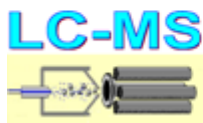


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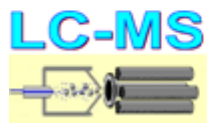
2011 Vancouver Post-ASMS Symposium

September 29, 2011, 3pm to 9pm
Holiday Inn Vancouver Centre
(711 West Broadway, Vancouver, BC V5Z 3Y2 Canada)

Meeting Chair: Professor Christoph Borchers

- 03:00pm-03:45pm: *Registration and Coffee Time – EXHIBITION*
- 03:45pm-04:10pm: "Proteinase K Non-Specific Digestion for the Comprehensive Identification of Interpeptide Crosslinks: Application to Prion Proteins" - Presented by **Jason Serpa** (Uvic - Genome BC Protein Centre)
- 04:10pm-04:35pm: "UHPLC-ESI-Q-TOF analysis of proanthocyanidins in grape seeds and red wine" - Presented by **Adeline Delcambre** (University of British Columbia Okanagan)
- 04:35pm-05:00pm: "Struggling with space charge in linear ion traps" - Presented by **Professor Donald Douglas** (University of British Columbia)
- 05:00pm-06:00pm *Networking Dinner - EXHIBITION*
- 06:00pm-06:25pm "Condensed Phase Membrane Introduction Mass Spectrometry (CP-MIMS) for Direct, Trace, On-Line Monitoring of Biomolecules and Environmental Contaminants in Complex Samples" - Presented by **Professor Christopher Gill** (Vancouver Island University)
- 06:25pm-06:50pm "Development of a Mass Spectrometry-Based Assay for Measurement of Angiotensin I and Plasma Renin Activity to Diagnose Secondary Hypertension" - Presented by **Professor Christoph Borchers** (Uvic - Genome BC Protein Centre)
- 06:50pm-07:15pm "An MRM study of EZH2 and its somatic mutations in follicular and diffuse large B cell lymphoma" - Presented by **Dr. Annie Moradian** (Genome Sciences Centre, BC Cancer Agency)
- 07:15pm-07:45pm *Networking Coffee Break - EXHIBITION*
- 07:45pm-08:10pm "Structural Proteomics Characterization of Prion Protein Aggregation" - Presented by **Professor Evgeniy Petrotchenko** (Uvic - Proteomics Centre)
- 08:10pm-08:35pm "Gas-Phase Ions of Human Hemoglobin A, F and S: Cross Sections, H/D Exchange and MS/MS" - Presented by **Yang Kang** (University of British Columbia)
- 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)

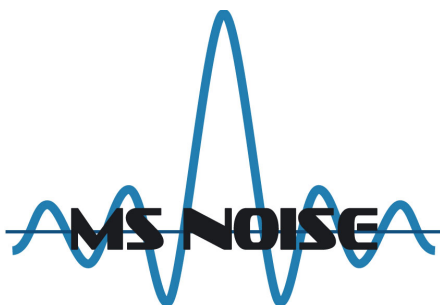
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2011 Vancouver Symposium Exhibitors



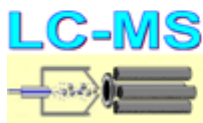
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Oral Sessions Abstracts & Speaker Biographies

Oral 01 - "Proteinase K Non-Specific Digestion for the Comprehensive Identification of Interpeptide Crosslinks: Application to Prion Proteins"

Jason Serpa (Uvic - Genome BC Protein Centre, Victoria, Canada)

Introduction:

The basis of crosslinking analysis is the chemical reaction of a bifunctional crosslinking reagent with functional groups on a protein, ideally leading to the formation of covalent bonds between the reagent and two amino acids. The distance between two crosslinked sites can be determined from the length of the spacer in the crosslinking reagent, and provides spatial information and distance constraints for the two connected amino acid residues.

In many proteins, however, there are only a few potential modification sites within the distance range of the crosslinker. Moreover, trypsin targets the same residues as commonly-used amino-directed crosslinkers, and cleavage will not occur at crosslinker-modified residues.

We therefore examined a non-specific protease, proteinase K, as an alternative to trypsin for crosslinking studies.

Methods:

A model peptide (Ac-TRTESTDIKRASSREADYLINKER) was obtained from Creative Molecules Inc. This peptide was crosslinked with our CBDPS crosslinker (CyanurBiotinDiPropionylSuccinimide), which is isotopically coded, biotinylated, and CID cleavable, and the modified peptide was then digested with a non-specific enzyme, proteinase K. The resulting peptide mixture was purified on avidin beads, eluted, and analyzed by reversed-phase HPLC using a Shimadzu spotter. CHCA matrix solution was applied to each spot, and MS and MS/MS spectra were analyzed on an AB 4800 MALDI TOF/TOF.

The native and β -oligomer prion protein isoforms (PrPC and PrP β , respectively) were obtained from PrionNet's PrP5 facility, and each protein isoform was crosslinked and digested in the same manner. The data was analyzed using the ICC-CLASS software suite (http://www.creativemolecules.com/CM_Software.htm).

Preliminary Results:

Results on our model peptide revealed that proteinase K, a non-specific enzyme, produced sets of related CBDPS crosslinks, all of which contain the same crosslinked pair, thus providing verification of the crosslinking results.

The procedure was next applied to native (PrPC) and β -oligomer (PrP β) prion proteins. Using proteinase K and CBDPS, we were able to differentiate the crosslinks from the very complex peptide mixture which is produced by this non-specific enzyme. This strategy, which requires all of the above-mentioned features of the CBDPS crosslinker, produced high sequence coverage and a mass distribution of the crosslinked peptides which is very suitable for mass spectrometry. We were able to detect over 60 total crosslinks, which included several sets of "confirmatory" crosslinks corresponding to linkages between the same two amino acids. Three of the 9 crosslinked pairs found in PrPC are exclusive to PrPC; 6 pairs are found in both PrPC and PrP β ; 6 of the 12 pairs found in PrP β are exclusive to PrP β . In comparison, previous crosslinking studies using trypsin produced no crosslinks in the 500-4000 Da mass range from prion proteins.

Included among the crosslinked pairs unique to PrP β is a K185-K220 crosslink from the C-terminal portion of the protein. In the native structure, the distance between K185 and K220 is over 26Å, and these residues are situated on opposite surfaces of the protein, separated by the aa127-aa165 loop. A conformational change involving unfolding of the aa127-aa165 loop, however, would expose residues K185 and K220, which would then become available for crosslinking with CBDPS, thus confirming this conformational change. We are currently in the process of determining whether this is an inter- or intra-molecular crosslink. In either case, this conformer-specific crosslink may be useful for future development of a method for detection of infectious prions in tissue samples.

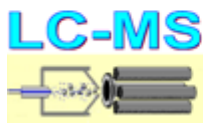
Novel Aspect

The use of a non-specific enzyme, proteinase K, for comprehensive determination of interpeptide crosslinks by MALDI MS and MS/MS.

Speaker Biography:

Jason J. Serpa received his B.Sc in 2005 from the University of Victoria, Canada. Currently a graduate student under Dr. Christoph Borchers. He has been an employee of the Uvic – Genome Proteomics Centre since 2008. His main focus of research is on combined approach of protein chemistry and mass spectrometry for structural proteomics.

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Oral 02 - "UHPLC-ESI-Q-TOF analysis of proanthocyanidins in grape seeds and red wine"

Adeline Delcambre (University of British Columbia Okanagan, Kelowna, Canada)

Introduction:

The proanthocyanidins or condensed tannins are polymers of flavan-3-ol units, e.g (-)-epicatechin or (+)-catechin. These compounds play a major role in the quality of wine contributing to sensory characteristics, such as color stability, astringency and bitterness. Moreover, proanthocyanidins have been shown to have anticarcinogenic and antioxidant effects. Numerous studies in HPLC-MS have been performed to analyze proanthocyanidins in wine and grape seeds. However, there has been a lack of data concerning the higher oligomers and polymers which are known to be very important in establishing characteristic mouthfeel in a particular red wine variety or origin. Thus, there is considerable scientific interest in developing improved methods for their analysis. An important characteristic of these compounds is their influence on taste due to their affinity towards salivary proteins. This property increases with molecular weight but unfortunately the ability to detect such large polymers decreases with size during MS analysis and co-elution of these compounds can make data interpretation difficult. In this abstract we discuss the optimization of MS fragmentation voltage and high mass isotopic pattern resolution in order to discriminate single and multi-charged proanthocyanidins oligomers in red wine and grape seeds by UHPLC-ESI-Q-TOF-MS.

Methods and Materials:

Merlot and Pinot Noir grapes were sampled from the 2010 harvest (Okanagan Valley, BC). Grape seeds were manually separated and extracted in acetone/water (70/30) to give a tannin crude extract (TCE). Red wine was simply filtered through a 0.45 μm membrane (Nylon, VWR) prior to analysis by UHPLC/ESI-MS. The samples (grape seed and red wine) were analyzed using UHPLC-ESI-Q-TOF-MS (Agilent 6530) in negative ion mode. The stationary phase was a Zorbax SB C18 column (2, 1 x 50mm, 1, 8 μm) from Agilent. UHPLC separations were accomplished using a binary gradient composed of water with 0.1% of formic acid (solvent A), and acetonitrile with 0.1% of formic acid (solvent B). The flow rate was maintained at 0.4 mL/min and the gradient was 0%-100% B over 10 minutes. UV detection was carried out at 280 nm wavelength. MS full-scan acquisition was set at m/z 50-3200 Da. Different parameters such as temperature of column, fragmentor voltages (100 to 300 V), collision energy (slope or fixed) and negative ionization mode were investigated.

Results:

The negative ion mode, which had been demonstrated by others researchers to be more sensitive and selective than the positive mode for proanthocyanidins, was used in these studies. First, to enhance detection of proanthocyanidins with high polymerization degrees, fragmentor voltage was optimized: The optimization of fragmentor voltage helped transfer larger multimers into the mass spectrometer for better detection; dimers of (-)-epicatechin was better observed at 100 V whereas the trimer was at 200V. Higher fragmentation voltage did not increase significantly the response. The compromise to distinguishing the proanthocyanidins (monomers and oligomers) was to fix the fragmentor voltage at 150V. In addition, as the degree of polymerization increased the abundance detected decreased.

The analysis of red wine or grape seeds was first achieved in MS-mode to identify a maximum of proanthocyanidin polymers over a short time. This identification was then further confirmed by performing MS/MS in targeted mode by varying collision energy. The targeted MS/MS mode was performed either with set collision energy (CE) mode or by using a CE slope mode. The slope mode helped us to determine the collision energy necessary for fragmenting the proanthocyanidins molecules. Knowing the optimum collision energy allowed us to use it in fixed mode in order to obtain of more informations on species of interest. A trend was seen between the size of the proanthocyanidin and the required collision energy: In the case of a monomer, collision energy of 16.6 V is needed whereas for a larger molecule such as a tetramer, energy 51.1 V is required. The sensitivity and high resolution of the mass spectrometer also allowed the detection of the multiply-charged series of overlapping oligomeric proanthocyanidins in the sample. Deconvolution of the patterns showed the presence of singly charged trimer, tetramer, pentamer and respectively doubly charged hexamer, octamer and decamer in red wine and grape seeds. An example for grape seeds of triply-charged octamer is illustrated on Figure 1 below

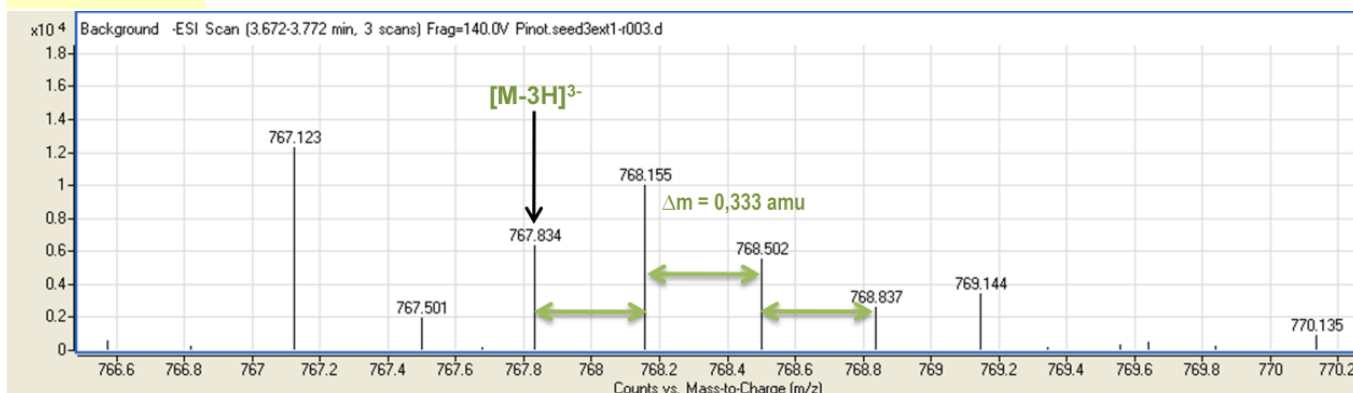
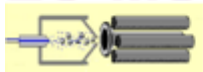


Figure 1: Negative ion mode, fragmentor voltage 140V, resolution of triply-charged ions (m/z theoretical = 767,8336 Da) of proanthocyanidins signals (DP 8), by ESI-Q-TOF

In some cases there can be also co-elution of compounds, but the high resolution of the QTOF can be used to clearly distinguish several proanthocyanidins with different degrees of polymerization. For a singly charged trimer, for example, we can easily distinguish the doubled charged hexamer from the triply charged nonamer (Figure 2).

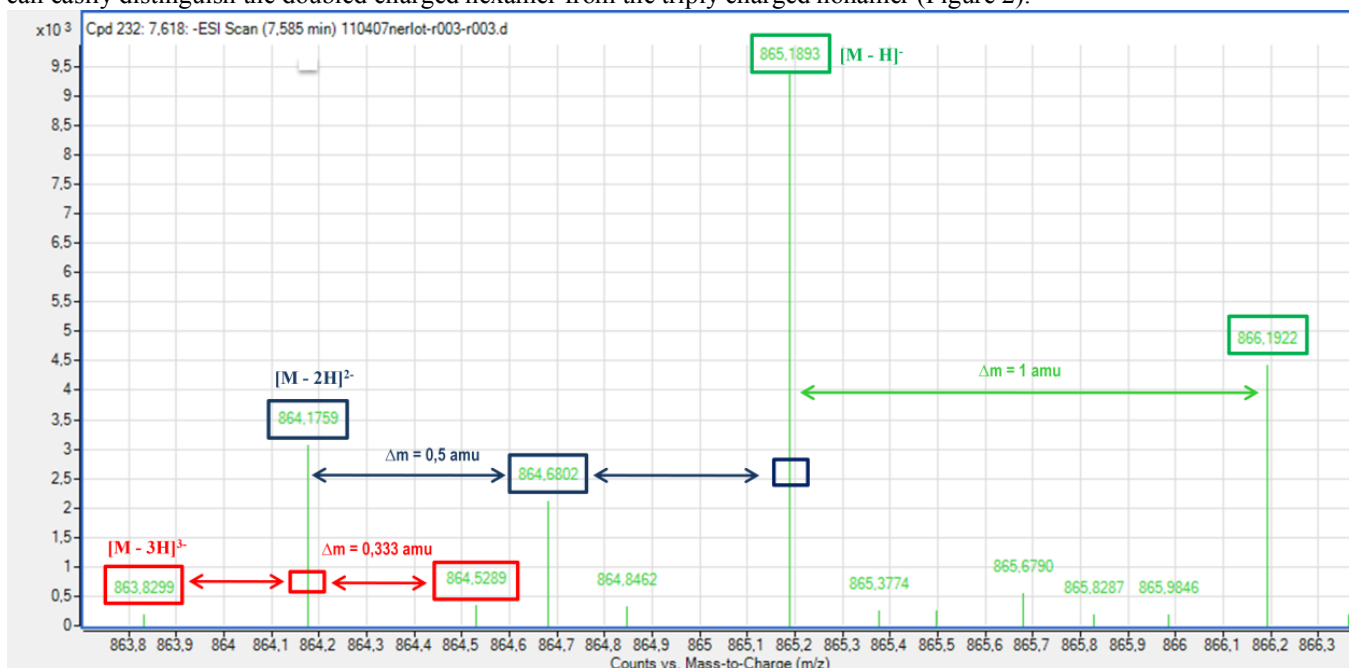


Figure 2: Negative ion mode, fragmentor voltage 150 V, resolution of triply, doubly and singly charged proanthocyanidins by ESI-Q-TOF

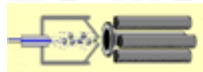
The MS/MS targeted mode was then applied to differentiate these three species. This method allowed us to identify the characteristic fragments of proanthocyanidins in red wine and grape seeds. The combination of the two MS modes (MS and Targeted MS/MS) in high resolution has enabled us to distinguish signals such as those coming from singly charged trimer and doubly charged hexamer, and to establish a fragmentation pathway specific for each proanthocyanidins.

Conclusion:

Some MS parameters (fragmentor, temperature, collision energy) were optimized to allow better detection of proanthocyanidin with high molecular mass. The multiply-charged species (doubly and triply charged) can create complex patterns that overlap with singly charged species if co-elution occurs during LC separation. However, the high resolution of the TOF in MS-only mode and MS/MS targeted mode allowed us to obtain a specific signature for each proanthocyanidins.

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This qualitative method will be optimized in the future to quantify the proanthocyanidins with high polymerization degree in red wine and grape seeds based on molecular ion and fragments. Deconvolution of the charged series should allow quantification of each oligomer of proanthocyanidin by using appropriate synthetic standard. The ratio of each oligomer could potentially be correlated to unique taste or mouthfeel characteristics of a wine or allow discriminating wine varieties or origins.

Speaker Biography:

Adeline Delcambre will be the instructor this winter (Session 2011/2012) for Enology I (BIOC 307 001). Her background is in mass spectrometry applying to enology.

Oral 03 - "Struggling with space charge in linear ion traps"

Professor Donald Douglas (University of British Columbia, Vancouver, Canada)

Linear quadrupole ion traps can store greater numbers of ions than 3D traps before space charge limits their performance. Nevertheless linear traps still show space charge effects that degrade the performance. Coulomb repulsion between the ions lowers the oscillation frequencies of the ions so that a greater radio frequency (rf) trapping voltage is required to bring ions into resonance with dipole excitation for ejection. Thus peaks in a mass spectrum shift to higher apparent masses. Space charge also produces nonlinear forces on ions and reduces mass resolution. In this first systematic study, we have investigated the effects of the operating conditions of a linear quadrupole trap with mass selective axial ejection on mass shifts caused by space charge.

Reserpine ions (m/z 609.3) formed by electrospray are stored in a linear quadrupole ion trap ($r_0=4.17$ mm, 1.0 MHz, length 20 cm) at 2×10^{-5} Torr. Ions, injected at $q=0.20$, are cooled for 100 ms. The trapping rf is then increased to place the ions at a higher q value just below the ejection q . Dipole excitation is applied and the trapping rf is scanned to bring ions into resonance for axial ejection. The effects of ejection q , excitation amplitude, scan speed, and quadrupole dc applied to the rods, on mass shifts caused by the storage of up to 800,000 reserpine ions were investigated.

With a scan speed of 50 Th/s, ejection at $q=0.85$, and no quadrupole dc, the space charge induced mass shift increases linearly with the number of stored ions to ca. 0.7 Th with 650,000 ions. Ejection at higher q values reduces the mass shifts from 1.1 Th at $q=0.60$ to 0.7 Th at $q=0.85$ (scan speed 50 Th/s), or from 0.65 Th to 0.35 Th (scan speed 1000 Th/s). Higher excitation voltages reduce mass shifts. Higher scan speeds decrease mass shifts, partly because higher scan speeds require greater excitation amplitudes.

The electric field from the space charge at the edge of a cloud of 700,000 reserpine ions (cloud radius = 0.417 mm, length 20 cm) is 24 V/m. This radial electric field might be partially countered by applying positive quadrupole dc in the direction of ion excitation. Thus the effects of applying dc were investigated. Ions were injected and cooled at $q=0.20$. The trapping rf was then increased to place ions at $q=0.84$, then scanned to eject ions near $q=0.85$. Applying 9 V of quadrupole dc reduces the mass shift caused by 600,000 ions from ca. 0.65 Th to 0.30 Th (scan speed of 50 Th/s) or from 0.35 Th to 0.20 Th (1000 Th/s). Surprisingly, switching the polarity of the applied quadrupole dc, so that the dc field adds to the space charge field, also reduces mass shifts by the same amounts. To understand this we are currently investigating the effects of the dc on the ion cloud shape and temperature. In summary, mass shifts caused by space charge can be reduced significantly by a proper choice of operating conditions.

Speaker Biography:

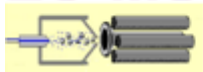
B.Sc., McMaster University 1971; Ph.D., University of Toronto (J. C. Polanyi, 1976); Post Doctoral Fellow, University of California, Berkeley (C.B. Moore, 1976-1978); Senior and Principal Research Scientist, SCIEX, 1979-1994; Barringer Award of the Spectroscopy Society of Canada, 1988; Fisher Scientific Award of the CIC, 1997, Noranda Award of the CSC 2001, FCIC; Fred Lossing Award of the Canadian Society for Mass Spectrometry, 2001; UBC Killam Research Award, 2003; elected Fellow of the Royal Society of Canada, 2007; NSERC-SCIEX Industrial Research Chair.

Oral 04 - "Condensed Phase Membrane Introduction Mass Spectrometry (CP-MIMS) for Direct, Trace, On-Line Monitoring of Biomolecules and Environmental Contaminants in Complex Samples"

Professor Christopher Gill (Vancouver Island University, Nanaimo, BC, Canada)

Introduction:

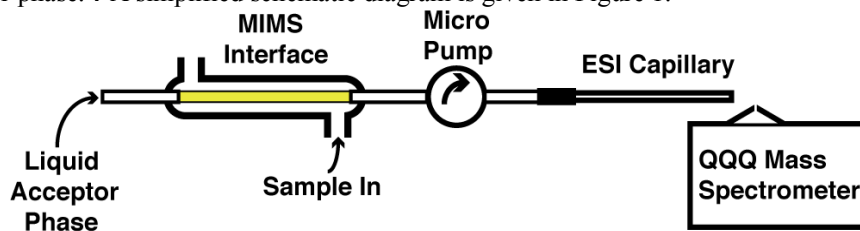
Membrane introduction mass spectrometry (MIMS) uses a membrane to transfer analytes from a sample to a mass spectrometer. For most MIMS systems, hydrophobic analytes are transferred to a vacuum or gaseous acceptor phase (e.g.



Helium), and are subsequently measured via EI-MS (e.g., modified GC/MS systems). Volatile/semi-volatile analytes are readily measured, but for low volatility, polar and/or charged analytes the gaseous transfer step limits the technique. We present an improved version of a capillary hollow fiber membrane based MIMS system using a condensed acceptor phase (e.g. methanol) to facilitate the transfer of analytes from the membrane to an ESI-MS/MS system. We report parametric studies, potential applications for bio-analytical/environmental measurements, and a comparison with direct infusion ESI-MS for complex samples with high salt content (e.g. artificial urine).

Methods:

For the presented work, 10cm long capillary hollow fiber membranes (HFM) composed of polydimethylsiloxane (PDMS) or Nafion® were coaxially mounted in 0.25" diameter stainless steel flow cells constructed with Swagelok™ fittings. A methanol acceptor phase was flowed through the lumen of the HFM at 500 μ L/min, and subsequently entrained to an ESI-MS/MS system (Micromass Quattro Ultima). The acceptor phase was transferred using a prototype in-line micro-pump system (VICI Valco Instruments). Aqueous samples at 25°C were flowed at 300 mL/min (re-circulated or single pass) over the outside of the HFM countercurrent to the acceptor phase. The system was equipped with two multiport valves to facilitate stopping the acceptor phase for greater analyte enrichments ('stopped-flow mode') and also to allow the direct infusion of standards and samples in the acceptor phase. . A simplified schematic diagram is given in Figure 1.

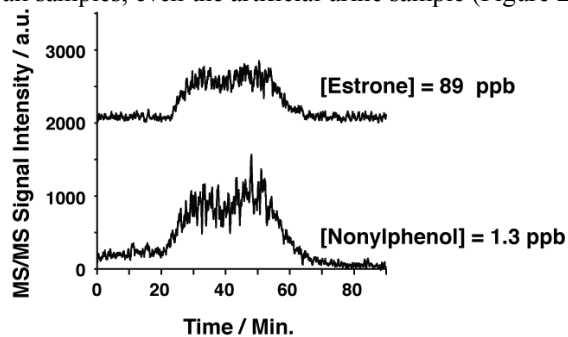


Results:

The HFM interface design presented was optimized using a PDMS membrane. Both sample and acceptor flow rates were examined, and the optimal flows for each were used in subsequent work. The in-line micro-pumping system improves upon earlier generations of CP-MIMS type experiments in the literature, as the higher acceptor phase linear velocities possible with the system allow effective on-line application, rather than flow injection analysis of sample plugs. We studied both peristaltic and prototype multi-head micro-pumps, and found the micro-pump produced better analytical signal stability and allowed a potentially much wider range of solvents. When the interface was operated in acceptor phase 'stopped flow mode', analyte enrichments (in the acceptor phase) were observed, yielding greater analytical sensitivity.

Nafion® HFMs were also examined as an alternative to PDMS for CP-MIMS. Nafion®, a commercially available copolymer of perfluoro-3,6-dioxo-4-methyl-7-octene-sulfonic acid and tetrafluoroethylene (Teflon®), has both high conductivity for ions (as a cation exchange membrane) as well as polar/hydroxylated species, and displays a propensity for water and alcohol transport. We observe complimentary analytical performance for hydroxylated analytes via Nafion® versus PDMS HFM (e.g. ppb level estrone has slightly better sensitivity using PDMS, whereas ethynylestradiol has 10X better sensitivity at the ppb level using Nafion®).

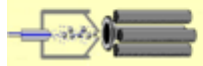
We studied the performance of CP-MIMS for direct measurements in deionized water, natural water and artificial urine samples for ppb level spikes of pharmaceutical (estrone) and environmental contaminant (nonylphenol) analytes. Satisfactory analytical performance was observed for all samples, even the artificial urine sample (Figure 2).



As a comparison, parallel CP-MIMS and direct infusion ESI-MS experiments were conducted for spiked artificial urine (a high salt content matrix). Signal suppression for direct infusion ESI-MS prevented successful ppb level analyte measurements

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that were readily obtained by CP-MIMS analysis. Furthermore, we demonstrate the use of CP-MIMS for the direct, on-line monitoring of a dynamic chemical system by following the chlorination of aqueous phenol at ppb levels, monitoring the formation and subsequent losses of polychlorinated phenol species.

Speaker Biography:

Dr. Chris Gill received his undergraduate degree in chemistry (BScH, 1989) from Acadia University in Wolfville, Nova Scotia. His graduate work was in analytical chemistry with Dr. Michael Blades at the University of British Columbia (UBC), developing new instrumentation for the direct analysis of solids by Laser Ablation Ion Trap Mass Spectrometry (PhD, 1994). Chris then spent two years as a post-doctoral researcher with the Advanced Chemical Diagnostics and Instrumentation group (CST-1) at Los Alamos National Laboratories (1994-1996) with Dr. Philip Hemberger and Dr. Nicholas Nogar (Laboratory Fellow). This work involved the development of new instrumental methods for direct, trace chemical detection in a variety of sample types, predominantly using hyphenated mass spectrometric methods (e.g. resonant laser ablation, metal ion CI, membrane introduction mass spectrometry, MALDI). This was followed by a year as a research associate with the Environmental Sciences Group (Dr. Ken Reimer, Royal Military College, Kingston Ontario and Dr. William Cullen, UBC). The work conducted included an assessment of the fate of PCBs in the Canadian Demolition waste stream (from old paints and building materials) as well as substantial contributions to environmental field assessments & analyses for Canadian & US Military exercises conducted at the Canadian Forces Maritime Experimental Test Range (CFMETR), Nanoose, BC. Currently, Chris is University-College Professor in the Chemistry Department at Malaspina (Nanaimo, BC) as well as co-director of the Applied Environmental Research Laboratories (AERL). The AERL (>\$1.5M) was established and is funded via peer reviewed funding (CFI, BCKDF, SCBC, NSERC) to conduct pure & applied research, including the use of membrane introduction mass spectrometry (MIMS) for environmental chemical analyses and the role of dissolved organic carbon in the photochemistry of natural waters. Because of MIMS low detection capabilities and direct, on-line measurement capabilities, current MIMS application research has been focused upon trace, real time analyses of dynamic chemical systems. The AERL has been involved in a number of inter-disciplinary projects involving industry, government agencies and academia including ultra-trace analysis of volatile compounds in complex matrices, bio- and photo-degradation of contaminated waters and water quality of natural and perturbed systems. Chris' current research interests involve the development of emerging analytical instrumentation and its applications for direct, real-time, ultra-trace environmental and bio-analytical analyses.

Oral 05 - "Development of a Mass Spectrometry-Based Assay for Measurement of Angiotensin I and Plasma Renin Activity to Diagnose Secondary Hypertension"

Professor Christoph Borchers (Uvic - Genome BC Protein Centre, Victoria, Canada)

Background:

Plasma renin activity (PRA), the quantitative measurement of the creation of Angiotensin I (Ang-I) in blood by renin, is an essential analytical tool for screening and diagnosis of secondary forms of hypertension. Typically, PRA is measured by competitive radioimmunoassay (RIA), but there are significant drawbacks to this technique which include the requirement for use of radioisotopes, non-specificity, and long -analytical times.

Methods:

We have developed and performed a method for PRA determination by immuno-MALDI (iMALDI) and compared this method with an established antibody-capture RIA in a population of 42 healthy adults.

Results:

The two methods showed a high correlation ($R^2=0.94$), and the correlation was linear. However, the numerical results were higher using the new iMALDI method due to differences in the buffer pH during Ang-I generation. No interferences from Ang-I related peptides (e.g., Ang-II or angiotensinogen) were observed.

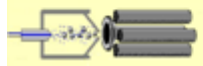
Conclusions:

The results of the PRA determination by iMALDI assay shows good comparison with RIA results, and offers the advantage of a shorter run-time, as well as a simplified analytical technique, free of radioisotopes. The short time-to-result and simple experimental set-up, combined with its improved specificity make this iMALDI-based a very promising as a clinical diagnostics tool.

Speaker Biography:

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Dr. Borchers received his B.S., M.S. and Ph.D. from the University of Konstanz, Germany. After his post-doctoral training and employment as a staff scientist at NIEHS/NIH/RTP, NC and he was the director of the Duke – UNC Proteomics Facility and held a faculty position at UNC Medical School in Chapel Hill, NC (2001-2006). Since then Dr. Borchers is employed at University of Victoria (UVic), Canada and with the current position of Professor in the Department of Biochemistry and Microbiology. He is also the Director of the UVic – Genome Proteomics Centre, which is one out of six Genome Canada funded Science & Technology Innovation Centre and the only one solely devoted to proteomics. His research is centred around the improvement, development and application of proteomics technologies with major focus on techniques for quantitative targeted proteomics for clinical diagnostics. For this research, multiplexed LC-MRM-MS approaches and the immuno-MALDI (iMALDI) technique are of particular interest. Another focus of Dr. Borchers' research is centred on technology development and application of the combined approach of protein chemistry and mass spectrometry for structural proteomics. Dr. Borchers has published more than 100 peer-reviewed papers in scientific journals and is the founder and CSO of two companies, Creative Molecules, Inc. and MRM Proteomics Inc. He is also involved in promoting proteomic research and education through his function as HUPO International Council Member, co-leader of the British Columbia Proteomics Network and President of the Canadian National Proteomics Network.

Oral 06 - "An MRM study of EZH2 and its somatic mutations in follicular and diffuse large B cell lymphoma"

Dr. Annie Moradian (Genome Sciences Centre, BC Cancer Agency, Vancouver, Canada)

Introduction:

The histone methyltransferase EZH2 tri-methylates lysine 27 of Histone H3 (H3K27me3). H3K27me3 is associated with gene repression and helps define gene expression programs during eukaryotic development. The aberrant expression and function of EZH2 in humans is associated with several cancers. Massively parallel short read sequencing of follicular lymphoma (FL) and diffuse-large B-cell lymphoma (DLBCL) showed the presence of frequent somatic heterozygous Y641 mutations in EZH2, occurring in 22% of cases of germinal-centre derived DLBCL and 7% of FL1. These mutations changed residue Y641 (wild type) in the active site of EZH2 to F, D, S, or H residues. We have developed a quantitative MRM assay to measure EZH2 wild type and mutant protein expression in cell lines.

Methods:

LC/MS/MS spectra of recombinant EZH2 were used to select three peptides; one common to both mutant and wildtype EZH2 (Common), and two peptides spanning the site of mutation, one each for wildtype (WT) and mutant Y641F (MutF) EZH2. MRM transitions were designed based on this information. The declustering potential for each parent ion and the collision energy for each transition were optimized using synthetic peptides. To quantify mutant and wildtype EZH2 in the WSU-DLCL2 (EZH2Y641F/WT) and DOHH2 (EZH2WT/WT) cell lines, EZH2 complexes were immunoprecipitated, separated by SDS-PAGE, and digested in-gel with trypsin. The relative amount of WT and MutF were determined using a standard curve. The analysis was performed on a 4000 QTrap using a nano-ESI source..

Preliminary Data:

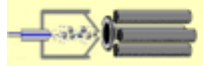
An external standard curve was constructed by processing mixtures of recombinant EZH2 WT and MutF at known ratios, and relating these ratios to the peak area ratio of the WT and MutF peptides. The curve was generated three times. An exponential curve was fit to the average of the three replicates spanning 0.0% to 100% EZH2 WT ($R^2 > 0.97$).

To determine the amount of mutant EZH2 in a DLBCL cell line, endogenous EZH2 complexes were immunoprecipitated from nuclear lysates derived from WSU-DLCL2 cells (EZH2Y641F/WT) and DOHH2 cells (EZH2WT/WT). Two independent experiments were performed. Gel electrophoresis fractions of the immunoprecipitates encompassing the size of EZH2 were analyzed for the peptides by MRM assay. Signals for the EZH2 WT, MutF and Common peptides were observed in the WSU-DLCL2 cell line, while only WT and Common peptide signals were observed in the DOHH2 cell line. This demonstrates that both EZH2WT and EZH2Y641F proteins were present in the WSU-DLCL2 cell line. The EZH2 WT/MutF peak area ratios were compared to the WT/MutF peak area ratios of the external standard curve. The percent EZH2WT was calculated from an equation fit to the standard curve. The comparison indicated that there was a 40:60 EZH2WT:EZH2Y641F protein ratio in the WSU-DLCL2 sample. Thus, approximately equal quantities of mutant EZH2 protein are present, showing that the mutations do not adversely affect the stability of the protein in vivo.

EZH2 is a member of the polycomb repressive complex 2 (PRC2) that trimethylates Histone 3 at residue K27. We have developed MRM transitions for relative quantitation of PRC2 complex proteins such as EED, SUZ12 and RBBP4 in WSU-

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DLCL2 and the DOHH2 cells lines to investigate the effect of mutation on protein complex stoichiometry and eventually its activity.

Novel Aspects:

We have developed an assay for the measurement of EZH2 proteins with single residue somatic mutations in lymphoma cell lines.

Speaker Biography:

Annie had the privilege to study and complete her graduate studies in Dr. Don Douglas's lab at Chemistry department in University of British Columbia. Throughout my studies towards her MSc. and then PhD degree in his lab she has gained invaluable experience in quadrupole mass spectrometry both in application and instrumentation areas.

Her PhD dissertation work involved instrument development for linear quadrupole ion traps with added octopole fields. She investigated new mass analysis methods for these quadrupoles and showed that axial ejection mass analysis of trapped ions in these quadrupoles is achievable with high resolution and high scan speed, similar to a conventional quadrupole rod set. Changing career directions She has joined Dr. Gregg Morin's proteomics group in BC cancer Agency's Genome Sciences Centre in Jan. 2008. At GSC's proteomics group they investigate the functional mechanisms of proteins and signaling pathways implicated in cancer progression. The group uses proteomics to map protein-protein interactions and to identify post-translational modifications for candidate cancer proteins. Recently part of their focus has changed to developing MRM based quantitative assays to measure the exact changes of expression in proteins. This could be the wild type, mutant and also different isoforms of proteins that prove to be expressed in different forms in cancer as well as other disease tissues. Today's talk is an example of this kind of study.

Oral 07 - "Structural Proteomics Characterization of Prion Protein Aggregation"

Professor Evgeniy Petrotchenko (UVic - Proteomics Centre, Victoria, Canada)

Introduction:

The central element in the development of the prion diseases is the conversion of the cellular prion protein (PrPC) into an aggregated pathological fibril-forming isoform (PrPSc), which leads to the accumulation of amyloid fibrils in the central nervous system and eventually to death. The exact molecular mechanisms which lead to the conformational change, as well as the final structure of the aggregates, are still unknown.

We are characterizing PrPC conformational changes and the 3D structure of the prion aggregates using a structural proteomics approach: a combination of protein chemistry and mass spectrometry (MS). We have applied a combination of limited proteolysis, surface modification, chemical crosslinking, and hydrogen/deuterium exchange with MS methods for the characterization of native prions and prion aggregates.

Method:

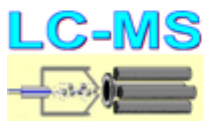
Native and β -oligomeric prion protein, PrPC and PrP β , respectively, were obtained from PrionNet's PrP5 facility (University of Alberta). For chemical surface modification, proteins were modified with our isotopically-coded amine-reactive reagent pyridinecarboxylic acid N-hydroxysulfosuccinimide ester (PCASS), then digested with trypsin, chymotrypsin, or pepsin and analyzed by MALDI MS. For limited proteolysis, native and β -oligomer forms of prions were digested with trypsin or pepsin. Digestion sites were determined by MALDI-MS and MSMS analysis of in-gel digests of SDS-PAGE separated proteolysis products. HDX using FTMS-ECD top-down analysis was performed on Bruker 12 Tesla FTICR mass spectrometer. Crosslinking analysis was done by LC-MALDI-MS and MS/MS of the crosslinked protein digests on an AB 4800 MALDI TOF/TOF.

Preliminary Results:

We detected several residues modified with our water-soluble PCASS reagent that were differentially-modified between the native and β -oligomeric forms. In addition to Y149 and Y150, we found differential modification of Y157 and Y162 in the second YYR motif in the β -oligomeric form.

We observed a different pattern of limited proteolysis between the native and β -oligomeric forms of the prion protein: with trypsin, limited proteolysis of the native soluble form of the protein rapidly removed the flexible N-terminus, while for the β -oligomeric sample, this cleavage was markedly slower. With pepsin, virtually no proteolysis was observed for the native form. However, for the β -oligomeric form, rapid accumulation of a C-terminal ~6 kDa product was detected.

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We also have characterized these PrP preparations using top-down FTMS with ECD. We have been able to achieve 100% sequence coverage, which ensures that we can determine the degree of exchange for every amino acid residue. Preliminary analysis of the native monomeric and β -oligomeric samples showed differences in the exchange rates of certain residues which are localized in the C-terminal portion of the protein.

Using BS3-H12/D12, we were able to detect several crosslinks that were different for the native and β -oligomeric forms of the PrP 90-232. These crosslinks were between the flexible N-terminal part of the molecule and the C-terminal portion of the molecule. Using our isotopically-coded, biotinylated, and CID cleavable crosslinker CBDPS, we were able to detect several crosslinks that were unique to the each form of the prion protein, including a K185-K220 crosslink in the C-terminal portion of the protein which is specific to the β -oligomeric form.

These various structural proteomics approaches have already provided valuable and complementary experimental data on the structure of prions. These approaches have already generated information on the conformational changes involved in the conversion of PrPC to PrPSc.

Novel Aspect:

The complementary data from multiple structural proteomics methods has been generated on prion protein conformational changes.

Speaker Biography:

Evgeniy V. Petrotchenko received his M.D. in 1986 from the Second Moscow Medical Institute, Moscow, Russia and his Ph.D. in bioorganic chemistry in 1996 from the Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus, Minsk, Belarus. He conducted postdoctoral studies at the National Institute of Environmental Health Sciences, National Institutes of Health in Research Triangle Park, NC and at the University of North Carolina at Chapel Hill, NC. Currently, he is a Research Assistant Professor at the University of Victoria-Genome BC Proteomics Centre, University of Victoria, British Columbia, Canada. Main research interest is a structural proteomics

Oral 08 – "Gas-Phase Ions of Human Hemoglobin A, F and S: Cross Sections, H/D Exchange and MS/MS" Yang Kang (University of British Columbia, Vancouver, Canada)

Introduction:

Hemoglobin (Hb) is a tetrameric protein. In adult humans, the predominant Hb is Hb A which consists of two α and two β chains ($\alpha_2\beta_2$). In newborns, 80% of human Hb is fetal Hb (Hb F, $\alpha_2\gamma_2$). The γ chains differ from the β chains in 39 amino acid residues, with 22 on the exterior. Another variant is sickle Hb (Hb S, $\beta\text{Glu6Val}$) which causes anemia. Because hemoglobin is of interest as a model protein-protein complex and has been extensively studied with MS, the physical properties of gas-phase Hb ions and any relation to the solution-phase properties of Hb are potentially informative. The availability of natural Hb variants also allows studying the effects of changing the individual chains on the properties of this gas-phase protein-protein complex. In this study, we have measured collision cross sections, H/D exchange (HDX) levels and MS/MS spectra of monomer, dimer and tetramer ions formed from freshly prepared human metHb A, F and S, to compare gas-phase conformations and binding strengths.

Methods:

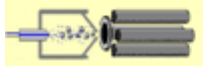
Collision cross sections are determined from the losses of axial kinetic energy of ions through collisions in Q2 of a home-built triple quadrupole system. Internal energies added to ions to cause dissociation, ΔE_{int} , are used to compare binding strengths and are determined from the kinetic energies of ions required to cause 50% dissociation of precursors in Q2. For gas-phase HDX, ions are confined in a linear trap in the presence of D2O vapor for 10 s, followed by mass analysis in a reflectron TOF. Hb samples are extracted from fresh human blood and diluted to 10 or 20 μM in 10% MeOH or ACN / 90% H2O (v/v), 10 mM NH4Ac, at pH 6.8.

Results and Discussions:

Fresh Hb A, F and S give similar mass spectra, and in 10% MeOH tetramer ions dominate. With 10% ACN, the abundances of monomer and dimer ions increase. We generally find higher levels of dimer ions in mass spectra than calculated from the solution K_d values. Solution conditions, including buffers and organic solvents, have an effect on the degree of dissociation. As well, mass spectra strongly depend on MS system operating conditions, making it difficult to compare ion abundances directly with calculated levels of species in solution. With all three hemoglobins, dimer ions give cross sections intermediate

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between monomers and tetramers (figure 1a) as expected from solution radii. Monomers of Hb S and F have similar cross sections, ca. 10% greater than Hb A. Cross sections of dimer ions of Hb S are 11% greater than those of Hb A and 6% greater than those of Hb F. Tetramers of Hb S are 13% larger than tetramers of Hb A or F. Relative exchange levels, %HDX (number of hydrogens exchanged/maximum number of exchangeable hydrogens, expressed as percent), are shown in figure 1b. Dimer ions give 31% HDX on average, slightly less than that of αh (ca. 34%) and tetramer ions. Monomers and dimers of all three Hb have similar HDX levels within the combined uncertainties. Tetramers of Hb S exchange 40% of hydrogens whereas Hb A and F tetramers exchange ca. 35% of hydrogens. While the cross sections show that Hb S tetramers are larger than Hb A and Hb F tetramers at times of a few milliseconds after ion formation, the HDX experiments indicate that they retain different and perhaps more unfolded conformations, for up to 10 s. In MS/MS of ferriHb (Fe^{3+}), holomonomers of Hb S and F require ca. 10% greater internal energy to lose a charged heme than Hb A (figure 1c). Under conditions where half of the dimer ions dissociate, Hb F (+11, +12) dissociates to two holo-monomers with nearly equal charges, while Hb A and S dissociate to both holo- and apo-monomers with asymmetric charge distributions. This may be due to the sequence difference of Hb F. Also Hb F requires less energy for dissociation than Hb A and S. Thus, in the gas-phase, Hb F dimers are slightly less stable than Hb A dimers. This differs from the binding in solution where $\alpha\text{h}\gamma\text{h}$ is more strongly bound. As for tetramers, Hb A and S dissociate to trimers ($\alpha 2\beta$ or $\alpha\beta 2$) and alpha or beta ions, while Hb F tetramers dissociate mainly to alpha ions and $\alpha\gamma 2$ trimers. Tetramers of Hb F require approximately 11% greater ΔE_{int} than Hb A or S. With tetramers, ΔE_{int} cannot be directly compared with ΔG_0^{sol} values for dissociation in solution because in solution two dimers are formed. Thus, in the gas phase, the order of the binding strengths of dimer ions is: Hb S > Hb A > Hb F; and the most stable tetramers are from Hb F. We do not find consistent relationships between the solution-phase stability and the gas-phase dissociation energies.

Novel Aspect:

These results provide new insights into the properties of gas-phase ions of Hb S and Hb F. The single localized mutation in Hb S evidently changes the conformation of monomer, dimer and tetramer ions in the gas phase. The different sequence of the γ chain of Hb F compared to the β chain of Hb A, also changes the physical properties of the gas-phase Hb complex

Speaker Biography:

Yang Kang received her BSc degree in Chemistry from Peking University, China, in 2008. Now she is a PhD candidate at University of British Columbia under the supervision of Prof. Don Douglas. Her current research focuses on mass spectrometry of gas-phase protein ions.

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).

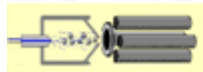
Methods:

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 $\text{CH}_3\text{COONH}_4$ and methanol performed on an XBridge C18, 50x2.1 mm, 5 μm column with a flow rate of 500 $\mu\text{L}/\text{min}$ 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:

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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 a QTRAP5500 System while maintaining adequate sensitivity, linearity, precision and accuracy. The same tacrolimus assay was used to evaluate the performance of a 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 μ L 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 7 μ L and 3 μ L 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.

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 – Evaluation of Dithranol as a MALDI Matrix for Tissue Imaging of Endogenous Metabolites by Fourier-Transform Mass Spectrometry

Cuong H. Le1, 2; Jun Han 1, 2; Christoph H. Borchers1, 2

1University of Victoria-Genome BC Proteomics Centre, Victoria, BC, Canada; 2Department of Biochemistry & Microbiology, University of Victoria, Victoria, BC, Canada

Poster 02 – Retrieval and Analysis of Isoform-specific Protein Information from Public Data Repositories provides Insights into Biological Complexity

Chengcheng Zhang; Juergen Kast

University of British Columbia, Vancouver, Canada

Poster 03 – Development and Evaluation of Atmospheric-Pressure Electron Capture Dissociation (AP-ECD) for the LC/MS Analysis of Protein Digests

Damon Robb; Davin Carter; Jason Rogalski; Juergen Kast; Michael Blades

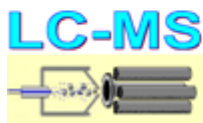
University of British Columbia, Vancouver, Canada

Poster 04 – Parametric Studies of Condensed Phase Membrane Introduction Mass Spectrometry (CP-MIMS): Membrane Types/Geometries, Acceptor Phase Modifications and Continuous On-Line Quantitation Techniques

Kyle D. Duncan2, 4; Bruce R. Todd3; Erik T. Krogh1, 4; Christopher G. Gill 1, 4

1Vancouver Island University, Nanaimo, BC, Canada; 2University of Victoria, Victoria, BC, Canada; 3The Instrument Works, Vancouver, BC, Canada; 4Appl. Env. Res. Labs.(AERL), Nanaimo, BC, Canada

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Poster 05 – Determining the Absolute Stoichiometry of Phosphorylation Sites by Multiplex MRM - Studying Phosphorylation Events of Estrogen Receptor in Breast Cancer

Dominik Domanski¹; Leigh C. Murphy²; Christoph H. Borchers¹

1UVic-GBC Proteomics Centre, Victoria, BC; 2University of Manitoba, Winnipeg, Canada

Poster 06 – Mobile Phase Decontamination of an Unexpected Isobaric Interference by Using On-line Filtration in LC-MS/MS Regulated Bioanalysis

Mathieu Lahaie, Milton Furtado and Fabio Garofolo;

Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Poster 07 – Hemolysis Effect on the Processed Reconstituted Stability of Morphine in Different Reconstitution Solutions

Eugenie-Raphaelle Berube, Milton Furtado and Fabio Garofolo;

Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Poster 08 – Severe Impact of Hemolysis on Fluvoxamine Long-Term Stability

Marie-Pierre Taillon, Milton Furtado and Fabio Garofolo;

Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Poster 09 – Impact of a Potential Newly Discovered Isobaric Interference of Fluvastatin on the Accuracy of Bioanalytical Data

Cynthia Cote, Marie-Pierre Taillon, Sylvain Latour, Milton Furtado and Fabio Garofolo;

Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Poster 10 – Avoiding Major Issues in the Bioanalysis of Prasugrel by LC-MS/MS: Thorough Investigation of Incurred Samples and Reference Standard Materials

Jean-Nicholas Mess, Milton Furtado and Fabio Garofolo;

Algorithme Pharma Inc., Laval (Montreal), QC, Canada

Poster 11 – Elimination of Selectivity and Quantification Issues during Ursodiol in Regulated Bioanalysis by Using a High Resolution Quadrupole Time-of-Flight Mass Spectrometer

Louis-Philippe Morin¹, Jean-Nicholas Mess¹, Suma Ramagiri², Mauro Aiello², Johnny Cardenas², Milton Furtado¹ and Fabio Garofolo¹;

1Algorithme Pharma Inc., Laval (Montreal), Quebec, CANADA ; 2AB SCIEX, Concord, ON, Canada

Poster 12 – Understanding formaldehyde cross-linking of proteins by mass spectrometry

Xuan Ding

University of British Columbia, Vancouver, Canada