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Rapid Determination of Drug Protein Binding Affinity Using Solid Phase Microextraction

Determination of free circulating drug is important in establishing the pharmacokinetic activity. In most cases, drug-protein complexes are formed, thus affecting the active level of circulating drugs. Techniques used for determining drug protein binding levels consist of ultrafiltration, ultracentrifugation and microdialysis. Automation can be used in the case of microdialysis, but processing may be greater than 6 hours for equilibrium to be reached.

In this study, a novel BioSPME microextraction device is evaluated as a rapid means of determining drug protein binding affinities from plasma. Here, the SPME LC Tips with C18 fiber chemistry were compared to ThermoFisher Scientific Rapid Equilibrium Dialysis (RED) device for speed and simplicity in measuring binding affinity in rat plasma samples.

Experimental

BioSPME is not an exhaustive technique and extraction is governed by distribution constants dependent on affinity of the analytes for the coating as compared to the sample matrix. After a given amount of time, equilibrium is achieved between the concentration of analytes in the matrix and the fiber coating.

In the case of the BioSPME fibers, the polymeric binder used to adhere the C18 functionalized particle onto the fiber core acts as a shield that prevents large molecular weight (i.e., proteins) from absorbing onto the fiber, thus allowing only the free fraction (unbound analyte) to be extracted by the fiber coating.

In this study, a model set of protein binding drugs were selected to compare the BioSPME approach with the equilibrium dialysis technique. Drugs with reference binding affinities ranging from 20%–99% were selected for comparison of the sampling devices.

Figure 4. Chromatogram of Binding Analytes

column: Ascentis® Express C18, 5 cm × 2.1 mm I.D., 2.7 µm particles
mobile phase: (A) 5 mM ammonium formate
(B) 5 mM ammonium formate in 90:10 acetonitrile: water
flow rate: 500 µL/min
column temp.: 40 °C
det.: MS/MS, ESI (+), MRM transitions
injection: 2 µL
gradient: 5%B to 70%B in 3 minutes, then to 90%B in 0.1 minute, hold 90%B for 0.9 minutes
instrument: Agilent® 1290 Infinity II with Agilent 6460 QQQ

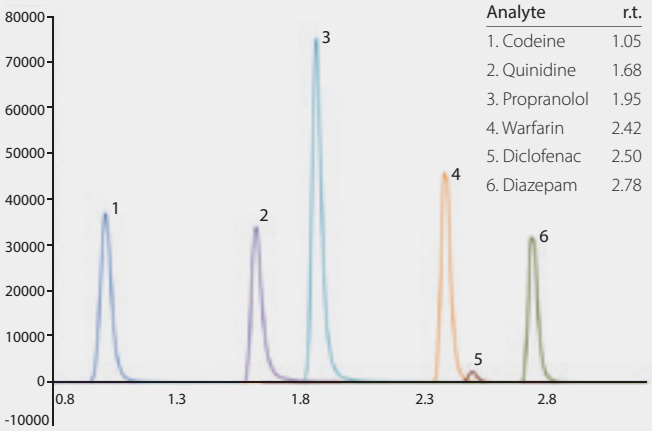


Figure 1. Single Use SPME

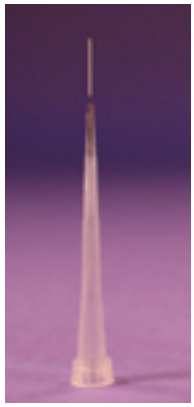


Figure 2. Analyte Uptake Onto BioSPME Fiber

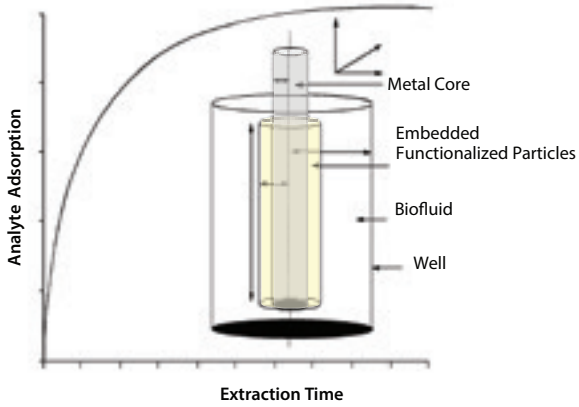
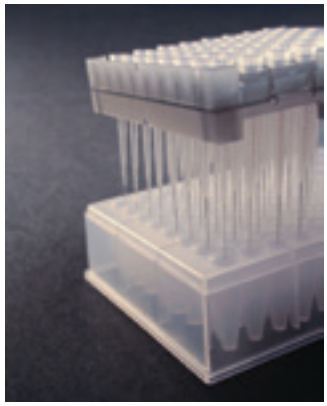


Figure 3. 96-Well Plate Format for Automation



Sample Preparation

Stock solution of binding analytes was prepared at 10 µg/mL in methanol. Rat plasma stabilized with K_2EDTA (BioReclamation, IVT, Hicksville, NY USA) was spiked at 200 ng/mL of binding analytes and allowed to equilibrate for 3 hours @ 37 °C prior to extraction studies. Phosphate buffered saline (PBS, pH = 7.4) was prepared at (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) concentrations. Spiked PBS was prepared @ 200 ng/mL of binding analytes and this was used for the SPME extraction studies. Blank PBS was used for the RED device binding studies.

SPME C18 Extraction

Supelco SPME LC Tips: 57234-U

Fibers were conditioned by soaking in methanol for 10 minutes and then soaked in water for 10 minutes to equilibrate. These fibers were placed in 2 mL 96-well plate with 800 µL of plasma and buffer samples and agitated for 30 minutes @ 500 rpm on automated shaker. The fibers were then transferred to 600 µL 96-well plate with 300 µL of internal standard desorption solvent (50 ng/mL in ACN) and agitated for 10 minutes @ 500 rpm on automated shaker. The SPME fibers were removed and the well plate was capped, vortexed and analyzed directly. Sample replicates N=5.

RED Device Protocol

Single-Use RED plate with inserts: Thermo PI90006

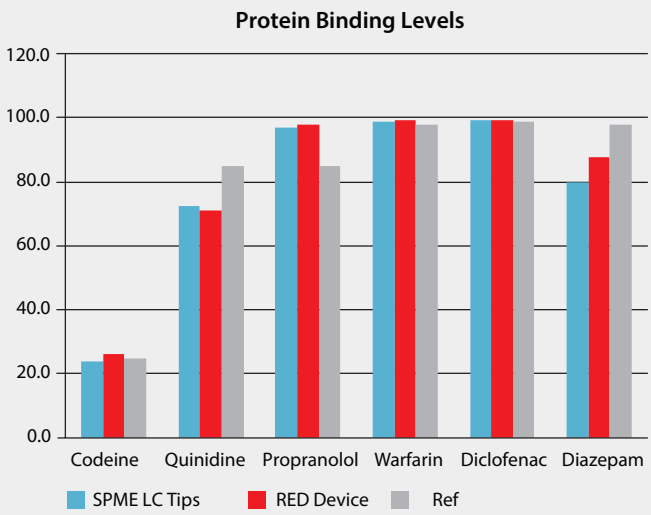
Prepare samples: Put 200 µL of plasma into plasma compartment of RED device and 350 µL of buffer into buffer compartment. Cap the device and mix @ 37 °C @ 250 rpm 4 hours. Take 50 µL aliquots from each compartment and add 50 µL of plasma into buffer compartment aliquot and 50 µL of buffer into the plasma compartment aliquot. Add 300 µL of ice cold acetonitrile with 50 ng/mL internal standard. Vortex samples @ 1200 rpm for 5 minutes and then centrifuged @ 15,000 rpm for 10 minutes. Decant supernatant into a glass HPLC vial for analysis.

Results

In the case of the BioSPME analysis, the free fraction of the analyte is measured in both the reference (PBS) and the plasma sample. This technique simplifies the calculation of determining protein binding level.

Protein binding affinities for both the BioSPME and the equilibrium dialysis devices closely matched the referenced range for all analytes. In the case of quinidine, a lower binding affinity was observed for both techniques as compared to the reference data. This may be specific to the plasma sample used in the study.

Figure 5. Binding Affinity Comparison



Drug binding levels were determined using the BioSPME approach in less than 60 minutes. Thus, there was a 4× reduction in analysis time over the equilibrium dialysis device. This was a significant time saving as compared to Thermo RED and other membrane techniques.

The BioSPME approach allows for direct sampling of the plasma sample, eliminating the need for protein precipitation as in the equilibrium dialysis device. This also minimizes concern associated with matrix interference.

The BioSPME approach for directly determining free fraction of drug within plasma proved to be a simpler and faster technique over traditional dialysis membrane techniques.

Additional studies are planned to further reduce the extraction time needed for the BioSPME method.

Reference

1. Journal of Laboratory Automation February 2011 16:56-67.

Ordering Information

Description	Cat. No.
SPME-LC Fiber Needle Probe, C18 coating, pack of 5 probes	57281-U
SPME-LC Pipette Tips, 96-tip array, C18 coating, pack of 96 tips	57234-U
SPME-LC Pipette Tips, 96-tip array, PDMS/DVB coating, pack of 96 tips	57248-U

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