On-line Microfluidic Extraction Enables Highly Efficient and Sensitive Direct Elution from Dried Blood Spots

Gary Valaskovic[1]; Christopher A. Evans[2]; Chester L Bowen[2]
[1]New Objective, Inc., Woburn, MA; [2]GlaxoSmithKline, King Of Prussia, PA

Introduction
Dried blood spots (DBS) have rapidly emerged in the pharmaceutical industry as a highly cost effective method for low volume sampling, storage, and retrieval of specimens prior to assay by liquid chromatography/tandem mass spectrometry (LC-MS/MS). Prior to analysis, a punch (typically 3mm) from the spot (typically 15 uL) is subjected to solvent extraction and subsequent analysis by conventional (mm bore) LC-MS/MS. Inefficiencies in extraction and volume handling limit assay sensitivity. Insufficient limits of quantification often preclude the use of DBS for high potency and/or inhaled compounds. Here we present a novel format and workflow utilizing micro DBS punches (< 0.4 mm) in a microfluidic flow-through extraction cell compatible with nanobore LC and nanoelectrospray MS/MS.

Methods
Rat blood aliquots (20 uL) containing sitamaquine were spotted on FTA or FTA Elute cards (Whatman) Micro-punches (0.35 mm ID) from the spot were obtained using a custom fabricated biopsy punch and transferred directly into an elastomer core extraction cell (0.4mm ID). Fused-silica tubing (50 um ID) was connected to the inlet and outlet of the cell. Compression of the elastomer core effectively sealed the inlet and outlet tubes in the cell to the micro-punch at zero dead volume. The extraction cell was placed in the injection loop of an autosampler connected to a nanoflow LC pump. The outlet of the autosampler was connected to a nanospray equipped ion trap mass spectrometer operated in (pseudo) SRM mode.

Preliminary Data
The micro biopsy tool proved to be an effective means to sample multiple punches from a single blood spot. Greater than 10 punches were readily obtained from the central (1/3) region of a typical spot, something not achievable in current DBS processing methodologies. After transfer of the punch, minor compression (< 20% by volume) of the DBS biopsy punch was observed during the transfer. With inlet and outlet tubes in contact with the punch, the core was compressed through externally threaded ferrules to form a leak tight conformal seal around the punch circumference. Mobile phase flow from the inlet tube was directly observed to pass through, rather than around, the DBS punch resulting in direct elution from surface. Once mounted in the loop of the autosampler injection valve, approx. 0.25 uL of extraction solvent (75% Acetonitrile) was transferred into the cell from a 10 uL gas-tight micro-syringe. The injection loop was switched from load to inject after a 120 sec extraction period. Once the valve was switched, the nanoflow pump (1 uL/min) pushed the extraction plug into the nanospray ionization source equipped with a junction style high voltage contact. Stable spray ionization was observed using a 20 um ID fused-silica emitter (sheath gas assisted) at 2.5 kV. Direct elutions from the DBS micro-punches were successfully analyzed. Using this novel micro extraction technique a substantial gain in sensitivity was achieved compared to existing UHPLC-MS/MS methodologies, with a pg/mL lower limit of sensitivity. Transfer of these methods to a triple quadrudpole mass spectrometer to evaluate quantitative performance is underway. This scale of this novel microfluidic direct elution device allows for increased assay sensitivity, as well as for the ability of multiple samples to be collected from the same individual DBS.