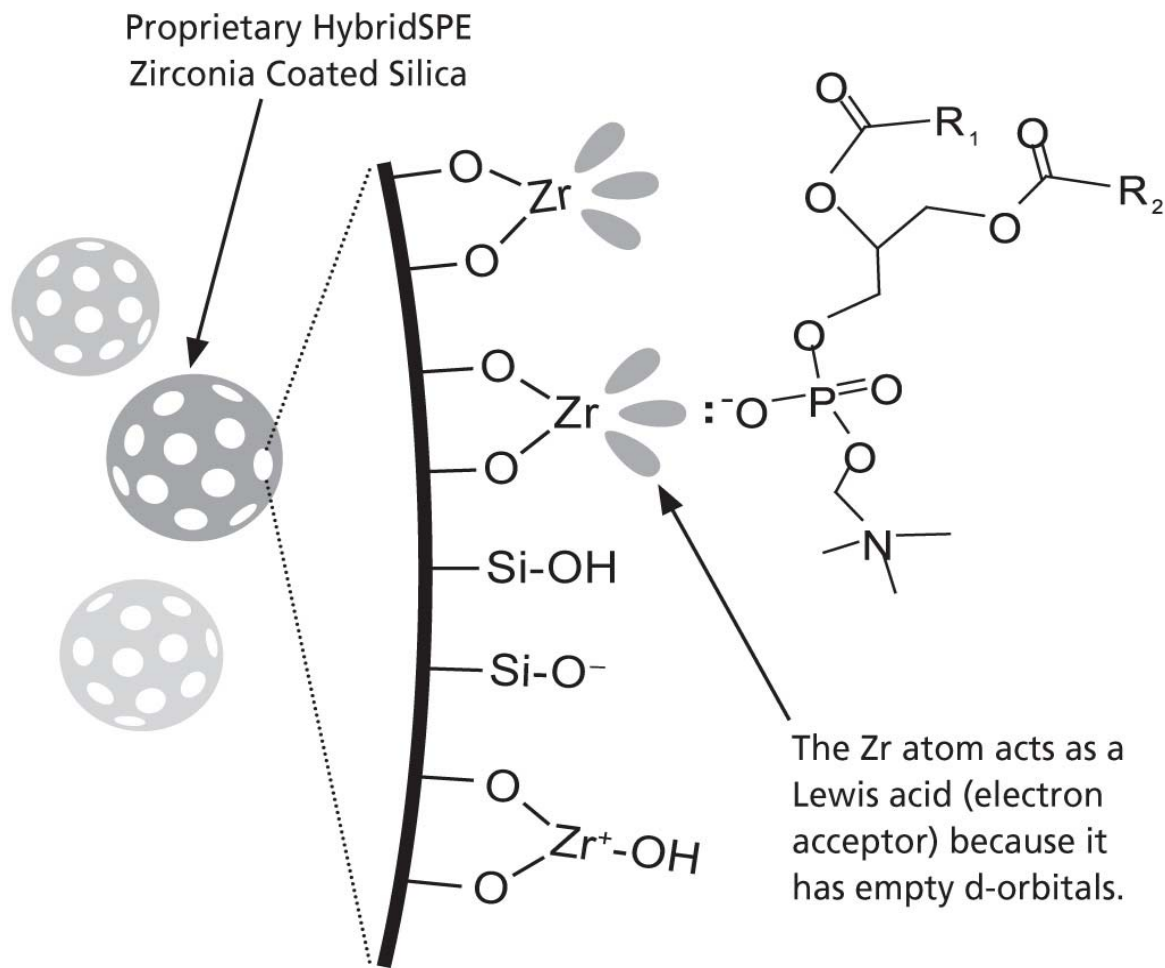
## Adaptation for Bioanalysis

- Screening protocols demonstrated the ability to perform chiral separation of multiple  $\beta$ -blockers in a single chromatographic run.
- The method was then transferred to a lower I.D. column dimension to enable better sensitivity for bioanalysis.
- The biological matrix evaluated in the study was rat plasma, requiring sample cleanup steps.
- Sample preparation techniques were evaluated for sample cleanliness and speed of processing.
- The plasma samples contain both proteins and matrix components such as phospholipids that can interfere with the analysis of compounds in biological matrices.
- The ability to remove or reduce matrix induced interference increases the robustness of the bioanalytical method.

## Adaptation for Bioanalysis (contd.)

- Sample preparation using standard protein precipitation was utilized along with novel HybridSPE technique to process spiked plasma samples.
- The HybridSPE approach for sample preparation, as depicted in Figure 2, was chosen for this study.
- The selective extraction of phospholipids is achieved using a novel zirconia-coated particle technology.
- The high selectivity towards phospholipids is achieved utilizing Lewis acid/base interactions between the phosphate group of the phospholipids and the zirconia surface.
- The zirconia-coated particle is not as Lewis “acidic” as pure zirconium oxide, thus enabling highly efficient extraction of phospholipids while remaining non-selective towards a broad range of basic, neutral and acidic compounds.

## Figure 2. HybridSPE Sample Preparation Approach



# Experimental Sample Prep Techniques

**Sample Prep protein precipitation:** To 100  $\mu\text{L}$  spiked rat plasma, add 300  $\mu\text{L}$  of 1% formic acid acetonitrile, vortex to precipitate proteins, then centrifuge at 15000 rpm for 2 min. Collect supernate and analyze directly.

**Sample Prep HybridSPE:** To 200  $\mu\text{L}$  spiked rat plasma, add 600  $\mu\text{L}$  of 1% formic acid acetonitrile, vortex to precipitate proteins, then centrifuge at 15000 rpm for 2 min. Collect 400  $\mu\text{L}$  of supernate and pass through HybridSPE 96 well plate using 10 mm Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

# Bioanalytical Method

column: Chirobiotic T, 25 cm x 2.1 mm I.D., 5  $\mu$ m particles

mobile phase: 15 mM ammonium formate; methanol

flow rate: 300  $\mu$ L/min.

temp.: 25 °C

injection: 1  $\mu$ L

sample/standard concentration: 1  $\mu$ g/mL each of  $\beta$ -blocker standards

system: Agilent 1200RR HPLC with 6210 TOF

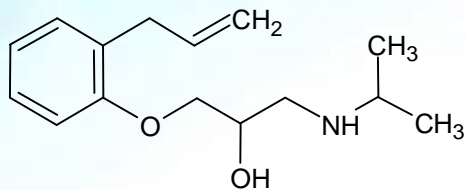
det.: ESI+

mass range: 50-2000 m/z profile scan

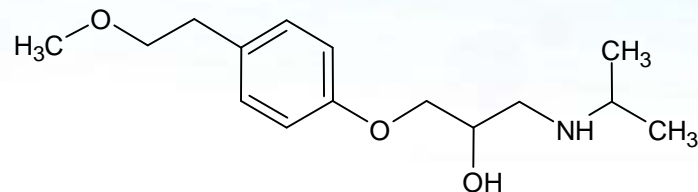
$\beta$ -Blockers monitored using accurate mass for each compound.

Phospholipid monitoring conducted using mass range 450-850 m/z.

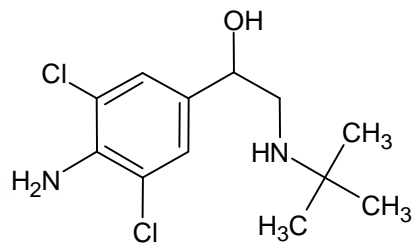
# Figure 3. Selected Set of $\beta$ -Blockers



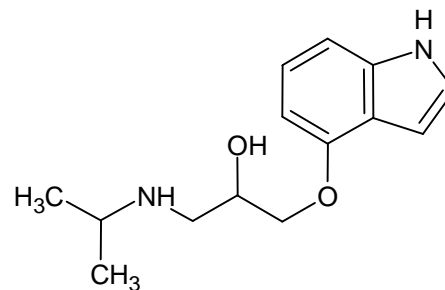
**Alprenolol**



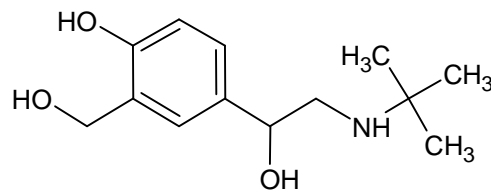
**Metoprolol**



**Clenbuterol**

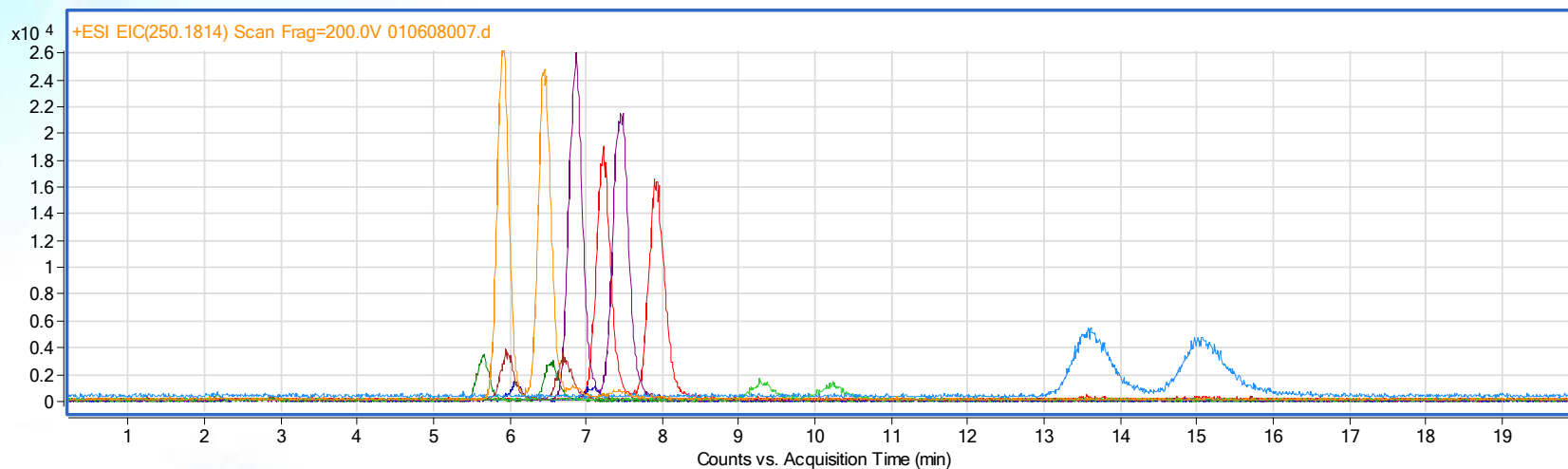


**Pindolol**

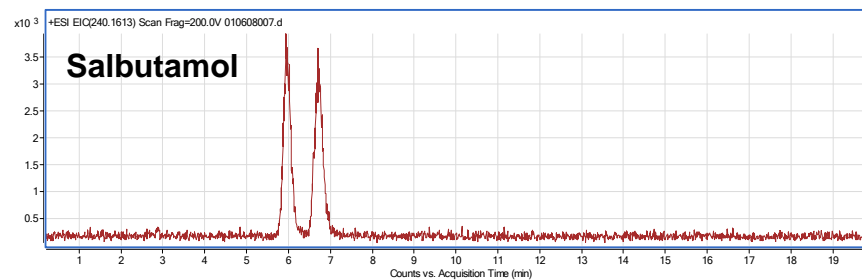
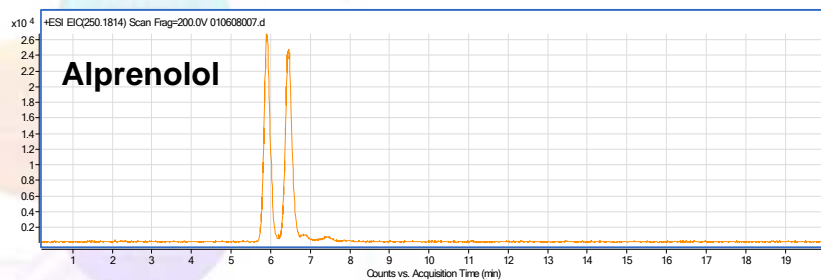
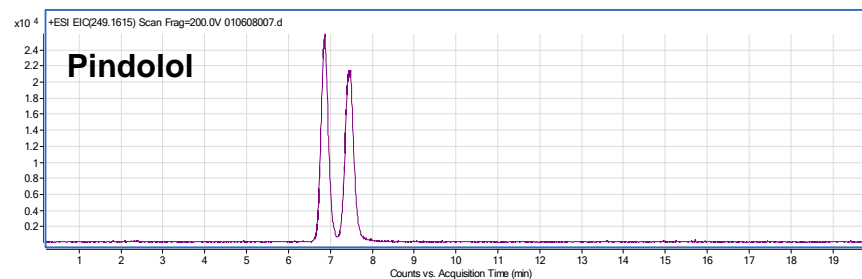
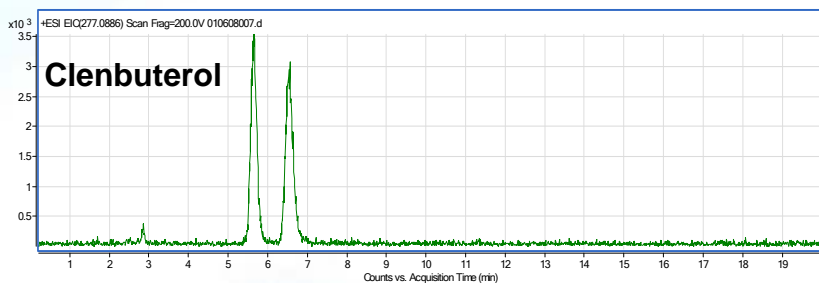
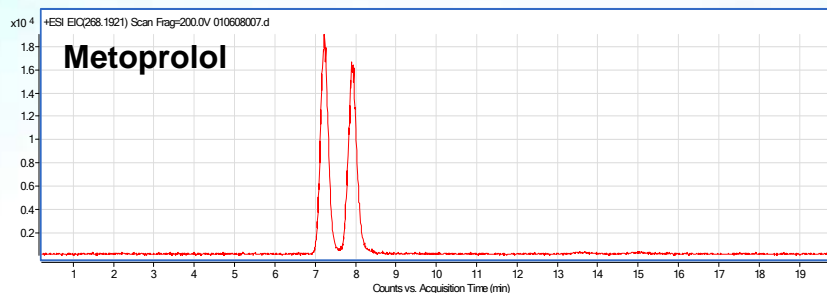


**Salbutamol**

# Figure 4. Composite Extracted Ion Currents

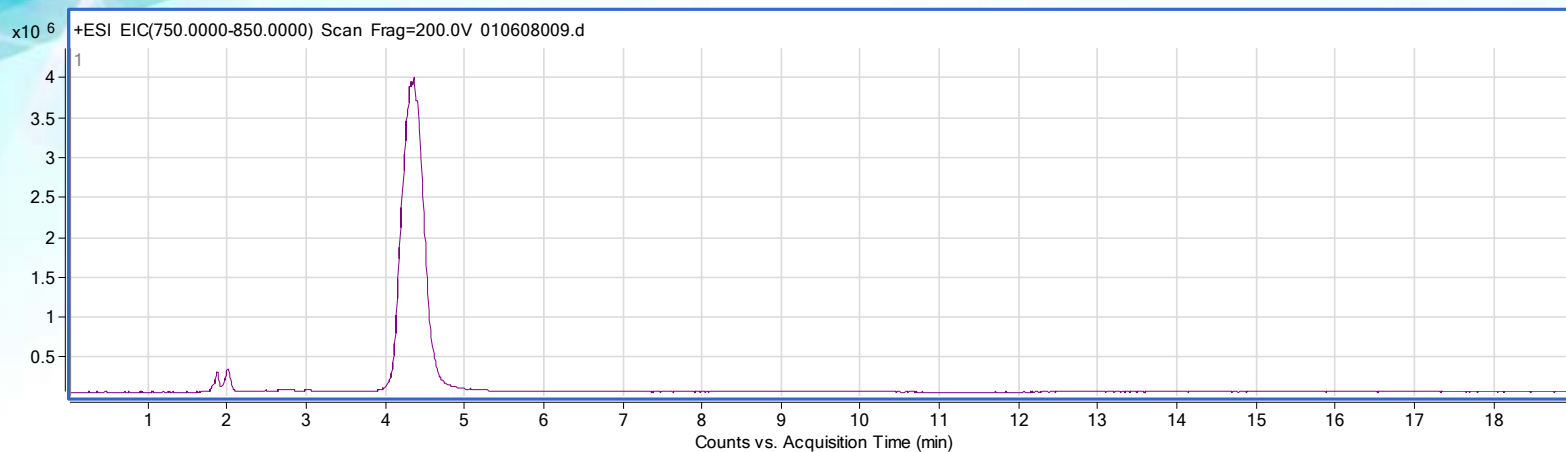


# Figure 5. Extracted Ion Currents of other $\beta$ -blockers

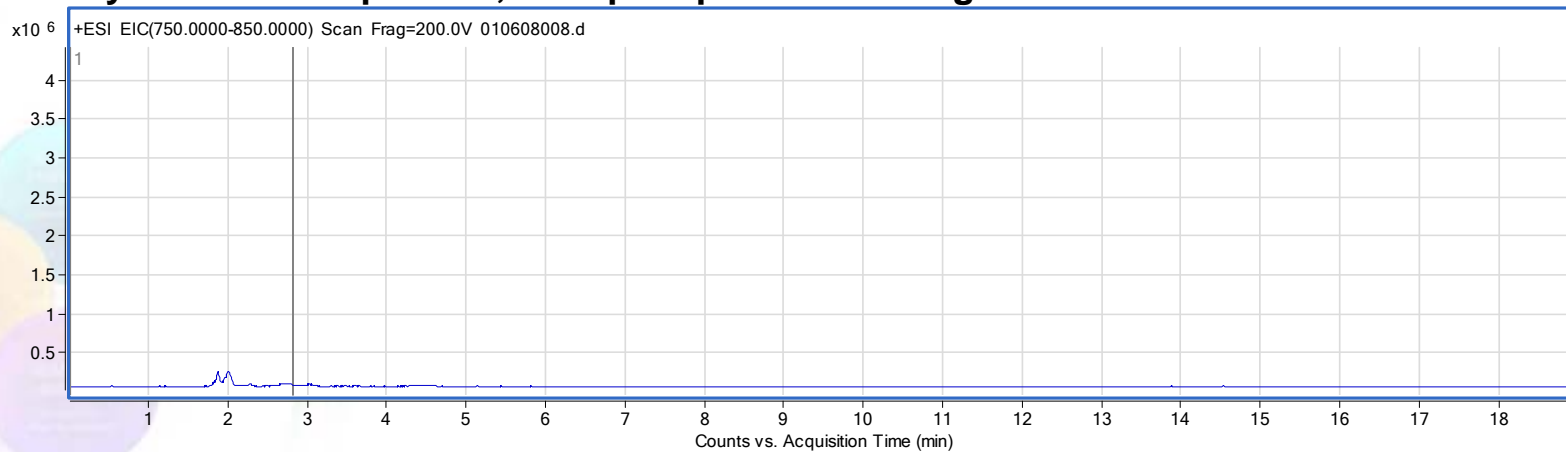


# Figure 6. Phospholipid Depletion

## Protein Precipitation Sample Prep, Phospholipids Monitoring



## HybridSPE Sample Pre , Phospholipids Monitoring



## Results and Discussion

- Figure 4 shows a composite of the extracted ion currents for the  $\beta$ -blockers analyzed in the rat plasma sample.
- Excellent selectivity and MS response of the respective analyte enantiomers is readily observed.
- Although coelutions exist between the different compounds, the added dimension of mass resolution allows for quantitation.
- Figure 5 presents the extracted ion current for the five individual  $\beta$ -Blockers in the spiked plasma samples.
- Though no chromatographic overlap of matrix phospholipids was present under these conditions, significant differences in extracted matrix are observed between the two sample prep techniques.
- Figure 6 depicts the effectiveness of the HybridSPE approach for the depletion of phospholipids.

## Conclusions

- Due to the enantiomeric selectivity exhibited by macrocyclic glycopeptide CSPs in polar solvents, they are highly amenable to LC-MS analyses.
- In this study it is demonstrated that these CSPs provide enantiomeric selectivity for a variety of  $\beta$ -blockers.
- Coupled with HybridSPE technology, the macrocyclic glycopeptide CSP Chirobiotic T was used to demonstrate methodology useful for clinical, PK and/or ADME/Tox type chiral LC-MS analyses.