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Poster Assignment # 1

Fine Isotopic Pattern Recognition for Small Molecule Identification

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Isotopic patterns arising from natural isotopic abundances have been utilized in mass spectrometric measurements since the beginning of mass spectrometry. With recent advancements in mass spectrometry, we can now routinely achieve very high resolution (> 100K) measurements. This increased resolution allows observation of the fine isotopic pattern, the separation of extremely close isobars in typically unresolved isotopic pattern members. This can be a powerful tool to confirm and aid in small molecule identification. The software tool to aid in accurate mass and fine isotopic pattern recognition is just as important as the instrument capability itself. Here we demonstrate the use of very high resolution to extract metabolites of a sulfur containing drug on a Q Exactive™ benchtop HR/AM instrument. Samples of omeprazole and metabolites in human urine were collected at 0-3hr, 3-5hr and 5-7hr post dosing. Sample preparation by SPE was followed by analysis by UHPLC-HRAM MS using a Q Exative instrument coupled to an Accela™ UHPLC system. Accurate mass data was acquired using alternating full scan MS (70,000 resolution FWHM at *m/z* 200) and all-ion-fragmentation (AIF) scans from which accurate mass NL triggered precursor ion selected MS^2 scans on potential metabolites were obtained. UHPLC separation was achieved with a gradient of ACN and Water with 0.1% on a Hypersil Gold aQ C-18 column (2x100mm, 1.9um). Data was analyzed using Compound Discoverer software to identify peaks displaying a one or two sulfur fine isotopic pattern. Analysis of Omeprazole metabolism samples on the Q Exactive served as a benchmarking study to prove the concept of using fine isotopic pattern recognition for small molecule identification. In this example, the A2 isotope was resolved to show both the one ^{34}S two ^{13}C components in the full MS scan. A fine isotopic search of the urine samples was perfomed using a fine isotopic search algorithm. Briefly, the search used the expected mass shift from any A0 to both the A2 of the heavy sulfur and the A2 of the two heavy carbons combined with the expected ratio contribution of both signals to search the full scan data. A small tolerance for the mass (in ppm) and the ratio (as a percentage of A0) were allowed. This search was performed looking for both single sulfur (omeprazole and phase I metabolites) and on two sulfur (sulfate meteabolites) components. The traces were then used to detect or confirm components that matched the pattern. The approach successfully identified multiple major and minor phase I metabolites of omeprazole including multiple oxidations, sulfone reduction, demethylation/dealkylation, and glucuronidations. The two sulfur trace successfully identified both sulfate conjugates of demethylated and oxidative metabolites of omeprazole. In addition, endogenous sulfur containing components were also identified in the searchs (urothione). Fine isotopic pattern recognition can be used as a new orthogonal way for finding and confirming new metabolites. It can also be applied in any small molecule workflows for detection and confirmation of compounds of interest.

Poster Assignment # 2

The increasingly recognized potential of ICP-MS and LC-ICP-MS in bioanalysis

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In today's bioanalysis, the vast majority of bioanalytical methods for PK and PD purposes are based on LC-MS/MS and immunochemical principles. However, an increasing group of compounds have molecular characteristics that open opportunities for the application of elemental detection by ICP-MS. This technique offers orthogonal properties to molecular detection and binding properties. Hence this may also provide superior capabilities in specific cases. This is also true for bioanalytical challenges for which bioanalysts would normally chose LC-MS/MS based methods, e.g. protein biomarkers and proteomics; in these domains elemental tagging provides these molecules with excellent characteristics for ICP-MS detection.
Recently technological improvements have been introduced by instrument manufacturers, thereby positioning ICP-MS more and more as a suitable technique for a wider range of drug candidates with more common elements (e.g. P, S, Br, I). In addition to that ICP-MS can also offer additional advantages for compounds where ELISA or LC-MS/MS would be the first methodology of choice due to the fact that ICP-MS provides elemental information, orthogonal or in addition to activity and/or molecular information.
In our bioanalytical laboratory ICP-MS is applied for pharmacokinetics, imaging purposes, mass-balance studies, food-effect studies and biomarker studies. Hyphenated to an LC-system, our ICP-MS systems are also used for speciation / metabolism studies. We have also applied ICP-MS as a final quantification step in ELISA using Europium-tagged antibodies.
Here, the analytical potential, the various modes of operation and the challenges of the application of ICP-MS in drug development applications are given, including an overview of recent applications in this area from our laboratory.

Poster Assignment # 3

Investigation of Quantitative Analysis of N-Glycans by MALDI-TOF MS with Matrix Pre-Spotted Plate

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It is now revealed that the alteration of glycoforms and the variation of N-glycan quantities in body fluids can reflect the progress of a cancer. Matrix assisted laser desorption and ionization time of flight mass spectrometry (MALDI-TOF MS) has been used in the investigation of N-glycans due to its high sensitivity, resolution and accuracy. Despite of the high performance of MALDI-TOF MS, it can be inadequate for the quantitative analysis due to the poor reproducibility. In this study, we developed the matrix pre-spotted (MPS) plate to increase the reliability on the quantification of N-glycans by MALDI-TOF MS. A MPS plate was prepared by pre-spotting of 2,5-dihydroxybenzoic acid (DHB) on a MALDI plate using an automatic matrix spotter prior to sample deposition. To increase the uniformity of co-crystallization, the spotted analyte was vacuum dried with aqueous acetonitrile solution. The capability of quantification by the MPS plate was evaluated by linear regression analysis with a N-glycan standard, Man5. The R^2 value of the linearity of the concentration (20 pmol/ μ L ~ 6.25 fmol/ μ L) against the signal intensity was 0.99. Human serum N-glycans were released by PNGase F and fractionated with carbon graphite column. The N-glycans of 10% acetonitrile elution were loaded onto 4 spots of the MPS plate and analyzed by MALDI-TOF MS. For each spectrum, 13 dominant N-glycans were selected and normalized by the sum of the 13 N-glycan peaks. The mean relative standard deviation (RSD) of the 13 peaks (n=4) was 2.5% with the minimum and maximum RSDs of 0.2% and 4.4%, respectively. Thirty samples of human serum N-glycans were analyzed by the same 4-repitition measurement. The RSD of the peak at *m/z* 1485.53 was in the range of 0.5% - 3.6%. The identical procedures were applied to a sample set composed of 169 ovarian benign tumor patients and 68 ovarian borderline tumor patients. RSD for all mass ion peaks calculated on the basis of quadruplicates from a single serum sample was 7.28% without normalization and 5.44% with normalized intensities. The results suggested that MALDI-TOF MS with MPS plate can provide reliable consistency in N-glycan quantification.

Poster Assignment # 4

What's Binding the Iron in that Glass of Wine or Beer You're Having?

Exploitation of the Characteristic Isotopic Signature of Iron to Discover Endogenous Iron-Binding Compounds in Wines and Beers

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Wine and beer are rich, complex mixtures of organic compounds. Iron is present in high concentrations in wine (ppm range) and beer (high ppb range), and a significant fraction of it is bound to organic ligands. Red wine, for example, contains a variety of known polyphenol antioxidants possessing catechol functionalities, which are often involved in iron binding. Iron is an important catalyst for oxidative processes, and its reactivity and bio-availability is altered by binding to organic ligands. Therefore, the speciation of iron in these beverages is not only important for human health, but also for beverage stability, quality, and developing taste character.

With recent rapid developments in high-resolution liquid chromatography coupled mass spectrometry (LC-MS), a molecular survey of pools of unknown iron-binding compounds in complex samples is now feasible. We exploit the characteristic naturally occurring stable isotopic fingerprint of iron to discover organic iron ligands and characterize them by MS. In this way, we have previously identified a variety of known and novel siderophores secreted by microorganisms into biological media.

Here we apply this approach to the detection and identification of organic molecules involved in iron complexation in wine and beer. Beverage samples were filtered and then subjected directly to nano-LC-MS on an LTQ-Orbitrap XL platform. We performed systematic interrogation of the tens of thousands of individual mass spectra collected during the LC-MS runs, using software, written in-house, capable of distinguishing iron isotopic signatures in raw MS data. Through these analyses, we were able to observe numerous distinct iron-binding compounds, some of which demonstrated complexation with multiple iron atoms, that appear to be differentially present across wines and beers.

We believe that the identification and measurement of iron-complexing compounds in wine and beer should be of great interest to the industry, due to the significant role that these compounds may play in beverage outcome.

Poster Assignment # 5

Beer-omics; analysis of the sugar composition of beer using cryo-^{13}C-NMR

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Beer-omics is a comprehensive, untargeted analysis of native beer samples, which can be done using a variety of analytical methods (NMR, MS, NIR, etc.). NMR has the unique advantage that it is quantitative by nature, is robust, and usually needs minimal sample preparation. The complexity of the sugar content and the sensitivity of mono- and various oligosaccharides make NMR especially useful for analysis of this family of components in beer. ^{13}C-NMR has the special advantage for this purpose as the spectrum carries only singlet resonances and has practically no overlap. With the introduction of optimized cryoprobes sensitivity has become sufficiently high to run a successful ^{13}C-NMR experiment in reasonably short time. Quantitative 1D ^{13}C, and ^{13}C-detected correlation experiments are most efficient to resolve and quantify resonances. Databases and sophisticated prediction tools can be truly helpful to identify the sugar components. The poster will demonstrate the use and usefulness of these tools through examples of local (Triumph Brewery, Princeton, NJ) and commercial beer samples.

Poster Assignment # 6

On-line Chip-based Strategy for 2D Fractionation – Comparing Peptides Found between 1D and 2D Proteomic Analysis

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On-line two-dimensional (2D) liquid chromatography is widely used for protein identification/quantification because of its increased peak capacity compared to 1D separations. A common workflow is the combination of strong cation exchange (SCX) as the first dimension, followed by reversed-phase (RP) chromatography for the second dimension. A more recently developed strategy has emerged where the first dimension is a high pH RP separation, which provides higher peak capacity. Peptides contain acidic and basic functional groups, and their retention time could be affected by changing the pH of the mobile phase, causing orthogonality between the RP separations at low and high pH.

A simplified chip based 2D-LC workflow has been developed using a high pH/RP first dimension and low pH RP secondary dimension coupled directly to the MS for proteomic analysis. Studies with a standard beta galactosidase digest confirmed good separation orthogonality in the different pH conditions. Next, the optimized workflow was applied to the analysis of E. *coli* cell lysates and a variety of workflow comparisons were performed to assess impact on proteomic workflows (1D, 2D-6 fractions and 2D-10 fractionations and multiple sample loads). We found a 1.8x and 2.1x increase in the number of peptides identified (5% local FDR) for 2D-6 and 2D-10 fractions versus using 1D chromatography. When the loading amount was increased by 10x, the number of detected peptides compared to a 1D workflow increased by 3.3x and 4x for the 2D-6 and -10 fraction workflows respectively. Analysis of peptide overlap between fractions in the 6 step workflow showed that only ~10% of peptides were found in multiple fractions, indicating good resolution in the first dimension.

Poster Assignment # 7

Exploring the Sensitivity Differences for Targeted Peptide Quantification in the Low Flow Rate Regime

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Introduction:
A common view of the protein/biomarker research pipeline is that one starts with high sensitivity global discovery and narrows the list of protein targets using high throughput targeted quantitation. While much focus has been on mass spectrometry innovations, the importance of the separation component for getting high quality data cannot be underestimated. Sensitivity, robustness and throughput of the LC strategy must also evolve as research progresses from discovery to targeted workflows. The nanoflow regime is used extensively for high sensitivity discovery experiments but more recently researchers are exploring the use of microflow for increased throughput and robustness for targeted quantification. In this study, sensitivity & throughput differences between nano and micro flow rates for targeted quantitation are explored.

Methods:
Separation of protein digests was performed on an Eksigent ekspert™ nanoLC 425 system and cHiPLC® system (Eksigent, USA) using four different flow regimes. Column diameters and flow rates used were: 75µm cHiPLC® column at 300nL/min, 200µm cHiPLC® column at 1µL/min, 300µm microflow LC column at 4µL/min, 500µm microflow LC column at 10µL/min. Gradient lengths were optimized for each flow rate, with faster run times utilized for the larger diameter columns run at higher flow rates. Concentration curves on a set of ten tryptic peptides were analyzed and the lower limits of quantification (LLOQ) for each peptide at each column size was measured using MRM acquisition on the QTRAP® 5500 System. Data was processed using MultiQuant™ Software.

Preliminary data:
The LLOQs for a set of ten tryptic peptides were measured using the 4 different columns and flow rates. A nanoflow source with glass capillary tips was used for the 75 and 200 µm ID column experiments and the high flow source with a low dead volume 25 µm ID hybrid electrode was used for the 300 and 500 µm ID column experiments. The average increase in LLOQ (or the decrease in sensitivity) relative to running a 75 µm ID column at 300 nL/min was measured for each peptide at each column diameter. For the 200 µm ID column running at 1 µL/min, a 2.5 fold decrease in sensitivity or increase in LLOQ was observed. Moving to the 300µm ID column at 4 µL/min, a 3x difference in sensitivity was seen. Finally, 4x difference in sensitivity was observed when the 500µm ID column at 10 µL/min was compared to the same experiment on a 75µm ID column. However the separation was performed in less than half the time with the 500µm ID column compared to the 75µm ID column (7 minutes @ 10 µL/min vs. 18 minutes @ 300 nL/min). Therefore, as flow rate increases, increased robustness and throughput can be obtained with just a small decrease in sensitivity. In addition, with the larger column diameters, more sample can often be loaded (if available) to offset this sensitivity difference.
This difference in throughput, while maintaining high sensitivity, is key to the continued advancement in the use of mass spectrometry in biomarker research applications, and offers an important alternative to antibody based assays, that typically take much longer to develop when a new antibody must be generated, or when antibody cross-reactivity is a concern.

Poster Assignment # 8

A novel microflow-UHPLC MS/MS to improve sensitivity and throughput for quantitation of 1α,25(OH)2-Vitamin D2 and D3 in human serum.

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The measurement of 1α,25-dihydroxyvitamin-D3 with LC/MS/MS normally presents several challenges . The low level of observed ionization means a low intrinsic sensitivity at the mass spectrometer level which, when coupled with a very low concentration in the plasma, creates difficulties in obtaining adequate functional sensitivity and levels of quantification. The analytical advantages of microLC (higher throughput, mass sensitivity and the small amount of sample required) make this technique attractive for challenging applications such as 1α,25-dihydroxyvitamin-D3 and other vitamin D assays. Using microflow LC and signal amplifying reagents, we have developed a sensitive and high throughput LC/MS/MS method to quantify diene-derivatized 1α,25-dihydroxyvitamin-D3 and D2 (DHVD3 and DHVD2) in human serum and plasma samples.

The LC/MS/MS method uses a micro flow binary gradient HPLC system coupled with a quadruple trap mass spectrometer through electrospray ionization source with a hybrid Peeksil and stainless steel electrode. Data were collected in a Multiple Reaction Monitoring (MRM) mode. Samples were derivatized using a specifically designed derivatization reagent, dried, and then reconstituted for LC/MS/MS injection. Isotope labeled internal standards and calibrants were also used for the assay. A linear gradient from 37% B to 55% within 2 minutes at a flow rate 35 µL/min was used in conjunction of 0.5 X 50 mm column packed with core shell 2.7 µm particles, maintained at 40 oC. A C8 300 µm guard column was used and the injection volume was 5 µL.

The results demonstrate a fast sensitive LC/MS/MS method based on micro flow UHPLC for quantification of DHVD3 and DHVD2 in human plasma. A mixture of d0-DHVD3/D2 (analytes & calibrants) and d6-DHVD3/D2 (Internal Standards) was derivatized with amplifyng-reagent and used as a system optimization solution to optimize the MS/MS (MRM) parameters. Initial results from the sample modified with the amplifying reagent show that DHVD3 and DHVD2 can be effectively separated from other matrix components with minimal interferences. Injections of 1500 fold diluted (by 1:1 MeOH: Water) sample provided better sensitivity (2-3 times) and less background interference when compared to the analytical flow (at 600 µL/min). Peak width measured at half maximum was much narrower when compared to the peak width of the peaks eluted using analytical flow LC. Micro flow LC low delay volume relative to column volume allowed the separation of critical isomers that co-elute using analytical flow rate LC.

MEASUREMENT OF PHOSPHOINOSITIDES (PtdIns) FROM STIMULATED THP-1 and PBMC CELL LINES USING LC-MS/MS.

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Purpose: To measure PtdIns levels in THP1 and PBMC cell lines with nanospray mass spectrometery. PtdIns have been found to be important regulators of many cellular events, such as intracellular signaling, migration, and vesicular trafficking. Previous studies have demonstrated that off-line nanospray provides unique advantages for analysis of PtdIns in prepared cell matrix samples, such as increased dynamic range and sensitivity. On-line nanospray has similar potential, but there are challenges in sample prep, loading, and analytical cycle time. A nanobore chromatography column-switching device has been developed for quantitation of various PtdIns from stimulated cell lines.

Methods: PtdIns (PI(5)P, PI(3,5)P2 and PI(3,4,5)P3 and a structural analogue valproic acid were used in this study. PtdIns were spiked directly into cell matrix. Invitro samples were directly injected into the nanospray MS/MS system. Quantitation was performed on samples obtained from the lab. The system was comprised of dual gradient HPLC pumps and dual 6-port switching valves. A trap column (25mm x 100-150um, C18 or polymer) is used to desalt samples and bump elute to a (10cm x 75um C18) packed-tip column. The first valve was used to switch the sample trap column out of the flow path; the second to switch the analytical column from the trap directly to the second LC. Quantitiation was performed on a triple quad instrument equipped with a nanospray interface.

Results: PtdIns were quantitated in cell matrix samples from 1 ng/mL to 1.0 ug/mL. %RSD were all below 20 % for duplicate injections.

Conclusions: Results showed the analysis of PtdIns using direct injection onto the nanospray MS/MS system, with a cycle time of 7.5 minutes. The analysis, which required lipid extraction for sample preparation and for spiking of a internal standard, could be validated for potential uses in measuring screening PtdIns levels in stimulated cell lines.

Poster Assignment # 10

Development of an Ultrasensitive Triple Quadrupole Mass Spectrometry Assay to Support BMS-791325 Microdosing Absolute Bioavailability Study

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In microdosing absolute bioavailability (μABA) studies, an intravenous (IV) microdose (< 0.1 mg) of an isotopically labeled drug is co-administered with an oral therapeutic dose of the unlabeled drug. This approach has been successfully applied to the determination of the absolute bioavailability of several BMS drug candidates. The preferred approach is to use a stable isotopically labeled (SIL) drug and LC-MS/MS for the quantitation of both the oral and IV drugs. An LC-MS/MS assay has been developed to support sample analysis for a planned BMS-791325 (Hepatitis C virus antagonist) μABA study, in which the SIL drug (0.1 mg) and the unlabeled drug (75 mg) will be concurrently dosed intravenously and orally, respectively. Theoretical isotope interference of the unlabeled drug to the labeled drug ($^{13}C_6$-BMS-791325) was minimal (0.5% impact to the labeled drug determination). However, experimental 187% impact was observed, which was thought to be due to an isotopomer impurity. This interference was decreased to 3% by choosing a different multiple reaction monitoring (MRM) detection channel. The lower limit of quantitation (LLOQ) were 10 and 500 pg/mL for the labeled and unlabeled drugs, respectively. The accuracy and precision data for both drugs were acceptable for future validation and sample analysis.

Poster Assignment # 11

Single Shot Kinetic Ranking of Carbonic Anhydrase II Inhibitors from Small Molecule Pools by Affinity Capture Mass Spectrometry (AC-MS)

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Kinetic analysis of molecular interactions have thus far been confined to binary or low complexity experimental systems due to concerns with non-specific binding of molecules to surfaces and the unambiguous identification of transiently bound compounds. The evaluation of biomarkers from patient plasma or the identification of small molecule binders from pooled libraries have therefore eluded analysis. Novel technology is now available to be able to both detect binding properties and to qualify that the binding partner is the correct binder. The nanoPore Optical Interferometry technology is ideally suited to interfacing with mass spectrometry since the affinity kinetics are measured in a flow system and hence can flow directly to a mass spectrometer. Here we demonstrate the concept of kinetic affinity capture mass spectrometry by identifying two sulphanilamides out of a library pool of eight equimolar small molecules that bind to and elute from immobilized carbonic anhydrase II with different rates.

Poster Assignment # 12

Development of a Chemoplexed LC-MS Method for Quantification of the Cyp 1A2 Enzyme System

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Cytochrome P450 (CYP) family of enzymes are the primary enzymes responsible for metabolism of xenobiotics. Given the importance of these enzymes it is clear why CYP catalytic efficiency is important in pre-clinical drug development. Unfortunately, consistently measuring the catalytic efficiency of enzyme-containing reagents such as liver microsomes has proved challenging. Activity assays are subject to the experimental conditions and direct protein measurements are not typically done.

We have developed a quantitative method for the determination of substrate, metabolite and enzyme in the same assay which allow us to make measurements of the complete enzyme system in a single analytical event. CYP1A2 activity was determined by metabolism of Phenacetin to Acetaminophen and CYP1A2 protein concentration was measured via a surrogate peptide approach in a combined assay using LC-MS/MS detection. Results from a small data set show a good correlation between enzyme concentration and activity.

The approach shows promise as a mass spectrometry-based analytical tool for quantifying complete enzyme systems rather than individual components. The ability to multiplex assays involving separate chemotypes such as small molecule substrates, metabolites and cofactors along with proteins and peptides in the same samples using a common analytical method may prove to be a valuable tool for studies in systems biology. Practical mass spectrometry-based, chemoplexed analyses may open the door to the use of enzyme systems as biomarkers and clinical diagnostic tools.

Poster Assignment # 13

Breaking the NanoLC-MS Throughput Barrier: High-Performance at 95% Duty-Cycle

Amanda Berg, Helena Svobodova, Gary Valaskovic

Nanospray is an essential tool in high-sensitivity mass spectrometry, but limited robustness, reproducibility, and ease-of-use have historically challenged the adoption of nanospray in quantitative applications. Recent trends toward MS-based biomarker quantitation have placed strict requirements on the analytical performance of nanobore LC-MS. Nanospray MS and nanobore LC-MS both rely heavily on nanospray source hardware for successful experiments. Nanospray source hardware has matured over the past ten years from simple homemade devices to sophisticated, application-specific instrumentation featuring stage automation, thermal control and high-resolution imaging. Many of these enhanced features provide robustness (automated tip rinsing, automated emitter change), throughput (multi-channel workflows), ease-of-use (multi-chip systems) or experimental flexibility. Here we present a novel nanospray source solution which delivers enhanced features of stage automation and multi-channel operation. Ease of use has been realized through the incorporation of an integrated nanobore LC-MS consumable and simplified plumbing scheme using a valve with a novel 3-slot rotor design. A four channel, three column version of the source enables an MS-duty cycle-time of 95%, compared to 40% for a single channel system. Using a HeLa digest, injection-to-injection reproducibility is demonstrated for 90 replicate injections across three different columns.

Performance Evaluation of a Flexible, Easy-to-Use Packed-Tip Column Device for Nanospray Enabled LC-MS

Helena Svobodova, Amanda Berg, Gary Valaskovic

Nanobore liquid chromatography is the method of choice for protein/peptide separation in the life sciences. Despite all the advances in nanospray product development, running experiments in nanospray mode remains challenging and requires specialized training and in-depth knowledge of instrumentation. Here we test the performance of a newly developed integrated nanobore column which combines a packed-tip column, high-voltage liquid-junction, column heater and transfer line into one easy-to-use device with universal connectivity. A self-guided positioning system ensures reproducible tip placement. Leak-free connections inside the integrated nanobore column guarantee good intra- and inter-column reproducibility while maintaining the sensitivity and separation efficiency of packed tip columns.

Tested columns were packed with 1.9, 3.0 and 5.0 µm C18 resins to 10 and 25 cm bed lengths. Using a 1 µl-loop, 300 fmol/µl and 1 pmol/µl bovine serum albumin digests (BSA, Waters) were injected directly onto the column. Spray stability, sample separation and signal intensity were evaluated at different temperature settings. Sample separation was achieved by a 30-min. 2-50% acetonitrile gradient at 300 and 500 nl/min. Analyses were conducted using a nanoLC•2D pump (Eksigent), linear ion trap mass spectrometer (LTQ, Thermo), customized nanospray source and custom packed-tip columns with embedded high-voltage liquid-junction and column heating (New Objective).

The performance of an integrated nanobore column was compared to a standard packed-tip column with the same dimensions. The peak width and peak asymmetry at 13.5% above baseline were calculated for four BSA peptide peaks (m/z 575.5, 569.7, 643.8 and 508.0) extracted from across the gradient elution profile. The peak widths were measured to be between 8.4s and 24s for the targeted peptides. The retention time for m/z 643.8 peak was 15.4 min for column 1, 15.6 min for column 2 and 15.5 for column 3. The peak area for the same peptide was integrated at 13.5 % above baseline and recorded to be 6.19E6, 6.62E6 and 6.57E6 respectively. The resulting RSD is less than 10% among these three tested columns.

Incorporating temperature control into the integrated nanobore column resulted in reduced column backpressures. The column pressure was recorded at 500 nl/min., 0.1% formic acid, 98% water and 2% acetonitrile. The pressure of a 10 cm column packed with 3.5 µm C18 particles decreased from 1575 PSI at 23°C to 1365 PSI at 50°C, a 13% pressure reduction. The pressure difference was more apparent on columns packed with 1.8 µm resin. The column pressure decreased from 4270 PSI to 3170 PSI, a 27% reduction in column pressure. In addition to decreasing the column pressure, heating the column also affected the elution order of some BSA peptides and improved the peak capacity. For example the elution order for peptides with m/z 740.8 and 954.8 was reversed when the temperature was increased from 23°C to 70°C. A 9% improvement in peak capacity was observed when the temperature of the column was increased from 23 to 50 °C. The temperature effect on different types of C18 resin remains under investigation and the data will be presented.

Poster Assignment # 15

Evaluating the Ruggedness of Nanospray on a Curtain Gas-Triple Quadrupole MS Equipped with Emitter Rinsing

Amanda Berg, Helena Svobodova, Ben Ngo, Gary Valaskovic

Abstract

Nanospray has become an essential tool in high-sensitivity MS, but limited robustness and reproducibility have historically challenged the adoption of nanospray in quantitative applications with triple quadrupole mass spectrometry. MS-based biomarker quantitation places strict requirements on the analytical performance of nanobore LC-MS not only due to the need for reproducibility and robustness but also the intrinsically complex nature of the samples used in these workflows. Automated emitter positioning with rinsing has been previously demonstrated to improve spray stability and analyte response on a 3D ion trap MS[1]. Here we investigate the utility of automated tip rinsing to improve emitter spray stability and data quality on a hybrid triple quadrupole/LIT MS equipped with a heated interface blanketed by a laminar flow of nitrogen gas.

Canine plasma was MTBE LLE purified, spin-filtered, evaporated to dryness and reconstituted in 25% MeOH/5%ACN/70% Water. Standards were added at concentrations of 250 ng/µl Caffeine, 5 pmol/µl Angiotensin I, 5 pmol/µl Neurotensin and 5 pmol/µl Bradykinin Fragment 1-7. The standard-spiked plasma was infused at 0.5 µl/min. Q1 MS data (4000 QTRAP/AB SCIEX) was collected in positive ion mode using a mass range of 400 – 1000 Da and a scan time of 0.1801 seconds. Using an uncoated pulled-tip fritted emitter (75 µm ID x 10 µm tip) ten-minute data files were collected using a constant set of parameters for ion-spray voltage, curtain gas and nebulization gas at an inlet temperature of 150°C.

Using a digitally controlled stage positioning system, the emitter was toggled between two sets of XYZ-coordinates functionally defined as the spray position and the wash position. At each sample injection, the emitter is toggled to the wash position diverting the emitter away from the MS inlet where a constant 50 µl/min gravity flow of solvent washes the exterior of the emitter. Upon initiation of the MS data acquisition, the emitter is toggled to the spray position and a spray image file is generated for spray stability validation at the start of each MS run. Comparative analysis of average TIC between data collected with washing and without reveals an obvious discrepancy. Using the RSD of the TIC as an indicator of spray stability, RSDs were calculated for a total of 650 injections for each emitter. Data collected with emitter washing produced consistent RSD values ranging from 4.29% to 8.37% with average TIC values of 1.37E9 and 1.50E9, respectively. Data collected without emitter washing produced RSD values ranging from 4.62% to 132.25% with average TIC values of 2.92E9 and 4.14E7, respectively. The TIC dropped almost two orders of magnitude for data collected without rinsing from matrix-associated particulate accumulation on the emitter. Data collected with washing produced consistent average TIC and RSD values over all 650 injections.

[1]Berg, AL; Marshall-Waggett, CJ; Valaskovic, GA; Proceedings of the 57th Conference on Mass Spectrometry and Allied Topics, Philadelphia, PA, May 29 - June 5, 2009.

Poster Assignment # 16

Development and validation of a new LCa MS/MS workflow for fast quantitation of PEth, an alcohol biomarker, in dry blood spots.

Authors
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Abstract
Phosphatidylethanol (PEth) is a glycerophospholipid homologue where ethanol by phospholipase D has been bound at the position that normally contains an amino-alcohol. As the formation of PEth is specifically dependent on ethanol, the diagnostic specificity of PEth as an alcohol biomarker is theoretically 100%. Quantitation of PEth in dry blood spot is of significant interest for forensic purposes. The published analytical LC-MS/MS methods and the one we have used routinely are slow. The multiplexed LC's coupled to MS/MS enable higher throughput but are complex to handle in routine labs.

We recently developed and implemented a fast and much simpler approach to quantitation of PEth using a Eksigent micro LC 200 and API 5500 triple quadrupole mass spectrometer system. A simple extraction followed by drying and reconstitution is used for sample preparation. A 2.0 min. analysis time was achieved using a single channel micro LC compared to approximately 9 min. on our standard 2.1mm ID column operation using UHPLC. Approximately 5x gain in LLOQ was also observed using micro LC. We will present the method, validation results and comparison data with standard multiplexed LC system.

Poster Assignment # 17

A sensitive and fast MicroLCa MS/MS quantitation of steroids in dry blood spots – Keeping it simple, keeping it clean!

Authors
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Abstract
Quantitation of steroids in dry blood spot is of significant interest in many disciplines, e.g. a panel of steroids is monitored in dry blood spots for Congenital Adrenal Hyperplasia (CAH) screening. The published analytical LC-MS/MS methods and others used routinely, to our knowledge, are generally slow and employ high performance MS/MS systems. Some labs have adopted multiplexed LC instrumentation coupled to MS/MS to achieve necessary throughput, automation and sensitivity. However, due to high complexity of the systems and/or high capital investment, these approaches are beyond the reach for most routine labs.

Here, we describe a sensitive, fast, yet simple approach to quantitation of Cortisol, 21 deoxycortisol, 11 deoxycortisol, Androstenedione and 17 Hydroxyprogesterone and preliminary data using a Eksigent ekspert™ micro LC 200 coupled to a cost effective API 3200™ triple quadrupole mass spectrometer system. A single step extraction followed by drying and reconstitution is used for sample preparation. The 2.5 min. micro LC-ms/ms method linearity was tested using CDC's DBS calibrators at concentrations 0, 10, 25, 50, 100, 150 and 500ng/mL. The isobaric steroids separation was also achieved without difficulty. The method accuracy was confirmed by excellent correlation of results with mean values of the results of participating labs on CDC proficiency test samples.

Poster Assignment # 18

Development and Validation of a Competitive ELISA Assay for Quantification of Cyanocobalamin (Vitamin B-12) in Human Plasma Samples

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Enzyme-Linked Immunosorbent Assay (ELISA) is widely used for quantification of large molecules. However, quantifying Cyanocobalamin (Vitamin B-12) has been problematic due to its small molecule size (~1.36 kDa) and limited number of epitopes. Though a competitive ELISA kit for human plasma samples is commercially available, in this kit, the vitamin B-12 standards were prepared in human serum albumin-based buffer and no quality control was included. The standards prepared in human serum albumin-based buffer were found not to be appropriate for quantifying vitamin B-12 in human plasma samples. In this method, the vitamin B-12 standards and quality control samples were prepared in pooled human plasma and the method was successfully validated for quantifying vitamin B-12.

In the validation, the standard curve was fitted with a 4-parameter regression model. The accuracy (Relative Error %) ranged from -8.8 to 4.7%, the intra- and inter-assay precision (CV %) ranged from 2.3 to 26.1% and 8.8 to 20.0%, respectively. The range of quantification was 200 – 2500 pg/mL in neat plasma with a LLOQ (sensitivity) of 200 pg/mL. Fourteen (82.4%) out of seventeen individual plasma samples spiked with 200 pg/mL vitamin B-12 demonstrated 80 to 120% recovery. The dilution linearity was acceptable up to 200 fold. The samples were stable for up to 19 hours at room temperature, up to 34 days at -20°C and 5 freeze/ thaw cycles. The extracted plasma samples could be stored for up to 4 days at -20°C.

These results demonstrated that the modified method performs well for quantification of vitamin B-12 in human plasma samples. This method has been successfully used in clinical sample analysis for a FDA-regulated bioequivalence (BE) study.

Poster Assignment # 19

Small Molecule Tof Screening and Characterization using UNIFI Scientific Information System

Authors, Mark Wrona, Yun Alelyunas, Craig Dorschel, Paul Rainville, Kevin Cook, Stephen McDonald Russell Mortishire-Smith
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As accurate mass LCMS platforms continue to mature there is much debate of how and why these platforms should perform quantitative assays in DMPK environments. This debate has been accelerated by the fact that the basic quantitative properties, including sensitivity, robustness and linearity of QTof technologies are beginning to rival with many of the tandem quadrupole platforms in routine use. This poster describes and demonstrates both the technical hardware considerations (sensitivity, linearity, dynamic range) as well as new software developments that enable routine quantitation on high resolution mass spectrometric (HRMS) instruments.
The major limitations of such an approach are clear, how do (and why would) we manage the large amount of extra information provided by HRMS platforms when triple quadrupoles get the job done (and are the gold standard for quantitative information). One of the key limitations is that many software tools have been developed to generate key metabolic information from compounds and equally towards generating bioanalytical results quickly and accurately, few informatics platforms have been developed to look at the data holistically. The UNIFI analytical system is built with an acquisition front end for instrument control, a unified processing system and application specific workflows all built upon an integrated data management system and database.

Data showing that the sensitivity of the Xevo G2-S is within striking distance of modern triple quadrupoles (low pg/mL levels, 3-24 pg/mL) with observed linear dynamic ranges of 3 to greater than 4 orders for several compounds in human plasma including Alprazolam, Buspirone, Clopidogrel, Ketoconazole, Propranolol, Reserpine, Verapamil and Warfarin. These levels were achieved in full scan mode scanning at > 32500 mass resolution for all components. The relationship of high resolution fast scan rates on Tofs at frequencies that support both UPLC resolved peaks and sufficient points across peak will also be discussed. The combination of these variables means that in theory Tofs are capable of handling the majority of assays normally reserved for the most sensitive triple quadrupoles. The missing piece of the puzzle is software which integrates both the quantitative elements of information with the qualitative workflows for which HRMS platforms have been primarily used for to date.
Software integration of these historically disparate workflows will be discussed. Using UNIFI to combine both the quantitative and qualitative information in a single application is one of the keys to moving forward with these workflows in practice.

Poster Assignment # 20

Metabolism of Dimethyl Fumarate in Rat Blood: Insight Into Pharmacokinetics and Metabolic Fate of Dimethyl Fumarate *In Vivo*

Venkatraman Junnotula and Hermes Licea-Perez

Abstract

Dimethyl fumarate (DMF) has been used for the treatment of inflammatory diseases such as psoriasis vulgaris and multiple sclerosis. In spite of its clinical efficacy, pharmacokinetic and metabolic fate of DMF is still poorly understood. After oral administration of DMF in pharmacokinetic studies no detectable amount of DMF has been shown in circulating blood; instead the hydrolysis product methyl hydrogen fumarate (MHF) was detected. It was hypothesized that DMF undergoes extensive hydrolysis under slightly basic pH conditions found in small intestine and by esterases found in circulating blood. Therefore, it has been believed that MHF is responsible for the pharmacological activity of DMF. However, this hypothesis is not consistent with much lower pharmacological activity of MHF as compared to DMF in various biological assays.

In this investigation, the objective was to gain insight into metabolic fate and mechanism of action of DMF *in vivo*. The metabolism of DMF in rat blood and in basic pH buffers (pH similar to that in small intestine) was investigated using UHPLC-MS/MS. The feasibility of quantifying the free fraction of DMF, its GSH conjugate adduct, and MHF was evaluated in rat blood. N-acetyl cysteine-D_3 was used as a trapping reagent to determine the concentration of DMF in rat blood. The trapping experiments confirm the lack of detectable amounts of free DMF available in rat blood after 20 minutes, suggesting quantification of free DMF is not feasible. To examine the formation of GSH conjugate and MHF in fresh rat blood and in basic pH buffers, DMF was fortified at 200 μM into rat blood and 100 mM ammonium bicarbonate buffer (pH 8 to pH9) and the formation of GSH conjugate and MHF were monitored over the time course (60 min). The data reveal that DMF forms both GSH conjugate and MHF in both fresh rat blood and in basic pH conditions. However, it is important to note that yield of GSH conjugate of DMF is much higher (~90%) than its corresponding hydrolyzed product MHF (~2%) in basic pH conditions found in small intestine (pH 8 to pH 9). In contrast, yield of GSH conjugate of DMF is lower (~15%) than its corresponding hydrolyzed product MHF (~20%) in rat blood. The higher yields of MHF compared to GSH conjugate of DMF in rat blood is consistent with proposed hypothesis that is involvement of esterases in the hydrolysis of DMF *in vivo*. Overall, data reported here clearly demonstrate that DMF forms both GSH conjugate and hydrolyzed product MHF under biologically relevant conditions. GSH conjugates of DMF known to be reversible and will be in equilibrium with free DMF. So, it is possible that small amount of free DMF is accessible to drug targets. Therefore, GSH conjugate of DMF may be partially or fully responsible for clinical efficacy of DMF.

Poster Assignment # 21

Abstract submission CPSA 2013

Submission Date	2013-08-22 10:32:02
Name	Marshall Siegel
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Message	Poster Title/Abstract Scoring Methods for Interpreting Mass Spectra of Unknown Structures Using the MASSPEC Algorithm Abstract MASSPEC,a novel algorithm, was designed to elucidate/correlate chemical structures with observed fragment ion spectra acquired in exact mass or nominal mass modes using any ionization method.The program generates in silico mass spectra for proposed structures and is capable of analyzing materials of unknown structures by finding the best scoring structure that matches the observed mass spectrum. The highest scoring compound has the highest degree of correlation with the unknown structure from among proposed structures. Enumerating the structure can generate newer structureswith elevated scores, thereby, correlating even more closely the predicted structure with the unknown. MASSPEC utilizes “superatoms” to describe chemical structures. Superatoms are connected sets of atoms that do not fragment further, and are connected together by “chemical” bonds to constitute the chemical structure.. By removing all possible combinations of “chemical” bonds n at a time, (where n = 0,1,2,3,4,…), all possible connected substructures for the molecule can be generated (fragment ions) and each one scored based on the number of bonds cleaved, the mass error between the experimental and theoretical masses and weighted by the ion abundances., Effectively, a theoretical mass spectrum is created from the fragment ions formed and the intensities either set to unity or to the inverse of the number of bond cleavages needed to generate the ion (related to the energy of ion formation) . The overall fit of a structure to a mass spectrum is the product of two factors: (1) the relative match between the proposed structure and other possible structures to the data, referred to as the Match Factor, and (2) the relative fit of the mass spectral data to the proposed structure, referred to as the Total Maxdat Score corrected by the Penalty Score for unmatched ions. The product of the two factors is referred to as the Total Score. The chemical structure with the highest Total Score in a library of structures is the most likely structure for the unknown. These results can then be enumerated to find additional structures with even higher scores, thereby, improving the correlation between the theoretical substructures and the experimental data. These principles are applied to a resolution enhanced EI mass spectrum of an unknown structure with a parent ion at m/z 239.980, containing two sulfur atoms.

Poster Assignment # 22

Abstract submission CPSA 2013

Submission Date	2013-09-06 14:28:58
Name	Joe Siple
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Message	Microsampling Techniques for Controlling Dried Blood Spot Volumes

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Purpose
A growing number of studies involving Dried Blood Spot sampling have raised concerns over the possible impact of hematocrit variation on sampling consistency and accuracy. If the sample spot dispersion pattern differs based on blood hematocrit levels, fixed-area samples punched from the within the circumference area of the dried blood spot will not represent a consistent “normalized” sample. A possible solution to this hematocrit effect is to punch or extract the blood spot in its entirety. To do this, accurate sample volumes are required. Here, a prototype microcapillary pipetting/dispensing system is evaluated for suitability.

Methods
Positive displacement capillary pipets were used to collect 40-45uL of whole sheep’s blood. Using a prototype DBS Multidispenser, 4 x 10uL and 3 x15uL aliquots of blood were dispensed from the capillaries and spotted onto FTA DMPK filter
cards. The precision and accuracy of the dispensed spot volumes was measured gravimetrically. To better understand the dynamics of quantitatively transferring microvolumes, several aspects of the sampling technique were examined: Blood vs water, hydrophobic treatment of capillary tip, capillary tip contact to card surface.

Results
Acceptable spot precision for 10uL plugged capillaries and DBS multi-dispensers (CVs ranging from 1.5 – 3.5%) was achieved, supporting the use of the Whole Spot Approach for quantitative DBS analysis.
Samples with higher viscosity (blood vs water) exhibited greater spot variation.
Pre-treating the capillary tip with hydrophobic ink did not improve spotting precision.
Touching the capillary tip to the surface of the DBS filter card resulted in better spot consistency.

Conclusion
The use of positive displacement capillary pipets and DBS multi-dispenser for spotting 10uL-15uL microsamples has been successfully demonstrated, thus supporting the approach of using the entire DBS sample for analysis. This system affords the ability to collect blood samples, and to accurately dispense onto DBS filter cards from a single device. In addition to producing accurate and precise microvolume samples, the prototype multi-dispenser does so efficiently (collects a single sample for dispensing 3-4 spots) and humanely (smaller samples drawn, fewer study animals needed).

Poster Assignment # 23

Abstract submission CPSA 2013

Submission Date	2013-09-08 12:49:59
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Message

Next Generation Plasma Collection Technology For Clinical and Pharmaceutical Applications.

Fred E. Regnier1, JinHee Kim1, Tim Woenker2, Jeff Dahl2, Jeremy Post2, Faith Hays2 and Scott Kuzdzal2. 1Novilytic Laboratories, West Lafayette, IN 47906; 2Shimadzu Scientific Instruments, Columbia, MD, 21046.

Mass spectrometry (MS) has become a major tool in drug discovery, clinical diagnostics, and personalized medicine. With the rapid progress being made in various modes of mass analysis, MS detection sensitivity, and analyte quantification it is surprising that the accompanying blood collection and sample preparation technologies are so antiquated. Acquisition of blood by venipuncture goes back hundreds of years while the dried blood spot approach of Guthrie is now a half century old. The work reported in this presentation will focus on a new technology that exploits the benefits of collection, drying, and transporting small samples on paper; but after the removal of blood cells and collection of a fixed volume of plasma. Subsequent to deposition of a blood drop on a small card composed of a laminated membrane stack (noviplex cards, Novilytic LLC), the sample rapidly spread laterally by capillary action in the first membrane layer and then proceeds as a front into a second membrane where cells are removed by a combination of adsorption and filtration as plasma is drawn down through the membrane matrix. Migration of plasma through the membrane system terminates in roughly 3 minutes with the collection of either 2.4 or 4.8 uL of plasma in a collection disc at the bottom of the membrane stack. The volume of sample collected between cards varied less that 2% over a hematocrit range from 20% to 71%. At this point the cell bearing upper layers of the membrane assembly were stripped from the card, exposing the plasma filled collection disc to the atmosphere. Within 15 min of air exposure the plasma loaded disc was sufficiently dry to be placed in an envelope for transport by mail or air courier. In both metabolomics and proteomic analysis plasma derived from the plasma extraction card was comparable to that obtained by conventional venipuncture methods.
This presentation will demonstrate the use of this new plasma extraction technology for rapid and reproducible plasma sample preparation and analysis using the latest generation of ultra-fast mass spectrometry. Noviplex cards were used to rapidly generate plasma from mouse whole blood samples for triple quadrupole MS peptide quantitation (Shimadzu LCMS-8050). Exceptional triple quadrupole scan speeds of 30,000 u/sec and the ability to perform polarity switching in 5 msec, combined with rapid and reproducible plasma preparation using noviplex cards, provides a rapid, accurate and reproducible plasma quantitation platform.

Poster Assignment # 24

Abstract Title: Intact Versus Signature Peptide Approach to Reach Optimal Sensitivity in Large Molecule Quantification by LC-MS: Exenatide Case Study

Authors: Jean-Nicholas Mess, Daniel Villeneuve and Fabio Garofolo

Affiliations: Jean-Nicholas Mess, Daniel Villeneuve and Fabio Garofolo

Abstract: Direct comparison of two different quantification approaches; intact versus signature peptide, for the quantification of Exenatide in human plasma. The exenatide amino acids sequence analysis revealed 3 theoretical trypsin cleavage sites which would result in the generation of 4 tryptic peptides. However, experimental digestion data performed in solution showed the presence of only 3 tryptic peptides, which only one showed to be more suitable for quantification. This peptide almost exclusively ionized to the +2 charge state (m/z 474.8) and clearly fragmented to one singly charged product ion (m/z 688.4). It was observed that the detection of exenatide tryptic peptide was 5 times more sensitive than the intact exenatide. The SPE extraction efficiency of exenatide was 61% and the digestion efficiency was 80%. Overall, the entire extraction process (SPE+digestion) efficiency was calculated to be 49%. The lower limit of quantification (LLOQ) achievable using the trypti!
c digestion approach was 10pg/mL, which is significantly lower than the reachable LLOQ for the intact exenatide. The calibration curve was linear (weighted 1/x2) with a coefficient of correlation of 0.9951. Accuracy for 3 levels of quality control samples (QCs) ranged between 102-103% and precision between 4-7%. The method was also tested for specificity, matrix effect, preliminary human plasma stabilities, which met acceptance criteria. This study demonstrates that the tryptic digestion approach is suitable for the quantification of exenatide in human plasma. On-going work is performed on the novel QTRAP6500 to further decrease the LLOQ to low pg/mL.

Poster Assignment # 25

Abstract Title: Glucagon Bioanalysis by LC-MS: “Unprecedented Level of Sensitivity (10pg/mL) for a Novel Formulation”

Authors: Jean-Nicholas Mess1, Louis-Philippe Morin1, Mauro Aiello2, Xavier Misonne2, Gary Impey2, Johnny Cardenas2 Josee Michon1, and Fabio Garofolo1

Affiliations: 1Algorithme Pharma Inc., Laval, Québec, CANADA
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Abstract: Development of LC-MS method more sensitive than LBA for challenging quantification of Glucagon at low pg/mL level in new formulation which is easier to administer than the currently available formulations. A bioanalytical method for glucagon in human plasma was validated on API5000. However, the LLOQ (100pg/mL) was not adequate to cover the pharmacokinetic profile of a novel formulation. Therefore, the method was re-developed on two different instruments to reach higher level of selectivity and sensitivity. TripleTOF5600 was tested in full scan TOFMS (30K resolution). The 3 most abundant isotopomers of+5 charge state of glucagon were summed. Moreover, MRMHS (15K resolution targeted approach), was also evaluated and the 3 most abundant isotopomers of the +5 charge state product ion (neutral loss of NH3) were summed. This experiment revealed that using a targeted quantification approach (MRMHS) led to a 10-fold increase in sensitivity over TOFMS. Hence, it was possi!ble to have on a TripleTOF5600 the same sensitivity of a API5000. QTRAP6500 was able to reach the LLOQ of 10pg/ml (10-time lower than validated LLOQ on API5000). The calibration curve was linear (weighted 1/x2) for the concentration range 10- 10000pg/mL with a coefficient of correlation of 0.9990. The accuracy for the LLOQ was 88% with 16% precision. The accuracy for quality control samples ranged between 91-101% while the precision between 3-9%. The outcome of this research showed that it possible the development an LC-MS method more sensitive than LBA for the challenging quantification of Glucagon at low pg/mL level for a novel formulation.

Poster Assignment # 26

Abstract Title: Advanced Use of High Resolution Mass Spectrometry (HRMS) to Overcome Triple Quadrupole Limitations in Large Molecules Quantification

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Authors: Louis-Philippe Morin, Jean-Nicholas Mess, Milton Furtado and Fabio Garofolo

Affiliations: Algorithme Pharma Inc., Laval, Québec, CANADA

Abstract: The use of HRMS (QTOF) to overcome limitation of triple quadrupole for development of Large Molecule bioanalytical assays. To achieve the lowest LLOQ possible during the method development of somatostatin and enfurvirtide, the use of HRMS was preferred over triple quadrupole. For somatostatin, a large cyclic peptide known for ineffective fragmentation, a parent-to-parent approach was selected to limit loss of sensitivity during the fragmentation process. This approach is practically impossible with a triple quadrupole due to high chemical noise generated. The LLOQ achieved on HRMS with the summation of the two most intense isopotomers was 12pg/mL (S/N 22 vs. 9 on triple quadrupole). For enfurvirtide, the product ion used was 1343, which is outside the normal mass range of the quadrupole (m/z 1200). When compared to the most sensitive fragment obtained with the triple quadrupole (465.1m/z) the LLOQ was easily decreased from 100 to 50pg/mL with S/N=15. Calibration cu! rves were found linear (weighted 1/x2) and coefficients of correlation were 0.9995 for somatostatin and 0.9987 for enfurvitide. The CV% calculated from five replicate injections of QCLLOQ was 6% for somatostatin and 9% for enfurvirtide. For both compounds, the precision of the QC samples were all within 15% and accuracy between 96-102%. This study demonstrated that HRMS instrument is more adapted and suitable than the regular triple quadrupole for overcoming of complex analytical challenges as in the case of Large Molecule quantitation. The HRMS was able in both case studies to allow more flexibility in the quantification to increase selectivity and sensitivity of the assays.

Poster Assignment # 27

Title:

DBS sampling and Immunocapture LC-MS/MS Quantitative Bioanalysis of Trastuzumab in Mouse Whole Blood

Abstract/description:

Dried blood spot (DBS) sampling involves the collection of small volumes of blood onto specialized collection paper (Ahlstrom). Advances in the sensitivity of analytical instruments, coupled with the benefits derived from obtaining small sample volumes have enabled DBS to be utilized for obtaining drug exposures. One such application for DBS sampling is for mouse studies conducted in Drug Discovery Oncology programs.

Trastuzumab (molecular weight of 146 Kda) is a monoclonal IgG antibody used for the treatment of breast cancers that was dosed recently in several Discovery mouse efficacy studies. In order to obtain full PK profiles from these mice during the in-life portion of the study and enable PK/PD modeling, DBS sampling was employed. However, with the much smaller sample volume available for analysis a more sensitive quantitative method was needed. A sensitive immunocapture method followed by trypsin digestion was developed to monitor values below 100 ng/mL. Selected surrogate peptides were detected by LC-MS/MS and used for the quantification of Trastuzumab. This poster will describe the method development and application of this method for PK studies.

Authors:

Lorell Discenza, Stuart Emanuel, Jennifer Brown, John Mehl, Celia D'Arienzo, Timothy Olah

Poster Assignment # 28

Intact 20kDa Extracellular Domain of APO2L/TRAIL Bioanalysis by HRMS: A Potential Cancer Therapeutic Protein

It was submitted by Dr. Fabio Garofolo (fgarofolo@algopharm.com) on Monday, June 24, 2013 at 19:18:33

Authors: Jean-Nicholas Mess and Fabio Garofolo* Affiliations: Algorithme Pharma Inc., Laval, Québec, CANADA

Abstract: Recombinant APO2L/TRAIL (Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand), typically analyzed by LBA, was successfully quantified as intact protein by High Resolution Mass Spectrometry (HRMS). The chromatographic separation of intact APO2L/TRAIL for mass spectrometric analyses was found to be challenging with silica columns. Whereas, polymer column greatly improved peak shape and reproducibility. Moreover, the column temperature had an important impact on the amount of APO2L/TRAIL recovered from column upon injection: Increasing the column temperature from 22°C to 70°C increased the recovery by at least 4-time and improved peak shape. Data processing method was optimized to avoid interferences, it was possible to quantitate only the most abundant charge state and isotopomer or the sum of each charge state and isotopomers. APO2L/TRAIL signal was found to have charge state distributions from +12 to +27 with the 6 most intense charge states being f! rom +18 to+23. Monitoring only one charge state (+20), with an XIC of 10mDa, led to variability and unacceptable peak shape. After summing the 6 most abundant multiple charge states with an XIC window of 10mDa around the most abundant isotopomer, a S/N=8 was observed at the LLOQ. The method showed very good results: dynamic range was linear (weighted to 1/x2) with a correlation of r=0.9960. The assay precision was below 5% and with accuracy between 101-108% for the LLOQ QCs and 3 additional QC levels. This data successfully confirmed the possibility to use HRMS for intact protein quantification without tryptic digestion.

Poster Assignment # 29

Model-based Analysis of the Relationship Between Dried Blood Spot and Plasma Pharmacokinetic Samples in Pediatric Patients and in Healthy Adults – Two Case Studies

Lihong Du, Matt Rizk and Wendy Comisar
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Plasma and dried blood spot (DBS) bridging studies where both plasma and DBS are collected simultaneously to increase the understanding of the relationship between plasma and DBS concentrations to assess the suitability of DBS as a matrix and to allow the pooling of DBS and plasma data for Population PK (popPK) analysis. Mixed effect modeling has been applied to two clinical programs to verify the feasibility of DBS PK sample collection in future clinical trials. In one program (raltegravir) simultaneous DBS and plasma samples were collected from pediatric patients, while in another program (MK-X) simultaneous DBS and plasma samples were obtained from healthy adult volunteers. Based on in vitro data including blood to plasma (B:P) ratio and unbound fraction both compounds appeared to be good candidates for DBS.

Raltegravir is a potent and effective HIV integrase strand transfer inhibitor for treating HIV positive adult patients. IMPAACT P1066 is the first study to examine raltegravir in pediatric patients in order to guide its usage in children. Venous blood and was drawn from Cohorts III - V children (ages 4 weeks to 6 years) to prepare plasma and venous DBS samples; in addition, a heel stick/finger stick (HS/FS) DBS sample was also prepared using HS/FS blood collected within five minutes of venous blood collection. A popPK model was previously established using plasma data obtained from children 4 weeks to 12 years of age (Cohorts IIB-V), as well as from healthy adults dosed with the same formulations. The existing PopPK model was fit to the venous DBS and HS/FS DBS individually with a linear bridging slope to correlate DBS to plasma. A mixed effect linear model was also employed to confirm the results of popPK model analysis. Overall, there is reasonable concordance between plasma and both types of DBS samples. The slightly lower bridging slope of HS/FS DBS than venous DBS was attributed to dilution of raltegravir by interstitial fluid during HS/FS collection. Due to interplay of model parameters, IIV and residue errors, comparison of using venous DBS or HS/FS DBS method should be based on the same structure model. Because of the exclusion of additional plasma data in popPK approach, the bridging slope may not the same as using mixed effect liner model and slope inter-individual variability (IIV) may not be able to be estimated.

MK-X is in clinical development. Simultaneous venous DBS, FS DBS and plasma were obtained for 200 and 400 mg doses from one study and simultaneous venous DBS and plasma were obtained for the 100 mg dose from another study. Both studies were conducted in healthy volunteers. A mixed effect linear model was fitted to the DBS versus plasma data with inter-occasion variability (IOV) included in addition of IIV to compensate the bridging slope difference between the two studies. The larger IOV suggested that bridging DBS and plasma should be collected and analyzed by multiple sites or labs to avoid systematic variations.

Furthermore, in 100 mg dose where up to 216hr postdose samples were collected, the B:P ratio appeared to increase with time and/or with decreasing plasma concentration. As a non-linear B:P ratio would be expected at high concentrations rather than low concentration due to binding saturation in either blood or plasma, a possible explanation for this unexpected B:P ratio are being explored although mean profile analysis seems supporting a time dependent B:P ratio.