



POSTER SESSION ABSTRACTS

Inspiration and Education

POSTER SESSION

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Applying Proximity Extension Assays for Proteomic Profiling of Dried Blood Microsamples to Enable Patient-Centric Sampling for Endogenous Proteins Levels in Clinical Trials

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*These authors contributed equally to the work

1 Pharmacokinetics, Pharmacodynamics, Drug Metabolism; 2 BARDS/Biometrics Research

Bioanalytical innovation and a desire to decrease patient burden has increased interest in microsampling and patient-centric sampling within the pharmaceutical industry. Microsampling has been successfully used at Merck to support Pharmacokinetic clinical studies. The relative ease of at-home sample collection and previous success in microsampling led us to investigate the use of dried blood microsampling (DBM) for proteins. In this study, the levels of proteins extracted from different microsampling collection devices using venous and capillary draws were examined. DBM results were compared to plasma protein levels. Extracted proteins were assayed in proximity extension assays (PEA) on two Olink protein panels, Cardiometabolic (CM) and Inflammation (INF). Each panel consists of 92 proteins. Results indicate that DBM proteomic analysis is possible using PEA. The % of CM proteins detectable for all samples was 93.5% for plasma and at least 95.6% for all DBM matrices. For INF proteins, the % detectable in all samples was 79.3% for plasma and $\geq 63.0\%$ for all DBM. DBM samples exposed to heat showed a $\sim 20\%$ decrease in protein levels. Broadly, results from capillary and venous collection showed similar protein levels for CM proteins. Although results varied, the correlation coefficient between plasma and DBM samples centered around 0.5. Most CM proteins measured 50% higher in the DBM samples than in plasma. Conversely, INF proteins measured in DBM often measured $>75\%$ lower than in plasma. Despite being biased high, the slope between the CM proteins measured in DBM and plasma was often close to one. For INF proteins, the slope of DBM vs. plasma was often less than one. The study showed that the relatively abundant proteins on the CM panel can be detected in plasma and in DBM using the Olink platform. Although biased, there is evidence that changes in CM panel proteins detectable in plasma may also be detectable in DBM.

Proteomic profiling of dried blood microsamples by LC-MS

Michelle R. Robinson¹, Daniel S. Spellman¹, Melanie Anderson¹, Brad R. Evans¹, Nana Wang², Daniel J. Holder², Kevin P. Bateman¹

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Keywords: Microsamples, Proteomics, LC-MS, Biomarkers, At-home sampling

Abstract

Biomarkers are valuable tools in drug development programs that can indicate safety and efficacy or identify appropriate patient populations for clinical trials. Plasma is the typical clinical sample because it is easily accessible and reflective of physiological state. Dried blood microsamples (DBM) offer these benefits while allowing at-home sampling which decreases patient burden and provides richer datasets through increased sampling frequency and broader enrollment. Liquid chromatography mass spectrometry (LC-MS) provides a flexible platform for biomarker analysis in plasma and DBM based on specificity, multiplexing capabilities, and small volume requirements, yet challenges related to high dynamic range and sample complexity in blood/plasma motivate the development of new tools. Presently, LC-MS is applied to measure a panel of proteins from plasma and DBM to determine the utility of DBM versus plasma for biomarker detection/quantitation and provide a baseline to compare new analytical platforms, specifically the Olink proximity extension assay.

Discovery proteomics was carried out for DBM, and a 20-protein panel was constructed by cross-reference with Olink's cardiometabolic panel and Merck's liver function panel. The panel was applied to a set of volumetric absorptive microsamples (VAMS), dried blood spots (DBS) and plasma from healthy subjects. Of 15 proteins detected, 13 were common to all sample types, and 1 was unique to either DBM or plasma. Protein abundance was generally greatest in plasma except for those associated with red blood cells. For DBS and VAMS, protein levels were not consistently higher in one device compared to the other, although several proteins were over 4x more abundant in VAMS relative to DBS. Protein levels in DBS did not exceed those measured in VAMS by more than 2x. Exposure to heat decreased protein levels by ~25% in DBM, underscoring the importance of sample handling. These LC-MS results provide a baseline for comparison to Olink technology.

Direct Analysis of Microglial Cells by Dual-Probe Microsampling Integrated with Ion Mobility-Mass Spectrometry

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Microglia, as the primary immune cells in the central nervous system, are responsible for constantly surveying their microenvironment. Changes within brain homeostasis can cause microglia to adopt either a neuroprotective or cytotoxic phenotype. In several neurodegenerative disorders, including Parkinson's disease (PD), microglia have consistently been found to be in an activated state secreting pro-inflammatory species, contributing to the neuroinflammation observed in patients with PD. A more in-depth understanding of the mechanism behind sustained microglial activation in PD is currently sought after. Therefore, an investigation into alterations within cellular metabolism may help to gain insight into underlying disease processes. To do this, a dual-probe microsampling device was designed for directly extracting and analyzing intracellular metabolites from adherent microglial cells *in vitro* by ion mobility-mass spectrometry (IM-MS). This technique provides the ability to analyze the metabolic profile of microglial cells in real-time, with time-dependent molecular changes detected after the presentation of pro-inflammatory stimuli. The dual-probe design allows for the simultaneous extraction and detection of chemically diverse compound classes by tailoring the extraction solvent mixtures utilized in each probe, increasing the metabolome coverage for each sample. Implementation of the dual-probe system to study metabolic changes in microglial cells exposed to a neurotoxin, mimicking PD progression, is demonstrated.

Investigation of Cationized Steroid Dimer Formation by Mass Spectrometry for Improved Analysis of Anabolic Steroids

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Athlete drug testing became routine following the 1968 Winter Olympics, and today athletes are routinely tested for over 200 prohibited substances, including anabolic steroids. This class of compounds is well known for their ability to enhance muscle development, shorten recovery time, and provide an unfair advantage to competitors using them. These compounds can be particularly challenging to detect due in part to the large number of isomeric species. However, the use of ion mobility to analyze sodiated steroid dimer complexes has been shown to enhance the separation of some isomeric steroid species. Furthermore, isomeric steroids have been observed to dimerize to different extents and to form mixed dimer complexes in the presence of other steroids. Therefore, this study investigates steroid dimer formation and factors that may affect dimer formation. Specifically, the role of concentration, other steroids, and relative sodium ion affinity and their effects on steroid dimer formation are explored.

Single-Use Reagent Kit for Real Time BTK Receptor Occupancy Assay: A New Bioanalytical Strategy to Simplify On-Site Biomarker Sample Collection for Clinical Studies

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Abstract

An innovative LC-MS/MS assay previously developed for real time BTK receptor occupancy determination has been successfully implemented in the Phase I study on branebrutinib (BMS-986195), a covalent, irreversible inhibitor of BTK that is being developed for the treatment of immune mediated diseases: rheumatoid arthritis (RA), lupus, and Sjogren's disease. This assay was used to establish the PK-PD relationship and for making dose escalation decision in Phase I clinical studies. The assay procedure requires on-site blood lysing during sample collection, which includes accurate pipetting and shaker-mixing of protease inhibitors, quencher, lysis buffer and water with blood. Such procedures could be difficult to implement in Phase II studies which will be conducted in multiple countries and numerous small clinics with potentially limited resources and varying operational capabilities. To ensure high quality samples are collected in all clinical sites, a simplified procedure with a user friendly single-use reagent kit is necessary. Therefore, the blood lysing procedure was systematically re-evaluated, which led to the successful elimination of unnecessary steps and equipment. With fewer samples to be collected at each site during Phase II studies, the use of pre-aliquoted reagents eliminates the need for accurate pipetting and avoids unnecessary reagent waste. Finally, the concerns on potential non-specific binding, stability and assay performance due to these changes were eliminated by additional stability and performance confirmation. The equivalency of the analytical results using the new and old procedures was successfully demonstrated. This new bioanalytical strategy has been successfully developed and will be used to support the Phase II study of branebrutinib worldwide.

**Development and Validation of a Patient-centric Volumetric Absorptive Microsampling- Liquid Chromatography
Mass Spectrometry Method for the Analysis of Cefepime in Human Whole Blood:
Application to Pediatric Pharmacokinetic Study**

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Abstract

Cefepime is a fourth-generation cephalosporin antibiotic with an extended spectrum of activity against many Gram-positive and Gram-negative bacteria. There is a growing need to develop sensitive, small volume assays, along with less invasive sample collection to facilitate pediatric pharmacokinetic clinical trials and therapeutic drug monitoring. The volumetric absorptive microsampling (VAMS™) approach provides an accurate and precise collection of a fixed volume of blood (10 µL), reducing or eliminating the volumetric blood hematocrit assay-bias associated with the dried blood spotting technique. We developed a high-performance liquid chromatographic method with tandem mass spectrometry detection for quantification of cefepime. Sample extraction from VAMS™ devices, followed by reversed-phase chromatographic separation and selective detection using tandem mass spectrometry with a 4 minute runtime per sample was employed. Standard curves were linear between 0.1 – 100 µg/mL for cefepime. Intra- and inter-day accuracies were within 95.4 – 113% and precision (CV) was < 11.3% based on a 3-day validation study. Recoveries were ≥ 45.5 % and the matrix effect was within 89.5 – 96.7 % for cefepime. Cefepime was stable in human whole blood under assay conditions (3 h at room temperature, 24 h in autosampler post-extraction). Cefepime was also stable for at least 1 week (7 days) at 4 °C, 1 month (39 days) at -20 °C and 3 months (91 days) at -78°C as dried microsamples. This assay provides an efficient quantitation of cefepime and was successfully implemented for the analysis of whole blood microsamples in a pediatric clinical trial.

Quantitative analysis of BMS-Drug-X in rat and monkey plasma by LC-MS/MS to support IND enabling toxicology studies: assay development, transfer, and validation

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Bioanalytical Sciences, Translational Medicine

BMS-Drug-X is a potent, irreversible inhibitor of Bruton's Tyrosine Kinase (BTK) that demonstrated rapid rate of inactivation and high selectivity. BTK plays a significant role in proliferation and activation of B cell receptor signaling pathway, and BTK inhibition show promising results in several hematologic cancers and autoimmune diseases. In support of IND enabling toxicology studies, bioanalytical assays using LC-MS/MS platform were developed to quantitatively measure BMS-Drug-X in toxicology species. One concern in accurate quantitation of BMS-Drug-X in circulation was that it could covalently bind *ex vivo* to free BTK present in whole blood, which might result in underestimation of drug levels in biological samples. Therefore, comprehensive stability experiments were designed and conducted in whole blood. The initial strategy was to deactivate BTK in biological matrices using a competitive quencher stabilizer. Since different stability performances were observed in different toxicology species, acidification to low pH was tested during sample pre-treatment to stabilize BMS-Drug-X in monkey whole blood. Meanwhile, the analyte was stable in rat whole blood for up to 2 hours without acidification. Therefore, different strategies of sample treatment were applied to different species based on stability results, which was critical to guide toxicokinetic sample collection. The developed assays were successfully validated in monkey and rat plasma, which ensured on-time delivery of high quality data in support of four GLP studies simultaneously. The assay development and validation results will be presented in this presentation.

Mass Spectrometry Applied for Automated Phenotyping of Clinical Trial Populations

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Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has been applied for the detection of genetic polymorphisms. This cost-effective, timely, mass spectrometry alternative approach has been validated to aid in the development of effective, safe drugs and dosages with respect to a person's genetic makeup (i.e. personalized medicine). Over the past three years it has been applied for screening based on metabolism characteristics to enroll clinical trial special populations. It has also been applied to characterize the overall clinical trials population, affording a head start in identifying phenotypic subsets of the population. It has also been used to identify individual subject differences should a PK anomaly arise. The approach has been applied to monitor broad panels comprised of as many as 69 genetic polymorphisms implicated in differential drug metabolism, as well as more specific assays.

The procedure describes a method for simultaneous analysis of multiple single-nucleotide polymorphisms (SNP) and copy number variations (CNV) from buccal swab samples. The assay is based on deoxyribonucleic acid (DNA) polymerase chain reactions (PCR) that utilize oligonucleotide primers designed to target specific loci in the genome. The PCR creates and exponentially increases (i.e. amplifies) the quantity of amplicons with molecular weights that correlate with those of known genotypes. The DNA is procured with buccal swabs from human subjects, and isolated by a magnetic particle processing robot (KingFisher Flex with a 96-well tip comb) into a stable buffer medium. Then, the DNA is amplified using PCR with primers designed to amplify large portions of the target areas (>1 kb) for CNV determinations and shorter areas adjacent to variable nucleotides for SNP determinations. A shrimp alkaline phosphatase (SAP) reaction follows, which deactivates the phosphate groups of unincorporated nucleotides from use in multiple-base extension reactions. Then, a single-base extension (SBE) reaction with thermosequenase adds the mass-modified dideoxynucleotide terminators to the amplicons according to their template strands. The various liquid handling steps are performed on a Tecan Genesis liquid handler outfitted with low volume tubing to operate in the 2-5 μ L range. The molecular weights of the amplicons are determined via an Agena Bioscience MassArray Analyser 4 MALDI-TOF with an automated chip prep module for spotting 30-50 nL samples onto the matrix arranged on a 96-position chip. The specific terminators added in the SBE reaction are determined by matching the molecular weights of the subject sample amplicons to those of known sequences from their respective loci. CNV quantities are calculated by comparing the ratio of amplicons detected from copy number variable regions to the amount of amplicons detected from regions with established copy number quantities.

