POSTER SESSION
& VENDOR EXHIBITION

September 19, 2018
6pm - 8pm

Bethesda North Marriott Conference Center
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Student Poster Award

Best student poster will receive the George Guiochon Chromatography Award and a travel grant to attend HPLC 2019 in Milan, Italy. Honorable Mention(s) and Winner will receive award certificates and opportunity to present at a WCDG meeting.

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Characterization of USP Oligosaccharide System Suitability Mixture A and Mixture B Reference Standards

Jingzhong (Tim) Guo, Huiping Tu, Maheswara Rao B, Anjali Kumari Chillara, Edith Chang, and Fouad Atouf
USP, Rockville, MD

Biologics represents a fast growing class of therapeutics. A majority of biologics manufactured by recombinant technologies contains post-translational modifications of asparagine (N-glycans) or serine/threonine residues (O-glycans) by the addition of glycans. The structures and compositions of these glycans play an important role in the bioactivity, stability, immunogenicity, pharmacokinetics and pharmacodynamics of the biologic therapeutics. As a designated critical quality attribute (CQA) of the product, glycosylation analysis has become necessary for the product characterization and release specifications. Therefore, USP has developed a series of general chapters published in the United States Pharmacopeia—National Formulary for characterization and assessment of protein glycosylation. Specifically, the chapter <212> Oligosaccharide Analysis provides several validated procedures and performance criteria for analysis of N-linked oligosaccharides released from therapeutic glycoproteins. To support these methods, USP has developed four reference standards (USP Oligosaccharide System Suitability Mixture A, B, C and D RSs) to assess the system suitability of these analytical procedures. The goal of the current study was to identify the glycan species in USP Oligosaccharide System Suitability (SS) Mixture A and B RSs that are not described previously and to compare these RSs with similar glycan libraries that are commercially available. The USP Oligosaccharide SS Mixture A RS consists of a mixture of mainly biantennary N-linked oligosaccharides that were released from human polyclonal immunoglobulin G (IgG) while USP Oligosaccharide SS Mixture B RS consists of a mixture of mainly high-mannose N-Linked oligosaccharides released from bovine ribonuclease B (RNase B). To identify the glycan species in both USP Oligosaccharide SS Mixture A and B RSs, 2-AB-labeld samples were analyzed by an LC-MS/FLR platform consisting of a UPLC system coupled with a fluorescence (FLR) detector and a Synap G2 QTOF mass spectrometer. For USP Oligosaccharides Mixture A RS, 32 glycan isoforms corresponding to 30 chromatographic peaks were identified. For USP Oligosaccharide SS Mixture B RS, 14 glycan isoforms

corresponding to 12 chromatographic peaks were identified. Furthermore, a quantitative comparison of USP Oligosaccharide SS Mixture A RS with similar glycan libraries from commercial vendors has been performed using the HILIC procedure 1 from General Chapter <212>. The results showed that USP Oligosaccharide SS Mixture A RS has slightly more uniform glycan distribution among partially sialylated, partially galactosylated, partially fucosylated biantennary and triantennary N-linked oligosaccharides while other N-glycan libraries were dominated by asialylated biantennary oligosaccharides. The results provide a framework for extending applications of the USP Oligosaccharide reference standards not only for system suitability assessment but also for identification purpose. In addition, these USP RSs can be used to ensure the quality of the critical reagents, assess the external quality exercises and train the new users.

Selectivity Characterization of Five Achiral Stationary Phases Using SFC and HILIC

Gay Lowden, Edward G. Franklin, Melissa Wilcox, Ted Szczerba
Regis Technologies, Morton Grove, IL

Supercritical fluid chromatography (SFC) is widely recognized as a preferred technique for preparative chiral applications. The use of compressed CO2 as the primary mobile phase provides many benefits: it is readily available, relatively inexpensive, and safe. It can also be recycled, thus leading to SFC's designation as a "green" technology. Additionally, the viscosities of compressed CO2 and mixtures with polar modifiers are much lower than those of aqueous mixtures. This allows for chromatographic run times which are approximately one-third to one-fifth as long as typical HPLC runs. These advantages are clearly applicable to a broad range of separations, beyond just chiral separations. Five achiral SFC stationary phases (2-ethyl pyridine, 4-ethyl pyridine, propyl-amine, polyethylenimine, and arginine) are described with respect to the selectivity characteristics observed in SFC analyses of acidic, basic, and neutral compounds. These results are compared to the selectivities observed when using these same stationary phases in hydrophilic interaction (HILIC) mode.

Development and Validation of a Stability-Indicating HPLC-UV Method for Triamcinolone Acetonide

Katya Petrova, Mitan R Gokulgandhi, Joshua Bhattacharya, Ren-Hwa Yeh, and Daren Tran
USP, Rockville, MD

Purpose: Triamcinolone Acetonide is a synthetic glucocorticosteroid with anti-inflammatory and immunosuppressive activity. The purpose of this study was to develop and validate a single stability-indicating HPLC-UV method for assay and organic impurities (OI) of triamcinolone acetonide in drug substance, and to modernize the USP Triamcinolone Acetonide monograph. Methodology: Chromatographic separation of triamcinolone acetonide and related impurities was achieved on a L1 column (Phenomenex, Inertsil ODS-3, 150 × 4.6 mm, 3µm) maintained at 40º, gradient elution with a mobile phase A consisting of 0.01% formic acid in water and acetonitrile (70:30 v/v) and mobile phase B comprised of 0.1% formic acid in acetonitrile and water (65:35, v/v). A gradient mode was operated at a flow rate of 1.2 mL/min and detection wavelength was at 240 nm. Results: The method separates triamcinolone acetonide and three impurities listed by the European Pharmacopoeia (Ph. Eur.) - triamcinolone, triamcinolone acetonide related compound (RC) C and triamcinolone acetonide RC B. Specificity of the method was evaluated by forced degradation studies on USP Triamcinolone Acetonide RS and it was observed that the base stressed condition formed degradants. The method was found to be specific; all degradation peaks >1% were separated from the triamcinolone acetonide peak by resolution of NLT 2.0 and from the three impurities by resolution of NLT 1.5. The peak purity studies of the triamcinolone acetonide peak by PDA and LC/MS confirmed that the peak is free of co-elution in all stressed samples. The method was robust as evaluated under a number of changes to the chromatographic parameters. The specified impurities were validated at the ICH limit of 0.1% (range of 0.05% - 1.0%) of the nominal sample concentration of 0.2 mg/mL. The assay of triamcinolone acetonide was validated in the range of 75-125% of the nominal sample concentration of 0.2 mg/mL. The data also demonstrate that the assay procedure for triamcinolone acetonide is suitable as an identification (ID) test by HPLC-PDA and HPLC-Retention Time. Both procedures (OI and assay) were found to be specific, linear, accurate, precise, and rugged.

Conclusion: A single stability-indicating LC/MS compatible HPLC method was developed and validated for the OI, assay and ID procedures for triamcinolone acetonide to modernize the USP Triamcinolone Acetonide monograph. The same HPLC method was further applied to modernize USP's triamcinolone acetonide related monographs with different pharmaceutical formulations – USP Triamcinolone Acetonide Cream monograph, USP Triamcinolone Acetonide Dental Paste monograph, USP Triamcinolone Acetonide Lotion monograph, and USP Triamcinolone Acetonide Injectable Suspension monograph.

Determination of Polycyclic Aromatic Hydrocarbons in Standard Reference Material 1597a via Normal-Phase Liquid Chromatography and Gas Chromatography/Mass Spectrometry

Hugh Hayes, Walter B. Wilson, Lane C. Sander, Andres D. Campiglia, Stephen A. Wise
National Institute of Standards and Technology, Gaithersburg, MD

Standard reference materials (SRMs) are complex natural matrix samples certified at the National Institute of Standard and Technology for the evaluation and validation of analytical methods for the determination of polycyclic aromatic compounds (PACs) in complex mixtures. PACs comprise a complex class of condensed multi-ring benzenoid compounds originating from a wide variety of natural and anthropogenic sources. The parent homocyclic species, which contain only carbon and hydrogen, are the familiar polycyclic aromatic hydrocarbons (PAHs). In this study, an analytical method was developed for the separation and identification of PAHs in SRM 1597a, which is a combustion-related mixture of PAHs isolated from a coal tar sample and dissolved in toluene. Due to the complexity of the sample matrix, the analytical method described requires a fractionation step using normal-phase liquid chromatography (NPLC) on an aminopropyl (NH2) stationary phase phase. SRM 1597a was characterized before and after fractionation by gas chromatography/mass spectrometry (GC/MS) using a 50% phenyl stationary phase. The NPLC-GC/MS method presented here allowed for the identification of 75 PAHs and 54 methyl-substituted PAHs.

Displacement of Monomer-Sized Bivalent Bispecific Antibody Charge Variants by Cation Exchange Chromatography

Lucas Kimerer, Timothy Pabst, Alan Hunter, Giorgio Carta
University of Virginia, Charlottesville, VA

Therapeutic applications of bispecific antibodies have seen growing interest in recent years due to their ability to engage two unique targets simultaneously. Bivalent bispecific antibodies (BiS) are commonly produced by recombinantly expressing a whole IgG and genetically fusing a single chain variable fragment (scFv) using a flexible polypeptide linker. Bivalent bispecific antibody downstream processing literature is scarce. In this work, a bivalent bispecific antibody with scFvs attached to the C-terminus of both heavy chains was evaluated on the cation exchange resin, Nuvia HR-S (Bio-Rad Laboratories). The BiS sample was purified by Protein A and hydroxyapatite chromatography. Analytical SEC HPLC showed the purified BiS sample to be homogeneous in nature, composed exclusively of monomeric protein. Equilibrium behavior of the BiS molecule was assessed using adsorption isotherms and linear gradient elution (LGE) chromatography. Adsorption kinetics were measured by fluorescently labeling the protein and visualizing transport through the resin bead using confocal laser scanning microscopy (CLSM). Two charge variants, accounting for ~20% of the sample, were identified from LGE and seen as displaced bands by CLSM. The main component and variants were preparatively isolated, fluorescently labeled with different dyes, and analyzed by multicomponent CLSM. Nuvia HR-S, the cation exchange chromatography resin used in this work, demonstrated sufficiently fast kinetics for displacement and separation of closely related bivalent bispecific antibody charge variants.

Biomarker Identification in Nonalcoholic Fatty Liver Disease via GC/MS

Brad Barrett,[1] Todd Richards,[1] David E. Alonso,[1] Xiang Zhang,[2] Joseph E. Binkley[1]
[1]LECO Corporation, St. Joseph, MI
[2]Department of Chemistry, University of Louisville, Louisville, KY

Non-alcoholic fatty liver disease (NAFLD) is a major medical problem in developed countries. NAFLD is associated with: increase obesity, insulin resistance, and metabolic syndrome. NAFLD is influenced by lifestyle: exercise, and diet. Research Samples & Study of 168 Rodent samples (original sample set, Pegasus 4D Study) were investigated for two critical factors: 1) Increased fructose consumption and 2) Inadequate Cu intake. Complementary investigation: Utilize a more sensitive instrument (20 fg) with extended linear dynamic range (105) to further characterize the samples. Analysis Objective: Identify potential NAFLD biomarkers in pooled rodent samples using high performance, benchtop GC-TOFMS.

Best Practices for Achieving Optimal Separations and Long Column Lifetimes in UPLC SEC of Proteins

Annie Landicho, Pamela Iraneta, Matthew Lauber, Susan Rzewuski, Bill Warren
Waters Corporation, Milford, MA

Size exclusion chromatography (SEC) is a technique commonly used throughout the development and commercialization of biotherapeutic proteins, such as monoclonal antibodies (mAbs). Ideally, these proteins and their molecular weight variants are separated based solely on their relative size in solution. ACQUITY UPLC Protein BEH SEC 200 Å, 1.7 µm columns are frequently used for the relative quantification of mAb monomers and associated high and low molecular weight species in less than 8 minutes. Several factors contribute to the generation of high quality and reproducible UPLC-based SEC separations of proteins. Included are the selection of an appropriately configured LC system, and the proper attachment of the column to the LC system. Particular attention will be given to practices for avoiding microbial contamination of the mobile phase and particulates from the samples.

Strategies to Evaluate and Monitor Forced Degradation Studies Using a Dual Detection (UV-MS) System

Annie Landicho, Paula Hong, and Patricia R. McConville
Waters Corporation, Milford, MA

Forced degradation studies are typically performed to understand the degradation pathway of pharmaceuticals. Mass balance is often part of this investigation and correlates the measured loss of a parent drug to the measured increase in degradation products, or impurities. Given the range of impurities and their chemical and physical properties, a single detection technique may not be adequate to accurately measure all of the degradants. Specifically, when ultra-violet (UV) detection is used alone, non-chromophoric species and/or co-elutions may be missed. Other issues can arise in the quantification of impurities observed in UV. The UV response of the impurity is typically assumed comparable to that of the active pharmaceutical ingredient (API); however, this is often times inaccurate. If the response of the impurity is incorrectly determined, over or under quantification of the impurity can occur. The result of any of these phenomena can lead to mass imbalance. To address the challenges of measuring and quantifying degradants, a dual detection system consisting of a photodiode array (PDA) and a mass detector (MS) will be used to analyze a stressed drug substance. While UV is typically used to assess and measure degradants, mass spectrometry allows for detection by an orthogonal technique and provides information to aid in characterization. For example, mass detection will be used to measure any non-chromophoric degradants that may be produced. The impact of missing degradants will be assessed. In addition, orthogonal detection will also be used to illustrate the impact of co-elutions on mass balance determinations. By assessing peak purity using both MS and UV, the final separation for the API and its degradants can be optimized to ensure no co-eluting peaks. The addition of MS information, whether for non-chromophoric species or co-elutions, will allow for a more complete mass balance evaluation and more comprehensive understanding of the degradation pathway.

Assessing Performance and Method Transfer of Monoclonal Antibody and Peptide Bioseparation Methods Using a Novel Biocompatible UHPLC System

Annie Landicho, Koshel, Zhang, Birdsall, Reed, Koza, Li, and Hong
Waters Corporation, Milford, MA

Many of the top-selling pharmaceuticals currently on the market and in the pipeline are biologics. Because biologics are more complex than small molecules, analytical methods for analysis and regulatory requirements tend to be less straightforward. To date, many of the methods used in development and quality control laboratories are HPLC-based. While this may be sufficient in some cases, there are noted advantages of updating legacy systems and methods with more modern instrumentation. Regulators also recognize and support the notion for improving process performance through lifecycle management in an effort to enhance product quality and patient safety. The product lifecycle includes development and manufacturing activities as well as technology transfers. When adopting new technology, it is of critical importance that instrumentation be robust and easily deployed. In this work, a new-to-market biocompatible UHPLC platform will be used to demonstrate the benefits of laboratory modernization in support of lifecycle management. Ion exchange, size exclusion, and peptide mapping are among the methods used to demonstrate equivalency across HPLC, UHPLC, and UPLC platforms. By updating from an HPLC platform, better resolution, shorter run time, and greater peak capacity can be achieved. As analytical assays are transferred to various in-house laboratories and contract organizations, it is imperative that results are consistent among sites. Method transfer across multiple instrument platforms is also demonstrated and assessed using retention time and peak area percent. By modernizing laboratory instrumentation, legacy methods can be successfully reproduced or updated to take advantage of new column technologies and lower dispersive systems for various improvements in performance.

Detection of Dopamine via Green Synthesis of Gold Nanoparticles Dipped Carbon-Fiber Microelectrodes

Dilpreet Raju, Alexander Mendoza, Pauline Wonnenberg, Sanuja Mohanaraj, Casey Culhane, Raquel Lara, and Alexander Zestos
American University, Washington, DC

HPLC was used to separate sugars (glucose, sucrose, fructose, and maltose) from raw honey samples to synthesize gold nanoparticles. Gold nanoparticles have increased focus due to their biomedical and material applications; however, gold nanoparticles produced from naturally occurring reducing sources and then used in application is understudied. Thus, the purpose of this study is to produce gold nanoparticles from naturally occurring honey, attach the gold nanoparticles to carbon fiber microelectrodes, and test the electrodes for dopamine detection. First, gold nanoparticles were produced at various ratios and with two different forms of honey: raw, untreated honey (referred to as AU) and grade A honey (referred to as PW). After production, the gold nanoparticles were either electrodeposited or dipped onto carbon fiber microelectrodes, followed by the testing for the detection of dopamine oxidation via fast-scan cyclic voltammetry (FSCV). The dopamine oxidative currents are higher with the Fructose (g4), AU Honey(g4) and PW Honey(g4) modified-CFMEs compared to the bare-CFME. This indicates that the modified-CFMEs possess larger electroactive surface area, leading to a higher rate of dopamine adsorption onto the electrode surface; thus, there is an increase in sensitivity towards dopamine oxidation with the modified-CFMEs.

Automated removal of phospholipids from membrane proteins for hydrogen deuterium exchange mass spectrometry workflows

Kyle W. Anderson, Elyssia S. Gallagher, Jeffrey W. Hudgens
Institute for Bioscience and Biotechnology Research, Rockville, MD
National Institute of Standards and Technology, Gaithersburg, MD

The majority of drug targets are membrane proteins; however, full-length membrane proteins are difficult to characterize by hydrogen deuterium exchange mass spectrometry (HDX-MS) due to their tendency to precipitate and aggregate in aqueous solutions. Membrane proteins can be solubilized in phospholipid bilayers, but phospholipids can impair liquid chromatography mass spectrometry (LC-MS) performance. We developed the first fully automated method to remove phospholipids from membrane proteins inline after deuterium exchange and prior to proteolysis and LC-MS. Denaturation, membrane removal, inline proteolysis, desalting, and analytical separation were all performed using a fully automated HDX PAL robot. Full-length FcγRIIa in liposomes was added to the base of a syringeless polyethersulfone nanofilter assembly (Thomson Instrument Company) containing quench buffer with 0.4 mg ZrO_2 to bind phospholipids. Nanofilter cartridge was pressed into filter base using a LEAP X-Press filtration station to separate denatured protein from phospholipids bound to ZrO_2 particles. Sample was digested by immobilized pepsin and analyzed by ultra-high performance liquid chromatography (UHPLC) MS/MS using a Thermo Orbitrap Elite. Only three lysophosphotidylcholines were detected after ZrO_2 treatment, demonstrating successful depletion of phospholipids. A 66% overall sequence coverage of FcγRIIa was attained with coverage across all domains. Presence of peptides from the transmembrane region demonstrated that phospholipids associated with this region were either not present or did not shield the transmembrane domain from digestion by pepsin. Our automated method provides higher throughput and lower measurement error than manual operation. Furthermore, this method enables efficient characterization of structural dynamics of full-length membrane proteins in lipid bilayers without truncation or mutation of membrane proteins for solubilization.

SEC/MALS/VISC/DRI study of mechanochemical copolymer degradation

Andre Striegel, Mallory Morris
National Institute of Standards and Technology, Gaithersburg, MD

Ultrasonic degradation is a convenient means of studying the degradation of macromolecules subjected to transient elongational stresses. Cavitation results in non-random, near-midchain scission of linear polymers, which ultimately reach a limiting molar mass Mlim beyond which degradation is no longer possible. The ultrasonic degradation of block, random, and alternating copolymer solutions of styrene-methyl methacrylate was studied, along with the degradation of their respective homopolymers, to isolate the effects of monomeric arrangement on copolymer degradation. Results from the degradation experiments were monitored by means of multi-detector size-exclusion chromatography. Decreases in both molar mass and size were observed for all polymers studied, except for those with molar mass near or below their Mlim. Differences in limiting molar mass were observed between the alternating and the other types of copolymers, indicating that monomeric arrangement influences ultrasonic degradation. Persistence length was identified as a key comparative parameter for a priori prediction of relative Mlim.

Size heterogeneity of NISTmAb RM 8671 by SEC

Katharina Yandrofski,[1] Alan Heckert,[2] Jim Fillibin,[2] John Schiel[1,2]
[1]Institute for Bioscience and Biotechnology Research, Rockville, MD
[2]National Institute of Standards and Technology, Gaithersburg, MD

The NISTmAb RM 8671 IgG1κ is intended to provide a well characterized, longitudinally available test material that is expected to greatly facilitate development of originator and follow-on biologics for the foreseeable future. Aggregation is a critical metric to establishing monoclonal antibody consistency and quality due to potential immunogenicity concerns. Therefore, a monomeric purity assay was optimized to evaluate and quantify the presence of aggregates using size exclusion chromatography (SEC). A central composite design optimization was conducted, resulting in a highly robust SEC assay. The optimized SEC method was used to (I) evaluate the homogeneity and stability of RM 8671; (II) assign monomeric purity reference values, and (III) establish the appropriate storage and handling conditions for the material.

Charge Heterogeneity Analysis of Intact Monoclonal Antibodies using CESI-MS

Melanie Juba, Esme Candish, St John Skilton, Marshall Bern, Bryan Fonslow
SCIEX Separations, Brea, CA

CESI-MS was employed to simplify the characterization of monoclonal antibodies (mAbs) at the intact level. Separating charge variants simplifies the spectra and facilitates the analysis of charge variants with small mass shifts. Using CESI-MS the charge heterogeneity, potential critical quality attributes (CQAs), and glycosylation profiles, can be determined in a single intact analysis.

Multidimensional Separation and HRMS Enhance Protein Identification from Limited Neuron Populations

Sam Choi, Camille Lombard-Banek, Pablo Munoz-LLancao, M. Chiara Manzini, Peter Nemes
University of Maryland, College Park, MD

Discovery proteomic characterization of single neurons raises new possibilities to understand cell-to-cell heterogeneity in the developing brain but requires sensitive detection technology to analyze proteins from limited population of neurons. High-resolution mass spectrometry (HRMS) is the current technology of choice for proteomic analysis due to its capability to identify proteins from large amounts of sample (usually from millions of cells). However, the technology is challenged in sensitivity to detect proteins from minute amounts of protein digest (~500 pg to 5 ng) that are afforded by a limited population of neurons, such as <10 neurons. Here, we present an ultrasensitive microanalytical platform that integrates multidimensional separation with HRMS to enable the characterization of proteins from a small population of neurons. We and others recently demonstrated that capillary electrophoresis (CE) HRMS enables trace-sensitive detection. However, CE analysis tends to produce compressed separation: majority of detected peptides separate across a 20–30 min window. This compressed separation challenges the MS/MS duty cycle, thus hampering sensitive peptide detection. To alleviate these complications, we implemented reversed phase fractionation prior to CE-nanoESI-HRMS platform to reduce sample complexity and enhance separation peak capacity. Briefly, protein digests (1–20 μg) from cultured mouse hippocampal neurons were fractionated on a C18 ZipTip cartridge using 10%, 20%, and 30% acetonitrile with 0.1% formic acid. From each resulting fraction, ~1/3 ng of protein digest was analyzed using CE-nanoESI-HRMS to evaluate the performance of our system. The hydrophobicity and electrophoretic mobility-based separations are orthogonal in nature, and therefore, combining these approaches improved peptide separation. Overall, this approach enabled the identification of 737 protein groups from 1 ng of digest, providing more than 31% improvement in protein identification compared to CE-nanoESI-MS without prefractionation. Additionally, the newly identified proteins belonged to the lower domain of the quantitative dynamic range, suggesting that this strategy increased detection sensitivity. Our results open a new possibility to characterize proteins from limited neuron populations.

Comparative Characterization of the Fc Domain N-Glycosylation in Monoclonal Antibody and Fusion Protein Therapeutics by CGE-LIF and UPLC-FL

Andras Guttman,[1] Marton Szigeti,[2] Robert Swart[1]
[1]SCIEX Separations, Brea, CA; [2]Horváth Csaba Laboratory of Bioseparation Sciences, University of Debrecen, Hungary

Comprehensive characterization of the N-linked carbohydrates of therapeutic antibodies and Fc-fusion proteins provides essential information about this important critical quality attribute. The most frequently used high performance liquid phase separation methods for carbohydrate analysis are ultrahigh pressure liquid chromatography (UPLC) and capillary electrophoresis (CE). Both methods require derivatization of the released sugars prior to analysis to support optical detection (commonly fluorescent). In case of capillary electrophoresis, a charged fluorophore should be used to support the electromigration process. One of the most frequently used fluorophore for carbohydrate labeling in CE is aminopyrenetrisulfonate (APTS) via a simple reductive amination reaction. This tag features excellent fluorescent characteristics and provides uniform labeling at the reductive end of the sugars only, i.e., one fluorophore per oligosaccharide. The same reductive amination based approach is used for UPLC analysis most of the time, however, in this instance the charge on the fluorophore is not of high importance. In most cases 2-aminobenazmide (2-AB) label is used for chromatography based analysis of carbohydrates. In this poster, the separation of fluorophore labeled N-linked carbohydrates, released from the monoclonal antibody therapeutics of adalimumab (Humira®) and the Fc-Fusion protein of etanercept (Enbrel®) is compared with the use of capillary gel electrophoresis with laser induced fluorescence (CGE-LIF) detection and UPLC with fluorescence (FL) detection. In both instances, standard industry sample preparation and separation parameters were used for the analysis.

Proteomic characterization of the embryonic neural ectoderm: understanding neural induction in Xenopus laevis

Aparna Baxi, Sally A. Moody, Peter Nemes
George Washington University, Washington, DC

When an embryo develops, parts of the embryonic ectoderm are induced to become the neural ectoderm, precursor of the central nervous system. Elucidating the molecular mechanisms that guide neural induction is essential to understand normal and disrupted development. In the South African clawed frog, Xenopus laevis, distinct cells of the 32-cell embryo have been mapped to have specific tissue fates. For instance, the midline dorsal-animal cells (named D111) are mapped to primarily form the neural ectoderm (NE). While neural induction has been studied at the gene and transcript level, the proteomic changes that shape the NE are still unexplored. Here we developed a bioanalytical framework to profile proteomic changes as these cells give rise to the NE. To characterize the protein expression of the NE, we integrated the classical embryological strategy of lineage tracing and dissection with high resolution mass spectrometry (HRMS). Our procedure began with the fluorescent labeling of the NE fated cells of the 32-cell embryo, followed by dissection of the resulting fluorescently labeled cell clone at gastrulation, when the NE is induced. The NE tissues were lysed, and abundant yolk proteins were depleted using density gradient centrifugation to enhance proteomic coverage. The remaining proteins were digested with trypsin. The resulting peptides were tagged with isobaric mass tags and fractionated using high-pH reversed phase fractionation. Each fraction was separated on a C18 nano-column coupled to an Orbitrap Fusion Lumos mass spectrometer for analysis. Acquired data were searched in Proteome Discoverer (version 2.2), peptide and protein identifications were filtered at <1% false discovery rate. From this measurement, we were able to identify ~2,800 protein groups from the developing NE tissues. Identified proteins included transcription factors that are involved in neural development. Our lineage specific approach to study proteins in the newly induced NE using high-resolution mass spectrometry promises to enhance our knowledge of proteins and signaling networks that regulate neural induction in embryonic stem cells during development.

Multiplexing Protein Quantification of Limited Yeast Digest using Capillary Electrophoresis ESI-HRMS

Vi Quach, Camille Lombard-Banek, Peter Nemes
University of Maryland, College Park, MD

High-resolution mass spectrometry (HRMS) is the technology of choice for the identification and quantification of proteins from typically tens-to-hundreds of micrograms of starting protein amounts. However, these experiments become challenging when protein amounts are severely limited, for example, from small biopsies, tissues, or populations of rare cells. In this study, we compared protein identification/quantification by conventional nano-flow liquid chromatography (nanoLC) HRMS to a custom-built CE HRMS platform. Proteins from a yeast lysate were reduced and alkylated before digestion by LysC. The resulting peptides were labeled with TMT 6-plex and mixed in predefined ratios (10:1 and 4:1). Analysis of ~20 ng of peptides by CE- HRMS identified 257 protein groups and quantified 238 proteins. In comparison, ~2 µg of peptides by conventional nanoLC-ESI-HRMS identified 698 protein groups and quantified 654 protein groups. It follows that detection of ~1/3 of the number of proteins from 100-times less starting material suggests ~30-times higher sensitivity by CE compared to nanoLC. Next, quantitative performance was evaluated based on the TMT ratios. Across n = 3 technical replicates, CE quantified 309 of 329 proteins with an expected 10:1 ratio (viz., 93.9% success rate) and 257 of 291 proteins with an expected 4:1 ratio (viz., 88.3 success rate). In comparison, nanoLC-HRMS quantified proteins with 93.6% and 91.0% success rate, respectively. These results suggest that CE-HRMS affords higher sensitivity for identifying and quantifying proteins from protein digests limited to nanograms of amounts, complementing the strengths of traditional nanoLC-HRMS for micrograms amounts of protein digests.

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WCDG holds regular meetings, typically on the third Wednesday of each month, from September to May at USP in Rockville, MD (unless otherwise noted). A dinner and social hour begin at 6PM, followed by a speaker at 7PM. We welcome you to join our discussions!

October 11	Melissa Phillips (NIST) at ACS HQ with Chemical Society of Washington
November 14	Andre Striegel (NIST) at USP
December 12	Speaker TBA at USP
January 16	James Grinias (Rowan Univ) at USP
February 20	Peter Nemes (UMD) at USP
March 13	Craig Byrdwell (USDA) at USP
April 15	TBA at Shimadzu Training Center with Washington-Baltimore Mass Spectrometry Discussion Group
May 15	Speaker TBA at USP

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