Utilizing Enantiomeric Separations in Bioanalysis

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Abstract

Enantiomeric separations are often restricted in bioanalysis due to incompatibility of biological samples with traditional mobile phases used with cellulose and amylose polysaccharide chiral stationary phases. Alternatively, macrocyclic glycopeptide chiral stationary phases perform using typical reversed phase mobile phases, making them highly amenable for bioanalysis. In this study, macrocyclic glycopeptide chiral stationary phases are utilized for enantiomeric bioanalysis. The properties of these chiral stationary phases allow for unique sample preparation techniques to be employed. The study evaluates the utility of these chiral stationary phases in combination with varied sample preparation techniques for speed and effectiveness in bioanalytical methods. Target drug and associated metabolites are utilized to evaluate the capability of the enantiomeric bioanalytical technique.

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.

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 studies is demonstrated through the analysis of selected β-blockers from rat plasma.

Introduction (contd.)

Macrocyclic glycopeptide-based chiral stationary phases (CSPs) operate best in polar organic solvents and aqueous-organic solvents – systems highly compatible with LC-MS.

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 peptide CSPs were screened for chiral selectivity toward a selected set of β-blockers. The subsequent chromatographic conditions were then adapted to LC-MS. Rat plasma, spiked with β-blockers, was prepared using a HybridSPE® approach and analyzed using the 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.

The LC-MS compatible mobile phase system enabled direct analysis using standard electrospray ionization (ESI+) without the need for more complex ionization sources (APCI, APPI) often required for normal phase chiral separations.

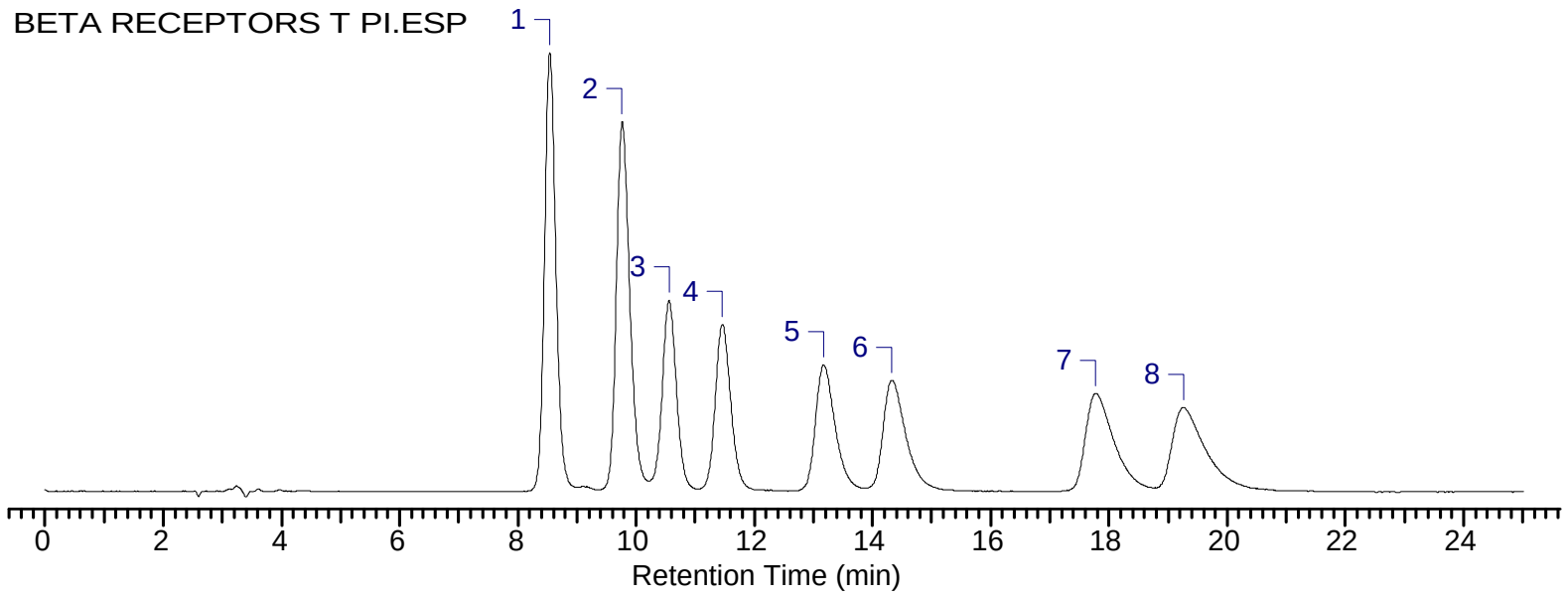
CHIROBIOTIC T was therefore utilized for further method development.

The final mobile phase combination enable direct injection of processed plasma samples without the need for evaporation and reconstitution, this greatly simplified the overall analysis.

Figure 1. β-Blocker Separation on CHIROBIOTIC T

column: CHIROBIOTIC T, 25 cm x 0.46 cm, 5 µm
mobile phase: methanol:15 mM ammonium formate
flow rate: 1 mL/min.
temp.: 25 °C
det.: UV (220 nm)
inj.: 3 µL

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	-



Both proteins and matrix components such as phospholipids can interfere with the analysis of compounds in biological matrices.

The HybridSPE approach for sample preparation, as depicted in Figure 2, was chosen for this study.

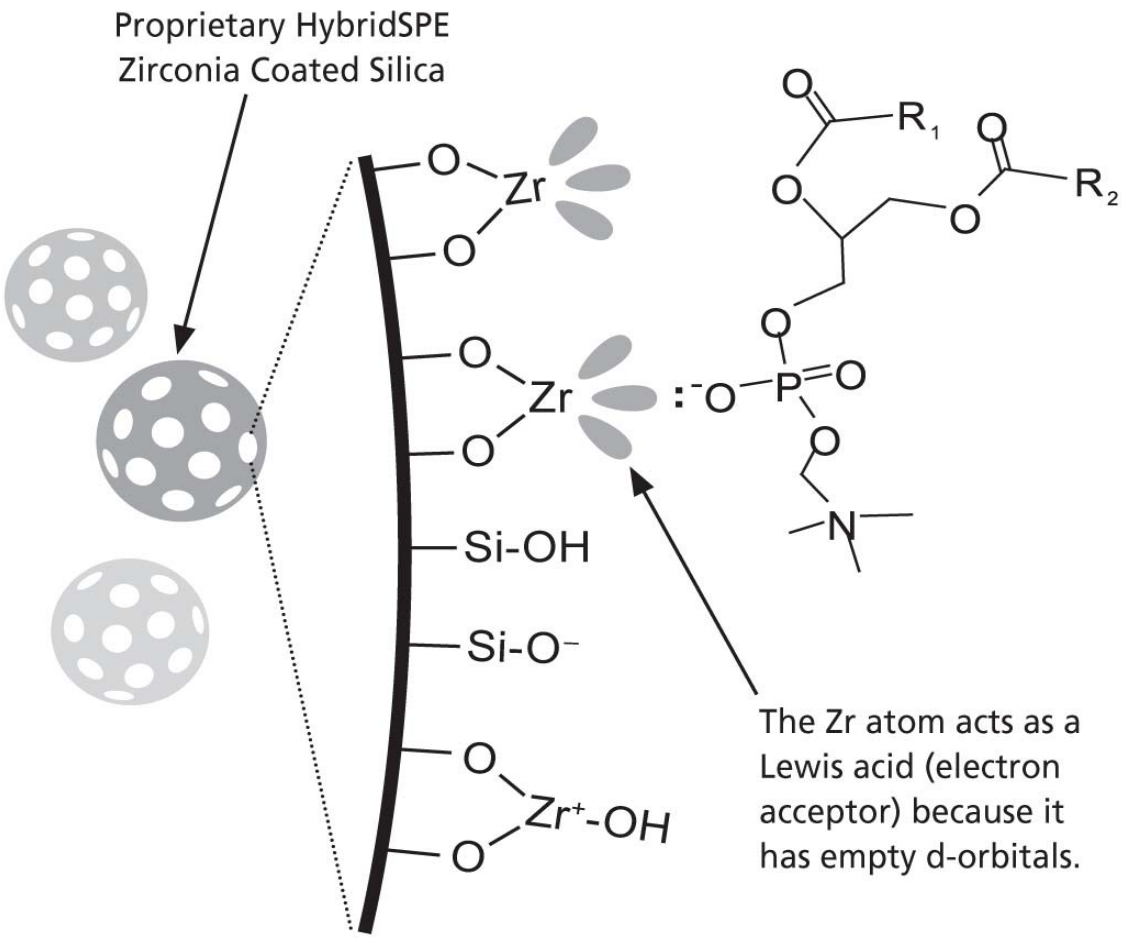
The selective extraction of phospholipids is achieved using a novel zirconia-coated particle technology.

The high selectivity towards phospholipids is achieved utilizing Lewis acid/base interaction 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. Phospholipid Interaction with Zirconia-Coated Particle of HybridSPE

- The high selectivity towards phospholipids achieved utilizing Lewis acid/base interaction between the phosphate group of the phospholipids and the zirconia surface.
- Modifier (1% formic acid) mitigate Lewis-acid base interaction between chelation/ acidic compounds.
- High organic content (75%+) acts as strong solvent when related to hydrophobic retention (acetonitrile, methanol).
- Aqueous content (25%) is strong solvent when related to HILIC retention.



Experimental

The set of β-blockers chosen for the study is shown in Figure 3.

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 for 2 min. Collect supernatant 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 for 2 min. Collect 400 µL of supernatant and pass through HybridSPE 96-well plate using 10 mm Hg vacuum for 4 minutes. Collect filtrate and analyze directly.

Instrument and Chromatographic Conditions

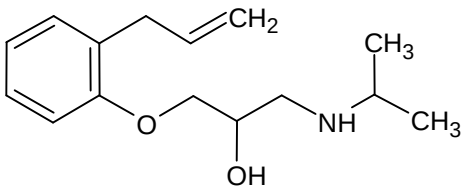
System:	Agilent 1200RR HPLC with 6210 TOF
det.:	ESI+
mass range:	50-2000 m/z profile scan

Beta Blockers monitored using accurate mass for each compound.

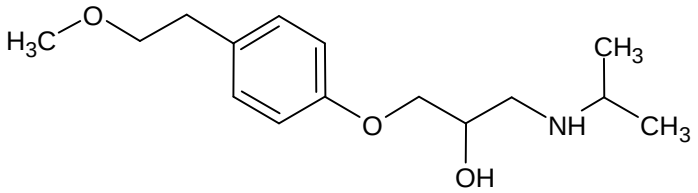
Phospholipid monitoring conducted using mass range 450-850 m/z

column:	Chirobiotic T, 25 cm x 2.1 mm, 5 µm
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 beta blocker standards

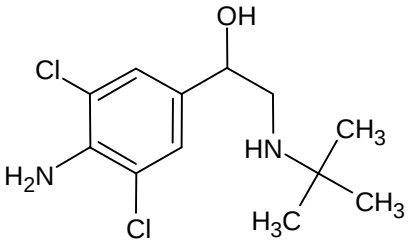
Figure 3. Selected Set of β-Blockers



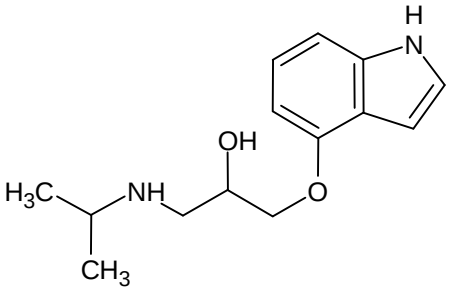
Alprenolol



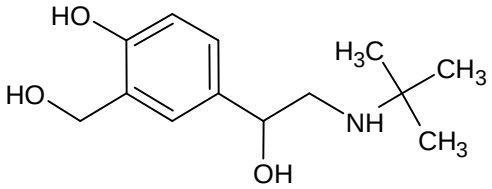
Metoperolol



Clenbuterol



Pindolol



Salbutomol

Results and Discussion

- Figure 4 shows a composite of the extracted ion currents for the β-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 metoprolol in the mix. No interference or ion-suppression is observed.
- Each of the compounds spiked could be observed in this manner, as shown in Figure 6.

Figure 4. Composite Extracted Ion Currents

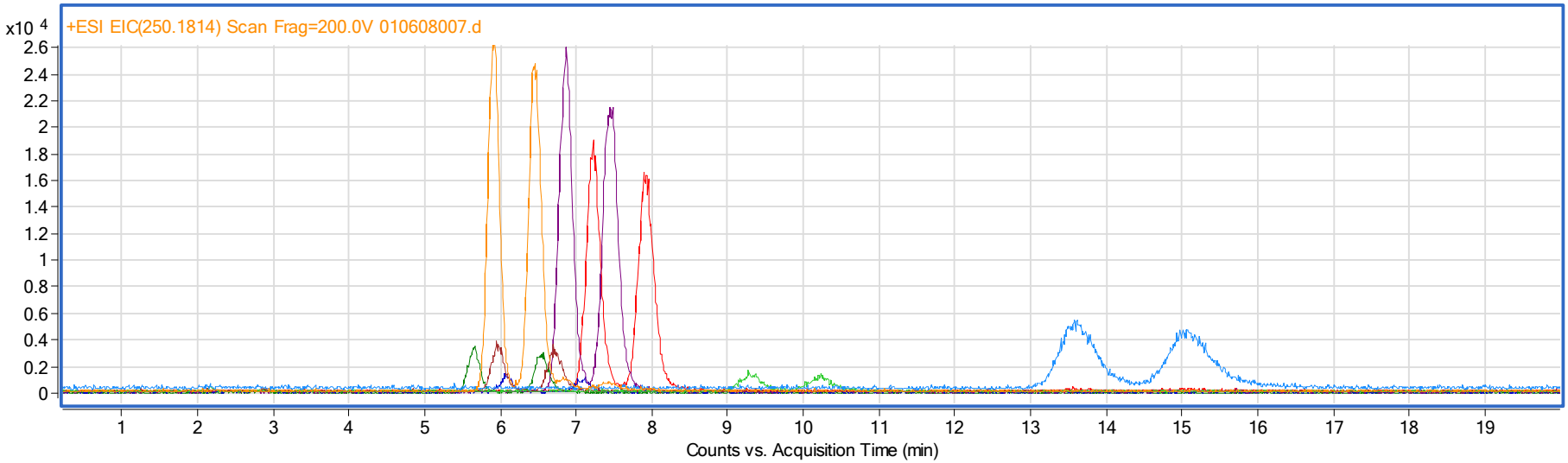


Figure 5. Extracted Ion Current: Metoprolol

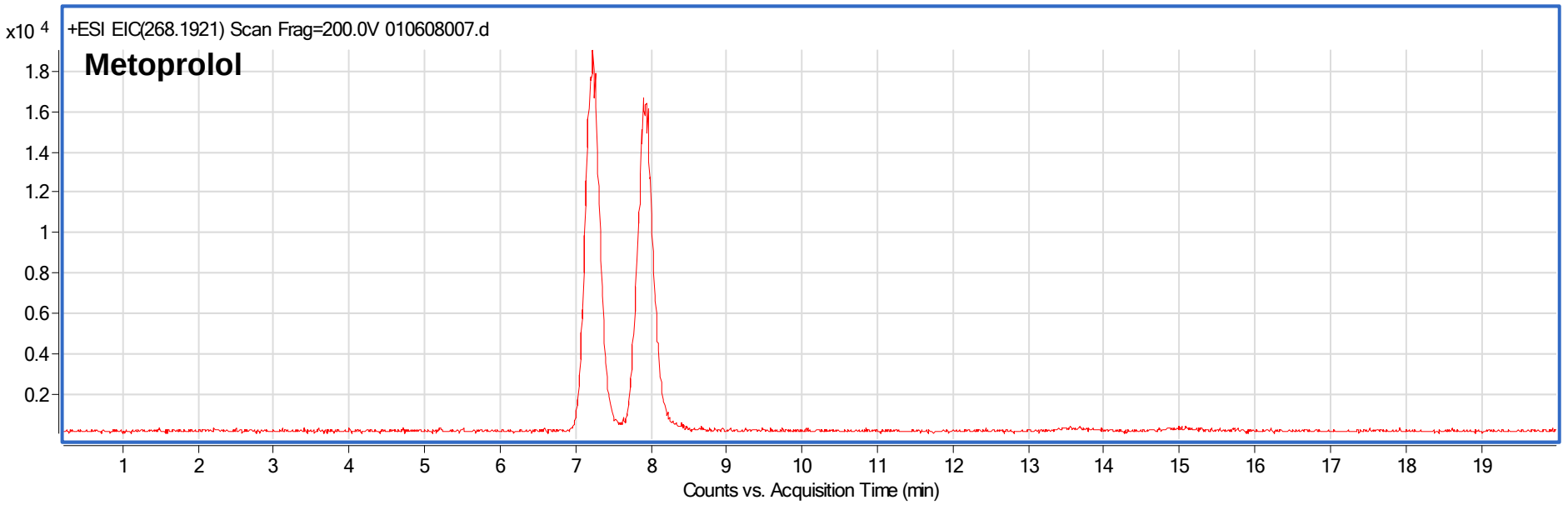
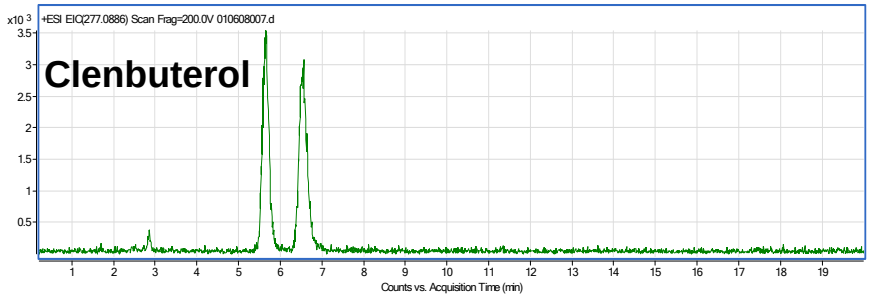
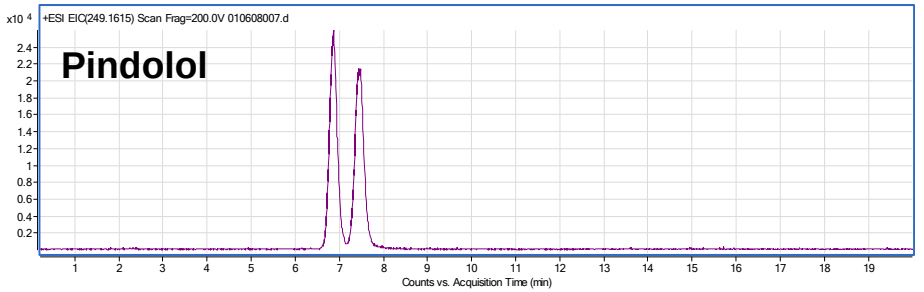
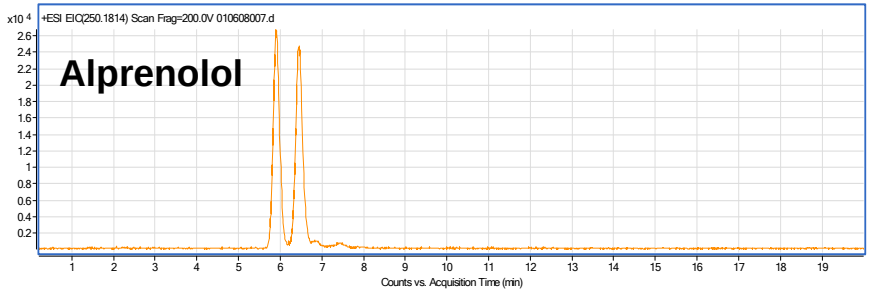


Figure 6. Extracted Ion Currents of other β-blockers







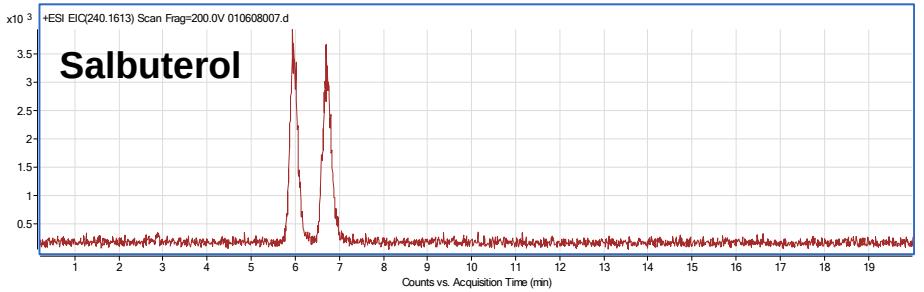
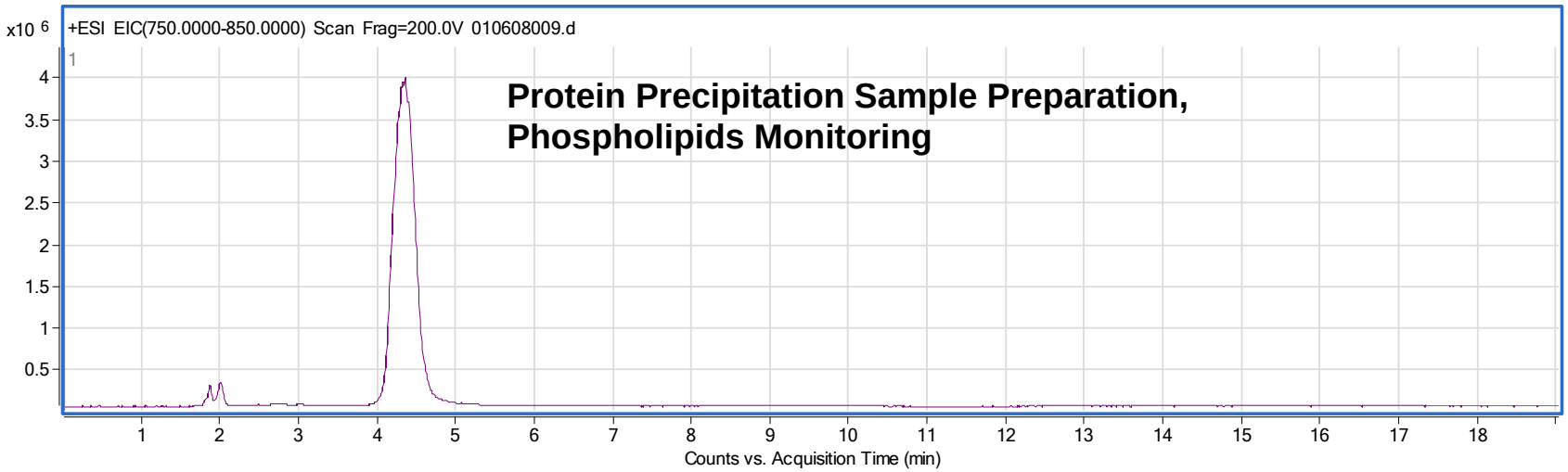
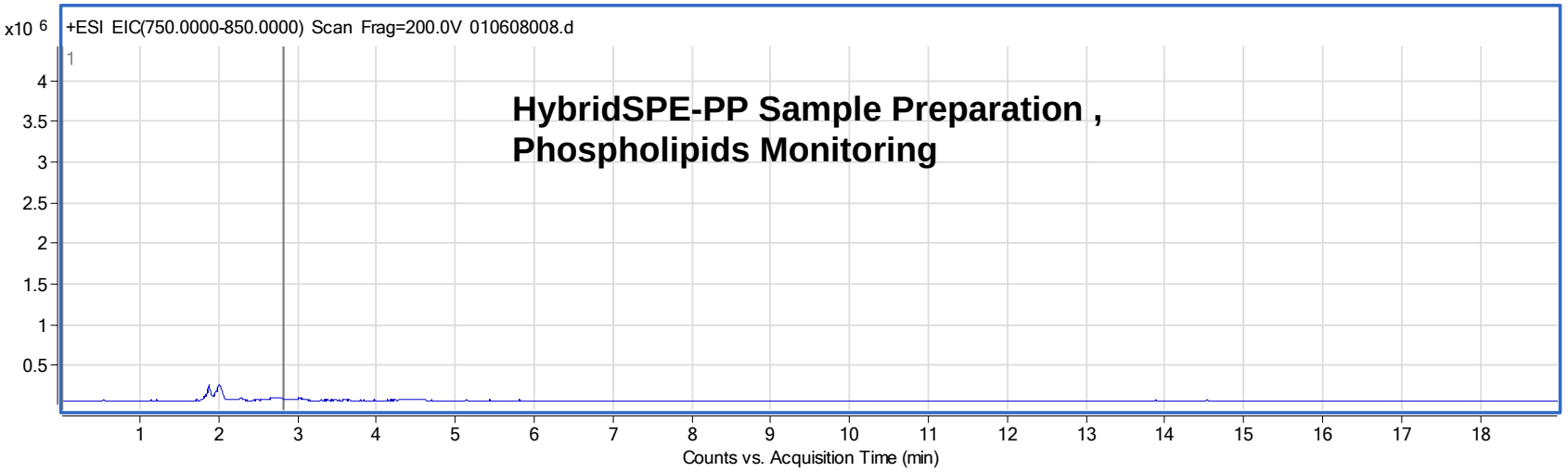


Figure 7 depicts the impact that sample preparation can have on co-extracted matrix. Here, samples processed using standard protein precipitation exhibited a large amount of phospholipids that can ultimately interfere with the quantitation of the early eluting analytes. In contrast, samples processed using the HybridSPE-PPT technique were depleted of phospholipids resulting in no analyte matrix effect. The simplicity and effectiveness of the HybridSPE-PPT technique makes this a valuable tool for sample preparation of biofluids. Cleaner sample extracts result in a more rugged method while increasing confidence in reproducible quantitative results.

Figure 7. Phospholipid Depletion





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.