POSTER SESSION ABSTRACTS

Inspiration and Education

POSTER SESSION
Tuesday, October 6, 1:00 pm - 3:00 pm

CPSA USA 2015
October 5 - 8, 2015
Sheraton Bucks County Hotel Langhorne, PA

POSTER ABSTRACT

#101

General Protein Analysis Using In-Source CID And SEC Chromatography

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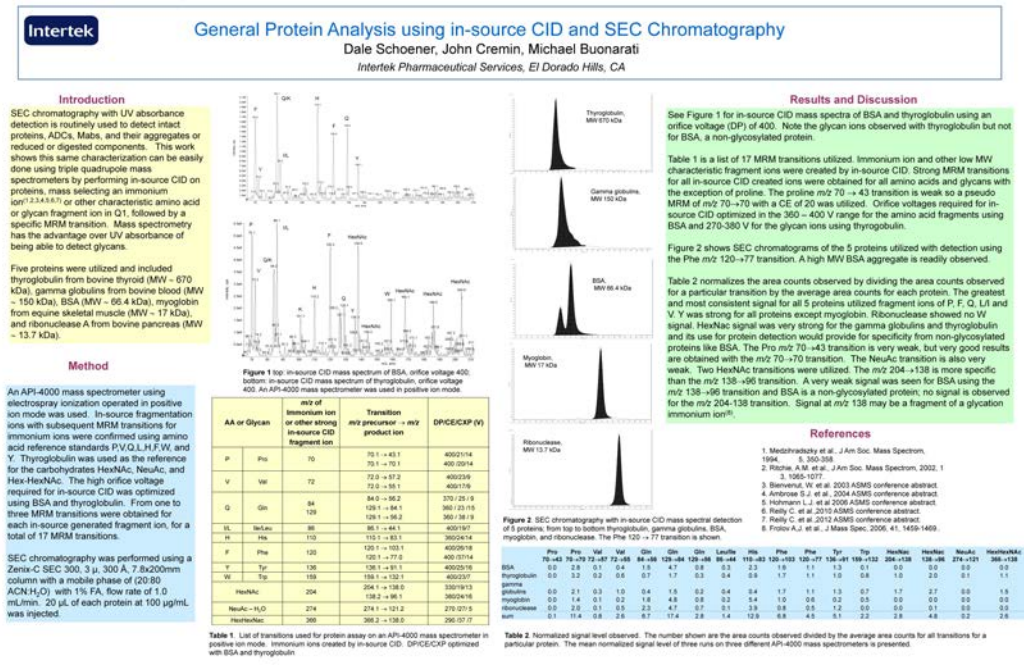
SEC chromatography with UV absorbance detection is routinely used to detect intact proteins, ADCs, Mabs, and their aggregates or reduced or digested components. This work shows this same characterization can be easily done using triple quadrupole mass spectrometers by performing in-source CID on proteins, mass selecting an immonium ion(1,2,3,4,5,6,7) or other characteristic amino acid or glycan fragment ion in Q1, followed by a specific MRM transition. Mass spectrometry has the advantage over UV absorbance of being able to detect glycans.
Five proteins were utilized and included thyroglobulin from bovine thyroid (MW 670 kDa), gamma globulins from bovine blood (MW 150 kDa), BSA (MW 66.4 kDa), myoglobin from equine skeletal muscle (MW 17 kDa), and ribonuclease A from bovine pancreas (MW 13.7 kDa).

An API-4000 mass spectrometer using electrospray ionization operated in positive ion mode was used. In-source fragmentation ions with subsequent MRM transitions for immonium ions were confirmed using amino acid reference standards P,V,Q,L,H,F,W, and Y. Thyroglobulin was used as the reference for the carbohydrates HexNAc, NeuAc, and Hex-HexNAc. The high orifice voltage required for in-source CID was optimized using BSA and thyroglobulin. From one to three MRM transitions were obtained for each in-source generated fragment ion, for a total of 17 MRM transitions.
SEC chromatography was performed using a Zenix-C SEC 300, 3 µ, 300 Å, 7.8x200mm column with a mobile phase of (20:80 ACN:H2O) with 1% FA, flow rate of 1.0 mL/min. 20 µL of each protein at 100 µg/mL was injected.



POSTER ABSTRACT
#102

Enzyme Activity Assay Of A Pegylated Arginase In Mouse Serum Using LC-MS/MS

Oanh Dang[1], Susan Alters[2], Scott Rowlinson[2], Everett Stone[3], John Bruce[1], Shannon Bryant[1], Mike Buonarati[1], Dale Schoener[1]

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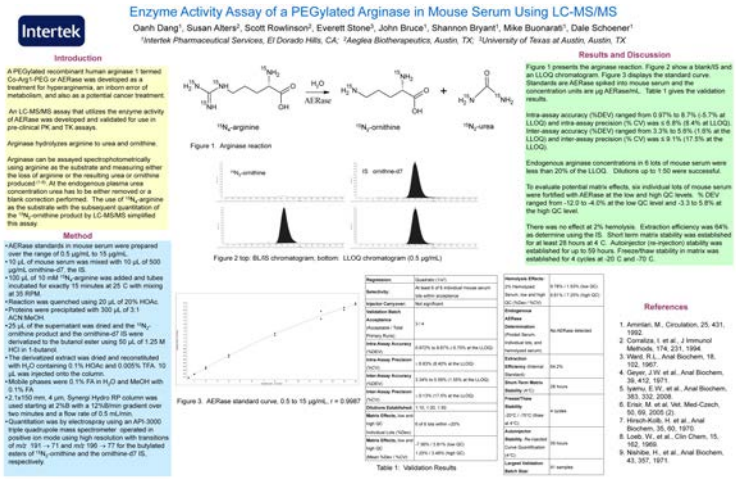
A PEGylated recombinant human arginase 1 termed Co-Arg1-PEG or AERase was developed as a treatment for hyperarginemia, an inborn error of metabolism, and also as a potential cancer treatment. An LC-MS/MS assay that utilizes the enzyme activity of AERase was developed and validated for use in pre-clinical PK and TK assays. Arginase hydrolyzes arginine to urea and ornithine. Arginase can be assayed spectrophotometrically using arginine as the substrate and measuring either the loss of arginine or the resulting urea or ornithine produced (1-9). At the endogenous plasma urea concentration urea has to be either removed or a blank correction performed. The use of 15N4-arginine as the substrate with the subsequent quantitation of the 15N2-ornithine product by LC-MS/MS simplified this assay.

•AERase standards in mouse serum were prepared over the range of 0.5 µg/mL to 15 µg/mL.
• 10 µL of mouse serum was mixed with 10 µL of 500 µg/mL ornithine-d7, the IS.
• 100 µL of 10 mM 15N4-arginine was added and tubes incubated for exactly 15 minutes at 25 C with mixing at 35 RPM.
• Reaction was quenched using 20 µL of 20% HOAc.
• Proteins were precipitated with 300 µL of 3:1 ACN:MeOH.
• 25 µL of the supernatant was dried and the 15N2- ornithine product and the ornithine-d7 IS were derivatized to the butanol ester using 50 µL of 1.25 M HCl in 1-butanol.
• The derivatized extract was dried and reconstituted with H2O containing 0.1% HOAc and 0.005% TFA. 10 µL was injected onto the column.
•Mobile phases were 0.1% FA in H2O and MeOH with 0.1% FA
• 2.1x150 mm, 4 µm, Synergi Hydro RP column was used starting at 2%B with a 12%B/min gradient over two minutes and a flow rate of 0.5 mL/min.
• Quantitation was by electrospray using an API-3000 triple quadrupole mass spectrometer operated in positive ion mode using high resolution with transitions of *m/z* 191 71 and *m/z* 196 77 for the butylated esters of 15N2-ornithine and the ornithine-d7 IS, respectively.



POSTER ABSTRACT

#103

Accuracy Of Endogenous Dilution As A Measure Of Biomarker Selectivity: Quantitation Of Cgmp And Camp In Human Plasma By LC-MS/MS

John E. Buckholz[1], Barry R. Jones[1], Raymond F. Biondolillo[1], Philip E. Joyce[1], Danielle J. Strong[1], Jennifer M. Cox[2], Bradley L. Ackermann[2]

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Introduction

Adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) are endogenous cyclic nucleotides with many biological functions. Accurate measurements of cAMP and cGMP in plasma and urine are useful biomarkers for development of therapeutics for a variety of indications.

As cAMP and cGMP are endogenous analytes, a surrogate matrix approach was taken to achieve accurate quantitation by LC-MS/MS. This approach adds additional assay characterization procedures including surrogate matrix qualification, which comprises parallelism and accuracy of endogenous dilution. Here we show that the accuracy of endogenous dilution experiment revealed a co-eluting interferent for cGMP, resolution of which required adjustment of LC parameters. This experiment, commonly referred to as 'parallelism' in ligand binding methods, is critical to establishing well validated biomarker methods.

Methods

cAMP and cGMP were isolated from 50 µL K_2EDTA plasma by protein precipitation followed by weak anion exchange solid phase extraction. Reversed phase HPLC chromatography over a shallow gradient were used to separate cAMP and cGMP from other endogenous analytes. An API5000 with positive ion electrospray ionization in SRM mode was used for MS detection.

Data were normalized by use of stable-labeled internal standards - [^{13}C,$^{15}N_2$]-cAMP, and [^{13}C,$^{15}N_2$]-cGMP. A surrogate matrix of 4% bovine serum albumin in phosphate buffered saline was used for the analyses.

For the accuracy of endogenous dilution experiment, plasma samples from several individual donors were diluted with surrogate matrix and assayed. The resulting calculated concentrations of cAMP and cGMP were compared to those of undiluted plasma for each lot.

Preliminary Data

Initially, a method was developed that demonstrated excellent statistical results and mean endogenous levels of 1.2 ng/mL for cGMP and 6.3 ng/mL for cAMP were established. It was observed during validation that dilution of unfortified control plasma with surrogate matrix yielded a positive bias for cGMP but not cAMP. Also, the calculated concentration of endogenous cGMP was higher than the previously observed inter-individual range. Upon closer inspection, some peak asymmetry and broadening were observed for cGMP compared to SIL IS, indicating an interfering peak. Further screening showed this interfering peak was present in 2 of 10 lots, and the retention time varied with analytical column.

Addition of 0.1% TFA to the mobile phase pinned the interfering peak to a specific retention time that was well resolved from the cGMP across all columns tested. Validation was restarted and successfully completed with this change implemented. Excellent endogenous dilution accuracy, linearity, sensitivity, parallelism, precision, and selectivity were demonstrated for the modified assay. Subsequent study sample data showed a time point and subject dependence on the presence and intensity of the resolved peak. This observation underlines the value of the endogenous dilution experiment as a probe of endogenous analyte selectivity in biological matrix.

CPSA USA

POSTER ABSTRACT

#104

Validation Of A Low-Volume Plasma LC-MS/MS Method Using A Capillary Microsampling (CMS) Technique

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Introduction: In recent years there has been much discussion related to the use of fewer animals for pre-clinical safety and efficacy testing. In addition, regulatory agencies have requested testing in pediatric patient populations for all new drug compounds. With the development of more sensitive instrumentation, the need for smaller blood sample size analysis, has been realized. Over the years, techniques such as Dried Blood Spot (DBS) have been utilized to sample less than 50 uL of blood. While DBS seems to work well for many analyses, the historical PK database for most clinical trials has been through the quantitation of drugs from plasma or serum.
Method: Recently a technique has been developed wherein a flexible plastic capillary tube, lined with K2EDTA, is used for the collection of 30 – 50 µL of blood. This tube is then spun down and approximately 15-20 µL of plasma is available. Small metered sections of the plasma section of the capillary are then cut and stored for analysis. Each section of the capillary is placed into a secondary container (e.g. Eppendorf tube or 96 well plate), diluted with a washout buffer and then plasma proteins precipitated using acetonitrile. The container is then spun down, the supernatant transferred to a fresh secondary container, further diluted with mobile phase, and analyzed by LC-MS/MS.
Results: The procedure with which this is performed is presented herein. The precision and accuracy is well within acceptable limits for validation, thus with this method a complete PK curve can be determined from a single mouse thereby greatly reducing the number of animals in each study. More importantly, in pediatric models, a heel stick can be used to extract very small volumes of blood for testing.

Conclusions

Utilizating this method allows for consistency with typical plasma bioanalytical methodology. Comparison of this method to the validated method indicates that the data are consistent. The standard curve is aliquoted using freshly prepared standards without the need for capillary tubing. Additionally, the bracketing QCs are similarly prepared for acceptance of the analytical run. With this method, atleast 3 sections of the capillary can be prepared and frozen for further analysis. Cross contamination and/or freeze thaw stability with be important attributes of this method. Future work will be performed to determine whether even smaller volumes of blood can be used, and whether standardization can be achieved with particular capillary

References (1) “Commentary: Bioanalysis Zone: DBS survey results”. Bioanalysis (2014) 6(3), 1-5. (2) Ove Jonsson, “Capillary Microsampling (CMS): Better Science – fewer animals”, “Hatching” EBF focused meeting, Brussels, June 2012.
(3) Kathryn Chapman, Simon Chivers, Dan Gliddon, David Mitchell, Sally Robinson, Tim Sangster, Susan Sparrow, Neil Spooner, and Amanda Wilson, “Feature: Overcoming the barriers to the uptake of nonclinical microsampling in regulatory safety studies”, Drug Discovery Today, 2014; 19(5): 528-532
(4) Ove Jonsson, Rodrigo Palma Villar, Lars B., Nilsson, Marie Eriksson, and Kristian Konigsson, “Validation of a bioanalytical method using capillary microsampling of 8 µL plasma samples: application to a toxicokinetic study in mice”, Bioanalysis (2012) 4(16): 1989-1998.
(5) Walter Korfmacher, Maria Fitzgerald, Yongyi Luo, Stacy Ho, Jie Wang, Zhongtao Wu, Gregory Snow, and Thomas O’Shea, “Capillary microsampling of whole blood for mouse PK studies: an easy route to serial blood sampling”, Bioanalysis (2015) 7(4): 449-461.

POSTER ABSTRACT

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The Effects Of Heat Stabilization And Euthanasia Methods On Free Fatty Acid Measurements In Rat Tissues

Oskar Karlsson[1]; Jörg Hanrieder[2]; Camilla Sivertsson[3]; Marcus Söderquist[3]; Fredrik Jernerén[4]

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Introduction

Lipidomics is a rapidly growing field, but the quality of research critically depends on recognition that the lipid pool is labile. Postmortem changes can cause accumulation of tissue free fatty acids (FFA). Many studies on brain lipids do not use adequate methods for sample fixation and there is a need for alternative methods that could stabilize various tissues including human samples. Conductive heat stabilization of tissues is an easy and controlled method of sample fixation, which has been shown to stop postmortem protein degradation and counteract post-sampling variation in the proteome. In animal research, the euthanasia protocol used may also influence the results. Here, we investigate the effects of heat stabilization, as well as the effects of different euthanasia methods, on FFA release and phospholipase A2 (PLA2) activity in the brain and liver.

Methods

Rat tissues were either instantly dissected from fresh tissue, or dissected after heat stabilization of the intact tissues using the Stabilizor T1 (Denator AB, Sweden). In short, heat stabilization was performed in Structure Preserve mode on the Stabilizor T1 which utilizes a combination of heat and controlled pressure to stop degradation while maintaining structural references. During the stabilization, conductive heating plates at 95 °C inactivate enzymes in the sample by a quick and strictly controlled increment of the temperature, resulting in rapid and uniform protein denaturation. The tissues from other animals were kept at room temperature for 10 and 20 min before the heat stabilization. Last group was directly heat stabilized and left at room temperature for 20 min. To compare the impact of euthanasia methods on FFA, rats were killed using three different protocols (decapitation, CO2 inhalation, and pentobarbital injection). All samples were stored at -80°C until analysis of lipids using liquid chromatography tandem mass spectrometry (LC-MS/MS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging. PLA2) activity was measured using the fluorometric EnzCheck PLA2 Assay kit.

Results

The results show the importance of considering preanalytical sample handling and euthanasia methods in lipid analysis. The analysis of brain homogenates clearly demonstrated PLA2 activity and time dependent post-sampling changes in the lipid pool of snap frozen non-stabilized tissue. There was a significant increase in FFAs already at 2 min, which continued over time. Heat stabilization was shown to be an efficient method to reduce PLA2 activity and ex vivo lipolysis. Post-sampling effects due to tissue thawing and sample preparation induced a massive release of FFAs (up to 3700%) from non-stabilized liver and brain tissues compared to heat stabilized tissue. The ToF-SIMS imaging of brain sections further demonstrated that the heat stabilization effectively inhibited postmortem lipolysis in the tissue. The lipolysis is also reflected by other changes in the lipidome (e.g. eicosanoids and docosanoids). It is therefore essential to use standardized protocols and preferably stabilize the tissue before analysis of any lipids or metabolites. This study demonstrates that conductive heat inactivation of enzymes responsible for the release of fatty acids can be utilized to minimize unintended consequences of post-sampling effects on experimental outcomes. The technique can be used on various tissues, both fresh and frozen, including human samples. Finally, our results show that it is important to also take the method of euthanasia in consideration when conducting as well as comparing previously published lipidomic studies. The studied FFA were decreased significantly (up to 44%) in the group of animals euthanized by pentobarbital injection compared with CO2 inhalation or decapitation.

POSTER ABSTRACT
#106

Unified Drug Testing By Online SPE-LC/MS/MS With Focus On Productivity Achieved Through Ease Of Use By Lab Technicians: One Totally Automated Method Measures ALL The Drugs In Urine And/Or Oral Fluids

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

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

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

POSTER ABSTRACT
#107

Bottom-Up Quantitative Measurement Of An Antibody Therapeutic In Human Plasma By LC-MS/MS Using Formic Acid Digestion

Lian Shan[1], Robert M. Sturm[1], Raymond F. Biondolillo[1], John E. Buckholz[1], Barry R. Jones[1], Ryan Faucette[2], Daniel J. Sexton[2]

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Introduction

DX-2930 is a human monoclonal antibody inhibitor of plasma kallikrein under investigation for long-term prophylaxis of hereditary angioedema. Although a ligand binding assay was ultimately successfully validated and used to assay PK samples, a "bottom-up" LC-MS/MS assay was developed due to early LBA method development issues. In-silico sequence homology searches utilizing various enzymatic digestion reagents (including trypsin) did not provide unique peptides for this therapeutic antibody. It has long been known that aspartyl peptide bonds can be cleaved with dilute acid and heat. Putative unique peptides were identified from in-silico formic acid chemical digestion of the DX-2930 amino acid sequence. A unique fragment was chosen as signature peptide and used to develop a quantitative assay for DX-2930 employing magnetic bead-based immunopurification, nano-flow chromatography, and selected reaction monitoring MS detection.

Methods

DX-2930 was isolated from sodium citrate plasma and SCAT169 plasma by magnetic bead-based antibody capture using Hamilton Star liquid handling robotics. Briefly, a biotinylated anti-DX-2930 anti-idiotyptic monoclonal antibody was incubated overnight at 10ºC with 100µL of human plasma diluted in 300µL phosphate buffered saline containing 0.1% bovine serum albumin. Streptavidin coated magnetic beads were then added to the samples, and the solution was mixed for 30 minutes at room temperature. The supernatant was removed to waste and the complexed beads washed several times. DX-2930 was eluted from the Ab/bead complex with 25mM HCl.

The eluted proteins were denatured with 8M guanidine in 100mM ammonium bicarbonate prior to addition of the stable-isotope labeled internal standard (IS) solution, which was an extended sequence version of the monitored formic acid fragment of DX-2930. The sample was reduced with tris (2-carboxyethyl)phosphine) (TCEP) and incubated at 56ºC for 60 minutes. Formic acid was added to a final concentration of 2%, and the solution was heated overnight at 95ºC.

Extracts were injected to 300µm x 5mm C18 trap column, and eluted to 75µm x 150mm C18 Thermo EASY-Spray column at 600nL/min flow rate. Thermo TSQ Vantage triple quadrupole mass spectrometer equipped with EASY-Spray nano source was used for MS detection.

Preliminary Data

The lower limit of quantitation of the assay was 100ng/mL, with linear limit to 1500ng/mL and extended 100 fold with dilution QC. Excellent assay performance was observed for DX-2930 in both citrated and SCAT169 plasma, with inter-day results within FDA BMV guidance for LCMS. This was accomplished in spite of the lack of IS normalization for the immunocapture procedure owing to the accuracy and precision of the Hamilton Star liquid handling robotics. Acceptable selectivity and accuracy were observed in human plasma from several individual donors. DX-2930 was found to be stable in citrated plasma for up to 24 hours at room temperature and for up to 3 freeze/thaw cycles.

POSTER ABSTRACT

#108

Evaluating The Potential For Cross Contamination When Performing 96-Well Sample Preparation Prior To LC-MS/MS Analysis

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Introduction

It is vital to ensure proper measures are in place to reduce or eliminate cross contamination between samples, which could result in false results. In many cases sample carryover in the LC/MS system is checked early on in the method development process. However, one area that can often be overlooked is during the sample preparation stages. This involves all aspects from pipetting, sample transfer, extraction protocol, evaporation and mixing steps. This poster evaluates various stages of the sample preparation process to determine the potential for cross contamination and present approaches to minimize and or eliminate the effect.

Methodology

All work was performed using the 96-well plate format due to the close proximity of samples and increased potential for cross contamination. Initial experiments investigated the use of a dye dissolved in multiple solvents with differing characteristics. Occurrence of cross contamination was investigated in the pipetting, transfer, extraction, evaporation and mixing steps. Dye evaporation experiments were repeated using various analyte suites spiked at high concentrations. All surrounding wells were spiked at the assay's LOQ. Evaporated samples were reconstituted in mobile phase prior to analysis. Samples were analyzed using LC-MS/MS.

Results

Dye experiments highlighted multiple areas that result in cross contamination if experiments are not structured in the correct way. We investigated the difference between positive pressure and vacuum processing. The former demonstrated less potential for cross contamination due to greater penetration of the luer tips (outlet nozzles) into the collection plate. This was the case for semi-automated processing using a positive pressure system and the automation platform Biotage Extrahera. For vacuum processing it is important to ensure adequate penetration of the luer tips into the collection plate, due to different manifold spacing and SPE plate design in terms of tip length.

Mixing was investigated using square and round well collection plates. Subtle differences were observed between the plates and care should be taken when selecting vortex speeds combined with solvent volumes for each.

Evaporation experiments used dye spiked into multiple positions in 96-well plates, while all surrounding wells were populated with blank solvent. The parameters investigated for evaporation studies were: gas flow rate, temperature, needle height and volume of solvent in the wells. All parameters were demonstrated to be important and need to be considered depending on experimental set up. Evaporation experiments were confirmed when investigating different analyte suites combined with LC-MS/MS analysis. Analyte suites were selected based on varying degree of volatility and hydrophobicity; amphetamines, opiates and benzodiazepines were investigated. Experiments were performed under "normal operating" conditions and replicated using a novel "Hot spot" plate adapter. The adaptor is designed to fit on top of the 96-well collection plate, while the needles of the evaporator penetrate through the chimneys during evaporation. This design reduces the open surface area of the top of the wells, directing evaporated solvent and gases away from adjacent well therefore substantially reducing the likelihood of contamination.

POSTER ABSTRACT

#109

A Novel Technique of Separating Polysorbate Mixtures from Large Protein Molecules

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Introduction

Polysorbates 20 (PS20) and 80 (PS80) are often used in large molecule biotherapuetic formulations as a means for preventing protein denaturation and aggregation. Understanding the molecular heterogeneity and stability of PS20/80 in a formulation is imperative as the degradation of polysorbates leads to a drop in pH which could lead to protein aggregation or denaturation (i.e. deamidation). The complex heterogeneity of polysorbates and protein interferences provides a clear challenge when trying to identify or quantitate either of these species when present in the same formulation.

Methods

An online separation technique known as turboflow is generally used to remove high molecular weight species from biological matrices when analyzing small molecules. Discussed herein is a new approach to the separation of polysorbates and large protein species using turboflow in conjunction with mass spectrometry. This methodology is a completely automated approach that increases the experimental throughput. The turboflow system operates in a two-dimensional fashion such that during the first stage the analyte is loaded onto the turboflow column then eluted with organic. Once eluted, the analyte is refocused onto a reverse-phase (RP) column. After analytical separation, the second stage occurs when the flow of the analyte is directed to the high-resolution Q Exactive mass spectrometer for detection. When a mixture of polysorbate and antibody molecules are introduced to the LC, the polysorbate molecules are retained by the turboflow column while the antibody molecules are washed through. Originally, an Accucore C18 column was used for the analytical separation post-elution of the polysorbate molecules from the turboflow column. However, due to the low binding affinity of the antibody of choice for the C18 packing material, a ProSwift RP-4H analytical column was substituted to retain and then elute the protein.

Preliminary Results

Using a combination of chromatographic separation and changing flow paths resulted separation of the polysorbate and antibody molecules. A calibration curve of the polysorbate was prepared with four orders of magnitude which allowed for the quantitation of individual polysorbate ions (single *m/z*) over a large linear range. Future experiments would be to couple the LC platform to a charged aerosol detector (CAD). Detection with a CAD will result in more precise quantitation of heterogeneous polysorbate mixtures. Smaller proteins were examined using this methodology and can be separated from their polysorbate mixtures as well.

USA CPSA

POSTER ABSTRACT

#110

Matrikleen: A New Generation Extraction/ Sample Cleanup Product For Plasma, Serum And Blood Samples

Xuejun Zang, Chhavi Bhardwaj, Slobodan Milasinovic, Anil Oroskar and Asha Oroskar

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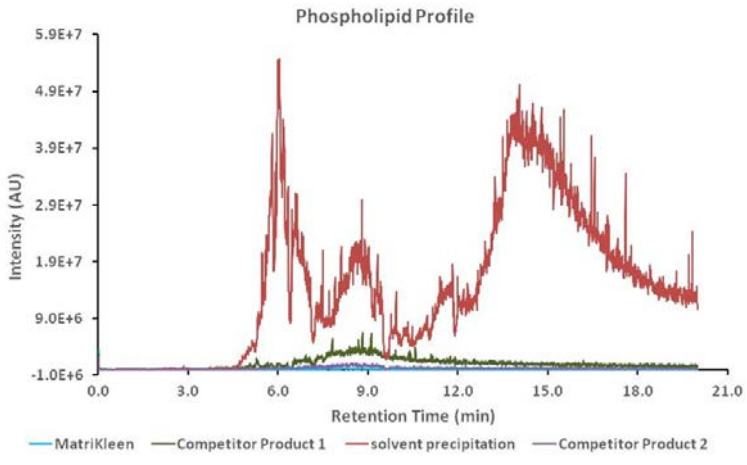
Scientists and researchers involved in studying biological matrices often face the biggest challenge at the sample cleanup and preparation step of the process. Analysis of biological samples is often hindered by unwanted interference from endogenous compounds in the matrices. These interferences could be from protein, phospholipids, salts or any other unwanted compounds inherent to the matrix. These compounds also affect the recovery and quantitation of the target analytes. Target analytes could be polar, non-polar, acidic, basic or neutral in nature, which makes it challenging to design an extraction/clean up technique or product which can be universally applicable to a wide variety of target compounds. In addition, the process must be simple to implement and must have fast process time for high throughput applications. There may not be any one type of universal extraction device or method available, but at least we want to divide the type of samples, and hopefully develop a "universal extraction device" for certain types of samples.

Pharmaceutical industries, CROs, and clinical diagnostic laboratories often are involved with one such type of biological sample matrices. For example, most samples are either blood original samples or urine/saliva original samples. For blood samples, most interferences are due to protein and phospholipids. At Orochem Technologies we developed a unique sample extraction product called MatriKleen, for effective removal of matrix-related interferences from such biological samples.

MatriKleen extraction plate is designed to eliminate the interfering compounds in plasma, serum and blood samples, and to provide high recovery for a wide range of analytes. A variety of extraction solutions with respect to the composition of the sorbents were tested before finalizing on a unique proprietary product with one step sample clean up capabilites. MatriKleen is designed for applications requiring high sample throughput such as CROs and clinical diagnostics laboratories, for example; total process time for 96 samples is less than 10 min.

Figure 1 shows a chromatogram comparing the phospholipid profile for serum sample extracts which were processed using standard solvent protein precipitation, MatriKleen and two other extraction plates available in the market.



As is evident in the figure above, compared to other extraction plates for plasma and serum samples, MatriKleen extraction plate sufficiently removes the unwanted interference from proteins and phospholipids and provides high recovery of a wide range of basic, acidic and/or neutral target analytes. Another key feature of the product is that the biological samples do not need to be acidified before extraction. Based on our study, mixture of methanol and acetonitrile is an excellent extraction reagent for MatriKleen plates. For one of the tests; acetaminophen, caffeine, hydrocortisone, and carbamazepine in serum samples were analyzed using above mentioned conditions and the recoveries of all four compounds were more than 73%.

Use Of High Carbon Load C18 UHPLC Column For Separation Of Clinical Analytes

Xuejun Zang, Chhavi Bhardwaj, David House, Anil Oroskar and Asha Oroskar

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Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has seen immense growth in clinical laboratories in recent times. LC-MS/MS method has emerged as a reliable method of choice for high throughput clinical laboratory due to its high specificity and high accuracy at low ng/mL level. However, even though mass spectrometry is more specific and selective than UV detection, it does not work effectively for the separation of isomers, which often require a good chromatographic separation. Clinical diagnostic screening methods usually cover tens of target compounds, many of which are isomers. Moreover, such screenings require high throughput and short turnaround time which poses a requirement of shorter run times in chromatographic separation/analysis. Use of UHPLC columns for LC-MS/MS applications addresses these requirements efficiently and is becoming increasing more popular.

For the work presented here we tested Orochem's Gazelle C18 UHPLC column for separation of pain management drugs panel. Gazelle C18 column is a high load C18, with particle size 1.7 µm. Four different compounds from Cannabinoid class were analyzed using Gazelle C18. The four target compounds were (-)-delta 9-tetrahydrocarnnabinol (THC), 11-nor-9-carboxy-delta 9 –THC, Cannabinol and Cannabidiol. Cannabidiol and (-)-delta 9-tetrahydrocarnnabinol (THC) have identical molecular mass and the precursor/fragmention transitions, consequently the two compounds could not be separated using MS. We were able to separate the two isomers efficiently using Gazelle C18 with two different conditions. With modifier, we can separate cannabidiol, (-)-delta 9-THC, cannabinol, and 11-nor-9-carboxy-delta 9 –THC in 40 sec. Without modifier, we can separate them in about 4 minutes. LC-MS/MS method combining with the efficiency of UHPLC column with MS detection offers significantly increased throughput, low solvent consumption, inline process monitoring, and improved LC-MS results.

Another application of Gazelle C18 is to separate vitamin D metabolites. There is increasing demand of analyzing vitamin D metabolites using LC-MS/MS. Our method is using a gradient program with ammonium formate buffer in methanol and water, total run time is about 4.6 min, mono- and di- hydroxyl vitamin D can be analyzed simultaneously.

A Lab Mobile Mass Spectrometer For High Fidelity Mass Analysis Around The Pharmaceutical Facility

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Integration of a lab mobile Direct Ionization in Real Time (DART®) ionization mass spectrometer systems is described. The adaptation of a state-of-the-art Waters Acquity® QDa detector to enabling ambient ionization with a commercial LC/MS is facilitated by development of a custom VAPUR® ion transport interface, new pulsed DART gas control to facilitate low gas consumption, and an industrial strength cart to permit rapid deployment of the system between laboratory and production sites.

Since the development of ambient ionization significant effort has been made to integrate the technology with a low cost, compact mass spectrometer system. Scientist at Purdue University have enabled a series of low performance API-MS systems by using custom vacuum systems and gas stream interrupting inlets (DAPI) to reduce the gas flow in order to make a very compact detector. Those systems have not been commercially viable in part due to cost of the customization. More recently the introduction of low cost, smaller footprint LC/MS systems by Advion (Ithaca, NY) and Waters (Milford, MA) spurred us to investigate the implementation of our real time ionization source with one of those systems in order to develop an instrument that might be used outside of the laboratory environment. Working with Waters we have developed and tested a custom DART interface for introduction of ions into the mass analyzer with good efficiency without flow interruption. The system utilized the Acquity QDa performance version in it normal configuration and MassLynx operating system for control and data processing. Integration of our DART source with the Acquity QDa on an industrial cart permits movement of the system between facilities, laboratories and even utilization in production environments. As DART does not require utilization of solvents for sample preparation or ionization the system can be deployed safely without requirement for transport of those solvents.

To facilitate more comprehensive analysis of liquids, tablets and powders we have developed novel sample prep devices including solid phase extraction probes SPE-it® samplers (Supleco, Bellefonte PA) and a simplified manual sample positioning device facilitate detection of trace contaminants and analysis of complex samples. The utility of the system for use in support of method development programs for drug formulations, troubleshooting synthesis and formulation validation applications will be discussed.

POSTER ABSTRACT
#113

Enabling Nanoliter Sample Size In Mass Spectrometry Analysis Using Laser Diode Thermal Desorption: Application To Pharmaceutical Matrices

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Introduction

Do more with less is the pharmaceutical laboratories operational goal. Reduction of sample volume used for screening and study analysis represents a major impact on research and development cost. Based on this sample reduction approach, a study of low volume sample extract analysis in the nanoliter range is performed. Improvements in mass spectrometer sensitivity enable the possibility of using nano-sample size. Two different matrices are evaluated as typical work platform: Precipitated Rat plasma and CYP inhibition buffer. LDTD-MS/MS offers the possibility of using very low volume samples for high-throughput applications.

Methods

Typical CYP inhibition assays are performed with a reaction stop solution using an internal standard mixture in acetonitrile:water (75:25). Solution is quenched with optimal ratio 1:9 volume/volume of matrix/internal standard mixture. The following metabolites were evaluated: OH-Midazolam, OH-Mephenytoin and dextrorphan. The mass spectrometer is operated in positive MRM mode. Low volumes were also tested for precipitated rat plasma. One part of plasma is crashed with four parts of acetonitrile containing internal standard. Clozapine is used as a test drug. The mass spectrometer is operated in positive MRM mode. Assays are performed with different laser patterns and 3 sample extract volumes of 2000, 200 and 100 nL spotted in a LazWell plate.

Preliminary data

Low volume experiment is tested in three different CYP inhibition assay. One part containing microsomes, buffer, substrate and NADPH is mixed and immediately stopped with 10 parts of a mixture of acetonitrile:water (75:25) containing internal standard. Three different spotting volumes were tested: 100, 200 and 2000 nL. Good accuracy (92.0 to 116.4 %) and precision (1.6 to 15.2 %) are obtained at LLOQ level with 100 nL. Signal reduction using 20 times less sample spotted in LazWell plate do not impaired the measurement LOQ with the same ratio as the signal to noise remain close to the one of larger volume. Excellent linearity (r= 0.9929 to 0.9999) with no signs of carryover is achieved with 100 nL. Blank interference is 1.1 to 20.0% at the LLOQ. The CYP experiments are pooled and 3 X 200 µL is spotted in a LazWell plate. Good accuracy (82.9 to 105.2 %) and precision (2.8 to 5.8 %) are obtained at LLOQ level for each metabolite in pooled test. For the rat crash plasmas experiment, a calibration curve has been prepared ranging from 15 to 3000 ng/mL. Three different laser patterns were evaluated: 3-45-0, 3-65-0 and 3-65-2. Two different spotting volumes are tested: 100 and 2000 nL. Good accuracy (105.8 to 107.5 %) and precision (1.2 to 3.6 %) are obtained at LLOQ level with 100 nL. Signal to noise ratio is conserved with minimal loss of factor 1.2 despite the loss of signal area count with 20 times less sample spotted in LazWell plate. Excellent linearity (r= 0.99956 to 0.99982) with no signs of carryover is achieved with 100 nL. Blank interference is 1.7 to 4.3% at the LLOQ.

Novel Aspect

Nanoliter sample size for analysis in different matrix extracts using LDTD-MS/MS, application to CYP and plasma assays.

POSTER ABSTRACT
#114

Utilization Of Paper Spray In Drug Development – Lipid And Metabolite Profiling And Drug Pharmacokinetic And Metabolism Screening

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The high throughput analysis of biological samples to facilitate the development of new therapeutics and for the improved understanding of disease is critical to the drug development process. Obtaining the maximal information with minimal sample preparation offers opportunity to progress research at a faster pace. Aside from speed and simplicity, minimal or no sample preparation provides the advantage of the bias of preparation techniques. Analytical techniques offer extended value as they “scale” from model systems where sample volume may be limited, through drug development to pre-clinical analyses where, again, sample amounts may be limited or precious. Paper spray is a technique for sample retention, deposition and introduction which accommodates this value statement. In this work paper spray is leveraged for sample introduction in both targeted and discovery mass spectrometric experiments. Whole blood, serum, plasma, cerebrospinal fluid and urine are examined and their metabolite and biochemical information content evaluated using paper spray. For all fluids little or no sample preparation is done with sample applied directly to the cellulose matrix. The stability of samples once deposited onto the emitters is evaluated as well. The analyses provide the facile and comprehensive profiling of the biochemical content of the samples with highly reproducible profiles with a minimal sample volume. A range of lipids including PCs, SMs and others along with their constituent free fatty acids are profiled along with a breadth of primary and secondary metabolites. Active therapeutic agents are also monitored in the matrices with highly reproducible results. Differential metabolite profiling is demonstrated between subjects, with treatment and associated with disease. Combined with high resolution accurate mass analysis (HRAM) this offers a high-information content tool to accelerate drug development.

Picofuze: Integration Enables High-Flow Nanospray/Microspray With Conventional LC-ESI-MS

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Advances in genomics data, mass spectrometry, and separation science places mass spectrometry at a unique time, one poised to make significant and sustained contributions to fundamental life science and applied human health. Liquid chromatography-mass spectrometry (LC-MS) has experienced significant technical evolution, having established trends toward decreasing column diameter, lower flow rates, and packing particle sizes. State of the art "conventional" LC has evolved from 4.6 mm inside diameter columns (ID), operating at ca. 1 mL/min, to 1 – 2 mm ID columns, operating at less than 200 µL/min. Even smaller micro- (0.2 – 0.3 mm ID) and nano-scale (< 0.2 mm ID) column formats, operating at 10 and 0.3 µL/min respectively, have strong application specific roles, particularly when high sensitivity is required and/or sample volumes are strictly limited. We have developed a nano/micro bore LC column ("PicoFuze" technology, 200 or 250 µm ID x 5cm length) integrated directly inside a conventional ESI electrode/probe assembly (SCIEX Turbo V). Advantages of this integrated approach include: ease-of-use, a new high-voltage contact with each column, control or elimination of pre-and post-column columns, a new ESI spray assembly with each column, and preservation of LC performance. Examples of this technology in the quantitative bioanalysis of small molecule drugs and targeted peptides/proteins in combination with triple-quadrupole mass spectrometry will be shown. This technology yields a sustainable sampling advantage, yielding the security of multiple injection volumes, an increase in analytical sensitivity, while preserving the culture of LC method development.

CPSA USA

POSTER ABSTRACT

#116

Picochip: Development Of Chip-Based Nanobore Column Platform With Universal Connectivity, Column Heating, Sheath Gas Capability, And Multi-Plex Operation.

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Packed-tip (PicoFrit) columns have successfully enabled nanoflow LC-MS applications by delivering optimal chromatographic performance at nanoflow rates. Assembling a high-performance system is at present time the domain of the expert user. Challenges in pre- and post-column plumbing, sample preparation of complex matrices, and mass spectrometer tuning requires training and persistence in method development. Incorporating short (10 cm) or long (25 cm) PicoFrit columns into a fully integrated device (PicoChip) enables ease-of-use while preserving the performance of this unique column format. Coupling this chip-based column solution to a heated and pneumatically enabled modular “docking station” source platform expands the functional application window, delivering a wider range of operating flow rates (100 nl/min. to 10 µl/min.) and column temperature (40 to 60 C) in a single device. Using pneumatically enabled source hardware and chip-based columns, the effect of pneumatically assisted ESI was evaluated for 150 µm ID chip-based consumables at 1 µl/min. to 5 µl/min. The effect of column heating is investigated for 75 µm ID and 150 µm ID columns.

Integration of nanospray in the PicoChip device enables automation and multi-channel operation. A novel 3-slot valve rotor design enables a linear flow path resulting in a simple, robust nanoLC-MS column switching system. A four channel, three-column version of the PicoChip source facilitates an MS-duty cycle time of 95% compared to 40% for a single channel system. Three PicoChip columns, 75 µm ID x 15 µm tip x 10.5 cm bed (New Objective), packed with ReproSil-Pur, 3 µm, 120 Å, C18-AQ (Dr. Maisch) were positioned on an automated PicoSlide source (New Objective). Using an HTC-PAL autosampler (Leap Technologies) equipped with a 6-port micro-injection valve (VICI), 0.72 µg of HELA digest (Thermo) were injected. A direct flow nanoLC pump with two gradient channels and one isocratic channel (Eksigent Ultra, AB SCIEX) was used for gradient delivery, column washing, equilibration and sample loading. Full scan MS data, with data dependent MSMS of the top three ions, were collected on an LTQ MS (Thermo). Extracted ion chromatograms of four HELA peptides (m/z: 416.4, 652.9, 839.3 and 655.9 Da), eluting across the gradient elution profile window (~13 minutes to 45 minutes) were used to evaluate retention time reproducibility. The retention time RSD was under 5% for all four peptides on all three columns (Column 1: 3.2%, 1.4%, 1.3%, 1.2%; Column 2: 2.8%, 1.5%, 1.2%, 1.1%, Column 3: 3.1%, 1.6%, 1.2%, 1.1% for m/z 416.4, 652.9, 839.9 and 655.9 Da, respectively). A 95% duty cycle was achieved for a 57 minute gradient separation, compared to a 66% duty cycle for a comparable gradient separation on a single channel system. This represents a roughly 30% improvement in efficiency. Further tests will evaluate different gradient and column lengths and optimal methods for maximum sample throughput.

POSTER ABSTRACT

#117

Microsampling And Micro LC: A 10 µL Acetaminophen Plasma Assay For Therapeutic Dose Or Microdose Levels

T Lloyd, K McManus, J Respondek, M Tan, A Harvey, B Ray, J Muehlbrad and S Unger

Improvements in mass spectrometers and smaller scale (micro LC and capillary LC) chromatography have allowed lower detection limits and a reduction of sample volume required for xenobiotic biofluid assays. Quantitative capillary devices offer a precision within 3%CV when performing single or repeated deliveries of 10-25 µL blood or plasma [Jonsson, Bowen]. In the latter approach harvesting plasma, whole blood is drawn into a capillary containing EDTA and a thixotropic gel. One end of the capillary has a self-sealing plug that enables the tube to be centrifuged. Upon centrifugation, the gel migrates based on density to form a stable barrier between the erythrocytes and plasma. The capillary is then loaded into a dispenser capable of delivering 10 µL aliquots of plasma or the entire volume of plasma. These microsamples can be stored and extracted conventionally in a liquid state or in a dried state.

Having previously demonstrated the utility of various dried matrix analysis approaches [Lloyd, Tan], in this application we stored and extracted the plasma microsample in a liquid state. Sample collection was via finger prick, filling a capillary to harvest 30 µL or more of plasma. In this approach, inter-subject variability and the need for bridging studies between blood-plasma and liquid-solid samples is eliminated.

A healthy male subject was dosed orally with 1000 mg of acetaminophen (McNeil-PPC, Inc.) and 8 samples were collected over a 24 hour period. Samples were drawn using contact-activated lancets (2.0 x 1.5 mm, Becton Dickinson) from different fingers, filling a 75 µL EDTA plasma collection capillary (Drummond Scientific) with blood. The capillary was centrifuged (Beckman Coulter) at 3000g for 10 minutes and the plasma forced from the capillary using either a Safepette dispenser (SafeTec) or a Wiretrol plunger (Drummond Scientific). A 10 µL aliquot was extracted by liquid-liquid extraction and analyzed by gradient reverse phase LC/MS/MS (API5000 and API6500, AB SCIEX). Assay range was 0.0250 – 15.0 µg/mL to match the therapeutic range, with all standards within +/- 6% of nominal (r=0.9994). The C_{max} of 13.4 µg/mL and a $t_{1/2}$ just over 2 hours (C24h = 0.325 µg/mL) agrees well with literature values. A microdose was then evaluated demonstrating the capability of micro LC to further lower the assay range proportionally.

References

Jonsson O, Rodrigo P, Nilsson L, et al. Capillary microsampling of 25µL blood for the determination of toxicokinetic parameters in regulatory studies in animals. Bioanalysis. 2012; 4(6): 661-74.

Bowen C, Licea-Perez H, Karlinsey M, et al. A novel approach to capillary plasma microsampling for quantitative bioanalysis. Bioanalysis. 2013; 5(9): 1131-5.

Lloyd T, Nunez S, Johnson R, Tan M. Full automation of dried blood spot/LC/MS/MS methods for topiramate, warfarin, acetaminophen, ritonavir, lopinavir and saquinavir in human whole blood. AAPS Annual Meeting and Exposition, November 10-14, 2013 San Antonio, TX, USA.

Tan M, Johnson R, Gus J, McManus K, Lloyd T, Unger S. Advantages and Limitations of Microsampling, Hematocrit and MicroLC in DBS: Quantitative Bioanalytical Assays for Immunosuppressants, Warfarin and Methylphenidate in Whole Blood. ASMS Annual Meeting, May 31 – June 4, 2015 St. Louis, MO, USA.

POSTER ABSTRACT
#118

Disulfide-Linked Cyclic-Peptide Substructures Automatically Assigned Using Exact Mass ESI MS/MS Data And The Masspec Algorithm

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Abstract
Peak assignments for the MS/MS mass spectra of cyclic peptides with multiple disulfide bridges are a challenge to analyze. In addition to the standard peptide cleavages, each disulfide linkage may undergo up to four possible fragmentation processes.
The MASSPEC algorithm was designed to exhaustively compute all possible substructures of ions consistent with mass spectral fragmentation rules. The MASSPEC algorithm correlates the predicted substructure masses with observed fragment ion masses and scores all assignments. In this way, the MASSPEC algorithm is a powerful tool for the elucidation of the complex fragmentation processes observed in the MS/MS spectra of disulfide-linked cyclic peptides.
The data for disulfide-linked peptides and related metabolites were acquired using a ThermoFisher Q Exactive Orbitrap mass spectrometer in the LC-MS/MS mode of the $[M+H]^{5+}$ parent ion under stepped collision energy (RP = 35,000, D = 3 ppm). MS^n experiments were conducted on an LTQ-Orbitrap. The MASSPEC algorithm is written in Visual Basic and is based upon advanced graph theory and combinatorial methods. User interface features used for the assays include a Data Input Module and an Output Graphics Display Module. Using the MASSPEC algorithm, the proposed structures for the fragment ions were obtained automatically by computing the highest scoring fragment ion substructures for the observed ions.

The MASPEC algorithm was applied to MS/MS data in the following manner. One and/or three superatom models were used to describe the peptide residues while the disufide linkages were re-expressed in superatom notation as C'-S'-HH-S'-C', where C' represents dehydroalanine, S' represents sulfur and HH represents two hydrogen atoms. The cleavage of the C'-S' bonds can result in the formation of persulfide and dehydroalanine groups and cleavage of the S'-HH bonds can result in the formation of cysteine and thialdehyde groups. In this manner, model grafted cyclotides (cyclic peptides with three disulfide bridges) were analyzed.
Orbitrap ESI-LC-MS/MS data for the parent grafted cyclotide and corresponding metabolites were acquired and evaluated. The MASSPEC Hydrolyzer Algorithm was used to generate all the possible metabolite structures for each parent compound. The highest scoring structure for the metabolite MS/MS data corresponded to the correct metabolite when checked against enzymatic data.

POSTER ABSTRACT
#119

Automated Structure Elucidation Of Unknown Metabolites In Pharmaceutical And Metabolomics Studies Using Tandem Mass Spectral Data And The Masspec Algorithm

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Abstract
A novel algorithm, called MASSPEC, was designed to elucidate/correlate chemical structures with observed tandem mass spectra acquired in exact mass or nominal mass modes using any ionization method. The program is ideally suited to analyze metabolomics and metabolite data since the product structures are based upon classes of known starting structures (natural products) or upon known structures (pharmaceuticals), respectively, each producing a variety of possible metabolic modifications that can be incorporated into the algorithm. The details for extracting the metabolite structure from the raw data will be described.
The underlying principle of the MASSPEC algorithm is that a proposed chemical structure is described by connected sets of atoms that do not fragment further, where each set is referred to as a superatom. The chemical structure is therefore a connected set of superatoms, where the connections are viewed as the chemical bonds linking the superatoms. A mass spectrum is generated from ionized substructures of the original parent molecule, whereby the connectivities of the superatoms in the original molecule remain intact, are broken or are modified by predicted rearrangements. In addition, substructures, referred to as floating superatoms, are incorporated into the calculation. These floating superatoms can be located anywhere within the molecule but are localized by determining their presence in each of the fragment ions. The floating superatoms can serve as additions or losses of substructures to a superatom, thereby behaving as metabolic changes to the parent structure. A scoring system, based upon the number of bonds cleaved and the mass accuracy of the observed vs. predicted masses are used to predict the best possible chemical structures for the fragment ions from a number of possibilities. Applications and strategies for use of the algorithm will be illustrated for two metabolomics problems of unknown metabolites related to norcardamine and tetracycline and for three primary metabolites from three different pharmaceuticals. The MASSPEC algorithm exhaustively computes all the possible substructures, limited only by the input constraints, and correlates them with the observed ions, a task which is virtually impossible to achieve by hand calculations.

Software-Assisted Screening For Gsh Conjugated Metabolites Using High Resolution Orbitrap Mass Spectrometry

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Chemically reactive metabolites have been implicated in many off-target adverse drug reactions including idiosyncratic drug toxicities. Identification of reactive metabolite potentials and elimination of bioactivation liability at the early drug discovery phase could prevent compound failure at a later stage.
Various LC-MS methods have been developed for high throughput GSH screening, including triple-quadrupole or Q-Trap-based neutral loss (NL) MS analysis, precursor ion scan (PI), multiple reaction monitoring (MRM)-based method, and high resolution mass spectrometry-based data dependent scans with mass defect filtering.
Much of the metabolite identification analysis in the past has been done with multiple LC-MS injections and manual data mining. With the introduction of Mass-MetaSite, targeted analysis can be used to reduce the number of LC-MS runs needed to acquire all data necessary. Additionally, following data acquisition, Mass-MetaSite provides data processing with metabolite identification, structural assignments, and an accompanying report.
The purpose of this study is to develop a more efficient GSH conjugate screening method by using high resolution mass spectrometry with MetaSite & Mass-MetaSite for reactive metabolite detection and semi-automatic data analysis. High resolution mass spectrometry with polarity switching will also be evaluated to improve detection and characterization of GSH conjugated metabolites.
By using Mass-MetaSite for targeted data acquisition and data processing, MS^2 data quality is improved and assay cycle time is reduced significantly. The faster scan rate of the Q Exactive allows for polarity switching data acquisition; complementary fragmentation data from positive ion/negative ion MS^2 spectra are valuable for structure elucidation. Data processing software for polarity switching MS data is highly desirable to enable such LC-MS techniques in high throughput analysis.

POSTER ABSTRACT
#121

A Universal Immunocapture-LC-MS/MS Workflow For Biological Compound Quantitation In Preclinical Studies

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Human monoclonal antibody (mAbs) quantitation in preclinical studies has become a very popular area and call for specific importance for mAbs therapeutics development in biopharmaceutical industry. While mass spectrometry has been proved to be an efficient, accurate and sensitive technique for protein quantitation, method development of LC-MS/MS based mAbs quantitation in preclinical assays still remains very challenging, due to high variety of antibody protein nature and complexity of biological matrix. In this poster, we introduce a universal solution for human IgG quantitation in preclinical assays, including immunocapture based protein enrichment by using BioBA sample preparation kit, signature peptide selection from high resolution peptide mapping data and MRM based LC-MS/MS quantitation method development. 5 ng/mL LLOQ of human mAb therapeutics has been solidly achieved, supporting a high-sensitive total solution for mAb quantitation in preclinical studies

POSTER ABSTRACT

#122

The Use Of Capillary Electrophoresis Coupled To Mass Spectrometry (CESI-MS) For Quantitation Of Nucleotides And Nucleosides With Minimal Sample Preparation

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Nucleotides and nucleosides are charged particles that are vital compounds in the biological processes. These polar compounds are usually difficult to analyze by traditional chromatography techniques, needing an extra derivatization step. Capillary Electrophoresis (CE) is a separation method that is ideal for highly charged and polar molecules, which can be notoriously challenging for liquid or gas chromatography. In addition, CE offers advantages of nano and picoliter sample-volume, high separation efficiency, and fast injection-to-injection times. CESI is a technology that integrates capillary electrophoresis (CE) and electrospray ionization (ESI) into a single process in a single device. With CESI, a stable ESI spray is generated at flow rates in the range of 5-30 nL/min. Operating at these ultra-low flow rates, it helps reduce ion suppression and increases assay sensitivity for optimal results. In this poster, we present a workflow using CESI-MS for the quantitative analysis of selected nucleotides using minimal sample volume, demonstrating baseline separation, reproducibility and detection at nanoMolar levels. A method for separation of nucleotides was developed using CESI with a short analysis time (< 10 min) and minimum required sample volume. In addition, it does not require derivatization for the analytes. Separation of challenging charged molecules: cytidine phosphates, ATP, GTP, UTP and, TTP have been demonstrated using the CESI-MS system. CESI could be applied for quantitative analysis with low detection limits (~ 200 nM) for the analyzed compounds. Application of this CESI-method is promising for metabolomics in biological areas (e.g. neurosciences, pharmaceutical industry) where almost all the samples are obtained at low volume and concentration

POSTER ABSTRACT
#123

Quantitative and Qualitative Metabolomics for the Investigation of Intracellular Metabolism

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Comprehensive knowledge of intracellular biochemistry is needed to accurately understand, model, and manipulate metabolism for industrial and therapeutic applications. Quantitative metabolomics has been driven by advances in analytical instrumentation and can add valuable knowledge to the understanding of intracellular metabolism. This work details a method that builds and extends upon existing reverse phase ion-paring liquid chromatography methods for separation and detection of polar and anionic compounds that comprise key nodes of intracellular metabolism by optimizing pH and solvent composition. In addition, the presented method utilizes multiple scan types provided by hybrid instrumentation to improve confidence in compound identification. The developed method was validated for a broad coverage of polar and anionic metabolites of intracellular metabolism. The work here describes the use of the QTRAP® 5500 System for quantitative and qualitative metabolomics investigations into the intracellular metabolism of microorganisms. We used both methods to successfully confirm and quantitate 100 metabolites in the metabolome of E. coli grown on two different growth media sources. The quantitative and qualitative workflows described here can be readily extended to other single cell and multi-cell organisms

Fast, Sensitive Derivatization For LC/MS Analysis Of Vitamin D With DBS Or DPS Cards

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Vitamin D is known to be critical for bone health but may have a role in both cancer and autoimmune diseases. In the US alone more than ten million vitamin D tests are done each year, but most tests use immunoassay methods which cannot distinguish vitamin D2 from vitamin D3. Only LC/MS can distinguish these forms but many diagnostic labs have switch from LC/MS back to immunoassays because they are less expensive.

Home testing for vitamin D is growing and this market relies on dried blood spot (DBS) cards with analysis performed by LC/MS. Both DBS and dried plasma spot (DPS) cards require just a drop of blood, which makes blood collection convenient and much less painful. The challenge with blood collection cards are the small sample size and poor ionization efficiency of vitamin D in LC/MS. Derivatization is the norm for measuring vitamin D by LC/MS and usually obligatory for vitamin D analysis with DBS cards. The new SecoSet reaction adds a permanent positive charge to vitamin D which greatly enhances the signal in LC/MS. Signal typically increases ten-fold and noise decreases because of the higher mass. It is a two minute, single pot reaction and does not require evaporation to dryness.

One drawback with DBS cards is the variability in the collected blood volume. This can translate into increased variability in the measured concentration of vitamin D. The Noviplex DPS card addresses this issue by collecting a consistent volume of plasma regardless of the amount of blood collected. The data will demonstrate that SecoSet is an effective derivatization technique for both DBS and DPS cards.

ingle-cell Mass Spectrometry Reveals Proteomic Heterogeneity in the 16-cell *Xenopus* Embryo

Camille Lombard-Banek, Sally A. Moody and Peter Nemes

Characterization of cell-to-cell differences in the developing embryo holds great potential to help better understand the mechanism of developmental diseases, which in turn can help the design of targeted therapeutics. This goal, however, requires technologies capable of measuring biomolecules in single embryonic cells (blastomeres), especially those that allow for the interrogation of proteins that provide a direct read on cell state and phenotypes. Here we extended single-cell capillary electrophoresis microflow electrospray ionization high resolution mass spectrometry (CE-μESI-HRMS) for single blastomeres to probe translational differences in individual blastomeres in the 16-cell *Xenopus* embryo.
Our custom CE-μESI-HRMS was capable of detecting peptides with a ~25-attomoles lower limit of detection, giving us confidence in this technology for proteomic analysis on single cells. The quantitative reproducibility was below 15% standard error of the mean for both technical and biological replicates. By iteratively revising the analytical steps of the workflow, we progressively increased the number of proteins that could be identified using the technology by 2 orders of magnitude, allowing us to measure proteins in single blastomeres. For the study, we chose the D11, V11, and V21 cells in the embryo because they give rise to different and specific tissue types in the frog. From these measurements we were able to identify 1,700+ protein groups that spanned ~4-to-5 log-orders of magnitude in concentration. Additionally, using exponentially modified protein abundance indexes (emPAI) as a read on relative protein amount in the cells, we were able to find translational differences. These results were confirmed by the extension of tandem mass tag quantitation to single cells which allowed us to quantify ~150 protein groups between the cell types. Combined, these results confirmed translational differences between the D11, V11, and V21 blastomeres at this early stage of development.
To conclude, our CE-μESI-HRMS allowed us to uncover translational differences between the different blastomeres in the 16-cell frog embryo. We expect this technology and workflow to be widely applicable to blastomeres at different stages and from different animal models that help recapitulate human developmental disease.