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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 levels and free circulating drug in biological systems is important in establishing pharmacokinetic 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. In this study, a comparison was performed between a novel sample preparation technique utilizing BioSPME (biocompatible solid phase microextraction) technology and RED (rapid equilibrium dialysis), which is considered the gold standard technique for plasma protein binding level determination. All experimental samples were analyzed via LC-MS/MS. Advantages of the BioSPME technique over current methodologies with respect to sample preparation time and workflow simplification will be presented, as well as a comparison of data accuracy and reproducibility.

Methods

The BioSPME devices utilized in this experiment were in 96-pin array format with a C18 functionalized particle coating. The 96-pin array was preconditioned by exposure to 2-propanol for 20 min. Analyte extractions were accomplished via direct immersion of the 96-pin array into spiked plasma and buffer samples for 30 minutes with agitation and heat at 37°C. Analytes were recovered via desorption within organic solvent.

The RED device wells were loaded with buffer and spiked plasma samples and incubated for 4 hours with agitation and heat at 37°C. A subsequent protein precipitation was performed prior to analysis.

All samples prepared by either the BioSPME or the RED techniques were analyzed using a SCIEX 5500 QTRAP® equipped with a Shimadzu HPLC system.

Preliminary Data

Free fraction concentrations of 3 compounds in human plasma (carbamazepine, rivaroxaban and apixaban) spiked at 100 ng/ml and 10 ng/mL were determined using either BioSPME or RED sample preparation methodology with analyses via LC-MS/MS. Good extraction efficiencies were achieved for all compounds as acceptable sensitivity of the LC-MS/MS method was observed for the studied spiking levels. All compounds showed comparable results in terms of protein binding values when comparing BioSPME and the RED technique versus accepted literature values. Using either the BioSPME or the RED technique, good precision out of plasma and buffer was demonstrated with %RSD values less than 15% for all compounds tested. The novel technique employing BioSPME within this study shows a reduction in sample processing time to just over one hour, compared with over 6 hours using the RED technique. These results show that the BioSPME 96-pin array technique offers good potential as a fast, simple method for the quantitation of plasma protein binding levels and is a viable alternative to RED and other techniques used in protein binding studies.

Novel Aspect

Biocompatible solid phase microextraction offers a faster and simpler sample preparation approach for determination of plasma protein binding via LC-MS/MS.