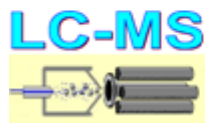


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2011 Montreal Post-ASMS Symposium



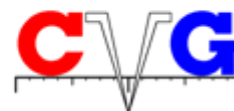
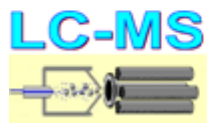
In Collaboration with CANADIAN SOCIETY FOR MASS SPECTROMETRY
SOCIÉTÉ CANADIENNE DE SPECTROMÉTRIE DE MASSE

September 22, 2011, 3pm to 9pm
Holiday Inn Pointe-Claire Montreal Airport
(6700 Trans Canada Hwy, Pointe Claire, QC H9R 1C2 Canada)

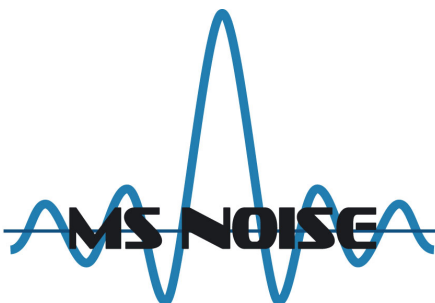
Meeting Chair: Professor Pierre Thibault

- 03:00pm-03:45pm: Registration and Coffee Time – EXHIBITION*
- 03:45pm-04:10pm:** "Utilization of online dilution techniques to improve quantitative and qualitative LC/MSn analyses for drug discovery" - Presented by **Ari Gritsas** (AstraZeneca R&D)
- 04:10pm-04:35pm:** "Kinetic Capillary Electrophoresis Coupled to Mass Spectrometry (KCE-MS) Reveals Stoichiometry and Binding Parameters of Drug-Cyclodextrin Noncovalent Complexes" - Presented by **Professor Maxim Berezovski** (University of Ottawa)
- 04:35pm-05:00pm:** "Characterizing the novel flagellin and pilin glycoproteins expressed by the archaeon, *Methanococcus maripaludis*" - Presented by **Dr. John Kelly** (National Research Council)
- 05:00pm-06:00pm Networking Dinner - EXHIBITION*
- 06:00pm-06:25pm** "Non-assumptive identification of protein modifications using high resolution-high speed quadrupole TOF-MS/MS analysis" - Presented by **Professor Lekha Sleno** (UQAM)
- 06:25pm-06:50pm** "Challenges and Achievements in Clinical and Research Mass Spectrometry Applications: the Sherbrooke Experience" - Presented by **Dr. Christiane Auray-Blais** (Centre Hospitalier Université de Sherbrooke/Faculty of Medicine and Health Sciences, Université de Sherbrooke)
- 06:50pm-07:15pm** "Validation of 25-Hydroxyvitamin D3 an Endogenous Biomarker in Human Serum using LC/APCI/MS/MS" - Presented by **Themis Flarakos** (Cirion Clinical Trial Services)
- 07:15pm-07:45pm Networking Coffee Break - EXHIBITION*
- 07:45pm-08:10pm** "New mass spectrometry approach to identify SUMOylated peptides from large-scale proteomics analyses using a LTQ-Orbitrap Velos" - Presented by **Professor Eric Bonneil** (University of Montreal)
- 08:10pm-08:35pm** "Going Negative in Metal Ion Chemistry" - Presented by **Professor Paul Michael Mayer** (University of Ottawa)
- 08:35pm-09:00pm** "Are the new QTOF Instruments the Next Generation of Mass Analyzers for Performing Difficult Quantifications in a Regulated Environment? Bioanalytical Case Studies & Problem Solving" - Presented by **Dr. Fabio Garofolo** (Algorithme Pharma)

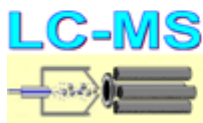
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Oral Sessions Abstracts & Speaker Biographies

Oral 01 - "Utilization of online dilution techniques to improve quantitative and qualitative LC/MSn analyses for drug discovery"

Ari Gritsas (AstraZeneca R&D Montreal, Ville Saint-Laurent, QC, Canada)

Introduction:

Generic assay conditions are the standard in drug discovery analyses. The conditions for most assays involve the precipitation of an in vitro/vivo sample with an organic solvent followed by ESI LC/MS/MS analysis using a reverse phase gradient. Chromatographic issues often arise with the high amounts of organic solvent in the injection solution. This problem is usually overcome using offline sample dilution prior to analysis. This is not always an option given the sensitivity requirements of the assay. Online dilution is an excellent alternative as it increases sensitivity by eliminating the offline dilution step and improves chromatography by enabling polar compound to "trap" at the head of the column prior to gradient elution. Online dilution thus was evaluated using various mixers and samples.

Methods:

A Waters Premier triple quadrupole mass spectrometer and a Waters 2777/1525 LC system were used for the quantitative experiments. An AB Sciex API4000 QTrap triple quadrupole mass spectrometer and a Shimadzu Prominence LC system were used for the qualitative experiments. The effects of online dilution were investigated with sample loops and static LC mixers. Samples from in vitro and in vivo testing using proprietary and non-proprietary compounds were used for the evaluation. Samples were precipitated with various amounts of acetonitrile and analyzed using mini-bore columns and generic LC gradient conditions.

Preliminary Data:

Preliminary results indicated that chromatographic improvement was achieved with both sample loops and static mixers. The static mixers, however, provided better results than the sample loops; especially at larger injection volumes. Quantitative method development for small polar compounds was accelerated and additional dilution sample preparation steps were eliminated thereby increasing efficiency for both in vivo and in vitro analyses. Qualitative metabolite profiling analyses were improved as the assessment of low amounts of very polar metabolites were now possible with the resulting trapping capacity from the online dilution. Low LOQ concentrations, not previously achievable, were now possible for the quantitative analyses. The use of static mixers showed promise for other scenarios where injection of samples with high organic content are necessary.

Speaker Biography:

Aristidis (Ari) Gritsas graduated from McGill University with a B.Sc. in Chemistry in 1994. He went straight to work in the pharmaceutical industry for Phoenix International Life Sciences, a contract research organization. Ari started in the Bioanalytical group operating LC/MS/MS instrumentation and eventually managed a group responsible for 15 systems. Phoenix became MDS Pharma Services and Ari moved on to pre-clinical drug development by joining MDSPS's Drug Metabolism and Bioanalysis group. His new role now focused on R&D to support various in vitro and in vivo studies. In addition to bioanalysis, Ari was responsible for metabolism work, specifically radiolabeled mass balance studies. In his 13 year career at MDS, Ari presented several ASMS posters investigating the use of new methodologies and technologies for the improvement of quantitative and qualitative LC/MS analyses.

Currently, Ari is a scientist at AstraZeneca R&D Montréal where he provides analytical support for discovery research into new therapeutics for analgesia. As a member of the site's DMPK group, he utilizes LC/MS/MS to investigate the pharmacokinetic properties of new chemical entities and their exposures in various pain models. His current research interest involve evaluating the use of mass spectrometry to improve current assays and solve additional issues in CNS and pain research.

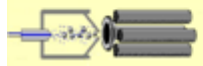
Oral 02 - "Kinetic Capillary Electrophoresis Coupled to Mass Spectrometry (KCE-MS) Reveals Stoichiometry and Binding Parameters of Drug-Cyclodextrin Noncovalent Complexes"

Professor Maxim Berezovski (University of Ottawa, Ottawa, ON, Canada)

Kinetic Capillary Electrophoresis (KCE) is an electrophoretic separation of molecules interacting during the separation. It establishes a new paradigm that separation methods can be used as comprehensive kinetic tools. KCE becomes highly

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attractive for selection and characterization of drug candidates and affinity probes, determination of rate and equilibrium constants. Coupling KCE online with mass spectrometry (MS) brings a lot of advantages in detecting multiple unlabeled analytes simultaneously, finding stoichiometry of complexes and binding sites of the interaction components. In my presentation, I will discuss how KCE reveals equilibrium and rate constants of weak and fast non-covalent interactions. I will show an approach to determine k_{on} , k_{off} and K_d of weak ($K_d > 1 \mu M$) and fast kinetics (relaxation time < 0.1 s) in quasi-equilibrium for small molecule drugs and cyclodextrins.

Speaker Biography:

Education: Ph.D. in Bioanalytical Chemistry, York University, Department of Chemistry, Toronto, Canada, 2005.

M.Sc. in Biochemistry, Novosibirsk State University, Russia, 1994.

Experience: 2009 - present: Assistant Professor, Department of Chemistry, University of Ottawa, Canada; 2005 - 2008: NSERC Postdoctoral Fellow, University of Toronto, Canada;

Scientific Interests: (1) Bioanalytical Chemistry — the analysis of biomolecules and their non-covalent interactions with Kinetic Capillary Electrophoresis and Mass Spectrometry; (2) Biopharmaceuticals & Biosensors — the selection and application of DNA/RNA aptamers in therapeutic and diagnostic purposes; (3) Biomarker Discovery for cancer and immune cells.

Oral 03 - "Characterizing the novel flagellin and pilin glycoproteins expressed by the archaeon, *Methanococcus maripaludis*"

Dr. John Kelly (National Research Council of Canada, Ottawa, ON, Canada)

Introduction:

Prokaryotes, both bacteria and archaea, express a variety of flagella, pili, fimbriae and even more unusual structures on their surface that have various functions including motility and adherence. In bacteria, these surface structures are often O-glycosylated whereas N-glycosylation predominates in archaea. However, information about archaeal flagellar glycosylation is sparse and practically nothing is known about the composition of archaeal pilins. We determined the structure of the novel flagellar N-linked glycan expressed by the methanogen, *Methanococcus maripaludis* using MS and NMR. Furthermore, we used an innovative top-down MS approach to identify the major pilin protein expressed by this archaeon. In addition, we show that pilin protein is modified with a glycan that is slightly different from that decorating the flagellin.

Methods:

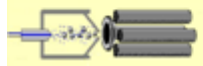
Flagellin from *M. maripaludis* was digested with trypsin and analyzed by nanoLC-MS/MS on a Q-TOF Ultima. Peptide MS/MS spectra were searched against the NCBI nr using Mascot 2.0.1. Glycopeptide MS/MS spectra were interpreted by hand. The flagellar glycan was isolated after pronase digestion by size exclusion and ion exchange chromatographies. NMR experiments (DQCOSY, TOCSY, NOESY, HSQC and HMBC) were performed on a Varian INOVA 600 MHz spectrometer. Pilin was isolated from a non-flagellated mutant, evaporated to dryness and dissolved in hexafluoroisopropanol/formic acid (9:1) and analyzed by ESI-MS and MS/MS on a Q-TOF2 MS. The pilin protein was also in-gel digested with Asp-N and the resulting digests were analyzed by nESI-MS/MS on the Q-TOF Ultima and a LTQ linear ion trap MS.

Preliminary data

NanoLC-MS analysis revealed that most, though not all, the flagellar N-linkage sites were modified with a 1036 Da glycan. The glycopeptide MS/MS spectra demonstrated that the glycan is a tetrasaccharide: a linking HexNAc (203 Da), a di-N-acetyl hexuronic acid (258 Da), a 358 Da sugar and a terminal 217 Da sugar. NMR analysis of the glycan determined the structure to be Sug- β -ManNAc3NAcA6Thr-4- β -Glc-NAc3NAcA-3- β -GalNAc-Asn where Sug is the novel monosaccharide, (5S)-2-acetamido-2,4-dideoxy-5-O-methyl- α -L-erythro-hexos-5-ulo-1,5-pyranose and Thr is threonine [1]. Next, we focused on the pilin. In-silico analysis suggested that three genes, MMP0233, 0236 and 0237, were likely the main pilin components and indeed deletion and complementation in-trans determined that the latter two are essential for pilin formation. But they were determined to be minor structural components. A non-aqueous top-down MS/MS approach identified the major pilin protein as MMP1685, the product of another pilin-like gene. Top-down MS/MS revealed that the pilin-like signal peptide was cleaved and the N-terminus was modified with a pyroglutamic acid. This result was validated by EM of an MMP1685 KO and by deletion/complementation studies. The protein sequence contains few Arg and Lys residues which explains why previous bottom-up analyses using trypsin failed. In-gel Asp-N digestion of the major pilin protein yielded glycopeptides originating from the C-terminus of MMP1685. Though only a small protein, MMP1685 has 4 sites of N-linkage, one of which is on the C-terminal Asp-N peptide. Q-TOF-MS/MS confirmed the peptide sequence and determined that the N-glycan was similar to that found on the flagellin proteins but with an additional hexose. MS/MS on the

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LTQ yielded simpler spectra composed of ions arising from fragmentation of the glycan and revealed that the extra hexose was appended as a side-branch to the linking GalNAc [2].

[1] Kelly et al, Carbohydrate Res. (2009), 344:648-653.

[2] Ng et al, J Bact. (2011), 193:804-814..

Speaker Biography:

John received a B.Sc. in Chemistry and Biochemistry from the National University of Ireland (Galway). He worked for a number of years as a product development chemist in the pharmaceutical industry before pursuing higher academic studies. John holds a M.Sc. in Analytical Chemistry from Memorial university and a Ph.D in Analytical Chemistry from Dalhousie university. He was an NSERC industrial postdoctoral fellow at Merck Frosst in Montreal before joining the NRC-Institute for Biological Sciences (NRC-IBS) in 1997 as a Research Associate. John is now a Senior Research Officer and Group Leader at NRC-IBS where he leads the Proteomics group. His research focuses primarily on mass spectrometry analysis of proteins, and especially protein PTM analysis, as well as the development of novel proteomics and glycoproteomics technologies for application in human disease research.

Oral 04 - "Non-assumptive identification of protein modifications using high resolution-high speed quadrupole TOF-MS/MS analysis"

Professor Lekha Sleno (UQAM, Montreal, QC, Canada)

Studying protein modifications, based on post-translational modifications (PTMs) or chemical reactions, represents an important application area in protein drug development as well as in depth protein characterization where mass spectrometry has a key role in the identification of modified species.

By studying four chemical modifications of the same protein, BSA, we have developed a new approach for grouping differentiating ions for each modification using a searching engine which is capable of searching for a multitude of modifications without loss in performance and using this data to target the extraction of signals from high resolution TOF-MS analysis

Speaker Biography:

2002 BSc Concordia University, Montreal (Biochemistry)

2006 PhD Dalhousie university, Halifax (Small molecule mass spectrometry) under supervision of Dietrich Volmer (NRC-IMB)

1 yr Post-doc University of Geneva, Switzerland (Reactive drug metabolites by mass spectrometry) with Gerard Hopfgartner

1 yr Post-doc University of Toronto (Analysing drug-protein interactions for target identification) with Andrew Emili (Donnelly CCBR)

Assistant Prof UQAM since 2008 (Bioanalytical Mass spectrometry: Covalent binding of reactive drug metabolites and Metabolomics).

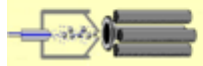
Oral 05 - "Challenges and Achievements in Clinical and Research Mass Spectrometry Applications: the Sherbrooke Experience"

Dr. Christiane Auray-Blais (Centre Hospitalier Universite de Sherbrooke/Faculty of Medicine and Health Sciences, Universite de Sherbrooke, Sherbrooke, QC, Canada)

Lysosomal storage disorders (LSDs) are caused by specific enzyme deficiencies resulting in the accumulation of different substrates in urine, plasma, and various tissues. We developed different tandem mass spectrometry methodologies for two groups of treatable LSDs: mucopolysaccharidoses and Fabry disease. Quantification and validation of these methods focused on specific biomarkers: 1) glycosaminoglycans (GAGs), dermatan sulfate (DS) and heparan sulfate (HS), in urine samples collected on filter paper for treatable mucopolysaccharidosis type I, type II and type III; and 2) glycolyngosphingolipids for Fabry disease. Fabry disease (FD) is an X-linked, multisystemic LSD characterized by the accumulation of globotriaosylceramide (Gb3) in biological fluids, vascular endothelium, heart, and kidney. Another biomarker was evaluated: lyso-Gb3 which is also excreted in biological fluids of Fabry disease patients. Other mass spectrometry applications concern the study of the metabolome (low-molecular weight molecules, typically <1000 Da) which was applied to two groups of patients: Fabry disease patients and women giving birth prematurely. For Fabry patients, a UPLC-QTOF metabolomic research study revealed novel biomarkers in urine and plasma. They were characterized as different analogs of lyso-Gb3. Finally, preliminary results of the other metabolomic study aimed to detect biomarkers in women giving birth prematurely. We developed a novel non-invasive approach which may allow the development of new strategies for the management of women

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at high risk for spontaneous preterm birth to reduce the incidence of preterm birth and perinatal morbidity. In conclusion, the Sherbrooke experience using tandem and time-of-flight mass spectrometry approaches will be presented for high risk screening, monitoring and follow-up of affected patients.

Speaker Biography:

Dr. Auray-Blais is the Director of the Quebec Provincial Mass Urinary Screening Program for hereditary metabolic disorders since its inception. She holds a Ph.D. in radiobiology from the Faculty of Medicine and Health Sciences at the Université de Sherbrooke and postdoctoral studies (fellowship) from Duke University Medical Center. She has a masters degree in Health Law from the Faculty of Law at the Université de Sherbrooke and a bachelors degree in biochemistry. She is the author of more than 145 publications, abstracts and articles. She is an associate professor in the Service of Genetics in the Department of Pediatrics at the Faculty of Medicine and Health Sciences at the Université de Sherbrooke. She is the Scientific Director for the Waters-CHUS Expertise Centre in Clinical Mass Spectrometry. She is the principal investigator and co-investigator in research grants, especially related to lysosomal storage disorders and other inborn errors of metabolism of micromolecules. She has received awards for her involvement in screening inborn errors of metabolism in newborns in Quebec.

Oral 06 - "Validation of 25-Hydroxyvitamin D3 an Endogenous Biomarker in Human Serum using LC/APCI/MS/MS"

Themis Flarakos (Cirion Clinical Trial Services, Laval, QC, Canada)

Introduction:

Vitamin D maintains normal blood levels of calcium and phosphorus and prevents conditions such as rickets or osteoporosis. 25-Hydroxyvitamin D is the major metabolite used to assess vitamin D status in humans. There are two major forms of vitamin D: plant derived ergocalciferol (D2) and cholecalciferol (D3) derived from animal sources or upon exposure to sunlight of 7-dehydrocholesterol. D3 is the more potent form. 25-Hydroxyvitamin D can also serve as a biomarker to diagnose conditions such as Crohn's disease, heart failure and even reduction of colon cancer risk. LC/MS/MS is becoming the technique of choice for various biomarker assays in reference laboratories due to its inherent selectivity. The method presented selectively quantifies 25-hydroxyvitamin D3 in human serum.

Methods:

Several sample preparation schemes as well as mobile phase and ion source combinations (ESI and APCI) were evaluated. Liquid-liquid extraction coupled with an APCI source using an aqueous formic acid and methanol mobile phase were found to provide the best combination sensitivity and selectivity. Under APCI conditions the ion transitions monitored and respective retention times were: m/z 383.3 > 211.2 at 2.1 min for 25-Hydroxyvitamin D3 (25-OHD3), m/z 395.3 > 377.3 (25-OHD2) and 2.2 min for 25-Hydroxyvitamin D2 and m/z 389.3 > 211.2 at 2.1 min for the Internal Standard (25-Hydroxyvitamin D3-d6).

Results:

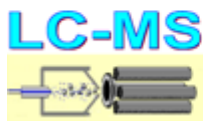
Analytical range is 5-100 ng/mL, linear curve fit (1/x) weighing. Endogenous levels of 25-OH D3 were assessed using ten donors. Matrix effect was minimized by the combination of a liquid-liquid extraction and APCI source. Validation consisted of Inter-run precision and accuracy across 6 batches and 5 levels of QC samples which met acceptance criteria with a CV \leq 15.0% and a Bias within \pm 15.0% except for LLOQ QC samples whose acceptance criteria were met at CV \leq 20.0% and a Bias within \pm 20.0%. Matrix selectivity was assessed by using 6 different donors at low and high QC levels. Data on Stability (Bench-Top and Freeze-Thaw 3 cycles) will also be presented.

Speaker Biography:

Themis Flarakos a native of Montreal, has presented over 40 posters at various venues in the areas of novel technologies to enhance the use of liquid chromatography / mass spectrometry, metabolite characterization and quantitative bioanalysis. He is a member of the American Chemical Society and the American Society for Mass Spectrometry. Themis received his B.Sc. in Biochemistry from Concordia University in Montreal Canada and has almost 20 years experience in bioanalysis. Themis started off his career in HIV research and then proceeded on to work with capillary electrophoresis at McGill University with Dr. Dave Lloyd (presently at BMS, Princeton, NJ) and Dr. Irv Wainer (presently at the NIH, MD).

In 1993, he entered MDS Pharma (formerly Phoenix International Life Sciences), where after 14 years his responsibilities grew and ultimately headed the Drug Metabolism and Bioanalysis group at MDS Pharma providing analytical support for discovery and preclinical development. The group focused on the use of LC/MS/MS in the support of in vitro metabolic profiling, pharmacokinetic assays and in-vivo metabolite profiling.

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In 2009, Themis moved to CIRION BioPharma Inc. to start-up and build the Mass Spectrometry Unit. A key focus of the group is to use LC/MS techniques in a GLP environment to compliment CIRION's existing R&D services in areas of drug development relating to Biosimilar therapeutics and Biomarkers (qualitative and quantitative analysis).

Oral 07 - "New mass spectrometry approach to identify SUMOylated peptides from large-scale proteomics analyses using a LTQ-Orbitrap Velos"

Professor Eric Bonneil (University of Montreal, Montreal, QC, Canada)

Introduction:

Small Ubiquitin-related MOdifier (SUMO) are proteins evolutionarily conserved from yeast to human that are involved in pathways such as intracellular trafficking, genomic integrity and signaling. Three main SUMO paralogs exist in human and their identification is challenging due to the limited number of substrates, the dynamic nature of this modification, and the complicated MS/MS spectra arising from long tryptic peptides with extended SUMO remnant chain. We produced human HEK293 cell lines stably expressing SUMO mutant proteins with a 6xHis tag and a strategically located trypsin cleavage site for convenient MS detection. This work will describe the analytical merits of CID, ETD and HCD activation modes and the development of a pseudo-MSE method to analyze SUMOylated peptides in large-scale proteomics studies.

Method:

cDNA His6-SUMO1-3 mutants were generated by PCR with forward primers containing His6 tag, KpnI and NcoI restriction sites and used to stably express in HEK293T cells the different His6-SUMO isoforms. Cells were lysed and centrifuged to isolate nuclear proteins. SUMOylated proteins were enriched on a Ni-NTA column, digested with trypsin and immunoaffinity purified with custom antibodies specific to SUMO1-3 stubs. 2DLC-MS/MS analyses were performed with either collision-induced dissociation (CID), electron-transfer dissociation (ETD) or higher energy-C trap dissociation (HCD). A pseudo-MSE method alternating between a survey scan and the simultaneous HCD fragmentation of all precursors between m/z 400 and m/z 1000 was developed to identify and sequence low-abundance SUMOylated peptides.

Results:

Our dual affinity method is based on the expression of functional SUMO isoforms that comprise a 6xHis-tag at the N-terminus and an arginine residue near the C-terminus. This arginine residue is strategically located to leave a five amino acid segment (paralog-specific SUMO stub) covalently attached to the modified lysine once SUMOylated protein targets are digested with trypsin. The corresponding modified tryptic peptides are subsequently enriched using antibodies specific to each SUMO stub. A further advantage of this approach is the formation of specific fragment ions for each SUMO paralogs under collisional activation or electron transfer dissociation. We first compared the LC-MS/MS analyses of 6 synthetic SUMOylated peptides spiked at various concentrations in a tryptic digest of cell lysates using each activation method. For the 50-fmol spike, HCD enabled the identification of all SUMOylated peptides with a detection limit of 50 fmoles. HCD fragmentation of SUMO3 tryptic peptides gave several characteristic fragment ions including b₂⁺* (NQ) at m/z 243.109, b₃⁺* (NQT) at m/z 344.157 and an internal fragment (GG) at m/z 132.077 that can be measured with high mass accuracy (> 5 ppm). However, the observation of these fragment ions using CID or ETD activation in the ion trap was compromised due to the low m/z cut-off limitation (1/3 rule). To facilitate the detection of these SUMO-specific ions, we developed a MS method that isolates all precursor ions above m/z 400 in the ion trap prior to their fragmentation in the HCD cell and the detection of the fragment ions in the Orbitrap analyzer. The integration of this MS acquisition mode with conventional high resolution MS spectra enabled the detection of SUMO peptides present at 10 fmoles in complex tryptic digests of cell extracts.

Novel Aspect:

Novel mass spectrometry approach for sensitive detection of SUMOylated peptides in large-scale proteomics experiments

Speaker Biography:

2000- Ph D Université de Montréal under the supervision of Prof. Karen Wadron

2000-2001: Post-doctoral position at Institute of Biological Sciences-NRC Ottawa under the supervision of Dr. Pierre Thibault

2001-2004: Senior Scientist Caprion Proteomics

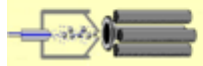
2004 to present: Proteomic Platform Manager IRIC-Université de Montréal

Oral 08 – "Going Negative in Metal Ion Chemistry"

Professor Paul Michael Mayer (University of Ottawa, Ottawa, ON, Canada)

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Metal anions, especially alkali metal anions, are well known in solution where they have been made in 'supra molecule complexes' (except Li-) in organic solvents. They are used in organic synthesis and the elucidation of charge transfer to solvent dynamics. Some transition metals also take on anionic character in solids. In the gas phase, many metal anions have been generated when a beam of positively charged alkali cations created from metal vapour undergoes double electron capture; from discharge and sputter ion sources and from dissociative electron. There have been two reports in the literature of the reactions of bare metal anions, generated by electron ionization of metal carbonyls. In my presentation I will describe the results of the reactions of a variety of bare metal anions with neutral substrates such as acetonitrile and nitromethane with a view to exploring the competition between electron transfer and bond activation.

Speaker Biography:

Paul Mayer is Professor of Chemistry at the University of Ottawa, where he has been since 1998. His research interests lie in the fundamental aspects of gas phase ion reactions and processes occurring in mass spectrometers. He obtained his BSc from the University of Manitoba in 1990, followed by a PhD with John Holmes at the University of Ottawa in 1994. He held postdoctoral positions with Tomas Baer (UNC Chapel Hill) and Leo Radom (ANU, Canberra, Australia), prior to his appointment at the University of Ottawa. He was the 2008 recipient of the CSMS F.P. Lossing Award and is currently the vice president of the CSMS.

Oral 09 – "Advanced Application of a High Resolution Quadrupole Time-of-Flight Mass Spectrometer to Resolve Chemical Noise in Regulated Bioanalysis"

Dr. Fabio Garofolo (Algorithme Pharma, Laval, QC, Canada)

Introduction:

Triple quadrupole mass spectrometers have been considered as the most reliable instruments for quantitative bioanalysis. However, the recent progress made on mass analysers such as linear ion trap (QTRAP5500) and time of flight (TripleTOF5600) may eventually change this perception. Contrary to a standard triple quadrupole both are able to increase the selectivity of an assay by either fragmenting in a second step (MRM3) or using higher resolution (TOF) while maintaining sensitivity. In this novel work, the new data generated for the bioanalysis of tacrolimus using the TripleTOF5600 were compared to the data previously generated by the API 5000 and QTRAP5500 System (Reference: ASMS 2010 poster MP 368).

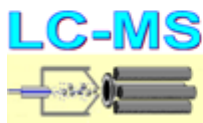
Methods:

Tacrolimus was extracted from human blood by liquid-liquid extraction using Chlorobutane:MTBE 80:20% (v/v) as extraction solvent. Calibration curve covered a range from 50.0 to 50 000.0 pg/ml. Detection on the API 5000, QTRAP® 5500 System and TripleTOF™ 5600 System was achieved in positive ion mode electrospray ionization. Liquid chromatographic conditions consisted of a gradient of 5mM CH₃COONH₄ and methanol performed on an XBridge C18, 50x2.1 mm, 5µm column with a flow rate of 500 µL/min for a total runtime of 5 minutes. Tacrolimus was successively injected onto the three different platforms and monitored at the transition m/z 821.5/768.5 for the API 5000, 821.5/768.5/718.1-718.5 for the QTRAP® 5500 System and 821.5/768.5 (10 mDa window) for the 5600 Triple TOF.

Preliminary Results:

As previously published by our group, a sudden increase in the baseline noise due to a ballistic gradient of MeOH was observed during the method development of tacrolimus on an API3000 and API5000 platform. This issue was completely resolved by using the MRM3 functionality of an QTRAP5500 System while maintaining adequate sensitivity, linearity, precision and accuracy. The same tacrolimus assay was used to evaluate the performance of an TripleTOF5600 System to eliminate the same issue. The results were positive on the TripleTOF5600 System with the MRM-HR (High Resolution) option. Indeed, it was possible to remove most, if not totally, the baseline noise caused by the use of a ballistic gradient. In terms of sensitivity, injection volume of 20 µL was necessary on the TripleTOF5600 System to reach the QCLOQ of 50 pg/ml which is slightly higher but still comparable to the injection of 7µL and 3µL necessary in MRM3 or standard MRM, respectively on the QTRAP5500 System. A coefficient of variance calculated from replicate injection of QCLOQ gave a %CV of 11.6 % and an accuracy of 109.2% also demonstrate that the LOQ concentration was suitable for quantification purpose. In addition, the quantification results generated by the TripleTOF5600 System were within acceptance criteria. Precision of the low, mid and high QC samples were respectively 9.2%, 2.5% and 5.0% whereas the accuracy of those QCs were successively 95%, 100% and 101%. Regression of the calibration curve generated from 50.0 to 50 000.0 pg/ml was 0.99870. In conclusion, the outcome of this research showed that the TripleTOF5600 System is able to provide good linearity, extra level of selectivity using the MRM-HR mode over a regular triple quadrupole with good sensitivity.

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Speaker Biography: Dr. Fabio Garofolo has been working in the pharmaceutical bioanalytical and LC-MS analytical fields for more than 20 years (1989-2010). He has also been heavily involved and committed to working as a volunteer for pharmaceutical and scientific non-profit organizations with the mission to promote the interactions among industrial, academic and regulatory bodies to provide education and forums for discussion in the pharmaceutical practices. Career Steps: 2005-present: Vice-President Bioanalytical Services at Algorithm Pharma; 2003-2005: Bioanalysis & Pharmacokinetic Head at Vicuron Pharmaceuticals; 2000-2003: Technical Manager at Lilly; 1998-2000: Laboratory Director at Biovail; 1994-1998: Laboratory Director at IAF. Accomplishments at a glance: Dr. Garofolo has over 100 publications & presentations in international conferences. He has developed around 250 innovative bioanalytical and analytical methods. He designed and invented 3 innovative bioanalytical approaches. He is the author and instructor of 50 courses and is the recipient of the following awards: Lilly Achievement Award (2001); Lilly Global Award (2002); Lilly Emmerson Award (2003).

Poster Session

Poster 01 – Isotope-labeled differential profiling of amine-containing metabolites in mesenchymal stem cells under hypoxic conditions

Leanne B Ohlund; Veronique Plante; Hesna Belainine; Tze Chieh Shiao; Sebastien Proulx-Bonneau; Borhane Annabi; Rene Roy; Lekha Sleno
UQAM, Montreal, Canada

Poster 02 – Accurate Mass Analysis of Coordination Assemblies

Marie-Christine Tang1; Karine Venne1; Marie-Pierre Santoni1, 2; Garry Hanan1; Alexandra Furtos1
1Universite de Montreal, Montreal, Canada; 2Universite Pierre et Marie Curie, Paris, France

Poster 03 – Development of a high-throughput scheduled MRM method for the detection of 218 drugs in human urine for doping control

Paule Emilie Groleau; Gerard Dussault
INRS-Doping control, Laval, Canada

Poster 04 – Interplay Between Protein Ubiquitylation and SUMOylation in Human Cells Unveiled Using a Novel Proteomics Approach

Chantal Durette1; Louiza Mahrouche1; Frederic Galisson2; Mathieu Courcelles1; Eric Bonneil1; Mounira K. Chelbi-Alix3; Pierre Thibault1, 4
1IRIC / Universite de Montreal, Montreal, Canada; 2CNRS UMR 5086, Lyon, France; 3CNRS FRE 3235, Universte Paris Descartes, Paris, France; 4Department of Biochemistry, Universite de Montreal, Montreal, Canada

Poster 05 – Quantitative phosphoproteomics and RNAi experiments using FAIMS-LC-MS/MS unveil unexpected CK2 targets in insulin signaling pathway

Gaëlle Bridon; Eric Bonneil; Olivier Caron-Lizotte; Pierre Thibault
Univ. of Montreal, Montreal, Canada

Poster 06 – Quantitative subcellular proteomics analyses of TNF- α activated mouse macrophages highlight the selective degradation of mitochondria by mitophagy

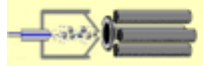
Christina Bell1; Luc English1; Matthias Trost 2; Magali Chemali1; Michel Desjardins1; Pierre Thibault1
1University of Montreal, Montreal, Canada; 2MRC Protein Phosphorylation Unit, Univ. of Dundee, Dundee, United Kingdom

Poster 07 – Energies of small polycyclic aromatic hydrocarbons; interstellar molecules

Brandi West; Paul Michael Mayer
University of Ottawa, Ottawa, Canada

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Poster 08 – Studying peptide fragmentation differences based on cysteine modifications on a quadrupole-linear ion trap instrument

Souade Ben Haddou; Andre Leblanc; Tze Chieh Shiao; Ren Roy; Lekha Sleno
UQAM, Montreal, Canada

Poster 09 – Untargeted metabolomics approach for studying *C. elegans* embryonic elongation using a combination of aqueous normal phase and reverse phase LC-ESI-TOF-MS

Fong Lam Chu; Sarah Jenna; Julien Schelpe; Lekha Sleno
UQAM, Montreal, Canada

Poster 10 – Optimization of purification protocol for LC-MS/MS analysis of click chemistry-modified peptides in complex protein samples

Andre LeBlanc; Tze Chieh Shiao; Rene Roy; Lekha Sleno
UQAM, Montreal, Canada

Poster 11 – An investigation of the energetics and conformations of polymer/substrate complexes studied with RRKM modeling, molecular dynamics and ion-mobility spectrometry

Justin Renaud; Paul Michael Mayer
University of Ottawa, Ottawa, Canada

Poster 12 – Application of mass spectrometry as a confirmatory tool for *Shigella* pathogen identification

Philippe Raymond; Karine Seyer; Sylvianne Paul
Canadian Food Inspection Agency, St-Hyacinthe, Canada

Poster 13 – Novel Data Acquisition and Processing strategies for Targeted Metabolome Profiling Using Differential Isotope Labeling and High Resolution Accurate Mass Spectrometry

Yasmin Boukhedimi²; Sarah Jenna²; Eva Duchoslav¹; Suma Ramagiri¹; Lekha Sleno²;
1AB SCIEX, Concord, Canada; 2UQAM, Montreal, QC

Poster 14 – Substantial Improvement in Dried Blood Spot (DBS) Technique by Using the "Pre-Cut DBS" Approach

Nikolay Youhnovski; Catherine Dicaire; Milton Furtado ; Fabio Garofolo;
Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Poster 15 – Impact of the Injection Volume on Matrix Effect in LC-MS/MS Bioanalytical Methods

Jean-Nicholas Mess; Mathieu Lahaie; Marie-Chantale Tremblay; Sylvain Latour; Milton Furtado ; Fabio Garofolo ;
Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Poster 16 – Advanced Application of a High Resolution Quadrupole Time-of-Flight Mass Spectrometer to Resolve Chemical Noise in Regulated Bioanalysis

Jean-Nicholas Mess¹; Louis-Philippe Morin¹; Suma Ramagiri²; Mauro Aiello²; Johnny Cardenas²; Milton Furtado¹;
1Algorithme Pharma Inc., Laval (Montreal), Quebec , Canada; 2AB SCIEX, Concord, ON, Canada

Poster 17 – DBS On-Card Derivatization: "An Easy & Alternative Form for Sample Handling to Overcome the Biological Matrix Instability of Thiorphan"

Marie-Pierre Taillon; Cynthia Cote; Jean-Nicholas Mess; Milton Furtado ; Fabio Garofolo;
Algorithme Pharma Inc., Laval (Montreal), QC, Canada