Development of an Integrated Microscale Ceramic Separation Device to Address Limited Sample Volumes in Bioanalysis

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Small sample volumes from tail-bled rodents and dried blood spot (DBS) cards present an extra challenge for the bioanalytical scientist. Microscale separations have shown potential for the high-sensitivity analysis of limited-volume samples in proteomics. In this paper, we will discuss the use of a capillary-scale ceramic separation device to achieve pg/mL levels of sensitivity for candidate drugs in a few microliters of biofluid.

Various drug compounds and associated metabolites were spiked into rat plasma and whole blood. Whole blood samples were prepared by spotting 15 µL of blood onto Whatman 903 ® Specimen collection paper, drying for two hours and extracting the blood spot with methanol. Samples were then injected onto a 0.300 x 100 mm channel packed with 1.7 µm BEH C18 and eluted using a linear gradient housed on a ceramic nanotile at 12µL/min. Mass spectrometry was performed on a tandem quadrupole MS operating in positive electrospray mode.

Utilizing samples derived from the protein precipitation of rat plasma and whole blood from blood spot cards, the reproducibility of the system was evaluated. The separation device showed excellent robustness with greater than 1,000 injections obtained from protein precipitated plasma. The average peak width for a small molecule was 2.3 seconds at base, giving a peak capacity of 130 for a 5 minute run. The assay sensitivity of the micro LC system was determined to be 25 times that of the standard 2.1mm system. Bioassays for various compounds were successfully developed from 15 µL DBS with LLOQs as low as 50 pg/mL utilizing only 1 µL of injected sample. For the analysis of the therapeutic peptide exendin a LLOQ of 100pg/mL was achieved whereas the LLOQ on a conventional LC/MS system was 10ng/mL.