POSTER ABSTRACTS

TP-1: Understanding Innate and Adaptive Immunity: A Large Scale Genomic and Proteomic Evaluation
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The objective of this study is to address immunological mechanisms involved in defense against infectious disease, with an emphasis on immune response gene polymorphisms and their effect on protein expression that contribute to inter-individual variations in immune response. The focus of this presentation will be on a Vi-polysaccharide vaccine used for *S. typhus*. We are correlating these genetic polymorphisms with response to phenotypes, such as severity of infection, or with vaccination outcome. Here we present the results to date from this study obtained from a variety of proteomic studies including Luminex bead assays, defensin analysis, 2-D DIGE, and shotgun proteomics. Here we present a summary of these large scale study results and their correlation with response to vaccination, demographic trends, and gene polymorphisms observed in this population. Their effect on innate and adaptive immunity will also be presented.

TP-2: Ruggedness of Nanobore LCMS for Qualitative and Quantitative Biomarker Analysis using an Automated Emitter Positioning and Rinsing System
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Nanobore LC-MS is a technique used for qualitative and quantitative analysis of complex proteomes extracted from biological matrices including plasma. Complex mixture analysis under nanoflow conditions presents significant challenges, including premature failure of nanobore components. For quantitative analyses system robustness and reproducibility are critical. A systematic investigation of emitter contamination resulting from the analysis of plasma was conducted. Standard spiked plasma were infused creating two data sets; one with emitter rinsing and the second without. Standard recovery was monitored throughout sequential injections and plotted. Improvement in the signal stability of each standard as a result of automated tip washing as compared to a traditional analysis with no tip washing is demonstrated. Using a digitally controlled stage positioning system, the emitter was toggled between two positions: the spray position and the wash position. At 0 kV the emitter was toggled to the spray position by a remote contact-closure control signal. A constant 80 ul/min 50% Methanol flow washed the exterior of the emitter. Comparative analysis of the intensity for each standard between data collected with washing and no washing reveals an obvious discrepancy. Data collected with washing produced consistent intensity values over 650 injections. Data collected without washing produced highly variable signal, changing more than 10-fold over the course of the experiment.

TP-3: Automated Data Analysis for LC-MS Metabonomics
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Continuous instrumentation advances in LC-MS have enabled better sample throughput and enhanced capabilities for the detection and quantitation of wide variety of molecules while producing enormous amounts of recorded signals. As a result, there has been a shift in research focus towards the automation of data analysis. The sheer size of LC-MS data sets precludes manual analysis even by a trained mass spectrometrist. A novel approach for automated data reduction of LC-MS profiles into lists of components with consecutive cross sample comparison has been proposed. The algorithms of the automated analysis include extraction of ion chromatograms, detection of charge states and grouping of isotope and adduct signals. Metabolite annotations are performed by matching ionization response, mass accuracy and retention times with corresponding synthetic standards. This approach has been successfully applied to metabonomics studies and has produced quantitation tables of annotated metabolites. An example of automated data analysis for an LC-MS metabonomics investigation of an animal fasting study has been presented.

TP-4: Strategies for Oligonucleotide Sequencing by High Resolution ESI Ion Trap Tandem Mass Spectrometry
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Oligonucleotides (oligos) and their application in diagnostic and therapeutic areas have expanded rapidly in recent years. New technologies in areas such as anti-sense, aptamers, and gene silencing (e.g., siRNA) have lead to this resurgence. Mass spectrometry has emerged as a primary characterization technique for oligo analysis as a proof-of-structure tool with determination of molecular mass as a key indication of product quality in oligo synthesis laboratories. The use of oligos in medical applications will increase the level of regulatory scrutiny and concomitant level of detail required for proof-of-structure. For example, molecular mass alone is not sufficient to demonstrate that a product has the correct linear sequence. Enzymatic digestion with phosphodiesterases followed by mass analysis of the fragments has been successfully used for sequence analysis of unmodified DNA and RNA. However, most of the oligos used in therapeutic applications have been chemically modified to be resistant to common enzymes. In this presentation, we demonstrate the

use of high-resolution (orbitrap) ion trap tandem mass spectrometry as a powerful alternative for oligo sequencing/confirmation applications. Unfortunately, MS/MS spectra of highly-charged negative ions result in complex product ion spectra that are tedious (at best) to interpret. Our approach uses high-resolution MS/MS data with deisotoping software and automated fragment ion prediction in an attempt to simplify the spectral interpretation process. Complete sequence information is not always obtained for all oligos with this approach. However, this is as a result of ion chemistry effects and the resulting "gaps" in sequence ion coverage, and is not due to the inability to interpret the data. The methods have been found to be generally applicable to many types of oligos (modified and unmodified) at lengths of up to 40 bases (~12 kDa).

TP-5: Meeting Genotoxic Impurity Regulatory Requirements for the Analysis of Alkyl Arylsulfonate Esters in Drug Substances and Drug Products

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With the recent publications of the EMEA guidelines and the FDA draft guidance for genotoxic impurities, the analysis of genotoxins in drug substances and drug products has become of prime concern to pharmaceutical manufacturers. Of particular interest is the analysis of alkyl esters of alkyl and arylsulfonic acids in the synthetic pathway and in final product of pharmaceutical materials. These sulfonic acid esters, are a common class of reagents used in the pharmaceutical industry as alkylating agents, catalysts, and in purification steps in the chemical synthesis of an API. In addition, the sulfonic acids are often used as the final salt form of the drug substance to improve chemical properties or bioavailability of the API. The presence of any residual alcohols from synthetic reaction or recrystalization steps may result in the formation of alkyl esters of the sulfonic acids. Many of these mesylate, besylate, or tosylate esters are known to be genotoxic while others are potentially genotoxic requiring monitoring in the final drug substance and drug product. The most common traditional analytical technique for monitoring alkyl sulfonate esters has been GC/MS or HPLC/UV/MS with derivatization using pentafluorothiophenol. More recently, HPLC/MS has been shown to give good results without the need for a complicated derivatization step, however, run times on the order of 20-30 minutes were required to achieve sufficient resolution from API. This paper describes a fast UPLC/UV/MSMS method for this analysis and compares detection methodologies with regards to regulatory requirements. UV detection was found to give sufficient sensitivity and selectivity for the analysis of genotoxins in some APIs, however, MS and MS/MS was shown to be a better general detection methodology for a wide range APIs due to the increased selectivity attainable with MS. Results of the analytical methods are shown for two commercially available pharmaceutical substances and the sensitivity, selectivity, and detection limits obtained using UV and MS/MS are compared.

TP-6: Simultaneous, Label Free Determination of *in vivo* Drug and Metabolite Tissue Distribution with High Resolution Mass Spectrometry

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Currently, most *in vivo* Drug Distribution studies require the preparation and administration of doses of expensive, custom synthesized radio-labeled parent drug compounds. One of the major drawbacks of this approach is that, in many instances, the radiolabel is present in both the parent drug and its metabolites, making it impossible to distinguish the unmetabolized parent drug from a metabolite. A method for easily obtaining this information of parent drug vs. metabolite distribution would be quite valuable to pharmacologists. One potential solution for better Drug Distribution studies involves the use of Mass Spectrometry. Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectrometry (MS) can be used to detect the presence, approximate amount and location of small molecules, lipids, peptides and proteins in tissue samples through in vitro molecular imaging, often referred to as MALDI imaging. In this presentation, examples of the use of both MALDI-TOF and MALDI - Fourier Transform Mass Spectrometry (FTMS) will be presented. In the first study, both instruments were used to detect and image the location of Erlotinib in various mouse tissues (liver, kidney, brain). The molecular imaging experiments not only indicated where in the mouse's body the drug accumulated, but it also gave insight regarding the different MALDI imaging technologies' respective capabilities for small molecule drug tissue imaging. A second study will detail the use of the MALDI-FTMS instrument to simultaneously measure and analyze the kidney distribution of therapeutic dose levels of Olanzapine at a series of successive time intervals after initial dosing. The studies presented here indicate that the distribution of small molecule drugs and their metabolites in tissues at therapeutic doses can be accurately and simultaneously measured utilizing a high resolution Mass Spectrometry system.

TP-7: Fast, Hyper Accurate Drug Metabolite Identification utilizing Fast Chromatography with High Resolution Mass Spectrometry

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In recent years, many ADME laboratories have been working actively to transfer metabolite identification and analysis methods to new fast, high resolution chromatography systems. However, this acceleration in separation techniques has created problems for the current generation of high performance mass spectrometers as many of them suffer from a dramatic loss in performance as the speed of the separation technique increases and render them unsuitable for metabolite identification studies. To answer the challenge of retaining high performance from a mass spectrometer, even at fast chromatography speeds, Bruker Daltonics has developed the maXis. This instrument is unique in its performance for high-resolution tandem mass spectrometry. The maXis delivers exceptional accurate mass (<1 ppm), high resolution

(>50K), high sensitivity (attomole levels), and possesses a wide dynamic range (~5 orders of magnitude). This presentation will demonstrate the results from the utilization of the maXis in conjunction with fast chromatography systems for both *in vitro* and *in vivo* metabolite identification studies. The presentation will demonstrate how the maXis was successfully able to measure and absolutely identify all expected metabolites from a number of small molecule parent drugs with high precision and accuracy. In many instances, the performance capabilities of the maXis were the key to identifying either low level or unexpected metabolites. Using combinations of high resolution extracted ion chromatograms with mass defect filtering and the application of Smart Formula 3D allowed for an easy highly automated workflow for these types of studies. These studies demonstrate that the maXis is clearly capable of providing high quality data in conjunction with fast separation techniques for metabolite identification.

TP-8: Use of MALDI Mass Spectrometry to Monitor DMPK Properties of Biotherapeutics
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With the ever increasing numbers of protein and peptide therapeutics moving through the Drug Development process at many companies, the need for fast, robust, effective, and powerful methods for detailed analysis of biotherapeutics has become acute. Current methods for protein analysis are often very slow, labor intensive, (PAGE Gels, ELISAs, Western Blots, etc.) and provide limited information. Much of the process of metabolizing a therapeutic protein or peptide involves proteolytic cleavage of the protein or processing of its modifications. To analyze the protein processing events often associated with DMPK of a biotherapeutics requires the ability to determine and monitor changes in protein size and composition as well as protein modifications. Many of these requirements can be met by high resolution MALDI-TOF/TOF Mass Spectrometry. In this presentation, the capabilities of MALDI-TOF/TOF Mass Spectrometry for the “Top Down” analysis of intact proteins will be detailed. This type of analysis allows researchers to simultaneously sequence and monitor the N- and C-Termini of proteins for any changes. Dozens of amino acids can be sequenced and monitored from either termini. Furthermore, this type of approach can be used to monitor PEGylated peptides or proteins for their stability to degradation. Several examples of the utilization of the top down analysis capabilities of a high performance MALDI-TOF/TOF MS to directly analyze recombinant proteins and antibodies for modifications, truncations, etc. will be presented.

TP-9: Software for the Vital Aspects of Metabolite Identification – Approaching the Target.
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Software modules that support the vital processes of metabolite identification are proposed to be emerging. While the goal of proposing structures and disseminating that across organizations is common, the processes and tools to accomplish the goal vary widely. Despite the absence of consensus, we have developed software enabling processing of low or high resolution mass spectrometry data and updatable databases for data and structures. Modules such as IntelliTarget and IntelliXtract made it possible to search for metabolites in targeted or nontargeted manner. It was also possible to analyze spectra showing fragment ions to assign structures. The structures, supporting data and additional information could be retained in databases. The databases were searchable in a variety of ways. Biotransformation schema created and saved databases offer metabolism groups and other colleagues a means to share and leverage the information from the analytical results.

TP-10: A Sensitive and Selective LC-MS/MS Method for the Determination of HMPMA and 3-HPMA in Human Urine
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The purpose of this research was to develop a method to measure metabolites of crotonaldehyde with an analytical range appropriate for measuring tobacco smoke exposure levels. The method developed and validated is a sensitive, selective, precise, and reproducible LC-MS/MS method for high throughput analysis of both 3-hydroxy-1-methylpropylmercapturic acid (HMPMA) and 3-hydroxypropylmercapturic acid (3-HPMA) human urine samples. An automated liquid handling device (Zymark/Caliper Sciclone) is used to perform the 96 well solid phase extraction. Urine samples (0.100 mL) are loaded on a conditioned Waters Oasis® HLB plate. Samples are washed with water and an acid solution and eluted with an ammoniated organic solvent. Mobile phase containing acetonitrile and ammonium formate buffer was used to achieve separation with a BioBasic AX precolumn followed by a BioBasic AX analytical column. An AB MDS Sciex API 4000 detected negative ions in MRM mode. Total acquisition time was 2.5 minutes. The bioanalytical assay for the quantitation of HMPMA and 3-HPMA in human urine met predefined acceptance criteria for precision, accuracy, sensitivity, selectivity, stability, and recovery. The method used calibration standards prepared in a surrogate matrix over the ranges 80.0 – 5000 ng/mL and 35.0 – 5000 ng/mL for HMPMA and 3-HPMA, respectively. Inter-batch precision (% CV) of quality control samples (LLOQ, low, medium and high) was less than or equal to 6.6% and 7.5% for HMPMA and 3-HPMA, respectively. The inter-batch accuracy (% Bias) was between +0.4% and -1.6% for HMPMA quality controls and between +1.0 and -3.2 for 3-HPMA quality controls. 15N, 13C3-HMPMA and d6-3-HPMA were used to determine recovery in human urine as both HMPMA and 3-HPMA are endogenous compounds with the recoveries measured at 81% and 78%, respectively. Short-term stability in human urine was established for 27 hours at 5°C under UV-shielded light. Freeze and thaw stability in human urine was established for six freeze (-20°C) and thaw cycles. Processed sample integrity in injection solvent (reinjection) was established for 155 hours at 5°C. All stability results refer to both HMPMA and 3-HPMA. The validated

method allows for high-throughput sample processing with a greatly reduced sample volume requirement and greater sensitivity than alternate methodologies.

TP-11: LC/MS/MS Method for Quantitation of Aldosterone and Related Steroids in In-Vivo Samples
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Aldosterone and its metabolites are steroid hormones produced by the adrenal cortex, which play important roles in the renin - angiotensin - aldosterone (RAAS) system. Aldosterone regulates the reabsorption of sodium, water, and secretion of potassium in the kidneys. Thus, aldosterone and its metabolites have been used as important biomarkers for cardiovascular drug discovery, especially in the hypertension models. An ELISA assay has been used for quantitation of aldosterone levels for in-vivo samples, include plasma and urine. Issues related to the aldosterone ELISA assay include matrix interference and cross-reactivity from aldosterone related metabolites. The first issue can be corrected by significantly diluting samples. However ELISA assay has exhibits high levels of interference from corticosterone, cortisol, deoxycortisol and deoxycorticosterone. The LC/MS/MS method developed has a limit of quantitation of 10 pg/ml in plasma and 25 pg/ml in urine. Waters Acquity UPLC BEH C18 column (1.7 µm, 2.1x50 mm) is used, mobile phases are methonal and water, aldosterone mass transition is 359.4→189, aldosterone is well separated from its related metabolites to improve assay selectivity chromatographically. In addition, in-vivo sample is extracted by acetonitrile:methonal (2:1), this process could be handled along with dosed compounds and is amenable to a high throughput automation process. The information generated using this one-shot LC/MS/MS method is critical for understanding compound-biomarker correlation.

TP-12: Quantitation of Plasma Angiotensin I in a Plasma Renin Activity Method for Ex Vivo Target Engagement
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Plasma Renin Activity (PRA) is a key target engagement assay in preclinical hypertension pharmacodynamic models. The measurement of renin activity is complicated by the difficulties in the quantification of Angiotensin I (Ang I), the product of renin-catalyzed reaction, which can be used to reflect the activity of renin-angiotensin-aldosterone system. Traditionally, the analysis of Angiotensin I is carried out by Radio Immunoassay (RIA). RIA is sensitive, but it has several issues. First, it requires large sample volumes, which is not amenable to serial sampling for pharmacokinetics and PK/PD. Second, it has very limited linear dynamic range, which sometimes requires samples dilution. Dilution schemes during sample processing can be quite complicated for unknown samples, as samples may be over or under-diluted and fall outside the linear range. An alternative approach to PRA measurement, reported in the literature, is LC/MS/MS analysis. Delivering sensitivity equal or superior to RIA is challenging to LC/MS/MS with small sample volume involved. One hurdle to a LC/MS/MS method is the sample extraction procedure, which is not only time consuming, but also prone to low extraction recovery of Ang I. In this report, we introduce a LC/MS/MS method to quantify endogenous levels of Ang I and Ang I produced in plasma (PRA) from treated in vivo samples. This method eliminates the sample extraction steps prior to LC/MS/MS analysis, providing high sensitivity, large liner dynamic range (0.043-282 ng/ml), as well high selectivity against Ang II (LLOQ= 0.03 ng/ml) and Ang III (LLOQ= 0.1 ng/ml). This LC/MS/MS method consists reverse phase chromatography using C18 column, gradient at 0.4 ml/min with 0.1% formic acid water and acetonitrile. Positive electrode spray mode with [Val5] internal standard presented. Quantitative data processing is carried out by Sciex analyst 1.4.2 software.

TP-13: From Sample to Spray: A High Performance Workflow for Top-Down Protein Analysis
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A new workflow for obtaining high quality mass spectrometric data on intact proteins from complex biological samples has been developed. The workflow includes sample fractionation using parallel Gel Eluted Liquid Fraction Entrapment Electrophoresis (GELFREE), advanced polymer phase nanobore liquid chromatography, and nanoelectrospray ionization coupled to FTMS for protein identification. This combination provides a powerful front-end solution to generate the highest quality top-down data. When coupled with ProSightPC for data analysis, these integrated tools comprise the most powerful, robust strategy to date. For high quality top-down protein analysis, biological samples must be pre-fractionated in order to reduce sample complexity. The GELFREE technology provides an elegant solution for the partitioning of up to eight samples (whole cell lysates, body fluids, etc.) in parallel based on intact protein molecular weight. Each fraction is extracted easily and with very high recovery in liquid phase. Following pre-fractionation, samples are subjected to high performance online nanobore liquid chromatography using polymeric chromatographic media for intact protein separation and nanoelectrospray ionization. In this work, the proteins fractionated into ~5kDa bins are subjected to high resolution tandem mass spectrometry using a 7 or 12 Tesla FTMS equipped with a digitally controlled nanospray source, providing a high level of ionization reproducibility and robustness. This workflow provides previously unattainable sensitivity and is suitable for characterization of protein targets in complex mixtures, biotherapeutics, or top-down implemented in high

throughput mode. The details of this optimized workflow for top-down proteomics will be presented along with discussion of the specific technologies.

TP-14: Novel and Robust ESI Sources for Efficient Bioanalytical Workflows at Capillary LC Flow Rates
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The diverse requirements of small and large molecule analysis has driven the bifurcation of electrospray ionization (ESI) into high (mL/min) and ultra-low (nL/min) flow regimes. Small molecule analysis has predominantly relied on high flow rates using millimeter diameter liquid chromatography (LC) columns. Fast gradient elution on short (≤ 5 cm) columns with sample-to-sample injection times measured in 1-5 minutes (or better) dominates the workflow. Large molecule (protein and peptide) analysis has relied on ultra-low flow ESI (nanospray) and the use of nanobore (≤ 75 um) LC columns. Workflows based on nanopsray/nanobore LC enable exceptional sensitivity and high separation power for complex mixtures. Long gradient elution LC with injection cycles of greater than 30-60 min dominates workflow. Biomarker validation of (endogenous) proteins and peptides demands that biomolecular LC-MS/MS transition from qualitative to quantitative analysis. Traditional small molecule workflows typically lack sufficient sensitivity and selectivity required for biomarker validation. On the other hand, large molecule workflows lack sufficient throughput for the quantification of large sample sets. Enabling efficient ESI in intermediate flow range, 1-20 uL/min, allows for the facile implementation of capillary scale (0.18 to 0.5 mm ID) columns. Capillary columns enables higher sensitivity than mm scale while delivering higher throughput, robustness, and ease-of-use than nanobore LC. Two different novel ESI source designs, based on high precision fused-silica components and microfluidic connectors, enable the integration of the concepts used in both high-flow ESI and nanospray. This precision emitter assembly enables stable ESI at flow rates from 1 to 20 uL/min at low (<2%) and high (>90%) organic mobile phase composition. Continuous flow and flow injection experiments demonstrate relative standard deviation of better than 5% for absolute ion intensity on a 3D ion trap mass spectrometer.

TP-15: High Resolution Dual nano-LC/MS Source for Increasing Sample Throughput of Gradient LC Methods
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Nanospray-LC/MS have mobile phase gradients that require significant re-equilibration times before a second analysis can be performed. A novel automated nanospray-MS source with a dual column configuration performs the gradient analysis of a sample on one capillary column while a second column is equilibrating. A previously published two-column, valve-based system for analytical scale LC applications has disadvantages for nanospray-LC/MS because substantial dead volume exists between the columns and the spray emitter. By spraying directly from the columns to the MS inlet, both increased throughput and high peak resolution are achieved. Two 75 µM I.D. C18 columns were placed on an automated motorized axis that alternates the positions of the columns on-axis to the MS inlet. Each column is connected to binary direct flow gradient pumps at flow rates of 300nL/min. 200nL injections were made with nano-electrospray initiated by applying both a 4kV high voltage spike and a nitrogen stream to the column exit to purge the emitter of droplets and excess mobile phase. Upon completion of analysis, the columns switch positions and a new analysis begins on the equilibrated column. This method not only increases throughput of routine analysis, but also LC/MS gradient method development. A neuropeptide standard solution was injected for six different LC/MS gradient analyses. Two C18 columns were used for the six injections with three analyses on each column. By performing six different gradients on the dual column system, peak widths as narrow as 9 seconds were achieved. The reduction in method development time was 65% over a single column system that requires time waiting before the mass spectrometer for column equilibration. The optimum method was repeated on both columns to demonstrate that small variations between the columns were not a factor in optimizing a nano-LC/MS gradient method. This work was partially funded by a NIH SBIR grant.

TP-16: Optimal Approach for Multiplexed Nano-LC/MS
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The growing use of LC/MS in drug discovery, biomarkers detection and proteomics research has made increasing sample throughput without compromising sensitivity extremely desirable. Multiplexing LC's for a single MS is a viable approach. Methods for multiplexing may range from the sequential input (one-on-at-a-time, or 1/N duty cycle, N=number of LC's), to intermediate duty cycle, e.g., Hadamard Transform, which has ~50% duty cycle, to one-off-at-a-time, i.e.,(N-1)/N duty cycle. This report evaluates nano-LC/MS multiplexing approaches with more than 50% duty cycle for signal detection, and the methods of deconvoluting the data. For N=3, the Hadamard approach and the one-off approach coincide. The experimental design described here is extendable to N=2n-1, n= 1, 2, 3…. Three clog-resistant polymeric spray devices were arranged in a circle so that the spray from each device was equivalent in position to the MS inlet. Each spray device was connected to a column and a pump. Samples used are two different peptide mixtures with gradient methods and one of the peptide mixtures with an isocratic method. The columns used were all 75 um i.d., 5 cm-long packed with 5 um C18 particles. The data were obtained using a sequence from a Hadamard S matrix of dimension 3 to be applied to the high voltage for spraying the eluate from each spray device. The sequences are in the form of 110, 101 and 011, where 1 is the "on" state and "0" is the "off" state of the spray voltage on each column. A software controlled electronic circuit switched the high voltage (HV) required for nanospray. The mass chromatographic data of the simultaneously separations were deconvoluted with a sequential "three-time-bin" deconvolution method, and an interpolation method. The resulted

chromatograms from each method did not differ greatly and gave satisfactory agreement to the separations that were taken individually. Comparison with the conventional sequential method and the one-off method will also be presented. This research has been partially supported by an NIH SBIR grant.

TP-17: High Throughput Nanospray Chip for Robust Molecular ID using Direct Infusion

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Nanospray mass spectrometry enables high sensitivity detection (at least 10x that of conventional electrospray), consumes less sample, and can tolerate samples in complex matrices or even DMSO. It does, however, suffer from being unstable because narrow-bore spray emitters clog, air bubbles cause spray interruptions, and optimized spray conditions vary for different experiments. In addition, the low flow rates make rinsing of the capillary tubing between the sample injector and the spray emitter time consuming. This report describes a nanospray nozzle chip that overcomes these shortcomings. The nanospray chip is made of 16 polypropylene nozzle/reservoir units, which may be stacked to form a 96 or 384-well format. Each nozzle has a 50-micron o.d. and a 20- micron i.d. and is connected directly to a sample well of up to 15 uL capacity. Sample is drawn through the nozzles by applying high voltage and is further assisted with free flowing nitrogen through a small punctured hole in the back sealing mat of the chip. Flow rates can be adjusted by varying the pressure of the nitrogen and the spray voltage. The constant spray is enabled by a current sensor placed close to the mass spectrometer inlet. The sensor converts a few percent of the total spray current into a negative feedback signal for a computer-controlled closed loop regulatory system. Several controls may be activated by the current sensor to bring the signal back to a set threshold. These controls include the coordinated timing of a high voltage ramp, a nitrogen pressure ramp, and a distance regulation between the spray nozzle and the mass spectrometer inlet. With these controls and an initial vacuum step, we demonstrate the routine spraying of samples from every nozzle of a 16-nozzle or 32-nozzle chip. The results of spraying diluted but unprocessed fetal bovine serum and erythromycin in DMSO automatically without manual adjustment is presented. This work is partially supported by an NIH SBIR grant.