POSTER SESSION ABSTRACTS

Inspiration and Education

POSTER SESSION
Tuesday, September 27, 1:00 pm - 3:00 pm

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Poster Abstract
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Development of a sensitive and selective GSH screening assay for drug discovery support

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Introduction: Bioactivation of drugs can form reactive intermediates, which can inactivate proteins and thus result in potential toxicities. Glutathione (GSH) is a universal scavenger of reactive intermediates and widely used in pharmaceutical drug discovery/development for trapping reactive intermediates as part of risk assessment. The formed GSH adducts are typically detected by using LC-MS/MS (liquid chromatography with tandem mass spectrometry) methods. In order to enhance the sensitivity of our current approach to prevent potential failure in detecting GSH adducts, a new LC/MS/MS method was developed by applying in-source fragmentation mass spectrometry.

Methods: Compounds are incubated with human liver microsomes and trapping reagents (1:1 of γ-GluCysGly (GSH): γ-GluCys^{13}C2^{15}N-Gly (GSX)). UPLC coupled with high-resolution mass spectrometry (HRMS) was used to detected GSH adducts. The mass spectrometry system is set up with in-source fragmentation at negative mode which allows monitoring two characteristic m/z peaks of 272.0888 and 275.0926 with data-dependent MS^n through isotopic matching (M:M+3=1:1). The system setup also allows monitoring neutron loss of 273 and 144.

Results: The newly developed method (termed XoPI-DDA-NL) was validated using 8 positive controls. The same GSH adducts reported in literature were observed in the new assay.
Comparing the previous in-house method with XoPI-DD-NL using positive controls and 26 in- house compounds from 4 different discovery projects, more GSH adducts were identified by the XoPI-DD-NL method; and 3 previous false negatives were correctly identified using this new approach. The methodology has been successfully applied to in-house projects to identify potential reactive moiety and contribute to structure activity relationship understanding.

Conclusions: Through application of in-source fragmentation, a new, sensitive, and selective XoPI-DDA-NL GSH screening assay has been developed and implemented to support drug discovery projects.

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Cytological and Histological Workflow Compatible Automated and Semi-Automated Sample Profiling and Analysis Methods

Mariam ElNaggar - Prosolia

Devices that enable quantitative characterization of chemical distributions facilitate the adoption and broader usage of mass spectrometry in health care environments. Molecular imaging can directly answer questions with respect to clinical outcomes. These questions tend to be restrictions in workflows and applications as diverse as basic research level microbial biomarker exploration and discovery, understanding of mechanisms of action in drug development, and real-time therapeutic and diagnostic measurements of human derived samples. Acquiring these results quickly and with minimized expense at the laboratory level—which in turn leads to patient care improvements and downstream health care cost reductions—is dependent on consistency and efficiency of analytical methodologies.

Commercial technologies have begun to address generating higher resolution data, but ablation and ionization systems can bias sampling. Liquid Microjunction Surface Sampling Probes can bypass some of these biases. Mass spectrometric analysis based on the impingement of fluid onto surfaces for extraction and ionization as an atmospheric pressure surface sampling workflow, also benefits from the obviated need for extensive sample preparation.

The continuous in situ microextraction provided by the flowprobe system also enables rapid, high sensitivity analysis with few limitations to sample type and versatile utility as regards experimental operation. Applying flowprobe analysis to various preparations, collected as part of an extant workflows, resulted in effective characterization of drugs, metabolites, and other biologically derived compounds of interest either as chemical images, time resolved intensity profiles, or mass spectra.

Data were acquired in order to characterize the profiling and imaging capabilities of the flowprobe utilizing various substrates and analytes and establish limits to imaging analysis. Evaluation of the mass spectral data and subsequent data from cells on cytospin slides revealed that fixation and analysis of the samples could be performed such that cells were preserved for subsequent morphological evaluation, even with spectra indicating the quantitative extraction of various phospholipids.

Tissue samples with endogenous compounds of interest and various biopharmaceuticals were characterized using flowprobe extractive analysis. Chemical images and profiles of dose response were effective in determining both the disposition and the time resolved intensities of drugs as well as other diagnostic compounds and metabolites of interest in various tissue and sub-tissue types. These data correlated well to other techniques pointing to the rapid and sensitive characteristics of the flowprobe analysis.

Monitoring the signal for calibrated concentration curves for drugs not bonded to tissue, it was noted that the integrated abundance for ion current could be linearly determined through tissue surfaces while lipid compounds associated with the tissue extracted from within the volume of the surface remained steady. Live-microorganism plate-based analysis has begun to generate a statistically significant number of species/strain profiles collected at various time points and stresses. Non-pathogenic colonies' spectra, yielded a few significant masses despite having the mixed assortment of spectral types with different solvents and media. A subset of these data can be applied toward strain-specific microbial classification. This specificity in profiling is of particular interest for medical diagnostics.

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Definitive Structure Elucidation of a Novel Rearranged Oxidative Metabolite of Doravirine

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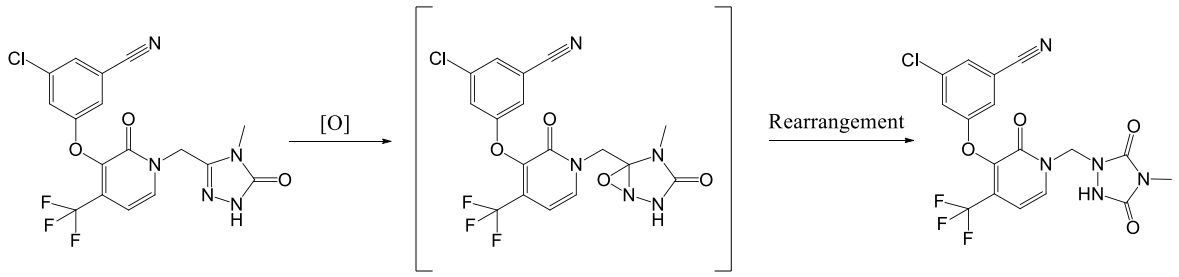
HIV-NNRTI is an extremely important program at Merck focused on the delivery of non-nucleoside reverse-transcriptase inhibitors which inhibit the replication of viral DNA by binding to a pocket near the active site of the polymerase. Doravirine (MK-1439) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) currently in Phase III clinical trials for the treatment of HIV-1 infection. It is a trifluoromethyl analog derived from a pyridone series with excellent potency against wild-type (WT) virus and a panel of 15 mutant viruses. Its potency, mutant profile and pharmacokinetics in preclinical species made doravirine a suitable candidate as a once-a-day drug for the treatment of HIV.

Human ADME studies using ^{14}C-labeled doravirine (MK-1439) revealed a circulating oxidative metabolite (M9) approaching the level of interest based upon the MIST guidance. It was, therefore, decided that definitive structure identification would be required and most likely potency/toxicity testing. It was determined by MS/MS that the site of metabolism was somewhere on the triazolinone ring (right side of the molecule). To gather enough metabolite for analysis, PPDM pooled recovered ^{14}C-labeled metabolite from human urine and purified it to >90%. The unexpected structure of the metabolite was determined by high-resolution solution NMR and believed to be the result of a *novel* intramolecular rearrangement (see figure below). The proposed oxidative metabolite was then synthesized affording > 1 gram of pure compound for testing.

This presentation will illustrate the definitive structure identification of the M9 metabolite with a specific focus on the various techniques used to examine low-level microgram quantities of metabolite by two-dimensional NMR. We will also discuss the proposed mechanism for the formation of this novel rearranged oxidative metabolite.



MK-1439 | Presumed Oxaziridine Intermediate | M9 Metabolite

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Practical considerations of using multiple spots for improving Dried Blood Spot LC/MS/MS Assay Sensitivities

Shenita Basdeo– BMS

Background:

Transitioning from supporting preclinical to clinical Phase 1 studies, bioanalytical sample analysis usually requires lowering the Low Limit of Quantification (LLOQ) of the assay significantly. For liquid samples such as plasma and blood lysate, lower LLOQ often can be achieved by simply increasing the sample volume of the assay. For DBS samples,-increasing sample volume involves using large spot size or multiple DBS spots. In this presentation, we will share our experience of combining two spots to increase assay sensitivity to support a BMS clinical program.

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An Improved High Throughput LC-MS/MS Method for the Determination of Phosphatidylethanol in Dried Blood Spots Using SelexION™ Technology

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Background: Phosphatidylethanol, with 18:1/16:0 being the most abundant species (PEth), is an abnormal phospholipid formed in the red blood cells after ethanol exposure with a half-life of approximately four days. With a cutoff of 20 ng/mL, PEth has been considered an alcohol biomarker to evaluate binge drinking behavior in a 3-week period. However, lowering limit of quantitation (LOQ) from 8 ng/mL may be warranted for testing other implications such as prenatal alcohol exposure; however, it is an analytical challenge due to chromatographic interferences from endogenous blood matrix.

Objective: To improve the sensitivity of a high throughput liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the detection of PEth in dried blood spots (DBS) with minimal sample preparation.

Method: Authentic blood samples were collected on Whatman®903 DBS paper, and three 3-mm punches of DBS were aliquoted for PEth analysis. DBS aliquots were incubated with d31-PEth internal standard in 1 mL of HPLC grade methanol at room temperature for 1 hour. The methanol extract was then evaporated under nitrogen at 40ºC, and the residue was reconstituted in 1 mL of mobile phase A. The LC was an Eksigent Micro-LC 200 Plus with Halo C18 column at flow rate of 50 µL/min and a 3.4 minute run. Mobile phase A was 50% 2mM ammonium acetate/25% acetonitrile/25% isopropanol, and mobile phase B was 40% isopropanol/60% acetonitrile. PEth was detected using a Sciex 6500+ QTRAP® with SelexION™. The modifier was 1-propanol with compensation voltage at -1.8V and separation voltage at 3600V. The MRM transitions with negative ion mode were 701→255/281 and 732→286/281 m/z for PEth and d31-PEth, respectively. The single calibrator of 20 ng/mL was made in two approaches and evaluated for equivalence by testing 102 whole blood or DBS samples: 1) PEth spiking solution directly added to three 3-mm punches of blank Whatman®903 paper; and 2) PEth solution made in whole blood from gray top tube then spotted at 40 µL on blank DBS paper.

Results: One hundred and two authentic samples were quantitated by a 20 ng/mL single-calibrator prepared with the two approaches resulted with equivalent quantitation (R^2 = 0.97, y = 0.97x – 0.46). The first approach to prepare controls and multi-point calibrators was thus determined acceptable to validate the method following standard guidelines. The calibration model was established 5 – 200 ng/mL, and both 20 and 8 ng/mL were proven acceptable single-calibrators to quantitate PEth within the range. The method was precise (between run and within run CV <12.4%) and accurate (bias +5 – +12.5% for controls at 25, 80, and 160 ng/mL). LOD and LOQ study was performed by fortifying decreasing amounts of PEth into negative DBS and quantitating using 8 ng/mL calibrator. The new SelexION™ coupled Sciex 6500+ QTRAP® instrument allowed LOD and LOQ to lower from 8 to 3.2 ng/mL with satisfactory separation from endogenous blood matrix. Matrix effect showed ion suppression for both PEth and d31-PEth by 30.6 – 41.2%.

Conclusion: The PEth sensitivity was improved due to the decrease in chromatographic interference by ion mobility separation of SelexION™.

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Prevalence Rates of Prenatal Alcohol Exposure from Detection of Phosphatidylethanol in Newborn Dried Blood Spots

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***Objectives*:** Prenatal alcohol exposure is the leading preventable cause of birth defects in the United States, producing an array of neurological, behavioural and physical abnormalities collectively known as fetal alcohol spectrum disorders (FASD). In the United States, the prevalence of FASD is estimated to be as high as 2 to 5% of school children. Identifying infants at risk for developing FASD requires confirmation of maternal drinking during pregnancy or detection of prenatal alcohol exposure. Phosphatidylethanol (PEth) is a direct biomarker of alcohol metabolism that has generated significant interest as a potential objective measurement of prenatal alcohol exposure. PEth is a unique phospholipid formed from phosphatidylcholine present in the membranes of red blood cells, and forms only in the presence of ethanol through a metabolic process catalyzed by phospholipase D. Our laboratory has developed and validated an assay for the extraction and detection of PEth from dried blood spots using liquid chromatography-tandem mass spectrometry. We have analyzed newborn dried blood spot cards for detection of PEth in both prospective and retrospective studies to examine the clinical utility of PEth as a biological screening test for fetal alcohol exposure in newborns and to determine prevalence rates of prenatal alcohol exposure in multiple populations.

***Materials and methods*:** Dried blood spot cards were collected and analyzed for detection of PEth in three different populations: (1) from 250 residual, dried blood spot cards from a Midwestern state health department; (2); from 487 newborns born at the Charleston Area Medical Center (CAMC) Women and Children's Hospital in Charleston, West Virginia and (3) from 135 newborns born at the National Social Security Perinatology Unit in Montevideo, Uruguay. PEth was detected in standard dried blood spot punches (3.1 mm) using an Agilent 6460 liquid chromatography-tandem mass spectrometry system following extraction into methanol.

***Results*:** In our analysis of 250 anonymous newborn dried blood spots from a Midwestern state, where reported alcohol consumption is less frequent, PEth was detected in 10 samples (4%). At the CAMC Women and Children's Hospital in West Virginia, where alcohol consumption during pregnancy is a high concern, PEth was detected in 112 samples (23%). In the public health care hospital in Montevideo where alcohol consumption during pregnancy is commonly reported, PEth was detected in 107 samples (79%).

Conclusion: Detection of PEth in dried blood spots is an effective method to screen for prenatal alcohol exposure during late pregnancy and results from these studies indicate that the prevalence rates of prenatal alcohol exposure vary significantly among populations with varying degrees of risk for alcohol consumption during pregnancy. Further studies are underway to fully examine the clinical utility of PEth as a biological screening test for prenatal alcohol exposure.

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Using Operational Analytics to Improve Lab Efficiency, Bottom Line Costs and Instrument Lifecycle Management

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Abstract

Pharma companies around the globe set up centralized support laboratories where large amounts of expensive analytical equipment are concentrated to serve multiple departments. The objective is to increase efficiency and capital utilization. The information generated in these laboratories are critical for compound selection and development. What is often underappreciated is the impact of operational instrument efficiencies on scientific output and the bottom line.

Operational efficiencies are defined here as the measurement of how instruments are behaving in the laboratory, particularly with regards to service activity, resulting in downtime and lower instrument utilization and throughput. Poor operational efficiencies translate to lack of instrument availability which in turn, impacts the drug research pipeline. This paper describes the transformation of operational discussions about laboratory instrument problems from anecdotal perceptions to real, actionable data. Raw data on instrument performance is transformed into easy to understand information using an innovative Analytics Dashboard. The Dashboard is part of the Merck LAMP program provided by PerkinElmer. This web based interactive tool provides clear visibility to instrument operational and instrument lifecycle data that previously was unavailable. Using this tool, a Lab Manager can quickly assess instrument behavior trends and identify both problematic instruments as well as symptoms that are a result of inadequate lab practices. It can also be used to manage the instrument lifecycle, providing actionable data to support instrument replacement as well as data to help ensure proper instrument inventory.

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Unified Drug Testing by Online SPE-LC/MS/MS: One Automated Method Measures ALL the Drugs in Urine and Oral Fluids

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Measurement of drugs of abuse in urine and/or oral fluids (OF) is common for pre-employment screening, DOT / federal mandated testing, law enforcement, and compliance / diagnostic determinations by physicians (with the latter two growing rapidly). While a variety of methods are available for these measurements, the fastest growing & preferred approach is LC/MS/MS because of the high degree of certainty it provides for simultaneous determination of both identification and concentration of drugs. While continued growth of LC/MS/MS for the measurement of drugs of abuse in urine and OF seems certain, there are several technical challenges that need to be met. These needs include being able to easily measure low dose drugs at or near 1 ng/g concentration (for medical purposes, Pesce, et. al. 2012 AACC conference, as well as zero tolerance testing), simplicity for performing measurements with lab technicians, and the ability to achieve high productivity for all work while minimizing the labor and number of workflows required.

In an effort to meet these needs, we have developed an automated on-line SPE-LC/MS/MS method. It uses SPE to clean and pre-concentrate samples so that low dose drugs in urine at 1 ng/ml concentration are easily measured at S/N ≥20. The method's design is balanced to address (identify / measure) all of the drugs (acidic and basic drugs as well as polar and non-polar drugs), as well as either urine or OF samples, all in one method, all in one workflow. Urine and OF samples even can be measured together on the same LC/MS/MS in the same run list. The method is simple, robust, and can be readily performed with a minimum amount of labor by lab technicians with MS familiarity. It is completely automated from sample plates/vials to results (with no change in work flow while still using only the native MS software) and can process two 96-well plates of samples overnight per LC/MS/MS. The results will be waiting for you in the morning.

Total automation is achieved using the PAL system LC autosampler which performs all sample preparation, including the SPE in parallel to LC/MS/MS analysis, & injects the sample into the LC/MS/MS. The cycle time achieved for on-line SPE-LC/MS/MS was 4.5 minutes for 71 drugs (opiates, metabolites, illicits, opioids, barbs, benzos, and THCA) in urine. The SPE methodology pre-concentrates the drugs so they are easily measured by the LC/MS/MS (high intensity, low background LC peaks) and high success rates are achieved for automatic integration of LC peaks. This allows the method to function well with any LC/MS/MS instrument (by simply adjusting sample volume loaded on SPE cartridge) and simultaneously allows highly proficient confirmation of low dose drugs (ca. 1 mg/day dosing) at 0.2 ng/ml concentrations in oral fluids.

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HSQMBC-TOCSY Structure Elucidation's Valuable Tool

Janine Brouillette – Merck West Point

HSQMBC-TOCSY is a relatively new NMR technique that enables the observation of very long-range heteronuclear coupling pathways. Whereas HSQC-TOCSY uses an out-and back transfer mechanism from a proton to its directly bound carbon via $^{1}J_{CH}$ followed by a TOCSY transfer via the H-H coupling network, the HSQMBC-TOCSY experiment employs an initial long-range heteronuclear transfer mechanism so that correlations to non-protonated carbons, which are not accessible in HSQC-TOCSY, are accessible in HSQMBC-TOCSY. Oligomycin A which is an inhibitor of ATP synthase is used as a model compound to show the utility of ^{1}H-^{13}C HSQMBC-TOCSY experiments in complex molecules.
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Development of an LC-MS/MS Assay for Simultaneous Quantification of ADMA and SDMA Using A Surrogate Matrix and Derivatization with Fluorescamine

Venkatraman Junnotula, Shelby Gorman - GSK

Abstract:

Arginine (ARG) and its methylated metabolites, monomethyl arginine (MMA), asymmentric dimethyl arginine (ADMA) and symmetric dimethyl arginine (SDMA) play an important role in the synthesis of nitric oxide (NO). In addition, circulating ADMA levels are known to be elevated in various diseases. Data on SDMA show that it is a useful marker for renal function. Therefore, accurate quantification of ADMA and SDMA is very useful in disease diagnosis and drug development. However, there are several analytical challenges associated with the quantification of ADMA and SDMA. These challenges include poor retention and separation on reverse phase chromatography columns, selectivity issues due low molecular weight, high polarity and structural similarity of ADMA and SDMA, and the presence of endogenous levels. To overcome these analytical challenges, derivatization of these analytes with fluorescamine and utilization of bovine serum albumin (BSA) as a surrogate matrix (analytes free matrix) was employed. This approach was successful over the concentration range of 5–1000 ng/mL for both ADMA and SDMA in human plasma. In addition, this method was successfully transferred to a variety of biological matrices including blood, urine, and tissue homogenates across multiple preclinical species and used in the support of several investigative studies. The data show that this simple method is selective, sensitive and robust. To our knowledge, this is the first LC–MS/MS based method that utilizes a surrogate matrix and derivatization with fluorescamine and provides adequate sensitivity for both ADMA and SDMA with a minimum of 10 µL of sample and a run time of 3.0 min.

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Disulfide Linked Linear Peptides Insulin, ANP, ShK : Automatic Assignment of Exact-Mass ESI-MS/MS Fragment Ion Structures Using the MASSPEC Algorithm

Authors

Marshall M. Siegel, Gary Walker (MS Mass Spec Consultants, Fair Lawn, NJ),
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Abstract

Fragment ion assignments in the ESI-MS & ESI-MS/MS mass spectra of linear peptides with multi-disulfide bridges are a challenge to analyze. In addition to the standard peptide cleavages (a,b,c,x,y,z) and small molecule losses (water, ammonia, etc.), the disulfide linkages may cause up to four additional fragmentation processes generating persulfide, dehydroalanine, cysteine and thioaldehyde products.

The MASSPEC algorithm was designed to exhaustively compute all possible ion substructures consistent with mass spectral fragmentation rules. The MASSPEC algorithm then correlates the substructure masses with observed fragment ion masses and scores the assignments. In this way, the MASSPEC algorithm is a powerful tool for the elucidation of the complex fragmentation processes observed in the ESI-MS & ESI-MS/MS spectra of disulfide linked peptides undergoing modifications such as chemical, metabolic and enzymatic modifications.

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A COMPARISON OF MICRO- AND MACRO- BLOOD SAMPLE COLLECTION FOR BIOANALYSIS OF A LARGE MOLECULE AND THE IMPACT ON HEMATOLOGY PARAMETERS

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A comparative assessment of toxicokinetic (TK) results obtained through use of standard macrosamples (0.4 or 0.5 mL) versus those from microsamples (32 or 80 µL) was conducted. Presented here is data from two separate studies, both involving IV and SC administration of a large molecule (biologic) to rats once weekly, for either 2 doses or 13 doses.

For the 2-dose study, TK microsamples were collected from all animals at all time points while reference macrosamples were collected for a limited number of predetermined time points. Microsamples were collected via tail vein and macrosamples by jugular venipuncture.

For the 13-week dose study, TK and immunogenicity (IG) blood microsamples were collected over a full timecourse from main study animals, the same animals from which hematology parameters were evaluated. Macrosamples were collected for a limited number of predetermined time points. Both micro- and macrosamples were collected via jugular venipuncture. A single microsample was used for assessment of both TK and IG.

Validated MSD assays (adapted for analysis of low volume serum microsamples) were used for assessment of TK and IG. Bioanalytical results obtained from micro and macro TK samples were considered comparable in both studies. IG results from the 13-week study correlated well with the TK parameters in individual animals. Consequently, the microsampling technique is considered to be adequate also for assessment of IG.

Hematology samples collected at usual volumes were within historical range for the species, strain and age of the animals, and were therefore considered unaffected by the repeat sampling. In conclusion, bioanalytical results obtained from micro and macrosamples were comparable at concurrent time points for the large molecule evaluated, the use of microsampling allowed a full TK profile evaluation from main study population with no impact on hematology parameters.

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Validation of a Hybrid LBA/LC-MS Assay for the Quantitative Determination of Conjugated-Payload of Antibody-Drug Conjugate (ADC) in Human Plasma.

Yulia Kim, Ang Liu, Rutika Patel, Shannon Chilewski, Robert Dodge, Renuka Pillutla, and Jian Wang
Bristol-Myers Squibb

Antibody-drug conjugate (ADC) described in this presentation consists of a fully humanized IgG antibody and a cytotoxic payload through a cleavable linker. A hybrid LBA/LC-MS assay was fully validated for the measurement of the conjugated-payload which is the payload molecule that is still conjugated to the antibody in the circulation and thus is available to bind to the tumor cell. This hybrid approach coupled the complementary analytical advantages of ligand binding (LBA) and LC-MS based techniques. Essentially there were three steps, immuno-capture, payload cleavage, and sensitive quantitation of the cleaved payload using LC-MS/MS.

The validation experiments included accuracy and precision, determination of the lower limit of quantification (LLOQ), selectivity, carryover, recovery, assay robustness, analyte stability, and target interference. The LLOQ was 0.270 ng/mL of conjugated payload which is equivalent to 20 ng/mL of ADC. The intra-day and inter-day precision (CV %) was within 5.3% and accuracy within 5.9% from nominal. The analyte was found to be stable for at least 28.5 hours in human plasma at room temperature and stable after 5 freeze-thaw cycles. There was no interference from soluble target at the estimated maximum level in patients. The method also demonstrated flexibility during the step of immuno-capture of the ADC from biological matrix, which can be conducted on both Agilent Bravo system using AssayMAP™ cartridges and TECAN Freedom Evo system using PhyTips™.

In this presentation, the validation experiments and results as well as the challenges encountered during method development will be discussed.