# **E CPSA**

### POSTER SESSION ABSTRACTS

Inspiration. Collaboration.

POSTER SESSION Tuesday, October 2 1:00 PM - 3:00 PM

> CPSA 2012 October 1 - 4, 2012 Sheraton Bucks County Hotel Langhorne, PA

Where Technology and Solutions Meet



Innovator Award Submission - Abstract Only

### Metabolite Identification: From data to information and from information to knowledge

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1) Lead Molecular Design, S.L.; 2) Molecular Discovery, Ltd; 3) University degli Study di Perugia

HRMS has openned a new era in drug metabolism. Nowadays it is common available in the pharmaceutical industry the capacity to generate high resolution data for a large number of compounds that can be used in qualitative and quantitative analysis.

Software like MassMetaSite is used to automatically process the data to find peaks and to perform an initial assignment of the potential chemical structure of the metabolites formed.

After this automatic process has been reviewed by an expert in a Web interface, the entire data (chromatogram, spectra, fragmentation), and information (metabolite structure, relationship of the area with analysis variables like time or matrix) is stored in a searchable database.

WebMetabase is the application that support this process, and it also provides an additional level of usage of the information like the visualization of the complex and information rich data from the Met ID analysis in a Structure metabolism relationship table that facilite the analysis of how the molecular changes in the parent compound modify the clearance and the metabolic pathway, facilitating the design of new molecules that may improve the metabolic properties.

The entire process from HRMS data to information (MassMetaSite driven) and from the Met ID report (WebMetabase) to design will be shown for a family of close related compounds.



#### ABSTRACT

Innovator Award Submission - Abstract Only

### An interdependent LC/MS/MS solution for urine testing for detection and quantification of drugs-of-abuse and pain management

Martin Steel McKinley Scientific

McKinley Scientific is an organization that does not design or manufacture products, or own traditional IP. McKinley Scientific's business is in equipment management and financing and being a conduit for our customers to more easily access technology. Our business model is to lease LC/MS/MS systems to allow users to refresh technology in a timely manner. We also marry other vendor technology with systems to support users in customizing configurations and performance. Instruments returned at lease-end are typically sold into R&D labs with a full compliment of support services provided by us or our partners.

Recognizing a need for LC/MS applications for drugs-of-abuse and pain management urine testing, it was clear that many of the businesses in this arena did not have the experience or resources to select an appropriate platform, develop and validate methods and effectively run high throughput testing services.

McKinley Scientific built a collaborative consortium to provide near turnkey testing capabilities to Clinical labs and Physician offices.

Due to the good availability of Thermo Quantum Ultra MS/MS, the Quantum platform was selected as the standard. Methods were developed by North Carolina based Opans, a contract analytical services lab, in conjunction with Georgia based Microliter Analytical Supplies, ITSP (Instrument Top Sample Prep), the manufacturer of a consumable device for solid phase extraction. The instruments are pre-configured and installed and serviced by North Carolina based Ion Technology Services. Onsite LC/MS training is provided by Opans and Validation coaching/consultation is provided by Molecular MS Diagnostics of Rhode Island who are clinically certified. McKinley Scientific is the program lead vendor, co-ordinating the services of the participating parties, and also provides financing options. Consequently our innovation is a targeted collaboration.



#### ICP-MS as a alternative detection technique in bioanalysis

Jaap Wieling, Fred van Heuveln QPS Netherlands, Hanzeplein 1-53, Groningen, The Netherlands

A relevant group of compounds have molecular characteristics that open opportunities for the application of elemental detection by ICP-MS. This technique offers a new range of properties and hence capabilities, in some cases superior to MS/MS detection. In addition to that ICP-MS can also offer additional advantages for compounds where ELISA or LC-MS/MS would be the first methodology of choice due to the fact that ICP-MS provides elemental information, orthogonal or in addition to activity and/or molecular information.

In our laboratory ICP-MS is predominantly applied as a methodology for pharmacokinetics, imaging purposes, mass-balance studies, food-effect studies and biomarker studies. An increasing number of applications have been developed, where ICP-MS and its various hyphenations, e.g. LC-ICP-MS, have been used for speciation / metabolism studies and for proteomics studies.

Here, the analytical potential, the various modes of operation and the challenges of the application of ICP-MS in drug development applications are given.



#### Simultaneous Quantitative and Qualitative Measurements for Primary Metabolism Investigations using a Quadrupole-Time-of-Flight Mass Spectrometer.

Don S. Richards<sup>1</sup>, Mark E. Savage<sup>2</sup>, Angus N. R. Nedderman<sup>2</sup>
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Recently there has been considerable interest in simultaneously performing both quantitative and qualitative DMPK analyses in support of small molecule drug discovery. This work describes an investigation into the possibility of using a Quadrupole-Time-of-Flight mass spectrometer to obtain clearance data, metabolite identification, structure elucidation and metabolite profiles from P450 microsomal incubations at a drug concentration of 1  $\mu$ M from a single sample set.

P450 microsomal incubations of commercially available drug substances including Pindolol, Verapamil and Haloperidol were prepared at 1  $\mu$ M concentration. The incubations were sampled and quenched, at intervals to provide a time course over a period of 60 minutes. The analysis of the samples was carried out by LC-MS using a Bruker Maxis Impact Quadrupole-Time-of-Flight mass spectrometer to obtain data suitable for measuring clearance and plotting metabolic profiles. Data dependent MSMS spectra were collected in order to identify and elucidate the structures of the observed metabolites. The clearance profiles and values are compared to those obtained using a triple quadrupole mass spectrometer to analyse the same sample set.



#### Development of a Mass Spectrometer Based, Derivatization Assay for Urea Assessment from Epithelial Lining Fluid Isolated from Human Bronchoalveolar Lavage Fluid

Chester L Bowen and Hermes Licea-Perez GlaxoSmithKline Pharmaceuticals

Abstract: A sensitive, selective, and quantitative method for the determination of urea has been developed and validated in human plasma. The method employs derivatization of urea with camphanic chloride to improve the chromatographic retention and separation. The derivatization was performed in epithelial lining fluid (supernatant from bronchoalveolar lavage) without prior sample clean-up. Ultra Performance Liquid Chromatography (UHPLC) technology on a BEH HSS-T3 stationary phase column with 1.7 µm particle size was used for chromatographic separation coupled to tandem mass spectrometry. The method was validated over the concentration range of 5-100 µg/mL, which suits well to the endogenous concentration in human subjects. The results from assay validation show that the method is rugged, precise, accurate, and well-suited to support pharmacokinetic studies. In addition, the relatively small sample volume (20 µL) and a run time of 1.5 min facilitate automation and allow for high-throughput analysis.

## POSTER ABSTRACT

### Proposed Plasma Micro-Sampling Device for Implementation in Pre-Clinical Studies

Authors: Chester L Bowen<sup>1,</sup> Molly Karlinsey<sup>1,</sup> Hermes Licea-Perez<sup>1,</sup> Kristen Jurusik<sup>1,</sup> Essaie Pierre<sup>1,</sup> Jim Kenney<sup>2</sup> and Joe Siple<sup>2</sup>

Affiliations: 1) GlaxoSmithKline; 2) Drummond Scientific

The benefits of micro-sampling to preclinical environments have already been recognized throughout the pharmaceutical industry through the implementation of Dried Blood Spot (DBS) technology. However, variable hematocrit levels in animal and patient populations could have an impact on accurate spotting and analysis due to differences in blood viscosity. Advances in LC/MS/MS technology, specifically instrument sensitivity, have allowed for a decrease in sample volume, facilitating the implementation of micro-sampling approaches in bioanalysis. Generally, plasma assays are preferable in the bioanalytical community as compared to DBS, whole blood, and blood/water assays. The use of plasma micro-sampling in preclinical toxicokinetic (TK) studies could facilitate the removal of satellite animals. As a result, blood sampling from the TK animals will allow direct correlation between the drug exposure and toxicity observed in the same individual animal as well as greatly reduce the number of animals required for each study. One of the current challenges of micro-sampling is generating plasma aliquots from a small volume of blood (<100  $\mu$ L). In this work, a prototype device is evaluated that will allow for isolation of a micro-volume of wet plasma (<50  $\mu$ L) from whole blood (<75  $\mu$ L).

#### POSTER ABSTRACT #5

#### AUTOMATED STRUCTURE ELUCIDATION OF METABOLITES AND NATURAL PRODUCTS USING THE MASSPEC ALGORITHM AND TANDEM MASS SPECTRAL DATA

Marshall M. Siegel, Gary Walker MS Mass Spec Consultants 39-24 Grant St. Fair Lawn, NJ 07410 201 791 1627

A novel algorithm was designed to elucidate/correlate chemical structures with observed mass spectra acquired in exact mass or nominal mass modes. The algorithm is based upon advanced graph theory and combinatorial methods and is written in Visual Basic. The underlying principle of the algorithm is that a proposed chemical structure is described as 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 metabolytic 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 a number of metabolites. Also, this methodology for elucidating chemical structures is applicable for all small molecules, natural products, peptides, saccharides, nucleotides and pharmaceuticals.

#### POSTER ABSTRACT #6

### Determination of Norepinephrine and Epinephrine in Minipig Plasma by LC-MS/MS

Huaibing He, Lucinda Cohen, and Sharon Tong PPDM,MRL, Merck

Norepinephrine (NE) and Epinephrine (EPI) are mediators of sympathetic nervous system, and play important roles in cardiovascular, hypertension and other therapeutic areas as important biomarkers for many drug discovery and development programs. Norepinephrine (NE) and epinephrine (EPI) are two extremely polar molecules, which have posted a significant challenge to develop a sensitive and selective assay with high throughput. Traditionally, LC-electrochemical detection method has been used with reasonable sensitivity, but is very labor intensive and low throughput. LC-MS could be an alternative approach, but the sensitivity and selectivity has never been comparable to LC-electrochemical detection method due to the poor ionization and large matrix interference on LC-MS.

A new LC-MS analysis approach has been developed in our lab, which employs weak cation exchange solid phase extraction and ion-pair LC-MS to enable retaining of NE and EPI on the column and reducing the interference from the matrix. Norepinephrine (NE) and epinephrine (EPI) was detected by API 5000 triple quadruple mass spectrometer with turbo-ion spray in positive ionization mode. A Thermo LX-2 system, equipped with a Fluophase column (PFP) and methanol/water mobile phase containing 0.1% heptafluorobutyric acid (HFBA), was used for LC separation. A surrogate matrix approach has been used for calibration curves by applying the stable isotope labeled D6-NE and D6-EPI as internal standards. A lower limit of quantitation of 0.025 ng/mL was achieved for NE and EPI, with the linear range at 0.025-100 ng/ml by our LC-MS approach.

This LC-MS/MS method requires smaller sample volume and provides comparable sensitivity and selectivity to traditional LC-electrochemical detection method, but more importantly with a significant improved throughput. Overall, this method has been successfully used for PK/PD-Biomarker synergy efforts for drug discovery and development.

#### POSTER ABSTRACT #7

Innovator Award Submission

#### PicoSlide Multichannel Nanospray: So easy a caveman can do it!

#### Gary Valaskovic<sup>1,</sup> Ben Ngo<sup>1,</sup> Amanda Berg<sup>1,</sup> Nathan Yates<sup>2</sup> 1) New Objective Inc. Woburn, MA; 2) University of Plttsburgh, Pittsburgh, PA

Nanospray ionization, in combination with nanobore liquid chromatography tandem mass spectrometry (nLC-MS/MS), has been the method of choice for protein/peptide biomarker discovery in the life sciences. These qualitative methods have typically featured nanobore columns running at flow rates typically between 300 to 1,000 nL/min. Columns are typically either self-made by the analyst or commercially available in the form of fused-silica tubing. The traditional laboratory solution has been a heavy investment in the education and skill set of the analyst. Key to success are critical skills in making "perfect" connections, tuning the nanoelectrospray source, and deep experience in system troubleshooting.

Injection-to-injection cycle times are typically long (> 30 minutes), so time lost to instrumental difficulties carries high economic cost. Given plumbing complexity, the ability to automate these processes has been limited. The growing need for analysis in the areas of protein quantification and biomarker validation places new demands on throughput, robustness, ease-of-use, and suitability for automation.

We have developed an integrated system for nanobore LC-MS that alleviates the need for specialized expertise in nano-scale separations. A novel design for a "PicoChip" based solution combines the functionality of the nanospray emitter, nanobore separation column, high-voltage contact, and autosampler transfer line into a single consumable device. The comfortable design of the consumable package makes it exceptionally easy to handle and eliminates the risk of emitter clogging or column breakage. A new high-voltage contact with every column change means consistent and stable spray ionization. Pre-assembly and testing of the assembled device in a production setting ensures results in the customer's lab.

The design of the PicoChip has a several-fold advantage for the end-user. PicoChip change-over can be accomplished by an in-experienced end user in seconds. The PicoChip design is readily adapted to front-end automation. A newly developed four channel PicoSlide source enables facile nLC-MS/MS workflow and improves instrument duty cycle from 40% to 95%. A radically simplified plumbing arrangement eliminates the complex multi-channel systems of the past. Furthermore, the PicoChip isolates the column from changes in ambient temperature and enables column heating and sheath-gas assistance for micro-spray compatibility.



### Study of small molecules captured in a pellet after protein precipitation of plasma sample

Zheng Ouyang<sup>1,</sup> Serhiy Hnatyshyn<sup>1,</sup> Petia Shipkova<sup>2,</sup> Michael Reily<sup>1</sup> 1) Bristol-Myers Squibb Company, Lawrenceville, NJ; 2) Bristol-Myers Squibb Company, Hopewell, NJ

Cold acidified methanol protein precipitation is routinely employed as the standardized method for preparing rat plasma samples in metabolomics studies. The supernatant from this procedure is subjected to targeted and untargeted measurements of endogenous metabolites using different analytical techniques while the remaining protein pellet is discarded. However, it is well known that some metabolites co-precipitate with the protein. In this study, we used rat plasma to investigate the recovery of endogenous metabolites from the pellet. We selected eight different solvents to re-extract the precipitated protein pellet and analyzed the extracts by high resolution accurate mass LC-MS spectroscopy. Our choice of solvents covered a range of: water, methanol, isopropanol, chloroform, methyl tert-butyl ether, ethyl acetate, hexane and n-butyl chloride and the LC-MS analysis was performed on an Exactive mass spectrometer (Thermo Scientific, San Jose, CA) interfaced with an Accela UHPLC (Thermo Scientific, San Jose, CA).

Our results reveal that a majority of measured metabolites are extracted predominantly into supernatant with standard acidified methanol protein precipitation method; however certain metabolites are still left behind in a significant amount in the pellet. This observation is important to consider when developing methodology for measuring absolute quantity of a metabolites in plasma samples.



#### Dextromethorphan Metabolism Profiling by the Analysis of Dried Matrix Samples Collected from Liver Microsomes In-Vitro Fluid and Analyzed by MFLC MS/MS

Chad Christianson<sup>1,</sup> Tarra Fuchs-Knotts<sup>2,</sup> Casey Johnson<sup>1,</sup> Shane Needham<sup>1</sup> 1) Alturas Analytics, Inc.; 2) Achaogen, Inc.

Introduction: Dextromethorphan is a safe, readily accessible, antitussive that has a well characterized metabolic pathway in humans. The CYP2D6 mediated O-demethylation to Dextrophan is well characterized. Typical invitro Dextromethorphan metabolism experiments require the addition of large volumes of organic solvents in order to stop the CYP2D6 transformation to Dextrophan. Experiments were conducted in order to determine if the benefits of Dried Matrix Spotting (DMS) techniques could be used to halt the CYP2D6 mediated activity. In order to develop a more sensitive method, micro-flow liquid chromatography coupled with a mass spectrometer (MFLC-MS/MS) was utilized for the determination of Dextromethorphan and Dextrophan in the in-vitro fluid.

Methods: The Dextromethorphan in-vitro incubation contained human liver microsomes, NaPi, NADPH Regenerating System A and B, and Potassium Phosphate/Magnesium Chloride buffer. This solution was incubated at 37°C prior to the addition of Dextromethorphan. Twenty-five uL of the solution was spotted onto a Whatman DMPK-C card and aliquoted into a tube. The spotted card was dried at 50°C under nitrogen for one hour prior to extraction and 500 uL of acetonitrile was added to the tube to stop the formation of the Dextrorphan metabolite. The analysis was performed on an ABSciex 5500® QTRAP coupled with an ekspert microLC system.

Data: In order to determine if the Dextrorphan metabolite formation from Dextromethorphan in human microsome in-vitro fluid can be stopped once spotted onto a DMS card, a metabolite formation time course experiment was conducted. The Dextromethorphan in-vitro solution was incubated at 37°C for 0, 15, 30, 45, and 60 minutes prior to either spotting on the DMS card or aliquotting into a tube. Once placed into the tube Acetonitrile was immediately added to stop the Dextromethorphan conversion to Dextrorphan. To determine if the DMS method is equivalent to the traditional organic crash method, the degradation of Dextromethorphan at each time point should be similar between each method. In addition, at T=0 no Dextrorphan should be detected in the DMS extracts. The results indicate that at each time point, the DMS metabolism of Dextromethorphan was within 3% of the metabolite formation in the acetonitrile crash method. Dextrorphan was not detected in the T=0 DMS samples. DMS is a suitable replacement for the organic crash method so the benefits of ambient storage and shipping can be utilized.

The column used for the binary gradient analysis was a ProntoSIL 120-3-C18 heated to 50°C (3 um, 50 mm X 0.5 mm)with a flowrate of 45 ul/min. In order to determine the signal gain of using the MFLC when compared to conventional HPLC flowrates, an extracted sample was re-analyzed on the ABSciex 5500® QTRAP using a Shimadzu HPLC system with a flowrate of 720 ul/min. The column used for the Shimadzu comparison analysis was a ProntoSIL 120-3-C18 heated to 50°C (3 um, 50 mm X 2.0 mm). The MFLC provided nearly a five-fold increase in Dextromethorphan response when compared to conventional HPLC.

Conclusion: The benefits of DMS can be utilized to perform in-vitro metabolite profiling experiments instead of the traditional organic crash method.



### Utilizing a Non-Targeted HR/AM-MS Method to Accelerate Quantitative Throughput for In-Vitro Metabolic Profiling

Keeley Murphy, Kevin Cook, Patrick Bennett, Timothy Stratton Thermo Fisher Scientific, San Jose, CA

As high resolution mass spectrometry becomes more widely implemented in drug discovery labs, experiments that better exploit generic full scan data collection are being developed. Preliminary information regarding drug metabolism collected early in the ADME screening stage can be used to better optimize compound design before comprehensive metabolite identification is performed. High resolution mass spectrometry can be used to simultaneously quantify the metabolism of the target compound and identify metabolites present in the sample as well collect data dependant MS/MS spectra, without significant MS method optimization. Analysis of fragmentation information for compound metabolites can provide additional confirmation for metabolite identification on metabolic hot spots in the target compound enabling more efficient drug design.

In-vitro incubations for Buspirone, Verapamil, were performed at two concentrations (3uM and 300nM). A time course study up to 45 minutes was designed to investigate the appearance of various phase 1 metabolites, and disappearance of parent compound. Sample reactions were quenched using a 3:1 dilution of acetonitrile followed by centrifugation. 5 uL sample injections were made onto a C18 column (50x2.1mm, 3um) and sample elution was accomplished using 0.1% formic acid in water and 0.1% formic acid in acetonitrile. MS analysis was performed using a Thermo Scientific Q Exactive benchtop quadrupole Orbitrap mass spectrometer.

Analysis for the 3uM incubation was performed using a shallow LC gradient over 11 minutes for separation of metabolites. MS data was collected in full scan data dependent MS/MS mode for quantitation of target compounds and relative quantitation of metabolites. Metabolite profiles were calculated for all six drugs and with results correlating to literature values. Additionally, the phase I metabolites identified for each of the six compounds were plotted for signal response over time using MetQuest software.

Analysis for the 300nM incubation was performed using both an 8 minute shallow gradient and a 3 minute fast gradient. MS data was collected in full scan data dependent MS/MS mode for quantitation of target compounds and relative quantitation of metabolites. Metabolite profiles were calculated for all six drugs using both LC methods, with results correlating to literature values. Metabolites were identified for both the 3 minute and 8 minutes LC methods and compared to the results obtained at the 3uM incubation. Metabolite identification at the lower concentration incubation and short LC analysis provides a good correlation between metabolites identification results at the 3uM incubation.



#### A Bench-Top Orbitrap Mass Spectrometer Based Workflow Solution for Intact Monoclonal Antibody Characterization

Zhiqi Hao, Yi Zhang, Nick Duczak, David Horn, Shiaw-Lin Wu\*, Patrick Bennett, Andreas FR Huhmer Thermo Fisher Scientific, San Jose, CA, USA, \*Barnett Institute, Northeastern University, Boston, MA

Intact monoclonal antibody (mAb) eluted from the column was analyzed using a bench-top Q Exactive quadrupole Orbitrap or a prototype second generation Exactive Orbitrap mass spectrometer. Top-down MSMS was performed using high energy collision dissociation with a unique spectrum multiplexing feature (msx HCD) in the Q Exctive instrument. Full MS spectra of intact or reduced mAb were analyzed using Protein Deconvolution 1.0. The top-down msx HCD spectra were analyzed using ProSight PC 2.0.

The full MS spectra of the mAbs show a complete charge envelope distribution of the mAb, with each charge state revealing five major glycosylation forms that are baseline separated. The measured ppm error on average molecular mass for data generated in 30 experiments using two different instruments is 6.9 ppm with a standard deviation of 6.4 ppm. Using the 140 K resolution setting, the isotopic peaks of the light chain was baseline resolved over the 1 min elution time, which resulted in monoisotopic molecular mass determination with an error of less than 5 ppm. For the top 5 glycoforms, the relative abundance reproducibility was within a few percent. To obtain amino acid sequence, top-down MS/MS was applied to the reduced mAb samples using the unique msx HCD approach. Besides the improved throughput from spectrum multiplexing, the advanced signal processing provides improved resolution and higher Orbitrap scan speeds, which is critical for on-line protein top-down sequencing. High resolution, information rich spectra were generated on the one minute LC elution time for reduced mAb samples. For the light chain, over 30% sequence coverage was achieved, including the N-terminal variable region, with a mass error of less than 5 ppm for fragment ions.

Both precise mass measurement and extensive, high confidence sequence information can be obtained for intact mAb using this workflow solution that combines high resolution MS, fast chromatography, high throughput msx HCD and accurate data analysis.



Innovator Award Submission

### Evaluation of a Microsampling System for Accurately Controlling Dried Blood Spot Volumes

Jim Kenney, Joe Siple Drummond Scientific: Jim Kenney and Joe Siple

A growing number of studies involving Dried Blood Spot (DBS) sampling have raised concerns over the possible impact of hematocrit variation on sampling consistency and accuracy. Typically, investigations have shown that, when placed onto the same or equivalent DBS card, blood samples containing higher hematocrit levels yield spots with smaller diameters compared to those containing lower hematocrits. The difference is spot diameter presents a problem when a uniform punch (e.g., 3mm or 6mm) is used to collect a fixed-area disk from within the diameter of the spot. The issue is that the amount of sample contained in the disk may not actually represent a true "normalized" sample.

An apparent solution to this hematocrit effect is to punch (or extract) the blood spot in its entirety. To support this "entire spot" approach, known amounts of sample, such as 5uL, 10uL, or 15uL, need to be delivered to the DBS card with definable and acceptable accuracy and precision. Once a known sample size is established, harvesting the entire spot should remove sampling bias associated with hematocrit.

The purpose of this study is to evaluate a positive displacement microsampling system that affords accurate and precise sample volume delivery to DBS cards. A fixed-volume "plugged microcapillary pipet" capable of collecting and delivering 5uL or 10uL spots is evaluated. A novel DBS dispenser for transferring multiple aliquots (3 x 15uL, 5 x 5uL, etc.) is also accessed.

#### POSTER ABSTRACT #13

Innovator Award Submission

### Proposed Plasma Micro-Sampling Device for Implementation in Pre-Clinical Studies

Chester L Bowen<sup>1</sup>, Molly Karlinsey<sup>1</sup>, Hermes Licea-Perez<sup>1</sup>, Kristen Jurusik<sup>1</sup>, Essaie Pierre<sup>1</sup>, Jim Kenney<sup>2</sup>, Joe Siple<sup>2</sup> 1) GlaxoSmithKline; 2) Drummond Scientific

The benefits of micro-sampling to preclinical environments have already been recognized throughout the pharmaceutical industry through the implementation of Dried Blood Spot (DBS) technology. However, variable hematocrit levels in animal and patient populations could have an impact on accurate spotting and analysis due to differences in blood viscosity.

Advances in LC/MS/MS technology, specifically instrument sensitivity, have allowed for a decrease in sample volume, facilitating the implementation of micro-sampling approaches in bioanalysis. Generally, plasma assays are preferable in the bioanalytical community as compared to DBS, whole blood, and blood/water assays. The use of plasma micro-sampling in preclinical toxicokinetic (TK) studies could facilitate the removal of satellite animals. As a result, blood sampling from the TK animals will allow direct correlation between the drug exposure and toxicity observed in the same individual animal as well as greatly reduce the number of animals required for each study. One of the current challenges of micro-sampling is generating plasma aliquots from a small volume of blood (<100  $\mu$ L). In this work, a prototype device is evaluated that will allow for isolation of a micro-volume of wet plasma (<50  $\mu$ L) from whole blood (<75  $\mu$ L).



### QUANTITATION OF RIBAVIRIN IN 0.010 ML OF HUMAN PLAMSA USING A FULLY AUTOMATED UHPLC/MS/MS METHOD

Andrew Glowacki and James Lewis Pharmanet USA, Princeton, New Jersey

In the highly competitive pharmaceutical industry the need to deliver high quality bioanalytical data quickly and with minimum sample volume becomes crucial to the success of many candidate drugs. As these demands have shaped the bioanalytical field various vendors have produced innovative solutions and equipment to enable laboratories to meet these needs. By combining several emerging technologies, we have set out to develop and validate a test method for the quantitation of ribavirin in human plasma. Our goal is to learn how best to develop a rugged, highly automated, rapid throughput method while consuming minimal sample volume.

This method will provide a template when developing future analytical methods to support a variety of projects. The high degree of automation is expected to increase the consistency of the assay procedure across multiple analysts, thereby increasing ruggedness. By increasing throughput, we expect to better be equipped to support large-scale projects, such as generic bioequivalence studies. By working with minimal sample volume, it is expected to become less challenging to support projects where sample volumes are limited, such as in pediatric or animal health studies

Although our assay is near the end stages of development and our plans are still subject to change, our methodologies have produced promising data thus far. We plan to begin our assay by using a Hamilton STAR line robot to aliquot 10 uL of sample into each well of a 384 well plate. The Hamilton robot will also add internal standard to appropriate wells of the plate. After gently mixing the samples, the Hamilton robot adds a protein precipitation solution to each well of the plate. The samples are mixed and centrifuged. The Hamilton robot then transfers some of the resulting supernatant to a new 384 well plate.

The plate is then analyzed by LC/MS/MS using a Waters Acquity UPLC system interfaced to a Sciex API4000 Mass Spectrometer (which for other compounds, may be upgradeable to an API5000 when necessary). The cycle time is approximately 45 seconds per sample resulting in an overall run time of about five hours per plate for a total of three plates run overnight. Assuming an excess of wells devoted to standards, QCs, and blanks, this would allow for a minimum of 335 samples per run. By these calculations, a small team of technicians (about three) would be able to produce over one thousand results in a single business day using a single LC/MS/MS system.

By combining several state-of-the-art technologies, such as the 384 well plate, the Hamilton STAR line, the Waters Acquity UPLC, and the Sciex API4000, a rugged, high throughput assay consuming minimal sample volume becomes a possible contender for a variety of challenging bioanalytical projects.



Innovator Award Submission

#### Alturas Analytics: Leading The Way In Bioanalytical Innovation

Shane Needham Alturas Analytics

In the changing arena of pharmaceutical research, contract research organizations must lead the industry. A CRO cannot be merely reactive to these changes. It must be proactive in anticipating and meeting complex bioanalytical challenges. Since its founding Alturas Analytics has been a research intensive laboratory, fostering bioanalytical innovation to provide our sponsors and industry the latest analytical methods and techniques.

#### Dried Blood/Matrix Spot Analysis (DBS/DMS)

Alturas was the pioneer and continues to be the industry leader in DBS. This technique is fit for purpose and ideal for programs where molecule stability is an issue. DBS also requires less blood, and once spotted the DBS cards can be shipped at ambient temperature.

Methods were soon developed at Alturas to adapt DBS to translucent liquids. Using a color indicating dye developed inhouse, it, s now possible to analyze tears, CSF, synovial fluid and other translucent, low-volume matrices. At that time in 2010 the term dried matrix spot (DMS) analysis was coined, and it, s common language in the industry today. In the last two years Alturas has given 14 presentations, published two white papers and contributed a book chapter on the subject of DBS/DMS.

#### Macromolecule Analysis

Alturas is currently conducting analysis of proteins, biomarkers and other macromolecules using LC/MS/MS. A "bottom up" approach is used to analyze these molecules in our laboratory. The sample is first prepared via immunoaffinity purification, solid phase extraction or protein precipitation. Proteases then digest the macromolecule into smaller surrogate peptides, which are then analyzed using tandem mass spectrometry. This technique provides excellent selectivity, and the sensitivity continues to improve as mass spectrometers evolve. Alturas Analytics, LC/MS/MS methods are now competing with ligand binding assays for quantitative analysis of large molecules.

#### Microflow-LC/MS (MFLC/MS)

The latest innovation at Alturas is the use of Microflow-LC/MS in a bioanalytical setting. This long available technology is being combined in the laboratory with DBS/DMS, macromolecule analysis and other bioanalysis techniques to great success. Alturas recently conducted an experiment that analyzed myoglobin, somatotropin and ceruloplasmin to compare HPLC and MFLC for macromolecule analysis. Using the same injection volumes and instrument condition, the sensitivity gains were more than 12X when compared to conventional LC/MS.

The same was true of an experiment where DMS was used to analyze Dextromethorphan in in-vitro fluid. The DMS approach was successful, especially when combined with MFLC-MS to give signal gains of more than 4X compared to traditional LC/MS. Microflow LC-MS will play a significant role in the future of bioanalysis to facilitate drug development.

#### Conclusion

Alturas Analytics continues to be the most innovative bioanalytical CRO in the industry. The demands of the pharmaceutical, biotechnology and medical device industries are always increasing. Alturas analytics is researching new methods and techniques to push the limits of sensitivity and selectivity for drug development.



### Development of a Novel Biocatalytic Approach for the Preparation of Significant Quantities of Oxidative Metabolites

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In vitro and in vivo metabolite generation and definitive structural characterization have historically proven to be both technically challenging and oftentimes extremely time consuming. While recent advances in nominal and accurate mass spectrometric approaches have allowed for increased detailed structural information, definitive and incontrovertible metabolite structure identification still remains quite elusive. One widely used method to unambiguously identify in vivo metabolite structures is to synthesize a wide variety of 'suspected' metabolites based upon MS-MS fragmentation data. Once the metabolite of interest is correctly identified via LC-MS, further resources may then be required to synthesize a sufficient quantity of metabolite to submit for testing (efficacy, PK, and a variety of functional assays). This general approach can be a very resource intensive process. As a result, the ability to obtain the correct metabolite in significant quantity with dramatically reduced effort would be extremely beneficial to all phases of drug discovery and development.

This presentation will provide an overview of a new paradigm using commercially available biocatalysts (available from Codexis) for the formation of up to milligram quantities (> 20 mg) of oxidative metabolites of interest. We will use a pertinent example conducted in our labs to illustrate the entire cross-departmental process used to generate and fully characterize a circulating metabolite of interest. We will specifically focus on the general use of the biocatalysts and the subsequent characterization of the metabolite of interest.



Innovator Award Submission

#### Rapid Analysis Using High Performance Ion Mobility Spectrometry

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Abstract: Electrospray ionization high performance ion mobility spectrometry (ESI-HPIMS) provides a high resolution separation for many general analytical and pharmaceutical applications. HPIMS is a gas phase technique that separates molecules based on their mobility in a gas medium. The measured drift time in milliseconds can be directly related to analyses molecular weight and structure. The recent development of superior resolution in our lab has enabled the ESI-HPIMS as an orthogonal separation method to HPLC and MS. This method reaches sub-microM sensitivity with a resolving power of 60-90. Its major advantage is that it can separate compounds in seconds. In addition, ESI-HPIMS can successfully separate isomers and molecules that are chromatographically sensitive, nonvolatile, thermally labile, or that lack a UV chromophore. Precision, sensitivity, resolution, linear response range and LOD/LOQ of the ESI-HPIMS method were carried out in order to determine the instrument's feasibility for use in many pharmaceutical applications. These applications include dissolution testing, content uniformity studies, reaction monitoring, and cleaning validation/verification.



### Matrix Independent Quantification of Proteins to Attomole Levels in Milligram-sized Samples

Stephen Dueker, Ann Hoffman, Saira Abidi and John Vogel Eckert Ziegler - Vitalea Science

Mass Spectrometry has rapidly become the tool of choice for quantification of peptides and proteins. This tool is applied for intact protein analysis, pharmacokinetic profiling and determination of degradation products. Variation in ionization, fragmentation and detector response across the protein mass range lead to complexity in quantitation. Combustive isotope ratio Accelerator Mass Spectrometry solves many of quantitative issues by providing standard free quantitation of lightly 14C-labeled large molecules without confounding matrix interferences. We illustrate the process for matrix independent quantification of proteins or protein-small molecule conjugates using combustive IR-AMS on a 14C labeled antibody injected into three test rats.

#### Methods

A 150,000 kDa antibody labeled in a sulfhydryl position(s) using 14C radiolabeled N-ethylmaleimide. The resultant specific activity was 55 mCi/mol, showing almost complete labeling at this single reactive site (1 14C per molecule = 62 Ci/mol). After cleanup by multiple dialysis steps, the purified antibody was delivered byn intravenous bolus injection into three rats (10 nCi per animal). Whole blood was serially harvested for 24 hr post dose by tail bleed into K3EDTA tubes. 15 uL aliquots were removed, dried, and combusted to gaseous CO2 in sealed quartz tubes using CuO as a solid oxygen source. The CO2 was reduced to graphite over an iron group catalyst and analyzed for 14C contents by AMS.

#### Preliminary Data

The 14C counts were normalized to the stable carbon-12 macroscopic current which in essence serves as the internal standard. Results are reported as pg-antibody equivalents per mL after factoring in the blood total carbon concentration and the specific activity of the antibody. Blood pharmacokinetics displayed a biphasic disappearance that closely mimicked a parallel dataset generated using established ELISA method. Limits of detection were order of magnitude greater for the AMS method (low femotmolar) than the ELISA method and repeatability was <3% CV on independently processed replicates. No method specific development was required. Speciation of 14C labeled fragments was not performed at the time of writing this abstract but is being planned for on-going analyses.



#### An Alternate Approach to Evaluating Whole Blood Stability in Bioanalysis

#### Ron Shoup, Tim Grever, Brian Beato, and Brian Engel AIT Bioscience

Establishing whole blood stability is a common but sometimes challenging aspect of bioanalytical method validation. The primary purpose is to provide evidence that target analytes are sufficiently stable in whole blood from the time the sample is drawn to the time the plasma portion is harvested and stabilized by freezing. The measurement can be complicated by the kinetics of distribution of the analyte between the plasma and red blood cells. Ideally, one would use an incurred sample already equilbrated with all of the analytes of interest, and split it into multiple portions. At specific time intervals, plasma from a portion of the whole blood sample would be harvested, and the analyte concentrations compared to those from plasma harvested immediately after the blood is drawn. Typically, the lack of availability of such incurred samples during method validation makes this ideal approach impractical.

There are numerous challenges when establishing whole blood stability in the absence of incurred samples. First, whole blood must be spiked with the analytes of interest much the same way that plasma QCs are spiked. However in whole blood, the analytes may require time to equilibrate with the red blood cells. Any amount of analyte that partitions into the red blood cells and remains there during plasma harvesting is not detected via the plasma assay. How long and at what temperature does one equilibrate the freshly spiked whole blood sample prior to initial plasma harvest? Additionally, during this time, analyte levels may actually be decreasing both due to instability and due to partitioning into the red blood cells. If plasma is harvested too soon for establishing initial analyte concentrations, that is, prior to full equilibration with the red blood cells, time points harvested later may yield reduced analyte concentrations that would obscure any conclusion as to analyte stability. This approach to establishing whole blood stability is problematic because it does not mimic incurred whole blood samples in which the analytes of interest are already in equilibrium with the red blood cells.

Proposed here is an alternate approach which focuses on analyte stability and avoids the confusion caused by analyte redistribution. A pool of whole blood is spiked with the analytes of interest and maintained at ice water or 37°C, as desired. Aliquots of whole blood are removed at various time points and are vigorously mixed 1:9 with acetonitrile. The red blood cells are lysed completely, releasing the analytes of interest. A portion of the acetonitrile supernatant is then used to spike analyte-free plasma for subsequent bioanalysis. Rather than using a semi-qualified method in whole blood, the plasma method already validated is used to assess the total content of analyte in the whole blood samples. The total analyte concentration is measured absolutely and can therefore be related back to the initial spiked concentration, with any decrease in analyte over time due only to instability in whole blood, without the complication of partitioning between the plasma and erythrocytes.



#### Identifying Abnormal Internal Standard Responses in LC-MS/MS Data

Ron Shoup, Tim Grever, Brian Beato, and Brian Engel AIT Bioscience

One challenge in evaluating LC-MS/MS data is identifying those samples which may have been mis-spiked with internal standard (ISTD). A common scheme is to only accept samples whose ISTD response is within 50-150% of the mean of the ISTD responses of the calibrators and QCs in that run. This approach can work when the ISTD response is relatively consistent throughout a run and is intended to screen out samples that were double-spiked or not spiked with ISTD. However, ISTD response in properly spiked samples can be highly variable for LC-MS/MS methods, making this approach unsatisfactory. For example, samples with analytes that ionize poorly and have co-eluting stable label ISTDs can yield ISTD response for samples with low analyte concentration of the analyte. In these situations, the ISTD response for samples with low analyte concentrations can be significantly greater than the ISTD response for samples containing high concentrations of analyte, due to ion suppression by the analyte. Even so, the calibration curves generated by response ratios (analyte response/ISTD response) in these situations may be quite linear. Acceptance criteria requiring ISTD responses to be within 50-150% of the mean of the ISTD responses of the calibrators and QCs is often not useful for these situations.

An alternative for identifying abnormal ISTD responses has been setting acceptance criteria for ISTD responses within 2-3 standard deviations of the mean of the ISTD responses from calibrators and QCs within a run. Unfortunately, this rule can be problematic for methods that yield highly precise ISTD responses throughout a run , because the acceptable ISTD response range is too narrow. The ISTD response from an incurred sample may differ slightly due to minor matrix effects specific to that individual sample, resulting in an absolute ISTD response that is outside the tight acceptance criteria arising in these situations. Clearly an alternate approach to identifying abnormal ISTD response is necessary for these and other types of situations that frequently arise in LC-MS/MS analyses.

Proposed here is a step-wise approach for determining abnormal ISTD response, which is greatly simplified by using functionality within the TSQ Module of Watson LIMS. After all samples in a run are integrated, Watson calculates the %CV of the ISTD responses of the calibrators and QCs. If this %CV value is >5.0%, samples with ISTD responses within of the mean of the ISTD responses of the calibrators and QCs are deemed acceptable. If the %CV of the ISTD responses of the calibrators and QCs is 5.0%, sample ISTD responses must be within jÀ 15.0% of the mean of the ISTD responses of the calibrators and QCs. Although all of these values are quickly calculated by Watson, the simple calculations can also easily be performed with common spreadsheet software or automated within electronic laboratory notebook workflows. The scheme also recognizes the industry's acceptance of 15% limits in bioanalytical methods as a reasonable cutoff for concern.



#### Quantitative LC-MS/MS Analysis of 15 Estrogens and Estrogen Metabolites in Human Plasma Using Supported-Liquid Extraction (SLE) Technique

Xi Chen, Daorong Guo, Suli Han, Hua Yang, David Jones, Bibo Xu Primera Analytical Solutions Corp., Princeton, New Jersey

Estrogens and estrogen metabolites (EMs) are a group of steroid hormones that are crucial in development of female sexual characteristics and reproductive process both in humans and animals. Abnormal levels of endogenous estrogen and EMs at different female developmental stages have been shown to associate with a variety of diseases such as breast cancer, osteoporosis, cardiovascular and neurologic disorder. It is crucial to have a sensitive and high throughout bioanalytical method to accurately quantify the estrogens and their metabolites. In this study, a simple and rapid HPLC-MS/MS has been developed and validated to reach a low limit of quantitation at 10 pg/mL for all 15 analytes, and the sample size required is only 300 uL.



### Fragment-based Drug Discovery: Comparison of Labeled and Label-free Approaches to beta-Amyloid Secretase (BACE-1)

Lauren E. Frick, William A. LaMarr, and Selena Larkin Agilent Technologies

Fragment-based screening offers advantages over traditional high-throughput screening by allowing more comprehensive coverage of chemical space, but the typical low potency of fragments leads to the frequent use of physical methods that detect binding. The few existing biochemical assays tend to employ optical methods, such as fluorescence spectroscopy (FS), which can be subject to confounding factors due to the high concentrations of compound needed to detect activity. Here we screen â-amyloid secretase (BACE-1) against a fragment library using two substrates, a labeled and an unlabeled peptide, which were detected either by FS or RapidFire high-throughput triple-quadrupole mass spectrometry (MS). Different kinetic parameters, hit rates, and hit sets were obtained depending on the substrate and detection method, suggesting that fluorescent labels and optical detection methods can result in follow-up of a set of compounds not inhibitory towards the unlabeled, more biologically relevant substrate.



### Pioneering approaches in biopharmaceuticals: analysis of a humanized monoclonal antibody (mAb) using a novel platform

Kristy J. Fraley, Lee Abberley, Thomas Mencken, Charles S. Hottenstein, Matthew Szapacs, and David Citerone PTS DMPK Bioanalytical Sciences and Toxicokinetics, GlaxoSmithKline, King of Prussia, PA, USA

Introduction: The Gyrolab workstation has been proposed to be a viable alternative to traditional immunoassays. The system benefits from a fully automated transfer of reagents and samples from a microplate onto a microstructure composed of a 15 nL streptavidin derivitized bead bed. As a result of the automated aspect of the instrument and the small path of diffusion inherent in the bead bed, a complete immunoassay can be completed for 112 samples in 50 minutes with a dynamic range generally extending to three orders of magnitude.

Results: The poster describes the development and full validation of a method for the determination of a humanized monoclonal antibody using the Gyrolab assay system (range of 5 to 250 ug/mL) in monkey plasma. The validation of this method assessed the following parameters: bias and precision, selectivity and minimum required dilution, dilution linearity and prozone effect, short term roomtemperature stability, stability due to freeze thaw cycles and processed diluted sample stability. The above parameters all met the acceptance criteria of +/- 20% nominal values. Finally, the method was used to support the toxicokinetic portion of a regulated preclinical toxicology study for the humanized mAb in monkeys.

Conclusion: A Gyrolab Immunoassay method was developed and fully validated for the determination of a humanized mAb in monkey plasma. The Gyrolab system proved to be a viable alternative to traditional immunoassay and was used to support a toxicokinetic study. The speed of analysis that the Gyrolab provides was beneficial in meeting timelines to complete this project as multiple assays can be completed in a single day.



### Proposed Plasma Micro-Sampling Device for Implementation in Pre-Clinical Studies

Chester Bowen and Hermes Licea-Perez Senior Scientific Advisor

The benefits of micro-sampling to preclinical environments have already been recognized throughout the pharmaceutical industry through the implementation of Dried Blood Spot (DBS) technology. However, variable hematocrit levels in animal and patient populations could have an impact on accurate spotting and analysis due to differences in blood viscosity.

Advances in LC/MS/MS technology, specifically instrument sensitivity, have allowed for a decrease in sample volume, facilitating the implementation of micro-sampling approaches in bioanalysis. Generally, plasma assays are preferable in the bioanalytical community as compared to DBS, whole blood, and blood/water assays. The use of plasma micro-sampling in preclinical toxicokinetic (TK) studies could facilitate the removal of satellite animals. As a result, blood sampling from the TK animals will allow direct correlation between the drug exposure and toxicity observed in the same individual animal as well as greatly reduce the number of animals required for each study. One of the current challenges of micro-sampling is generating plasma aliquots from a small volume of blood (<100  $\mu$ L). In this work, a prototype device is evaluated that will allow for isolation of a micro-volume of wet plasma (<50  $\mu$ L) from whole blood (<75  $\mu$ L).



### The quest for the best brew. Cryoprobe-assisted NMR analysis of brewed coffee

Eleanor Munger<sup>1,\*</sup> and Istvan Pelczer<sup>2</sup>

1) Carleton College, Northfield, MN; 2) Princeton University, Department of Chemistry, Princeton, NJ

As a water-based solution, brewed coffee provides an interesting subject for NMR mixture analysis both for component identification and quantitative measurements. Very high sensitivity cryoprobe-based NMR technology allows qualitative and quantitative analysis of components even at very low concentration in a complex mixture. In this study, two aspects of the coffee brewing process were investigated: whether filtration in the brewing method would change the composition of the coffee, and reuse of grounds.

Excitation sculpting water solvent suppression allowed taking 1H NMR spectra of the native brews without any modification [1]. 1HNMR, 2D J-resolved, and 13CNMR spectra were collected as a means of identifying the components of the coffee. Based on simple spectrum analysis and comparison to previous research [2], twenty-four components were identified in the 1H NMR spectra. Spectral overlays allowed for scaled comparison of the different conditions described. Based on these comparisons, we could conclude that filtration does not remove or modulate significantly the concentration of any of the identified compounds. As for brewing the same grounds multiple times, after the first brew, all peaks showed significantly lowered intensities, although in a differential manner. Finally, further coffee research could be performed in the future, expanding not only the variety of brewing methods, but also using NMR as a means for quality control, and to distinguish different roasts or plant origins.



### Are you running on clean energy?!... Comparative quantitative 1H and 13C NMR analysis of energy drinks

Bradley J. Gorsline (<sup>1,\*</sup>) and Istvan Pelczer(2) 1) Princeton University, Princeton, NJ; 2) Princeton University, Department of Chemistry, Princeton, NJ

Energy shots, such as 5-Hour Energy have come into widespread popular use in recent years, yet their composition and quality has been called into question. Using cryoprobe-assisted very high sensitivity 1H (with high efficiency water suppression), 13C, and 2D J-resolved NMR we could verify and quantify the molecular composition of the ingredients in a large variety of 5-Hour Energy samples with relative ease and efficacy. For this analysis we used the original samples in their native condition with no other modification than adding a little D2O for lock just before the experiment was run. Using quantitative NMR techniques, we attained accurate absolute concentration measurements of the components, most importantly a credible measure of the caffeine in the three strengths of 5-Hour Energy and that of sugar-replacement substances. Quantitative 13C experiments, made possible by the 13C-optimized cryoprobe, were especially helpful. This project extends the growingfield using NMR for food and beverage analysis and quality control.



### Composition of a fashion-drink; NMR analysis of a variety of coconut water products

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1) Princeton University, Princeton, NJ; 2) Princeton University, Department of Chemistry, Princeton, NJ

Coconut water has become quite popular, even fashionable in the U.S. and abroad lately due to their high electrolyte content and rehydrating properties. Over the last couple of years it has showed up on the market in a great variety of forms, flavors, and packaging, also from several geographical sources. The main selling point of coconut water products is their authenticity, with products described variably as "Natural", "100% Pure", and "Real".

NMR spectroscopy is a natural born analytical tool for qualitative and quantitative assessment of complex mixtures. We have conducted a detailed study, which examines the validity of these claims, how these samples compare and what their real composition is. We have surveyed original coconut samples from different geographical locations and several packaged and flavored versions with more in line for future studies.

High-sensitivity, cryoprobe-assisted 1H, 13C, and 2D-J-resolved spectroscopy, in combination with extensive use of database information and literature data, helped to identify and quantify a large number of components. All samples were run in their native condition adding only a small, measured amount of D2O for lock. 1H NMR spectra were run using high-efficiency solvent suppression. 13C-detection optimized cryoprobe technology was essential to complete this assessment, especially for sugar components. Absolute concentration measurements were also possible using a portable capillary insert method. The poster presents a summary of these results; some of the samples show characteristic and significant differences and deviations, even sugar components may vary. As a general conclusion, NMR spectroscopy provides a reliable and powerful tool for comparative, qualitative and quantitative analysis of natural juices, such as coconut water.



### A Validated Method for the Quantitation of Olanzapine by Direct Injection of Plasma onto an LC-MS/MS System

Katrina Emilia Nizzi; Timothy L. Shoaf; John W. Torchia BASi, 2701 Kent Ave, West Lafayette, IN 47906

Direct injection of plasma samples and subsequent on-line separation of analytes from matrix offers several advantages over traditional sample preparation / samples analysis techniques. These include significantly reducing the time required of laboratory personnel and the chances of errors during sample preparation. This poster presents a validated method for quantifying the anti-psychotic drug olanzapine in human plasma using direct injection onto a Shimadzu CoSense 2D chromatography system configured for on-line sample extraction from matrix using a specialized trap column followed by separation on an analytical column. The method shows comparable performance to a previously validated method using a liquid/liquid extraction followed analysis by LC-MS/MS.

The method used the Shimadzu CoSense system comprising a SIL-20AC refrigerated autosampler, four SIL-20AD pumps, a CTO-20AC column oven, and CBO-20A controller attached to and controlled by a Sciex 4000 triple-quadrupole mass spectrometer with a Turbo-Ionspray source operated in negative ion mode. The plasma samples were aliquotted into a 96-well plate and spiked with labeled-isotope internal standard. The plate was then vortexed, centrifuged, and sealed. The samples were then injected onto the Shimadzu CoSense system and extracted on a MAYI-ODS column. After washing for two minutes, Olanzapine was back-flushed from the trap column and onto a Phenomenex Polar RP column for analytic separation. Once loaded onto to the analytical column, a conventional gradient eluted Olanzapine to be detected by MS/MS. Total run time was four minutes. Total preparation times ranged from 45 to 60 minutes; approximately one-quarter to one-third the time required of the conventional assay.

The method was validated in compliance with FDA GLPs and guidances. Validation experiments included inter/intra-day accuracy and precision, blank selectivity, between subject accuracy and precision, carry-over, dilution integrity, multi-plate, autosampler re-injection reproducibility, processed sample stability, and matrix influence. The validation had acceptable results for all experiments. Results were similar to the conventional method. Relative accuracy ranged from +8.4% to +0.5% and precision ranged from 3.4% to 9.8% RSD. Carryover was assessed by observing the response of blank samples without internal standard placed after the highest standard calibrators. Carryover greater than 20% the lower limit of quantitation was not observed. The method also showed robustness towards hemolyzed samples at high and low QC concentrations. Dilution integrity up to fifty-fold was demonstrated. Matrix influence was also assessed and indicated a response enhancement in plasma versus neat samples.

Direct injection of plasma samples and subsequent on-line separation of analytes on a Shimadzu CoSense system saves analyst time and gives comparable results to traditional manual sample preparation.



#### A Bioanalytical Method for Therapeutic Peptide PK Study

Xuejun Zang, Igor Gavin, Asha Oroskar Orochem Technologies, Inc

Up to date, about 70 synthetic therapeutic peptide drugs have been approved in US, Europe and Japan, and there are many more therapeutic peptides in preclinical pipelines. We studied and developed a bioanalytical assay for our client, s therapeutic peptide. This peptide can be extracted from mouse plasma using Sagacity HL SPE cartridge, and recovery is 87%. Peptide extract is further analyzed by LC-MS/MS with Reliasil C18 HPLC column, the linear range is 10-500 ng/mL. This assay is successfully used for this peptide, s mouse PK study.



### 1JCC-Edited HSQC-1,n-ADEQUATE: A New Paradigm for Establishing Molecular Structure

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Undesired 1JCC correlations unavoidably "leak" into 1,n-ADEQUATE spectra because of the oscillatory nature of the amplitude transfer functions of the 1JCC and nJCC coupling constants. Making the amplitude transfer delays asymmetric in duration provides a means of selectively inverting 1JCC correlation responses in 1,n-ADEQUATE spectra. The modified pulse sequence, in addition to editing the spectrum, also provides a cleaner noise floor than the original 1,n-ADEQUATE experiment.

Generalized Indirect Covariance (GIC) processing of a non-edited GHSQC spectrum with an inverted 1JCC 1,n-ADEQUATE spectrum affords a 1JCC-Edited HSQC-1,n-ADEQUATE spectrum. Direct (1JCC) correlations between pairs of protonated carbons are diagonally symmetric and have negative phase; 1JCC correlations between protonated and adjacent non-protonated carbons are diagonally asymmetric and negative; long-range (typically via 3JCC) correlations between pairs of protonated carbons are diagonally symmetric and positive; long-range correlations between protonated and non-protonated carbons are diagonally asymmetric and positive. Strychnine is used as a model compound to illustrate the results obtained. The resulting 1JCC-Edited HSQC-1,n-ADEQUATE spectrum provides unequivocal access to 1JCC correlation data equivalent to 2JCH correlations in a GHMBC and predominantly 3JCC long-range correlations equivalent to 4JCH GHMBC correlations. When used to complement a readily acquired GHMBC spectrum, the synergy of these data provides a powerful new paradigm for establishing molecular structures.



#### Steroid Panel Analysis of H295R Cells Using LC-MS/MS

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Steroid hormones regulate metabolism, neurotransmission, intracellular signaling and much more. Regulation of these hormones produces a wide range of clinical effects including sedation, seizure prevention, oncology and reproduction. The biological process of producing steroid hormones is called steroidogenesis. Our clients are investigating the effect of man-made chemicals (industrial, agricultural, pharmaceutical) on steroidogenesis. The H295R human carcinoma cell line is the primary in-vitro model for steroidogenesis.

This paper will describe the assay we have developed on the lonics 3Q 200 series triple quadurpole mass spectrometer for the measurement of cortisol, corticosterone, 11-deoxycortisol, androstenedione, deoxycorticosterone, testosterone, 17a-hydroxyprogesterone, dehydroepiandrosterone, 17a-hydroxypregnenolone, progesterone, pregnenolone, estrone, and estradiol from H295R cell culture media. The analytical measurement range has been optimized for forskolin stimulated H295R cells, but is also applicable for quantification of steroids in unstimulated cells.



#### Steroid Panel Analysis of H295R Cells Using LC-MS/MS

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