



POSTER SESSION ABSTRACTS

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POSTER SESSION
Tuesday, October 4 1:15 PM - 3:00 PM

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Langhorne, PA

Where Technology and Solutions Meet

QUANTITATION

Bioanalysis

ADDA: A new LC/MS/MS sample delivery platform for drug discovery bioanalysis

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The service model that most resembles drug discovery bioanalysis may be a fast food restaurant, that serves breakfast, lunch and dinner all day, is located at a busy intersection and is strictly take out. That is, the number of new chemical entities (NCE), types of samples and variety of in vitro and in vivo studies that require analysis is large and quite diverse. There is rarely time for formal method development since data turnaround is the highest priority. These circumstances engender the need for a triage approach wherein samples are directed toward the optimal method within a well-defined set of bioanalytical options. The ADDA instrument is an LC/MS/MS sample delivery system that features flexible hardware design coupled with unique software automation to enhance throughput in LC/MS/MS bioanalysis drug discovery. The instrument is designed to perform high-throughput LC/MS/MS (8-10 sec/sample) for screening and in-vitro bioanalysis, as well as, multiplexed LC for traditional gradient or isocratic LC approaches applied for in vivo sample analysis. The instrument control software is designed to integrate with DiscoveryQuant software (AB Sciex) and a global database of MS/MS conditions, this approach allows seamless linkage of MS platforms, users and methodologies. This combination of hardware and software technology is well suited to address the broad range of bioanalytical tasks encountered in drug discovery and provide the highest quality data in a time and resource efficient manner. The details of ADDA and related software automation, including application examples will be described.

POSTER ABSTRACT

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QUANTITATION
Bioanalysis

Qualification of a Robotic Liquid Handler to Perform Bioanalytical Validation or Sample Analysis Runs

Ron Shoup

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Programs based on Hamilton's Venus One software were developed to analyze Watson LIMS Worklists and build processing sequences for a Hamilton Microlab Star liquid handler. The program was based on 3 modules: barcode recognition of sample ID's and comparison to Watson worklists, the construction of a matrix standard curve, and the creation and transfer of aliquots of each type of sample to 96-well plates. The liquid handler was first verified to factory specifications for precision and accuracy of liquid transfers. An Operational Qualification Plan was then written as a series of 10 scripts based on 4 analytical runs that covered every type of validation or study sample expected from current regulatory guidance. The samples were based on a validated method for methadone and its EDDP metabolite in human plasma. The execution of the scripts involved LC-MS/MS or colorimetric measurement.

The test scripts evaluated the performance of the system towards recognizing the requirements of each Watson Worklist. System-generated standard curves covering 3 orders of magnitude demonstrated mean bias less than 5%, with most values less than 2%. Barcoded sample positions were recognized and diluted correctly for transfer to plates. Quality control pools were measured with CV's ranging from 3% to < 1%. Carryover and control negative samples were never contaminated in any machine transfers. Analytical runs up to 250 samples were interpreted and processed correctly.

Validation samples were identified by the system according to Watson Sample ID or barcodes relating to each type of special sample. Samples were correctly aliquotted by the program, according to: the required base material (blank matrix, neat solvent, or prepared matrix sample), any dilution factor, and the use of a normal or surrogate internal standard.

Automated processing of samples on the Hamilton system gave results that were more accurate and precise than manual means. The test scripts provided a reasonable confidence that the system would improve lab efficiency and eliminate inter-analyst dependencies on assay performance. The operating program permitted the complete construction of assay validation run sets without human intervention.

QUANTITATION

Bioanalysis

Achieving Regulatory Approval: Overcoming Bioanalytical Challenges in an Onglyza™ Micro-tracer Absolute Bioavailability Study with Accelerator Mass Spectrometry

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Characterization of absolute oral bioavailability (BA) is useful for oral drug development and is a requirement of some Health Authorities. Here we present results from an absolute BA study in which a microdose of intravenous (IV) [¹⁴C]saxagliptin was administered concomitantly with an efficacious oral dose of saxagliptin (BMS-477118, Onglyza™). Orally administered saxagliptin was measured in plasma using a validated protein precipitation method with LC-MS/MS detection. Intravenously administered [¹⁴C]saxagliptin was determined by Accelerator Mass Spectrometry (AMS) after protein precipitation, UPLC-fractionation, and graphitization. The graphitization process results in a loss of structural information, therefore, chromatographic separation to fully resolve a drug from its metabolites is required to achieve assay specificity. Many practices involved in LC-MS/MS method development and validation are less appropriate for the AMS method. Challenges related to suitable sample preparation methodology for AMS and their solutions will be presented. A technique-appropriate validation was conducted that fully demonstrated the accuracy, precision, stability, specificity and recovery of the AMS method across the concentration range of 0.025 to 15.0 DPM/mL (equivalent to 1.91 to 1144 pg/mL). The raw AMS measurement, ¹⁴C/C ratio, expressed as fMC (fraction Modern Carbon) and known DPM/mL of [¹⁴C]saxagliptin standards, were used to construct a weighted (1/y²) least-squares, linear regression model and used to predict the concentration of unknowns. The absolute bioavailability of saxagliptin was calculated at ~50%. Pharmacokinetic results indicated good agreement in the terminal phase half-life values between the intravenous and oral routes demonstrating that the IV radioactive microdose provides a sound approach to determine absolute bioavailability.

POSTER ABSTRACT

#4

QUANTITATION
Bioanalysis

Comparison of Microsampling Techniques for Quantitative DBS and DPS Preparation

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Purpose:

Recent studies concerning the possible impact of hematocrit and other matrix effects on dried blood spot (DBS) sampling points to the need for accurate and precise volume control for dried matrix spot (DMS) applications. A number of microsampling techniques can be used to transfer samples taken for spotting; however, most require significant user effort and few produce multiple spots. Here, we evaluate the precision of a prototype device that is easy to use and produces multiple spots from a single sample draw.

Methods:

Five - 15 μ l aliquots of whole sheep's blood were spotted onto Whatman FTA DMPK paper using Drummond's prototype DBS Incremental Dispenser with plugged microcapillary tubes. The precision of the aliquot volume dispensed was measured gravimetrically and compared to DBS samples prepared in a traditional manner (Drummond Microcaps with bulb dispenser). The Drummond prototype dispenser was also tested for precise spotting of 10 μ L plasma spots, using a novel self-sealing microhematocrit capillary tube.

Results:

Preliminary studies demonstrate that sample volume variations, based on SD and CV calculations, can be significant (CVs from 3.2% - 4.8%) and somewhat technique dependent when spotting with Microcaps, whereas Drummond's DBS Incremental Dispenser with plugged microcapillary tubes offers better consistency and control (CVs from 2.6% - 4.2%) over the range of aliquot volumes tested. Similar results (CVs from 2.4-2.9%) were also obtained for 10 μ L plasma spots, using Drummond's self-sealing plasma separating capillary tubes.

Conclusions:

The prototype Drummond's DBS and DPS dispensers introduced herein afford the ability to perform collection of blood sample, plasma separation (if necessary), and dispensing of multiple quantitative aliquots, all of a single tube. In addition to producing sample aliquots that are highly accurate and repeatable, the prototype dispensers do so in a manner that is both efficient (no time lost changing tips), cost effective (1 tube versus 3-5 tubes), and humane (reduces the number of draws taken from each animal). Further microsampling studies for quantitative DBS are soon to be completed, and evaluation of similar techniques for dried plasma spot (DPS) applications is planned.

QUANTITATION

Bioanalysis

Development of a bioanalytical DBS assay using a tandem quadrupole with a novel collision cell design

Joanne Mather, Paul Rainville, Debadeep Bhattacharya, and Rob Plumb
Waters Corporation, Milford, MA

Dried blood spots (DBS) have been used for over 20 years in neonatal testing. In DBS, a paper blotted heel or fingerprick is dried and shipped for analysis. This approach has now been applied to the field of bioanalysis in preclinical, toxicokinetic, and clinical studies. DBS' benefits include low sample volumes, improved study recruitment, toxicokinetic data from one animal, reduced animal usage, storage and transport expenses. However, the small volumes typical of the DBS samples require highly- sensitive LC/MS/MS assays. In addition, the cards can present matrix issues. Here we present the use of an UPLC/MS/MS technology for high sensitivity analysis of pharmaceutical compounds in DBS cards.

The DBS cards are available in treated and untreated formats. The antiviral-antiinfective chemicals on the treated cards can dissolve during extraction and can interfere with the analyte signal in LC/MS. In order to amend for any background signal from the card, special care should be taken during chromatographic method development. In this study, a new type of tandem quadrupole MS with a novel collision cell design was used that allowed simultaneous collection of full scan and MRM data. The data was collected for three types of blood spot cards spiked with Alprazolam.

The chromatography data shows significantly lower background signal for the untreated cards compared to the treated cards. The full scan MS data show that the treated cards have an increasingly intense ion current as the organic concentration of the LC gradient increases. This is also reflected in the response of analyte ion where the signal response is reduced with the treated cards. The Alprazolam assay was shown to be linear over the calibration range of 100pg/mL - 500ng/mL. This UPLC/MS/MS approach was used to analyse Sitamiquine from blood spots with an LLOQ of 50pg/mL and a linear dynamic range of 4 orders of magnitude. The QC data shows the low concentration QCs had RSD of 2.08 % and the highest QC a reproducibility of 3.8 %. This data shows that high sensitivity assays can be developed with good reproducibility using DBS cards and UPLC/MS/MS.

POSTER ABSTRACT

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QUANTITATION
Bioanalysis

Comprehensive Study Examining Interfering Compounds from Various Dried Blood Spot Cards and Extraction Solvents with ESI Positive Tandem Quadrupole MS

Jennifer Simeone¹; Chester L Bowen²; Joanne Mather¹; Robert Plumb³; Debadeep Bhattacharya¹, and Paul Rainville¹

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Dried Blood and Plasma Spots (DBS) in rodents eliminate any terminal procedures necessary for adequate sample volume and also supports use of fewer animals, better data quality, and cost-savings in shipping and storing samples. However, potential interferences may arise from these cards including the additives that denature proteins, lyse cells, and stabilize DNA. This study illustrates the most common interferences seen from a variety of cards and extraction solvents.

DBS cards (Whatman DMPK-A, DMPK-B, DMPK-C and 903) were investigated. Methanol, acetonitrile, water, formic acid and zinc sulfate in varying combinations were used for extraction. Methyl-tert-butyl ether and acetone: acetonitrile were also tested to simulate liquid-liquid extractions (LLE). Cards (blank or spotted) were punched followed by addition of solvents, vortex-mixed, and centrifuged. The supernatant was transferred directly into injection vials. For LLE, the supernatant was transferred, evaporated and then reconstituted with methanol: water. Samples were then injected onto an UPLC column and eluted using a linear gradient followed by MS using a tandem quadrupole MS operating in positive electrospray mode.

The extracted samples from different cards showed differences in the spectra when analysed by MS scan over the range of 100 - 1000 m/z. Each card type showed unique patterns of interfering ion transitions and the noted transitions and retention times varied based on the organic mobile phase. All card types showed identical spectra of biological interferences based on matrix. For extraction solvents containing water, increased matrix components were observed compared to solvents without water. Multiple punches taken from each individual card type and punches from multiple cards showed no discernable variation in results. It is important to identify regions of potential interference with the analyte(s) of interest to avoid ion suppression and ensure overall reproducibility of an assay. DBS shows decreased abundance of biological interferences leading to improved assay reproducibility. Considering each card type provides a unique set of interfering transitions and differences based on mobile phases, it is possible to choose a particular card based on the known retention time of the analyte(s).

QUANTITATION

General

LC/MS/MS Quantification of Fluticasone Propionate in Human Plasma at the fg/mL Level

Joanne Mather, Kendon Graham, Debadeep Bhattacharya, and Rob Plumb
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Fluticasone propionate is a trifluorinated corticosteroid that has potent anti-inflammatory activity and is used to treat asthma and chronic obstructive pulmonary disease. As Fluticasone undergoes metabolism, there is negligible systemic exposure. For accurate measurement of pharmacokinetics, it is necessary to detect this compound in the sub- pg/mL level. Historically, LC/MS/MS instrumentation has not provided sufficient sensitivity to quantify the plasma concentrations of this compound at the fg/mL level. In this paper, we demonstrate the quantification of fluticasone at the sub pg/mL level using LC/MS/MS.

As Fluticasone propionate is dosed by the inhaled route, the majority of the drug is directed to the lungs and the remainder is eliminated in the liver via hydrolysis of the S- fluoromethyl carbothioate function to form the inactive 17-carboxylic acid metabolite. These factors result in circulating levels of the drug in the 0.5pg/mL - 1pg/mL level. The high protein binding ability also presents a challenge when developing a sensitive assay.

The samples were prepared by solid phase extraction. 375L of plasma was diluted with aqueous solution containing Stable Label Isotope (SLI) internal standard for both analytes and mixed well. The samples were applied to an OASIS HLB Elution plate, washed with an organo-aqueous solution and eluted in a solution of methanol- acetonitrile. This eluted solution was diluted with aqueous buffer prior to injection onto the ACQUITY UPLC/Xevo TQ-S system. The sub 2m separation resolved the active compound from endogenous interferences and metabolites. The novel ion guide optics in the MS resulted in sufficient sensitivity to detect and quantify fluticasone propionate at 0.75 pg/mL. The peak width of the fluticasone analyte was calculated to be approximately 3 seconds at the base, allowing for a high resolution separation of the analyte from the endogenous material in the sample. The calibration was shown to be linear over the range of 0.75 - 15pg/ mL, with the signal to noise ratio of the 0.75 pg/mL being in excess of 5:1. This level of sensitivity allows for the accurate determination of the pharmacokinetics of the Fluticasone propionate in plasma.

POSTER ABSTRACT

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QUANTITATION

General

A Direct LC-MS/MS Method for Determination of β -Alanine in Human Plasma

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Beta-alanine, a non-essential amino acid, is an important factor in building up muscle mass and strength. Abnormal beta-alanine levels in human diet have been reported to cause diseases such as cardiovascular disease, or symptoms such as paraesthesia, resulting from high blood-plasma concentrations of beta-alanine. Therefore, monitoring beta-alanine levels in human plasma samples may play a role in disease diagnosis and prevention. An LC-MS/MS method has been developed for use in this monitoring. The method can detect beta-alanine at level of detection (LOD) of 2 ppb in water. The limit of detection and quantification (LOQ) in plasma will be presented.

Quantitative analysis of molecules below 100 daltons can be a challenge. Most of the existing methods for amino acid analysis involve a derivatization step, which is time-consuming. When electrospray ionization (ESI) is used as the ionization method, plasma samples present an additional challenge as they contain phospholipids which can result in matrix effects, specifically ion suppression.

The rapid resolution method presented here uses an internal standard rather than derivatization for quantitative analysis. The instrument utilized is an Agilent 1200 HPLC and 6410 Triple Quadrupole MS equipped with a hot box upgrade operated in positive mode.

QUANTITATION

General

The Separation of Basic Analytes on a Polar-modified C18 HPLC Stationary Phase

David W. House, Asha A. Oroskar, and Anil R. Oroskar

Orochem Technologies, Inc.

The separation of basic organic compounds on typical C18 HPLC stationary phases is not always straightforward. Gradients, buffers, modifiers, and column temperature are some of the variables that are often used in the quest to separate closely related compounds with reasonable peak asymmetry. Orochem Technologies has introduced a new C18 HPLC stationary phase that contains specially designed polar sites, which permit excellent separations of difficult amine and amide mixtures. These separations can often be carried out using mobile phases based on blends of water and alcohols or water and acetonitrile without the need of buffers or modifiers. Although these stationary phases contain polar sites they retain their high hydrophobicity. These stationary phases, trade named Orosil[®], μ Polar C18, are available in particle sizes of 1.7 micron for UHPLC applications; 3, 5, and 10 microns for typical analytical applications; and from 20 microns up to 300 microns for semi-prep, preparative, and SMB (simulated moving bed) applications.

POSTER ABSTRACT

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QUANTITATION

General

“Mass Spec friendly” Plastics Safe for Quantitative Mass Spectrometry Analysis

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The use of high-quality materials in sample preparation is critical for analytical applications using mass spectrometry. Leaching of chemicals from plastic and/or rubber may contaminate samples resulting in appearance of additional peaks in mass spectra which interfere with analyte analysis. We developed a quality control procedure for plastic consumables used in mass spectrometry and evaluated plastic products manufactured by a number of vendors. We detected significant contamination in methanol, acetone or acetonitrile washes from plastic products acquired from some vendors. The contamination was also present when consumables were pre-washed with water suggesting that chemicals were extracted from plastic by the organic solvents. To develop a product line of consumables for mass spectrometry applications we identified ways of effectively removing contamination from plastic. These “mass spec-friendly” plastics do not require conditioning and do not contaminate samples and therefore can be safely used in all analytical protocols including mass spectrometry analysis.

CHARACTERIZATION & IDENTIFICATION

General

A Novel High Throughput Method Using Full Scan HRAM and Online Extraction for Plasma Protein Binding Determination

Kevin Cook, Mark Dreyer, Keeley Murphy, Nicholas Duczak

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The ability of a drug to reach its intended therapeutic target as well as its ability to be eliminated from the body is affected by the reversible binding to plasma or serum proteins. Equilibrium dialysis is the accepted method for estimating protein binding. Rapid equilibrium dialysis speeds throughput when compared to the traditional method through its automation friendly footprint and because of high surface area to volume ratio of the membrane compartment which reaches equilibrium within four hours. To further increase throughput, the need to optimize each compound for QQQ analysis is eliminated by taking advantage of full scan HRAM analysis.

Plasma containing drug was added to one chamber while buffer was added to the second chamber separated by a semi-permeable membrane (8K MWCO). The samples were incubated at $\sim 37^\circ\text{C}$ while shaking at 100 rpm for 4 hours. Afterwards, an aliquot from each chamber (200 μL plasma, 300 μL buffer) was removed and equal amounts of fresh plasma and buffer were added to respective incubated aliquots. The protein/buffer mixtures were precipitated using an acidified organic ISTD cocktail solution, thoroughly mixed, centrifuged, and the supernatant was transferred to a 96-well plate for subsequent analysis.

Protein binding determination was conducted in both human and rat plasma for a wide spectrum of low to high binding compounds such as Gabapentin (literature value: <3%), Levofloxacin (24-38%) and Warfarin (98-100%). In an effort to create a generic workflow, incubations were performed at a single concentration for the compound library analyzed. The total time to analyze the entire 16 compound sample set ($n=4$), for both human and rat, was approximately 5 hours. Overall, the data acquired agreed with literature values with acceptable reproducibility using either a simple mean or well-to-well calculation. The time savings provided with this workflow is useful in both a discovery and development environment due to the elimination of off-line sample preparation, chromatographic multiplexing, and removing compound optimization required for triple-stage quadrupole instruments.

POSTER ABSTRACT

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QUANTITATION

General

Quantitation of metabolites in plasma samples by UV-MS correction using a dual-cell-linear ion trap mass spectrometer

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The quantitative assessment of metabolites in samples from development in vivo studies has become an area of increasing study with the release of guidances from regulatory bodies (MIST and M3 R2) and their subsequent interpretation and implementation. We have applied a UV correlation to assign concentrations to metabolites from in vitro generated samples and used the results to quantify plasma samples on the same instrument.

Initially, metabolites of dextromethorphan were generated in vitro through incubations for 2 hours at 100 μM Dextromethorphan for 2 hours to assure the creation of significant concentration of metabolites. Samples were analyzed by UHPLC-UV along with a standard curve of dextromethorphan prepared in microsomal matrix (50 μM to 1 μM) to assign absolute metabolite levels. The molar absorptivity of dextromethorphan and its metabolites was assumed to be the same. The in vitro sample was diluted to create a standard curve in plasma at known concentrations for UHPLCMS analysis on a Velos Pro dual pressure linear ion trap. The significant difference in formation rates in vitro required two separate dilutions be prepared to prepare standard curves of the appropriate expected range for each analyte. Plasma samples for UHPLCMS analysis were prepared by spiking known amounts of dextrophan and methoxymorphinan (between 750 and 5 ng/mL) into plasma extraction matrix followed by mixing with in vitro microsomal matrix. A total of 12 spiked concentration values were prepared for each analyte. Quantification of the spiked plasma samples against the UV-generated metabolite standard curve was compared to the known spike values and also confirmed for two metabolites by standard curves created with synthetic standards. For all the samples analyzed, the calculated concentration was within 2X the known value when quantifying based on the in vitro UV-MS correlation standard curve. Quantitation was performed by targeted selective reaction monitoring (SRM) as well as full scan MS2 on dextromethorphan, dextrophan, and methoxymorphinan without the use of timed segments.

QUANTITATION

General

Optimization of Nanospray Voltage and Spray Stability: Impact on Chromatographic Peak Area for Analyte Quantitation

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State-of-the art liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis uses a constant electrospray (ESI) voltage for data acquisition. Modern qualitative and quantitative LC-MS/MS methods depend on highly efficient gradient elution chromatography. The changing chemical composition of mobile phase during gradient elution results in an inherent disconnect with single point ESI voltage optimization. A constant ESI voltage limits spray stability and compromises chromatographic peak area quantification, limiting total peak area and increasing peak area relative standard deviation (RSD). Using a nanospray source equipped with a digitally controlled stage and software for precise and reproducible emitter positioning for data acquisition we investigate the relationship between spray stability and data quality at flow rates of 200 nL/min, 500 nL/min and 1000 nL/min.

POSTER ABSTRACT

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QUANTITATION
Peptides & Proteins

Evaluation of 150 μm ID Packed Tip Columns for Quantitative Peptide Analysis by LC-MS/MS

Stanley Durand; Helena Svobodova; Amanda Berg; Gary Valaskovic
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A predominant workflow for qualitative proteomics has been "GeLC-MS," a combination of 1- (or 2-D) gel electrophoresis with reverse-phase nanoflow liquid chromatography mass spectrometry (nLC-MS/MS). The limited protein quantity isolated from a single gel band coupled with column loading capacity maximums necessitate the use of 75 μm ID packed columns for optimal sensitivity. However, limitations on sample injection volume, gradient and flow characteristics, and excessive delay volume hinder throughput. Novel methods for fractionating complex biological samples with higher loading capacities and more efficient recovery, such as novel solution phase tube-gel fractionation and others, demand a column format which maximizes on the extended dynamic range of these emerging techniques. Packed tip columns with a larger ID (150 μm to 200 μm) facilitate higher sample loading capacity and enable higher flow rates for improved cycle time while maintaining the optimal sensitivity realized in the nanobore packed tip column format. Using peptide standards, single protein digests and whole yeast digests improvements in cycle time and sample loading capacity using 150 μm ID packed tip columns are demonstrated.

QUANTITATION

Peptides & Proteins

Development and Validation of a Method for the Determination of a Genetic Fusion Protein in Human Plasma using UHPLC-MS/MS for First Time in Human (FTIH) Study Support

Bonnie Orr, Matthew Szapacs, Jonathan Kehler, Chester Bowen
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Currently, immunoassay is considered the 'gold standard' for detection and quantitation of biopharmaceuticals for the support of pharmacokinetic exposure studies as it provides an extremely sensitive platform to develop quantitative assays in biological matrices. The use of LC-MS/MS has gained considerable attention as a method to quantitate proteins from biological matrices as a result of its increased dynamic range and selectivity compared with immunoassay methods.

Data will be presented detailing development and validation of the GLP compliant method to support the FTIH study. The validated analytical method is based on protein digestion using the endoproteinase enzyme Lys-C. A specific peptide fragment is then extracted by solid phase extraction, followed by UHPLC/MS/MS analysis. The lower limit of quantification (LLQ) is 50 ng/mL, using a 50 μ L aliquot of human plasma with a higher limit of quantification (HLQ) of 10,000 ng/mL. Additionally, the assay needed to take into account any interference caused by concomitant drug during study (acetaminophen).

The data will show selection of the precursor ion for monitoring based on the analysis of the peptide map generated using the endoproteinase enzyme Lys-C. Selectivity, sensitivity and linearity of the method in addition to stability in human plasma and blood at various temperature conditions will be presented.

POSTER ABSTRACT

#16

QUANTITATION
Peptides & Proteins

Multiplexing SIMs on a Novel Benchtop Orbitrap MS with a Quadrupole Mass Filter to Increase Sensitivity for Peptide Quantitation

Kevin L. Cook, Patrick Bennett, Nicholas Duczak, Louis Maljers
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In bioanalysis, triple quadrupole MS systems have been predominantly used for peptide quantitation. Although triple quadrupole systems generally offer the most sensitive and robust quantitation, the poor fragmentation and multiple charge states of large peptides can result in weak selected reaction monitoring (SRM) signals. In this investigation, we demonstrate the accurate and precise quantification of peptides in a biological matrix using a new approach that utilizes multiplexed SIMs capability provided on a novel benchtop quadrupole-Orbitrap mass spectrometer. Multiplexed SIMs refers to multiple fills of the instrument's C-trap with quadrupole-filtered ions, prior to a Orbitrap mass analyzer scan. We employed a ten-minute LC method at 150 $\mu\text{L}/\text{min}$. Mass spectrometry analysis was performed using a quadrupole-Orbitrap LC-MS/MS systems equipped with a heated electrospray ionization (HESI) source. A generic method development approach was used to analyze Insulin, Exendin-4 and GLP-1 peptides, between 10 $\text{pg}\cdot\text{mL}^{-1}$ and 10 $\mu\text{g}\cdot\text{mL}^{-1}$ with good linearity and reproducibility.

QUANTITATION

Biomarkers & Metabolomics

Analysis of a Therapeutic Antibody and a Biomarker in Parallel Utilizing Immunocapture and LC-MS/MS

Neha Akella, Jonathan Kehler, Matthew Szapacs and Scott Hottenstein

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A method was developed to measure a therapeutic antibody and its corresponding antigen in a single LC-MS/MS method. Immunoassay was not viable as there were selectivity and reagent availability issues. The method was needed for support of a safety assessment study planned using transgenic mice. Because of limited sample volume a dual LC-MS/MS assay measuring both the therapeutic and SAP (PD biomarker) needed to be developed. The poster will show method development and validation work done to develop both antibody and antigen methods for safety assessment support.

POSTER ABSTRACT

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QUANTITATION
Biomarkers & Metabolomics

A Sensitive Assay for the Measurement of Endogenous Levels of Angiotensin Peptides as a Biomarker Utilizing HPLC-MS/MS Detection

Jonathan Kehler, Matthew Szapacs and Neha Akella

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Angiotensin is a potent vasoconstrictor. When circulating in the body Angiotensin I is converted to Angiotensin II by cleavage of the dipeptide histidine-Leucine from the C-terminus of Angiotensin I by the Angiotensin converting enzyme (ACE). Angiotensin II is converted to Ang 1-7 by Angiotensin converting enzyme 2 (ACE2) which is further broken down to Ang 1-5.

A method for the determination of Ang II, Ang 1-7 and Ang1-5 was developed using LC-MS/MS and a one run validation was successful for the range of 5 to 1000 pg/mL for Angiotensin II and 1 -7 and 10 to 1000 pg/mL for Angiotensin 1-5. All precision and bias values were +/- 20% which meets acceptance criteria. Angiotensin II and 1-7 were quantitated to support two toxicology studies which provided critical biomarker data for the project. The method is sensitive, selective and robust which successfully analyzed in vivo study samples

QUANTITATION

Biomarkers & Metabolomics

Overcoming the challenges in targeted and non-targeted metabolite profiling of myxobacterial secondary metabolites using UHR-Q-TOF-MS analysis

Daniel Krug¹; Nina Cortina¹; Gabriela Zurek²; Aiko Barsch²; Verena Tellström²; Rolf Mueller¹

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2 Bruker Daltonik, Bremen, Germany

Introduction

Several secondary metabolites produced by Myxobacteria exhibit potent biological activities and are currently under investigation as potential leads for novel drugs. Hence, these bacteria are a promising source of natural products. However, the number of metabolites identified to date is significantly lower than expected from genome sequence information. Thus, the discovery of novel secondary metabolites from genetically proficient myxobacterial producers presents an on-going challenge. Wildtype and mutant myxobacteria strains were analyzed to determine the production patterns of known metabolites and to discover new metabolites.

Data

Here, we present an ESI-UHR-Q-TOF based analysis of myxobacterial secondary metabolites, which enables several challenges frequently encountered in metabolite profiling studies to be solved. The challenges comprise the simultaneous need for fast, robust, and sensitive analysis with high resolution, accuracy and excellent reproducibility. Analytical solutions for targeted and non-targeted metabolomics experiments by means of ESI-UHR-Q-TOF-MS are discussed.

Since mass accuracy and resolution of TOF instruments are independent of the acquisition rate, they are perfectly suited for a coupling to U-HPLC separations. These hyphenations enable a reduction of analysis time in combination with high chromatographic resolution and therefore increasing sample throughput. Acquisition rates of up to 20Hz were achieved without any compromise in mass accuracy or resolution.

Targeted and non-targeted metabolite profiling: Acquisition of full scan accurate mass spectra enable the targeted screening of known compounds e.g. from the class of DKxanthenes based on very selective, high resolution EIC (hrEIC) traces with small mass windows of 1.0 - 0.5 mDa. A comparison of several datasets following a comprehensive extraction of molecular features combined with statistical analysis enabled the discovery of novel biomarkers using a non-targeted approach from the same data files used for the targeted analysis.

Identification

Mass accuracies of 0.1 ppm are often not sufficient for unambiguous formula identification for m/z values above 500. A combination of accurate mass data and isotopic pattern information in MS and MS/MS spectra can extend this m/z range for reliable formula suggestions. The metabolites (?) Myxalamid A and DK-Xanthen-548 were identified using this approach as compounds that differ in abundance between the myxobacterial strains analysed in this study.

POSTER ABSTRACT

#20

QUAL / QUAN

Comparison of Accurate Mass and Nominal Mass MSMS for the Simultaneous Acquisition of Qualitative and Quantitative Data in DMPK Studies

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A crucial phase of the drug discovery process is quantitative and qualitative analysis of candidate pharmaceuticals and their metabolites in biological fluids. Typically, a triple quadrupole (TQ) mass spectrometer is used for quantitative analysis, while qualitative data is derived from hybrid quadrupole time of flight (QToF) or ion trap instrumentation. In this study, compare the benefits and drawbacks for the simultaneous acquisition of qualitative and quantitative LC/MS/MS data using either a QToF or a TQ instrument. The throughput, sensitivity, and spectral quality were compared using 20 structurally diverse compounds in blood products.

The TQ mass spectrometer was used to simultaneously acquire LC/MS data in MRM mode for quantitative analysis and full scan mode for qualitative analysis of metabolites. The QToF was operated in alternating low and elevated energy mode allowing simultaneous collection of precursor and product ion data. QToFs can acquire data at fast acquisition speeds without reduction in mass accuracy or spectral quality, allowing a narrow mass window for data processing. This feature allowed use of a 0.5mDa mass window around the target analytes thereby offering a high degree of selectivity and excellent signal with 50pg/mL standards. The use of new detector electronics allowed for stable mass measurements and a wide dynamic range.

The average limits of detection (LOD) for the TQ mass spectrometer in MRM mode was ~10pg/mL while that of the QToF was ~50pg/mL for all 20 compounds. The fast data capture rate of the TQ mass spectrometer ensured that the LOD was not changed by the use of very fast gradient analysis. Compared to the 5 mins gradient, the QToF LOD was reduced ~5 fold with 2 minute gradients owing to the faster sampling rate affecting the ion statistics. Whilst the TQ mass spectrometer was more sensitive for quantitation, the full scan data used for metabolite detection with this instrument was ~50 times less sensitive than the QToF data. The QToF also provides more spectral information than the TQ mass spectrometer. Both instruments were found to be ideal for DMPK studies, with the TQ mass spectrometer being better for high throughput quantitation.

QUAL / QUAN

Simultaneous Determination of Metabolic Stability, Metabolite Identification and Profiling Using the Agilent 6550 iFunnel Q-TOF LC/MS System

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Timely and rapid assessment of metabolic stability, metabolite identification and profiling is critical for accelerating lead optimization and enhancing the success rate of drug candidates entering into drug development. Triple quadrupole LC/MS instruments using multiple-reaction-monitoring (MRM) have been the workhorse for quantitative analysis such as metabolic stability and profiling. However, this platform is optimized for high sensitivity target quantitation and not well suited for non-targeted qualitative analysis. For these reasons, metabolite identification (qualitative) is often performed in a separate analysis on different types of mass spectrometers. Furthermore, due to the limitation of sensitivity on traditional tandem mass spectrometers, a relatively high substrate concentration (i.e. 10-20 μM) is often required in order to identify metabolites with a broad coverage. The ability to obtain quantitation and identification in a single analysis makes metabolic stability, metabolite identification and profiling studies much more efficient. This also has the potential to increase assay productivity and decrease costs in drug discovery and development.

Herein, we present an integrated Qual/Quan workflow. The utility of the Agilent 6550 iFunnel Q-TOF LC/MS system for determination of metabolic stability, metabolite identification and profiling in a single experiment is demonstrated in the *in vitro* buspirone (1 μM) metabolism study in rat liver microsomes.

POSTER ABSTRACT

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CHARACTERIZATION & IDENTIFICATION
Metabolite Identification

Using UPLC-QuanTOF technologies and chemically intelligent software tools for confident metabolite identification and biotransformation localization in discovery and development

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Increasingly rich datasets collected by HRMS instrumentation are rapidly becoming standard methodology in the drug development arena. Generating meaningful qualitative and quantitative information from the raw data is a complex process. Understanding, curating and reporting this information for use both within a drug metabolism group and externally to other departments is laborious. To ensure unbiased and thorough analysis, Met ID is becoming increasingly reliant on software tools to aid expert users in their analysis. Tools such as Metabolyx have gathered many parts of this process into a workflow that allows improved identification and characterization of the metabolites in a more comprehensive and efficient manner. Within this package, the utilization of chemical information of the parent compound aids assignment and understanding of the parent compound using advanced in silico tools. This identification, characterization and use of structural and metabolic prediction to aid assignment and reporting will be demonstrated using rat liver microsomal incubations of Nefazodone. These chemically intelligent structural assignment tools are used to automate the structural assignment of the parent. Recently, these tools have been further applied to the localization of the site of the biotransformation for all of the identified metabolites through automated structural interpretation and visualization of the datasets. Once the metabolites have been characterized, additional predictive tools are available to the researcher. Improved integration with Meteor (Lhasa Inc) will be demonstrated, showing the ability to export the interpreted dataset directly from Metabolyx and further validate the pathways using thorough literature based, mechanistic understanding and filtering of plausible biotransformation structural possibilities. Continued software tool enhancements ultimately enhance and unlock the capabilities of HRMS instrumentation and allow scientists to make more confident analyses.

CHARACTERIZATION & IDENTIFICATION

Metabolite Identification

Identification of a complicated metabolite for a GSK drug candidate using the structure identification tool box

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Metabolite identification is an important and sometime challenging part of the drug development process. The use of accurate mass high resolution mass spectrometry and other tools in the structure identification tool box is very useful for the more challenging metabolites. Several of the tools in our structure ID tool box were used to identify a complex structure of a metabolite for a GSK drug candidate. . Generally full scan accurate mass and data-dependent MS_n from Orbitrap would provide enough data for identifying most of metabolites. A significant amount unknown metabolite M of a GSK drug candidate was observed in dog samples (urine and feces) from a mass balance study. The sample was very dilute due to the very low dose. A preparative LC separation provided concentrated samples to infuse. Nano mate infusion gave us the opportunity to acquire both positive a negative mode data. CID/HCD in both modes provided different and useful information. We also obtained useful data by changing the collision energy and isolation width to narrow down the location of the conjugation.

POSTER ABSTRACT

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CHARACTERIZATION & IDENTIFICATION
Metabolite Identification

High-resolution accurate mass-measurements and metabolite identification: an automated approach using fragment prediction in combination with fragment ion search (FISh)

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A novel metabolite screening and identification process combining high-resolution and accurate-mass data in combination with novel software-tools has been outlined. In order to evaluate an automated and software-driven pathway for metabolite screening and identification we processed high-resolution accurate mass data using the fragment ion search approach (FISh-approach) of the software Mass Frontier 7.0 (Thermo Fisher Scientific). Data-Dependent-MSⁿ-data (n = 2-3) was used for component detection and identification based on theoretically calculated fragments of the metabolite structures. The investigated metabolites were derived from Ticlopidine, a drug which is known to be extensively metabolized. Ticlopidine was incubated with human liver S9 enzymes at 10 μ M Ticlopidine, 3mM NADPH, and 3 mM S-Adenosyl-L-methionine chloride. Aliquots of the reaction solution were withdrawn at consecutive time points and the reaction was stopped by the addition of ACN. Analyses were performed on a LTQ-Orbitrap XL mass spectrometer. High-resolution accurate-mass data was directly exported to the Mass Frontier 7.0 software. After the automated removal of noise and baseline signals and the theoretical calculation of possible fragments of Ticlopidine, a general list of possible phase I and II biotransformation was applied and the LC-MS chromatogram was processed by a component detection algorithm. Fragment ion search (FISh) was then used to screen the detected components and the corresponding spectral trees to identify putative metabolites of Ticlopidine. The FISh approach was able to generate possible structures of metabolites by displaying the relationship between detected components with the matched m/z values and the corresponding fragment structures based on the calculated fragments of Ticlopidine. In order to control the quality of our results and to test the recovery of the automated FISh process in biological fluids, we add individual samples to different biological matrices and compared the final results in terms of spectral quality, detection of possible metabolites and proposed structures of the metabolites.

CHARACTERIZATION & IDENTIFICATION

Metabolite Identification

Application of a Novel Bench-top Orbitrap Mass Spectrometer with a Quadrupole Mass Filter for Metabolite Profiling in Drug Discovery

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We present the application of a novel bench-top quadrupole-Orbitrap MS for rapid drug metabolite profiling and identification. For in vitro drug metabolite identification and reactive metabolite screening by LC/MS, a bench-top ion trap MS or comparable low-resolution instruments that can perform polarity switching are routinely employed. The current study investigated the utility of a novel bench-top Orbitrap mass spectrometer equipped with a quadrupole mass filter and a collision cell in fast analysis of metabolic soft spots. This new platform was designed to meet needs for routine metabolite identification tasks by taking key advantages of polarity switching and HR-MS. Timolol, propantheline and rantitidine (10 μ M) were incubated in rat liver microsomes (1 mg/mL) fortified with NADPH (1 mM) for 30 min. Metabolites in the incubated samples were analyzed on a Q-Exactive benchtop Orbitrap mass spectrometer. High resolution full scan MS and MS/MS data were collected in a data-dependent fashion with polarity switching. Multiple data mining tools, including mass defect filter (MDF), were employed for metabolite detection. UV responses of metabolites provided semi-quantitative information. HR-MS and MS/MS data acquired in both positive and negative ion modes were utilized for structural elucidation. A number of major and minor metabolites of the model compounds formed in the rat liver microsomal incubations were quickly detected by processing of the accurate MS data using HR-MS data-mining tools. Several metabolites not reported in literature were identified through the analysis. For example, the two metabolites derived from dealkylation of the morpholine moiety of Timolol (P-C2H₂ and P-C4H₆O) were found in the incubation sample. In addition, polarity switching in one duty cycle of acquisition significantly enhanced metabolite identification capacity. For example, positive and negative MS/MS spectra of Rantitidine metabolites provided different, but complementary structure information of the two major demethylation metabolites, which led to quick identification of the difference in modification positions.

POSTER ABSTRACT

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CHARACTERIZATION & IDENTIFICATION

Biomolecule

By-products in recombinant TAU protein production characterized by LC-MALDI Top-Down Sequencing (LC-MALDI-TDS)

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In Strong polarity drug purification , the two problems were that the drug is usually convert to salt for alkalinity is unstabled,and the drug was in ionic form. There was no retention in reversed phase chromatography, such as C18, and short life of column efficiency would be found in normal phase chromatography suce as silica or NH₂. In order to solve the problems to save time and cost, a novel media material – Amide Bonded Silica - was made by Bonna-Agela Technologies, Amide series media was worked in the hydrophilic interaction chromatography mode, significantly efficiency was gain when purificating the strong polarity drugs. Especially strong water-soluble drugs. An application of slimming drug showed the advantages of the Amide series media when compared with the traditional silice media under the same detection conditions: (1) Sample loading was up to 35 mg/g (sample/ media weight ratio) while 15 mg/g of Silica media; (2) Recovery up to 48% while 23% of Silica media;(3) Column life up to 50 times while 5 times of Silica media. We can draw some conclusions from that:Amide series media can be got good performance,such as sample loading,recovery, column life, when purificate the strong polarity and water soluble compounds.

CHARACTERIZATION & IDENTIFICATION

Biomolecule

Development of a Methodology for Rapid Characterization of Biological Therapeutics

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By 2014, it is expected that six of the top world's top ten best selling drugs will be biopharmaceuticals. The characterization of these products presents many challenges due to their high molecular weight, the heterogeneous nature of proteins, and the complexity of the production process. Subtle changes in the manufacturing steps can introduce unexpected and unwanted modifications to the product. Therefore, a solution is required for fast, reliable, and quantitative characterization of biopharmaceuticals in a high throughput, automated manner. Fortunately, many of the tools used in classical small molecule Qual/Quant workflows are applicable to biopharmaceutical analysis. We describe a method based on LC-MS technology, combining ultra-high-resolution chromatography (U-HPLC), ultra high-resolution mass spectrometry (UHR-TOF-MS), and newly developed software –BioPharma Compass– to provide complete automation of the characterization workflow.

Results

20 samples of human IgG1 were analyzed using an automated workflow where LC-MS data were acquired on a UHR-TOF mass spectrometer and the spectra analyzed with the Maximum Entropy Deconvolution algorithm. The resulting peak lists were qualitatively and quantitatively compared to a "gold" reference standard. Using this approach any differences in the samples compared to the reference were automatically highlighted. Results show the high spectral mass accuracy and resolution of the platform is clearly capable of determining small differences in mass and intensity indicating differences in glycosylation.

The peptide mapping workflow was tested by analyzing 96 tryptic digest of BSA and transferrin by LC-MS. Data were automatically matched to the theoretical protein digests considering possible modifications. Sequence coverage of up to 95% was obtained for most samples. Future work will investigate the use of alternative proteolytic enzymes to obtain full sequence coverage.

POSTER ABSTRACT

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CHARACTERIZATION & IDENTIFICATION
Biomolecule

Automatic MS/MS characterization of N-linked Glycopeptides

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Glycosylation is the most abundant posttranslational protein modification. Involved in many relevant biological processes and pathways, it is crucial to the understanding of many diseases. However, glyco-peptide analysis is still challenging. Due to high glycan heterogeneity and ion suppression effects, abundance of glycopeptides in tryptic digests is low and may require enrichment and separation techniques. In addition, interpretation of MS/MS spectra is difficult as classical database search approaches cannot be used when the peptide sequence and the glycan molecular weight are unknown. The proper determination of the peptide mass, i.e., the aglycone, is the key for automated glycan database searches. We developed a software approach evaluating the peptide mass in MALDI-TOF/TOF MS/MS and ESI LC-MS/MS spectra of N-linked glycopeptides.

A simple and effective tube construct for salty samples in cryoprobes

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High ionic (usually salt) concentration seriously reduces the efficiency of cryoprobes (cold probes) degrading sensitivity and extending the 90 degree pulse width and good quality tune/match can be difficult. There are various solutions which have been proposed, some are quite sophisticated using special tubes (of oval shape, for example) or double smaller concentric inserts. A “poor man’s” choice can be simply taking a tube with smaller OD than that of the probe, introducing an air gap and increasing the distance between the body of the sample and the coil itself.

We describe a simple and inexpensive refined version of this latter approach which also utilizes the concept of separating lock and shim. It is most useful for (biological) samples in water but can be beneficial for all samples of high ionic strength. A relatively large OD (typically 4.1 mm) tube is used to carry the sample while the lock solvent is between the walls of the 5mm OD container tube and the insert. Other size combinations are also possible, of course. This tube construct largely resolves the above issues, retains the highest sensitivity, and makes it possible to avoid any dilution of the sample itself, next to additional smaller benefits.

POSTER ABSTRACT

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INSTRUMENTATION

Fundamental Characteristics of Small Molecule Analysis using Ultra Low Dynamic Flow

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Ultra-low flow rate (< 20 nL/min) nanospray ionization has demonstrated reduced ion suppression, a trend toward equimolar response, and high ionization and utilization efficiency for small molecule analytes. The majority of these experiments feature the use of offline (static) nanospray. Typically static nanospray is operated in a regime whereby the applied voltage generates or controls the effective through-emitter flow rate of mobile phase. Such static experiments are often difficult to control because the flow rate is a function of applied voltage, mobile phase composition, and emitter geometry. By decoupling through-emitter flow rate from applied voltage, it is possible to retain the benefits of static nanospray in combination with the robustness and repeatability of pumped flow (dynamic) nanospray.

High Throughput Screening for Lead Discovery using Multiplexed LC with High Resolution Accurate Mass LC/MS

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High Throughput Screening is a valuable part of identifying new leads and directing drug design in pharmaceutical research. The ability to quickly perform large numbers of analyses is critical to the success of any HTS assay. Traditionally the quantitative components of these analyses are performed using Fluorescence, Luminescence, Absorbance, or Radiolabel techniques. These techniques provide rapid sample analysis but also contain inherent limitations, such as the occurrence of false positives, increased time for method development and complex sample preparation. High resolution accurate mass LC/MS coupled with multiplexing technology provides a selective and sensitive alternative to traditional methods without the need for labeled substrates and at rapid analytical speed. Samples were made to simulate an enzymatic screening assay and injected onto a C18 column (10x2.1mm, 3.5 μ m). Chromatographic separation was accomplished through a generic two step gradient using 0.05% trifluoroacetic acid in water and 0.05% trifluoroacetic acid in Acetonitrile. Mass spectrometry analysis was performed by heated electrospray in positive ion mode, and analyzed using generic MS conditions. Samples were injected to demonstrate reproducibility and calibration curves were analyzed to measure the dynamic range of the analytical method, along with the limits of detection and quantitation. The experiment data set containing 10,000+ sample injections provides results demonstrating CV's of less than 20% for all sample replicates, linear signal response for all points across each calibration curve, and reasonable LOD and LOQ of 10nM and 25nm respectively, with an estimated analysis time of 2.25 hours per 384 well plate. This novel combination of hardware and software provides the unique capability to rapidly analyze large samples sets while retaining the ability to chromatographically remove unwanted. Through the implementation and integration of this technology, high quality MS data can now be collected at speeds capable of supporting the demands of large scale high throughput screening.

POSTER ABSTRACT

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INSTRUMENTATION

Rapid Chemical ID using a Direct Analysis in Real Time Mass Spectrometry using a Heated Sample Screen in the ID-CUBE™

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A strategy for open access direct analysis in real time (DART®-MS) using an electrically heated sample stage to create more rapid and through desorption ionization is discussed. The combination of DART with an ultrahigh resolution Thermo EXACTIVE mass spectrometer (>100,000 resolving power) in combination with a consumable metal screen that can be heated from room temperature to >500C in ten to 15 seconds is shown to provide capability for mass determination of both high and low vapor pressure molecules in under 20 seconds per sample is demonstrated. This new DART technology packaged in the so called, "ID-CUBE" source reduces the time per analysis primarily by replacing the traditional heated carrier gas of the DART with a more direct heater that uses variable electrical current running through the sample laden wire screen to desorb molecules from it. Eliminating the inefficient cartridge heater from the design using this direct current heater decrease the time required for the experiment by minutes.

A disposable paper card with embedded screen is used to reduce the potential for memory effect that sometimes occurs in LC/MS based systems. Rapid vaporization is enabled by directing an electrical current through the sample coated conductive metal screen while unheated ionizing gas flows from the DART source through that screen. By positioning the heated metal screen between the DART source exit and atmospheric pressure inlet (API) of the mass spectrometer molecules enter the ionizing gas as they enter the gas phase. The experiment, characterized as "transmission-mode" DART, decreases the time necessary for analysis by enabling near instantaneous desorption of sample since the metal screen can be heated in seconds to temperatures exceeding 400C. The method also uses less gas since it is no longer necessary to use heated gas to desorb the sample molecules.

Examples of the use of this technology for chemical identification of small molecules and complex mixtures will be described.

Application of a Novel Bench-top Orbitrap Mass Spectrometer with a Quadrupole Mass Filter for Metabolite Profiling in Drug Discovery

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Use of electronic laboratory notebooks (ELNs) for regulated bioanalysis requires validation of numerous ELN workflows. Presented here is a strategy for using common ELN functionality to validate workflows such that subsequent reviews are more efficient and comprehensive than for typical software validation. It involves execution of scripts similar to those employed during typical software validation, but with a twist. Traditionally, successful execution of scripts is affirmed by signing and dating a brief note stating that the expected results were observed. An ELN, however, can provide advantages when the electronic results of each script execution are saved as a unique, retrievable version of an ELN experiment. This way, during review of the workflow validation, the result of each and every executed script is available for scrutiny exactly as it appeared during testing.

An ELN test plan is written to include instructional scripts to be executed during validation. The test plan and the workflow being validated are executed simultaneously in separate ELN experiments. Script-designated functions are described in the test plan experiment, and performed in the workflow experiment, which is then version-saved, rendering it forever retrievable. For each individual script in the test plan experiment, the saved version of the corresponding workflow experiment is documented, along with any comments.

Testing a specific ELN workflow in this manner results in a complete electronic validation "package", including supporting experiments, that is accessible for review by Quality Assurance and approval by Management. This review process is efficient, yet comprehensive, as every executed script result is a click away, and yields exactly what the tester observed during validation, as opposed to a traditional "Observed expected results" comment that cannot be confirmed during review. The entire resulting electronic validation "package" corresponding to each validated workflow can be conveniently archived for future reference and review.

POSTER ABSTRACT

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DATA ANALYSIS

Strategy for Designing Electronic Lab Notebook Workflows Both Flexible Enough for R&D and Comprehensive Enough for Regulated Work

Brian Beato, April Pisek and Jessica White

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Electronic laboratory notebook (ELN) workflows are typically designed for either research or regulated use. Documentation requirements for regulated sample analysis are unnecessarily comprehensive and rigid for free-form research. Presented here is a widely applicable strategy for designing practical ELN workflows for use across the full range of regulatory requirements, from non-regulated R&D to fully regulated sample analysis. Logic is embedded into each workflow to turn on and off specific functionality based upon the type of work being performed. For example, for non-regulated R&D work, user entries are not mandatory, and very limited error checking occurs. This strategy provides research chemists access to the same useful ELN workflow functionality available for regulated work, to any extent desired at the time, but without also imposing any impractical regulatory documentation burden. Despite using the same validated workflows, however, data obtained in such a non-regulated manner is electronically segregated from use to support regulated studies via automatically applied nomenclature. Because of the embedded logic, these same workflows are also sufficiently comprehensive for use during validation and regulated sample analysis to record, and provide real-time QC for, a litany of mandated items. The logic embedded in a single workflow via this design strategy is sufficiently flexible that it allows the level of documentation mandated and real-time QC performed to be finely tuned across several types of work, including method development, method validation, long term stability assessment, and sample analysis.

Efficient Strategy for Generating Method-Specific Workflows in an Electronic Lab Notebook

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One common concern among scientists regarding implementing an electronic laboratory notebook (ELN) is that method-specific workflows may either lack sufficient detail to be useful, or require repeated validation exercises for each new method. With the strategy for designing ELN workflows outlined here, a detailed, method-specific workflow can be written for any method in less than a few minutes without requiring additional workflow validation. As an example, a workflow for instrumental analysis is designed to include a hidden table for listing method-specific details such as mobile phases, column, temperature, wash solvents, and system suitability criteria. This table remains empty in the primary ELN workflow, which then goes through a very extensive validation process to ensure that its functionality and links to other databases are accurate. From this validated primary workflow, many unique secondary workflows can be written by including method-specific information in the aforementioned hidden table. Entries made in the hidden table in these method-specific secondary workflows are automatically copied into fields visible to the end user, thus providing specific details necessary for performing a given method. Generating secondary workflows by merely adding information to the hidden table in the primary workflow does not alter functionality, so no additional validation is required. During method development, as various parameters are updated, the hidden table in the validated instrumental analysis workflow is updated with the method-specific information. Additionally, screen captures of LC and MS/MS parameters such as the mobile phase gradient, tune parameters, and m/z transitions may be brought into the secondary workflow, providing more instructional detail without impacting previously-validated workflow functionality. Taking merely minutes to create, such secondary workflows provide all necessary method-specific detail without requiring additional validation.

POSTER ABSTRACT

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DATA ANALYSIS

Advances in the Automated Data Analysis of High Resolution Accurate Mass LC-MS Metabolomics

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High resolution accurate mass (HRAM) LC-MS metabolomics provides a unique approach for evaluation of perturbations in biochemical pathways. HRAM LC-MS couples high sensitivity detection with measurement accuracy and wide dynamic range, thus producing an enormous amount of information. Previously presented at CPSA 2010, our approach for reduction of the complexity of HRAM LC-MS dataset and condensing the results into biologically meaningful information* has been amended with many improvements and expanded to the new Thermo Fisher Q-Exactive platform.

The improved workflow for automated data analysis using Component Elucidator (CE), in-house software written specifically for data processing of HRAM metabolomics data, is illustrated with the metabolomics experiment investigating the effects of fasting on metabolic changes in male rats. All HRAM LC-MS data were collected on a Thermo Fisher Q-Exactive mass spectrometer coupled to a uHPLC Acella chromatographic system. Raw-files containing HRAM LC-MS profiles were automatically processed with CE software which includes data reduction, signal annotation and statistical analysis. The data reduction module converts raw data points into tables of components, where each pair of accurate mass and retention time corresponds to a unique analyte. Subsequent quantifiability filtering, alignment and annotation further reduces the number of components and assigns molecular identities, resulting in an output table of annotated components along with relative abundances in each sample. Finally, in the statistical module, univariate statistics were applied to reveal changes and trends which correlate with the duration of animal fasting.

* Serhiy Hnatyshyn, Tom McClure, Michael Reily, Mark Sanders. Component Elucidator the Software for Automated Analysis of High Resolution Accurate Mass LC-MS Datasets in Metabolomics. CPSA 2010, Longhorn, PA, October 2010

Automated Deisotoping and Charge Deconvolution of High-Resolution LCMS Data

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Since mass spectrometers do not measure mass, but mass-to-charge (m/z) ratios, software algorithms must be employed to convert the raw m/z data to useful mass information. This is particularly important for processing and interpretation of biomolecule LCMS data where multiply-charged ions are observed. For example, ProMass deconvolution software allows entire biomolecule LCMS data sets to be processed directly from the MS sample run list and reported in a simplified web-based format. Unit resolution mass spectrometers such as ion traps and quadrupole instruments result in unresolved isotopic clusters above mass ~ 2000 - 3000 Da. Therefore, the charge deconvolution process for 'normal resolution' data cannot reliably utilize the isotopic pattern to determine uncharged mass. In these cases, the deconvolution process returns a result that is closest to the isotopic average mass of the species being analyzed. With the widespread proliferation of high-resolution mass spectrometers, new deconvolution algorithms need to be utilized to take advantage of the mass resolution and the information contained in the experimentally observed isotopic patterns. A deisotoping approach allows determination of exact (monoisotopic) mass and offers a marked increase in mass accuracy relative to average mass determination methods. In this presentation, we demonstrate the use of a new data processing package that utilizes the Positive Probability, Ltd. (PPL) deisotoping algorithm integrated into the ProMass software workflow. Once an isotopic unit cell formula and instrumental peak shape have been modeled within the PPL software, the deisotoping method is saved and referenced by a ProMass parameter file. The ProMass software is operated in the normal way, with the only change that a PPL-enabled parameter set is utilized during processing. Data will be shown that demonstrates the utility of the software for automated exact mass of determination of peptides, small-medium sized proteins, and oligonucleotides.

POSTER ABSTRACT

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DATA ANALYSIS

Mass Spectral Structure Elucidation and Correlation Algorithm Based Upon Advanced Graph Theory Methods

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A novel algorithm was designed to elucidate/correlate chemical structures with observed mass spectra acquired in exact mass or nominal mass modes. The algorithm is based upon advanced graph theory methods and written in Visual Basic 2005. The underlying principle of the algorithm is that a mass spectrum is generated from ionized substructures of the original parent molecule, whereby the connectivities of the atoms or superatoms of the original molecule remain intact or are modified by predicted rearrangements. A scoring system, based upon the number of bonds cleaved and the mass accuracy of the observed vs. predicted masses, is used to predict the best possible chemical structures for the fragment ions from a number of possibilities. Application of the algorithm will be illustrated for elucidating the structure proposed for the antibiotic Aspartocin A (MW 1317.8) from the ESI-MS/MS spectrum of the doubly charged parent ion. Other structures for the antibiotic proposed by Fujino and by Bodanszky will be analyzed as well as random permutations in the amino acid residues of the proposed structures. The highest scoring structure was found to be consistent with the correct structure for Aspartocin A. This methodology for elucidating proposed structures applicable for all small molecules, metabolites, natural products, peptides, saccharides, nucleotides and pharmaceuticals.