POSTER SESSION ABSTRACTS

Inspiration and Education

POSTER HALL OPEN
Tuesday, October 24 1:00 – 3:00 PM
University I/II Lobby

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POSTER ABSTRACT
#101

Quantitation and Identification of Polysorbates in Protein Matrix

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Introduction

Polysorbate 20 (PS20) and 80 (PS80) are used in the formulation of biotherapeutic products for both preventing surface adsorption and as stabilizers against protein aggregation. The polysorbates used in the formulation of biopharmaceuticals are mixtures of different fatty acid esters with the monolaurate fraction of polysorbate 20 making up only 40-60% of the mixture and the monooleate fraction of polysorbate 80 making up >58% of the mixture. Polysorbate stability and degradation are of interest to formulation scientists.

In this poster, we present a novel methodology to quantitate and identify polysorbates simultaneously which used two detectors in parallel: a charged aerosol detector (CAD) for quantitation and a mass spectrometer (MS) for identification.

Methods

A Thermo Scientific™ Transcend™ II TLX-1 chromatography system was used to remove protein matrix from polysorbate with Turboflow™ technology. Sample was transferred to detectors directly after eluting from the Turboflow column to avoid compound separation because the total amount of polysorbates in the sample was measured in this experiment.

Two detectors, consisting of a Thermo Scientific™ Corona™ Veo™ charged aerosol detector and a Thermo Scientific™ TSQ Vantage™ triple quadrupole mass spectrometer, were connected to a Transcend II TLX-1 system in parallel by using a tee-connector and PEEK tubing. The length of PEEK tubing was adjusted to keep the retention times acquired from the two detectors the same. The parameters of the two detectors were default values based on the LC flow rate without any optimization. SII control software was used to communicate between the Transcend II and detectors. Thermo Scientific™ Xcalibur™ software was used for data acquisition and processing.

Preliminary data

A novel methodology of using the Transcend II system to remove protein matrix was presented in Clinical & Pharmaceutical Solutions through Analysis (CPSA) 2015 in

Langhorne, PA. As follow-up work, the polysorbate quantitation after removing protein matrix became our research interest. The charged aerosol detector was connected to the Transcend II to measure the total amount of polysorbate.

Six PS80 calibrants with concentration ranging from 0.0005% to 0.008% PS80 in H2O (v/v) were injected and analyzed as well as four samples in varied protein concentration to study the matrix effect. Solvent blank sample was injected as well to estimate the system carryover.

A single peak of PS80 with good peak shape was observed at RT of 2.68 min on the CAD which could be auto-integrated by software. A linear calibration curve was achieved from the range of 0.0005% PS80 in H2O (v/v) to 0.008% PS80 in H2O (v/v) with R^2 equal to 0.995. Each calibrant was injected three times and the calculated average was less than 5.5% different from theoretical amount and the standard deviations of three injections were within 10% without internal standard correction. System carryover was ignored because the calculated PS80 concentration from solvent blank sample was negative which indicates polysorbates were washed away from system and column very efficiently.

Four PS80 samples having varied protein concentration were analyzed to mimic the formulation complexity in biotherapeutic products. The measured concentrations were very close to the weighted amounts which proved again that the Transcend II has high yield on removing protein matrix but trapping PS80 on the Turboflow column. Additionally, protein absorption was observed from PS80 samples having high protein concentration, and formulation scientists can use the system to study the protein absorption.

TSQ Vantage was connected to a Transcend II to identify the eluted compound and gave the necessary chemical information that was lacking from the CAD data. The typical pattern of polysorbates in the mass spectrum proved that CAD was measuring polysorbates exclusively.

Novel aspect

The use of charged aerosol detection to quantify polysorbate, and the use of charged aerosol detection and a mass spectrometer in parallel for polymer quantitation and identification.

POSTER ABSTRACT
#102

Outsourcing of Biomarker Analysis in Exploratory Clinical Development

Arkady Gusev

Early clinical development programs often utilize biomarkers to identify novel targets, confirm mode of action (MOA), efficacy and accelerate drug candidate evaluation. Sponsors are increasingly relying on outsourcing and vendors to keep up with the rapid pace of drug development and meet organizational strategic goals. Effective implementation of exploratory biomarkers in early clinical development requires constant evaluation of the external vendor landscape. Here we present a framework for outsourcing exploratory biomarker analysis in early clinical development, and discuss the advantages and disadvantages of different strategic vendor models. Strategic vendor management approach is illustrated by benchmarking data from CROs.

POSTER ABSTRACT
#103

Monitoring Brain Tissue Metabolites *Ex Vivo* During Electrical Stimulation Using Liquid-Microjunction Surface Sampling

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Aim

Here, we demonstrate the use of liquid-microjunction surface sampling (LMJ-SS) for the *ex vivo* analysis of brain tissue. Thereafter, we electrically stimulate the tissue, monitoring the response of dopamine before and after the stimulation event, using mass spectrometry.

Introduction

Liquid-microjunction surface sampling probe (LMJ-SSP) is based on the continuous flow of solvent using an in situ micro-extraction device interfaced with electrospray ionization. In contrast to traditional mass spectrometry imaging techniques, it operates under ambient pressure and requires no sample preparation, making it ideal for sampling thicker tissue sections for electrophysiological studies. Parkinson's disease (PD) is a neurological disorder characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta of the brain. Here, we demonstrate the use of LMJ-SSP for sampling 1 mm mouse brain tissue sections, sectioned fresh post-sacrifice and maintained in artificial cerebral spinal fluid (aCSF) to create near-physiological conditions. Dopamine, the key neurotransmitter implicated in PD, was identified by comparison with a standard and monitored before and after electrical stimulation.

Method

An adult C57BL/6 mouse was sacrificed and the whole brain were harvested immediately from the skull fresh, without perfusion. A zinc "rodent matrix" with 1mm spacing was used to section the brain immediately post sacrifice. The sections were placed into wells of aCSF, pH 7.4 before being transferred onto glass slides for direct sampling. To assist with identifying dopamine in the tissue sections, a 10 µL aliquot of a

dopamine standard was pipetted directly into the liquid-junction. To assess background interference, a 10 µL aliquot of aCSF was also pipetted into the junction. A Thermo Scientific Velos Pro linear ion trap mass spectrometer was used for all experiments. The LMJ-SSP used was the commercially available Flowprobe from Prosolia.

A Stanford Research System DS345 and stainless steel electrode with a 0.005" diameter tip were used to stimulate the striatum region of the tissue.

Results and Discussion

This study reports the use of LMJ-SS for the *ex vivo* analysis and electrical stimulation of brain tissue maintained under near-physiological conditions. Following electrical stimulation of the striatum the intensity of dopamine increased; this observation has been shown using *in vivo* deep brain stimulation, a therapeutic treatment for PD. LMJ-SS coupled to MS offers rapid analysis, and minimal sample preparation. Furthermore, freezing of the tissue is not necessary for sectioning, maintaining cell viability. Finally, the ambient nature of the LMJ-SSP allows near-physiological conditions to be maintained during sampling, a feature not available in traditional vacuum sources. Overall, this methodology could translate to other real-time investigations in which tissue viability and neural networks are of interest and thus highlights the potential of LMJ-SS for contributing to real-time *ex vivo* neurological studies.

Novel Aspect

This methodology highlights the potential of LMJ-SS for contributing to real-time *ex vivo* neurological studies.

POSTER ABSTRACT
#104

Novel Plasma Card for Shipping Clinical Samples

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Clinical trials often collect blood at a medical center and spin down the plasma. But the plasma has to be frozen for shipment to a LCMS testing lab. The growing trend is to conduct clinical trials in China, India or other some other foreign country and ship frozen plasma samples to the LCMS lab by priority air. The cold chain logistics for such deliveries cost hundreds of dollars. Temperature monitoring and shipping delays can greatly complicate the management of clinical trial samples. The better approach is to dry the plasma sample quickly and ship a stable and safe (non-infectious) sample at ambient temperature for tens of dollars. Novilytic has developed a collection device that can hold up to 10 microliters of plasma or urine, dries in minutes, and ships worldwide at ambient temperature.

A plasma sample is prepared for shipment by pipetting up to 10 microliters of plasma onto the plasma collection disc. The collection disc is mounted on a base card in a manner that prevents plasma from leaving the collection disc. The material in the collection disc air dries quickly. Analytes can be recovered by transferring the collection disc to a vial, adding extraction solvent, and vortexing for a few minutes. Recoveries are nearly 100% for some analytes but typically are around 80% to 90%. Repeatability in the ng/mL range typically exhibits less than 10% variation with CVs of 4% to 8%.

The current approach to shipping clinical samples is truly wasteful of resources and makes little sense. Why ship hazardous water with kilos of coolants, when new technology enables the shipment of a few grams of stable and safe materials at ambient temperature.

POSTER ABSTRACT
#105

Regulated LC/MS/MS Bioanalysis of Therapeutic Antibodies Based on Nano-Surface and Molecular-Orientation Limited (nSMOL) Proteolysis Method Using a New Reagent Kit

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In preclinical and clinical trials of therapeutic monoclonal antibodies (mAbs), the construction of a measurement method for the drug concentration in blood or disease tissue is essential for its pharmacokinetic (PK) evaluation. While ligand-binding assays have been used historically, alternative LC/MS/MS platforms have emerged. One such platform is LC/MS/MS quantitation based on nano- surface and molecular-orientation limited (nSMOL) proteolysis. It is designed to keep antibody specificity with minimizing sample complexity and simplify a sample preparation protocol, thus delivering robust quantitation with higher sensitivity. Immunoglobulin (IgG) fractions containing therapeutic antibodies were collected from human plasma with IgG collection resin. The IgGs immobilized on the inside of the pores are proteolyzed with trypsin-immobilized nanoparticles on the surface. Because Fc is immobilized to the pore, outside the reach of the nanoparticles, Fab oriented towards the solution is predominantly reacted with trypsin. Thus, nSMOL proteolysis minimize the sample complexity by excluding both tryptic fragments other than those around the Fc region and trypsin enzyme, allowing a selective quantification of target mAb peptides with higher efficiency ans selectivity in combination with MRM measurement.

POSTER ABSTRACT
#106

An Accelerator Mass Spectrometry-enabled Microtracer Study to Evaluate the Human Mass Balance of KD101, an Anti-obesity Drug under Development

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Background: In clinical drug development, it is important to understand the absorption, distribution, metabolism and excretion (ADME) properties of a drug in humans. The microtracer study based on the accelerator mass spectrometry (AMS) is an ultrasensitive technique to obtain human ADME profiles with a negligible radiation dose. KD101 is a novel compound under development to treat obesity. The aim of this study was to investigate the absorption, metabolism and excretion properties of KD101 in obese subjects.
Methods: A randomized, open-label, single-dose, one-treatment, one-period, one-sequence study was conducted in six males with a BMI ≥27, who received KD101 at 400 mg with 3.52 μg of [^{14}C]-KD101 (180 nCi) in the fed state. Plasma, urine and feces samples were collected up to 288 hours post-dose for mass balance and metabolite profiling. Plasma concentrations of KD101 was determined using a validated GC method. Total radioactivity in the samples was determined using AMS. Safety and tolerability was evaluated based on vital signs, adverse events, clinical laboratory tests, and electrocardiography.
Results: All of the subjects completed the study with no clinically significant safety issue. Mean total recovery rate (range) was 85.21 % (75.36-99.01 %), consisting of 77.96 % (68.31-92.33 %) for urine and 7.26 % (5.91-8.51%) for feces, which differed greatly from the pre-clinical data. Oral absorption of [^{14}C]-KD101 was rapid with the peak plasma concentration reaching at 5.83 h post dose, which was consistent with the previous report. In the urine radiochromatogram, five large peaks were identified including a peak represented by the parent compound.

Conclusion: KD101 is excreted predominantly through the urine in humans. Many of the excreted materials in the urine were considered metabolites. This study demonstrated effectiveness of the microtracer study enabled by AMS in humans to investigate the ADME property of KD101, which hugely differed from that seen in the preclinical animals.

Conflict of interest: This study was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI14C1234). All of the other authors declare no conflict of interest in this study.

POSTER ABSTRACT
#107

MICRODOSING/MICROTRACING CLINICAL TRIALS USING ACCELERATED MASS SPECTROMETRY IN CLINICAL DRUG DEVELOPMENT

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Microtracing/microdosing is an innovative technology that can revolutionize the current paradigm of clinical drug development. Typically, a very small amount of the drug, i.e., '*micro*dose', which is less than 100 micrograms (or 30 nmoles for proteins), is administered to humans. Since this is much smaller than 1/100 of the pharmacologically active dose, microtracing/microdosing technology can be employed at a very early stage of clinical drug development even when there is limited animal toxicology data. Furthermore, in order to trace minute doses, an accelerator mass spectrometer (AMS) is required and the compound should be labeled, typically with ^{14}C. The microtracing/microdosing study allows clinical drug development scientists for generating the intravenous pharmacokinetics, mass balance, metabolite profiling, and absolute bioavailability data much easier, faster, and at a significantly lower cost. Based on this understanding, this study investigated the current status and employment of AMS-based microtracing/microdosing studies in actual drug development. To achieve this objective, we performed an extensive search of the literature and public information, Delphi focus group interviews, surveys, and personal communications with the key players in the field. The number of the clinical studies that used ^{14}C and AMS dramatically increased from only 3 in 2001-2005 to 59 in 2011-2015. The survey showed that 31.6% of new drug development scientists were planning to perform microtracing/microdosing studies. Furthermore, 73.7% of survey responders replied that they would consider AMS-based microtracing/microdosing studies if there is a well-established service provider. This study confirmed that the frequency of AMS-based microtracing/microdosing studies for drug development has been in a steady increase

for the past decade or so. This increase was partly because several issues of AMS application in the previous era, such as dose-linearity, sample pre-processing, and high cost, have been adequately addressed. In conclusion, AMS-based microtracing/microdosing studies have been steadily employed in actual drug development, which is expected to increase further in the future.

POSTER ABSTRACT
#108

Accelerator Mass Spectrometry-enabled Microtracer Study to Evaluate the Human Mass Balance of KD101: Method challenges for analysis of a volatile oil.

Sinae Lee, HyeJin Yoon, Jun Gi Hwang, Sang Won Lee, Anhye Kim, Stephen Dueker, and Howard Lee

The large majority of drugs and not volatile and amenable to standard analytical techniques. Volatile compounds pose a different set of challenges, requiring changes to the total sample preparation procedure and often analytical method. We encountered such situation during a human microtracer study (180 nCi human oral dose) of compound KD101, a single isomeric oil, (-)α-Cedrene (206.23 g/mol). A total of 6 subjects were administered 400 mg of unlabeled KD101 admixed with 180 nCi of radiolabeled drug as a tracer. Detection of the radiolabel was performed using graphite based Accelerator Mass Spectrometry (AMS), with support from Liquid Scintillation Counting (LSC) for early urinary time-points. Post- administration, cumulative urine and fecal voids were collected for 288 hr post-dose. Serial blood collections were taken frequently post-administration and then daily for the duration of the study.

Preliminary work showed the KD101 could be completely removed under vacuum concentration in the absence of any trapping agent or biological matrix. This problem was exacerbated by the AMS processing method where samples are also evacuated under vacuum for torch-sealing inside quartz combustion (oxidation to gaseous carbon dioxide, prior to reduction to graphite). We found an acceptable technique to limit volatility losses through the pretreatment of all samples with an excess of tributyrin prior to concentration. The tributyrin served thus as both a carbon source and a chemical trapping agent. Sucrose was also tested but showed little ability to “capture” the compound during dry-down. We showed that parent compound recovery could be improved from <10 to 89% recovery using 2.0 mg of tributyrin per sample or LC fraction, and a minimum dry-down time (20 min).

The overall results of the study validated the dilution method. Mean mass balance recovery was 85.21% (77.96% in urine, 7.26% feces). This is considered a good mass balance recovery given the fact that it was difficult to control for losses due to compound

volatility. The concentration decay in the plasma was largely bi-exponential after the absorption period, with a wandering baseline out to 288 hr post-dose, with occasional "jumps" in concentration, which was attributed to the displacement of compound in fat depots. Metabolite radiochromatograms from urine and plasma showed intact parent in plasma with metabolites exclusively explaining the urinary components.

In summary, we achieved satisfactory results for the mass balance and metabolism of a volatile oil using a chemical trapping agent. The detriment was a lowering of overall detection sensitivity (e.g. 0.88 dpm/mL of plasma vs. 0.05 dpm/mL of unmodified plasma), but sensitivity was still sufficient to achieve mass balance and perform metabolite profiling using highly sensitive AMS detection.

POSTER ABSTRACT
#109

Determining the Source of Matrix Effects During the Quantitation of Phosphate Anabolites in Rhesus Monkey PBMC Samples

Justina Thomas

The ongoing interest in the HIV market for compound development has increased the need for analysis of drugs in peripheral blood mononuclear cells (PBMCs). The analysis of PBMCs is paramount for nucleoside reverse transcriptase inhibitors (NRTIs). In general, NRTIs are inactive but, once inside the cell, the inactive nucleoside is phosphorylated to the corresponding phosphorylated anabolites (mono-, di-, and tri-phosphates) where the triphosphate is the active species. The primary site of action of NRTIs is in PBMCs, and understanding the uptake and intracellular distribution of these anabolites in cells is critical for characterization of the overall disposition of compounds from this class.

During support of a preclinical study testing Compound A, significant anomalous internal standard (IS) responses were observed during the quantitation of the mono- and tri-phosphate anabolites of Compound A. A sample is characterized as having an anomalous IS response when the area response of the IS in the sample is less than 50% or greater than 150% of the lowest or highest response, respectively, observed in the accepted standards and quality control samples. One of the primary contributing factors to the anomalous IS responses was found to be the matrix. For this study, and as a routine practice for quantitative bioanalysis, standards and quality control samples are generated using blank matrix derived from subjects not associated with the study, and in most cases the matrix is purchased. In addition, in the case of PBMC analysis for this assay, cell count for each sample was another notable variable. Study samples are routinely quantitated using a calibration curve containing a defined amount of cells per millimeter of diluent whereas the cell count in the study samples are normalized post-analysis during toxicokinetic parameter generation. In the case of the preclinical study with Compound A, the cell count per milliliter of diluent ranged from $21x10^6$ to $99x10^6$, whereas the standards and quality control matrix contained 4.5x106 cells per milliliter of diluent. Collaborative efforts were undertaken to determine the source of the matrix effect and determine if cell count played a significant role.

POSTER ABSTRACT
#110

A Rapid, Automated Sample prep workflow for Immunoaffinity LC-MS/MS: Enrichment and digestion in less than 3 hours

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Michael Rosenblatt

The Use of LC-MS/MS for the analysis of Protein Drugs, particularly IgG's is continuing to advance in its utility and methodology. Characterization of these molecules throughout the entire drug development pipeline is now common and continues to grow in its implementation. One key step in the development process is the analysis of drug metabolism. For protein molecules this is done by quantifying "surrogate" signature peptides produced after proteolytic digestion. In a typical workflow, serum or plasma from animal models (preclinical analysis) dosed with the target IgG family drug are obtained. The IgG target is then enriched using a magnetic streptavidin bead coated with a biotinylated Goat anti-human IgG which recognizes the Fc region of the Humanized IgG. Following a binding and wash step, the IgG is eluted using a low pH buffer. The eluate is then denatured, reduced, alkylated, digested and desalted prior to LC-MS/MS analysis. The entire sample prep workflow, typically takes 4-18 hours and, in general, implies at least 24 hours in order to generate analytical LC-MS/MS results. The sample prep, is often the bottleneck step. We have revisited the sample preparation workflow and dramatically reduced sample prep time. In our workflow we are using magnetic, high-capacity streptavidin coated with a capture antibody. These beads have a binding capacity of ~15 mg/mL of settled beads and thus have the ability to analyze IgG samples over a very wide range of concentrations. Following the enrichment and wash step as described previously, the samples are either eluted and digested using Promega's Rapid Trypsin/Lys-C step (with or without the reduction/alkylation step) or digested "on bead". The on-bead digestion step appears to be very efficient and results in less sample processing as well. Typical digestion times are 10-30 minutes. This revised protocol reduces the total sample preparation time to about 2.5 hours, or less. The procedure is robust and flexible and has been automated on a Tecan Freedom Evo liquid handling workstation. In short, we have developed a highly efficient, rapid sample preparation workflow. The procedure is highly flexible and adaptable and should suit the overwhelming majority of Bioanalysis Immunoaffinity LC-MS/MS sample prep applications.

POSTER ABSTRACT
#111

Assuring Quality and Consistency with HRMS during the preparation of Critical Reagents

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Matthew Scisone

Successful Ligand Binding Assays (LBAs) are dependent on the use of quality critical reagents. Lot-to-lot inconsistencies can cause changes in assay reproducibility, robustness, and accuracy. By ensuring uniformity throughout the life cycle of a critical reagents use we can effectively eliminate the need for tedious and costly assay bridging and perhaps, revalidation. By employing Preparative SEC, Analytical SEC, High Resolution Mass Spectrometry, and Tangential Flow Filtration our lab has established a rigorous and flexible method by which aggregation, purity, and incorporation ratio (label:protein) can all be accurately quantitated and reported during the production and life cycle of the reagent, ensuring the best quality and highest consistency of critical reagent.

POSTER ABSTRACT
#112

Application for nanofluidic devices towards single cell proteomics and study *Xenopus laevis* embryonic development

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Anumita Saha-Shah, Melody Esmaeili, Peter S. Klein, Benjamin A. Garcia

Nanofluidic technologies have enabled mass spectrometric analysis of single cells and study of inter-cellular heterogeneity. However, most of these technologies have a limitation in that highly ionizable species such as lipids and small molecules predominate the mass spectra and suppress the signals from peptides and proteins. As a result most of these technologies are focused towards metabolomic and lipidomic studies. Here, we have combined nanofluidic sample collection strategy with offline sample preparation and LC-MS/MS for analysis of > 1200 proteins per cell. *Xenopus laevis* embryos were chosen as a model system for this study because 1) large cell sizes enable easy manipulation of sample, 2) the embryos divide after fertilization without increase in size, as a result, cell sizes vary from 1.2 mm to < 100 μm during development and serves as a good system for sample limited studies such as single cell analysis, 3) enables study of inter-cellular heterogeneity and relate it to development of various parts of a developed organism. Preliminary studies have shown the applicability of our method towards study of heterogeneity among cells at developmental stage 3. Embryonic development was also studied by analyzing synthesis of new proteins and protein turnover through metabolic labeling with stable isotope labeled amino acids. Further studies are currently underway to study the dynamics of protein synthesis throughout embryonic development.

POSTER ABSTRACT
#113

Introduction of a microsampling device , MSW^2

Ippei Takeuchi

We introduce a new microsampling device, $MSW^2{}_{TM}$.
Microsampling method is contribute to reduce human's (and animal's) burden of blood sampling, and developed by trial and error in pre-clinical and clinical studies. In these days, many kinds of microsampling devices are launched, $MSW^2{}_{TM}$ that Shimadzu has developed is one of liquid microsampling devices too. $MSW^2{}_{TM}$ is a tool to quantify constant exact volume of plasma without technical difficult. $MSW^2{}_{TM}$ comprises 2 kinds of component, Microsampling Wing $_{TM}$ and Microsampling Windmill $_{TM}$. Microsampling Wing $_{TM}$ is a handy device which has a microchannel with a hydrophilic and EDTA coated inner surface. The total capacity of microchannel is 23μL. Also, a small tip like part of the Microsampling Wing $_{TM}$ carrying 5.6μL aliquots of plasma can be snapped off with fingertips. The Microsampling Windmill $_{TM}$ can hold up to 14 Microsampling Wings $_{TM}$ to be centrifuged for plasma separation without any other process.
In this poster, we evaluated the sampling accuracy and precision of $MSW^2{}_{TM}$ by determining the concentration of caffeine spiked in milk.

POSTER ABSTRACT
#114

Drug Monitoring by the Volumetric Absorptive Microsampling Approach for Dried Blood Spots: Method Development Considerations to Mitigate Hematocrit Effects

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Kasie Fang

Background: GSKA is a compound that was in development in Phase I clinical trials. A robust analytical method has been developed and validated using a Volumetric Absorptive Microsampling (VAMS) sampling device. Methodology: After accurate sampling 10 μL of blood, VAMS tips were air dried approximately 18 hours and desorbed by an aqueous solution containing internal standard (IS). The recovered blood underwent liquid-liquid extraction (LLE) in ethyl acetate to minimize matrix suppression. Assay accuracy, precision, linearity, carryover, selectivity, recovery, matrix effects, hematocrit (HCT) effects and long- term QC stability were evaluated. Results: HCT-related assay bias was minimized in 30-60% blood HCT range and all validation parameters met acceptance criteria. The method is suitable for quantitative analysis of GSKA in human blood.

POSTER ABSTRACT
#115

Development of a novel LC concept for clinical proteomics

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Background

Mass spectrometry based proteomics and metabolomics are fast growing and powerful technologies, with the potential to revolutionize health care and precision medicine. However, available separation technology has so far limited throughput and robustness and thereby prevented omics technologies from being fully integrated and routinely used in a clinical setting. Here we describe a conceptually novel chromatography system that significantly increases robustness and sample throughput while maintaining the sensitivity of current nano-flow LC.

Methods

The new system uses four low-pressure pumps in parallel to elute samples from a disposable trap column while also forming a chromatographic gradient. The sample and gradient are moved into a holding loop that subsequently is switched in-line with a single high-pressure pump and a separation column. Thus elution from the disposable trap column and gradient formation become de-coupled from the high-pressure separation.

Results

We have characterized the performance of the new system regarding cross contaminations (<0.05%, total TIC), retention time shifts and peak width (<8.5 sec) in over 1,500 HeLa runs. The short overhead time of approximately 2 min allowed us to measure 60 samples per day (22 min gradient, 2 min overhead time) compared to 40 samples with a standard nano-LC systems (22 min gradient, 15 min overhead time). The 100 human plasma samples were analyzed 40 hours and each resulted in several hundred quantified plasma proteins, including more than 50 FDA- approved biomarkers

with high reproducibility.

Conclusion

We have designed a new separation technology for LC-MS applications with the sensitivity, robustness and throughput required for clinical workflows and that will allow uninterrupted analysis of thousands of clinically relevant samples for biomarker discovery studies as well as for analysis of patient samples in clinical laboratories.

Keywords: HPLC, separation, clinical proteomics, LC-MS, biomarker

POSTER ABSTRACT
#116

Accessing High Performance Microflow on a Chip-Based Platform

Helena Svobodova, Aaron Dewberry, Amanda Berg, Mike S. Lee, Gary Valaskovic

Novel Aspects: A chip-based column with integrated heating and sheath gas functionality capable of operating at 10μL/min.

Introduction: Traditionally, an experimental method must choose higher output or sensitivity. Modern LC/MS solutions are attempting to bridge the gap between the short runtimes of high flow applications and the sensitivity of low flow applications, by targeting the microflow range. Flow rates, in this range, allow nanoflow users to collect data faster. Capillary flow users can expect more sensitivity and less solvent use. Here, we present a highly efficient chip-based solution with integrated column heating and sheath gas support capable of operating at flow rates between 1-10μL/min. This newly developed platform enables higher throughput while maintaining the high sensitivity of low flow applications.

Methods : A standard consisting of 8 synthetic peptides (PicoSure, New Objective, Inc.) was direct injected on a chip based column using an HTC-PAL auto-sampler (Leap Technologies). The 150μm ID chip-based columns (PicoChip, New Objective, Inc.) were packed to 5cm long bed with 3μm (Reprosil-PUR, C18 AQ, Dr. Maisch GmbH) and 5μm (HALO Peptide C18, Advance Material Technologies). Sample separation was achieved by a 2min. 2-35% acetonitrile gradient which was delivered by a nano LC pump (Eksigent, Sciex) operating at 10μL/min. Data was collected in full MS positive mode on LTQ mass spectrometer (Thermo) at 2.4kV with sheath gas setting of 5. The sheath gas delivery and column heating to 50°C were realized through a specialized chip-based platform.

Preliminary data:
Previous studies showed that the chip-based technology can be successfully utilized at flow rates ranging from nanoflow to low microflow[1]. Here we present a chip based column capable of operating at 10μL/min on a platform, which can support nanoflow as well a microflow applications.

150μm ID chip-based columns were packed to 50mm bed lengths with 3 and 5μm particle C18 resins. The pressures of these columns measured at 10μL/min,2% acetonitrile,98% water at 50°C were 7085psi for the 3μm resin and 5184psi for the 5μm resin (36% pressure difference) indicating that the developed hardware currently capable of operating at pressures up to 8,000psi can support both column types. Replicate injections of 500fmol/μL mixture of 8 synthetic peptides were analyzed with a 2min gradient of 2-35% acetonitrile at 10μL/min on a column packed with 5μm resin. Consistent reproducible peak widths of less than 3sec were observed for all 8 peptides, in three replicate injections. The average peak capacity varied from 38.8 for MH2+ peptides 592.3 and 494.3m/z to 44.3 for MH2+ peptide 550.3m/z. Injection reproducibility was evaluated based on the retention time and peak area RSD. The retention time RSD was measured to be less than 3 for all 8 evaluated peptides. The peak area RSD was less than 10% for all peptides with an exception of MH2+ peptide 550.3 m/z with RSD 11.2%.

This initial study demonstrated the feasibility of using the chip-based device for high performance microflow. Further testing will evaluate the performance of different resin types at 10μL/min and optimize the run conditions to further improve sample throughput and separation.

[1] Dewberry AD; Svobodova H; Berg, AL; Valaskovic GA: Maximizing Flexibility: A Gas and Temperature Enabled Chip-Based Solution for Nanoflow and Microflow LC-MS, Proceedings of the 64th Conference on Mass Spectrometry and Allied Topics, San Antonio, June5-9, 2016

POSTER ABSTRACT
#117

Comparison of Intact Level and Surrogate Peptide Quantification of a 150 kDa mAb in Plasma using HRMS and Tandem Quadrupole MS

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Purpose:
Comparison of high resolution MS and tandem quadrupole for quantification of Trastuzumab in plasma using intact level and surrogate peptide approaches, including measurement of detection limits and linear dynamic range.

Methods:
For the surrogate peptide approach Trastuzumab was spiked into rat plasma along with an infliximab internal standard and immuno-purified using Protein A plate. Samples were then prepared for LC-MS analysis using commercially available digest and SPE kits following supplied protocols. Resulting signature peptides were analyzed using tandem quadrupole (Xevo TQ-XS) and high resolution (Xevo G2-XS QTof) MS. For quantification at the intact level, Trastuzumab was spiked into mouse plasma and purified using magnetic bead based anti-human FC immuno-purification. The prepared sample was analyzed using the Vion QTof platform.

Results:
Using the surrogate peptide approach the LLOQ ranged from 10-25 ng/mL using tandem quadrupole MS and 25-50 ng/mL for high resolution MS. The linear dynamic range was >4 orders of magnitude for both platforms. Using the intact approach, the LLOQ was 3 ng/mL in BSA solution with a linear dynamic range of >2.4 orders of magnitude. In mouse plasma a LLOQ of <250 ng/mL and a linear dynamic range of >2 orders of magnitude was achieved.

Conclusions:
Sensitive detection of mAb concentration in plasma was achieved using both the surrogate peptide and intact approach. The importance of sample preparation for achieving high sensitivity, linearity and accuracy is highlighted. While high resolution mass spectrometry offer excellent sensitivity and flexibility to study large molecules at both the peptide and intact level, tandem quadrupole MS remains the most sensitive

platform for surrogate peptide based quantification. Recent progress in intact level analysis suggests that the sensitivity gap between surrogate peptide and intact level quantification could be closed further with improved sample preparation and MS technologies.

POSTER ABSTRACT
#118

Metabolomic Analysis of *Drosophila melanogaster* Organs during Overnutrition

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Louis Searcy

Abstract:

Matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) was used to investigate metabolic differences between high and normal glucose diets in *Drosophila melanogaster* (fly) organs. This high carbohydrate diet models overnutrition, which is associated with metabolic diseases including obesity, type 2 diabetes, and heart disease. Chronic overnutrition challenges homeostasis of cells and can lead to accumulation of unnecessary lipids.[1] Principal components analysis was used to determine features that varied between the two diets. The most variation was observed in heart and central nervous system tissues. Heart dysfunction has previously been induced by high glucose diets;[2] This MS approach aims to identify metabolite imbalances in the dysfunctional heart tissues.

Methods:

Flies were aged for three weeks on 1 M or 0.15 M glucose feed. The organs were dissected and placed on microscope slides and frozen at -80°C until analyzed by MALDI MS. The slides were coated in matrix consisting of 40 mg/mL 2,5-dihydroxybenzoic acid and 10 mM sodium acetate dissolved in 90:10 methanol:water. The matrix was sprayed onto the slides using a glass Type A Meinhard Nebulizer (Golden, CO) with a flow rate of 3 mL/min.

The fly organs were then analyzed in a Thermo Scientific LTQ XL linear ion trap mass spectrometer (San Jose, CA) with a MALDI ionization source, consisting of a Lasertechnik Berlin MNL 106-LD N2 laser (λ=337nm) (Berlin, Germany). MALDI parameters included a laser energy of 14 µJ, 4 laser shots/spot, and a step size of 75µm.

The mass spectral data was processed with Thermo ImageQuest v1.0.1 software (San Jose, Ca), MSiReader[3], and MetaboAnalyst[4]. ImageQuest and MSiReader provide MS images. MSiReader allows for background subtraction and region of interest selection, while MetaboAnalyst provides a comprehensive statistical platform.

Discussion:

Principal components analysis shows variation in the heart and central nervous system (CNS) of the flies, but not in the fat body or gut. This might be caused by the fat body reaching a maximum storage capacity leading to lipotoxicity in the heart and CNS tissue. Other comparisons were made with PCA showing differentiation between the content of the tissue types studied.