

# Effects of Matrix Age on Protein Binding Results Extracted with a Prototype Phospholipid Removal Plate and Analyzed using MFLC-MS/MS

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## Overview

### Purpose

To demonstrate if the age of plasma or serum will impact the protein binding of Propranolol.

### Methods

Eight hour dialysis incubation at 37°C and extracted with a prototype phospholipid removal plate, followed by MFLC-MS/MS (ekspert™ microLC 200 System coupled with an ABSciex 5500® QTRAP).

### Results

Plasma that is one month old provides significantly lower protein binding values when compared to fresh plasma. The prototype phospholipid removal plate provided clean extracts with adequate extraction efficiency (79.0%). The data generated by the MFLC-MS/MS was reproducible and the analyte response was approximately 4X greater than conventional HPLC.

## Introduction

The pharmacological interaction between plasma proteins and pharmaceuticals is a relationship that will impact the systemic effects the drug will have in-vivo. In order to determine how much of the drug remains unbound to the plasma proteins equilibrium dialysis is commonly used. The accuracy of the equilibrium dialysis protein binding results is critical for some programs that rely on this data to move ahead with future studies or abandon the new drug or treatment entirely. Here, we report on the protein binding effects of plasma/serum age and the plasma anti-coagulant when propranolol is spiked in plasma/serum and the percent protein binding is determined. A prototype phospholipid removal plate was used for the extraction and MFLC-MS/MS was used for the analysis.

## Methods

### Protein Binding

- Matrix: Human Plasma and Serum collected fresh and one month old
- Compound: Propranolol 100 ng/mL
- HTDialysis HTD96b dialysis device
- 12-14 kDA MWCO membrane
- Matrix/PBS volume: 100 µL
- 8 hour incubation at 37°C

### Extraction

- Propranolol extracted from plasma and serum
- Sigma Aldrich Prototype Phospholipid Removal Plate
- Sample volume: 50 µL
- Solvent: 200 µL Acetonitrile

### MFLC

- ekspert™ microLC 200 System
- Gradient using acetonitrile and water with 1% formic acid
- Flow rate: 30 µL/minute
- Column: ProntoSIL 120-3-C18 (MAC-MOD)
- Column temperature: 50°C

### MS

- ABSciex 5500® QTRAP operating in MRM mode
- ESI
- Positive ion mode
- MRM transition (m/z):  
Propranolol: 260 → 116  
Phospholipid: 182 → 182

Propranolol

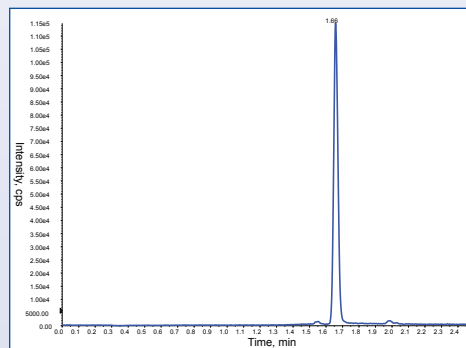
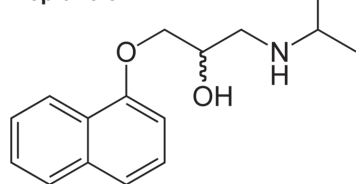


Figure 1. MFLC-MS/MS Analysis of Propranolol from Buffer Chamber

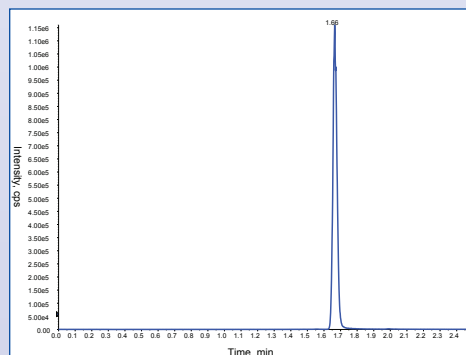


Figure 2. MFLC-MS/MS Analysis of Propranolol from Human K<sub>2</sub>EDTA Plasma Chamber

## Conclusions

- The age of the matrix used to perform protein binding determination will affect the reported protein binding results.
- The Sigma Aldrich Prototype Phospholipid Removal Plate provides adequate extraction efficiency and excellent phospholipid removal.
- The MFLC provided ~4X increase in Propranolol response when compared to conventional HPLC and can be utilized to perform routine bioanalysis.

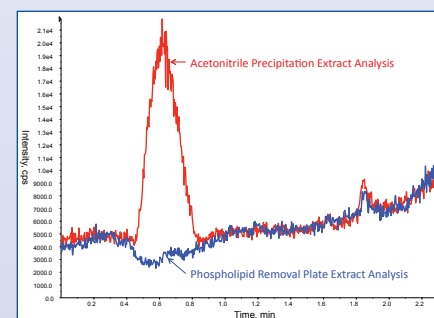


Figure 3. Acetonitrile Precipitation and Phospholipid Removal Plate Plasma Extract Analysis for Phospholipids

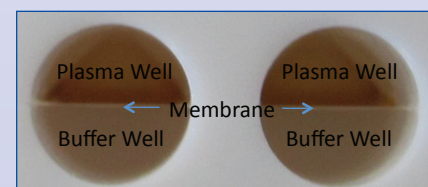


Figure 4. Two Wells of the HTD96b Dialysis Device

Table 1. %Protein Binding Results for Fresh Matrix and One Month Old Matrix

Human Matrix	% PB Fresh Collection	%PB ~ One Month Old	% Difference
K <sub>2</sub> EDTA Plasma	89.1	74.6	16.3
Sodium Heparin Plasma	92.0	71.0	22.8
Sodium Citrate Plasma	88.2	71.1	19.4
Serum	91.6	82.9	9.5