Alturas Analytics, Inc.
The LC-MS Experts

A comparison of Rapid Equilibrium Dialysis with Biocompatible Solid Phase Microextraction for Plasma Protein Binding Determination using LC-MS/MS

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INTRODUCTION

Determination of protein binding (%PB) levels and free circulating drug in biological systems is important in establishing PK activity. The portion of drug that binds to protein within the body is rendered inactive and unavailable to work against the intended target of the drug. Currently RED (rapid equilibrium dialysis) is considered the gold standard technique for plasma %PB level determination but this labor intensive technique usually takes several hours in order for equilibrium to be reached prior to extraction and LC-MS/MS analysis. A novel and simple sample preparation technique utilizing BioSPME (biocompatible solid phase microextraction) technology in order to determine the %PB has been developed. A %PB comparison of three compounds carbamazepine, rivaroxaban and apixaban was performed utilizing BioSPME and the RED device in human plasma.

OVERVIEW

Purpose

Compare the plasma protein binding values obtained from BioSPME and the RED device for three compounds at two concentrations

Methods

Human plasma spiked with carbamazepine, rivaroxaban and apixaban was loaded onto the BioSPME 96-pin array and RED devices and the compartments were extracted and analyzed using LC-MS/MS

Results

The plasma protein binding results were comparable between the two devices and the equilibrium time was reduced from >6 hours (RED device) to 1 hour using the BioSPME 96-pin array

METHODS

BioSPME

- Precondition pin array with 2-propanol for 20 min
- Wash pin array with 10 second submergence in water
- Submerge pin array into spiked plasma or spiked buffer (at equivalent concentration) for 15 min at 37°C and vortex mix
- Wash pin array with 60 second submergence in water
- Desorb compound from pin array by submerging in in 8/2 MeOH/Water for 20 min
- Add internal standard, dilute with water and analyze by HPLC-MS/MS
- Quantify compounds by extracting and analyzing an 8 point standard curve in duplicate from plasma/ buffer

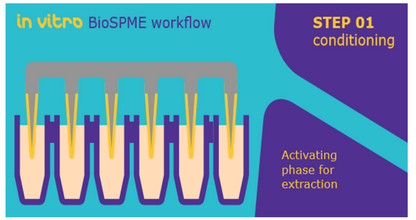
RED Device

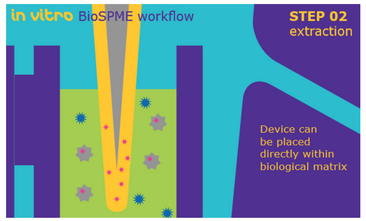
- Load device with spiked plasma and blank buffer
- Incubate for 4 hours at 37°C
- Remove an aliquot of the plasma and buffer chamber
- Extract and analyze by HPLC-MS/MS
- Quantify compounds by extracting and analyzing an 8 point standard curve in duplicate from plasma/ buffer

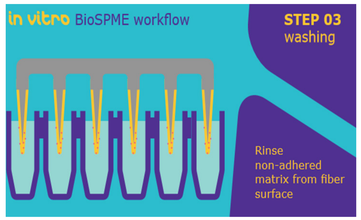
HPLC-MS/MS

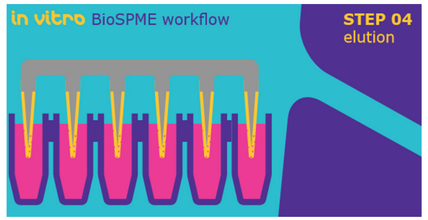
- SCIEX QTRAP® 5500
- Shimadzu HPLC System

BioSPME WORKFLOW









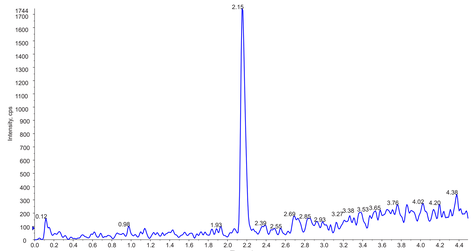


Figure 1: Carbamazepine 10.0 ng/mL in plasma extracted from SPME

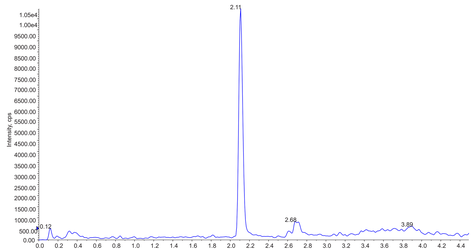


Figure 2: Apixaban 10.0 ng/mL in plasma analyzed from SPME

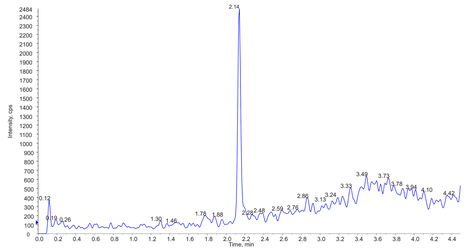
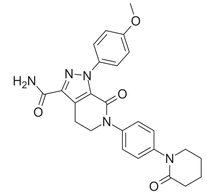
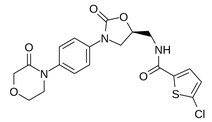


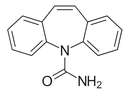
Figure 3: Rivaroxaban 10.0 ng/mL in plasma analyzed from SPME



Structure 1: Apixaban (LogP 2.7)



Structure 2: Rivaroxaban (LogP 1.5)



Structure 3: Carbamazepine (LogP 2.45)

	% PB BioSPME		% PB RED	
	10 ng/mL	100 ng/mL	10 ng/mL	100 ng/mL
Carbamazepine	60	54	61	61
Apixaban	84	81	89	86
Rivaroxaban	83	85	88	88

Table 1: Comparison Data Between BioSPME and RED Devices

% PB Calculation

$$\frac{\text{amount extracted from buffer} - \text{amount extracted from plasma}}{\text{amount extracted from buffer}} \times 100\%$$

CONCLUSION

- A novel method has been developed to determine the %PB of three compounds from human plasma using BioSPME followed by HPLC-MS/MS analysis
- The BioSPME method developed is simpler and faster than the RED device and provides comparable data
- BioSPME method can be fully automated