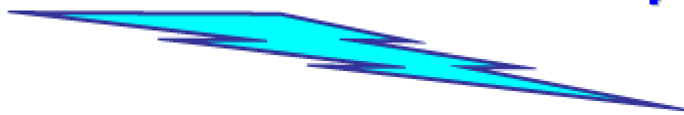
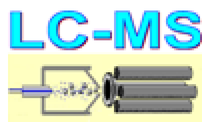
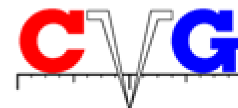






FINAL Program

The 8th Mass Spectrometry CVG Annual Symposium

(Analytical, Bioanalytical, Environmental, Proteomics, Forensic and Instrumental Topics)

Montreal, Canada, September 23th, 2009, 8am to 5pm

(Holiday Inn Point Claire)

PROGRAM AT A GLANCE

08:00am-08:30am: Registration & Breakfast (Morning Posters Set-up)

08:30am-08:40am: Canadian MS Group Updates - Dr. Fabio Garofolo - Algorithme Pharma, Laval, Canada (**Afternoon Session Chair**)

08:40am-09:00am: Opening Remarks: **"Enhanced Mass Accuracy and Resolution; a Paradigm Shift in Mass Spectrometry"** - **Prof. Pierre Thibault** - Université de Montreal, Montreal, Canada **(Morning Session Chair)**

09:00am-09:20am: Presentation No.1: ***"Accurate and Sensitive All-Ions Quantitation Using a New Ultra High Resolution LCMS and its Application to Endogenous Metabolite Profiling"*** – **Dr. Mark Sanders** - Thermo Fisher Scientific, USA (**Keynote** Speaker invited by the 2009 Gold Sponsor: **Thermo Scientific)**

09:20am-09:40am: Presentation No.2: **"Dioxin Analysis by Gas Chromatography-Fourier Transform Mass Spectrometry"** **Dr. Vincent Taguchi** - Ministry of the Environment, Toronto, Canada (**Keynote** Speaker invited by **CVG) -**

09:40am-10:00am: Presentation No.3: – **"Quantitation of Several Antiretroviral Drugs in Human Plasma by LC tandem MS"** **Prof. Bernard F. Gibbs** - McGill University, Montreal, Canada (**Keynote** Speaker invited by **CVG)**

10:00am-10:20am: Presentation No.4: - ***"Improved detection of reactive drug metabolites with bromine-containing glutathione analog using mass defect and isotope pattern matching" –*** **Prof. Lekha Sleno** - UQAM, Montreal, Canada (Speaker invited by Agilent Technologies)

10:20am-11:20am: Networking Coffee Break / **EXHIBITION** / **Morning POSTERS No. 01AM-40AM**

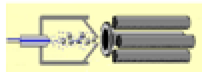
11:20am-11:40am: Presentation No.5: - ***"Rapid analysis of catecholamines and metanephrines in biological fluids by automated online solid-phase extraction LC/MS/MS"*** – **Dr. Sylvie Beaudet** - MDS Analytical Technologies, Concord, Canada (Speaker invited by Spark Holland)

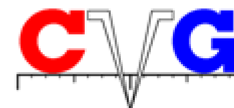
11:40am-12:00pm: Presentation No.6: - ***"Aqueous-Normal Phase/HILIC in LC-MS Separations: Retention Mechanisms and Their Impact on Retention and Selectivity" –*** **Dr. David S. Bell** - Sigma-Aldrich/Supelco, Bellefonte, USA

12:00pm-12:20pm: Presentation No. 07 – ***"High-Field MALDI FTMS for Direct Pre-Clinical Imaging of Drug Distribution and Metabolism"*** – **Dr. Katherine A. Kellersberger** - Bruker Daltonics, Inc., Billerica , USA

12:20pm-01:40pm: Networking Lunch
12:20pm-12:50pm: **EXHIBITION** / **Morning POSTERS No. 01AM-40AM**

Canadian LC-MS Group





12:50pm-01:00pm: Morning Posters removed
01:00pm-01:10pm: Afternoon Poster set up
01:10pm-01:40pm: **EXHIBITION** / **Afternoon POSTERS No. 01PM-40PM**

01:40pm-02:00pm: Presentation No.08: ***"Increasing the Success Rate of Quantitative LC-MS/MS Bioanalysis in a Discovery Environment"*** - **Garnet McRae** - Sussex Research Laboratories, Ottawa, Canada (Speaker invited by mSPEC)

02:00pm-02:20pm: Presentation No.09: ***"From Single Cells to Whole Body Sections: Multiscale Imaging of Phospholipids by MALDI MS"*** **– Prof. Pierre Chaurand -** University of Montreal, Montreal, QC, Canada (Speaker invited by Prof. Pierre Thibault, Chair)

02:20pm-02:40pm: Presentation No.10: ***"Development of a Metabolite Identification Workflow using MALDI-QTof and Multivariate Statistical Analysis"*** - **Dr. Andrew Baker** - Waters Corporation, Milford, USA

02:40pm-03:00pm: Presentation No.11: ***"Systematic and Expanded Investigation of High and Unexpected Positive Deviation for QC Samples during GLP Incurred Samples Analysis by LC-MS/MS" -*** **Georges El-Kadissi** - Algorithme Pharma, Laval, Canada

03:00pm-03:40pm: Networking Coffee Break / **EXHIBITION** / **Afternoon POSTERS No. 01PM-40PM**

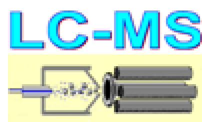
03:40pm-04:00pm: Presentation No.12: ***"A Simple Way to Remove Phospholipids from Bioanalytical Samples." –*** **Dr. Ben Yong** - Varian, Inc., Lake Forest, USA

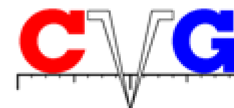
04:00pm-04:20pm: Presentation No.13 –**"Evaluation of a New Electrospray Ion Source and Interface Combination for Ruggedness and Sensitive in LC-MS/MS"** – **Dr. Dragan Vuckovic** - Ionics Mass Spectrometry Group, Bolton, Canada

04:20pm-04:40pm: Presentation No.14 – ***"A Rapid, Easy Sample Cleanup Process utilizing Supported Liquid Extraction Plates Prior to LC-MS/MS Analysis"*** – **Dr. Lee Williams** - Biotage GB Limited, Ystrad Mynach, UK.

04:40pm-05:00pm: Presentation No.15 – ***"Mass Defect Trigger IDA to improve selection of candidate ions for MSMS confirmation of metabolites from in-vivo samples."*** **Dr. Yves Leblanc** - MDS Analytical Technologies, Concord, Canada

Canadian LC-MS Group





Oral Sessions Abstracts

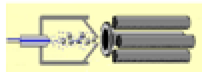
(To see list of posters, please go directly to page 17)

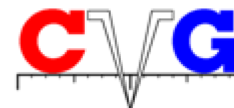
Chair (Morning): Prof. Pierre Thibault (Université de Montreal)
Chair (Afternoon): Dr. Fabio Garofolo (Algorithme Pharma)

Opening Remarks: "Enhanced Mass Accuracy and Resolution; a Paradigm Shift in Mass Spectrometry"
Pierre Thibault
Université de Montreal, Montreal, Canada
Speaker Biography: Prof. Thibault is a renowned bioanalytical chemist specialized in mass spectrometry and proteomics with more than 20 years experience as a principal investigator in academic, government and industry laboratories. Before joining IRIC in 2004, Dr. Thibault was a founding director at Caprion Pharmaceuticals (2001-2004) where he developed an innovative proteomics platform together with bioinformatic tools to identify and quantify proteins differentially expressed in cancer cells as part of immunotherapy programs in partnership with pharmaceutical companies. He was also a Senior Research Officer with the National Research Council of Canada's Institute of Marine Biosciences in Halifax (1990-1996) and Institute of Biological Sciences in Ottawa (1996-2002). He pioneered the use of sensitive high resolution separation methods and microfluidic devices coupled to mass spectrometry and their applications in protein chemistry and cell biology. His scientific achievements in this area have been recognized by numerous awards and distinctions including the National Research Council Outstanding merit award for scientific innovation and the Canada Research Chair in bioanalytical mass spectrometry and proteomics. He regularly serves as member of peer review committees, consultant for instrumentation companies and Scientific Advisor to funding agencies and research institutes.

Keynote Speaker invited by the 2009 Gold Sponsor (Thermo Scientific)

Oral Presentation No.01 - Accurate and Sensitive All-Ions Quantitation Using a New Ultra High Resolution LCMS and its Application to Endogenous Metabolite Profiling
Mark Sanders1; Kevin J. Mchale1; Chunang (christine) Gu2; Petia Shipkova3
1Thermo Fisher Scientific, Somerset , NJ; 2ThermoFisher Scientific, San Jose, CA; 3Bristol Myers Squibb, Princeton, NJ
Novel Aspect: Accurate and sensitive multi-component quantitation achieved by utililizing high resolution accurate mass LC/MS data
Introduction: Endogenous metabolite profiling (metabolomics) is an extreme example of multi-component quantitation. Even though in most cases the quantitative comparisons between samples are relative, the key to a successful study is high quality quantitative results. In some studies the differences between groups can be quite subtle and high analytical reproducibility is required to identify potential biomarkers . Once potential biomarkers have been found, more rigorous, absolute quantitative assessments are usually made to validate the initial observations. Here we demonstrate the use of ultra high resolution LCMS to provide sensitive, high quality quantitative data. This then provides a simple, relatively unbiased and highly quantitative assessment of metabolomics samples and negates the need for multiple MS platforms.
Methods: Protein precipitated plasma was analyzed by full-scan MS acquired from m/z 85-1000 at resolutions up to 100,000 FWHM on the Exactive (Thermo Scientific) orbitrap mass spectrometer. Separation was achieved on a 2.1x150 mm, 1.9 um Hypersil Gold column via a 15 min uHPLC gradient elution method. Data analysis was performed using Sieve software and statistical approaches to identify potential biomarkers. The identities of the components of interest were confirmed using fragmentation data generated by passing ions through a collision cell (HCD cell) on the Exactive instrument. The quantitative performance was validated using stable labeled standards spiked into rat plasma and analyzed with the same chromatography on a TSQ Quantum Access (Thermo Scientific) triple quadrupole mass spectrometer.
Results: High resolution LC/MS platforms are often used for metabolomic screening to allow for a relatively unbiased view of the sample and the ability to deconvolute the many components of a complex biological sample. However, the triple quadrupole mass spectrometer is considered the gold standard for quantitation and so





typically, the validation of potential biomarkers are performed with LC/MS SRM techniques. The problem with this approach is that MS/MS methods need to be developed for each analyte of interest and that as the number of analytes increases the advantages of using a triple quadrupole diminish. The move to smaller SRM dwell times to accommodate more components across narrow uHPLC peaks compromises both sensitivity and precision. In addition, the more analytes the more labor intensive the SRM method development becomes. In contrast, the instrument set up for high resolution quantitation is very simple, with a full-scan over the mass range of interest and no prior knowledge of the analytes needed. Selectivity for the quantitative measurement is provided by high resolution MS and the use of narrow mass windows (3ppm) around the analyte of interest. This provides a high degree of selectivity without losing ion current, which occurs when MS/MS techniques are used. Quantitative performance was comparable to that of a triple quadrupole when multiple analytes were monitored. A pooled control sample was used to ensure sample integrity throughout the study and comparison of this with individual animal data gave a comparison between analytical and biological variability. For a series of amino acids the analytical variability (~5%) was well within the biological variability observed (~15%). Using this approach the metabolomics experiment is greatly simplified and all stages of the workflow from screening, to identity confirmation, to absolute quantitative measurements can be performed on a single ultra high resolution LC/MS platform.

Speaker Biography: Dr. Sanders is the Director of the Demonstration and Applications Laboratories for Life Sciences Mass Spectrometry at Thermo Scientific. The labs in San Jose, CA and Somerset, NJ provide demonstration capability, applications development and support for Thermo Scientifics' ion trap, triple quadrupole and Orbitrap technologies over a wide range of LC/MS applications. Prior to joining Thermo Scientific in Jan 2008, Mark was a Senior Principal Scientist and Leader of the Discovery Analytical Sciences Mass Spec Group at Bristol-Myers Squibb in Princeton, NJ. During his 11 year tenure Mark's research focused on developing new applications of mass spectrometry based technologies to enhance the drug discovery efforts at Bristol-Myers Squibb. Mark received his B.S. in Chemistry (1983) and Ph.D. in Synthetic Organic Chemistry (1987) from the University of Nottingham in England and continued with postdoctoral research at UC Berkley where he studied pesticide metabolism and mode of action. Mark's research interests include Metabolomics and the development of automated systems and software based on MS technology.

Oral Presentation No. 02 - Dioxin Analysis by Gas Chromatography-Fourier Transform Mass Spectrometry

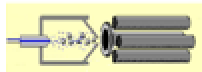
Vincent Y. Taguchi[1]; Ray E. Clement[1]; Stefan Krolik[3]; Robert Nieckarz[1,2]; Robert Williams[4]
[1]Ministry of the Environment, Toronto, ON; [2]University of Waterloo, Waterloo, Canada; [3]Consultant to Varian Inc, Montreal, Canada; [4]Varian Inc, Lake Forest, CA

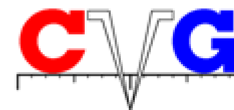
(Keynote Speaker invited by CVG)

Novel Aspect: GC-FTMS analysis of brominated- and chlorinated-dioxins at ultrahigh resolution

Introduction: The monitoring of chlorinated-dibenzodioxins/furans (CDDs/CDFs) is an important environmental priority. Monitoring of brominated-dibenzodioxins/furans (BDDs/BDFs) and mixed brominated-/chlorinated-dibenzodioxins/furans (BCDDs/BCDFs) is also important because brominated compounds are expected to be generally of comparable or greater toxicity than chlorinated compounds.The standard method of analysis of CDDs/CDFs involves the targeted use of high resolution mass spectrometers operating at 10,000 resolution. Using this method, BCDDs/BCDFs cannot be differentiated from CDDs/CDFs because ultrahigh resolution is required. Fourier transform mass spectrometers (FTMS) can provide ultrahigh resolution and ultrahigh mass accuracy, capabilities that can be used to expand the range of analyzable BCDDs/BCDFs, improve selectivity and, more significantly, potentially provide a rapid screening method for detecting total CDDs/CDFs, BDDs/BDFs, BCDDs, BCDFs in a single method.

Methods: Analyses were performed on a Varian 920-MS equipped with a 9.4T superconducting magnet, a triple quadrupole front end, a CP-3800 GC and a CP-8400 autosampler. The instrument was operated in electron ionization mode at 80,000 to 100,000 resolution with a cycle time of 1.5 seconds over the mass range 200-650 Th. The GC conditions were optimised to give a total run time less than 20 minutes. CDD/CDF standards consisting of CS1 to CS5 were used to assess the method in terms of sensitivity and reproducibility. Samples taken from a





fire were used to assess the viability of using this as a screening method and results were compared to those obtained using the standard GC-HRMS method.

Results: The preliminary data obtained from CDD/CDF and BCDD/BCDF standards showed excellent reproducibility and precision compared with the standard GC-HRMS method. Isotope ratios obtained were well within the acceptable range. The sensitivity obtained was approximately 20 times less than that using the standard method. Several factors that could improve the sensitivity were identified and are being addressed. Resolutions in the range of 80,000 to 100,000 were obtained on the capillary GC time scale. The mass accuracy on standards was better than 0.5 ppm using external mass calibration and was better than 2 ppm on samples. Where mass accuracies were out of this range, analyte interferences could be observed. A significant benefit of the method developed was in the domain of screening. Results of total CDDs and CDFs from "real-world" samples showed a good correlation between those obtained using the GC-FTMS and the standard GC-HRMS method. Moreover, due to the acquisition of full mass range, ultrahigh resolution spectra using the FTMS, as opposed to SIM using the GC-HRMS method, it was possible to analyze the data and screen for other possible sample components such as the mixed BCDD/BCDF in the same analysis.

Speaker Biography: Dr. Taguchi received a B.Sc. in chemistry in 1969 and a Ph.D. in synthetic organic chemistry in 1973 from McMaster University. He completed post-doctoral fellowships in the Pathology Department in the McMaster Medical Centre, the Pharmacy Research Division of Merck Frosst Laboratories, the Biomedical Mass Spectrometry Unit at McGill University and the Division of Clinical Pharmacology at Toronto Western Hospital. After several years as a research chemist at Polysar Limited, he moved to a mass spectrometry scientist position at the Ministry of the Environment, then to a supervisor's position and eventually to a manager's position with the Mass Spectrometry Section of the Laboratory Services Branch. This section has a complement of 27 staff and single & triple quadrupole mass spectrometers, time-of-flight mass spectrometers, quadrupole/time-of-flight mass spectrometers, double-focusing magnetic sector mass spectrometers, a sector-quadrupole tandem hybrid mass spectrometer and a Fourier transform (ion cyclotron resonance) mass spectrometer [FT(ICR)MS]. The FT(ICR)MS is equipped with a 9.4 Tesla superconducting magnet, an LC-Qq vacuum cart and a GC-QqQ vacuum cart. Vince is a member of the American Society for Mass Spectrometry, American Chemical Society, Chemical Institute of Canada, Order of Chemists of Quebec, Association of the Chemical Profession of Ontario, Canadian Society for Analytical Sciences and Spectroscopy, Canadian Society for Mass Spectrometry and is the current chair of the Toronto Area Mass Spectrometry Discussion Group. Vince has 35 years experience in analytical chemistry and has 25 publications and 89 presentations.

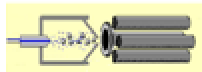
Oral Presentation No. 03 - "Quantitation of several antiretroviral drugs in human plasma by LC tandem MS"

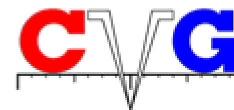
David W. Blank; Brian J. Gilfix; Marcos DiFalco; Line Roy; Bernard F. Gibbs
McGill University, Montreal , Canada
(Keynote Speaker invited by CVG)

Novel aspect: Identification and quantitation of several HIV antiretroviral drugs in body fluids

Introduction: The human immunodeficiency virus (HIV) is responsible for causing acquired immuno-deficiency syndrome (AIDS), a devastating and potentially fatal disease. Infection can be treated as a chronic illness with antiretroviral drugs (ARVs). Therapeutic drug monitoring is the standard procedure to optimize the effectiveness of antiretroviral drugs used to treat infected patients with. Several ARVs are commonly prescribed simultaneously in order to control the virus and their concentration levels in the blood stream is critical Ideally, the analytical method should be able to monitor any combination of commonly used ARV drugs simultaneously in body fluids. The method should possess excellent specificities and precision with short analysis times. An LC tandem MS method was developed and implemented to monitor several ARVs.

Methods: Calibration curves (0.1 - 10.0 µg/mL) were prepared in heparinized plasma. Samples were heated for 30 min. at 56°C to deactivate the virus. One hundred µL of plasma were precipitated with 400 µL of acetonitrile containing 1 µg of cimetidine (IS). A similar procedure was used for the preparation of quality control samples at 0.4, 4.0, 8.0 µg/mL. QC samples were prepared with a separate weighing of reference standards. Tubes were capped, vortexed 30 s and centrifuged at 14,000 g for 10 min. Supernatants were injected onto a C18 column in





Agilent 1100LC connected to an ABI/Sciex QSTAR MS.. Data acquisition time was 2.0 min with a 2.5 min injection cycle time.

Results: Eighteen approved ARVs were monitored in several patients. Results from these analyses were crucial in allowing physicians to adjust dose regimens appropriately. Sample preparation was relatively straightforward and analysis time required was 3 min for simultaneous detection and quantitation of all analytes. Calibration standards, QC samples and study samples were injected in a randomized fashion to ensure that the analytical system was performing properly Calibration curves were analysed by linear regression with a 1/concentration weighting. A correlation coefficient (R2) of > 0.69 was established for quantitation of each analyte. Within run and between run precision (CV) was approximately 4 %. The method was linear for each analyte over an excellent dynamic range (four orders of magnitude. Precision (CV) for the various analytes with this method varied from 2.0 to .6 % for within run data while CVs for between run data varied from 2.1 to 5.8 %. The robustness of the system was tested with several analyses were performed in triplicate. As opposed to a previous method developed in this laboratory, this study utilized a modern instrument with better sensitivity and response time. The number of drugs quantitated was increased from 12 to 18 (50%) and the analysis tine was simultaneously decreased from 5 to 2.5 min.

Speaker Biography: Prof. Gibbs was educated in Montreal and has two Masters' degrees and his doctorate. He consults and collaborates with universities, hospitals, biotechnology companies and government laboratories.

He has published 95 papers, 2 book chapters and US patents. His present appointments include: Manager, McGill University Sheldon Biotechnology Center Mass Spectrometry Unit; CEO and Principal Consultant, Bivan Consultants, Montreal, Canada; Adj. Professor, Faculty of Medicine, McGill University, Canada; Medical Scientist, Biochem Dept. of Medicine, McGill University Health Center (MUHC); Member of the Medical Biochemistry Exec. Committee, MUHC.

Oral Presentation No. 04 - Improved detection of reactive drug metabolites with bromine-containing glutathione analog using mass defect and isotope pattern matching

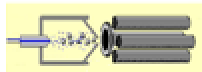
Lekha Sleno; André LeBlanc; Tze Chieh Shiao; René Roy
UQAM, Montreal , Canada

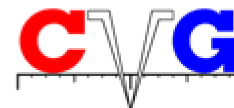
(Speaker invited by Agilent Technologies)

Introduction: Reactive metabolite detection represents a crucial step in assessing potential toxicity of pharmaceutical compounds. The most common method for screening the formation of these unstable, electrophilic species is by trapping them in vitro with glutathione followed by LC-MS analysis. GSH adducts can then be screened by neutral loss and precursor ion scanning, or mass spectral filtering using the mass defect of the supposed adduct. Certain methods have used analogs of GSH with fluorescent tags, quaternary amines or stable-isotope labels in order to increase sensitivity or decrease false positive rates. This presentation will describe the use of a brominated analog of GSH for determining reactive metabolites by LC-MS.

Methods: Rat microsomal incubations were performed to characterize reactive metabolites in vitro using glutathione and the 2-bromo-carbobenzyloxy (CBZ) derivative of GSH. Acetaminophen, clozapine, diclofenac, fipexide and estradiol were incubated under oxidative conditions, with and without trapping agents. Liquid chromatography was performed on a 1200 series Agilent rapid resolution LC system using a Thermo BetaBasic-18 150 x 2.1 mm column filled with 5 µm particles with 0.1% formic acid/acetonitrile gradient. Adducts were screened by LC-MS on an Agilent 6210 time of flight (ESI-TOF). MassHunter Mass Profiler software (version B.02, Agilent Technologies) was employed for comparing incubations and filtering the data based on isotope patterns and mass defects of the formed adducts.

Results: The 2-Br-CBZ derivative of GSH was synthesized by reacting oxidized glutathione (GSSG) with N-(2-bromo-benzyloxycarbonyloxy)-succinimide under alkaline conditions, followed by reduction with DTT, and extraction with ethyl acetate. The purity of the final product was verified by LC-MS and NMR. Following in vitro microsomal incubations with GSH and Br-CBZ-GSH, all adducts were screened using LC-MS with accurate mass measurements on an ESI-TOF instrument, followed by mass spectral filtering and statistical comparison of control vs. samples using Mass Profiler software. This software allowed the specific detection of Br-CBZ-GSH adducts using the isotope pattern of Br (with additional isotope contributions from the parent drug, if necessary) as well as





the removal of all "molecular features" not consistent with the mass defect calculated for the studied adducts. The data were compared for the unlabeled and bromine-containing trapping agents.

Conclusions: We have combined mass defect and isotope filtering for screening reactive metabolites by accurate mass LC-MS with a novel bromine-containing GSH analog. Due to its increased hydrophobicity, this new analog increased the chromatographic retention of GSH adducts resulting in higher sensitivity, since they elute at higher organic content. The preparation of this analog was also very simple and inexpensive; in comparison to previously reported analogs (or stable-isotope labeled compounds) used for this type of work.

Speaker Biography: Dr. Sleno earned her B.Sc. in Biochemistry at Concordia University in Montreal, and then went on to complete her PhD in 2006 in the field of small molecule mass spectrometry at Dalhousie University and the National Research Council in Halifax, NS under the supervision of Dr. Dietrich Volmer. Next, she went abroad for a post-doctoral appointment at the University of Geneva in Geneva, Switzerland with Prof. Gérard Hopfgartner, focusing on the analysis of reactive metabolites by LC-MS. Her second post-doctoral stay was in back in Canada at the Centre for Cellular and Biomolecular Research (CCBR) at the University of Toronto with Prof. Andrew Emili, where she worked on detecting drug target-ligand interactions by mass spectrometry. She is now an Assistant Professor in the Chemistry Department at the Université du Québec à Montréal, where her research focuses on covalent binding of reactive drug metabolites and metabolomics applications of mass spectrometry.

Oral Presentation No. 05: Rapid analysis of catecholamines and metanephrines in biological fluids by automated online solid-phase extraction LC/MS/MS

Sylvie Beaudet1; Martin Sibum2; Luce Boulanger3
1MDS Analytical Technologies, Concord , Canada ; 2Spark Holland Inc.,, Emmen, NETHERLANDS; 3CHUM St-Luc Hospital, Montreal, Canada
(Speaker invited by Spark Holland)

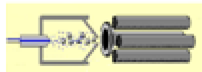
Novel Aspect: This high-throughput SPE/LC/MS/MS method enables automatic extraction, concentration and separation of plasma catecholamines in a very short analysis time.

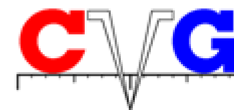
Introduction: Quantitation of plasma free catecholamines has been used for clinical diagnosis of phenochromocytoma, paraganglioma and neuroblastoma, which are tumors that produce excessive amounts of catecholamines. A rapid, sensitive and fully automated high-throughput online solid-phase extraction/Liquid Chromatography/Tandem mass spectrometry (SPE/LC/MS/MS) method is proposed. This online method enables extraction, concentration and separation of plasma catecholamines in a very short analysis time. It also reduces potential for human error associated with labor intensive steps usually required in offline solid-phase extraction while maintaining sample quality and throughput.

Methods: We used an automated online solid-phase extraction system coupled to a hybrid triple quadrupole/linear ion trap mass spectrometer. The following catecholamines were studied: norepinephrine, epinephrine, normetanephrine, metanephrine, dopamine, 3,4-dihydroxyphenylalanine, vanillomandelic acid, 3,4-dihydroxyphenylacetic acid and homovanillic acid. Plasma samples and deuterated analogs were picked up separately by the autosampler and injected onto the SPE cartridge in one injection operation. The eluted sample was then transferred onto an analytical column for chromatographic separation and subsequent MS/MS detection. The acquisition was performed in multiple reaction monitoring (MRM) mode using positive electrospray ionization (ESI) and structural confirmation obtained with MRM triggered Enhanced Product Ion (EPI).

Results: This method is specific and allows rapid testing for disorders associated with increased catecholamine concentrations. The use of SPE led to significant cleaner samples and was highly effective in decreasing interferences present in the matrix that can cause problems with many HPLC-based methods. Several SPE materials were tested and C18 sorbent was selected based on the recovery, reproducibility and peak shape. HILIC chromatography was used because metanephrines are polar analytes that are not well-retained on most reverse-phase columns. By using HILIC chromatography, excellent separation was achieved and the presence of higher organic composition as the analytes elute provides increased response, resulting in better sensitivity.

Integrating Information Dependant Acquisition (IDA) into the automated LC/MS/MS workflow provides increased information versus the MRM-only catecholamine quantitation. The ability of the system to automatically trigger full scan MS/MS and MS3 spectra from the MRM transitions can be used to distinguish the





target compounds from their metabolites. This additional degree of confirmation helps eliminate erroneous metabolite identifications and confirm the presence and structure of the respective products.

The combination of the online solid-phase extraction with the hybrid-triple quadrupole/Linear Ion Trap mass spectral detection provide an automated, sensitive and selective method to identify and quantitate metanephrines in these complex samples in a single run. The automated sample preparation, combined with fast LC/MS/MS runtimes, is compatible with the high-throughput demands in bioanalytical and clinical research labs.

Speaker Biography: Dr. Beaudet did her undergraduate studies at Université du Québec à Montréal. Sylvie performed her doctorate study at Ecole Polytechnique Fédérale de Lausanne in Switzerland where she obtained her Ph.D. in 1993. Since the last 5 years she is working at MDS Analytical Technologies as an application chemist in the Product Application Laboratory. Previously she worked at Dephy Technologies in Montreal as an application lab manager and as a research associate in the Environmental Analytical Chemistry sector at the Biotechnology Research Institute (NRC).

Oral Presentation No. 06 - Aqueous-Normal Phase/HILIC in LC-MS Separations: Retention Mechanisms and Their Impact on Retention and Selectivity

David S. Bell, Carmen T. Santasania and Craig R. Aurand
Sigma-Aldrich/Supelco - 595 N. Harrison Rd. Bellefonte, PA 16823 USA

Introduction: A significant interest in normal-phase chromatography using aqueous-organic mobile phases (aqueous normal-phase, ANP) coupled with mass spectrometry has been observed in recent years.[1,2] In this mode of chromatography, analyte retention increases monotonically with an increase in the organic component of the mobile phase. The high organic content of the mobile phases used in this arena have been shown to enhance electrospray-interfaced ionization and provide retention for polar analytes relative to traditional reversed-phase LC-MS. The underlying mechanisms of retention in ANP/HILIC are not well understood. The aim of this study was to enhance our understanding of the governing retention mechanisms in this interesting mode of chromatography and employ this information for the development of robust ANP/HILIC-LC-MS analyses.

Methods: Through a combination of mass spectrometric selectivity and modern software tools, retention and selectivity data for a wide variety of analytes were obtained on bare silica and polar, bonded phases in ANP/HILIC mode. Mobile phase variables thought to be important in this mode, such as buffer type and concentration have been contrasted and compared on each of the phases.

Results: Evidence of strong contributions from ion-exchange as well as HILIC partitioning mechanisms have been obtained on both bare silica and polar bonded phases. The bonded phases, in contrast, show little HILIC character.

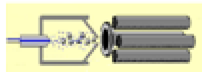
Conclusions: Since the variables that control these contrasting ionic and partition based retention mechanisms are very different, it is of great importance to recognize the driving forces responsible for retention and selectivity. Insights into LC-MS method development strategies as well as parameter considerations often neglected in reversed-phase processes are highlighted.

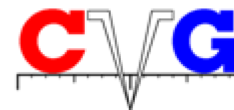
[1] W. Naidong, J. Chromatogr. B 796 (2003) 209.
[2] D.S. Bell, Jones, A. Daniel, J. of Chromatogr. A 1073 (2005) 99.

Speaker Biography: Dr. Bell. With a B.S. degree from SUNY Plattsburgh and a Ph.D. in Analytical Chemistry from The Pennsylvania State University, Dave spent the first decade of his career within the pharmaceutical industry performing analytical method development using various forms of chromatography and electrophoresis. During the past 12 years, working directly in the chromatography industry, Dave has focused his efforts on the design, development and application of stationary phases for use in HPLC and hyphenated techniques. In his current role of Analytical Research and Services Manager at Supelco, Dr. Bell's main focus has been to research, publish and present on the topic of molecular interactions that contribute to retention and selectivity in an array of chromatographic processes.

Oral Presentation No. 07: - High-Field MALDI FTMS for Direct Pre-Clinical Imaging of Drug Distribution and Metabolism

Katherine A. Kellersberger1; Michael L. Easterling1; Santosh Kesari2; Claire M. Sauvageot2; Jeffrey N. Agar3; Nathalie Y.R. Agar4





1Bruker Daltonics, Inc., Billerica , MA; 2Dana Farber Cancer Institute, Harvard Med. School, Boston, MA; 3Brandeis University, Waltham, MA; 4Harvard Medical School, Neurosurgery, Boston, MA

Novel Aspect: Direct assessment of drug distribution, metabolism, and blood-brain barrier crossing in animal xenografts using a MALDI-FTMS-based platform for preclinical imaging.

Introduction: Mass spectrometry imaging (MSI) has proven to be a powerful technique for the direct analysis of chemical species in tissue. The combination of spectral and spatial information derived from MALDI-MS experiments provides a workflow which does not rely on labeling strategies. Instead only mass based information is used for selectivity, facilitating both targeted and non-targeted analysis. As the spectral figures-of-merit associated with the MS detector used for molecular imaging increase, so does the confidence in elemental assignment of small molecules. In this work, we show the advantages of using MALDI-FTMS for drug and metabolite imaging of anti-cancer targeted therapy administered at therapeutic levels in mice with brain tumor xenografts.

Methods: Two swiss male nu/nu mice of 12 weeks of age were administered therapeutic doses of erlotinib by oral gavage. Both animals were sacrificed up to 4 hours later. The brain, kidneys, and liver from both animals were dissected immediately and flash frozen in liquid nitrogen. Frozen mouse organs were sectioned using a Microm HM525 cryostat from Mikron Instruments Inc, and thaw mounted on indium tin oxide (ITO) coated glass slides. Samples were matrix coated with DHB using piezoelectric nebulization of the matrix solution. Mass spectrometry was performed on an ApexUltra 12.0 T FTMS consisting of a hybrid Qh front-end and dual stage ion funnel source equipped for both MALDI and ESI.

Results: Using an in-situ xenobiotic assay by MALDI-FTMS imaging, erlotinib was detected directly from brain, liver, and kidney tissue sections. In the liver section, the drug as well as 6 of the known first- and second-generation metabolites were spatially resolved in the tissue and identified using the high mass accuracy of the detector. Metabolites were also identified in kidney and brain sections, in some cases localized to tumor regions. The high resolving power associated with the FTMS experiment (>150,000) allowed discrimination between isobaric peaks from the tissue matrix and the low-level metabolites, as shown for two of the identified species. Furthermore, the ability to visualize heme provides a means to evaluate the ability of the drug to cross the blood-brain barrier, which can be a considerable limiting factor in the development of effective brain tumor treatment.

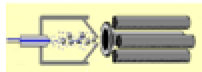
Speaker Biography: Dr. Kellersberger is currently the LCMS Applications Manager for Bruker Daltonics, based in Billerica, MA. Prior to taking her current position, Dr. Kellersberger was a member of the FTMS applications group at Bruker, specializing in high-resolution mass spectrometry. Dr. Kellersberger began her career as an analytical chemist at DataChem Laboratories in Salt Lake City, UT where she spent several years analyzing environmental contaminants using a variety of techniques, including UV-VIS, GC, GC-MS, IC, and LC. She left this position to complete her Ph.D. in Analytical Chemistry at Brigham Young University, specializing in ion-molecule reactions utilizing Fourier-transform mass spectrometry (FTMS). Following completion of her degree, she did a NIH-sponsored research fellowship at the University of Maryland, Baltimore County, studying RNA and RNA:protein complexes using high-resolution mass spectrometry, and joined Bruker shortly thereafter. For the past year, she has focused on developing high-resolution small-molecule imaging using MALDI-FTMS.

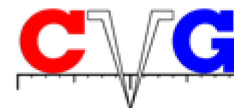
Oral Presentation No. 08 - Increasing the Success Rate of Quantitative LC-MS/MS Bioanalysis in a Discovery Environment

Garnet McRae
Sussex Research Laboratories, 100 Sussex Drive, Ottawa, Ontario, Canada, K1A 0R6
(Speaker invited by mSPEC)

Novel Aspect: An approach to fast, accurate LC-MS/MS analysis in a discovery environment. The use of generic methods and useful tips for more difficult compounds combined with discovery-stage appropriate Standard and QC preparation and acceptance criteria help to increase the success rate of analysis the first time.

Introduction: In a discovery environment, many New Chemical Entities (NCE's) are seen in a given day, week or month with many of them seen only once. It is imperative to provide timely, accurate analysis in a very short amount of time to allow go-no go decisions to be made quickly. A generic approach to discovery-grade LC-MS/MS analysis is required to permit fast, accurate analysis of in-vitro and in-vivo samples with little or no prior method development. Standard and QC preparation as well as acceptance criteria are not as stringent as GLP





requirements at the discovery stage, however we need to ensure that these criteria are set appropriately for the stage where a particular compound is in the discovery process.

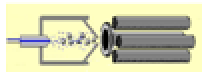
Methods: Compounds were spiked via serial dilution into the appropriate matricies (Caco-2 buffers, microsomal matrix, plasma etc) at the appropriate concentration for the in-vitro or in-vivo test. Extraction was by protein precipitation with MeOH containing internal standard followed by direct injection onto the LC-MS/MS system.

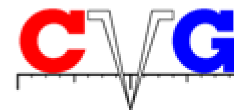
LC-MS/MS Conditions: A Shimadzu Prominence LC System with an Ace 3-C18 30 x 2.1mm, 3µm column and mobile phases consisting of: MP-A: 100:0.1;water:formic acid, MP-B: 100:0.1;ACN:formic acid were used. The gradient was: 2 to 90%B in 1.0 min, 90%B until 1.6 min at a flow of 0.5 mL/min with a total run time of 2.5 min. An MDS/Sciex API-2000 mass spectrometer was used in ESI positive and negative modes.

Results: Typical results using the generic LC-MS/MS method approach to discovery are normally within GLP acceptance criteria for Precision and Accuracy (+/- 15%) as well as specificity and carryover (≤20% of LLOQ). Depending on the NCE, application and calibration curve range, carryover may exceed the 20% of LLOQ limit, however this is permissible in a discovery environment where curves are injected in ascending order and samples injected in time-point order. The generic method should be characterized with respect to where known potentially problematic endogenous compounds elute. The Lysophosphatidyl choline and phosphatidyl choline elution times were evaluated relative to NCE and other small molecule retention times using the generic gradient and protein precipitated rat plasma. Retention times of NCE's can be monitored to determine if they are co-eluting with phospholipids to give a warning of potential method issues. Atenolol, Pindolol, Propranolol, Digoxin and Digitoxin elute prior to the phospholipids, however Domperidone elutes at the start of the phospholipid region indicating possible method issues. MS/MS compound optimization is a necessary step in the analysis of NCE's. For discovery stage methods, optimize on multiple product ions during infusion to allow a choice of transitions for injection of the run. Inject the LLOQ and Blanks with all transitions and then choose the best one to inject the run. Some compounds are problematic due to low ionization efficiency or poor fragmentation. A few tips can help to increase sensitivity issues in short order. Compounds should be infused into mobile phase at normal method flow rate and mass spec temperatures to get a good idea of sensitivities. Ion Spray voltage should be evaluated as low voltages (mixed mechanism ionization) (1500 V) can provide a signal boost in some cases over higher voltage values (4500 V). When injecting the extracted LLOQ samples, evaluate high and low voltages to ensure that the S/N gain is real ie) that the noise does not increase at the same rate as the compound signal. An ISV of 1500V was chosen in this case to provide a S/N increase of up to 2.5-fold. During infusion, be aware of typical adducts ie) Neg mode: formate or acetate, Pos mode: Na, K, NH_4. Some compounds may show low ionization efficiency for the M+H due to high adduct formation and may also show poor fragmentation, resulting in low sensitivity. In these cases, survivor ion scans or adduct to parent transitions may be useful to maintain high sensitivity. Digoxin is an example of a Negative mode formate adduct (m/z 825) fragmented to molecular ion (m/z 779) to overcome poor fragmentation efficiency. The S/N of an LLOQ for Digoxin were 4, 7 and 2 respectively, for formate adduct survivor ion scan (825-825), formate adduct to molecular ion scan (825-779) and molecular ion survivor scan (779-779). The formate adduct to molecular ion scan was chosen in this case as it provided the best S/N. Discovery-grade standard and QC samples are not usually prepared the same as for GLP-grade methods. The number of concentration levels, whether QC's are included or not and the number of replicates used depends on the stage of the NCE. -New NCE: Curve only (6 concentration levels); -NCE Hit: Curve (5-7 concentration levels) + QC's (3 concentration levels); -Lead Compound: Curve (7-8 concentration levels) + QC's (3 concentration levels); -Curve and QC's (if used) should bracket samples in all cases. Discovery grade acceptance criteria are often more lenient than for GLP methods. The acceptance criteria depend on the stage of the NCE. -New NCE: Precision and Accuracy (P+A) +/- 25%, Specificity: ≤50% of LLOQ, carryover: no criteria (use blanks after high standards and inject samples in order); -NCE Hit: P+A, +/- 25%, specificity: ≤20% of LLOQ, carryover: ≤50% of LLOQ; -Lead Compound: P+A, +/- 15%, specificity: ≤20% of LLOQ, carryover: ≤20% of LLOQ.

Stabilities and Matrix Effect are normally not evaluated in early discovery, however should be evaluated as a compound progresses: -New NCE: No stability or matrix effect; -NCE Hit: Room Temp (RT) plasma stability, no matrix effect; -Lead Compound: Stock stability, Plasma stability (RT, F/T, L/T at -80 or -20C) and matrix effect

Summary: To increase the success rate of quantitative LC-MS/MS bioanalysis, the following steps should be performed: -Generic Extraction and LC Parameters;-Use Specificity of MS/MS; -Use tips to increase chances of





immediate success; -Prepare curves and QC's and set acceptance criteria based on stage of NCE; -Evolution of method from Discovery-grade to GLP-grade.

Speaker Biography: Garnet McRae, B.Sc., began his career in the pharmaceutical industry in 1993 with Apotex Fermentation Inc. in Winnipeg, MB. In 1997, he moved to Montreal where he held research positions performing HPLC and LC-MS bioanalysis with Phoenix International/MDS Pharma Services and CTBR Bio-Research. In 2004, he relocated to Ottawa where he has held positions with Ionalytics Corp. as FAIMS Applications Specialist, with PainCeptor Pharma as Research Manager responsible for HPLC and LC-MS labs and currently with Sussex Research as the Director of Bioanalytical Services. He is a frequent contributor to scientific conferences with primary interests in the areas of HPLC and LC-MS. His extensive experience includes bioanalytical method development, validation and sample analysis. He currently specializes in discovery-stage bioanalytical support using HPLC and LC-MS techniques and holds a B.Sc. in Chemistry from the University of Manitoba.

Oral Presentation No. 09 - From Single Cells to Whole Body Sections: Multiscale Imaging of Phospholipids by MALDI MS

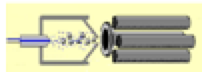
Pierre Chaurand1; Peggi Angel2; Richard M. Caprioli2
1Presently at University of Montreal, Canada 2Vanderbilt University, Nashville , TN
(Speaker invited by Prof. Pierre Thibault, Chair)

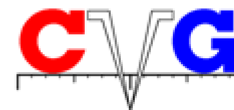
Novel aspect: High resolution imaging MS of phospholipids from tissue sections down to cellular dimensions

Introduction: For the past decade, MALDI MS has been used to image the composition and spatial arrangement of biomolecules in thin tissue sections. One of the major challenges of the technology is to image features down to the dimension of a single cell. This is only possible after both instrumentation (laser focus) and sample preparation (matrix disposition) considerations. We have recently built and characterized a MALDI source capable of focusing laser light on regions <5 µm in diameter. In parallel, advances in matrix deposition via sublimation allow generation of homogenous matrix films on sections. Using this sample preparation strategy, we report MALDI MS imaging of phospholipid (PL) distribution on a variety of tissue sections with spatial resolutions ranging from 5-200 µm.

Methods: Sections were cut at 10-12 µm from a freshly frozen 2-day old mouse pup, adult mouse brain, or adult mouse heart and thaw-mounted on conductive target plates. Serial sections were also cut and H&E stained. Matrix (2,5-DHB) deposition was performed using a sublimation apparatus coupled to a rough vacuum pump (5x10-2 Torr) and heated to ~100ºC. MS data were acquired by MALDI-TOF MS using either a Bruker Ultraflex II mass spectrometer operated in reflectron mode. High spatial resolution (5-25 µm) data was acquired on a custom-built MALDI-TOF instrument operated in linear mode. Ion images were assembled using FlexImaging or Biomap software.

Results: Tissue sections destined to be analyzed by IMS come in a wide range of dimensions with different analytical requirements. Whole body sections (from mouse, rat, or other species) are typically surveyed at low spatial resolution to obtain global distributions of molecular content. Small tissue sections or specific anatomical features may require acquisition of higher resolution data down to the cellular level to obtain biological information distinct to a feature or pathology. For all tissue sections, sublimation of 2,5-DHB produced homogenous coatings yielding excellent PL signals in both positive and negative polarities. These high quality coatings allowed us to investigate molecular composition down to a spatial resolution of 5µm. Sagittal whole body sections were cut from a 2-day old mouse pup measuring 1x3cm. PL distributions were surveyed over the sections with a spatial resolution of 200µm. Numerous organ specific signals were detected. For example, m/z 844.5 was highly abundant in the liver, whereas m/z 810.6 was unique to the adrenalin gland. Coronal adult mouse brain sections were investigated with a range of resolutions. A first section was surveyed at 100µm to assess the PL expression of brain substructures. Numerous signals, such as m/z 826.7, were specific to the corpus callosum, while other signals such as m/z 734.7 were distributed within the cerebral cortex and striatum. Higher resolution data acquired at 25, 10 and 5µm demonstrated PL signals, including m/z 673.7 and 886.3, localized within striatum bundles. PL distribution of pulmonary valve from adult mouse heart, a 10x600µm structure, was imaged at a 10µm resolution. Molecular composition was found to involve numerous ions, such as m/z 723.7 and 848.7. These data demonstrate that matrix deposition via sublimation produces homogenous coatings that allow high resolution MALDI imaging MS down to the cellular level.





Speaker Biography: Prof. Chaurand obtained his Ph.D. in 1994 from the Université de Paris Sud, Orsay, France (Yvon Le Beyec) and Post doc, (1994-1998) at University of Düsseldorf, Germany (Bernhard Spengler). He continued his research (1998-2009) at the Dept of Biochemistry, Vanderbilt University, Nashville TN, USA (Richard Caprioli). Presently, he is Professor Agrégé, at the Dept of Chemistry, Université de Montréal, Québec, Canada. For the past 20 years, Dr. Pierre Chaurand has contributed to the development and characterization of various aspects of matrix-assisted laser desorption ionization (MALDI) mass spectrometry.During his Ph.D. years (Université de Paris Sud, Orsay, France), his contributions ranged from the fundamental understanding of ion production via the MALDI process and subsequent detection by impact on various surfaces including microchannel plate detectors. During his post-doctoral training (University of Düsseldorf, Germany), Dr. Chaurand's efforts were forwarded towards the design and construction of MALDI time-of-flight mass spectrometers optimized for peptide sequencing by post-source decay. Dr. Chaurand has spent the last 11 years of his professional career has research faculty at Vanderbilt University (Nashville TN, USA) contributing to the development of a technology named 'imaging mass spectrometry', which allows the profiling and mapping of biomolecules including proteins, lipids and other metabolites, as well as administered pharmaceuticals, by MALDI mass spectrometry by the direct analysis of thin tissue sections. This technology allows to directly probe the molecular content of the different cell comprising tissues without having to perform any kind of extraction, while preserving the spatial arrangement. The protein profiles and images recovered have been found to be very tissue specific. This technology is being applied to the study of numerous diseases including cancer, to better understand the onset and progression but also to bring an aid to diagnosis and prognosis. Indeed, the recovered molecular profiles and images are a direct reflection of the health status of patients. In a broader context, imaging mass spectrometry can be used to study the organization of biomolecules in any plant of animal tissues to enhance molecular understanding of its development or metabolic processes. Dr. Pierre Chaurand has very recently joined the Dept of Chemistry at the University of Montreal, where his research efforts for the development of imaging mass spectrometry will be continued.

Oral Presentation No. 10 - Development of a Metabolite Identification Workflow using MALDI-QTof and Multivariate Statistical Analysis

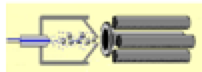
Andrew Baker[1]; Stephen Mcdonald[1]; Henry Y. Shion[2]
[1]Waters Corporation, Beverly, MA; [2]Waters Corporation, Milford , MA

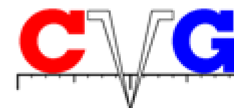
Novel Aspect: Implementation of MALDI-Q-Tof Mass Spectrometry with Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) for in-vitro metabolite identification

Introduction: In pharmaceutical drug discovery it is essential to identify all metabolites of a new chemical entity to assess the possible toxic effects; typically this work is performed with LC-MS/MS analysis. We present a method of determining presence and relative abundance of metabolic biotransformations using solid phase extraction, accurate mass MALDI Q-Tof MS analysis and OPLS-DA multivariate statistical analysis. This approach provides complimentary information and allows users to unambiguously target and identify these metabolites, in a quick and simple fashion.

Methods: Verapamil, trimipramine, and propranolol were incubated at 10 µM with rabbit liver microsomes for thirty minutes and extracted using a 96 well HLB µElution plate. The extracts were mixed with α-cyano-4-hydroxycinnamic matrix and analyzed using a Waters MALDI SYNAPT mass spectrometer. Data was acquired in triplicate for control (t=0) and incubated samples (t=30). Spectra were filtered using a chemically intelligent mass defect filter to remove unrelated matrix ions from the analysis prior to statistical analysis using OPLS-DA. An S-Plot identified ions that differ as a function of their covariance and contribution across the sample groups (Control and Incubation). These results were submitted for elemental composition analysis to confirm their identity. Identified metabolites were analyzed by MALDI MS/MS for further characterization.

Results: The application of chemically intelligent mass defect filters were effective in removing interfering mass spectral peaks from the data set and resulted in reduced spectral complexity. Advanced statistical methods such as orthogonal partial least squares (OPLS-DA) and the visualization techniques provided in MarkerLynx XS allow facile identification of components that lead to class separation between metabolized and control samples. MassFragment was used interpret MS/MS spectra for characterization and localization of the observed biotransformations. The results obtained using the MALDI-TOF approach correlate well with results obtained





using LC/MS based metabolic profiling techniques and reports in the literature, however at a substantial saving of instrument time (1-2 minutes per sample by MALDI analysis as opposed to 10 minute standard LC/MS methods) and laboratory resources.

Speaker Biography: Dr. Baker is currently Principal Mass Spectrometry Applications Specialist at Waters Corporation. He received his Ph.D. in Analytical Chemistry from Indiana University in 1998 under the direction of Milos Novotny. Starting in 1998, Andrew worked in the Micromass demonstration lab on triple quadrupole applications and then moved into the small-molecule metabolite identification and profiling applications space in 2000. Recent research interests involve utilizing high resolution chromatographic and ion mobility techniques combined with time of flight mass spectrometry for characterizing complex samples.

Oral Presentation No. 11 - Systematic and Expanded Investigation of High and Unexpected Positive Deviation for QC Samples during GLP Incurred Samples Analysis by LC-MS/MS

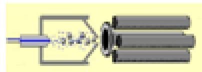
Georges El-Kadissi; Mireille Nohra; Natasha Savoie; Ericka Franco; Véronik Gill; Milton Furtado; Chantal Menard; Mary Carbone; Fabio Garofolo
Algorithme Pharma Inc., Laval (Montreal), Quebec , CANADA

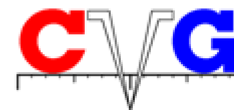
Novel Aspect: Importance of structured investigation by using of multiple reference standards to explain the salient high deviation for quality control samples.

Introduction: Montelukast is used in the treatment of prophylaxis, chronic asthma and seasonal allergic rhinitis. A bioanalytical method, able to quantify Montelukast, was successfully validated in human plasma. During incurred samples analysis, some high positive deviations for quality control samples were noticed. Consequently, an investigation was started. A preliminary investigation was first conducted to eliminate all general possible causes to this phenomenon due to error or oversight during sample preparation. However, as no assignable cause could be identified following this preliminary investigation, an expanded investigation was started. Hypotheses were listed: 1) Matrix effect causing response enhancement, 2) contamination of stock solutions, 3) solubility problems and impurities/alteration in reference standards. Then, a series of confirmatory investigations were conducted.

Methods: Montelukast-d6 was used as internal standard (IS). Montelukast method employed protein precipitation extraction using MeOH/Acetone 50/50%v/v. The extracted was injected on a Agilent Technologies 1100 pumps and autosampler coupled to a AB/Sciex-API3000 MS/MS equipped with heated nebulizer source in positive mode. The gradient mobile phase consisted of 0.1% HCOOH and Acetone on a Zorbax, SB-C18, 5μ, (50X2.1)mm. Montelukast and Montelukast-d6 (IS) eluted at approximately 0.83 and 0.82 minute, respectively. During validation, the method showed an intraday precision range of 2.9% to 4.3% and an accuracy range of 96.1% to 112.5% and an interday precision range of 3.8% to 7.0% and accuracy range of 95.1% to 113.9%.

Results: Verification of the first hypothesis (Matrix effect) yielded no significant enhancement from the matrix. Prior to sample analysis, matrix effect testing was conducted during method validation with different donors and no matrix effect was observed. Results from several tests suggested a problem with a particular stock solution (not due to contamination) used to prepare the quality control samples and/or the reference standard lot-B used to prepare this particular stock solution. Several tests were rigorously designed to investigate solubility problems and impurities/alteration in the reference standard. A new lot-C of reference standard was ordered and compared with reference standard lot-A used during method development and validation and with reference standard lot-B used during sample analysis. Results demonstrated a solubility problem of reference standards lots B and C in MeOH:H2O 50/50 % v/v for a period of time exceeding 2 days versus reference standard lot-A which was perfectly soluble in this solvent. During method development, reference standard lot-A showed acceptable solubility in MeOH:H2O 50/50 % v/v versus other solvents. Reference standards lots B and C were more soluble in pure MeOH. After further investigations on the synthetic procedures used to prepare the different lots, it was discovered that the reference standard lot-A went through an extra step of purification which explains the differences in solubility with lots B and C. As a remedial action, stock solutions prepared in MeOH:H2O 50/50 % v/v were only used between 0 and 2 days. However, as a long term corrective action, the solvent was changed to MeOH and the method went through a partial validation. In conclusion, rigorous tests, meticulous trend analysis and mainly investigating different lots of reference standard were able to reveal a severe and unusual analytical phenomenon that was not definitively apparent during method development and validation.





Speaker Biography: Mr. El-Kadissi is a professional cumulating over 5 years of experience in the clinical trials environment under Canadian, American and European regulatory agencies requirements, and covered most of the aspects of bioanalysis. He has a M.Sc in chemistry from the Lebanese University and he taught chemistry, physics and mathematics in high school during 3 years. Mr. El-Kadissi started at Algorithme Pharma Inc. in 2005 as an analyst and he was promoted to QC auditor in 2005 than to Team Manager. He received a Certificate in Management from Concordia University / Montreal in 2006 and he is currently pursuing an MBA degree at Concordia University, John Molson School of Business / Montreal.

Oral Presentation No. 12 - A Simple Way to Remove Phospholipids from Bioanalytical Samples.

Ben Yong1; David Jones1; Ritu Arora1, Paul Boguszewski2
1Varian, Inc., Lake Forest , CA, 2Varian, Inc., Essex Road, Church Stretton, UK

Novel Aspect: Phospholipid removal from protein precipitation in a 96 well plate.

Introduction: Bioanalytical analysis requires removal of matrix which interferes with LC/MS analysis. Phospholipid removal has received much attention in literature recently. Phospholipids can cause ion suppression, and shorten column lifetime. Removal of Phospholipids is generally achieved by SPE or liquid liquid extraction. A new development of lipid depleted protein precipitation allows the user to remove lipids with a simple general methodology, saving both costs and method development time. This filtration based, depletion approach gives improved cleanliness over protein precipitation, yet avoids additional method development or sample processing time.

Methods: Captiva NDLipids 96 well plates are packed with a non-drip membrane, a protein filtration membrane and a proprietary sorbent in order remove proteins and lipids. Typical precipitation conditions are 3:1 acidified MeOH to plasma although alternate solvents and ratios are also discussed. Samples are filtered under vacuum and analyzed by LC-MS/MS. Lipid removal is monitored by measuring the 184-184 MS transition and sample cleanliness monitored using post-column infusion.

Results: Good recoveries were achieved for a range of pharmaceutical compounds with a range of logP values. The protein and lipid removal are dependant on the exact precipitation conditions used as well as several other key parameters. Using these results we are pleased to present a set of generic conditions for protein precipitation on the Captiva NDLipids 96 well plates under which the majority of proteins and phospholipids can be removed.

Speaker Biography: Dr. Yong did both his batchelors and his PhD at Cardiff University. Dr. Yong's PhD is in the field of synthetic inorganic chemistry and homogeneous catalysis. After a one year postdoc at the University of New Orleans he started in the field of liquid chromatography and spent six years working in sales and applications. He has been in his current position as a chromatography specialist for Varian for a little over a year.

Oral Presentation No. 13 - Evaluation of a New Electrospray Ion Source and Interface Combination for Ruggedness and Sensitive in LC-MS/MS

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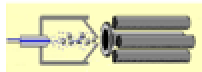
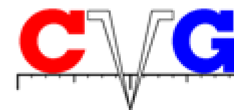
1Ionics Mass Spectrometry Group, Bolton, ON; 2IONICS Mass Spec Group, Inc., Bolton, ON

Novel Aspect: The new method of transporting ions into a MS minimizes optimization procedure, prevents instrument contamination, and increases the sensitivity

Introduction: The triple quadrupole mass spectrometer (MS) coupled with HPLC is a universally used technology in the pharmaceutical industry as a result of its sensitivity, ease of use, speed and robustness. One of the deficiencies of the systems today is long term robustness, often required cleaning as the result of contaminating. Most of the MS's today have one pass of orthogonality to the entrance interface to decrease the contamination entering the MS. Here a heated interface is used with an entrance and exit orthogonal to both, which in combination with a coaxial flow ion source and low velocity flow sampling, significantly decreases the contamination, increasing long term robustness, and improves ease of use to the instrument.

Methods: A mass spectrometer with a new interface is characterized using an electrospray coupled to a tandem MS using a variety of chemical standards. The standards are used to characterize the sensitivity and signal/noise of the system with respect to: solvent flow (0.2 to 2ml/min), solvent composition (100% aqueous to 100% organic), ion source position, compound fragility, thermal sensitivity (degradation), and compound mass (mass discrimination). The parameters optimized are: position, electrospray voltage, temperature of the interface and





electrospray probe, desolvation gas flow, curtain gas flow, nebulizer gas pressure and declustering voltage. A variety of matrices (10mM ammonium acetate and plasma) are used to determine the robustness of the interface via long term stability LC-MRM runs of the standard compounds.

Results: Characterizations of the following compounds are complete: acetaminophen, fluorouracil, minoxidil, testosterone, dexamethasone, salometosone, fluticasone, taurocholic acid, and reserpine with respect to sensitivity. The physical parameters explored are solvent flow and composition, ion source position and gas flows, source and interface temperatures, and the declustering potential. There is a broad optimization of the physical parameters for all the compounds tested allowing one set of values to be used for all. The only parameter which required optimization was the interface temperature (200 to 300C). As a result of the ions being transferred from atmosphere to the mass spectrometer by flow dominated regime which was very soft, the declustering voltage was the same for all compounds. This was unexpected, usually the fragile compounds or compounds with low molecular weights (<200) require a lower potential. The ease of use and roughness has been demonstrated to be a result of the unique orthogonal design of the interface source combination. The sensitivity of the compounds doubled as the solvent flow increase from 0.2 to 2 ml/min of a 1/1 water/acetonitrile. The lowest level of detection for these compounds was found to be in the 10 to 100 femtogram level.

Speaker Biography: Dr. Vuckovic was born in Belgrade, former Yugoslavia on March 8, 1952. He obtained BS in Physical Chemistry at University of Belgrade, Faculty of Physical Chemistry, in 1976. Dragan obtained a Masters Degree in Theoretical Physical Chemistry in 1978 (it was prerequisite for Ph. D.) and Ph. D. in Theoretical Physical Chemistry, University of Belgrade, Faculty of Physical Chemistry in 1982. Post doctoral education: 1982-1983 in Institute for Organic Chemistry, University of Erlangen, Erlangen, Germany, worked with Prof. Paul for Rague Schleyer. 1985-1986 spent as visiting scientist at Cornell University, Department of Chemistry in the group of Prof. Roald Hoffmann. Visiting Professor at Temple University, Department of Chemistry, Philadelphia, 1988. 1978- 1993 worked in the Institute for Nuclear Sciences "Vinca", Department of Chemistry, University of Belgrade, in the field of Theoretical Chemistry. 1989-1993 Head of the Department of Chemistry and 1991-1993, Scientific Director of the Institute of Nuclear Sciences "Vinca". Immigrated to Canada in 1993. 1993-1995 worked at the Department of Chemistry at York University, Toronto. 1995-1997 worked at Hypercube Inc (Theoretical Chemistry software provider), Waterloo, Ontario. 1997-2000 President and owner of ETC Inc, Hamilton, Ontario (designed theoretical chemistry educational software for high schools and entry level university chemistry – EduChem). 2000-present, Vice President of Ionics Mass Spectrometry Group, Inc., Concord, Ontario. Has more than 20 scientific papers, many oral and poster presentations in the field of Theoretical Chemistry and Mass Spectrometry.

Oral Presentation No.14 -A Rapid, Easy Sample Cleanup Process utilizing Supported Liquid Extraction Plates Prior to LC-MS/MS Analysis

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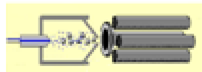
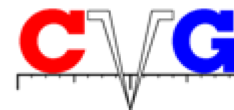
Introduction: It is well known that traditional liquid-liquid extraction (LLE) provides very clean extracts prior to LC/MS analysis. Supported liquid extraction is analogous to traditional LLE, however, analyte partitioning takes place using an inert support material, rather than two immiscible liquids. This provides excellent extraction efficiencies while alleviating many of the tedious liquid handling issues associated with LLE.

This talk shows how more generic methods for the extraction of various acidic, basic or neutral drugs can be utilized, therefore simplifying the whole SLE+ method development process. Extraction screening protocols based on the combination of two loading pHs and four extraction solvents are compared. The loading pH is dictated by the types of analytes being tested: For acidic analytes the plasma loading pHs are approximately 3.2 and 6.1; for neutral analytes the pHs are approximately 6.1 and 8.0; and finally for basic analytes 8.0 and 10.5. Full buffer details are given in the experimental section.

Methods: Reagents: Non-steroidal anti-inflammatory drugs (NSAIDs), β-blockers, corticosteroids and formic acid were purchased from Sigma Chemical Co. (Poole, UK). Blank human plasma was obtained through the Welsh Blood Service (Pontyclun, UK). All solvents were HPLC grade from Fisher Scientific (Loughborough, UK). Sample Preparation: Supported Liquid Extraction Procedure. Plate: ISOLUTE SLE+ Supported Liquid





Extraction Plate 200 mg, part number 820-0200-P01. Sample pre-treatment: Acidic analytes (NSAIDs):- Plasma pre-treatment 1:1 v/v with either 1% formic acid or 0.1% formic acid aq. This sample dilution results in loading pH's of approximately 3.2 and 6.1, respectively; Neutral analytes (Corticosteroids):- Plasma pre-treatment 1:1 v/v with either H2O or 0.1% formic acid aq. This sample dilution results in loading pH's of approximately 6.1 and 8.0, respectively; Basic analytes (β-blockers):- Plasma pre-treatment 1:1 v/v with either H2O or 0.5M ammonium hydroxide. This sample dilution results in loading pH's of approximately 8.0 and 10.5, respectively. Sample Application: The pre-treated plasma was loaded onto the plate, a pulse of vacuum applied to initiate flow and the samples left to absorb for 5 minutes. Analyte Elution: Addition of 1 mL of various water immiscible extraction solvents. The extraction solvents evaluated were DCM, 95:5 (v/v) DCM/IPA, MTBE and EtOAc. Post Extraction: The eluate was evaporated to dryness and the analytes reconstituted in 500 µL of appropriate H2O/MeOH mixtures prior to analysis. HPLC Conditons: Instrument Waters 2795 Liquid Handling System (Waters Assoc., Milford, MA, USA). Column: Zorbax Eclipse XDB C18 3.5 µm analytical column (100 x 2.1 mm id, 3.5 µm) (Agilent Technologies, Berkshire, UK). Guard Column: C8 guard column (Agilent Technologies, Berkshire, UK). Mobile Phase: 0.1% formic acid aq and MeCN (acetonitrile) at a flow rate of 0.25 mL/min using various gradients. Mass Spectrometry: Instrument: Ultima Pt triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Positive and negative ions were acquired in the multiple reaction monitoring mode (MRM). Desolvation Temperature: 350 °C; Ion Source Temperature: 100 °C; Collision Gas Pressure: 2.3 x 10-3 mbar.

Results: Analytes were selected to give a broad range of polarities (LogP) and pKa values within each suite. The appropriate screening procedures listed above were then carried out. Figures 2-7 show the respective recoveries obtained for the three analyte suites using various combinations of pH loading and extraction solvent conditions.

Conclusion: The acidic analytes, NSAID, recoveries for equivalent extraction solvents were generally higher for the lower pH loading conditions, while the Neutral Corticosteroid recoveries were consistently high using all extraction solvent and pH loading combinations. Triamcinolone, however, only gave 50% recoveries when using DCM as the extraction solvent. Six out of Nine β-blockers showed consistently high recoveries for all extraction solvents at both loading pH's. For the 3 most polar analytes (atenolol, sotalol and nadalol) better recoveries were obtained when using combinations of more polar extraction solvents at a higher loading pH. By using the suggested protocol (screening two pH loading conditions combined with four extraction solvents) good recoveries and RSD's were obtained for the majority of analytes used in this study. For very polar analytes, assuming they have adequate solubility in the extraction solvents, more precise pH control is required (analytes must be in their unionized forms).

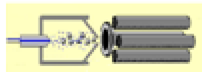
Speaker Biography: Dr. Williams earned his Chemistry degree in 1996 from the University of Wales, Swansea, with a minor in business studies. He then went on to get his Ph.D in 2000 from the University of Wales, Swansea. His Thesis was the Analysis of Pharmaceuticals and Agrochemicals by LC-MS and SFC-MS Using Atmospheric Pressure Ionization Techniques with Prof. Dai Games. He continued his education with a Post Doc from 2001-2004 at the Division of Biochemical Toxicology, National Center for Toxicological Research (NCTR), FDA, Arkansas, USA. His studies focused on various LC/MS work combined with sample cleanup. From there he joined Biotage and is currently a Bio-analytical Project Manager.

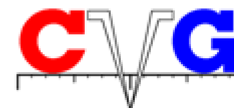
Oral Presentation No. 15 - Mass Defect Trigger IDA to improve selection of candidate ions for MSMS confirmation of metabolites from in-vivo samples.

J.c. Yves Leblanc1; Eva Duchoslav1; Nic Bloomfield2
1MDS Analytical Technologies, Concord, On , Canada ; 2MDS Analytical Tech- Sciex, Concord, ON

Novel Aspect: Real Time Selection of Precursor Ion based on Mass Defect, More Selective Detection and Confirmation of Metabolite

Introduction: Instrument manufacturers offer the capability to automatically collect MS and MSMS using the following principle; 1) perform a survey scan , 2) select precursor ion (s), 3) perform MSMS on the precursor(s) and 4) repeat process for duration of LC. The challenge in such application is to determine on an LC time scale what are the ion of interest, especially when dealing with complex biological mixture. Mass Defect Filtering is a widely accepted post-acquisition data processing method that enables selective detection of metabolites in the presence of high chemical noise or endogenous species signal. IHere we report on the use of Mass Defect





Filtering as a criteria to select precursor ions in real-time with Information Dependant Acquisition (MDf Trigger-IDA)

Methods: Rat and Hhuman liver micorosome incubation of clozapine, buspirone, haloperidol and talinolol were diluted in various matrix to mimic in-vivo metabolism samples (urine, plasma and bile). All standards were obtained from Sigma and used without any additional purification. Separation was performed on a Luna C18 (2) 2.1x100mm, 2u column (Phenomenex) using a Prominance 20-AD XR system (Shimadzu). All analysis were preformed on a research hybrid quadrupole time of flight (QqTOF) system operation equipped with a TurboVTM ionspray source and operated at 30,000 resolution

Results: The Mass Defect of unmodified clozapine and that of the GSH-clozapine conjugate were used with a window of 40mmu as filters for precursor selection in IDA. This approach was compare to more traditional IDA criteria such as dynamic background subtraction (DBS), mass range and intensity threshold. The MDt-IDA method enable detection of all previously reported metabolites of clozapine (total of 11) where as the conventional IDA methodology only detected 6. It was also found that comparison of the post-acquisition Mass-Defect filtering of the survey scan yielded a chromatogram that was identical to the TIC of the precursor ion experiment in the MDf Trigger-IDA, suggesting that all ions detected in post-acquisition processing were effectively selected in real time to perform MSMS with unit resolution selection of the precursor ion. This is a significant improvement over conventional automated MSMS approaches taken in metabolism and data for the other compounds will be shared in the present work.

Speaker Biography: Dr. Le Blanc is a Senior Research Scientist as part of the Applied Research Group at MDS Analytical Technologies. His main area of interest are application of hybrid quadrupole linear ion trap (QqLIT) and quadrupole time-of-flight (QqTOF) technology in qualitative analysis (proteomic and metabolism), he still shows strong interest in quantitative applications using triple quadrupole instrumentation primarily due to his 2 years experience as the manager of the LC-MSMS laboratory at Phoenix International (now part of MDS Pharma Services). As part of the MDS-AT division, Dr Le Blanc has been directly involved in the development and marketing of the API 3000, API 4000 and QTRAP based systems though is former role of lab manager and technical marketing group lead.