Chiral LC-MS Analysis of β-Blockers from Plasma using Macrocyclic Glycopeptide Chiral Stationary Phases

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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.
In this study, enantiomeric separation for several β-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 β-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 β-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 β-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 β-blockers – See Figure 1.
• Chirobiotic T was therefore utilized for further method development.
Figure 1a. Comparison of Several Macro cyclic Glycopeptide Columns for Metoprolol Enantiomer ic Resolution

Chirobiotic T
Chirobiotic R
Chirobiotic TAG
Chirobiotic V2
Figure 1b. β-Blocker Separation on Chirobiotic T

- Column: Chirobiotic T, 25 cm X 4.6 mm I.D., 5 µm particles
- Mobile phase: 15 mM ammonium formate; methanol
- Flow rate: 1 mL/min.
- Temp.: 25 °C
- Det.: UV (220 nm)
- Injection: 3 µL

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Adaptation for Bioanalysis

• Screening protocols demonstrated the ability to perform chiral separation of multiple β-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

Proprietary HybridSPE Zirconia Coated Silica

The Zr atom acts as a Lewis acid (electron acceptor) because it has empty d-orbitals.
Experimental Sample Prep Techniques

Sample Prep protein precipitation:  To 100 µL spiked rat plasma, add 300 µL 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 µL spiked rat plasma, add 600 µL of 1% formic acid acetonitrile, vortex to precipitate proteins, then centrifuge at 15000 rpm 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.
Bioanalytical Method

column: Chirobiotic T, 25 cm x 2.1 mm I.D., 5 µm particles
mobile phase: 15 mM ammonium formate; methanol
flow rate: 300 µL/min.
temp.: 25 °C
injection: 1 µL
sample/standard concentration: 1 µg/mL each of β-blocker standards
system: Agilent 1200RR HPLC with 6210 TOF
det.: ESI+
mass range: 50-2000 m/z profile scan
β-Blockers monitored using accurate mass for each compound.
Phospholipid monitoring conducted using mass range 450-850 m/z.
Figure 3. Selected Set of β-Blockers

- **Alprenolol**
- **Metoprolol**
- **Clenbuterol**
- **Pindolol**
- **Salbutamol**
Figure 4. Composite Extracted Ion Currents
Figure 5. Extracted Ion Currents of other β-blockers
Figure 6. Phospholipid Depletion

Protein Precipitation Sample Prep, Phospholipids Monitoring

HybridSPE Sample Prep, 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 β-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.