



POSTER SESSION ABSTRACTS

Inspiration. Collaboration.

POSTER SESSION
Tuesday, September 30th 1:00 PM - 3:00 PM

CPSA 2014
September 29 - October 2, 2014
Sheraton Bucks County Hotel
Langhorne, PA

Where Technology and Solutions Meet

Poster Title

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3. Assessment of the ionKey/MS™ technology to reach ultra high level of sensitivity for bioanalysis quantification of large molecules in plasma; Louis-Philippe Morin¹, Sukhdev Bangar², Paul Rainville² Erin Chambers², Mary Lame², Fabio Garofolo^{1*} ¹Algorithme Pharma Inc., Laval, Québec, Canada ²Waters Corporation, 100 Cummings Center, Beverly, MA, USA

4. A Novel Metabolomics-Based Assay for Pre-Analytical Quality Control of Human EDTA Plasma; Schiewe, H.-J. (2), Kamlage, B.(1), Bethan, B.(1), Peter, E. (1), González Maldonado, S. (1), Schmitz, O. (1), Schatz, P.(2); (1) metanomics GmbH, Tegeler Weg 33, 10589 Berlin, Germany; (2) Metanomics Health GmbH, Tegeler Weg 33, 10589 Berlin, Germany

5. Advanced NMR Strategies for the Elucidation of Complex Molecular Structures; Janine N. Brouillette¹, Michel Frederich², Luc Angenot², Alembert T. Tchinda³, Alexei V. Buevich¹, R. Thomas Williamson¹, and Gary E. Martin¹; ¹ Merck Research Laboratories, Process & Analytical Chemistry, NMR Structure Elucidation Group, West Point, PA. ² Universite de Liege, Departement de Pharmacie, Belgium ³ Institute of Medical Research and Medicinal Plants Studies (IMPM), Ministry of Scientific Research and Innovation, Yaounde, Cameroon

6. Rapid Global Proteomic Analysis of Acetaminophen induced protein changes in Liver Tissue: Identification of Biomarkers for (potential) early detection of Toxicity in 3D Human Liver Microtissues; Claudia Escher¹, Roland Bruderer¹, Reto Ossola¹, Patrina Gunness², Magdalena Bober¹, Lukas Reiter¹, Yulia Butscheid¹, Jens M. Kelm², Oliver Rinner¹, Simon Messner²; ¹Biognosys AG, Schlieren, Switzerland, www.biognosys.ch; ²InSphero AG, Schlieren, Switzerland, www.insphero.com

7. Trans-mitochondrial ¹³C-flux analysis supports an important role of non-oxidative metabolism in insulin secretion in human islets.; Tiago Alves, Rebecca Pongratz, OrLando Yarbrough, Gary Cline, Graeme Mason, Richard Kibbey; Yale University School of Medicine, New Haven

8. Using ToF MRM, Target Enhancement and Ion Mobility for Quantitation using a Time-of-Flight Mass Spectrometer; Mark Wrona, Yun Alelyunas, Paul Rainville, Russell Mortishire-Smith

9. Combining On-Bead Conjugation and MS Analysis following IdeS Digestion Simplifies the Production and Characterization of ADCs; Chris Hosfield¹, Philip Compton², Becky Godat¹, Kevin Cook¹, Sergei Saveliev¹, Archer Smith², Nidhi Nath¹, Paul Thomas², Neil L. Kelleher², Michael Rosenblatt¹ and Marjeta Urh¹ (¹ Promega ² Northwestern)

10. Quantitation of Cholesterol and 4β Hydroxycholesterol in Human Plasma using Surrogate Matrix and ESI-LC/MS/MS; Barry R. Jones¹; John E. Buckholz¹; Kathlyn McCardle¹; Emily King¹, Parya Nouri², Jenny Zhang² ¹Quintiles Bioanalytical and ADME Labs, Ithaca, NY; ²Clinical Assay Group, Global Innovative Pharma Business, Pfizer, Groton, CT

11. Metabolite profiling reveals druggable metabolic distinctions between basal-like and non-basal-like triple-negative breast cancers Jeffrey R. Peterson*, Alexander Beatty*, Lauren Fink*, Alexander Strigun[§], Erik Peter[§], Hajo Schiewe[§], Ulrike Rennefahrt[§]*Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111; § metanomics GmbH & [§]Metanomics Health GmbH, Tegeler Weg 33, 10589 Berlin, Germany

12. Development of Chip-based Nanobore Column Platform with Universal Connectivity, Column Heating and Sheath Gas Capability; Helena Svobodova, Amanda Berg, Gary A. Valaskovic; New Objective, Inc., Woburn, MA

13. Maximizing Nanoflow Spray Stability and Sensitivity Using Automated Emitter Rinsing; Amanda Berg, Helena Svobodova, Gary Valaskovic;New Objective, Inc.

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15. Alternatives to Traditional Bioanalytical Techniques; Lori Payne, Intertek Life Sciences, USA; Andrew Brotherhood, Sophie Rothwell, Ashleigh Wake, Lorna Kettle, Intertek Life Sciences, UK

16. Multiple Aliquot Extraction Of Blood Plasma; ¹Fred Regnier, ¹Tim Woenker, ¹JinHee Kim, ²Jiri Adamec, and ¹Tim Schlabach. ¹Novilytic, West Lafayette, IN 47906 and ²University of Nebraska, Lincoln, NB 68598.

17. Development of a High-throughput LC-MS/MS Assay for Pain Management Panel from Urine; H. Qiao, J. Ye, E. Majdi, L. Cousins. IONICS Mass Spectrometry Group Inc., Bolton, ON, Canada

18. A Novel 6x5 LC-MS/MS Peptide Reference Mixture of Instrument Performance Monitoring; Michael Rosenblatt, Ethan Strauss, Kevin Cook and Marjeta Urh - Promega Corporation, Madison, WI

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22. Minimize the Cyber Attack Surface of Medical Devices; Mark Janeczko; Dedicated Computing, Waukesha, WI

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25. In-Vitro ADC Linker Stability Investigations in Biological Matrices, Kristen Jurusik, Sherry Wang, Matthew Szapacs, Yanwen Qian, Christopher Evans, GlaxoSmithKline, King of Prussia, PA

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27. A Rapid and Reproducible Immuno-MS Platform from Sample Collection to Quantitation of IgG, Rachel Lieberman¹, David Colquhoun¹, Jeremy Post¹, Brian Feild¹, Scott Kuzdzal¹, Fred Regnier², ¹Shimadzu Scientific Instruments, Columbia, MD, USA ²Novilytic L.L.C, North Webster, IN, USA

28. Application of Unique Stationary Phases for Effective RPLC Method Development, Thomas J. Waeghe, Carl L. Zimmerman, Geoffrey Faden, MAC-MOD Analytical

Poster # 1

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Poster # 2

Combining Laser Ablation Electrospray Ionization Mass Spectrometry Imaging with Collision Cross Section for Improved Metabolic Profiling from Clinical Samples

Peggi M. Angel²; Callee Walsh²; Greg Kilby; Pamela Cantrell²; James Langridge¹; Giuseppe Astarita¹, ¹Waters Corporation, Milford, MA; ²Protea Biosciences, Morgantown, WV

Laser Ablation Electrospray Ionization Mass Spectrometry (LAESI-MS) is an innovative technology that allows imaging mass spectrometry and molecular signature profiling on complex clinical samples ranging from tissues to high throughput well plate analyses. The “sweet spot” for LAESI analysis is in the small molecule range, targeting pharmaceutical and metabolite distribution within samples. However, a main problem in any platform for metabolic analysis is that there often are numerous candidates under one isobaric peak, which limits a complete definition of the metabolite. Here, we discuss analytical strategies using collision cross sections (CCS) generated by traveling wave ion mobility mass spectrometry (TWIMS) to increase confidence in metabolic identifications from complex samples. We show that coupling LAESI with TWIMS generates CCS that match CCS collected using traditional liquid chromatography coupled to electrospray ionization. Comparison of LAESI-TWIMS imaging data generated from normal and Alzheimer’s brain tissue sections shows that CCS is a highly useful tool in generating biologically relevant metabolic information from complex clinical samples.

Methods

Travelling-wave ion mobility mass spectrometers (Waters Synapt G2-Si HDMS) located in different laboratories, were used to derive CCS values for a variety of lipid and metabolic standards. Direct infusion and LAESI-TWIMS analysis in well plate format were performed for lipid standards to evaluate LAESI-generated CCS versus ESI CCS. Frozen brain samples of grey matter from Alzheimer’s disease and healthy control subjects were sectioned in a cryostat and maintained frozen at -15°C during analysis by LAESI-TWIMS. LAESI-MS parameters consisted of 15 laser pulses per pixel at 5 Hz and 800 uJ of laser energy. Data were collected in negative and positive ion mode over a mass range of m/z 50 to 1200 using traveling wave ion mobility. Data were lockmass corrected and identifications made by accurate mass via the METLIN database (metlin.scripps.edu) with CCS values study-derived from analysis of standards. Ion distribution maps were visualized using ProteaPlot v2.0.1.3.

Results

Ion mobility-derived CCS values were not affected by instrument settings or chromatographic conditions (RSD <3% for 98% of over 250 CCS measured) and were highly reproducible across instruments located in independent laboratories. The use of CCS typically dropped the number of candidates under one near-isobaric or isobaric peak from around 25 candidates to 3 or less. Analysis of CCS from a polyalanine standard showed a correlation coefficient of 1.0000 between LAESI-TWIMS to direct infusion ESI-TWIMS in both positive and negative mode. A total of 89 lipid standards compared between methods revealed a correlation coefficient of 0.993. On-tissue

analysis of human brain tissue sections by LAESI-TWIMS identified 165 molecules including lipids and metabolites. Our results indicate the CCS is useful as an additional analytical measurement for metabolic analyses that is robust across laboratories and mass spectrometry platforms, significantly improving the accuracy of metabolic identifications within complex clinical samples.

Poster # 3

Assessment of the ionKey/MS™ technology to reach ultra high level of sensitivity for bioanalysis quantification of large molecules in plasma

Louis-Philippe Morin¹, Sukhdev Bangar², Paul Rainville², Erin Chambers², Mary Lame², Fabio Garofolo^{1*}, ¹Algorithme Pharma Inc., Laval, Québec, CANADA ²Waters Corporation, 100 Cummings Center, Beverly, MA, USA

Purpose

Quantification of large molecules such as peptides and proteins by LC-MS/MS can be challenging. These compounds generally involve laborious extraction and chromatographic procedures in order to achieve low quantification limits. In this research, the ionKey/MS™, an integrated microfluidic LCMS system was used to reach ultra low level of sensitivity for the quantification of Glucagon and Desmopressin.

Methods

Glucagon and Desmopressin samples were spiked in human plasma within a range of 10pg/mL to 2500pg/mL and 0.5pg/mL to 2000pg/mL respectively and extracted via solid phase extraction technique (SPE). Chromatographic separations for both methods were obtained onto an Acquity UPLC M-Class through a “trap & elute” injection. Trapping was performed on a Symmetry C18 300umX50um column with a flow rate of 25uL/min. A gradient elution was completed onto a Waters Acquity BEH PST C18 iKey™ with a flow rate of 2uL/min. Detection was performed on a XEVO TQ-S operated in positive ESI. Glucagon $[M+5H]^{5+}$ was monitored at the mass transition m/z 697.5 → 694.0. Desmopressin $[M+2H]^{2+}$ was monitored at the mass transition m/z 535.4 → 328.2.

Results

Glucagon curve was found linear, weighted 1/x, from 10 to 2500.0pg/mL with a coefficient of correlation of 0.9993. Signal-to-noise ratio (S/N) obtained for the lower limit of quantification (LLOQ) of 10pg/mL was over 5 and coefficient of variation (%CV) for 5 replicates injection was 7.0%. Desmopressin curve was found linear, weighted 1/x, over the concentration range of 0.5-2000.0pg/mL with a coefficient of correlation of 0.9965. Signal-to-noise ratio obtained for the LLOQ of 0.5pg/mL was over 5 and %CV for 3 replicates injection was 11.6%.

Conclusion

The results shown demonstrate that the use of an integrated microfluidic LC/MS platform can achieve robust performance and reach high levels of sensitivity for large molecule applications

Poster # 4

A Novel Metabolomics-Based Assay for Pre-Analytical Quality Control of Human EDTA Plasma

Schiewe, H.-J. (2), Kamlage, B.(1), Bethan, B.(1), Peter, E. (1), González Maldonado, S. (1), Schmitz, O. (1), Schatz, P.(2), (1) metanomics GmbH, Tegeler Weg 33, 10589 Berlin, Germany; (2) Metanomics Health GmbH, Tegeler Weg 33, 10589 Berlin, Germany

Background

Clinical research and development studies depend on high-quality specimens. Access to high quality samples, collected and stored along standardized procedures, minimizes the risk of errors in the pre-analytical phase which may jeopardize successful drug target or biomarker identification and validation as well as reliability and reproducibility of clinical results. Metabolites are well-suited as biomarkers for the quality control of biobank samples due to the response of the metabolome to physiological and chemical processes.

Methods

Based on our previously reported metabolomics results, a targeted GC-MS assay was developed for quantitative analysis of quality markers in human EDTA plasma samples. Human EDTA plasma samples obtained after applying defined pre-analytical confounding factors (e.g. wrong tube type, blood- and plasma storage, freeze-thaw cycles) were subjected to mass-spectrometry based metabolomics or to a targeted assay developed to control for such pre-analytical confounders.

Results

The quality control assay detected plasma samples of a poor pre-analytical quality due to prolonged storage of blood or plasma or incubation at improper temperatures. Pre-analytical confounders resulted in significant and reproducible changes of the human plasma metabolome with blood storage having the highest impact. Samples with insufficient pre-analytical quality were identified with high sensitivity and specificity. A score is calculated for each sample that indicates its pre-analytical quality.

Conclusions

The plasma metabolome is influenced by the pre-analytical phase. The newly developed assay enables pharmaceutical R&D, clinical research organizations, and biobanks to better understand the actual condition of human plasma samples, efficiently monitor SOP compliance in multicenter trials, deliver superior quality samples, and support evidence-based decisions for sample selection.

Advanced NMR Strategies for the Elucidation of Complex Molecular Structures

Janine N. Brouillette¹, Michel Frederich², Luc Angenot², Alembert T. Tchinda³, Alexei V. Buevich¹, R. Thomas Williamson¹, and Gary E. Martin¹, ¹ Merck Research Laboratories, Process & Analytical Chemistry, NMR Structure Elucidation Group, West Point, PA; ² Universite de Liege, Departement de Pharmacie, Belgium; ³ Institute of Medical Research and Medicinal Plants Studies (IMPM), Ministry of Scientific Research and Innovation, Yaounde, Cameroon

There have been a number of advanced NMR experiments described in recent reports which have included pure shift HSQC, dual-optimized inverted $^1J_{CC}$ 1,n-ADEQUATE for establishing direct and long-range carbon-carbon correlations, and LR-HSQMBC for very long-range 1H - ^{13}C and 1H - ^{15}N correlations. As molecules become structurally more complex, the potential for overlapped resonances in the proton spectrum increases, which can, in turn, make it considerably more difficult to make unequivocal assignments of proton and carbon resonances. To illustrate the application of some of the contemporary new NMR methods that have become available, applications of them to the spectral assignment of the complex “dimeric” *Strychnos* alkaloid sungucine will be shown.

Poster # 6

Rapid Global Proteomic Analysis of Acetaminophen induced protein changes in Liver Tissue: Identification of Biomarkers for (potential) early detection of Toxicity in 3D Human Liver Microtissues

Claudia Escher¹, Roland Bruderer¹, Reto Ossola¹, Patrina Gunness², Magdalena Bober¹, Lukas Reiter¹, Yulia Butscheid¹, Jens M. Kelm², Oliver Rinner¹, Simon Messner²,
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Determination of mode of action remains a challenge in preclinical phases of drug development. Here we report on how global proteomic analysis allows for a faster and more thorough understanding of the mode of action and mechanism of toxicity induced by drug treatment using acetaminophen as the model drug. 3D Human Liver Microtissues (InSphero) were co-cultured with Kupffer cells and were exposed to 3 subtoxic concentrations (4.6, 13.7, 370.4 μ M) and one toxic concentration of acetaminophen (3333.3 μ M) for 72 hours. We then applied a novel mass-spectrometric approach - Hyper Reaction Monitoring (HRM) – to quantify changes in protein expression. HRM combines DIA (Data Independent Acquisition) with comprehensive spectral libraries to identify and quantify all detectable proteins in a given sample. This process is facilitated by a software platform (Spectronaut) developed for high content proteomics analysis. Through the HRM approach it was possible to profile and quantify 2788 proteins and evaluate the response to different concentrations of acetaminophen over time and subsequently identify acetaminophen-adducts on specific sites in six proteins. The outcome of this study also illustrated that minimal sample quantity is required for a large-scale proteomic study (~12,000 cells/sample) and that identification of proteins relevant to toxicity in acetaminophen metabolism (unbiased discovery approach) was achieved. This targeted analysis of known proteins of interest can further lead to specific pathway analysis.

Poster # 7

Trans-mitochondrial ¹³C-flux analysis supports an important role of non-oxidative metabolism in insulin secretion in human islets.

Tiago Alves, Rebecca Pongratz, Orlando Yarbrough, Gary Cline, Graeme Mason, Richard Kibbey, Yale University School of Medicine, New Haven

Background and aims: Non-oxidative mitochondrial metabolism has previously been implicated glucose-stimulated insulin secretion (GSIS). In particular, pyruvate carboxylase (PC) and mitochondrial PEPCK (PEPCK-M) have been strongly associated with GSIS. Recent reports suggested that in contrast to rodent islets, human islets have relatively little PC flux. The positional flux of [¹³C₆]glucose carbons through each reaction provides important information regarding its relevance to the GSIS without perturbing normal metabolism. Current NMR-based methods are limited by sensitivity and cannot resolve the different anaplerotic fluxes.

Materials and methods: A novel quantitative LC/MS-based technique was developed to track ¹³C-labeled substrates and directly measure positional step-wise metabolic fluxes in INS-1 cells and human islets. Metabolic flux analysis was performed under metabolic steady-state but isotopic non-steady-state conditions. Absolute flux was calculated with the program CWAVE using differential equations to model mass and isotope balance.

Results: Metabolism included oxidative (glycolysis, pyruvate dehydrogenase (PDH) and TCA cycle) and non-oxidative (PC, malic enzyme (ME) and PEPCK-M) reactions. There was significant isotope dilution between sequential steps in glycolysis and TCA consistent with very active metabolic cycling. Similar to oxygen consumption, TCA cycle flux increased incrementally with glucose (1.27±0.02, 2.31±0.06, and 5.02±0.1 fold, P <0.001, going from 2.5 to 5 to 7 to 9 mM glucose) while the contribution of PDH to the TCA cycle (~90%) remained flat. PC flux was nearly evenly divided between ME and PEPCK-M flux, though remarkably, glucose carbons flowed exclusively through PEPCK-M and not ME. PC flux increased with glucose starting at 2% to more than 50% the rate of the TCA cycle (fold increases of 3.73±0.36, 9.89±0.52, and 28.91±2.2 above background, P<0.001) and closely correlated with insulin secretion. PEPCK-M flux increased linearly with insulin secretion. Similar rates of PC relative to PDH fluxes were observed in islets from human donors.

Conclusion: This technique has broad applicability to the study of intracellular metabolism in normal and diabetic human islets as well as other tissues. Quantification of discrete trans-mitochondrial fluxes beta-cells supports a very significant contribution of non-oxidative metabolism to the mechanism of insulin secretion.

NIH R01-DK092606

Poster # 8

Using ToF MRM, Target Enhancement and Ion Mobility for Quantitation using a Time-of-Flight Mass Spectrometer

Mark Wrona, Yun Alelyunas, Paul Rainville, Russell Mortishire-Smith, Waters Corporation, Milford, MA

Purpose

To investigate alternative quantitative modes of operation for Synapt HDMS QTof. Investigation includes ToF-MRM with target enhancement and ion mobility dynamic range enhanced modes of operation to achieve low pg/mL sensitivities and/or higher dynamic range in human plasma.

Method

Standard curves of pharmaceutical compounds (Verapamil, Buspirone, Clopidogrel and Alprazolam) were prepared in human plasma and data acquired using an ion mobility equipped mass spectrometer. Data was collected using ToF-MRM with target enhancement (high transmission modes) pure quantitative mode of acquisition and assessed for use in bioanalytical PK (quantitative) workflows. ToF-MRM was compared to MS and MSE (quan/qual type). In a second arm of the study, non-ion mobility data was compared with full scan ion mobility (with dynamic range enhancement) modes of operation.

Results

ToF-MRM based selective modes of acquisition achieved 5-10x more ion counts more than generic full scan approaches. This ultimately yielded better detection limits, 2-5 fold better signal to noise and simultaneous file size reduction and data simplification appropriate for bioanalytical workflows. The availability of ion mobility in "HD" analysis modes can also extend this approach by also providing selectivity through an additional measure of discriminating the ion(s) of interest from the noise (matrix). In this work, we show that enhancements using DRE (dynamic range enhancements) improve the dynamic range for IMS considerably providing a path to practical use in quantitative environments. We discuss the differences in limits of detection using various modes of acquisition and the impact in both typical microsomal based assays. Further improving limits of detection and quantitative performance for plasma based assays using HRMS platforms are also discussed.

Poster # 9

Combining On-Bead Conjugation and MS Analysis following IdeS Digestion Simplifies the Production and Characterization of ADCs

Chris Hosfield¹, Philip Compton², Becky Godat¹, Kevin Cook¹, Sergei Saveliev¹, Archer Smith², Nidhi Nath¹, Paul Thomas², Neil L. Kelleher², Michael Rosenblatt¹ and Marjeta Urh¹ (¹Promega ²Northwestern)

Antibody-drug conjugates (ADCs) are a growing class of targeted therapies designed to deliver cytotoxic drugs to cancer cells. The process to make ADCs can be tedious, involving optimization of numerous variables including linker design, attachment chemistry and loading density. We have developed an improved method to produce ADCs utilizing antibodies immobilized on high-capacity magnetic protein A or G particles. We employed this method to label Rituximab with a fluorescent dye, and characterized the conjugate using mass spectrometry techniques including intact mass and subunit-level analyses after digestion with IdeS protease. Conjugation of AlexaFluor to Rituximab immobilized on high capacity magnetic protein A or G particles was accomplished after mild reduction of the hinge disulfides followed by covalent attachment using maleimide chemistry. Intact mass analysis of PNGase F-treated naked and conjugated Rituximab demonstrated characteristic mass shifts representative of different payload distributions of the dye. Full MS of the IdeS-digested ADC revealed mass shifts consistent with loading on both the Fd and light chain. The combination of on-bead conjugation followed by IdeS digestion and subsequent MS analysis simplifies the workflow for production and characterization of ADCs.

Poster # 10

10 Quantitation of Cholesterol and 4 β Hydroxycholesterol in Human Plasma using Surrogate Matrix and ESI-LC/MS/MS

Barry R. Jones¹; John E. Buckholz¹; Kathlyn McCardle¹; Emily King¹, Parya Nouri², Jenny Zhang², ¹Quintiles Bioanalytical and ADME Labs, Ithaca, NY; ²Clinical Assay Group, Global Innovative Pharma Business, Pfizer, Groton, CT

Introduction

The ratio of 4 β hydroxycholesterol (4 β -OHChol) to cholesterol is an endogenous index on CYP3A activity. Accurate quantitation of 4 β -OHChol and cholesterol by LC-MS/MS is necessary for confident assessment of CYP3A induction/inhibition and insight into those effects on drug metabolism.

There are several challenges to the quantitation of endogenous cholesterol and 4 β -OHChol by LC-MS/MS. Both require a surrogate matrix or surrogate analyte approach, which adds additional assay characterization procedures to ensure accurate quantitation. Further challenges include 1) the large disparity of endogenous concentrations between the two analytes, 2) the propensity for sterols to convert to oxysterols during sample processing, 3) poor ionization characteristics, 4) presence of both esterified and unesterified circulating analytes, 5) solubility of cholesterol, and 6) potential interferences from similar endogenous species.

Methods

Lithium heparin human plasma was pretreated with 50 μ g/mL butylated hydroxytoluene before sample analysis. For analysis of 4 β -OHChol, 80 μ L of plasma was saponified via base hydrolysis followed by liquid/liquid extraction with hexane. UHPLC chromatography and shallow gradient were used to separate 4 β -OHChol from other endogenous analytes, such as 4 α -OHChol and other oxysterols. MS detection was done using an API5500 with positive ion ESI ionization in SRM mode.

For the cholesterol assay, 10 μ L of plasma was diluted with surrogate matrix prior to saponification, derivatization with picolinic acid, and subsequent liquid/liquid extraction. Cholesterol was eluted under isocratic conditions at 0.8 mL /min with a total cycle time of 2.5 min. MS detection was done using an API4000 with positive ion ESI ionization in SRM mode.

Data were normalized by use of stable-labeled internal standards - [²H₇] 4 beta hydroxycholesterol or [²H₇]-cholesterol. 4% Bovine serum albumin in 10 mM phosphate buffered saline was used as the surrogate matrix for both analyses.

Preliminary Data

Derivatization of cholesterol to a picolinyl ester increased ionization efficiency by >2 orders of magnitude. This allowed for up-front dilution of the biological matrix, which improved peak shape. Further, the calculated concentration of cholesterol increased with dilution factor, until reaching a plateau. The assay performance at any given dilution factor met rigorous accuracy acceptance criteria, and the underestimation of

endogenous cholesterol levels was only evident with endogenous dilution linearity experiments.

4 beta hydroxycholesterol was analyzed without derivatization, as sufficient sensitivity was achieved to quantify the lowest expected concentrations under down-regulated conditions. A shallow gradient and UHPLC conditions were necessary to separate 4 β -OHChol from other low-level components.

Accurate and precise quantitation (RE < 15%, RSD < 15%) of extracted plasma QC samples and diluted endogenous plasma samples was demonstrated for both cholesterol and 4 beta hydroxycholesterol when measured from the surrogate matrix curves. Excellent linearity, sensitivity, parallelism, accuracy, precision, and selectivity are demonstrated and presented for these assays.

Metabolite profiling reveals druggable metabolic distinctions between basal-like and non-basal-like triple-negative breast cancers

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Triple-negative breast cancer (TNBC) is an aggressive and prevalent form of breast cancer that is not responsive to current targeted therapies. Development of targeted therapies for TNBC is hindered by its genetic heterogeneity and a lack of common oncogenic drivers.

To elucidate druggable metabolic alterations in TNBC, we performed metabolite profiling on 12 well-characterized TNBC cell lines representing all TNBC subtypes previously defined by gene expression (Lehmann et al. J Clin Invest. 2011) as well as control primary and non-transformed breast epithelial cells. Hierarchical clustering based on intracellular metabolite levels clearly and reproducibly segregated TNBC cell lines from non-transformed lines. Alterations in energy utilization, lipid metabolism, and other pathways of importance to proliferating cells differed significantly from controls. In addition, TNBC cells segregated into two discrete groups that correlated with basal-like vs. non-basal-like gene expression status. Metabolites participating in druggable pathways including amino acids (glutamate, glutamine, serine, glycine, and trans-4-hydroxyproline) and lipids (specific phosphatidylcholines & sphingomyelins) differed significantly between TNBC metabolic subtypes. Ongoing studies are evaluating whether these differences represent dependencies with therapeutic relevance. Finally, metabolite profiling was used to examine metabolic alterations associated with treatment of TNBC and control cells with clinical kinase inhibitors rapamycin, sorafenib, imatinib, and lapatinib. Our findings suggest that co-targeting kinases and metabolic targets may offer an opportunity for synthetic lethality with a reduced likelihood for the development of drug resistance.

Development of Chip-based Nanobore Column Platform with Universal Connectivity, Column Heating and Sheath Gas Capability

Helena Svobodova, Amanda Berg, Gary A. Valaskovic, New Objective, Inc., Woburn, MA

Introduction

Nanobore column chromatography has become a method of choice when analyzing a wide variety of peptide and protein samples. The complex nature of biological samples requires frequent testing of different separation methods on a variety of nanobore columns to determine the best separation conditions. Here we test a newly developed easy-to-use chip based system with universal connectivity, column heating and sheath gas capability. The introduction of sheath gas to the column design enables platform flexibility, improved spray stability at aqueous conditions and higher flow rate capabilities ($> 1 \mu\text{l}/\text{min}$). The flexible design of the chip columns facilitates method development, providing columns with different inner diameters, bed lengths and a wide selection of stationary phase materials.

Methods

Nanobore columns (75 μm ID, 15 μm tip, PicoFrit, New Objective) were packed to 10 and 25 cm bed lengths with 3 and 5 μm C18 resins. These columns were assembled into a chip based device with embedded column heating (New Objective). Bovine Serum Albumin (1 pmol, Waters) and 6-Bovine Protein (50fmol, Michrom Bioresources) digests were injected (HTC PAL, Leap technologies) directly on column using a 1 μl sample loop. Sample separation was achieved by a 2-50% acetonitrile gradient delivered by a nanoLC pump (Eksigent Ultra, AB Sciex) at 300 nl/min and 500 nl/min flow rates. Full scan MS data (350 – 1500 Da) were collected on a LTQ mass spectrometer (Thermo) equipped with a thermally controlled chip based nanospray source (New Objective).

Preliminary Results

Packed Tip columns with 10.5 and 25 cm long beds packed with 3 μm and 5 μm C18 resins were assembled into chip columns enabled with column heating. The initial column evaluation was performed at 500 nl/min, 2-50% acetonitrile gradient at four different temperature settings – ambient, 40°C, 50°C and 60°C. Peaks for four BSA peptides (m/z 575.5, 569.7, 643.8 and 508.0) were extracted at 13.5 % above baseline. The extracted peaks were selected across the whole gradient to cover peptides with different affinity to the stationary phase. The average peak capacity for the extracted peptides increased 47% when the temperature increased from ambient to 40°C. Less than 10% change in the peak width of BSA peptides m/z 575.5, 646.8 and 508 was detected when the temperature was increased further to 50°C and 60°C. While the peak capacity for m/z 575.5, 643.8 and 508.0 ions improved with the increased temperature, the peak width of m/z 569.7 peak remained almost constant at ambient and 40°C (9.6 s and 10.8 s respectively).

The pressure of the chip columns was monitored at the ambient and at elevated temperatures. The pressure of the 10 cm long columns packed with 5 μm and 3 μm resins decreased 39% and 30% when the temperature was changed from ambient to 60°C.

Maximizing Nanoflow Spray Stability and Sensitivity Using Automated Emitter Rinsing

Amanda Berg, Helena Svobodova, Gary Valaskovic, New Objective, Inc., Woburn, MA

Introduction

Nanobore LC-MS has demonstrated improved sensitivity over traditional higher-flow LC-MS workflows in quantitative applications. Reproducibility, robustness and sensitivity are the hallmarks for successful quantitative assays. While nanoflow LC-MS enables greater sensitivity, reproducibility and robustness have historically limited its adoption to quantitative LC-MS/MS workflows. Here we present data demonstrating significantly improved spray stability and reproducible analyte response using automated emitter rinsing for flow injection experiments. The benefits of automated tip rinsing will be further explored for nanobore LC-MS/MS data using stable isotope dilution MRM-MS.

Methods

Flow injection experiments were collected at 300 nl/min. using a direct flow nano pump (Eksigent) at 90% A (0.1% Formic Acid in Water, JT Baker) and 10% B (0.1% Formic Acid in Acetonitrile, JT Baker). Samples were loop injected (1 μ l, HTC PAL) and twenty-minute full scan data files (Q1MS 400 – 1000Da) were collected on a 4000 QTRAP (AB SCIEX). A 500 fmol/ μ l 4-peptide mixture in 70% mobile phase A/30% mobile phase B was monitored for analyte response and spray stability over a series of 418 replicate injections. Using an uncoated emitter (360 μ m OD x 20 μ m ID x 10 μ m tip, New Objective) and a nanospray source with automated stage actuation and software control (DPV-450, New Objective) two data sets were collected.

Preliminary Data

The automated stage and software control enable the emitter to be diverted away from the inlet in between each injection to a defined position at which a gravity flow of solvent rinses the exterior of the emitter. The effect of regular tip rinsing was evaluated by comparing a data set collected with automated tip rinsing to a data set collected without tip rinsing. Replicate injections were collected, totaling 418 injections equaling 139 hours run time for each data set. Thirty-second segments were extracted from each injection. The average TIC and average TIC RSD were calculated and plotted for each data set. The no-rinsing data set showed a total change in the average total ion current from 1.09E8 for injection 7 to 4.32E6 for injection 366, representing a 25 fold decrease in analyte response. In comparison the rinsing data set showed a total change in the average TIC from 1.16E8 for injection 4 to 5.69E7 for injection 335, a 2 fold decrease in analyte response. Both data sets showed scatter in the plot of average TIC per injection, but the data set collected without rinsing showed a general trend towards decreased analyte response. The RSD for the no-rinse data set, had a spread of 1.25% for injection 98 to 42.83% for injection 384. In comparison, the data collected with rinsing showed a spread of 0.78% for injection 150 to 4.87% for injection 413. The no-rinse data showed a

34-fold change in RSD compared with a 6-fold change for the rinse data. Automated emitter rinsing resulted in consistent analyte response and spray stability (supported by reproducible RSD values for the average TIC) for this data set. Further studies will be conducted to evaluate the benefits of tip rinsing for improving the RSD values of peak area ratios for stable isotope dilution MRM-MS experiments.

Poster # 14

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Alternatives to Traditional Bioanalytical Techniques

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Purpose

Methods such as ELISA and LC-MS/MS have long been established as the techniques of choice to support pharmacokinetic (PK) studies of small and large molecules from clinical and pre-clinical programs. With the advancements in technology other non-traditional techniques such as quantitative NMR, ICP-MS and CGE can present viable alternatives for bioanalytical applications. Presented in this review will be a general introduction to such techniques with emphasis placed on providing, not only an overview of the principle behind these approaches, but also on how, in the right situation, these techniques may offer advantages over the current method of choice.

Methods

Inductively Coupled Plasma Spectroscopy with Mass Spectrometry (ICP-MS) is used to determine trace levels of inorganic materials in biological matrices, such as platinum containing anti-cancer drugs. Capillary Gel Electrophoresis (CGE) can be applied to bioanalysis of oligonucleotide therapeutics. Quantitative Nuclear Magnetic Resonance Spectroscopy (NMR) is suitable for the determination PEGylated molecules in biological matrices.

Results

All three techniques have been successfully used to generate data that meets regulatory requirements for drug development equivalent to traditional techniques such as LC-MS and ELISA. A case study using each technique will be presented illustrating that for compounds that are not suitable for mass spectrometry (not easily ionizable) or where there is an absence of a chromophore or where sample matrix interferences can be reduced these techniques may offer an advantage.

Conclusions

Non-traditional techniques such as quantitative NMR, ICP-MS and CGE have evolved over the years and have been used successfully in bioanalysis to generate data accepted by regulatory agencies. In certain situations with suitable molecules, these non-traditional techniques may be more desirable than traditional LC-MS and ELISA for pharmacokinetic studies.

Multiple Aliquot Extraction Of Blood Plasma

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Blood sampling is at the heart of clinical diagnostics and DMPK studies. Determining metabolite, drug, and protein concentrations in the various blood compartments is complicated by biological variation. A volume (C) of freshly drawn blood is composed of a volume (A) of cells and the liquid volume (B) of plasma; i.e. $A + B = C$. Although C can be determined easily, A and B vary between subjects. The equation still has two unknowns. Determining the amount of an analyte in a volume C of blood has no meaning unless the liquid volume in the biological compartment in which it is dissolved is known. The most widely employed solution to this problem is to separate the cellular and liquid components of blood. New, miniaturized modes of extracting plasma from finger-stick derived blood by using membrane filtration to remove cells have recently been introduced that eliminate the two unknown dilemma.

Although these new plasma extraction methods have many advantages, a weakness is that collection of additional samples for archiving and further analyses require additional finger-stick sampling. This introduces the possibility of inter-sampling variability. A new, miniaturized sampling technology is being reported here in which multiple plasma samples are extracted from a single blood sample simultaneously by microfluidic transport through channels in a stack of membranes; split ratio and flow velocity in channels being determined by the shape, size, and porosity of membranes through which the sample flows. The driving force for liquid flow in all cases is capillary action. The system laterally splits a drop of whole blood into a series of identical fractions using a single membrane before cell removal by longitudinal filtration begins in a second membrane. Use of a single, high porosity membrane in the initial splitting process increases the probability that multiple samples of plasma will be derived from the same, unfractionated blood sample. It is important to recognize that the rate of plasma flow into a collection vessel (generally a paper disc), and potentially the degree of disc loading is a function of the distance from the site of blood deposition. This is why it is important that a reservoir of blood be rapidly placed over collection vessels before cell removal to assure equal transport distances during filtration and collection. Data will be presented for inter-vessel variation in collection volumes within a device and across multiple fractionation devices. Extraction of 2.5 μ L plasma aliquots in a set of collection vessels is generally achieved within 3-5 min. Dried plasma samples are obtained by removing the upper two membrane layers and allowing the collection vessels to air dry.

Development of a High-throughput LC-MS/MS Assay for Pain Management Panel from Urine

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Background:

The widespread use and the potential abuse of opiates, sedatives, and stimulants drugs have increased the need and in some cases the requirement to screen patients on a routine basis. Pain panels continue to grow in complexity as more prescription and non-prescription compounds are added. This has made the job of toxicological analysis even more challenging. To fulfill these requirements, a fast, reliable, and accurate LC-MS/MS method has been created for the analysis of a pain panel comprised of 30 drugs on an IONICS 3Q 120 triple quadrupole mass spectrometer.

Methods:

First, the mixed drug standard solution was spiked into the urine matrix, then diluted with the mobile phase A (100% H₂O, 0.1% formic acid) to make a series of concentrations ranging from 0.016 to 16 ng/mL. The internal standard concentration used was 10 ng/mL. The calibrator solutions will be directly injected without further treatment. IONICS 3Q 120 mass spectrometer equipped with a heated coaxial flow ion source and "Hot Source-Induced Desolvation" interface was used. The time-managed MRM in MolanaTM software was used to optimize the dwell time for each MRM transition based on the retention times and the number of MRM transitions within given experiments. The separation was performed on a Shimadzu Prominence LC system. A 10 μ L sample was loaded onto a Restek Ultra II Biphenyl column (50 x 2.1 mm, 5 μ m) kept at 40 °C. A gradient method was created with a flow rate of 600 μ L/min and a total LC cycle time of 7.5 minutes. Solvent B was composed of 0.1% formic acid in 100% methanol.

Results:

A total of 57 MRM transitions were used to monitor 30 drugs including internal standards. No matrix interferences were observed. LC system carryover was checked to ensure the validity of the data. An overlay of the extracted chromatograms of 30 drugs in a 7.5 minute LC run showed that all of the analytes were clearly separated. The calibration curves showed good linearity for all the analytes across the whole concentration range with a coefficient $R^2 > 0.99$. All calibration curves used a linear weighting regression of $1/x$. The LLOQs for the 30 drugs were in the range of 0.032 to 2 ng/mL. At LLOQs, the accuracy was between 84-114%, and CVs were $< 10\%$ for all analytes.

Conclusion:

The results in this study show that in a 7.5-minute LC run, this LC-MS/MS method can effectively separate the 30 pain panel drugs. The quantitation results also indicate that this method is accurate, precise, and reproducible. The LLOQs for all the 30 drugs is in the range of 0.032 to 2 ng/mL, which is 2 to 3 orders lower than the typical screening cutoff concentration (300 ng/mL), and much lower than the typical confirmation cutoff concentration (50 ng/mL) for most of the drugs of abuse. Therefore, this LC-MS/MS method with IONICS 3Q 120 mass spectrometer is an effective combination for clinical pain management to monitor patient drug use and program adherence or for drugs of abuse or other workplace drug testing.

Poster # 18

A Novel 6x5 LC-MS/MS Peptide Reference Mixture of Instrument Performance Monitoring

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For the Characterization of Biologics, consistent and optimal monitoring of instrument performance continues to be challenging and has yet to be standardized across all MS laboratories. Towards this end, we have prepared a novel peptide mixture. In combination with an accompanying software tool, this mixture reports on LC-MS instrument performance. The novelty of the mixture is based on the following: The mixture contains 6 peptide sequences in which each sequence is a mixture of 5 isotopologues. Each isotopologue is mixed at different molar ratios so that a linear curve, based on mass and intensities can be produced to assess instrument sensitivity and dynamic range.

In addition to reporting on all critical instrument parameters, the software can also report on parameter history, compare instruments, and display XIC traces for the peptides. This is the first example of a peptide mixture designed to give a reporting of all critical LC and MS parameters in a single run. All of the peptide sets resolve with identical chromatography and are distinguished only by molecular mass. In doing so, we have the advantage of verifying the sensitivity and dynamic range of MS detectors in a single run. In our studies, based on Orbitrap and Q-TOF type instruments, we have detected peptide abundances as low as 20 amole (200 fmole of the heaviest peptide loaded on column). The software tool is also able to detect the peptides spiked into a complex background.

The use of pure shift gHSQC nmr as a vital tool in the structure elucidation of sub-nanomole range compounds

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The ability to accurately interrogate complex natural products, drug metabolites, degradates, trace impurities, and other sample-limited compounds has become quite challenging as sample size is reduced down to the nanomole range or below. Consequently, new analytical techniques to address this issue have become increasingly important and can have an immediate and dramatic impact on the analysis of minute quantities of sample. To that end, we have established the routine use of the pure shift HSQC experiment as a tool to interrogate the structure of scarce compounds. The pure shift variant of the gHSQC pulse sequence allows for the reduction of homonuclear vicinal (^1H - ^1H) couplings to afford one-bond ($^1J_{\text{CH}}$) ^1H - ^{13}C correlations with increased sensitivity and resolution. We present here a detailed report on the routine use of the sensitivity-enhanced pure shift HSQC experiment, coupled with 1.7 mm MicroCryoProbe™ technology, to probe nanomole scale analytes. A complex glucocorticoid steroid (betamethasone) and the antineoplastic compound (taxol) will be used to illustrate the general utility of the sensitivity-improved pure shift HSQC experiment

LC-MS/MS Method for the Detection of Free Thyroxine and Free Triiodothyronine by Using an Ultra-High Sensitive Triple Quadrupole Tandem Mass Spectrometer

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Background:

The majority of routine clinical laboratories perform Free Thyroxine (FT4) and Free Triiodothyronine (FT3) measurements on immunoassay (IA) platforms. These IA's are affected by changes in binding protein concentrations and have a weak inverse linear log relationship to Thyroid Stimulating Hormone (TSH) level in hypo- and hyperthyroid individuals. Also, IA's have poor performance at the upper and lower values of the reference intervals. The gold standard for free thyroid hormone analysis involves preparation of sample using equilibrium dialysis. This is a time consuming and technically difficult technique. However Liquid Chromatography-tandem Mass Spectrometry (LC-MS/MS) following ultrafiltration of the sample at 37°C (method as previously described by Gu et al. Clin Biochem. 2007;40;1386-1391) has been shown to perform better than IA in the above described circumstances and involves a simpler and more convenient sample preparation than that by equilibrium dialysis. Our objective was to improve on the sensitivity of this initial method. Here, we describe our 3rd generation LC-MS/MS method with improved sensitivity over the initial mass spectrometry method.

Method:

Sample preparation was performed by ultrafiltration of 500 µL of serum using a 30 kDa centrifugal filter (Centrifree YM-30, Millipore). Following the addition of the sample to filtering device, samples were centrifuged in a temperature controlled centrifuge at 1113 g for 30 minutes at 37 °C. The ultrafiltrate of 150 µL was added to 450 µL of methanol containing deuterium-labeled Internal Standards (IS) for FT4 and FT3 and was centrifuged. Further, 350 µL of the supernatant was diluted with water, vortexed and 200 µL was injected into LC-MS/MS. FT4 and FT3 were detected by electrospray ionization in negative mode with the following transitions: 775.6/126.7 m/z (for FT4) and 649.9/126.7 m/z (for FT3). The LC-MS/MS setup consisted of a Shimadzu UFLCXR HPLC system interfaced to the IONICS 3Q 320 Triple Quadrupole tandem MS. Chromatographic separation was performed using Poroshell 1.7µm C18 column (100mmx2.1mm) with a gradient mobile phase (A: 2% Methanol in water containing 0.01% acetic acid; B: 98% Methanol, at the flow rate of 0.5 mL/min). Run time per injection was 13 minutes.

Results:

The method described displayed good linearity over a concentration range of 0-25 pg/mL (FT3) and 0-5 ng/dL (FT4) with $r^2 > 0.995$. Between day precision CVs across the concentration range were: 4.8-8.8 % (for FT3) and 7.5-7.8% (for FT4). Lower limit of Quantitation (LLOQ) at signal to noise ratio (S/N) of 10 was 0.2 pg/mL for FT3 and 0.05 ng/dL for FT4 at S/N ratio of 20. The comparison r values with our first generation method were 0.87 and 0.82 for FT4 and FT3, respectively.

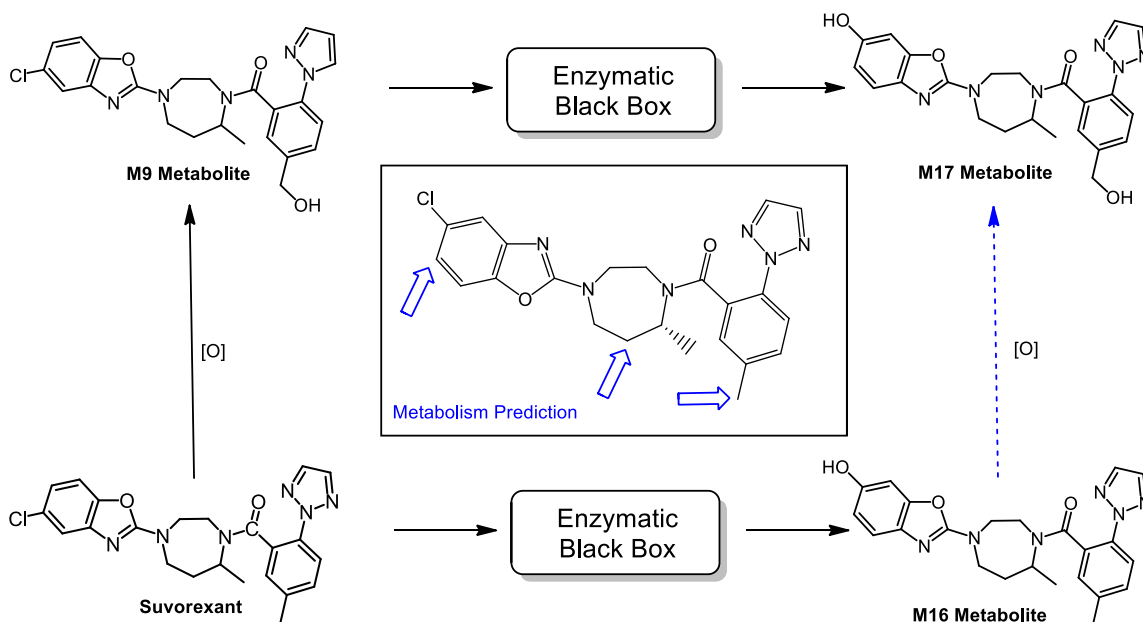
Conclusion: The sensitivity of the 3rd generation FT4/FT3 method described above is greatly enhanced due to highly sensitive mass spectrometer (IONICS 3Q 320) and column technology. LLOQ is now 10 fold lower than that found for previous reported 2nd generation FT4/FT3 LC-MS/MS methods.

Synthesis and Structure Elucidation of Interesting Oxidative Phase I Metabolites of Suvorexant

James Small, Steven Pitzenberger, Chris Cox, Paul Coleman, Semhal Berhane, Ray McClain, Dan Cui, Tammie Cabalu, Bo Liu, Jingjun Yin, John Limanto, and Debra Wallace

Over the past decade a significant amount of research has focused on the use of potent dual orexin receptor antagonists (DORA's) as targets for the treatment of insomnia and other sleep-related disorders. During that time, Merck has discovered and optimized a unique family of substituted diazepanes culminating in the development of our recently approved sleep medicine suvorexant.

The objective of the research discussed here was to synthesize and determine the structure of the unknown metabolites of interest (M9, M16, and M17). While the project team was able to clearly identify M9 via the direct synthesis of the oxidative product of the benzylic methyl group, the preparation of the expected direct substitution product of suvorexant at the chlorine did *not* give the desired metabolite. This poster presentation will give a detailed account of the structure elucidation of key *in vitro* metabolites of suvorexant using high resolution NMR and LC-MS. In addition, this presentation will also introduce the use of novel biotransformation platforms (such as Codexis Enzymes) to generate metabolites of sufficient quantity to characterize by state-of-the-art analytical techniques (NMR and HRMS).



Poster # 22

Minimize the Cyber Attack Surface of Medical Devices

Mark Janeczko, Dedicated Computing

Healthcare in the U.S. is transforming into one of the most technology-intensive industries. Advances in medical devices, for instance, enable those devices to collect more data and deliver more advanced care than ever. Many devices are networked so that they can communicate with electronic health records and remote storage, delivering new benefits. But as medical device manufacturers are learning, along with new capabilities come new cyber-security risks. . Medical device manufacturers and the computing suppliers that work with them are in a position to stay ahead of threats. It's a matter of taking the right steps.

1. **Design** with security in mind
2. **Remove** unnecessary components in software stack
3. **Keep** a current, ready-to-validate software stack ready for faster patch development
4. **Whitelist** applications, restricting what can run
5. **Freeze** the operating system to block unwanted changes

As healthcare enters the digital era, patient information is becoming increasingly vulnerable, it's critical to be proactive.

Automated Structure Elucidation of Unknown Metabolites in Pharmaceutical and Metabolomics Studies using Tandem Mass Spectral Data and the Masspec Algorithm

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

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.

Poster # 24

Analysis of polysorbates in biotherapeutic products using two-dimensional HPLC coupled with mass spectrometer

William Hedgepeth, Kenichiro Tanaka; Shimadzu Scientific Instruments, Inc., Columbia MD

Polysorbate 80 is commonly used for biotherapeutic products to prevent aggregation and surface adsorption, as well as to increase the solubility of biotherapeutic compounds. A reliable method to quantitate and characterize polysorbates is required to evaluate the quality and stability of biotherapeutic products. Several methods for polysorbate analysis have been reported, but most of them require time-consuming sample pretreatment such as derivatization and alkaline hydrolysis because polysorbates do not have sufficient chromophores. Those methods also require an additional step to remove biotherapeutic compounds. Here we report a simple and reliable method for quantitation and characterization of polysorbate 80 in biotherapeutic products using two-dimensional HPLC.

In-Vitro ADC Linker Stability Investigations in Biological Matrices

Kristen Jurusik, Sherry Wang, Matthew Szapacs, Yanwen Qian, Christopher Evans, GlaxoSmithKline, 709 Swedeland Road, UW2110, King of Prussia, PA 19406, USA

The increasing prevalence of Antibody Drug Conjugates (ADCs) in cancer research has sparked an interest by both the industry and regulators to understand the ability of the ADC molecule to shed the linker and payload *in vitro*. A recent enquiry from the FDA regarding the stability of the maleimidocaproyl linker for one of GSK's ADC assets currently in development prompted a series of experiments to characterize this linker. The initial experiment involved fresh rat, monkey and human plasma and human blood spiked at pre-determined concentrations with the targeted ADC and incubated under various conditions with subsequent quantification of the cys-mc-monomethyl auristatin F (MMAF) using validated LC-MS/MS assays. Storage of the spiked ADC samples at -20°C was also included in the stability test. Following the acquisition of the incubation time-course and whole blood stability data, further experiments were developed and conducted in human plasma, blood and buffer solutions to investigate the role temperature and pH have on the ADC-linker-payload stability and possible matrix cysteine binding to residual linker-payload which lead to the generation of free cys-mcMMAF. The results of these *in vitro* experiments presented allow an in depth look at the importance of conducting a thorough analysis of an ADC to accompany *in vitro* GLP data prior to the start of a first-time in human (FTIH) study. This approach proved to be a viable experiment in assessing the ADC linker behavior and provides a potential application for the evaluation of future ADCs.

Poster # 26

Determination of Drugs in Serum using Heart-cutting Two-dimensional Ultra High Performance Liquid Chromatography

Kenichiro Tanaka, William Hedgepeth_Shimadzu Scientific Instruments, Inc., Columbia, Maryland

HPLC separation and quantitation are often used for measurement of drug concentrations in the blood. When analyzing the concentration of a drug in blood using HPLC, it is necessary to separate analytes from other compounds. However, the separation of analytes and other compounds in blood is not easy because of the complex matrix. Sample preparation is useful for these cases to reduce matrix, but it can take a long time. In this poster, we analyzed propranolol in serum using heartcutting two-dimensional ultra high performance liquid chromatography. This method was developed on our UHPLC system equipped with a high-pressure switching valve. Separation is conducted sequentially by the 1st column and then the 2nd column in this analysis. Valve switching and other operations are set within the software time program, thereby enabling automated, continuous analysis. Five different levels of propranolol standard solution ranging from 0.1 to 10 mg/L were used for the linearity evaluation. The coefficients of determination were more than 0.999. The peak area reproducibility for a concentration of 1 mg/L was 0.98 % (n=6).

Poster # 27

A Rapid and Reproducible Immuno-MS Platform from Sample Collection to Quantitation of IgG

Rachel Lieberman¹, David Colquhoun¹, Jeremy Post¹, Brian Feild¹, Scott Kuzdzal¹, Fred Regnier², ¹Shimadzu Scientific Instruments, Columbia, MD, USA ²Novilytic L.L.C, North Webster, IN, USA

Dried blood spot analysis (DBS) has provided clinical laboratories a simple method to collect, store and transport samples for a wide variety of analyses. However, sample stability, hematocrit effects and inconsistent spotting techniques have limited the ability for wide spread adoption in clinical applications. Dried plasma spots (DPS) offer new opportunities by providing stable samples that avoid variability caused by the hematocrit. This presentation focuses on an ultra-fast-immuno-MS platform that combines next generation plasma separator cards (Novilytic L.L.C., North Webster, IN) with fully automated immuno-affinity enrichment and rapid digestion as an upfront sample preparation strategy for mass spectrometric analysis of immunoglobulins. Combining the sample collection technique of next generation plasma separator Noviplex cards for quick plasma collection from whole blood, with the automated affinity selection and trypsin digestion of the Perfinity workstation coupled to LCMS-8050, provides a very rapid and reproducible Immuno-MS platform for quantitation of IgG peptides. Furthermore, this rapid immuno-MS platform can be applied to many other peptide/protein applications.

Application of Unique Stationary Phases for Effective RPLC Method Development

Thomas J. Waeghe, Carl L. Zimmerman, Geoffrey Faden, MAC-MOD Analytical

There are several important requirements for fast, effective RPLC method development. First, it is important to have columns from a manufacturer whose products have a reputation for quality, reproducibility, and performance. Second, it is prudent to have method objectives clearly defined and documented, and to implement a systematic approach for evaluating and optimizing those important parameters that affect retention, selectivity, and resolution. Finally, it is extremely useful to have a set of complementary stationary phases that have a variety of different retention mechanisms for analyte interactions.

In this poster we will demonstrate the usefulness of a set of new stable, low-bleed stationary phases with unique, complementary selectivities as part of an overall method development scheme. These phases include alkylphenyl, alkylpentafluorophenyl, polar-embedded amide, a broad-pH-range octadecyl, and a unique long-chain alkylcyano phase. An example of such an overall method development strategy using these phases will be presented.