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Title: Strategy for Conducting Equilibrium Dialysis of Plasma Samples and Subsequent LC-MS/MS Analysis of the Resultant Samples

Purpose: To evaluate the application of 96-well equilibrium dialysis system combined with LC-MS/MS-based analysis in the ex-vivo plasma protein binding determination.

Methods: Equilibrium dialysis is the preferred method to determine the free drug fraction. Recently, the throughput of equilibrium dialysis has been improved by implementing a 96-well format plate without compromising the quality of the data. The present poster illustrates the successful application of a 96-well format equilibrium dialysis plate combined with Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) in the determination of protein binding of BMS compound A in human plasma.

During the validation, 300 μ L control human plasma spiked with BMS compound A and 500 μ L pH 7.4 phosphate buffer were added into the opposite side of the wells in a 96-well equilibrium dialysis plate according to the pre-described dialysis plate map. Plasma samples were added into the top half wells and buffer samples were added into the bottom half wells. The filled plate were then incubated at 37°C for 16 hours. Portion of plasma and buffer samples were then taken out of the wells and diluted to the appropriate concentrations with either buffer or plasma to generate plasma:buffer mixed matrix environment. The mixed matrix samples then went through solid phase extraction using C2 cartridges, the extract samples were injected onto an LC-MS/MS system and were monitored by MRM mode in a positive TurboIonSpray ionization. Protein binding data were calculated based on the ratio of BMS compound concentrations in buffer side and plasma side. The LC-MS/MS method was validated in the mixed matrix at the curve range of 1-1,000 ng/mL.

Results: The validation of the combined process indicates that the apparent free fraction obtained by this method correlates well with the reported values determined by the original equilibrium dialysis devices at BMS: Tandem got $86.0 \pm 5.0\%$ bound (combining all concentrations), while the BMS data (combining all concentrations) came out to $86.5 \pm 5.3\%$ bound. Data from both BMS and Tandem showed no concentration effect. This method was then applied to the analysis of clinical plasma samples taken from

HIV infected pregnant women who had received Compound A oral dose. The sample throughput was increased almost 5 fold in the 96-well format device comparing to the traditional device (96-well vs 20 or less well). The ex vivo binding data from pregnant and non-pregnant women showed no significant difference.

Conclusion: The 96-well format equilibrium dialysis device is able to provide high throughput measurements for the free and bound concentration of small pharmaceutical molecules and generates comparable data to that obtained from the traditional equilibrium dialysis system.

Key words: protein binding; equilibrium dialysis; LC-MS/MS, high throughput; clinical samples