EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION 2023

Better life with Analytical Chemistry

Crowne Plaza Princeton
Conference Center
Plainsboro, NJ
November 13–15, 2023

ABSTRACT BOOK

eas.org

2024

CALL FOR PAPERS

November 18–20, 2024

EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION

Partners in Problem Solving

Crowne Plaza Princeton Conference Center, Plainsboro, NJ

EAS invites YOU to be a part of the Technical Program next year.
Contribute a paper for oral or poster consideration via our website:
Online submission opens March 1, 2024
www.eas.org/asubmit

EAS seeks contributions from scientists in many areas of analysis, which makes its program uniquely strong. Areas of interest include but not limited to:

Bioanalysis
Capillary Electrophoresis
Chemometrics
Conservation Science
Environmental Analysis
Forensic Analysis
Gas Chromatography
Liquid Chromatography
Immunochemistry
IR/NIR/Raman Spectroscopy
Mass Spectrometry
Laboratory Management
Laboratory Automation
NMR Spectroscopy
Pharmaceutical Analysis
Process Analytical
Quality by Design
Industrial Hygiene
Regulatory/Compliance
Sample Preparation
Science Education
Sensors
Separation Sciences
SFC & SEC
Science Surface Science
Vibrational Spectroscopy

2023 EAS Abstracts

This volume contains the final abstracts for the oral and poster presentations which take place Monday, November 13, through Wednesday, November 15, 2023. If an abstract is not provided in this volume or the Addendum, then the presenting author did not supply an abstract. For each abstract provided, a complete mailing address for the presenting author is shown. Additional authors are indicated, however, their mailing addresses are not provided.

More Information

To obtain answers to EAS-related questions after the meeting:

EAS Hotline	732-449-2280
EAS E-mail	askEAS@EAS.org
EAS Web Site	www.EAS.org

Eastern Analytical Symposium & Exposition, Inc.
P.O. Box 185
Spring Lake, NJ 07762

Save the Date

The 2024 EAS
November 18 - 20, 2024
Crowne Plaza Princeton – Conference Center
Plainsboro, NJ

We want you to be a part of the
63rd Eastern Analytical Symposium!

2024 Call for Papers opens March 1

1 Plenty of Room at the Top: From Molecular to Macroscopic Chemical Patterning and Characterization of Soft Materials

Shelley Claridge, Purdue University, Department of Chemistry and Weldon School of Biomedical Engineering, West Lafayette, IN 47907

Many early successes in nanoscience leveraged hard, crystalline materials and rigorous environmental control (e.g., vacuum, cryogenic temperatures) to achieve precise control over structure and function; such control also provides important advantages for characterization. However, many emerging materials applications, ranging from tissue scaffolds for regenerative medicine to wearable electronics, rely on soft, heterogenous materials and operate in complex real-world environments. Both aspects make structure more difficult to control and to characterize. Here we discuss a tandem approach to material design and characterization rooted in classic surface science. The interfacial assembly approach is scalable and robust, providing access to precise structural elements that can be integrated with conventional hydrogels and other soft materials to create new function. Importantly, high-resolution characterization is possible for the initial nanometer-resolution chemical patterning steps carried out on a hard crystalline surface, providing greater information about designed chemical environments in the final soft material product. We discuss applications of our new approach in tissue engineering and inorganic nanocrystal assembly as well as implications for generating new basis sets of hierarchical material design. Throughout, we also connect to themes from Mary Wirth's remarkable contributions to the understanding of materials and heterogeneity in chromatographic separations.
References: Bang, JACS 138, 4448 (2016); Villarreal, JACS 139, 11973 (2017); Porter, Chem 5, 2264 (2019); Davis, ACS Nano 15, 1426 (2021); Lang, ACS Nano 15, 10275 (2021); Shi, ACIE 60, 25436 (2021); Shi, ACS Nano 15, 15429 (2021); Arango, ACS AMI 14, 43937 (2022); Singh, JACS 145, 1668 (2023)

2 Drug Discovery Using Mass Spectrometry for Synthesis and Analysis

Graham Cooks, Purdue University, Brown Labs, Chemistry Dept., 560 Oval Dr, West Lafayette, IN 47907

We have explored accelerated organic synthesis in ESI microdroplets [1] Reaction mixtures analyzed by ESI give analytical information on the constituents but in small droplets the solvated ions react and products are detected in the mass spectrum. Rate constants increase inversely with droplet size and acceleration factors (vs. bulk) of ca. 104 are common. Reaction acceleration is an interfacial phenomenon favored in small droplets because of their high surface/volume ratios and by highly reactive species (water ion, hydronium ion, etc.) formed by the strong interfacial electric field. Products are formed in microdroplets of reagents as they fly (millisecond time scale) to a mass spectrometer. Their super-reactivity means that high throughput measurement of reactions in nanovolumes can be followed at rates of 1 sample/second in arrays of 6144 samples using automated MS equipment.[2] Reaction acceleration is the basis for (i) reaction screening, identifying the conditions that favor product formation, (ii) small scale synthesis, simply by collecting the small droplets in which reactions are accelerated and (iii) bioassays in which the products of synthesis interact with a protein or other drug target. High throughput (HT) reaction screening can be followed by HT synthesis and then by HT bioassays, including enzyme kinetic measurements.[3] Automated instrumentation is used for this drug discovery protocol.

References:
[1] Xin Yan et al. Angew. Chem. Int. Ed. 55 (2016) 12960–12972.
[2] Nicolás M. Morato et al. SLAS Technology (2021) 26 555–571.
[3] R. G. Cooks, et al. Israel J Chem. (2023) e202300034.

3 Interfacial Chemistry within Porous Chromatographic Silica: The Inside Story Revealed by Confocal Raman Microscopy

Joel M. Harris, University of Utah, Department of Chemistry, Salt Lake City, UT 84112, Jay P. Kitt, Grant J. Myres, David A. Bryce

In the development of stationary-phase materials, it is critical to understand how their interface structure relates to functioning as separation media for selective retention. To that end, we have adapted confocal Raman microscopy to examine the interior surfaces of porous chromatographic silica, reporting structural information about the stationary phase and its interactions with solvent and solute molecules. The spatial resolution of confocal microscopy can selectively probe the liquid-solid interface inside an individual porous particle, yielding quantitative information on populations of molecules on its interior surfaces. We have applied this methodology to investigate reversed-phase separations, to quantify interfacial populations of surfactants and solutes in ion-interaction retention, and to determine the role of n-alkyl chain order in shape selectivity of reversed-phase separations. Recently, we have extended these investigations to employ chromatographic silica as a high-surface-area support for selective separation and quantitative Raman scattering detection of molecules concentrated from solution. This concept can be applied to detecting trace-level PAH compounds from nanomolar solutions into 60-fL particle collectors. By depositing supported-lipid bilayers onto wide-pore silica surfaces, the association of unlabeled cell-signaling proteins with lipid-bound recognition sites can be quantified at model-membrane surfaces. Immobilization of DNA within porous silica particles offers a quantitative means of screening of small-molecule therapeutic candidates that bind to specific DNA sequences. Finally, detecting association of proteins with silica-immobilized DNA aptamers indicates that Raman microscopy is structurally-informative and label-free means of investigating DNA-protein interactions that are critical to biological regulation and function.

4 Surface Science Underlying Protein Chromatography

Mary Wirth, Purdue University, 560 Oval Dr., West Lafayette, IN 47907

The efficient separation of proteins is as important as it is difficult. The importance owes to the different biology of the various proteoforms, and the difficulty is from the large number of proteoforms and the limits of mass spectrometry in the analysis of overlapped bands. This presentation deals with the limits to separation of efficiency determined not by mobile phase mass transport but rather by the nature of the interactions with the surface that lead to slow sorption kinetics. The separation techniques of reversed-phase, hydrophobic interaction, and hydrophilic interaction liquid chromatography (HILIC) and ion chromatography will be discussed, with emphasis on therapeutic monoclonal antibodies.

5 Distribution and Transport of Microplastics and Associated Pollutants in New York Harbor Waters

Beizhan Yan, Lamont-Doherty Earth Observatory of Columbia University, 61 Rt. 9W, Palisades, NY 10964

Varying levels of microplastics were observed in NYC waters, with the highest concentration observed in Newtown Creek and the lowest in the Hudson upstream of New York Harbor. Additionally, in-situ adsorption experiments conducted in Newtown Creek and Brooklyn Navy Yard demonstrated the potential of microplastics to transport pollutants and pathogens including tuberculosis. Notably, flame retardant compounds such as PCBs and PBDEs were found on plastic pellet surfaces, with significantly higher adsorption on HDPE compared to PVC. Gene analyses revealed significant differences in microbial community between water and plastic samples. Using a modeling approach, we estimated that the residence time of microplastics in the Harbor-estuary region is approximately two weeks, with a predominant transport pathway down the New Jersey coast, eventually entering the Atlantic Ocean.

6 Rapid Single-Particle Chemical Imaging of Nanoplastics by SRS Microscopy

Naixin Qian, Columbia University, 405 West 118th St., Apt. 43, New York, NY 10027, Wei Min, Beizhan Yan

Micro-nano plastics originating from the prevalent usage of plastics have raised increasingly alarming concerns worldwide. In particular, nanoplastics are believed to be more toxic since their smaller size renders them much more amenable, compared to microplastics, to enter the human body. However, detecting nanoplastics impose tremendous analytical challenges on both the nano-level sensitivity and the plastic-identifying specificity, leading to a huge knowledge gap in this mysterious nanoworld surrounding us. To address these challenges, we developed a hyperspectral stimulated Raman scattering (SRS) imaging platform with an automated plastic identification algorithm that allows micro-nano plastic analysis at the single-particle level with high chemical specificity and throughput. We first validated the quantum enhancement of the narrow band of SRS to enable high-speed single nanoplastic detection below 100 n¬m. We then devised a data-driven spectral matching algorithm to address spectral identification challenges imposed by sensitive narrow-band hyperspectral imaging and achieve robust determination of common plastics polymers. With the established technique combining the best detection sensitivity and chemical specificity, we studied the micro-nano plastics from bottled water as a model system. We successfully detected and identified nanoplastics from major plastic types. Micro-nano plastics concentrations were estimated to be about $2.4\pm1.3\times10^5$ particles per liter of bottled water, about 90% of which are nanoplastics. This is orders of magnitude more than the microplastic abundance reported previously in bottled water. High-throughput single-particle counting revealed extraordinary particle heterogeneity and nonorthogonality between plastic composition and morphologies; the resulting multidimensional profiling sheds light on the science of nanoplastics.

7 Characterization of Microplastics by Using a Novel Method of Pyrolysis GC-MS

Ashok Deshpande, NOAA Fisheries, Sandy Hook, 74 Magruder Rd., Highlands, NJ 07732

Microplastics are contaminants of emerging concern that enter the aquatic environments from the breakdown of larger plastics and also from cosmetics, synthetic clothing, and industrial processes. Microplastics are a cause for concern because their size range overlaps with the preferred particle size ingested by the animals at the base of the aquatic food webs. The knowledge of polymer composition is important in the understanding of sources and risk assessment. We tested the utility of a novel method of pyrolysis GC-MS for the characterization of microplastic polymer types. In this method, a small piece of microplastic sample, less than 1 milligram in weight, is placed in a narrow quartz tube, which is then placed in a platinum coil and

heated to 750 degrees C. The intense heat breaks down the large plastic polymer chains into smaller fragments. These fragments are then transferred to, separated on a GC column, and identified using a mass spectrometer. The pyrolytic fragmentation patterns appear to be reproducible and unique to a given polymer type. The two-tier approach of peak fingerprints and mass spectra of marker peaks provides higher confidence in the data quality. In addition to polymer typology, the presence of additives and other chemicals in the plastics can be determined in the analytical run. The proof-of-same concept of pyrolysis GC-MS was tested in a variety of new plastic items and in the weathered plastic samples from the littoral and aquatic environments.

8 **Comprehensive Two-Dimensional Gas Chromatography: The Future of Nontargeted VOC Analysis**

Katelynn Perrault Uptmor, William & Mary, Department of Chemistry, P.O. Box 8795, Williamsburg, VA 23187

Multidimensional separations were originally developed in order to separate, identify, and characterize chemical molecules in highly complex samples due to compound quantity, class breadth, and dynamic range. Specifically, comprehensive two-dimensional gas chromatography (GC×GC) was first applied in the complex characterization of petroleum products but has since flourished into a commercially available technique widely demonstrated for many applications. Nevertheless, many industries still view this technique as being largely a research tool rather than a widespread tool for routine analysis. This presentation discusses the basic history, requirements, and utility of GC×GC as well as research conducted to reconfigure pre-existing GC systems to GC×GC systems for routine analysis. The fundamental setup of GC×GC with preexisting quadrupole mass spectrometry (qMS) systems will be shown as equivalent for quantifying target molecules while performing non-targeted analysis. In addition, we demonstrate key considerations on the presentation of GC×GC output to non-specialist users for decision-making purposes (e.g., juries, policy-makers, industry quality control, regulatory testing). Current results indicate that GC×GC is perceived similarly to one-dimensional GC output and is not considered to be more complex for comparison of samples to one another, providing a promising gateway for this type of data in routine analyses within our society.

9 **Adventures in Two-Dimensional Liquid Chromatography Separations of Therapeutic Oligonucleotides**

Dwight Stoll, Gustavus Adolphus College, Department of Chemistry, 800 West College Ave., Saint Peter, MN 56082, Daniel Meston, Maria Sylvester, Ajit Ghimire, Matt Sorensen, Todd Maloney

The analysis of therapeutic oligonucleotides is becoming increasingly important in the pharmaceutical industry, with several prospects for new drug products being in the pipeline. Thorough characterization of these materials at the level needed to ensure safety and efficacy is challenging, and it is becoming increasingly clear that conventional liquid chromatography (i.e., one-dimensional methods) separations are unable to fully resolve all possible impurities present in these samples. Two-dimensional liquid chromatography (2D-LC) is an attractive potential solution wherein critical pairs can be sampled from a first-dimension separation involving a solvent gradient steep enough to both retain and elute diverse species in the sample, followed by further separation in the second dimension with more selective elution conditions. In this presentation we discuss our initial efforts in this space where we have implemented a systematic approach to method development for 2D separations using ion-pairing reversed-phase (IPRP) separations in both dimensions. We find that the second dimension of the 2D system not only provides additional selectivity to enhance the resolving power of the first dimension but is also sufficiently sensitive to enable detection of impurities down to the 0.1-0.3% level. Finally, we will also discuss preliminary work aimed at the development of 2D separations using IPRP conditions in the first dimension and hydrophilic interaction liquid chromatography (HILIC) conditions in the second dimension. These separations have characteristics that complement ion paired reverse-phase (IPRP)-IPRP separations and deserve a place in the toolkit for oligonucleotide characterization by liquid chromatography.

10 **Trapping Mode Two-Dimensional Liquid Chromatography for Quantitative Low-Level Impurity Enrichment in Pharmaceutical Development**

Ziqing Lin, Bristol Myers Squibb, One Squibb Dr., New Brunswick, NJ 08903, Qinggang Wang, Yiyang Zhou, Jonathan Shackman

Trapping mode two-dimensional liquid chromatography (2D-LC) has recently found applications in pharmaceutical analysis to clean, refocus, and enrich analytes. Given its enrichment capability, 2D-LC with multiple trappings is appealing for low-level impurity monitoring that cannot be solved by single dimensional LC (1D-LC) or unenriched 2D-LC analysis. However, the quantitative features of multi-trapping 2D-LC remain largely unknown at impurity levels from parts-per-million (ppm) to 0.15% (w/w). We present a simple heart-cutting trapping mode 2D-LC workflow using only common components and software found in typical off-the-shelf 1D-LC instruments. This robust, turn-key system's quantitative capabilities were evaluated using a variety of standard markers, demonstrating linear enrichment for up to 20 trapping cycles and achieving a recovery of over 97.0%. Next, the trapping system was applied to several real-world low-level impurity pharmaceutical case studies including (1) the identification of two unknown impurities at sub-ppm levels resulting in material discoloration, (2) the discovery of a new impurity at 0.05% (w/w) co-eluted with a known impurity, making the undesired summation above the target specification, and (3) the quantification of a potential mutagenic impurity at 10-ppm level in a poorly soluble substrate. The recovery in all studies was better than 97.0% with RSD lower than 3.0%, demonstrating accuracy and precision of the 2D-LC trapping workflow. As no specialized equipment or software is required, we envision that the system could be used to develop low-impurity monitoring methods suitable for validation and potential execution in quality-control laboratories.

11 **LCxMS*y*: Exploring Different Combinations of Multi-Dimensional Liquid Chromatography with Multiple Parallel Mass Spectrometry**

William Byrdwell, USDA, ARS, BHNRC, MAFCL, 10300 Baltimore Ave., Bldg. 307C, Beltsville, MD 20705

Multi-dimensional liquid chromatography (MD-LC) is becoming more available and commonly reported. The conventional approach to comprehensive MD-LC is to place a detector at the outlet of the second dimension, ^{2}D, and to elute all analytes from the ^{2}D column in one modulation period. We have published multiple examples of unconventional approaches to MD-LC in which up to four mass spectrometers were used simultaneously in parallel for detection across two or three dimensions, LC*x*MS*y*. In LC2MS2 experiments, or LC1MS2 × LC1MS2, we employed dual parallel mass spectrometers, using different and complementary ionization techniques, (ESI, APCI, and APPI) in each of the two dimensions of 2D-LC. This was the first use of silver-ion UHPLC as the second dimension in 2D-LC and was used to separate triacylglycerols (TAGs) in seed oils containing *cis*- and *trans-fatty* acids. Next, we demonstrated LC3MS4, or LC1MS2 × (LC1MS1 + LC1MS1), which was 3D-LC with parallel second dimensions that employed four mass spectrometers using ESI and APPI distributed across the three dimensions of separation. We compared conventional single modulation elution to multi-cycle elution ("controlled wraparound"). Most recently, we combined the best aspects of the previous LCxMSy approaches for LC3MS3, or LC1MS1 × (LC1MS1 + LC1MS1), that used the Ag^{+} LC as the first second dimension, ^{2}D(1), and a multi-cycle separation as the second second dimension, ^{2}D(2). We demonstrate quantitative lipidomic analysis in both the first and second dimensions of separation of infant formula TAGs and pulse (dried legume seed) TAGs.

12 No abstract submitted by the author.

13 **Detecting and Quantifying Solid-Phase Impurities by Leveraging Morphological Differences Using Image-Based PAT**

Hossein Salami, Merck & Co. Inc., 2025 E Scott Ave, Rahway, NJ 07065

Detecting and quantifying impurities in a process such as synthesis of an active pharmaceutical ingredient (API) is an important task. Many processes constitute several side reactions producing various impurities, the concentration of which might be an important process and quality attribute impacting downstream steps. For example, unlike a single-phase reaction system, in a crystallization process impurity concentration might be referring not just to the solution phase, but also to the solid phase. The impurity might be present in the crystal phase forming, for example, co-crystals, or form a second solid phase by itself. Both cases of which are likely to be highly undesirable as crystallization is usually used as a purification method. Detecting solid impurities in such systems can be done using spectroscopic methods such as a Raman probe, provided a sufficiently strong signal. However, this might become challenging if there is not a strong signal, or if peaks from various components overlap. In this talk, we describe the potential of using image-based process analytical technologies (PATs) as an alternative for detecting solid-phase impurities or anomalies. Discussing few examples, including reactive-crystallization or spray drying processes, we describe how an image-based PAT can provide, potentially near real-time, information about the formation of impurities or anomalies in a system, provided that the impurity solid phase has morphologically distinct features. We also examine the potential of going beyond just detection and quantify the amount of such particles.

14 **Scalable Continuous Photochemical and Electrochemical Reactions: Reactors and PAT Challenges and AI: From Picoseconds to Tonnes**

Michael George, University of Nottingham, School of Chemistry, University Park, Nottingham, Nottinghamshire, NG7 2RD, United Kingdom

Photochemistry and electrochemistry are potentially very powerful tools for manufacturing not least because energy is delivered to reacting molecules far more selectively than by bulk heating in an atom efficient manner. Indeed, more than a century ago, Ciamician, presented a very powerful vision of where photochemistry could lead us [Science 1912, 36, 385-394]. Since then, photochemistry has become a major strand of chemical research in academia. By comparison, its penetration into

chemical manufacture remains comparatively modest because of a whole series of issues, mostly centred on the problems of carrying out large-scale photochemical reactions both efficiently and safely. In recent years we have been addressing some of the challenges of making photochemistry and electrochemical synthesis greener, more energy efficient and more widely accessible. This presentation will cover our activity particularly aiming for generic approaches for linking and scaling up multi-step processes in the context of photo-, electro- and thermal- chemistry on the kg/day scale with emphasis the synthesis of the anti-malarial drug Artemisinin as well as recent advances in simple reactor designs enabling 1-10 kg/day productivity in Taylor Vortex reactors with a very small footprint. Existing and new PAT approaches that exploit autonomous flow reactors and self-optimisation will be discussed with the aim of improving sensitivity, specificity, dynamic range and the speed of data acquisition, to be coupled with AI innovations.

15 **Codifying Tacit Knowledge for Data Modeling**

Brandye Smith-Goettler, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486

In pharmaceutical manufacturing, root cause analysis of anomalous production or laboratory events require extensive resources. Technical operations experts are diverted from their daily drug product delivery tasks to assist with the analyses. This redirection of resources can lengthen end-to-end lead times, alter long-range operating plans, and, thus, affect our ability to reliably supply medicines. The knowledge that these experts relay during an investigation is often already documented in reports, laboratory notebooks, and batch records. This presentation focuses on the use of data science to leverage existing information and to transform it to knowledge via contextualization. Use cases of natural language processing (NLP) and other advanced modeling techniques are discussed.

16 **Detection of Yellow Fever Virus in Human Remains Using Mass Spectrometry-Based Protein Analysis of Dental Pulp**

Kyra Miller, Rutgers University-Camden, 315 Penn St Camden, NJ 08102, Kimberlee Moran, Carla Cugini

The goal of this project was to determine if the yellow fever virus could be detected in historic remains by analyzing the proteins found in the dental pulp of the remains. Typical yellow fever diagnostic techniques rely on blood or liver tissue so when these tissues are not recoverable, yellow fever detection is currently limited. In order to meet the objectives of the study, a retrograde collection method was used to extract dental pulp on modern teeth and will be used on teeth from a known yellow fever victim and teeth from a historical cemetery site. The three different populations serve as a negative control, positive control, and the unknown population for the purpose of yellow fever detection, respectively. Following the dental pulp extraction, the pulp was subject to a protein extraction methodology and was analyzed via liquid chromatography-mass spectrometry (LC-MS). The aim of using mass spectrometry technology is to detect both blood proteins (in order to validate the dental pulp extraction method) and to detect yellow fever specific proteins (with the capsid protein being the primary protein of interest). If yellow fever specific proteins are detectable in the dental pulp, this research could prove valuable for the fields of archeology and paleomicrobiology.

17 **Determining the Variability in Color in Human Head Hair**

Emma Redman, Cedar Crest College, 100 College Dr., Allentown, PA 18104, Lawrence Quarino

Color has traditionally been used as a microscopic comparison parameter in human head hair analysis, despite the extent of natural color variation within an individual and between individuals not being known. This study is designed to measure inter and intrapersonal variation of color in head hair and to determine if color can be reliably used as a parameter in microscopic hair examination. Fifty hairs were collected from several people with visibly similar head hair. Each hair was mounted on glass slides with DPX mounting media (nD - 1.521) and examined at 200x using an Olympus BX53 polarizing light microscope under standardized illumination. Color measurements in the RGB system (red, green, blue) was performed using CellSens® software. Color measurements were taken at the most consistent points in the cortex, a region still under the skin. Median RGB values for each participant were grouped together and compared using principal component analysis and linear discriminant analysis. Results showed data overlap between individuals with little to no discriminate value. Similarly, overlap between individuals was observed qualitatively using color charts based on median hair values. Given the data generated both qualitatively and quantitatively in this study, color may have limited utility in microscopic hair comparisons.

Reference:

[1] Robertson J., Brooks E. A Practical Guide to the Forensic Examination of Hair From Crime Scene to Court, 2st Edition, 2021.

18 **A Novel FTIR Method for the Detection and Quantitation of Ruhemann's Purple in Latent Fingerprint Analysis**

Kira Bochard, Arcadia University, 8480 Limekiln Pike, Apt. 101, Glenside, PA 19095, Kimberlee Moran, Cynthia Tidwell, Heather Harris

The ninhydrin to Ruhemann's purple reaction is a commonly used forensic technique to detect fingerprints on porous surfaces. The deep purple color produced allows friction ridge imaging to be compared to various databases. Most forensic uses for ninhydrin only focus on the visual comparisons of prints; however, fingerprints also leave behind valuable organic content that should not be discounted in analysis. A novel method of Ruhemann's purple based detection was developed to determine whether age could be differentiated along with biological sex. This study explored the use of Fourier-transform infrared spectroscopy to detect the Ruhemann's purple molecule for latent prints treated with ninhydrin. Utilizing an Fourier transform infrared spectroscopy attenuated total reflection (FTIR-ATR) allowed for a non-destructive method that retained the image of the print. The target peak position for the Ruhemann's purple molecule was determined to be 1572 cm^{-1}. Fingerprint development and the deepness of the Ruhemann's purple coloration had no direct effect on the detected absorbance. Prints developed from younger donors had better resolution compared to prints from older donors, and male fingerprints developed more consistently than female prints.

19 **Evaluation of Gas Chromatography-Triple Quadrupole Mass Spectrometry for the Identification of Organic Gunshot Residues from Known Shooters and Non-Shooters**

Thomas Ledergerber, West Virginia University, Department of Chemistry, 1600 University Ave., Morgantown, WV 26509, Monica Joshi, Luis Arroyo, Tatiana Trejos

Gunshot residue (GSR) analysis is critical in the investigation of crimes involving a firearm. The forensic community has recognized the value of complementing the standard identification of particles of inorganic gunshot residue (IGSR) with organic gunshot residue (OGSR). Special attention has been given to liquid chromatography mass spectrometry (LC-MS) and gas chromatography (GC)-MS methods, due to their prevalence in forensic laboratories. However, single quadrupole GC methods have limitations to reach the required sensitivity. Therefore, this study evaluates gas chromatography-triple quadrupole mass spectrometry as an alternative technique. Standard solutions of compounds commonly included in smokeless powder formulations including 2-N-diphenylamine (2-NDPA), 2,4-dinitrotoluene (2,4-DNT), 4-N-diphenylamine (4-NDPA), Akardite II (AKII), diphenylamine (DPA), ethyl centralite (EC), methyl centralite (MC), and nitroglycerin (NG) were used to optimize and validate the method and were monitored during authentic sample testing. Twenty authentic OGSR samples were collected from the hands of a shooter immediately following firing. Additionally, 20 samples were taken from volunteers who had not fired or handled a firearm within 24 hours of sampling. Aluminum SEM stubs with carbon adhesive were used to lift residues from the subjects' hands. In this presentation, the method's optimized parameters and figures of merit are reported and compared to GC-MS and LC-MS-MS. The gas chromatography-triple quadrupole- mass spectrometry (GC-QqQ-MS) method detected various OGSR compounds collected from the known shooter's hands, producing a 100% true positive rate for authentic shooter samples. Moreover, the results yield 95% true negative rate for background samples taken from non-shooters. This method was shown to be sufficiently sensitive for analysis of OGSR recovered from skin specimens.

20 **Proteomics Analysis of Breast Milk for Early Detection of Breast Cancer: A Mass Spectrometry Approach**

Aneeta Arshad, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13676, Danielle Whitham, Brian T. Pentecost, Kathleen F. Arcaro, Costel C. Darie

Breast cancer (BC) is a leading cause of cancer-related deaths in women worldwide. Early detection of breast cancer is crucial. Invasive ductal carcinoma (IDC) is the most common subtype of BC, accounting for 85% of all new diagnoses of BC. It is a malignant tumor that starts in the milk ducts of the breast and invades the surrounding breast tissue. Human breast milk contains secreted proteins, immune cells and exfoliated epithelial cells, and can be collected non-invasively during an important time in breast development. Mass spectrometry-based (MS) proteomic experiments are ideal for the investigation of human breast milk proteins as potential BC biomarkers. Additionally, the MS-based methods allow for quantitation of the observed protein differences. In this study, we performed proteomics analysis on 10v10 human breast milk samples using mass spectrometry to identify protein biomarkers to aid in early breast cancer detection. Samples are collected from women who are actively lactating for both the BC and their age-matched controls. In-gel and in-solution digestions were preformed followed by nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) to identify proteins which are dysregulated from women with BC and the matched control. Proteins were found to be differentially expressed between breast milk samples from women with BC and without BC. This study will be confirmed with a larger cohort of breast milk samples for validation. If these proteins are found to be consistently dysregulated between BC

and the matched controls, a draft protein biomarker could be determined to aid in earlier detection of BC.

21 Proteomic Analysis and Comparison of Stage IIA T1N1 ER/PR Negative Breast Cancer Serum to Controls for Identification of Potential Biomarkers for Breast Cancer

Pathea S. Bruno, Clarkson University, Box 5810, 8 Clarkson Ave, Potsdam, NY 13699, Brian T. Pentecost, Costel C. Darie

Breast cancer (BC) is among one of the leading causes of death in women. BC tumors are classified by the presence of estrogen receptors (ER), progesterone receptors (PR), or human epidermal growth factor 2 (HER2) and by anatomic features (size and involvement of axillary lymph nodes). The early stages of BC can indicate dysregulation of proteins which can be characterized as biomarkers that can be used in early detection and include younger women as well. Using Breast Serum allows for detection via breast tissue directly, and analysis of intracellular and secreted proteins from the tumor. Mass Spectrometry is important for the study of proteomics because of its high sensitivity and ability to detect low abundant proteins. In this study serum samples from 5 women with ER PR negative breast cancer were compared to 5 age matched control counterparts. The 1N1 tumors were less than 20 mm in size with tumor cells found in up to 3 axillary lymph nodes. Samples were prepared via both in-gel and in-solution proteomic techniques followed by Nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) to identify proteins that are dysregulated between the matched pairs. This was performed in biological replicates with a total of 15 cancer samples and 15 control samples digested & analyzed. Raw data were then analyzed using ProteinLynx Global Server (v 2.4) Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. Any dysregulated proteins will be compared to those found in various cancer case studies in this project.

22 A Proteomic Investigation of Human Serum from Donors with Stage IIB Breast Cancer and Matched Controls to Identify Protein Biomarkers for Earlier Breast Cancer Detection

Danielle Whitham, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Panashe Mutsengi, Brian T. Pentecost, Costel C. Darie

Breast cancer (BC), is a leading cause of death for women globally. Invasive ductal carcinoma (IDC) is one of the most common subtypes, accounting for 85% of all BCs. Early diagnosis and treatment of BC is crucial. This study focuses on 8 women with stage IIB BC with tumors between 30-50mm across (T2) with invasion into 1-3 nearby lymph nodes (N1), and 8 age-matched controls. Lymph node involvement is the first stage of metastasis. Samples were chosen on the basis off the TNM anatomic staging system. One way to detect BC in its early phase is through identification of proteins that are dysregulated due to the onset of BC (i.e., protein biomarkers). Mass spectrometry (MS)-based proteomic methods are ideal for the investigation of serum protein biomarkers, as serum is an idea 'tissue' for screening studies. Utilizing MS-based proteomic methods, we are able to quantify protein differences from women with BC and without, which could lead to a protein biomarker for BC, therefore aiding in earlier diagnosis and treatment. A preliminary study using serum from women with BC and the matched controls, we use both in-gel and in-solution based proteomic methods followed by nano-liquid chromatography tandem mass spectrometry(nanoLC-MS/MS) to identify proteins which are dysregulated between the matched pair, but also quantify these proteins. If significant proteins are found to be consistently dysregulated between a large cohort, a protein biomarker could be determined to aid in the diagnosis, prognosis, and treatment of BC.

23 A Proteomic Investigation of Human Serum from Donors with Triple Negative Breast Cancer and Matched Controls to Identify Potential Protein Biomarkers for Breast Cancer detection

Norman Haaker, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Isabelle Sullivan, Danielle Whitham, Brian T. Pentecost, Costel C. Darie

Breast cancer (BC), one of the most common cancers, is a leading cause of death for women in the United States. An estimated 1 in 8 women in the United States will develop BC in their lifetime. Triple negative breast cancer (TNBC) is defined as cancer cells with less than 1% of all receptors such as estrogen, progesterone and HER2 receptors, thus being the most aggressive type of BC. Therefore, early diagnosis and treatment is crucial, and protein biomarkers could be a solution for this if significant dysregulations are found in the sera. Serum provides insight into what's happening in the body at the time of collection, making it an ideal 'tissue' for BC screening studies. Mass spectrometry (MS)-based proteomic methods are ideal to investigate protein biomarkers and are used in this study to search for protein differences in the serum between women with BC, their matched controls and biological replicates. 16 serum samples from women with TNBC and matched controls (8 vs 8) were investigated by an in-gel and in-solution digestion, followed by nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. The raw data was analyzed using ProteoWizard MSConvert (v. 3.0) Mascot Daemon server (v. 2.5), and Scaffold 4.3 software. The dysregulated proteins found in this study include apolipoproteins, alpha-1-antitrypsin family proteins and serpin peptidase inhibitors, which are both proteins previously found in breast milk studies. Other proteins include a variety of glycoproteins, and immune proteins.

24 A Proteomics Investigation of Human Sera from African American Donors with Invasive Ductal Carcinoma Breast Cancer and Matched Controls

Logan Seymour, Clarkson University, 10 Clarkson Ave, Potsdam, NY 13699, Panashe Mutsengi, Danielle Whitham, Brian Pentecost, Costel Darie

Cancer is among the top 5 causes of death in the United States and Breast cancer (BC) is the second most frequently diagnosed cancer with 287,250 new BC cases in women 2022. African Americans have the highest mortality and lowest survival rates of any ethnic group, with a 34% lifetime risk of developing cancer to be 34%, and a 41% likelihood of dying from breast cancer. There are two main types of invasive BCs: Invasive Ductal Carcinoma (IDC) and Invasive Lobular Carcinoma (ILC). In IDC, the cancer cells begin in the ducts, invade other parts of the breast tissue and may migrate to the axillary lymph nodes & beyond. Comparison of IDC serum samples with matched controls could identify dysregulated proteins that can potentially be used as protein biomarkers. In this investigation, we used a proteomics approach to compare sera from 3 African American donors with IDC and 3 matched healthy controls. The IDC samples were estrogen and progesterone receptor positive, and the tumors were classified as having lymph node involvement (pN1) or without lymph node involvement (pNX).The samples were prepared according to in-gel and in-solution digestion protocols and analyzed by nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. The resulting raw data were analyzed using ProteinLynx Global Server (v 2.4), Mascot Daemon server (v. 2.5), and Scaffold 4.3 software. The dysregulated proteins that were identified are currently being interrogated as potential biomarkers for breast cancer.

25 A Case Study Investigation for Biomarker Discovery: Proteomics Analysis of Sera from anAsian American woman with Triple Negative Breast Cancer and a Matched Controls

Hailey Morrissiey, Clarkson University, Department of Chemistry & Biomolecular Science, 49 Market St., Apt. 3, Potsdam, NY 13676, Panashe Mutsengi, Danielle Whitham, Brian Pentecost, Costel Darie

Breast cancer (BC) is a leading cause of death in women in the United States. Nearly 13% of women will develop BC in their lifetimes. Triple-Negative Breast Cancer (TNBC), in particular, is an incredibly aggressive form of cancer, due to its lack of specific receptors commonly observed in breast epithelium and BC. This form of BC expresses less than 1% of estrogen receptors (ER), progesterone receptors (PR), and HER2 receptors, making it the hardest BC to treat. It is imperative to diagnose BC efficiently and quickly; using a human serum test that utilizes protein biomarkers would allow for earlier diagnosis and treatment. We are using mass spectrometry (MS) based proteomics methods, to identify serum biomarkers that differ between a control and breast cancer case. In this study, serum samples from one Asian American woman with TNBC (classified as pT2 pN0), and a race- & age-matched control (1 vs 1) were analyzed using in-gel and in-solution trypsin digestion, followed by nano-liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) analysis, using a NanoAcquity UPLC coupled with a QTOF Xevo G2 MS. The in-solution based study was done with three biological replicates, to confirm the proteins identified. The raw data was then analyzed using ProteoWizard MSConvert (v. 3.0), Mascot Daemon server (v. 2.5), and Scaffold 4.3 software. Some dysregulated proteins include immune proteins, apolipoproteins, alphs-1-antitrypsin family proteins and serpin peptidase inhibitors, which all have roles in cancer/ tumor development.

26 Time- and Spatially Resolved Observation of Molecular Adsorption and Transport at Living Cell Membranes

Hai-Lung Dai, Temple University, Department of Chemistry, Philadelphia, PA 19122

Nonlinear light scattering in the form of second harmonic generation, due to its symmetry properties, has been proven effective for observing molecular adsorption and transport at the surfaces of colloidal objects, including living biological cells. This method affords membrane specificity, real time resolution, and the ability to image single cells in examining molecule-membrane interactions. This talk lays out the basic principles of second harmonic light scattering (SHLS) and illustrate how SHLS can be applied to examine molecular adsorption and transport at cell membranes. This method has been used to determine the fundamental mechanism of the century-old Gram stain for classifying bacteria. Examples illustrating effects of molecular structure and membrane structure in influencing molecular adsorption and transport at living cell membranes will be presented. SHLS applied in the imaging modality shows that transport rate varies greatly from regions to regions at a cell membrane. Furthermore, it will be shown that this second harmonic microscopic tool can be used to determine membrane phase transition and membrane asymmetry.

27 Second Harmonic Investigations of Bacterial Membrane Complexity

Tessa Calhoun, University of Tennessee, 418 Mossman, 1311 Cumberland Ave., Knoxville, TN 37996

The rise of antibiotic resistance requires new tools to probe the crowded and heterogeneous microenviroment of living membranes, the cell's physical barrier. Second harmonic scattering can quantitatively assess the adsorption and transport of small molecules through the membranes of living cells. With this approach, our group is gaining new insight into both the intrinsic and extrinsic factors that influence how small molecules traverse bacterial membranes. Specifically, this includes the small molecule structure, membrane fluidity and activity of efflux pumps.

28 Investigating the Influence of Chaperones on Desmin Fragment-Related Cardiomyopathy via Two-Dimensional Infrared Spectroscopy

Ariel Alperstein, University of Delaware, 163 The Green, Brown Laboratory Department of Chemistry & Biochemistry, Newark, DE 19716

Desmin is a key muscle protein found throughout the body that is implicated in heart disease. Under certain stress conditions, desmin fragments into amyloidogenic peptides that can then seed the breakdown of healthy desmin filaments. Chaperone proteins normally serve to sequester these misfolded fragments and prevent further aggregation. However, the native chaperone system can quickly become overwhelmed, leading to a buildup of damaged and/or misfolded proteins and cardiac dysfunction. Here, we investigate how the presence of chaperone proteins affects aggregation kinetics and aggregate structure. Using two-dimensional infrared spectroscopy (2DIR), we measured desmin fragments under aggregation conditions with and without isotope labeling to determine oligomer and amyloid β-sheet secondary structures. Isotope labeling revealed kinetics of a particularly amyloidogenic sequence. Results were verified with transmission electron microscopy and thioflavin T fluorescence assays. We then used 2DIR to measure these fragments in the presence of chaperone proteins found in the heart to determine their effect on the aggregation kinetics. Both αB-crystallin and heat shock protein 27 changed aggregation kinetics. These results will inform future imaging work on aggregate heterogeneity.

29 The Interactions of Electrolyte Solutions with Charged Monolayer Films at the Air/Water Interface

Paul Cremer, Penn State, Department of Chemistry, University Park, PA 16802

Infrared-visible sum frequency generation vibrational spectroscopy is a powerful technique for probing interfacial water structure adjacent to charged lipid and surfactant films. Information on the ordering of the alkyl chains as well as the headgroup hydration can be obtained. This presentation focuses on the roles that surface charge density, pH, headgroup identity, and salt concentration in the adjacent subphase play in organizing water molecules in the vicinity of the interface. Specifically, the chemical specificity of the cation plays an important role in interactions at negatively charged surfaces, while the identity of the anion can do the same at positively charged interfaces. The water layer and headgroup vibrational resonances are additionally perturbed based on the nature of the ion pairing interactions. These interactions can either involve contact ion pair formation or solvent shared ion pairing, depending on the particular conditions. Moreover, the interfacial water structure is dependent on the surface potential beyond the first hydration layer and can be analyzed via Gouy-Chapman theory.

30 Dairy Ingredients Safety for the Global Market

Erin Aungier-Markoff, Cayuga Milk Ingredients, 15 Eagle Dr., Auburn, NY 13021

With growing concerns for global food supply comes interest in food safety, in particular in the dairy industry. Dairy powder is used for infant formula which met a shortage of product this past year and brought to focus monitoring and testing. Infants are susceptible to contaminates, one being chlorate which inhibits the absorption of iodine, and both chlorates and perchlorates cause thyroid problems. Therefore, ingredient manufacturers are required to follow government regulations and/or customer specifications to control chlorate and perchlorate contaminants that can enter a product before or during manufacturing. Perchlorates tend to originate from environmental sources; whereas, chlorates originate from disinfectant by-products. These disinfectant by-products usually enter the product through water used during processing. CIP systems are essential and use an enormous amount of water. Disinfection is of paramount importance in the manufacturing of milk products due to milk's inherent risks of bacteria and potential foodborne illnesses. Utilizing ultra-pressure liquid chromatography tandem mass spectrometry (UPLC-MS/MS) for detection is an effective and essential technology for monitoring and quantifying chlorates and perchlorates in dairy products. As analytes, chlorate and perchlorate are small and negatively charged, making them challenging to detect and quantify. Hydrophilic interaction liquid chromatography (HILIC) is uniquely suited for the separation of these polar analytes. One needs the ability to adapt methods to different matrixes for continuous monitoring during the manufacturing of concentrated dairy powders to monitor the complex processing from feed to farm, to plant, to finished product.

31 Analysis of PFAS, Pesticides, and Mycotoxins in Food and Agriculture

Volker Bornemann, Avazyme, Inc., 2202 Ellis Rd., Ste. A, Durham, NC 27703

Mycotoxins are naturally occurring toxins that have contaminated foods for thousands of years. Europe has implemented some strict regulations for certain mycotoxins which has recently created some import issues for United States (US) grown peanuts. Our liquid chromatography mass spectrometry (LC/MS/MS) based analytical method can reliably measure 40 different mycotoxins at ppb levels in a large variety of foods and feeds. A similar approach allows us to accurately measure more than 400 man-made pesticide residues in raw agricultural commodities as well as processed food, feed, and beverages down to levels of 10 ppb or lower. Pesticides and their uses are strictly regulated by the US Environmental Protection Agency (EPA), enforced by the Food & Drug Administration, as well as the US Department of Agriculture (USDA) in organic food, who monitors 180 different pesticides in USDA Organic food production. A reactively new and fast-growing concern is centered around the man-made per- and polyfluoroalkyl substances (PFAS), which are a diverse group of thousands of chemicals used in hundreds of types of products. PFAS in the environment can enter the food supply through plants and animals grown, raised, or processed in contaminated areas. PFAS are also a concern in drinking water as well as irrigation water. PFAS degrade very slowly in the environment, which has led to the nickname "forever chemicals". Several PFAS molecules can be accurately measured at ppt levels and some even at single digit ppq (parts-per-quadrillion) levels with modern-day LC/MS/MS equipment. A major current limitation is the availability of certified reference standards.

32 Flexible Natural Toxin Methods for Food to Meet Current and Future Regulatory Requirements

Emily Britton, Waters Corporation, 34 Maple St, Milford, MA 01757, Narendra Meruva

Changing climates have resulted in an increased demand for natural toxin testing to ensure that products are compliant with various global regulations. In addition, many food and feed companies are becoming interested in performing due diligence testing for identifying trends and emerging threats. Industry standard methods exist for sets of mycotoxins and alkaloids in certain commodities, and advances in sample preparation and tandem mass spectrometry have enabled the development of sensitive and reliable multi-analyte methods. Sample matrix, detection requirements, number of analytes, and instrument sensitivity play critical roles in determining a fit-for-purpose workflow. This presentation includes an overview of general mycotoxin and alkaloid test methods, flexible options for sample extraction and clean up, and innovations in liquid chromatography and mass spectrometry that enable low level, reproducible, and streamlined workflows capable of meeting current and future regulatory requirements.

33 Multi-Residue GC-MS/MS Analysis of Pesticides in Infant Food

Douglas Stevens, Waters Corporation, 34 Maple St., Milford, MA 01757, David Gould, Stuart Adams, Simon Hird, Frank Dorman

Gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS) is a powerful analytical technique used for the detection and quantification of pesticide residues in food and environmental samples, traditionally using helium as a carrier gas. There has been difficulty sourcing helium worldwide in recent years, leading to supply instability and significant spikes in cost. This has created demand for alternative carrier gases for gas chromatography. Nitrogen is readily available, inexpensive, inert and safe compared to other options such as hydrogen. This work demonstrates the transfer of GC- atmospheric pressure chemical ionization (APCI) MS/MS methods run with helium carrier gas to nitrogen using an atmospheric pressure gas chromatography (APGC™) ionization source. APGC demonstrates improvements in selectivity and sensitivity when compared to electron ionization (EI) analysis due to the generation of abundant molecular ions. Furthermore, APGC achieves consistent chromatographic performance when using helium or nitrogen carrier gas. A routine method of >200 pesticides was run on a Xevo™ TQ-S micro System using both carrier gases. Extracts of infant food (cottage pie and cucumbers) were also prepared for pesticide analysis using nitrogen as the carrier to show the equivalent performance of each gas when using APGC. Switching between carrier gases while maintaining separation efficiency and sensitivity is achieved using a scaled column of smaller diameter combined with lower carrier gas flowrate for nitrogen.

34 Determining the Time Since Deposition (TSD) of Variable Heated Bloodstains Utilizing Raman Spectroscopy and Chemometrics

Alexis Weber, University at Albany, SUNY, 1400 Washington Ave., LSRB 1113 Albany, NY 12205, Igor K. Lednev

Blood traces are commonly found at crime scenes and can provide substantial information about the event that occurred and individuals involved. Determining the time of crime is an important goal for crime scene investigations, which can be achieved by estimating the time since deposition. If crime scenes contain multiple sets of bloodstains, the TSD allows for the selection of bloodstains relevant to the crime;

and therefore, reduce the number of samples which would be collected. Vibrational spectroscopy has shown provide reliable, rapid, and non-destructive methodologies to determine the TSD of bloodstains. However, research with these techniques so far have analyzed the aging of bloodstains, specifically the degradation of hemoglobin, under ambient conditions. However, crime scenes are not always in such pristine environments and degradation rate of hemoglobin is commonly affected by the surrounding environment. Therefore, it is necessary to develop a model that is capable of estimating the TSD of bloodstains in different environments. There are infinite varieties of environmental conditions. Our goal is to determine how potentially high temperature conditions affect the aging mechanism of bloodstains. For this purpose, fresh blood samples were collected so that no anticoagulants were present, which potentially can affect the ex vivo aging mechanism of blood. The bloodstains were then aged in a controlled heated environment and tested at numerous time points post deposition. After the spectra were collected, they were loaded into statistical software for preprocessing and modeling. The reproducibility of heated blood analysis and TSD determination model are discussed.

35 Investigating the Dynamics of Soil Chemistry and Its Related Microbiome Through Liquid Chromatography and Mass Spectrometry

Jessica Grace Prudence Hay, Deakin University, School of Life and Environmental Sciences, 75 Pigdons Rd., Waurn Ponds Victoria, Australia 3216

This project aimed to address some challenges facing forensic soil analysis by examining both the chemical and microbial methods used for the discrimination of soil samples. A comprehensive chemical analysis approach, focusing on ultra-high-performance liquid chromatography, UV-visible spectroscopy and mass spectrometry was used to develop a directly translatable method for the modern forensic testing laboratory, coupled with modern genomic approaches to correlate the chemical and microbial data. Extraction and analysis of soil samples taken from the southeast of Australia from March 2020 to May 2023 occurred with a focus to interrogate key questions surrounding soil evidence including the effect of time, storage conditions, and different geographical features. Through principal coordinates analysis of the UV-visible chromatograms and mass spectra, it was found that this method could discriminate between locations, timepoints, and age points, and therefore, for the first time, shows that the method may offer a quick and reliable method for forensic soil analysis. Soil microbiome changes were investigated through the burial of 192 5 cm2 pork skins, wrapped with different fabrics (synthetic and natural) or without fabric, buried at the four locations, and excavated at 5, 19, 54, and 252 days after burial. It was found that fabrics directly influence the microbial diversity on pork skins, and was accompanied by variable biofilm formation, mould, and staining patterns. The data generated here highlights the importance of both soil chemistry and soil microbiome investigations for forensic analysis.

36 The Application of Particle-Correlated Raman Spectroscopic Analysis of Soils to Mock-Casework Scenarios

Samantha Gong, University of New Haven, 241-90 Oak Park Dr., Douglaston, NY 11362, Marisia Fikiet, Peter De Forest, Brooke Kammrath

Soil is a continuous but complex mixture, reaching across geological bodies in combination with its highly transferable nature, the complexity of soil composition makes it valuable material for forensic trace evidence and object-to-scene association. One analytical method with excellent potential for forensically relevant samples is Raman spectroscopy, which has a demonstrated history of use for mineral identification. Particle-correlated Raman spectroscopy (PCRS) is a novel method that combines automated image analysis with Raman spectroscopy, providing microscopic-morphological and chemical information about samples non-destructively. When applied to soil, this information includes mineral identification, microscopic-morphological characteristics, and particle-size distributions. In this project, PCRS is used to analyze soil particles collected from simulated evidence samples. Mock-evidence was collected from three geographical locations. Known soil samples were also collected to serve as references. The mock-evidence items were prepared in a method detailed by Stoney et al. for the analysis of very small particles. The collected adhering soil was then cleaned to isolate the mineral grains per the method described by Palenk. The particles in the range of 90nm-180nm in diameter were then dispersed onto a Raman-inactive microscope slide and analyzed using PCRS. The results were compared to the reference samples that were treated and analyzed with the same method. Source consistency could then be determined using a set of match criteria that includes mineral identity, particle morphology, and ¬¬¬composition percentage. Determining the source of soil collected from mock-evidence by using PCRS was demonstrated to be possible when reference samples from suspected sources are available for comparison.

37 Capabilities and Limitations of Particle Correlated Raman Spectroscopy (PCRS) for the Analysis Forensic Soil Minerals

Jasmine Kaur, University of New Haven, 300 Boston Post Rd., West Haven, CT 06516, Joshua Christensen, Ella Galvan, Ethan Groves, Skip Palenik, Chis Palenik, Peter De Forest, Marisia Fikiet, Virginia Maxwell, Brooke W. Kammrath

This study evaluated the capabilities and limitations of Particle Correlated Raman Spectroscopy (PCRS) for the analysis of soil samples. Given the long-stated criticisms of forensic soil analysis (e.g., subjective and time consuming), there is a need for an automated system which can provide an efficient and statistically comparable approach to the interrogation of soil samples. PCRS, an integrated technique that combines image analysis with Raman spectroscopy, has the ability to provide morphological and chemical information from a mixture of discrete particles. To develop PCRS for inclusion in a forensic soil workflow, the limitations and advantages of the method need to be evaluated, which has been the goal of the research that will be presented. For the evaluation of PCRS as a tool to analyze soil minerals, single-blind PCRS was completed on four unknown, four-component mixtures of comminuted minerals and an additional ten soil samples. Following the dispersion of particles on a Low-e slide, 90-180 mm fraction of minerals was analyzed using image analysis and Raman spectroscopy using two lasers excitations (532 nm and 785 nm). The resulting Raman spectra were identified via spectral library searching of the RRUFF2 mineral database. The results of the PCRS method were then compared to those obtained using traditional methods for mineral identification, including polarized light microscopy and scanning electron microscopy equipped with energy dispersive X-ray spectroscopy. Similarities and distinctions of the results between these approaches have been evaluated to explore the utility of the present PCRS method for use in forensic soil casework.

38 Transport and Deposition of Emerging PFAS Through Rainfall

Jennifer Faust, College of Wooster, 943 College Mall, Wooster, OH 44691, Yubin Kim, Kyndal Pike, Jameson Sprankle, Donald Conley, Rebekah Gray, Paul Edmiston

PFAS, or per- and polyfluoroalkyl substances, are anthropogenic chemicals that are ubiquitous in the environment, toxic to humans and wildlife, and highly resistant to degradation. PFAS released into the atmosphere can undergo regional and long-range transport, and they have been detected in rainwater all over the world. Here we characterize PFAS profiles in rainwater across a regional network in the central United States. Using liquid chromatography triple quadrupole mass spectrometry and liquid chromatography quadrupole time-of-flight mass spectrometry, we identified and quantified or semi-quantified ten PFAS from EPA Method 1633, as well as 23 PFAS not included in United States Environmental Protection Agency (EPA) methods; we classify this latter group as emerging PFAS. Notably, hexafluoropropylene oxide dimer acid (trade name GenX), which is a replacement for legacy PFAS, was detected at all sites at concentrations up to 5 ng/L. The dominant species measured across all sites was trifluoroacetic acid (CF3COOH), which reached concentrations as high as 1200 ng/L. Non-targeted analysis revealed series of H-substituted perfluorocarboxylic acids and fluorotelomer carboxylic acids present with highly branched isomeric distributions. The isomeric profiles varied from site to site, providing indirect evidence that distinct sources influenced PFAS deposition across the geographic region. Overall, these results show that a significant fraction of PFAS in environmental samples will be missed if monitoring occurs only for linear isomers of PFAS listed in EPA methods. Our ongoing measurement campaigns seek to assess connections between atmospheric and terrestrial PFAS profiles through concurrent measurements of PFAS in rainwater and surface water.

39 Developing and Testing of Passive Samplers for Dissolved PFAS

Rainer Lohmann, University of Rhode Island, Graduate School of Oceanography, South Ferry Rd., Narragansett, RI 02882, Jitka Becanova, Matthew Dunn, Jarod Snook

Several poly- and perfluorinated alkyl substance (PFAS) have become a global concern due to their persistence, long-range transport, bioaccumulation and adverse effects. Recent regulatory decisions have resulted in maximum contaminant levels (MCLs) in drinking water range from 2 ng/L in Denmark to tens of ng/L for several PFAS combined in several states in the US. The United States Environmental Protection Agency (EPA) has proposed maximum concentration levels for several PFAS in drinking water (PFOA and PFOS at 4 ng/L each, with PFNA, PFHxS and Genx at 9-10 ng/L and PFBS at 2,000 ng/L), which highlights the analytical challenge of measuring such low concentrations. Passive sampling (a process relying on diffusion to enrich PFAS in a sorbent of choice) might provide a means of obtaining representative concentrations of PFAS in surface, groundwater and ocean water. The use of passive samplers enables the determination of time-weighted concentrations, which is often more relevant compared to snapshot sampling. The presentation discusses the different approaches and tools being currently developed and used to measure PFAS, and their applications. Recently developed, characterized and published samplers include those operating in the linear uptake phase (“kinetic samplers”) and equilibrium samplers. For example, we presented an equilibrium

passive sampler based on nanographene, which enriches PFAS by several orders of magnitude. In contrast, a polyethylene-based tube passive samplers operates in the kinetic uptake phase for weeks. In addition, results from passive samplers in water will be used to assess their utility to correlate and predict concentrations in biota.

40 Investigating the Role of Coastal Wetland on Per- and Polyfluoroalkyl Substances (PFAS) Transport

Mi-Ling Li, University of Delaware, 15 Innovation Way, Newark, DE 19711

PFAS are a group of anthropogenic chemicals linked to adverse effects on wildlife and humans. Few studies have assessed how PFAS are transported and distributed in coastal watersheds. Salt marshes have the potential to remove many contaminants, but little information is available on their role in mediating PFAS transport. Here, we assess the spatial distribution and composition of PFAS in a tidal salt marsh and investigate how geochemical and hydrological conditions affect their fate and transport. We use St. Jones Reserve, a tidal marsh positioned between a military base and Delaware Bay, as the study site. We collect groundwater, porewater, and sediment from seven sites that span across three marsh zones with contrasting geochemical and hydrological conditions, and surface water from the tidal creek at high and low tide. The results show composition profiles of PFAS vary across the different environmental media and marsh zones. We find that dissolved organic carbon content can largely explain the spatial variability of PFAS in marsh waters. The groundwater from the unconfined aquifer next to a tidal creek generally has higher PFAS concentrations than that in the surface water of the tidal creek. In contrast, the groundwater samples from deeper aquifer exhibit very low levels of PFAS contamination. These results indicate the history of recent PFAS contamination in the area. The findings of this study will improve our understanding and PFAS mobility in the environment and the factors that affect their fate and transport as they migrate to the ocean.

41 PFAS Method Development and Bioaccumulation in Long Island Sound

Kaitlyn Campbell, University of Connecticut, 3107 Horsebarn Hill Rd., Storrs, CT 06269, Jessica Brandt, Christopher Perkins, Anthony Provatas

Per- and polyfluoroalkyl substances (PFAS) are a group of organic chemicals with anthropogenic origins that are commonly detected in seafood and can lead to human exposure via dietary consumption. The United States is the third largest producer of shellfish; thus, it is vital to develop a straightforward and simplistic method to monitor concentrations in seafood products. The present study used a quick, easy, cheap, effective, rugged, and safe (QuEChERS) method followed by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) to determine 28 PFAS compounds in Eastern oyster tissue. This method was validated using 12 unknown composite samples collected from a recreational shellfish bed with suspected sources of PFAS pollution to the aquatic environment. Oysters were suspended in lantern nets at four locations, including one reference site, for two months during the summer of 2022 and bioaccumulation was calculated. Method detection limits (MDLs) ranged from 3.7 to 20.5 ng g^{-1} at the 25 ng mL^{-1} level and recoveries spanned 52.1 to 105.9% at the 100 ng mL^{-1} analyte level. Targeted analysis of unknown samples revealed concentrations up to 8.4, 7.2, 5.2, 3.0, and 2.2 ng g^{-1} for PFHpA, PFPeS, PFHxA, PFOA, and PFOS, respectively. Mean sum concentrations were two times higher at sites near suspected PFAS sources (12.4 – 12.7 ng g^{-1}) compared to the reference site (6.2 ng g^{-1}). This validated method proved to be an inexpensive and efficient way to quickly screen oyster samples and could help mitigate human exposure by providing highly accurate and rapid results.

42 Deoxyguanosine Monophosphate In-Vitro Reaction with Artemisinin Derivatives: LCMS Identification of Reaction Products

Emilio Roman-Flores, Rowan University, 311 Spruce St., Absecon, NJ 08201, Amos Mugweru

Artemisinin and its derivatives are popular in the treatment of malaria. New literature suggest they could be used in management of different forms of cancer. In this study, Artesunate was incubated with Deoxyguanosine Monophosphate nucleobase and the reaction monitored using liquid chromatography-time-of-flight (LC/TOF) mass spectroscopy. This reaction was also monitored in presence of iron, a common element found in vitro. The reactants were incubated at 37° Celsius and was analyzed at different time periods. Artesunate and the selected nucleobases peaks appeared at different retention times. As time progressed peak heights decreased and new peak signatures corresponding to new product formation was observed. Inclusion of iron in the reaction mixture heavily influenced the rate of product formation. Information about specific molecules formed is discussed.

43 Simultaneous Determination of Naphazoline Hydrochloride and Pheniramine Maleate along with their Related Compounds by High Performance Liquid Chromatography (HPLC)

Fadi Alkhateeb, Waters Corporation, 34 Maple St., Milford, MA 01757

In recent years, the United States Pharmacopeia (USP) has undertaken a modernization effort to update outdated analytical methodologies in its monographs. The focus is on the main sections of monographs, which include identification, assay, and organic impurities. A key element of the modernization process is minimizing the use of hazardous solvents and reagents in the analytical procedure. One way to address this concern is to use a single chromatographic method to analyze multiple active materials and their related compounds. In this study, we demonstrate the combination of three USP chromatographic methods into a single HPLC method for analyzing two APIs (naphazoline hydrochloride and pheniramine maleate) and their related compounds. A comprehensive evaluation of system suitability, range, accuracy (recovery), intraday and interday precisions are presented as a part of this study. The application of the method to the analysis of samples obtained from commercially available ophthalmic and nasal solutions are also demonstrated and discussed.

44 Re-Development of a Fit-For-Purpose Assay Degradation HPLC Method Using Open Lab Software

Deliana Arias, Merck & Co., Inc., Small Molecule Analytical Research & Development, 126 E. Lincoln Ave., PO Box 2000, Rahway, NJ 07065, Zack Zhiqiang Guo

Phase 1 clinical compound A is a small molecule compound with good oral bioavailability and long half-life A fit-for-purpose high-performance liquid chromatography (HPLC) Assay/Degradation method was developed during the clinical release for first in human (FIH) studies. However, it was noted that later the assay method has robustness issues. Unknown peaks were identified above International Conference on Harmonization (ICH) reporting threshold which were later identified to be non-active pharmaceutical ingredient related. Method optimization is warranted to assure correct stability assay and deg level quantitation at each time point. ACD/Labs and fusion quality-by-design (QbD) HPLC column screening tools allowed us to evaluate the chromatographic conditions comprised of six stationary/mobile phase combinations. The mobile phases utilized ranged from mass spectrometry (MS) compatible to non-MS compatible, and cover a wide range of pH, and organic solvent strengths. The selection of the mobile phase had a significant impact on the baseline performance. Screening tools offered information on critical method parameters that allowed a selection of the optimized chromatographic conditions. Optimization of the gradient condition using these column screening tools was rapid and significantly reduced the cost, labor, and waste production.

45 GC-FID Analysis of an Industrial Perfluorinated Alkylamine in Novel Vehicles to Support In Vivo Studies

Fabricio Beltran, Charles River Laboratories, 1407 George Rd., Ashland, OH 44805, Nikita Navalkar, Bo Tokarski, Geoff Clay

Per- and Polyfluoroalkyl Substances (PFAS) are chemicals that have been used to make industrial coatings for a variety of applications. PFAS compounds are considered “forever chemicals” due to the amount of time it takes for these compounds to break down. Because of their widespread use for industrial applications and release into the environment, PFAS concentrations have increased globally, and exposure to these chemicals has been linked to health concerns in humans and animals. To better understand the effects of these compounds, in vivo toxicology studies are needed and conducted. The class of PFAS compounds includes thousands of molecular subtypes, including perfluorinated alkylamines (PFAA). PFAA compounds can be used in a variety of industrial applications, including coolants and fuel cells. To help support in vivo toxicology studies for a recently investigated industrial PFAA, two novel vehicles were designed to provide effective suspensions for two different animal species. These vehicles provided better suspensions than what had been previously employed for in vivo toxicology studies. Further, a novel gas chromatography-flame ionization detection (GC-FID) method, coupled with headspace sampling, was developed to facilitate dose formulation analyses for these two vehicles. This poster presentation reports novel vehicles and analytical methodology which could potentially be applied for dosing and/or analysis of low-solubility PFAA compounds in other in vivo studies.

46 Liquid Chromatographic Gradient Method Allowances Provided by General Chapter, USP <621> Chromatography

Catharine E. Layton, Waters Technologies Corporation, 34 Maple St., Milford, MA 01757, Paul D. Rainville

The United States Pharmacopeia (USP) portfolio of solutions addresses quality assurance, enhances regulatory predictability, and helps manufacturers distribute quality medicines, dietary supplements and foods. On Dec 1, 2022, a harmonized standard for General Chapter <621> Chromatography was released. This standard incorporates <621> Chromatography (USP), 2.2.46. Chromatographic Separation Techniques (EuPh) and 2.01 Liquid Chromatography (JP) texts. New additions to the chapter provide the limits of flexibility when using validated, gradient LC method

separation parameters such particle size, flow rate, gradient slope, and injection volume. In this work, we utilize the gradient method adjustments described in USP Chapter <621> to demonstrate the method modernization provided for an antiviral drug impurities monograph.

47 Improvements in the Analysis of Synthetic Peptide Enfuvirtide and HAART Drugs Utilizing MaxPeak™ High Performance Surfaces (HPS)

Brianna Clements, Waters Corporation, 34 Maple St., Milford, MA 01757

HIV is a virus that affects millions of people every year. If left untreated, it can progress into AIDS, which causes those infected to be further immunocompromised and susceptible to various ailments and cancer. AIDS is the cause of death for hundreds of thousands of people every year. There have, however, been advancements in treatment options for HIV. These treatments include Highly Active Anti-Retroviral Therapy (HAART) combined with synthetic peptides such as enfuvirtide. To address the need for a rapid, modern analytical method capable of separating the current standard of HIV treatment, we developed a linear and reproducible HPLC-UV/MS method that facilitates separations nearly 4x faster than previous methods. Further, we demonstrated the benefits of using MaxPeak HPS, when compared to traditional stainless-steel systems, for synthetic peptide analysis. The work presented here showed that MaxPeak HPS proved to be more reproducible when compared to traditional stainless steel systems. In addition, MaxPeak HPS offers increases in peak area and height signals for enfuvirtide, leading to lower limits of quantitation. MaxPeakTM is a trademark of Waters Corporation.

48 Dissolution Method Development for Anisotropic Dosage Forms: Quantitating Release Rate for 3D Printed Capsules

Michael Barrett, Merck & Co., Inc., 126 E Lincoln Ave., Rahway, NJ 07065, Joseph Della Rocca, Derrick Smith, Ashish Punia, Andre Hermans, Melanie Marota

3-dimensional printed (3DP) dosage forms can be used to control the API release rate in a clinical setting. Using a fused deposition modelling technique, we generated a simple 3DP dosage form consisting of water insoluble polymer components with water soluble slits and a liquid fill containing active. During development, the symmetry of the dosage form led to anisotropic behavior during dissolution, which in turn, lead to highly variable dissolution profiles. As a result, we developed non-compendial dissolution to fully quantify the effects of formulation and dosage form changes. Here, we show the development of a robust USP4 dissolution method to quantify the dissolution profiles of the 3DP dosage forms. These methods allow reliable and reproducible data to compare various formulations, printing parameters, and printer run to printer run variability of the capsules, advancing the development of 3D printed dosage forms.

49 Strontium Ion Assay for Coral Tank Monitoring via Ion Chromatography with Suppressed Conductivity Detection

Kayry Segarra, University of Maryland Baltimore County, 1000 Hilltop Cir., RM 100 Meyerhoff Chem Building, Baltimore, MD 21250, William LaCourse

Coral reefs are at risk due to an increase of coral bleaching events brought on by anthropogenic causes. Corals provide a biodiverse habitat for roughly 25% of marine life and are critical for the survival of coastal regions, therefore, strategies to mitigate risks to coral survival are of great importance. Part of the efforts to conserve coral include rehabilitation in aquatic tanks. Literature is limited regarding coral tank monitoring; assays for coral tank monitoring could provide information about coral health. Strontium is one of the conservative cations in seawater and is chemically similar to calcium found in the coral calcium carbonate skeletal structure, however, its detection is complicated by the overwhelming presence of the other conservative cations. Here we present an improved method for the assay of strontium utilizing ion chromatography with suppressed conductivity detection (Dionex ICS 2100). The method resulted in full resolution of Sr^{2+} from the other conservative cations. A calibration curve was made in the range of 1-20ppm; the LOD and LOQ were found to be 0.4ppm and 1ppm, respectively. The improved method was then utilized to analyze coral tank samples demonstrating the ability to detect Sr^{2+} at low levels.

50 Proteomics Analysis of Sera from a Hispanic Woman with Triple Negative Breast Cancer and a Matched Control: A Case Study Investigation for Biomarker Discovery

Angiolina Hukovic, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Danielle Whitham, Brian T. Pentecost, Costel C. Darie

Breast cancer (BC), found most commonly in women, is a leading cause of death in women in the United States. Nearly 13% of women will develop BC in their lifetimes. Triple-Negative Breast Cancer (TNBC) in particular, is an aggressive form of cancer due to its lack of specific receptors commonly observed in breast epithelium and BC. This form of BC lacks estrogen receptors (ER), progesterone receptors (PR), and HER2 (human epidermal growth factor receptor 2) receptors, making it the hardest BC to treat. It is imperative to diagnose BC efficiently and quickly. A human serum test, utilizing protein biomarkers would allow for earlier diagnosis and treatment. We are using mass spectrometry (MS) based proteomics methods, to identify serum biomarkers that differ between controls and breast cancer cases. In this study, serum samples from one Hispanic woman with TNBC (classified as PT2 pN0), and a race- & age-matched control (1 vs. 1) were analyzed using in-gel and in-solution trypsin digestion, followed by nano-liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) analysis, using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. The raw data was then analyzed using ProteoWizard MS Convert (v. 3.0) Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. The dysregulated proteins found in this study are being compared proteins from a previous discovery biomarker experiment using human breast milk. The dysregulated proteins are also confirmed to be true positives by comparison to a larger cohort of serum from women with BC and without.

51 Proteomics Analysis of Sera from an Asian American woman with Triple Negative Breast Cancer and a Matched Control: A Case Study Investigation for Biomarker Discovery

Celeste A. Darie, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Danielle Whitham, Panashe Mutsengi, Brian T. Pentecost, Costel C. Darie

Breast cancer (BC) is the most common cancer among women, and a leading cause of death in the United States. Triple negative breast cancer (TNBC) is an aggressive form of BC devoid of receptors that are found in the breast epithelium. TNBC lacks expression of estrogen receptors(ER), progesterone receptors (PR) and HER2 . The lack of receptors makes TNBC difficult to treat and for women with TNBC struggle to stay in remission. A test for BC biomarkers using human serum will be a useful supplement to mammography in diagnosing and monitoring BC. We are using mass spectrometry (MS) based proteomics methods, to identify serum biomarkers that differ between controls and breast cancer cases. In this study, a serum sample from one Asian American woman with TNBC (classified as PT2 pN0), and a race- & age-matched control (1 vs 1) were analyzed using in-gel and in-solution trypsin digestion, followed by nano-liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) analysis, using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. The raw data was then analyzed using ProteoWizard MS Convert (v. 3.0) Mascot Daemon server (v. 2.5) and Scaffold 4.3 software. Dysregulated proteins found in this study could aid in diagnosing a protein biomarker in human serum for women with BC. We note that this is a small case study, and will be compared to our larger ongoing serum studies such as a 48v48, and previous breastmilk studies to confirm dysregulated proteins identified here.

52 A Proteomic Analysis of Human Serum from Donors with Triple-Negative Breast Cancer and Matched Controls to Identify Protein Biomarkers for Breast Cancer Detection

Jerome Strong III, Clarkson University, Box 5810, 8 Clarkson Ave., Potsdam, NY 13699, Danielle Whitham, Panashe Mutsengi, Brian T. Pentecost, Costel C. Darie

Breast Cancer is one of the most common cancers among women, with approximately one in eight women developing it in their lifetime. One of the most aggressive forms of BC is Triple Negative Breast cancer (TNBC). These cases are difficult to treat as the cancer cells score negative for estrogen receptor (ER), progesterone receptor (PR), and a human epidermal growth factor receptor 2 (HER2). Because of this, early detection is important for efficient treatment. Early stages of BC can show dysregulation or proteins, leading to potential use as biomarkers. Mass Spectrometry (MS) Is highly specific, which allows it to be reliable in identifying protein dysregulation. This study uses MS-based proteomics to identify differences between the proteins found in human serum from 8 women who have TNBC and their 8 control counterparts. Samples were analyzed via in-gel and in-solution digestion, subsequently by Nano liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. Raw data are under analysis using ProteinLynx Global Server (v 2.4) Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. Dysregulated serum proteins will be compared to other dysregulated proteins found in a number of breast cancer studies from this lab that have focused on breast milk and serum of affected women.

53 Monitoring the Estrogen-Inducible Proteins in Lake Trout (Salvelinus namaycush) from Great Lakes upon Exposure to Environmental Contaminants

Taniya Jayaweera, Clarkson University, Biochemistry & Proteomics Group, Department of Chemistry & Biomolecular Science, 8 Clarkson Ave., Potsdam, NY 13699, Bernard Crimmins, Sujan Fernando, ThomaS Holsen, Costel C. Darie

A major goal of the current project is to monitor expression of some liver proteins in lake trout (Salvelinus namaycush; collected from the Great Lakes) upon exposure to environmental contaminants. Some of these contaminants, like polychlorinated

biphenyls (PCBs), have estrogenic activity (estrogenic disrupting compounds or EDCs). Two important fish proteins whose levels change upon exposure to EDCs are vitellogenin and zona radiata proteins. These are prime candidates for our study in lake trout for several reasons: a) the levels of these proteins are increased in lake trout relatives such as atlantic salmon (Salmo salar) and rainbow trout (Oncorhyncus mykiss) upon exposure to EDCs, b) Both proteins are produced in male fish only upon exposure to EDCs, c) both proteins are produced only in the liver (and secreted in the blood). Here, we use proteomics to analyze fish homogenates from three Great Lakes (Erie, Huron & Michigan) and to identify the vitellogenin and zona radiate proteins, the two major liver proteins whose expression changes upon EDC exposure. We have good identification of vitellogenin in all three samples, but we did not yet identify the zona radiate proteins. The study is ongoing and once we find the zona radiate proteins, we will monitor the two proteins in a larger number of fish, to identify the lake trout that expresses these proteins because of the exposure to EDCs. This, in turn, will allow us to monitor the influence of EDC contaminants in the lake trout from Great Lakes.

54 Targeted Lipidomic Analysis of Fatty Acids in Marine Dietary n3-PUFA Supplements

Yifan Li, Columbia University, 175 Payson Ave 4B, New York, NY 10034, Fereshteh Zandkarimi

Marine dietary supplements such as fish oils are in high demand as a source of omega-3 polyunsaturated fatty acids (n3-PUFA) mainly eicosapentoneic acid (EPA) and docosahexaenoic acid (DHA). Consuming a reasonable dose of dietary supplements can reduce inflammation, decrease the chance of cancer, reduce anxiety, and protect the heart. While omega-3 fatty acids take the spotlight, fish oil also contains a certain amount of saturated fatty acid which has an impact on health. Considering the growing markets for marine dietary supplements as functional foods, comprehensive profiling methods are highly needed. We developed a targeted mass spectrometry-based lipidomics analysis in combination with liquid chromatography to quantify the fatty acid compositions of different fish oil supplements. The result exhibited noteworthy differences in the content and the concentration of fatty acids among various types of fish oil. Overall, this fatty acid analysis may provide us with a better insight into the origin and quality of n3- PUFA oils supplements.

55 Determination of Illegal Dyes in Chili Powder Using UPLC-MS/MS

Stephanie Nauth, University of Central Florida, 4000 Central Florida Blvd, Orlando, FL 32816, Andres Campiglia, Christian Febres Collazo

Under the Federal Food, Drug, and Cosmetic act, only color additives approved by the Food and Drug Administration (US FDA) are legal for use in foodstuffs. Unfortunately, despite FDA regulations, illegally added, hazardous synthetic dyes have been shown to be present in adulterated food products. Numerous chromatographic methods have been developed for the analysis of hazardous dyes in food and beverage products. When performing chromatographic analysis on dyes, different conditions are required for different chromophores such as triphenylmethane, xanthene, anthraquinone, and azo dyes. However, many dyes are not suitable to use ultraviolet spectroscopy or fluorescence spectroscopy due to their low optical activity and the ones that are still face interference from the matrix itself which can cause problems with identifying the dyes in the spices. Herein, we investigate the chromatographic behavior of four xanthene dyes (Acid Red 87, Acid Red 51, Rhodamine 101, and Basic Red 1), one quinone imine dye (Basic Red 2) and one triphenylamine dye (Basic Red 9) for their determination in chili powder samples via Ultrahigh Performance Liquid Chromatography-Mass Spectrometry/Mass spectrometry (UPLC-MS/MS). Chili powder is a very common spice used around the world. Part of its popularity stems from chili powder being a mixture containing other spices such as cumin and oregano and powdered peppers such as cayenne and/or paprika. Our studies will focus on chili powder and seasoning mixes obtained at local markets in the Orlando, FL area.

56 Assessments and Comparisons of Chiral Chromatography with Fully Porous Particles and 2.7-um Superficially Porous Particles in HPLC and SFC

Edward Franklin, Regis Technologies, Inc., 8210 Austin Ave., Morton Grove, IL 60053, Melissa Wilcox, Gay Lowden

Chiral chromatography is an effective means to separate enantiomers and other stereoisomers using various modes of chromatography (e.g., normal phase, reversed-phase, supercritical fluid, etc.) at both analytical and preparative scales. In recent years, new column technologies have improved chiral analysis speeds and efficiencies and sought to address challenging separations with innovative chiral stationary phase chemistries. In this work, we assess the enantioseparation performance of fully porous particles (FPPs) and superficially porous particles (SPPs) with the Whelk-O1® chiral stationary phase (CSP). Specifically, we compare 1) kinetic performance, 2) retention characteristics, and 3) sample loadability.

57 Fractionation of Complex RNA Mixtures for Enhanced Modification Mapping via LC-MS/MS

Cassandra Herbert, University of Cincinnati, 312 College Dr., Crosley Tower 404, Cincinnati, OH 45219, Jennifer Kist, Patrick Limbach

Mass spectrometry, especially LC-MS/MS, has become one of the most prominent methods for identifying and mapping post-transcriptionally modified nucleosides to their respective RNA sequences. With the development of oligonucleotide mapping programs, -our lab- among others, has shown that it is possible to map modifications on previously unknown RNAs in complex mixtures, specifically tRNA. However, higher level organisms transcribe hundreds of tRNAs increasing sample complexity and making it difficult to map low abundance tRNAs. To overcome this challenge, we seek to reduce sample complexity before LC-MS/MS analysis via fractionation. The method we have developed utilizes an offline anion exchange chromatography separation step. These tRNA fractions are then digested enzymatically to oligonucleotides that are amenable to analysis via LC-MS/MS. The developed method has been applied to full-length commercially purchased yeast tRNAs. After fractionation, an RNase T1 digestion was performed before oligonucleotide separation using ion-pair reverse phase chromatography tandem mass spectrometry. Unique digestion products were identified and detected in higher abundance in specific fractions compared to others indicating an abundance of the observed tRNA within the fraction. Mapping results, obtained from RNAModMapper, demonstrate a significantly higher number of digestion products mapped to their respective tRNA sequences after fractionation in comparison to the unfractionated sample. Overall, we demonstrate that our method can enhance modification mapping via LC-MS/MS and confidence in alignment using oligonucleotide mapping software.

58

Withdrawn by the author.

59 Synthesis, Characterization, and Electrochemical Properties of Derivatized Naphthoquinone Electron Acceptors

Brinda K. Narayana, Rensselaer Polytechnic Institute, Department of Chemistry and Chemical Biology and The Baruch ’60 Center for Biochemical Solar Energy Research, 110 8th St., Troy, NY 12180, Megan Schlogl, Patrick Landry, Vasily Kurashov, John H. Golbeck, K.V. Lakshmi

Quinones are naturally occurring isoprenoids that are widely exploited by photosynthetic reaction centers (RC) (e.g., the bacterial RC, photosystem II (PSII) and photosystem I (PSI)). The versatility and functional diversity of quinones is in part due to the diverse redox potentials that are controlled by the substituent effects and by ‘smart matrix effects’ of the protein-binding pocket. For example, both the Type I PSI and Type II PSII RCs contain quinone cofactors that serve very different functions as the redox potential of similar quinones can operate at up to 800 mV lower reduction potential when present in PSII. However, the factors that determine quinone function remain unclear. It is thought that the structure and location of the quinone cofactor, the geometry of its binding site, and ‘smart’ matrix effects from the surrounding protein environment greatly influence the redox potential. To develop a systematic understanding of the effects of substituents on the electronic structure of quinones, we investigate a series of 2-methyl-1,4-naphthoquinone (menadione, MD) models linked to viologen acceptors using phytyl tethers of varying length. By examining the changes in redox potential using cyclic (CV) and differential pulsed voltammetry (DPV) of the MD models in aprotic and protic solvents, we correlate the electronic structure of the derivatized quinone with the nature of the molecular substituents and hydrogen-bonding interactions. This provides direct insight on the tuning and control of quinone cofactors in biological solar energy conversion through photosynthesis.

60 Analytical Impacts on the Belzutifan Next-Gen End-game Chemistry Development

Hong Li, MRL, Merck & Co., Inc., 126 E Lincoln Ave, Rahway, NJ 07065, Rekha Gangam, Wenjun Liu, Diane Lee, Clara Hartmanshenn, Timothy Wright, Jiaxuan Yan, Daniel Dirocco, Jonathan Mcmullen

Belzutifan is a first-in-class oral small molecule HIF-2α inhibitor, approved in several markets for the treatment of Von Hippel–Lindau (VHL) disease associated with Renal Cell Carcinoma (RCC). In an effort to improve manufacturing efficiency and reduce costs, a next-generation chemistry has been developed to support additional indications including RCC. This new chemistry utilizes elegant biocatalytic steps that enable the shortest possible route from commodity chemicals to API. This presentation will focus on the analytical challenges faced during the development of the end-game chemistry to support next-generation chemistry. During development, a mass balance was observed, where a thorough investigation revealed that the mass balance gaps were caused by polymerization /oligomerization during the SNAr step. These oligomerizations presented a unique challenge to the analytical design to develop a world-class method to monitor all process-related impurities and accurately determine mass balance while supporting the route development and implementation of processes to eliminate and control the oligomerization. In combination with mass balance gaps, the team noted the coloration of the resulting API associated with the oligomer content. To address the coloration and provide measurable an-

alytical data a color of solution method was developed to monitor and assess the oligomer content as a complementary method. The challenges and conclusions presented here-in describe how the analytical tools were employed to drive the team to an optimized synthetic route generating high-quality API.

61 Analysis of WCU Andean Headwear Collection

Sadie Patterson, West Chester University of Pennsylvania, Allegheny Hall 737-A, 121 W. Rosedale Ave., West Chester, PA 19383, Zachary Voras

This project investigated the chemical composition of a collection of indigenous Andean hats and headdresses in the West Chester University Museum of Anthropology and Archaeology. Utilizing micro-x-ray fluorescence (XRF) and Fourier-transform infrared (FTIR) spectroscopy for elemental identification and molecular analysis, respectively, a combination of natural and synthetic materials were found in the headwears' production. The bases of the hats tend to have wool or cotton present, while additional decor such as ribbons and embroidery tends to be synthetic. More information is needed to determine whether many synthetic decorations were added during or after the production process. Areas of further interest and investigation are located in beadwork, sweatbands, and powder residue. The data gathered during this project will be used for advising museum studies students and faculty on proper handling of artifacts, best practices for caring for and storing the collection, and for the creation of a database to make museum collections more accessible to university and non-university agents.

62 AQbD and Green Chemistry Principles Driven Method to Determine Mycophenolate Mofetil Impurities-Identification of Degradation Products by QToF LCMS

Siva Krishna Muchakayala, Catalent Pharma Solutions, 1100 Enterprise Dr., Winchester, KY 40391, Naresh Kumar Katari, Vishnu Murthy Marisetti

Developed a systematic and eco-friendly analytical method utilizing quality-by-design (QbD) and green chemistry principles. Initially, the critical method parameters (CMPs) were screened using a D-optimal design. The robust final method conditions were optimized using a systematic central composite design (CCD). Through graphical and numerical optimization, the method conditions were augmented. The pH of mobile phase buffer (25 mM KH_2PO_4) (MP-A), initial gradient composition (% MP-A), flow rate (mL min^{-1}), and column oven temperatures (°C) are 4.05, 87, 0.4, and 30, respectively. The best possible separation between the critical pairs was achieved while using the Waters Acquity UPLC BEH C18 (100 × 2.1) mm, 1.7 µm analytical column. A mixture of water and acetonitrile in the ratio of 30:70 (v/v) was used as mobile phase-B for the gradient elution. The analytical method was validated in agreement with ICH and USP guidelines. The specificity results revealed that no peaks interfered with the impurities and MPM. The mean recovery of the impurities ranged between 96.2 and 102.7%, and the linearity results r >0.999 across the range of LOQ – 150%. The precision results (%RSD) ranged between 0.8 and 4.5%. The degradation products formed during the alkali degradation were identified as isomers of mycophenolic acid and sorbitol esters using Q-ToF LCMS and their molecular and fragment ion peaks. The developed method eco-friendliness and greenness were assessed using analytical greenness (AGREE), green analytical procedure index (GAPI), and analytical eco score, and found it is green.

63 Cipher Software as a Tool for an Improved Data Integrity for Dissolution Testing in GMP Space

Monika Baraniak, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903

Data integrity (DI) reaffirms the pharmaceutical industry's commitment to manufacture drugs that are safe, effective and fulfil quality standards. At the same time, DI is a crucial tool for regulatory authorities to use in protecting public health. In order to improve the data integrity of standalone bench top instrument used for dissolution operation in a GMP laboratory, a commercially available software developed by Distek was evaluated and implemented to eliminate the manual entry or copy/paste of instrument operation raw data into Electronic Laboratory Notebooks (ELN). Distek Cipher software is a Dissolution Control Software that captures real time dissolution and sampling raw data. Furthermore, an automatically generated report by the software after each dissolution run can be transferred electronically into ELN via a secure data path from a designated data repository, thus providing a solution for an increased DI and Compliance as well as improved efficiency for dissolution testing in GMP space.

64 Replacing Electropolished Stainless Steel Tubing with a Silicon-Based CVD Coating for Higher Inertness, Lower Wet-Up and Dry Down Performance, and Greater Corrosion Resistance for Gas and Fluid Transfer

Jesse Bischof, SilcoTek Corporation, 225 PennTech Dr., Bellefonte, PA 16823

The lead times and cost for electropolished tubing rose rapidly during the COVID-19 pandemic. There were two key drivers for this price increase. First, many supply chains broke down and it became very difficult to purchase non-electropolished stainless steel tubing, let alone quality electropolished tubing. Second, the large domestic growth in the semiconductor and pharmaceutical industries along with their demand for electropolished tubing placed strain on an already stressed market. This led to many organizations seeking alternatives to electropolished tubing. Here we show how a silicon-based chemical vapor deposition (CVD) coating on non-electropolished tubing can offer improved inertness for trace level transfer of sulfur compounds, ammonia, and *volatile organic compounds (VOCs)*, reduced wet-up and dry down times for moisture analysis, and improved corrosion resistance in acidic, high salt, and other aggressive environments.

65 To Mix or Not to Mix: Effect of Using Solvent Blends as Modifier in Chiral SFC

Daipayan Roy, Amgen, One Amgen Center Dr., Thousand Oaks, CA 91320, Larry Miller

SFC is extensively used in both academia and industry for resolution of enantiomers for analytical and preparative applications. Hundreds of new chiral compounds are being synthesized daily and with limited arsenal of chiral stationary phases newer ways to separate said compounds on existing columns must be developed. In this study we look at using solvent blends as modifiers in chiral SFC. A variety of solvents including methanol, ethanol, isopropanol and acetonitrile were mixed in different proportions to elucidate the effects of the blends. A total of seven stationary phases including coated stationary phases Chiralcel OJ, OD, and immobilized phases Chiralpak AD, AS, IC, IG, and (S,S)-Whelk-O 1 were used for this study. We have tested a wide array of commercially available analytes and report the differences in selectivity when using different modifier blends to answer the question whether such blends result in enough novel selectivities which would warrant their use in chiral SFC screening.

66 Polysaccharide-based Chiral Stationary Phases for the Separation of Atropisomers

John Ferraro, Daicel Chiral Technologies, 1475 Dunwoody Dr., Ste. 310, West Chester, PA 19380, Weston Umstead

Atropisomerism is a form of chirality which can arises from an axis of rotation within a molecule. This rotation allows for two distinct conformational forms, which like enantiomers, can have different effects on the body when administered as a pharmaceutical. Often the energy of these two conformational forms is similar and makes the synthesis of a single atropisomer challenging. Chiral chromatography can offer a fast and effective way to isolate each enantiomer for biological testing and can scale to commercial scale production to meet demand. This work focuses on the analytical and preparative method development and optimization of several relevant examples currently available on the marketing, including Sotorasib, the first-in-class KRAS inhibitor, and Telenzepine, a selective M1 antimuscarinic, along with others.

67 Gefapixant Citrate (MK-7264) Sulfonamide Step Speciation Study: Investigation into Precipitation-Dissolution Events During Addition of Chlorosulfonic Acid

Nelo Rivera, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Ryan Cohen, Si-Wei Zhang, Zachary Dance, Holst Halsey, Siqing Song, Xiaodong Bu, Mikhail Reibarkh, Hong Ren, Alfred Lee, Darryl Chang, Sachin Lohani

Sulfonamidation is the pre-penultimate step in the commercial synthesis of gefapixant citrate. Mechanistic understanding of this step was critical for impurity control in the drug substance. Several stress studies were performed during process characterization for this step to ensure process robustness and to mitigate potential upset scenarios. One parameter explored was the slow addition of chlorosulfonic acid (CSA) to form the sulfonic acid intermediate. During the extended addition, an unexpected series of precipitation – dissolution events were observed. To better understand the reaction mechanism and accommodate the CSA tank switch during the reaction, a detailed investigation using a variety of analytical techniques was performed which revealed the formation of various protonated species of both the diaminopyrimidine starting material and sulfonic acid intermediate with stark differences in their solubility properties. These speciation events explained the changes in solubility behavior observed in the reaction system during the course of CSA addition. The full understanding of step 4 speciation supported the CSA addition procedure that was ultimately adopted for commercial process to ensure operation robustness.

68 Novel End-Capping Method with Silyl-Reagent Including Ethylene Chain

Norikazu Nagae, ChromaNik Technologies Inc., 6-3-1 Namiyoke, Minato-ku, Osaka, 552-0001, Japan

Trimethylsilyl reagents (TMS) have been widely used as end-capping reagents for silica-based reversed-phase liquid chromatography packing. As a new concept, 1,2-bis(dimethylchlorosilyl)ethane, which was bonded the methyl groups of two

trimethylchlorosilane reagents, was thought to function as a denser end-capping reagent than trimethylchlorosilane. In this study, we evaluated a reversed-phase packing with both 1,2-bis(dimethylchlorosilyl)ethane as an end-capping reagent and a high temperature reaction to convert the silanol groups into siloxane bonds. A core-shell silica was used as the silica base material. An ethylene chain is expected to provide high stability of its packing material under basic pH condition and 1,2-bis(dimethylchlorosilyl)ethane as an end-capping reagent contains an ethylene chain in the molecule, so that the proposed core shell C18 column was compared with hybrid type core shell C18 columns. As a result, the proposed C18 column not only exhibited almost same stability as hybrid type core shell C18 columns under basic pH conditions, but also showed much higher stability than hybrid type core shell C18 columns under acidic pH conditions. Regarding inertness towards basic and metal chelating compounds, the proposed core shell C18 column exhibited almost the same peak shape as hybrid type core shell C18 columns. However, for formic acid as an acidic compound, the proposed core shell C18 column showed a symmetrical peak while hybrid type core shell C18 columns showed a terrible tailing peak.

69 The Determination of Nutritional and Toxic Elements in Plant-Based Foods Using ICP-MS

Andrea Palpini, PerkinElmer, 710 Bridgeport Ave, Shelton, CT 06484, Liyan Xing

Plant-based foods are gaining increasing popularity with consumers. Nutritional values, environmental benefits, sustainability, and ethical concerns are some reasons driving this market trend. Manufactured from ingredients such as fruits and fruit derivatives, nuts, vegetables, grains, and legumes, nutritional gains marketed by a plant-based diet include lower cholesterol levels, improved gut health, rich source of vitamins, and decreased risk of chronic diseases caused by inflammation. In addition to these potential benefits, challenges may arise in relation to their formulation, nutritional content, and safety. Primary concerns include lower levels of micronutrients such as iron, zinc, and calcium compared to traditional animal-based foods, and the potential of heavy metals uptake from plant-based foods grown in contaminated soils or using contaminated water, fertilizer, and pesticides. Given these potential health concerns, elemental testing is necessary to ensure plant-based foods are safe for consumption. Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) is a common and suitable technique for this type of testing. With the capabilities to detect both trace levels of toxic elements as well as high levels of nutritional elements, ICP-MS can be used to obtain accurate results while meeting the growing demands for lower detection capabilities, high sample throughput and matrix tolerance. In this work, microwave-assisted sample digestion is used for sample preparation of a variety of plant-based sample types including certified reference materials (CRMs) for method accuracy and validation. Following EAM 4.7 criteria, methods for sample preparation and analysis are shown to be suitable to meet requirements while obtaining good accuracy, reproducibility and stability.

70 LC and LC MS Methods for Analysis of mRNA Poly(A) Tail

Martin Gilar, Waters Corp, 34 Maple St., Milford, MA 01757, Catalin Doneanu

We describe three methods for measurement of mRNA poly(A) tail length and heterogeneity. The poly(A) tail was cleaved from mRNA with RNase T1 enzyme and the liberated ~120 nt long poly(A) species were analyzed with (i) size exclusion chromatography method (SEC), (ii) ion-pair reversed-phase liquid chromatography method (IP RP LC), and (iii) with liquid chromatography mass spectrometry (LC-MS) method. SEC is a simple and robust method for measurement of poly(A) tail average length. IP-RP-LC resolves poly(A) tail species differing a single nucleotide in length, providing information about poly(A) tail heterogeneity. LC with high-resolution mass spectrometry (HRMS) directly measures the molecular weight of multiple poly(A) species. LC-MS poly(A) tail length and heterogeneity measurements were in agreement with SEC and IP-RP-LC results.

71 Lego® Blocks as 'Standard' Samples for Evaluation of Fluorescence

Richard Crocombe, Crocombe Spectroscopic Consulting, 300 Boston Post Rd., West Haven, CT 06516, Brooke Kammrath, Pauline Leary

Fluorescence interference in Raman spectroscopy is a well-known problem and is especially significant in portable instruments where the availability of a variety of exciting wavelengths is unlikely. A number of fluorescence avoidance schemes are described in the literature, and implemented by Raman spectrometer manufacturers, but there does not appear to be a standard method for evaluating their performance. In addition, some samples shown in instrument descriptions, like 'dark rum' and 'sesame seed oil' are not reproducible. Therefore, we propose a set of colored Lego blocks as 'standard' samples for this purpose; they have the attractive properties of being very low cost, rugged, non-toxic, easy to transport and ship, and appear to be manufactured using a standard process. This paper is the first step, and shows the Raman spectra of a set of blocks at different exciting wavelengths, along with their visible-near-infrared spectra, so that we understand the origins of the observed fluorescence.

72

Withdrawn by the author.

73 A Qualitative Comparison of the Fast Fourier Transform and the Morlet Wavelet Transform for Potential Depression Diagnosis Using Resting-State Electroencephalographic Data

Eliana Du, West Windsor Plainsboro High School South, 346 Clarksville Rd., Princeton Junction, NJ 08550

The diagnosis of Major Depressive Disorder (i.e., "depression") has long relied on subjective measures, such as patient self-evaluation and doctor analysis. With few quantitative factors, this leaves depressed patients liable to be misdiagnosed and to not receive the necessary help, should they require it. To improve depression diagnosis, more quantitative methods must be explored. One such method is the analysis of electroencephalographic (EEG) data, which has a long history of usage in large-scale neurological assessment and, when taken from a resting-state patient, has potential applications in clinical settings. However, there are many different signal processing methods that can be used to extract features from EEG data. Thus, it is important to choose the most accurate method for depression-specific features. In this paper, two signal processing methods, the Fourier transform (FT) and the continuous wavelet transform (CWT), are used to analyze actual continuous, resting-state EEG data at well-established frequency bands to create similar topographic maps of a subject's brain. The transforms and topographic maps are created using the most recently updated MATLAB software as of September 9th, 2023, the 2023.0 version of the EEGLAB software, and the most recently updated FieldTrip toolbox software as of August 17th, 2023. The transforms' respective topographic maps are then assessed qualitatively to detect depression-specific outputs to ultimately determine the strengths of each transform in potential quantitative depression-diagnosis.

74 Investigation of the Effects of Overexpression of Human Jumping Translocation Breakpoint (JTB) Protein Using In-Solution Digestion-Based Proteomics

Krishan Weraduwage, Clarkson University, Department of Chemistry and Biomolecular Science, 8 Clarkson Ave., Potsdam, NY 13699, Madhuri Jayathirtha, Danielle Whitham, Costel C. Darie

Human JTB (hJTB) is a gene located on the human chromosome 1 at q21 which is involved in the unbalanced translocation in various types of cancer. JTB protein is found to be overexpressed in many types of cancer including prostate and breast cancer and is also present in normal cells. The biological function and the pathway through which this protein causes increased cell growth and proliferation are not fully deciphered. Upregulated and downregulated JTB conditions can be a good approach to understanding the function of the protein and its interacting partners, as well as the biological pathways and metabolic processes through which it functions. MCF7 breast cancer cell lines were transfected with the sense orientation of the JTB cDNA in HA, His, and FLAG-tagged CMV expression vector as well as with shRNA plasmids. Proteins extracted from transient and stable transfected cells were separated using in-solution digestion-based Proteomics. These samples were analyzed by a Nano Acquity UPLC coupled with QTOF Xevo G2 Mass Spectrometer. Data processing was done using Mascot 2.4 server and Scaffold 4.1 software. We found several proteins such as HSP's, Actin, and tubulin proteins and pathways which are closely associated with hJTB function. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a biomarker for early diagnosis and treatment of breast cancers.

75 Assessing the Memory Effect of Trifluoroacetic Acid (TFA) on Various Reversed-Phase HPLC Stationary Phases

Matthew Swoyer, GSK, 1250 S. Collegeville Rd., Collegeville, PA 19426, Colleen Dugan

Trifluoroacetic acid (TFA) is a universally utilized acidic mobile phase additive in HPLC. TFA lends many chromatographic advantages, however it is hypothesized to leave a "memory effect" on the column, altering its subsequent performance. This has been demonstrated in literature with coated chiral columns in normal phase and polar organic mode, and it is reversible by washing the column. The impact of TFA on achiral reversed-phase columns remains unclear. Some speculate that TFA strongly adsorbs to the stationary phase, changing the column's selectivity. Due to the supposed memory effect, many chromatographers dedicate these columns for use with TFA only. This study aimed to determine if there was a change in performance of reversed-phase analytical columns after exposure to TFA, in the scope of achiral small molecule separations. Eight columns were assessed, representing a variety of stationary phase chemistries. A 13-component test mix was used for the assessment, typically run in-house as a performance check. The test mix was run on each column, using o-phosphoric acid as an additive. The columns were then washed and run using TFA as an additive. The columns were washed once more and run under initial o-phosphoric acid conditions. The chromatograms obtained from both o-phosphoric acid runs were compared and assessed against pre-defined acceptance criteria to determine if a change in chromatography could be observed after the column was exposed to TFA. The results of this assessment will inform future best practices with regards to column dedication and washing procedures.

76 Structural Characterizations of Multiple Strawberry Fiber Fractions by NMR

Gary D. Strahan, USDA/ARS, Eastern Regional Research Center, 600 E Mermaid Lane, Wyndmoor, PA 19038, Hoa K. Chau, Arland T. Hotchkiss Jr.

Strawberries are considered a healthy food that is consumed fresh, or often in jams, jellies and as a food ingredient flavor. They are a low caloric food, containing many antioxidants, vitamins, and fibers, which are said to strengthen the immune system, protect the heart and boost mental function. In order to better understand the structure-function relationships of these health claims, multiple strawberry fiber fractions were analyzed using several sophisticated analytical methods. It was found that the commercial pomace fraction contained dietary fiber, pectin, xyloglucan, xylan, β-glucan andglucomannan. This report will primarily focus on obtaining detailed compositions and fine structures of the strawberry fiber fractions using nuclear magnetic resonance spectroscopy (NMR). These results will assist food companies and consumers interested in healthy food ingredients.

77 Rapid Timeframe Biophysical Characterization of Vaccine Intermediates Under Accelerated Stability Conditions

Donald Kotowski, Analytical Research and Development, MRL-Merck and Co., Inc., 770 Sumneytown Pike, West Point, PA 19486, Andrea Gomez, Walter Wasylaschuk, Michael McNevin

Accelerated and real-time stability studies are an integral part of vaccine development to evaluate aggregation risk, formulation composition and process conditions. Discoveries of detrimental formulation performance or aggregation during these long duration stability studies could delay product development if on critical path. In this study, conditions to rapidly assess the impact of process changes on stability of vaccine like particle (VLP) were compared to a 28-day accelerated stability study. The investigation techniques included biophysical characterization tools such as dynamic light scattering (DLS) and field flow fractionation (FFF). Aggregates of VLPs can readily form by altering salt concentrations and/or buffer pH through dialysis and when followed by exposure to elevated temperatures. Exposure to more extreme temperatures for 4 hours can also produce similar particle sizes as a 28-day study without the need for buffer alterations. These accelerated stress conditions were studied concurrently with process variations such as batches produced in Teflon versus stainless stain containers and processes including or excluding EDTA to determine potential causes of aggregation. The two conditions most comparable to a 28-day study for VLPs were 45°C for 4 hrs. in original buffer and dialysis into low salt and high pH buffer and incubating for 2 days at 37°C. Extrapolation of data gathered for other VLPs or vaccine process intermediates under these or similar conditions can facilitate the rapid identification of process conditions which mitigate aggregation risk and can be leveraged when assessing process alterations.

78 Universal Ion Chromatography Method for Anions in Active Pharmaceutical Ingredients Enabled by Computer-Assisted Separation Modelling

Dolee Merai, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Tianyu (Kelvin) Yuan, Matthew J. Gunsch, Ryan Peters, Sachin Lohani, Frank Bernardoni, Michael A. Zompa, Imad Haidar Ahmad, Erik L. Regalado, Christopher A. Pohl

Ion Chromatography (IC) is one of the most widely used methods for analyzing ionic species in pharmaceutical samples. A universal IC method that can separate a wide range of different analytes is highly desired as it can speed up the method development and validation processes. Herein we report the development of a universal method for anions in active pharmaceutical ingredients (APIs) using computer-assisted chromatography modeling tools. We have screened three different IC columns (Dionex IonPac AS28-Fast 4µm, AS19 4µm and AS11-HC 4µm) to determine the most suitable column for universal IC method development. A universal IC method was then developed using an AS11-HC 4µm column to separate 31 common anionic substances in 36 mins. This method was optimized using LC Simulator and a model which precisely predicts the retention behavior of the 31 anions was established. This model demonstrated an excellent match between predicted and experimental analyte retention time. To validate this universal IC method, we have studied the stability of sulfite and sulfide analytes in ambient conditions. The method was then validated for a subset of 29 anions using water and organic solvent/water binary solvents as diluents for commercial APIs. This universal IC method provides an efficient and simple way to separate and analyze common anions in APIs. In addition, the method development process combined with LC simulator modeling can be effectively used as a starting point during method development for other ions beyond those investigated in this study.

79 Using Novel Stationary Phase Selectivity to Address Potential NDMA Over-Quantification due to Isobaric Interference in the LC-MS/MS Analysis of Nitrosamines

Geoff Faden, MAC-MOD Analytical, 103 Commons Court, Chadds Ford, PA 19317, Matt James, Anthony Edge

Detection of mutagenic nitrosamines in drug substance and product is an area of global concern. The polar nature and low molecular weight of some nitrosamines makes achieving chromatographic retention by LC challenging and presents possibilities for interference from other low molecular weight impurities. During development of an LC-MS/MS method for eight target nitrosamines in API, significant over-quantification of N-nitrosodimethylamine (NDMA) was observed. Spiking experiments confirmed this was due to isobaric interference from co-eluting DMF. At a spiking level of 100 ppm, 307% over-quantification of 1.0 ng/mL NDMA was recorded, highlighting the implications for quantification in pharmaceutical samples. Alternative MRM transitions were assessed, although accuracy improved, DMF co-elution remained problematic. Several stationary phase chemistries were found to provide enhanced retention and separation of NDMA and DMF. Additionally, fully porous particles, as opposed to solid core, were found to enhance hydrophobic retention of NDMA, sufficient to resolve it from DMF. Two alternative methods were developed and both demonstrated excellent linearity ($R2 \geq 0.99$), accuracy (93.3 to 109.1%) and precision (%RSD 0.3 to 9.5%). Limits of detection ranged from 0.02 to 0.55 ppb with respect to drug substance (0.04 to 1.5 pg on column), significantly lower than limits specified by regulatory authorities. The improved chromatographic selectivity successfully improved NDMA quantification accuracy in the presence of DMF (accuracy = 104.0% and 102.7% at the 100 ppm spiking level). Highly selective MRM transitions for DMF were established to further safeguard against potential for NDMA over-quantification.

80 Building a Project Centric Database for Immediate Use or in a Low Resource Environment

Michael Barrett, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Anna Shternin, Hannah Dvorak, Shari Sellers, Graciela Terife

Centralized repositories for cross-functional project data have many benefits. It breaks down silos, allows for trends to be rapidly assessed, enables rapid investigations, and gives steakholders instant access to all project data. Typically, data for pipeline programs is distributed across TeamSite's, email, ELN, local files, etc. resulting in inefficient knowledge sharing and decision making. Here we highlight the collaboration across functional areas and their efforts to build and curate an interdisciplinary database for development projects that contains over 400 unique attributes accessible to the entire team. Such databases enable Spotfire visualizations and statistical analysis of data collected by various departments with a few clicks of a mouse and can act as a data management roadmap. The solutions presented within make up a framework that is flexible, quickly implementable, forward compatible, and does not require IT support. The storage, basic architecture, applications, and project impact of this database will be described.

81 Modifying the Method for Industry-Wide Content Analysis of Polysorbate 80 (PS80) on HPLC-ELSD

Kennedy Guillot, GSK, 1250 Collegeville Rd., Collegeville, PA 19426, Daniel Steyer, Katie Carnes, Sina Mortazavi, Suraj Hettiarachchi, Michelle Ward, Lee Oliver

Polysorbate 80 (PS80) plays an integral role in the pharmaceutical industry, where it acts as a stabilizer and emulsifier for a variety of drug products. In biopharmaceuticals, PS80 stability is a major concern. Studies have shown that residual enzymes from drug production processes can catalyze the degradation of PS80, which can negatively affect drug efficacy and shelf life. To mitigate PS80 degradation risk in drug products, it is critical to improve drug production processes and have accurate methods for measuring PS80 concentration. Across the pharmaceutical industry, PS80 content measurements are commonly performed by HPLC-ELSD analysis. In recent studies at GSK, the employed HPLC-ELSD analysis was found to substantially underreport PS80 content in one of our biopharmaceutical assets. In this presentation, we bring forward the results of our investigation into this issue and the improvements made to the analytical method. It was found that employing a higher ID column improved PS80 retention by increasing solvent tolerance from the injected samples and that injection volumes influenced the molar response of PS80. Employing the results of this investigation into a revised method, PS80 spike recovery was improved in 3 separate GSK antibody assets. The initial method gave spike recoveries of 90.0-92.6%, while recoveries ranged from 99.7-103.6% with method revisions in place.

82 Using Microfluidic Droplet Technology to Improve Throughput for Chemical Analysis

Robert Kennedy, University of Michigan, Department of Chemistry, 930 N. University Ave, Ann Arbor, MI, 48109

Manipulating samples as droplets within microfluidic devices has emerged as an interesting approach for chemical analysis and screening. In segmented flow, one embodiment of this technology, nanoliter samples are manipulated in microfluidic channels as plugs separated by an immiscible fluid, such as air or fluorinated oil. These plugs serve as miniature test-tubes in which reactions can be performed at high throughput. Microfluidic tools have been developed to split, dilute, extract, and filter such plugs at rates >1000 samples/s. Most experiments have been limited to fluorescence detection, but we have developed methods to analyze droplet content

by mass spectrometry (MS), chromatography, and ion mobility spectrometry. A natural application of this technology is for high throughput experimentation. One area where we have applied this technology is for catalyst discovery. Biocatalysts can be developed by screening enzyme variants to identify active enzymes for a given reaction. Similarly, traditional organic catalysts require extensive exploration of reaction conditions and substrates to develop. We have developed droplet based approaches that has the potential to decrease the time required to develop such catalysts using droplet-MS. Droplet technology can also be used for chemical monitoring or sensing applications. In this approach samples emerging from a miniaturized sampling device are segmented for later analysis. We have used this method to monitor neurotransmitter dynamics in the brain. The technology and application to studies of neurotransmission are be demonstrated.

83 Small Separations and Big Data: Using Analytical Chemistry to Address the Challenges of Cancer Detection

Rebecca Whelan, University of Kansas, Multidisciplinary Research Building, 3020 Becker Dr., Lawrence, KS 66047

Capillary electrophoresis (CE) offers many compelling analytical attributes including compatibility with biomolecules, high resolving power, small sample requirements, and the ability to be combined with many informative detectors. An analyte class of particular interest are serum proteins that can serve as biomarkers of cancer development. This presentation will highlight recent efforts in our lab to apply the analytical power of capillary electrophoresis separations to cancer biomarker detection and analysis. In one area of effort, we use CE in a non-standard preparative mode to perform fractionation of tryptic digests as part of a bottom-up proteomics workflow. Protein and peptide identification of diverse input samples (including bacterial cell lysate, human serum, and conditioned cell media) are enhanced in this approach. In particular, the coverage of ovarian cancer biomarker protein MUC16 (CA125) is enhanced by a factor of 3. In another area of ongoing work, CE serves as the fractionation step in nucleic acid aptamer selection (SELEX). We have identified single-stranded DNA aptamers that bind the ovarian cancer biomarker protein HE4 with high affinity using this approach. We describe lessons learned from applying high-throughput DNA sequencing to the CE-SELEX process, and bioinformatics tools for mining the rich giga-byte-scale data sets that result.

84 Two-Dimensional Liquid Chromatography (2D-LC) for Advanced Characterization of Industrial Polymers and Sustainable Materials

Peilin Yang, Dow Chemical, 230 Abner Jackson Pkwy., Lake Jackson, TX 77566

The chemical industry faces unprecedented challenges and opportunities to develop new materials that are more affordable, sustainable and have better performance. Novel polymer design, incorporation of recycled contents, and bio-sourced raw materials are among the top innovative sustainable solutions. A common theme of challenges in these innovation areas is the generation of high-complex mixtures covering a broad range of molecular weight and molecular structures. Given the ever-increasing regulations and society's demands for sustainable materials, more detailed characterization of highly complex chemical mixtures has become essential to supporting new product development in the chemical industry. Multi-dimensional chromatographic separations coupled with information-rich mass spectrometric detection are often required to resolve and identify large numbers of unique chemical constituents in these complex mixtures which often contain both large and small molecules. In this talk, we discuss the application of 2D-LC coupled with multiple detectors for the characterization of highly complex mixtures including novel synthetic polymers, biodegradable polymers and recycled materials using various combinations of separation modes and detector schemes.

85 Low-Cost, Open-Source Tools for Chemical Analysis

James Grinias, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Samuel Foster, Deklin Parker, Christopher Piccolo, Joeachin Obasi, Catherine Seltzer, Matthew Will

Over the past decade, the use of low-cost, open-source microcontrollers and single board computers in analytical instrumentation has grown tremendously. Based on their simple design and wide flexibility, it is easy to implement them for a wide variety of instrument control purposes (including the user interface). In our lab, we have focused on using the Arduino Uno and Raspberry Pi platforms to reduce the cost and complexity of various separation tools. They have most prominently been applied for data acquisition as a replacement for more expensive commercial DAQ systems. By combining the Arduino Uno with a simple analog-to-digital converter chip, a voltage reader was developed for under $50 that can be coupled to any chromatography or electrophoresis detector that provides an analog voltage data output. To further increase the functionality of the open-source DAQ, a Raspberry Pi-based device was created that eliminates the need for an external operating computer and adds chromatographic data processing to the acquisition program (filtering, baseline subtraction, and calculation of figures of merit). More recently, these devices have been expanded for full instrument control of high-throughput LC platforms, multi-tip electrospray ionization sources, microfluidic devices for real-time monitoring of bacterial biofilm formation, and automated derivatization devices for gas chromatography mass spectrometry (GC-MS) analysis of fatty acids, all of which are described here.

86 AI, Machine Learning, Chemoinformatics, and Chemometrics: What's the Deal

Peter Harrington, Ohio University, 133 University Ter., Athens, OH 45701

Since the advent of the computer, it has been tasked with solving problems. With advances in electronics came more powerful computers capable of solving more difficult problems. With the larger storage capacities, bigger data collections allowed algorithms to learn from the data. The algorithms sought to emulate the way a scientist would solve the problem. However, in the 1990s, a new approach was devised that mimicked the microstructure of the nervous system as an artificial neural network. Analytical chemists are confronted with wide (i.e., more measurements than objects) and not tall data (i.e., more objects than measurements) that typifies big data. Regularization used by the elastic net and support vector machines allows robust models to be built from wide data while minimizing the risk of overfitting. Many advances in modern society are due to embedded machine intelligence. These innovations include voice recognition in phones and personal assistants, image recognition for identifying faces in photos, language translation, and digital transcription of handwriting. These advances all rely on deep neural networks (DNNs). A history from single neuron through shallow to deep learning networks is covered. Discussion on database quality is presented, emphasizing the "garbage in leads garbage out" principle. Techniques for understanding the decision-making processes of the trained models are important because they put the chemistry back into the chemometrics and allow for making new discoveries. Still, more importantly, it ensures that the network is not making decisions on artifacts in the data and flaws in the experimental design.

87 Temporal Surface Mode Decomposition for Transient Kinetics Analysis

Matthew Kunz, Idaho National Laboratory, 570 Belair Ave, Idaho Falls, ID 32953, Debtanu Maiti, Rebecca Fushimi

Catalysis is inherently a dynamic process due to the accumulation and or depletion of active sites over a given reaction. Understanding these dynamics allows for the development of next generation catalysts and strategies for enhancing the efficiency of catalysts used today. However, this task is not trivial as modeling such a dynamic system is computationally infeasible and steady-state techniques often only describe a snapshot of information. We propose an alternative way of studying a catalysts behavior through the use of statistical learning methodologies combined with dynamic catalysis instruments. More specifically, this talk will focus on the application of the data-driven Dynamic Mode Decomposition (DMD) to transient kinetic information measured by the Temporal Analysis of Products (TAP) reactor to reveal surface reaction modes. This work fuses data-driven optimization principles of dynamic systems, temporal transient kinetic information, and physics based constraints to reveal the latent variables of the reaction-diffusion mechanism. Due to the statistical nature of the approach, the decomposition is able to account for temporal experimental noise while dealing with mass spectrometer calibration between measured gas species. This methodology provides a way to decouple the transient kinetics of a reaction system to elucidate the catalytic performance of complex materials and design better dynamic reaction systems.

88 Photonic Data Science: Model Transfer for Raman Spectra and FAIR Data Storage for Vibrational Spectroscopic Data

Thomas Bocklitz, UBT, Nürnberger Str. 38, D-95440 Bayreuth, 95440, Germany

Photonic techniques are optimal tools to characterize samples in various research disciplines like remote sensing, material characterization, life science and medicine. Photonic characterization techniques with many advantages are vibrational spectroscopic measurement techniques like infrared (IR) absorption spectroscopy and Raman spectroscopy. To utilize the full potential of these techniques, the whole data life cycle of the vibrational spectroscopic data needs to be investigated and optimized. This photonic data life cycle starts with the data generation and the planning of the corresponding study/experiment, then the data modelling suing AI techniques like chemometrics, ML and DL including model evaluation and model interpretation is following and finally data storage and archiving are needed to allow a FAIR usage of the data. In photonic data science, all the techniques within the life cycle are researched and finally sequentially combined in a data pipeline, which standardizes the vibrational data and extracts reliable high-level information from it. In this contribution, we highlight our studies aiming at a standardized data analysis pipeline for biomedical Raman spectra, as well as studies about the generation of correction procedure and model transfer techniques so spectral data and models can be used across devices and conditions. Furthermore, we highlight our research activities towards a repository for sharing vibrational spectroscopic data (VibSpecDB) which is embedded in the national research data infrastructure initiative in Germany (NFDI) and its chemistry consortium (NFDI4Chem).

89 Rashomon Effect on Model Interpretability and Improving Model Generalizability

John Kalivas, Idaho State University, Dept. of Chemistry, 921 S.8th Ave., Pocatello, ID 83209

A chemometric calibration goal is to form a prediction model useable for accurately analyzing new samples. Another objective is the ability to interpret the accurately predicting model. For example, once a calibrated regression vector is obtained with spectral data, such as with partial least squares (PLS) and Raman spectra, the user sometimes wants to interpret the meaning of the regression coefficient values relative to the spectral wavelengths and calibrated prediction property. However, for numerous reasons, model interpretation is generally not possible. Studies have shown that diverse model regression vectors can be formed with different shapes and magnitudes and all accurately predict target samples spanned by the calibration set used to form the model. This situation has been labeled the Rashomon effect with the Rashomon set consisting of the collective set of useful models. For example, a variety of modeling methods properly tuned, such as PLS, deep learning, or support vector machine (SVM), applied to an appropriate data set will all sufficiently predict the analyte. In this case, a large Rashomon set is said to exist. In a large Rashomon set, simple models exist in this collection of all possible accurately predicting models in addition to complex models. This presentation discusses the Rashomon effect in conjunction with the feasibility of strict model interpretability. Demonstrating the Rashomon effect are two mathematical approaches from the chemometric literature characterizing the multitude of diverse models that can be achieved and predict accurately. Both approaches establish useful models deviating from the orthogonal net analyte signal (NAS) model.

90 Strategies and Tools to Simplify and Support Method Development in Two-Dimensional Liquid Chromatography - A Progress Report

Dwight Stoll, Gustavus Adolphus College, Department of Chemistry, 800 West College Ave., Saint Peter, MN 56082

Two-dimensional liquid chromatography (2D-LC) is recognized as a versatile and powerful tool applicable to diverse analytical challenges ranging from resolution of molecules with multiple chiral centers to complex mixtures of biomolecules. However, a major barrier that prevents more users from realizing these capabilities in practice is the paucity of easy-to-use strategies and tools to support development of 2D-LC methods. In this presentation I will provide a progress report on several facets of our effort to address this need. This includes: 1) the development of a large, freely available database of retention data to support development of modeling and simulation tools; 2) the development of a freely available web-based simulator for 2D-LC that leverages our retention database; and 3) development of generic method development strategies that simplify decision-making during the 2D-LC method development process. I also briefly touch on the role of machine learning in these efforts. Our aim with all these activities is to enable a more systematic approach to 2D-LC method development, so that we can lessen our reliance on trial-and-error experimentation and user experience compared to what has been done in the past. In the process of reporting on these activities, I discuss their use in the development of methods for applications ranging from small molecules to therapeutic peptides and therapeutic oligonucleotides. We expect that the proliferation of easy-to-use method development tools and strategies will both expand the application space for contemporary 2D-LC and increase the number of users engaged in development of 2D-LC methods.

91 Pharmaceutical Portfolio Delivery by Benefit of Strategic Method Development and Automation for Large and Small Molecule Separations

Kaitlin Grinias, GSK, 1250 S Collegeville Rd., Collegeville, PA 19426, Colleen Dugan, Mark Sleeper, Daniel Steyer, Katie Carnes, Sina Mortazavi, Stephanie Lehman, Justin Shearer

Delivery of safe and efficacious therapies to patients relies on a well-designed chemical manufacturing and control (CMC) Strategy. Drug product and drug substance manufacturing controls are confirmed by content and purity testing using chromatographic analysis. Robust separation methods, modeling, automation, and miniaturization can reduce overall cycle time, environmental impact, and the resource necessary to develop analytical controls. The Analytical Development department at GSK has undertaken a variety of efforts to be at the forefront in these areas. We have leveraged the hydrophobic subtraction model and completed a column robustness study to design a selective reverse-phase high-performance liquid chromatography (RP-HPLC) column toolkit for our method development strategy in the small molecule space. Our platform analytical methods are deployed on a global fleet of 150+ HPLCs with automated system suitability monitoring. Additionally, with an objective to reduce the environmental footprint, demonstration of a compact LC applied to primary process development showed a 1400-fold reduction in solvent consumption and 3-fold reduction in analysis time. Finally, the application of a sensitive and selective polysorbate assay by HPLC- charged aerosol detection and orthogonal proteomics study by HPLC-mass spectrometry to inform biopharmaceutical process development is shared.

92 Coupling Separation and Sample Preparation Methods for Pharmaceutical Analysis

Jared Anderson, Iowa State University, 1605 Gilman Hall, Ames, IA 50010, Danial Shamsaei, Shu-An Hsieh

Chromatographic separations remain a vital component of ensuring the purity of active pharmaceutical ingredients (API). In some cases, sample preparation approaches are needed in order to ensure adequate sensitivity is achieved prior to performing separations. This talk discusses on-going efforts to develop new approaches in coupling sample preparation methods to fast separations using gas chromatography (GC) and high-performance liquid chromatography (HPLC). On-going efforts in analyzing genotoxic impurities in APIs using GC as well as improving the separation and analysis of boronic acids and pinacol esters using HPLC will be highlighted. The talk also discusses approaches to miniaturize chromatographic systems through the development of portable smartphone-based fluorescence detectors for HPLC which enable positive peak identification and peak purity analysis through the customization of dual-channel flow cells.

93 Development of Limit Test for Residual Spermine and Putrescine in Monoclonal Antibody In-Process Samples

Sina Mortazavi, GSK, 1250 S Collegeville Rd., Collegeville, PA 19426

Chemical residuals are produced or introduced during the production of biopharmaceuticals. The biopharmaceutical manufacturing process is comprised of two components: upstream and downstream manufacturing. During the upstream process, cells are cultured, grown to scale, and products harvested; in the downstream process, protein is purified using a series of polishing steps. Since residuals are often hazardous, their clearance must be demonstrated to ensure patient safety. If they remain following downstream purification, it must be proven they are below safe levels. The focus of this presentation is spermine and putrescine. These two residuals presented unique challenges: (1) they are chromophore lacking analytes. (2) spermine and putrescine are highly polar and poorly retained on reverse phase columns. (3) The biopharmaceutical portfolio and dosing structure required clearance to be demonstrated at very low levels. Several detection methods were explored for derivatized and underivatized samples, including fluorescence, charged aerosol detection (CAD), and mass spectrometry. An ICH-compliant limit test was developed using derivatization with benzoyl chloride, followed by separation on a reverse phase (Agilent C18) column and detection using CAD.

94 Small Molecule HPLC Method Optimization Using an Acidic, Basic, and Neutral Panel and Superficially Porous Particles

Conner McHale, Advanced Materials Technology, 3521 Silverside Road, Quillen Building, Ste. 1-K, Wilmington, DE 19810

High-pressure liquid chromatography (HPLC) is one of the most popular laboratory techniques in the world. Choosing the right column and operating conditions is by far the most important part of developing an HPLC method that works on all brands of HPLC instrument. HPLC method optimization plays a serious role in obtaining separation goals and should not be overlooked. Initial choice of the correct stationary phase, pore size, particle size, mobile phases, and column dimension all are critical in achieving the best separation as quickly as possible. Valuable time can be wasted when one or more of these variables is chosen incorrectly. Whether the goal is speed, resolution, or a combination of both, certain selections should be made for each of the above variables in order to achieve the most rugged separation possible for any given sample. This presentation reviews proven small molecule method development steps that lead to rugged analytical separations. Acidic, basic, and neutral compounds are screened to show the effects of many different important variables that comprise an HPLC method.

95 Nonstoichiometric Pseudoprotic Ionic Liquids as Media for Metal Separations

Mark Kobrak, Brooklyn College-CUNY, Dept. of Chemistry, 2900 Bedford Ave., Brooklyn, NY 11210

Nonstoichiometric pseudoprotic ionic liquids (NPPILs) are a recently-emerged class of ionic liquids with unusual physicochemical properties. We have recently begun exploring NPPILs composed of mixtures of amines and organic acids for use as agents for the liquid-liquid extraction of metals from aqueous phase. Our results indicate that not only can these systems promote extraction in high yield, their solvation properties can be controlled in intriguing ways through variation of the acid/amine ratio. We explore the relationship between their physicochemical properties and their extraction behavior, and discuss what this suggests about their adaptation for use in gels or other formulations suitable for chromatography and other separations techniques.

96 **The Benefits of Reducing Metal Ion Introduction into HPLC Flow Paths via Silicon-Based CVD Coatings**

Jesse Bischof, SilcoTek Corproation, 225 PennTech Dr., Bellefonte, PA 16823

As separations and purifications move toward higher sensitivity and higher throughput, the need for a robust, inert fluid pathway has become more critical. A typical high-performance liquid chromatography (HPLC) instrument is made from stainless steel, PEEK, and ceramic components. The steel in these systems will have active sites on the surface that require routine passivation or priming to be effective when analyzing reactive compounds, especially at low detection limits. Additionally, stainless steel is not favorable for biological analysis as the material is not considered "bio-inert". Metal ions can leach into the flow path causing issues. For instance, oligonucleotides can suffer oxidation or degradation, proteins can experience irreversible aggregation, fermentation processes result in lower yields all due to elevated concentrations of various metal ions. Titanium typically replaces the stainless steel, but the metal surface can still be a source of difficulties when analyzing metal loving compounds. PEEK is often used as a bio-inert, metal free surface; however, there are pressure and machinability issues involved with this material. Here we investigate a different approach that would allow the utilization of metal alloys while reducing the amount of metal that can interact with solution: chemical vapor deposition (CVD) coated metal hardware. We quantify the amount of metal that leaches into water, methanol, and acetonitrile when in direct contact with stainless steel, titanium, MP35N and Hastelloy via inductively coupled plasma mass spectrometry (ICP-MS). We also show how CVD coatings can bring the number of metals leached down to zero. Examples of why this is important are shown in various HPLC situations.

97 **Modeling and Visualizing Mass Transfer of Monoclonal Antibodies (mAb) in Size Exclusion Chromatography (SEC) Columns**

Sornanathan Meyyappan, Waters Corporation, 34 Maple St., Milford, MA 01757, Fabrice Gritti

Most models of mass transfer inside a liquid chromatography (LC) column fully ignore bed microporous structure (complex randomized packing of spheres, um-scale) and the internal structure of the particles (complex distribution of interconnected mesopores, nm-scale). High and low molecular weight impurities in manufactured mAb samples are critical quality attributes and full characterization of these often requires longer columns. The resolution provided by conventional 2.1X300 mm SEC columns is insufficient as these columns operate under a sub-optimal dispersion regime due to inherent wall effects induced by heterogeneous packing density across the column cross-section. 4.6mm columns are therefore preferred for better resolution and performance despite increased solvent consumption. This work demonstrates that the modeling and visualization of mass transfer/diffusion of monoclonal antibodies (mAb) in size-exclusion chromatography (SEC) columns is achievable with the internal molecular dynamics of an LC column. The modeling is based on adaptive numerical computation of two-dimensional advection-diffusion mass transfer kinetics for mAb samples integrated with wall-affected velocity profiles derived from a reconstruction of the packed bed structure from focused ion-beam scanning electron microscopy. GPU-based parallel computing for iterative matrix inversion (using rapid-biconjugate gradient stabilized method) was employed to solve a non-uniform unsteady finite volume model. The modeling integrates heterogeneous diffusion characteristics of mAb and presents an in-depth understanding of how interfacial column dynamics affect macroscale mass transport. The modeling is directly scaled to a 2.1X300mm SEC column with 1.95um particles and parallelly computed at a very high level to predict the retention, and sensitivity of polymer analytes in SEC applications.

98 **The Systematic Screening Protocol: A Streamlined Way to Develop Fast and Robust Reversed-Phase Liquid Chromatography Methods**

Kenneth Berthelette, Waters Corp, 34 Maple St., Milford, MA 01757, Kim Haynes

Method development activities can be challenging, even with relatively simple mixtures. This is due to the wide variety of available stationary phases, and possible testing conditions. Performing method development without a set plan can lead to lengthy studies without a guarantee of an acceptable method. However, employing a structured method development approach can streamline the process by setting specific rules and guidelines for even novice analysts to follow. This can not only reduce the time needed to develop a suitable method but improves traceability of the method development process and reduces the needed decision making that can accompany method development. Coupling the systematic screening protocol with the best columns further elevates the success of the approach. Using columns that are specifically designed to reduce un-wanted secondary interactions, like MaxPeak Premier Columns which include MaxPeak™ high-performance surfaces (HPS), ensures the data collected during method development and optimization is of the highest quality, eliminating the doubt in method robustness that can exist in these activities. This presentation documents a systematic method development process for the analysis of structurally similar triazine herbicides. By implementing a systematic method development process, a suitable method was found within two days of analytical runs. The best conditions obtained from method development were subsequently optimized to further improve the overall separation performance. Final method conditions used an acetonitrile mobile phase modified with ammonium hydroxide on a high pH stable hybrid silica MaxPeak Premier Column.

99 **Materials Analysis and Art Historical Research on a 16th Century Painting of St. Catherine of Alexandria**

Jeffrey Taylor, New York Art Forensics, 1027 Grand St., Brooklyn, NY 11211

Art Forensics endeavors to analyze art objects from a triangulated approach that involves scientific methods combined with art historical and provenance research. This paper considers a sixteenth century painting on wood panel and the process that led to the development of a proposed authorship. The painting in question was delivered to New York Art Forensics without any knowledge of its subject, title, or author. Following a thorough documentation and materials analysis that places the work in the sixteenth century, likely of Italian origin, our researchers then began considering who might have been the painter and the painting's subject. This research required a comprehensive understanding Catholic Church iconography as well as familiarity with Italian renaissance masters. The techniques employed required an ability to utilize traditional connoisseurship skills. The figure in the painting was identified, through the objects next to the sitter, as Saint Catherine of Alexandria. After considerable examination of potential candidate artists, New York Art Forensics was able to narrow the focus onto a handful of followers of Leonardo da Vinci, and eventually to the studio of Bernardino Luini.

100 **Science in the Museum: How Analytical Techniques Inform Art History, Conservation, and Museum Practice**

Marco Leon, The Metropolitan Museum of Art, 1000 Fifth Ave., New York, NY 10028

In the Spring of 1882, following accusations in the press that two of its ancient statues from Cyprus were forgeries, The Metropolitan Museum of Art took the unprecedented decision to exhibit the two sculptures outside of their cases and to invite the citizens of New York to examine them with their own eyes...and more. As one contemporary account puts it "Full advantage of this invitation was taken, and during the following weeks thousands examined the discredited statues. Again, every indignity was heaped upon defenseless stone; visitors washed, chiseled, cut, scraped, treated with caustic potash and other chemicals, brushed with wire brushes, and examined microscopically to their hearts' content". The verdict: authentic! Thus, forensic examination of works of art started at the Met. In the 1920s, Columbia University electrochemistry professor Colin Fink introduced metallography and electrochemical methods to the study of antiquities, and Met curator James Rorimer pioneered the use of UV illumination to identify restorations and forgeries. More recently, a whole array of advanced analytical techniques, including scanning electron microscopy, X-ray fluorescence spectrometry, Raman and infrared spectroscopy, and liquid chromatography – mass spectrometry has become standard at the museum. This talk will highlight their application to the study of works of art.

101 No abstract submitted by the author.

102 **The Authentication of Untitled: Red, Black, and Silver by Jackson Pollock**

Nicholas Petraco, John Jay College CUNY, 524 West 59th St., New York, NY 10019

A lecture given on July 10, 2022, for Stonybrook University and The Pollock-Krasner House and Study Center's 2022 Lichtenstein Lecture series entitled: "Trace Evidence: Analyzing Ruth Kligman's Purported Pollock." The lecture covered the extensive scientific analysis and forensic authentication of a painting known as Untitled: Red, Black, and Silver (RBS). This painting created by Jackson on the ground, outside his studio, in Springs, for his lover, Ruth Kligman, in the summer of 1956, three weeks before his tragic death. A review of the plausible provenance evaluation by the Pollock/Krasner authentication committee and their recommendation to place RBS in the Supplemental Catalogue Raisonné as well as the scientific analysis performed on the painting prior to the author's involvement which included paint analysis and fractal evaluation, will be followed by a detailed report of the trace evidence removed from the painting, its analysis and findings, and a brief review of the statistical evaluation. Finally, the author sums up the overwhelming circumstantial evidence which, in his opinion, proves conclusively that RBS is by Jackson Pollock, and painted in Springs, at the now Pollock-Krasner National Historic Site.

103 **Investigation of the Effects of Human Jumping Translocation Breakpoint (hJTB) Protein Using Mass Spectrometry Based Proteomics**

Taniya Jayaweera, Clarkson University, Box 5810, 8 Clarkson Ave., Potsdam, NY 13699, Madhuri Jayathirtha, Danielle Whitham, Costel C. Darie

Human JTB (hJTB) is a gene located on human chromosome 1 at q21, which is

involved in the unbalanced translocation of various types of cancer. JTB protein is ubiquitously present in normal cells and is found to be overexpressed in various types of cancer including prostate and breast cancer. Hence, the protein could be used as a biomarker for treating different malignancies and serve as a drug target for their treatment. The biological mechanism of the protein or the pathway through which it increases cell proliferation is not fully deciphered. Here, we applied different proteomics approaches to study the protein dysregulation patterns in overexpressed and downregulated JTB conditions in both MCF7 cells (cancer) and HEK293 cells (normal) that could indicate the function of the JTB protein and its interacting partners. We further employed different protein digestion approaches, such as gel-based (1D-PAGE) and gel-free methods coupled with mass spectrometry-based proteomics to investigate the entire cellular proteome. Gene set enrichment analysis (GSEA) was also performed to identify the pathways associated with the dysregulated proteins and identify the signaling mechanisms through which the JTB protein contributes to cancer. We identified several proteins involved in mitotic spindle assembly, cell proliferation, metastasis, and anti-apoptosis to be dysregulated, confirming the JTB protein's involvement in tumorigenesis.

104 Development of an Automated Matrix Assisted Laser Desorption Ionization Mass Spectrometry Workflow for Formulation Risk Assessment of Novel, Engineered Cytokine Proteins

Gregory Pirrone, Merck & Co., Inc., 33 Ave. Louis Pasteur, Boston, MA 02115, Erik Munsell, Alexey Makarov, Heidi Ferguson, Suman Luthra, Mohammad Al-Sayah

Biological therapeutics are major contributors to the pharmaceutical pipeline and continue to grow in sales and scope. Additionally, the field's understanding of cancer biology has advanced such that biopharmaceuticals can harness the power of the immune system for oncology treatments. Several of these novel therapeutics are engineered versions of naturally occurring proteins in order to improve therapeutic properties including, potency, target engagement and half-life extension. Cytokines, such as interferons and interleukins, are a broad class of signaling proteins. They modulate the body's immune response, and engineered cytokines have entered the clinic as promising new immuno-oncology therapies. While these therapies hold great promise, their additional complexity introduces analytical challenges, and traditional analytical platforms may be ill suited. Further, the pharmaceutical industry relies on streamlining approaches for high-throughput experimentation to achieve speed and efficiency in the discovery and development of new modalities. These demands necessitate the use of state-of-the-art techniques to rapidly characterize these new modalities and guide process development and optimization. Matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) is a rapid, sensitive and automatable technique amenable for high-throughput analysis of proteins. In this work, we have developed an automated platform to sample, prepare, acquire and analyze molecular fragmentation within engineered cytokines samples following a short stability study. This screen was used to inform the final formulation strategy for clinical development by providing unambiguous identification in situations where chromatographic peaks are not fully resolved. This workflow can be used to accelerate formulation decisions critical for biopharmaceutical development.

105 Optimization of the In-Gel Sample Preparation for Mass Spectrometry-Based Proteomics

Danielle Whitham, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Costel C. Darie, Brindusa Alina Petre

Proteomics is becoming increasingly popular in modern applications. The current experimental techniques for gel-based proteomics experiments need to be both accurate and efficient. Currently, protein digestion and peptide extraction method from a sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is both time consuming and labor-intensive. Optimization of this method could save time and effort while yielding similar or acceptable results in terms of protein identification compared to the controls. To optimize the digestion protocol, we used two model proteins: bovine serum albumin (BSA) and lysozyme. Both proteins vary in their molecular weights and number of disulfide bonds, identifiable easily from as Mascot Daemon search, which make them ideal proteins for this investigation. The trypsin digestion variations include various digestion times and temperatures. Peptide extraction variations include shaking and sonicating with varying times and steps. Several concentrations of both proteins were also tested to assess the sensitivity. The peptide mixtures were then analyzed by nano liquid chromatography liquid chromatography (LC-MS/MS) using a NanoAcquity ultra-pressure (UP)LC coupled with a QToF Xevo G2 MS, and the raw data was analyzed using Mascot Daemon (v. 2.5) server. Both the protein score and type of protein found was taken into consideration with data analysis. Variations in the parameters (digestion time, extraction time and number of steps, extraction method, etc.) for protein digestion and peptide extraction allows us to determine which method yields acceptable protein identification scores. Currently, the trypsin digestion and extraction with shaking variations seem to be the most promising yielding similar, if not better mascot scores than controls.

106 Evaluation of LC-MS Instruments for Multi-Attribute Method (MAM) Implementation

Catherine O. Brown, GSK, 1250 S. Collegeville Rd., Collegeville, PA 1942, 6 Kelsey T. Morgan, Matthew D. Maust, Justin W. Shearer, Nicole A. Schneck

Within the biopharmaceutical industry, it is well-known that multiple analytical techniques are used to confirm structural integrity and key attributes of a biotherapeutic product. Although liquid chromatography mass spectrometry (LC-MS) is primarily used as a characterization assay, that landscape is changing as the multi-attribute method (MAM) concept has gained traction over the past decade. LC-MS technology for MAM focused on protein/product attribute monitoring and quality control has become an emerging talking point across the industry as early adopters face challenges related to platform selection, software, and implementation strategy. In this talk, we discuss the evaluation of two LC-MS systems to apply MAM peptide mapping workflows to support activities from forced degradation to process development. For the MAM system evaluation, mAb1 was used to monitor selected post-translational modifications (PTMs): C-terminal lysine loss, oxidation, deamidation, isomerization, succinimide formation, and glycosylation. mAb1 identity was confirmed by MS1 mass profiles using four unique, signature peptides. PTMs of interest were selected based on previous characterization and structure-function relationship data sets using the mAb1. PTM relative quantitation was calculated using the peak area of the modified peptide in relation to its wildtype. Additionally, a cross-over study from a characterization LC-MS platform to the LC-MS systems being used for MAM and monitoring applications is discussed. Lastly, MAM data sets were correlated with other quality assay readouts including cIEF and HILIC. Key strengths will be outlined for each MAM platform evaluated, in addition to highlights on ease of deployment and training for end users.

107 Moving into PROTACs Drug Discovery: Considerations for LC-MS Analysis

Sharon Tentarelli, AstraZeneca, 35 Gatehouse Dr, Waltham, MA 02451

In early-stage drug discovery, chemistry groups that have typically focused on traditional small molecule research are now often exploring PROTACs (proteolysis-targeting chimeras) compounds, which link a small molecule that binds a protein target to a ligand recruiting an E3 ubiquitin ligase. For these conjugated molecules, the molecular weights often range from 800-1500 amu and solubility can be quite low. Analytical chemists accustomed to working with conventional small molecules may need to adjust various instrument parameters and assay methodology to accommodate this new modality. Multiple detailed examples and practical suggestions will be presented on how LC-MS based assays can be optimized for working with PROTACs. For open-access LC-MS, adjustments to data processing can assist the synthetic chemists in confidently interpreting their data. For higher-end instrumentation, fragmentation settings for HRMS-MS structural elucidation and options selected for automated parameter optimization for MRM method development should also be modified. One case study will address hydrolytic stability assays, where compounds may experience aggregation or agglomeration, resulting in trend curves that differ from those seen with typical precipitation. Examples illustrate how to identify and mitigate this issue. A second case study addresses forced degradation studies in a formulation matrix, where the higher molecular weight of PROTACs can overlap with the molecular weight range of excipients, making it difficult to distinguish between compound degradation and interaction with the excipients. In this example, appropriate analytical conditions served to identify an unexpected matrix interaction.

108 Do You Really Understand Your Crystallization – The Value of PAT

Norman Wright, Mettler Toledo AutoChem, 6708 Alexander Bell Dr., Columbia, MD 21046

Fundamental to successful process analytical technology (PAT) has been improvements to the capabilities of in-line and on-line instruments for real-time analysis. This can be seen by the increasing number of applications developed over a broad range of academic and industrial syntheses specifically in pharmaceutical and biopharmaceutical sciences. The push to investigate increasingly complex chemistries has followed often requiring innovative reaction analysis technologies to help describe both the reaction mechanism and intermediates. Advanced particle size characterization along with infrared and Raman spectroscopies provides a set of tools that when used in concert can provide a greater degree of reaction and process understanding, often necessary for challenging chemical reactions. The many advantages that real-time spectroscopic analysis provide include readily integrated analytics that yield data rich, structurally specific information from reaction intermediates and final products in an accessible format. This capability is increasingly important in the drive to gain more information from fewer experiments, reducing time and saving money. This paper presents examples of real-time reaction analysis to address PAT challenges, such as how to easily extract and combine information from multiple sources, thus enabling the identification of control parameters that are required for optimizing a reaction without compromising product quality and critical when optimizing and scaling up reaction processes.

109 Development of Adaptable and Scalable Quantitative Mid-Infrared Spectroscopy Models for In-line Monitoring of the Continuous Synthesis of Furosemide Using Dynamic Calibration Methodology

Roudabeh Sadat Moazeni Pourasil, Virginia Commonwealth University, 506 E Jackson St., Richmond, VA 23219, Yuma Miyai, Matthew Glace, Rachel Vallejo, James Rydzak, Thomas D. Roper

In this study, we established an in-line process analytical technology (PAT) platform to monitor the continuous synthesis of furosemide across different scales and conditions. The platform incorporates a dynamic calibration approach using a design of experiment (DoE) method, referred to as DoE-PAT. By systematically varying key DoE factors such as starting material concentrations, temperature, and residence time, we enhanced the robustness of the global PAT model. We combined mid-infrared (Mid-IR) spectroscopy and partial least squares regression (PLS) to develop a smart PAT platform capable of real-time furosemide concentration measurements in complex crude reaction mixtures. To optimize the statistical results, we iteratively constructed quantitative models by adjusting the pre-processing and variable selection procedures of the spectroscopic data. The performance of the 94 constructed models was evaluated using multiple independent external validation sets, employing statistical measurements such as root mean square error (RMSE), bias, and correlation coefficient (R2) values. The PAT platform was successfully deployed and tested during the initial DoE development, laboratory-scale synthesis, and final large-scale synthesis. Analytical metrics, including accuracy, precision, robustness, and reproducibility, were assessed to evaluate the performance of the real-time monitoring procedure. Our developed DoE-PAT platform exhibited excellent prediction capacity for furosemide and demonstrated durability across various reactor types and process conditions, without requiring further mathematical manipulation or recalibration. Overall, this study establishes a reliable and efficient PAT platform for real-time monitoring of furosemide synthesis, offering significant advantages in terms of accuracy, versatility, and adaptability to different manufacturing scales and conditions.

110 Expanding the Role of In-line Spectroscopy in the Pharmaceutical Environment

John Wasylyk, Bristol Myers Squibb Co., One Squibb Dr., New Brunswick, NJ 08901, Robert Wethman, Ming Huang

The application of in-line spectroscopy as a PAT (Process Analytical Technology) tool to monitor various processes during the development cycle provides insight into real time kinetic behavior. In-line studies involving vibrational techniques has been utilized to follow reaction and reagent stability, as well as crystallization processes. The crystallization processes often include following polymorph transformations. Raman technology, including low frequency Raman, is a valuable tool in distinguishing different polymorphs, and can also be used in the study of solvate forms, as well as the kinetics of multiple polymorphic transitions during crystallization and storage. Near Infrared has also been applied as an option to Raman Spectroscopy. Sometimes overlooked, is the advantage that in-line spectroscopy brings to the table, namely sustainability. Benefits often include real time analysis with no sample preparation. Likewise, grab samples can also be analyzed via the sample spectroscopy-based techniques. Once integrated in an appropriate control strategy, PAT tools can enable enhanced process step control and help achieve improved product purity and yield. This presentation will cover a range of studies covering in-line and off-line polymorph transformations as well as reagent stability studies, both of which are key to driving sustainability in analytical analyses.

111

Withdrawn by the author.

112 Multivariate Regression of Chili Capsaicinoids for Absorbance-Transmittance Excitation Emission Matrix (A-TEEM) Spectroscopy and UPLC-DAD data

Adam Gilmore, HORIBA Instruments Inc., 9 East View Ct., Flemington, NJ 08822, Uwe Nienaber

Analysis of capsaicin (C), dihydrocapsaicin (DC), nordihydrocapsaicin (NDC) and their sum (MC) from methanol extracted dried chilis by UPLC-DAD yielded RSD for repeat extractions around 3 % with 4 min run times. The extraction solvent included DMBO ((±)-3,4-dimethoxybezyl 4-methyloctanoate) as an internal standard. Major relative limitations of UPLC-DAD include Capex and Opex and complexity for lab operators. Split samples (diluted 31 x) were analyzed by A-TEEM spectroscopy scans requiring < 1 min plus 2-3 min for autosampling. Significant A-TEEM correlations were measured for C, DC, NDC and MC using partial least squares (PLSR) and extreme gradient boost regression (XGBR). The calibration/validation data included 298/97 samples with [MC] ranging from 0.087 to 6.93 weight %. Subtraction of the DMBO-blank caused baseline noise deviation of ±0.242% of the maximum [MC] and ±1.92% of the minimum [MC]. The r^2 values for the validation set for MC by PLSR/XGBR were 0.99426/0.99368 with RMSE values of 0.2304/0.2417 weight % equating to relative errors around 4.0 %. Another MC validation set (n=24) ranging from 0.281 to 4.108 wt. % (excluding the blanks) yielded r2/RMSE values of 0.98726/0.157 for XGBR. A-TEEM analyses used fixed conditions and were normalized to an external Water Raman Scattering Unit value. We discuss how A-TEEM correlations may be improved by increasing the integration time for lower [MC] samples and or by eliminating the DMBO. We conclude the PLSR and XGBR model's RMSE results (± 4%) were on par with the UPLC RSD (±3 %).

113 Accurate and Reliable Analysis of Food Samples Using ICP-MS with Argon Gas Dilution

Andy Fornadel, Thermo Fisher Scientific, 104 Quinn Rd., Severna Park, MD 21146, Sukanya Sengupta, Bhagyesh Surekar, Richard Fussell, Daniel Kutscher

Food is subject to regular analysis for potentially harmful contaminants, including screening for pesticides, typical contaminants and also toxic metals. This task is commonly fulfilled by analytical testing laboratories, often specialized particularly on the analysis of foods, which turn over a large number of different sample types to provide rapid answers to manufacturers and regulatory authorities. For elemental analysis, the use of inductively coupled plasma mass spectrometry (ICP-MS) is widespread due to the outstanding detection limits that can be achieved. Although ICP-MS is known as an analytical technology unaffected by matrix effects, this may still be an issue when testing large amounts of potential different sample types. Matrix effects could be observed when samples contain differing amounts of major elements, or when the digestion procedure may result in different acid concentrations. Although internal standardization provides a means to account for the changing responses, due to the stringent quality control requirements applicable in regulated laboratories, even small differences in the samples may cause the need to isolate a particular sample, dilute and re-run, which creates additional labor and cost for the laboratory. This presentation highlights how inductively coupled plasma mass spectrometry (ICP-MS) can be used for the high throughput analysis of food samples using automated dilution of the samples with argon gas directly in the instrument. The method was fully characterized and initially validated using a certified reference material as well as spiking experiments, and has proven to provide accurate and reliable results.

114 Analytical Testing Solutions for Method Validation Studies on PFAS Testing of Drinking Water and other Samples Matrices by UHPLC/MS/MS.

Cole Strattman, Perkin Elmer, 286A Old Coach Rd. Charlestown, RI 02813

Growing environmental and health concerns about Per- and Polyfluoroalkyl Substances (PFAS) have led to stricter and more extensive regulations of these substances in drinking water, ground water, soil, and food over the past decade. PFAS are man-made chemicals used in a wide variety of commercial products like non-stick cookware, food packaging, paints, clothing, fire retardants and surfactants since the 1940's. Due to their inert nature, PFAS are persistent and have been found to accumulate throughout the environment. Originally considered biologically inactive, recent research has revealed their toxicity to humans and wildlife leading to stricter global regulations restricting their levels in food, water, air, and soil. As regulation increase, the need for PFAS testing continues to grow in environmental and state laboratories all over the United States as well as the world. PFAS standard and sample preparation are a challenging and time-consuming part of any laboratory scientist job. Method validation and then subsequent routine analysis of PFAS samples using liquid chromatography with tandem triple quadrupole mass spectrometry (LC-MS/MS) is a common practice and can be accomplished easily with some experienced tricks and tips. With ever changing Federal and State regulations on PFAS compounds in our environment, it is helpful to understand the various test methods available from the United States Environmental Protection Agency (EPA) including 537.1, 533, 1633, and 8327. Although these methods have fundamental differences, they all share common target analytes and dynamic linearity ranges. Method validation results and data calculations, specifics of sample preparation, SPE, standard preparation are discussed.

115 Determination of Cannabinoids in Animal Feeds by Liquid Chromatography–Tandem Mass Spectrometry

Xin Xu, University of Pennsylvania, New Bolton Center Toxicology Lab, 382 W Street Rd., Kennett Square, PA 19348, Lisa Murphy

Hemp is a variety of the Cannabis sativa plant species, which contains a wide range of cannabinoids. There is growing interest in using hemp materials as animal feed ingredients, which may raise safety concerns because of the potential transfer of active cannabinoids to the resultant products of animal origin. Hence, the detection and identification of cannabinoids in feeds would be useful. In this study, a simple, fast, and sensitive method was developed for simultaneous quantification of 4 major cannabinoids in animal feeds by liquid chromatography–tandem mass spectrometry (LC-MS/MS). A simple solvent extraction and dilution approach was used to extract cannabinoids from the feed matrix. The method was validated in 2 types of cattle feeds with acceptable intra-day and inter-day accuracy (87.5–116%) and precision (<15%). The limit of detection was 0.05 µg/g, and the limit of quantification was 0.1 µg/g. Furthermore, the method was able to identify and quantify cannabinoids

in cattle feeds mixed with hempseed cake as well as in several different hempseed materials, demonstrating its potential in veterinary laboratory applications.

116 Wet Chemistry Automation for Increasing Laboratory Productivity in Environmental, Food & Beverage Testing

Gary He, Thermo Fisher Scientific, 1214 Oakmead Parkway, Building 10, Sunnyvale, CA 94085

Automation of wet chemistry workflows not only improves the reliability, reproducibility, and sensitivity of results compared to manual techniques, but it also boosts laboratory productivity by freeing staff to walk away and work on other value-added tasks. This presentation will discuss the use of automated discrete analyzers and regulatory-compliant system methods for the determination of nutrients, enzyme activities, sugars, organic acids, fermentation critical parameters, and pH in routine environmental, food & beverage analysis. Participants will also learn that multi-parameter tests can be consolidated onto the high-throughput platform which provides a faster, easier, turnkey solution to traditional wet chemistry approaches for productivity improvement and cost reduction.

117 Transitioning from the Bench to Lab Leadership

Scott Hanton, Lab Manager Magazine, 26458 Harrow Court, South Lyon, MI 48178

Most lab managers are trained as scientists and earn their position through excellence at the technical bench. However, most of these new lab managers never studied management, business, or psychology. While most successful scientists have developed a range of people skills during the course of their technical career, like teamwork, cooperation, and flexibility, they now have a steep learning curve to become competent at delivering the leadership and management activities required in the new role. In addition, most lab managers are promoted from within, and they need to transition from being an important peer in the lab to being the manager. In this talk, we discuss: Important leadership principles; important management principles; elements of people management; Ways to address issues and conflict; importance of learning new skills.

118 How to Recruit, Hire and Onboard New Staff

Dwayne Henry, Montgomery College, 7600 Takoma Ave., Takoma Park, MD 20912

One of the issues many lab managers deal with is the high turnover rates of their lab staff, which unfortunately can also lead to issues of understaffing and low morale in the workplace. This can be due to a variety of reasons, however many times it is due to ineffective recruitment, ineffective hiring practices, and/or even more importantly ineffective onboarding of new staff. In this session we discuss the most current and effective methods of recruitment (both within and outside of the organization), as well as successful and proper hiring techniques to give you the best chance of hiring the right individual for the job. Lastly, we discuss productive onboarding strategies that will not only give new employees the proper foundation needed for a successful start, but also for continued success throughout their employment.

119 Improving Laboratory Processes Through SharePoint

Veronica Godley, SAWS, 2800 U.S. Hwy 281 North, San Antonio, TX 78212

Tracking various processes, such as controlled documents, corrective action preventive actions, and demonstrations of capabilities, poses challenges for many laboratories. To enhance efficiency, accessibility, and overall productivity, the San Antonio Water System Environmental Laboratory harness the powerful capabilities of SharePoint, a versatile digital collaboration platform to track DOCs. SharePoint offers an extensive range of tools and functionalities that facilitate streamlines workflows, seamless communication, and efficient information management. This session aims to shed light on the key benefits and strategies for maximizing SharePoint potential to optimize business processes, specifically in the context of laboratories. In addition, laboratories seeking to address challenges in tracking Demonstration of Capabilities (DOCs) can find SharePoint as a valuable tool. The session will also delve into the San Antonio Water System Environmental Laboratory Services outcomes of adopting SharePoint for tracking DOCs and highlights the key steps involved in setting up this process. This session serves as a practical guide for laboratories contemplating the transformation from paper-based processes to SharePoint-enabled workflows. Laboratories can leverage their DOC process by gaining an understanding of SharePoint capabilities as well as foster a culture of continuous improvement and collaboration.

120 How to Manage the Budget

Tarshae Drummond, Fayetteville State University, 1200 Murchison Rd., Fayetteville, NC 28301

Managing the budget plays a huge part in the success of a laboratory. It is financially allocating and distributing funds to manage the resources essential to its operation. Personnel, equipment, supplies, ect. . . are some components of budget allocations. Future goals and missions play a part in how to manage the budget. Therefore, knowing the objectives, doing research, asking questions, and planning are some ways to help manage the budget. If the budget is not handled and allocated properly the result can be inefficient operations, profit loss, misuse of resources, and other negative results. The talk features steps to create, manage, and maintain the budget to reflect how to effectively manage the resources.

121 Dietary Pathways and Routes of Human Exposure to PFAS

Dana McCue, EHS Support, 150 Church St. Northborough, MA 01532

Per- and polyfluoroalkyl substances (PFAS) are a manmade class of environmentally-persistent organofluorine chemicals widely used across various industries over the past seventy years. Toxicological evidence suggests that certain PFAS can pose a human health risk, particularly long-chain varieties. Most regulatory and scientific focus has concerned the drinking water ingestion pathway of human exposure to PFAS and not dietary. This is attributed, in part, due to analytical constraints in foods and an evolution in the understanding of potential risks posed by PFAS. Proportionally less focus has been put on ingestion exposure pathways through the consumption of food items, despite evidence suggesting that diet is the primary pathway of human exposure to PFAS for most populations. In this presentation, we will review a conceptual exposure model for PFAS that describes sources, transport processes, and exposure to human receptors. Particular attention will be focused on food and beverages as exposure media to PFAS. More research is needed to fully understand where and in what magnitudes PFAS enters the food supply, but multiple government initiatives have been underway to fill in these data gaps.

122 Using Non-Targeted Analyses to Probe PFAS Exposure from Sources Ranging from Nonstick Pans to Fish

Erin Baker, University of North Carolina, 131 South Rd., Caudill Laboratory 017, Chapel Hill, NC 27514, James Dodds, Anna Boatman, Kaylie Donelson, Greg Kudzin, Ashlee Falls

Per- and polyfluoroalkyl substances (PFAS) are a class of manmade organofluorine chemicals used in a variety of household and industrial applications. Due to the regulation of some historical PFAS over the last 15 years, the number of emerging PFAS has skyrocketed. To date, the United States Environmental Protection Agency's PFAS Master List contains over 14,000 entries with additional PFAS being discovered regularly by non-targeted screening (NTS). While the ability to elucidate chemical structures with NTS relies on producing informative fragments, typical data-dependent acquisition approaches often do not fragment important features due to their low abundance or co-eluting matrix molecules. To address this limitation, a NTS approach employing liquid chromatography (LC), ion mobility spectrometry (IMS) and mass spectrometry (MS) was coupled with data-independent acquisition (DIA) to fragment all detectable ions. This presentation details how the NTA workflow utilizes LC retention times, IMS collision cross sections, m/z values, fragmentation information, mass defects, and homologous series characteristics to confidently identify previously proposed PFAS, while also uncovering novel structures that have not been reported.

123 Bioaccumulation of Per/polyfluoroalkyl Substances (PFASs): What We can Learn Through High-Resolution Mass Spectrometry Analysis of Complex Mixtures

Carrie McDonough, Carnegie Mellon University, 4400 Fifth Ave., Pittsburgh, PA 15213

Humans and all other living things are continuously exposed to mixtures of per/polyfluoroalkyl substances (PFASs) via drinking water, diet, indoor dust, and commercial products (e.g., textiles, food wrappers, and cosmetics). Over time, these exposures have led to widespread accumulation of PFASs in human serum, and in the tissues and fluids of virtually every other animal investigated. Biomonitoring efforts have successfully established the global occurrence of perfluoroalkyl acids (PFAAs) and several other PFASs in human blood. However, despite considerable efforts to expand analyte lists to encompass more of the thousands of known PFASs, studies continue to report a significant gap between total organofluorine and total quantifiable PFASs in human blood. Identifying the PFASs contributing to this unidentified organofluorine (UOF) gap is essential to understand predominant exposure pathways and will enable a more complete understanding of total body burden and associated health impacts. Here I discuss progress toward characterizing PFAS mixtures in biological samples, including high-resolution mass spectrometry (HRMS) efforts and bioanalytical approaches for the identification and prioritization of novel PFASs.

124 PFAS in Foods - Method Expansion and Results

Susan Genualdi, United States Food & Drug Administration, 5001 Campus Dr., College Park, MD 20740, Wendy Young, Elsie Peprah, Cynthia Srigley, Stacey Wiggins, William Limm, Christine Fisher, Brian Ng, Lowri deJager

The development and expansion of analytical methods for per- and polyfluoroalkyl substances (PFAS) in food is essential for the continued monitoring of the United States (US) food supply and assessments of dietary intake. In March of 2022, the European Union Reference Laboratory (EURL) for halogenated persistent organic

pollutants (POPs) in Feed and Food released a guidance document covering priority PFAS of interest, including analytical method parameters and limits of quantification (LOQs). As a result, the Food and Drug Administration (FDA) began method extension work to incorporate ten new additional analytes to method C-010.02 including long chain perfluorosulfonic acids, fluorotelomer sulfonates, and perfluorooctane sulfonamide. Four long chain carboxylic acids were also validated across all foods, which were previously added to C-010.02 but only validated in seafood. In December of 2022, The European Union published Commission Regulation 2022/2388, establishing maximum levels for perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) in certain foodstuffs, primarily fish, molluscs, crustaceans, and eggs. As a result, the FDA method was evaluated for performance in reaching EU required LOQs for both compliance and monitoring purposes. The FDA method was found to be able to reach all required LOQs for analytes in matrices with established maximum levels. This method has been used for the analysis of PFAS in FDA total diet study samples, seafood surveys, and agricultural samples. The results from these samples are discussed.

125 Exposome and Human Disease: From Neurological Disorders to Diabetes and Cancer

Vasilis Vasiliou, Yale University, 451 College St, New Haven, CT 06511

The exposome, encompassing the totality of environmental exposures throughout an individual's lifetime, plays a pivotal role in shaping human health and disease. This emerging field of research sheds light on the intricate relationship between environmental factors and the development of various diseases, including neurological disorders, diabetes, and cancer. Neurological disorders, such as Alzheimer's disease, Parkinson's disease, and autism spectrum disorders, are multifaceted conditions with complex etiologies. The exposome framework provides a comprehensive approach to understanding how environmental factors, ranging from air and water pollution to chemical exposures, lifestyle choices, and psychosocial stressors, interact with genetic susceptibilities to influence neurological health outcomes. Diabetes, a global epidemic affecting millions, is influenced by a myriad of environmental factors. The exposome perspective underscores the significance of early-life exposures, such as prenatal and childhood exposures to endocrine-disrupting chemicals, dietary factors, and urban environments, in shaping diabetes risk later in life. Moreover, the interplay between the exposome and the gut microbiome adds a new dimension to our understanding of diabetes pathogenesis. Cancer, a leading cause of morbidity and mortality worldwide, is influenced by a complex interplay of genetic and environmental factors. The exposome paradigm emphasizes the impact of diverse environmental exposures, including tobacco smoke, occupational hazards, chemical pollutants, radiation, and lifestyle factors, on cancer development and progression. Unraveling the exposome-cancer relationship holds great promise for improving prevention, early detection, and therapeutic strategies. Advancements in exposome research methodologies, including biomonitoring, omics technologies, and data analytics, are providing valuable insights into the intricate web of exposures and their effects on human health. Longitudinal cohort studies, combined with sophisticated exposure assessment tools, are uncovering the cumulative and interactive effects of multiple environmental exposures over time.

126

No abstract submitted by the author.

127 Advanced Molecular Phenomics in Systems, Synthetic, and Chemical Biology

John McLean, Vanderbilt University, Department of Chemistry, 7330 Stevenson Center, Nashville, TN 37235

The human genome project (HGP) is recognized as one of the most successful big science projects in modern history. One of the primary motivations to undertake the HGP was to better understand what made us human and healthy - and how to use this code to improve the human condition by better understanding disease and potential treatment. While our knowledge expanded dramatically, we also uncovered profound biological complexity that we could not understand. This led to the current frontier of molecular phenomics, to catalog the broad-scale changes in the molecular inventory in cells, tissues, and biological fluids at a specific biological state. In phenomics, we seek to characterize the comprehensive molecular basis of biology in both space (e.g., at a cell, tissue, and organismal level) and time (e.g., healthy versus disease state). This places enormous demands on measurement technologies and computational approaches to organize the millions of potential species present in vanishingly small spatial coordinates. One of the promising strategies in molecular phenomics is ion mobility-mass spectrometry (IM-MS). IM-MS provides rapid (ms) gas-phase electrophoretic separations on the basis of molecular structure and is well suited for integration with rapid (us) mass spectrometry detection techniques. This report describes recent advances in IM-MS integrated omics measurement strategies in the analyses of complex biological samples of interest in systems, synthetic, and chemical biology. New advances in artificial intelligence and machine learning from an unbiased and untargeted perspective are described to quickly mine these massive datasets for actionable information.

128 Identifying and Quantifying Cellular and Media Metabolites Predictive of Mesenchymal Stromal Cell Potency by Metabolomics Coupled to Machine Learning

Facundo Fernández, Georgia Institute of Technology, 901 Atlantic Dr. NE, Atlanta, GA 30302

Mesenchymal stromal cells (MSCs) have shown promise in regenerative medicine applications due to their ability to modulate immune cells. However, MSCs demonstrate significant functional heterogeneity in terms of their immunomodulatory function because of differences in MSC donor/tissue source, as well as non-standardized manufacturing approaches. As MSC metabolism plays a critical role in their ability to expand to therapeutic numbers ex-vivo, we comprehensively profiled intracellular and extracellular metabolites throughout the expansion process to identify predictors of immunomodulatory function (T cell modulation and indoleamine-2,3-dehydrogenase (IDO) activity). Here, we profiled media metabolites in a non-destructive manner through daily sampling and nuclear magnetic resonance (NMR), as well as MSC intracellular metabolites at the end of expansion using mass spectrometry (MS). Using a robust consensus machine learning approach, we were able to identify panels of metabolites predictive of MSC immunomodulatory function for 10 independent MSC lines. This approach consisted of identifying metabolites in 2 or more machine learning models and then building consensus models based on these consensus metabolite panels. Consensus intracellular metabolites with high predictive value included multiple lipid classes (such as phosphatidylcholines, phosphatidylethanolamines, and sphingomyelins) while consensus media metabolites included proline, phenylalanine, and pyruvate. Pathway enrichment identified metabolic pathways significantly associated with MSC function such as sphingolipid signaling and metabolism, arginine and proline metabolism, and autophagy. Overall, this work establishes a generalizable framework for identifying consensus predictive metabolites that predict MSC function, as well as guiding future MSC manufacturing efforts through identification of high potency MSC lines and metabolic engineering.

129 Ion Mobility-Mass Spectrometry for High-Throughput Multi-Omics

Kelly Hines, University of Georgia, 302 East Campus Rd., Athens, GA 30602

There is growing interest and appreciation in the use of mass spectrometry-based multi-omics approaches to study biological processes and diseases from a systems-level perspective. The performance of discovery-level multi-omics typically involves the partitioning of a complex sample in its individual components, followed by thorough analysis of each "ome" under optimized liquid chromatography (LC) and mass spectrometry (MS) conditions. However, these multi-omics experiments must be streamlined before their findings can be implemented into diagnostic or prognostic applications. The rapid gas-phase structural separations afforded by IM-MS provides an opportunity for high-throughput measurements of biological samples containing mixtures of lipids, metabolites, peptides, and other biochemicals. We are developing methods for single injection high-throughput multi-omics based on ion mobility-mass spectrometry (IM-MS). The feasibility and advantages of IM-MS coupled to liquid chromatography and flow injection are demonstrated for microorganism identification to the species and strain levels using integrated lipidomic and metabolomic features.

130 Advancing Lipidomic Measurements and Informatics Tools to Enable Better Environmental and Health Assessments

Erin Baker, University of North Carolina, 131 South Rd., Caudill Laboratory 017, Chapel Hill, NC 27514, James Dodds, Jessie Chappel, Jack Ryan, Amie Solosky

Lipidomic measurements provide important information about the health of biological systems due to the vast presence of lipids in cellular membranes and their roles in signaling and energy storage. However, lipidomic evaluations are extremely difficult due to the numerous isomers possible and the great concentration range of the lipid species present in complex biological samples. The development of powerful analytical techniques and computational tools is therefore essential. To analytically evaluate lipids, the integration of liquid chromatography, ion mobility spectrometry, collision induced dissociation, and mass spectrometry (LC-IMS-CID-MS) techniques for lipidomic studies has recently been implemented. These multi-dimensional measurements improve the separation of lipid species and provide valuable polarity, structural and mass information simultaneously for each feature detected. While LC-IMS-CID-MS measurements enable better lipid characterization and identification, they also result in extremely large and complex datasets which are difficult to process. To face these challenges, we have developed cheminformatics tools and machine learning pipelines for the rapid and automated processing of lipidomic data. We also manually curated a lipid database containing experimental LC, IMS, MS and MS/MS information for over 1000 lipids. This presentation details how LC-IMS-CID-MS measurements for various sample types were combined with the newly developed library and informatic tools to gain novel insight about different biological systems.

131 SubVision: Deep Learning to Accelerate Biopharmaceutical Formulation Development

Yue-Ming Chen, Merck & Co., Inc., 126 East Lincoln Ave., Rahway, NJ 07065

Controlling particulate matter in finished injectable drug products is crucial to both a successful manufacturing process and regulatory approval. Regulatory agencies require such data to examine critical quality attributes, such as subvisible particle counts from formulation studies, for demonstrating stability of drug products during transportation, storage, and administration. Micro-Flow Imaging (MFI) has been widely employed to identify subvisible particulates for sterile liquid protein formulation studies. However, the current standard software possesses only limited rudimentary data analysis capability, such as aspect ratio filters, and cannot effectively distinguish classes of particles. For instance, it struggles to differentiate low-risk particles such as air bubbles and silicone oil droplets from high-risk protein aggregates. To address these shortcomings, we have developed SubVision, a cloud-based, AI-assisted automated analytics platform to classify particles into different classes, enabling more fine-grain analysis of MFI data. SubVision delivers an end-to-end solution that meets the challenge of efficiently accelerating particle analysis of MFI data from an entire formulation experiment. Moreover, our system automatically produces a comprehensive and standalone analysis report, providing in-depth detail on particulate matter characterization. More importantly, by leveraging learned image features from our deep learning model, scientists can gain deeper insights into subvisible particle populations on the experiment. This capability opens up possibilities for developing new methods for testing or stressing formulation samples, such as high-concentration subcutaneous products.

132

No abstract submitted by the author.

133 Automated Data Pipeline for the High-Throughput Screening of Organic Solubility in Pharmaceutical Drug Development

Michael Rerick, GSK, 8 York Road, Douglassville, PA 19518

The adoption of high-throughput screening has alleviated major challenges associated with small molecule drug development by readily evaluating large libraries of potential therapeutic compounds. Comprehensive analytical testing of physical properties, formulations, and process conditions can be efficiently carried out to allow for data driven decisions at an accelerated rate. Commonly, the results of these screens are used to make informed decisions on individual projects, but what innovations in knowledge management are required to leverage all historical data for decisions in future drug development efforts across the organization? With emerging advancements in predictive modeling and machine learning becoming more accessible, adoption of streamlined data management throughout the pharmaceutical industry is paramount to developing new medications more efficiently, reliably, and cost-effectively. The primary focus of this presentation is to review the steps GSK has taken to bridge the gap between analytical chemistry and data science in an effort to modernize small molecule drug development. An end-to-end data pipeline has been developed and applied to small molecule organic solubility workflows, encompassing experimental design using LibraryStudio, instrument integration with Agilent UHPLC systems, and visualizations in Spotfire. The outcome of this approach to data generation is a standardized result set contributing to a centralized database for predictive modeling of solubility. Combining this with mixed-reality training modules using HoloLens technology, analysts are trained more uniformly and efficiently to lower error frequency and expedite characterization of therapeutic candidates.

134 Investigating Oral Solid Dosage Excipient Compatibility via Automation and High-Throughput Experimentation

Alexander Chin, Merck & Co., Inc., RY801-200, 126 E. Lincoln Ave., Rahway, NJ 07065

Excipient compatibility is a necessary step in the development of new drug products to ensure the safety and efficacy of our products for patients. While most excipients are universally accepted for delivery, stability, and manufacture of our active pharmaceutical ingredients (APIs), there remains a risk of physical and chemical interactions that can impede rapid product development. The process to interrogate the entire formulation space can be a tedious and time-consuming endeavor. This forces formulators to spend unnecessary and excess resources generating samples to evaluate performance, and in turn inundate analytical chemists with samples with an unknown chance of success. For over a decade, high-throughput automation has been utilized for excipient compatibility at Merck. By collaborating with our formulators and applying high-throughput preparation, analytical characterization, and data analysis tools, we have been able to accelerate the excipient compatibility process, saving time and resources during product development. Standard experiments consist of 96-well plates, which will generate approximately 500 liquid chromatography injections and 700 x-ray powder diffraction scans. In the span of one month, we can probe the physical and chemical risks of an API in the presence of our excipient catalog. In collaboration with information technology partners, specialty software was created to manage and examine all the data generated efficiently. The insight from our excipient compatibility screen provides necessary information to our formulators, so they may carefully triage troublesome excipients and focus on ones with the highest probability of success for primary market formulation development.

135 Getting the Most Out of Portable Instrumentation: Handheld LIBS Method Development for Timber Analysis

Caelin Celani, University of Delaware, 104 Brown Lab, The Green, Newark, DE 19716, Erin McClure-Price, James Jordan, Tyler Coplen, Helder Carneiro, Maria Delgado, Karl Booksh

The illegal timber trade has significant impact on both the survival of endangered tropical hardwoods like Dalbergia spp. as well as substantial economic impacts. Due to minimal governmental intervention at the source, fast and reliable solutions which are deployable at ports of entry are a necessity for policing the illegal timber trade. Handheld laser induced breakdown spectroscopy (LIBS) coupled with chemometric classification algorithms have established a framework for successfully classifying over a dozen species of Dalbergia and its lookalikes. While this task is effective for a moderate number of species, hundreds of unique species will need incorporated into models for proper field implementation. For this reason, substantial work has been done to optimize the LIBS signal prior to analysis to maximize model performance. In this work, LIBS signal and modelling are optimized through analysis of Signal-to-noise (S/N) versus delay time, investigation of the effects of gating, and maximization of net analyte signal prior to analysis via a number of different classification algorithms. Additionally, practical modelling strategies for classification tasks with many classes are discussed.

136 Academics to Industry: Becoming a Pharmaceutical Forensics Chemist

Brittany Handzo, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901

Pharmaceutical forensics is a unique industry field, where samples of unknown identity, composition, quality, or origin are analyzed. Every investigation differs in nature, ranging from counterfeit drug products to patient complaints. Being a scientist in this field not only requires various analytical skills such as microscopy and spectroscopy, but also involves other elements such as problem solving, integrity, creativity, and urgency. The objective of this presentation is to highlight the journey of becoming a pharmaceutical forensics chemist, starting from academic studies, through early career experiences, and ending with current job responsibilities.

137 PCR-Less, Enzyme-Free Methods for Sensitive Detection of Disease Biomarkers

Samuel Mabbott, Texas A&M University, 600 Discovery Dr., College Station, TX 77843

In the rapidly evolving field of point-of-care (POC) diagnostics, it is crucial to develop methods for sensitive and specific biomarker detection that can be adopted in resource-constrained areas. Our group's research focuses on the utilization of DNA hairpin assemblies, exploring their potential to enhance detectable signals in the presence of specific biomarkers. Our approach exploits the unique properties of DNA hairpin structures. These structures exhibit dynamic behavior that can be harnessed to create intricate, self-assembled formations when interacting with specific targets. This interaction is facilitated by the process of toe-hold induced hairpin opening, a spontaneous mechanism enabling enzyme-free, isothermal amplification of DNA. The strategic design of these DNA sequences, distinguished by short single-stranded regions known as "toe-holds", facilitates hairpin unfolding. In essence, this acts as a biomarker-sensitive switch, triggering an amplification event without the need for enzymatic intervention or fluctuating thermal conditions. This intuitive process amplifies the measurable signal providing a means for visual or spectroscopic detection - a crucial attribute for POC diagnostics. In my presentation, I elaborate on our work regarding the synthesis of spontaneous hairpin assemblies for the highly sensitive POC detection of biomarkers associated with myocardial infarction and preeclampsia. Our methodologies leverage the potential of colorimetry and surface-enhanced Raman scattering (SERS) for detection, maximizing the sensitivity and specificity of our assays. This integrative approach allows an exceptional level of sensitivity in biomarker detection, greatly enhancing the effectiveness and efficiency of POC diagnostics.

138 From Crime Scenes to Clinics: Raman Hyperspectroscopy of Body Fluid Stains and Chemometrics for Forensic Purposes and Diseases Diagnostics

Bhavik Vyas, University at Albany, 1400 Washington Ave., Life Science Building-LSRB1114, Albany, NY 12222, Lenka Halamkova, Igor Lednev

Raman hyperspectroscopy, in combination with advanced chemometrics, emerges as a powerful and non-destructive technique for studying micro-heterogeneous systems, particularly body fluids. This method offers a comprehensive understanding of the structures and biochemical components of samples at a microscopic level, opening up practical applications in disease diagnostics and forensics evidence analysis. The unique capability of Raman hyperspectroscopy lies in its capacity to integrate the influence of multiple biomarkers into a specific spectroscopic signature,

even at low concentrations. Hyperspectral imaging plays a pivotal role in this context, providing detailed spectral information for individual pixels in a sample image, thereby enabling the identification of components and their respective concentrations. In scenarios like distinguishing between healthy and diseased samples or classifying different body fluid stains for forensics, the spectral differences offer critical insights, such as disease biomarker discovery and body fluid categorization. However, the subtle spectral changes caused by different components can be challenging to discern visually. To address this, chemometrics or advanced statistics become instrumental. We leverage the potential of Raman hyperspectroscopy and chemometric analysis to develop a universal test for identifying major body fluids, including urine stains commonly encountered at crime scenes. Additionally, we demonstrate the successful determination of the donor's race through Raman spectroscopy and a random forest classification model. Building on this success, we extend our approach to disease diagnosis, focusing on Raman spectroscopy of saliva, with the promise of revolutionizing medical diagnostics.

139 Applications of NMR and Statistical Methods in Establishing Analytical Comparability and Process Consistency for mAbs

Igor Dikiy, Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Rd., Tarrytown, NY 10591, William Matousek, Simona Horsa, Polat Abdubek, Dylan Howie, Cody Secor, Michael Rosconi, Erica Pyles

During development of biological drugs such as monoclonal antibodies (mAbs), it is important to establish comparability of key physicochemical properties, including higher-order structure (HOS), following changes and/or improvements to the manufacturing process. NMR spectroscopy is a non-destructive and data-rich analytical method that can report on higher-order structure, as well as dynamics, of macromolecules in solution at atomic resolution. Recent advances in NMR acquisition and analysis, including non-uniform sampling schemes, have allowed for the collection of highly reproducible fingerprint spectra of therapeutic mAbs. However, certain challenges in using NMR to analyze GMP-produced, natural abundance mAb samples remain, including the difficulty of making accurate peak assignments to get site-specific information. Here we show several different phase-appropriate applications of NMR spectroscopy in characterizing therapeutic mAb comparability and process consistency, using unbiased statistical approaches to obviate the need for residue-specific assignments. Computational comparisons using ECHOS-NMR and PCA of 1D and 2D NMR spectra establish comparability between different manufacturing processes, as well as intra-process consistency, while revealing small differences in HOS. Additionally, we demonstrate initial steps toward assignment of protein and glycan-derived signals allowing for preliminary quantitative and qualitative structure-function analyses of critical quality attributes.

140 Leveraging Quadrupolar Nuclei in Solid-State NMR

Alexander Paterson, UW-Madison, 433 Babcock Dr., Madison, WI 53706

Solid-state nuclear magnetic resonance (SSNMR) spectroscopy is a powerful tool for the study of systems ranging from the small-molecule organics, to biosolids such as proteins, to inorganic materials such as ceramics, semiconductors, battery materials, and interfaces. The wide range of chemistries involved in inorganic chemistry motivates the use of SSNMR to target quadrupolar nuclei, i.e., nuclei with nuclear spin greater than ½. Some nuclei, such as 11B or 27Al, are sensitive targets that easily provide a wealth of information. Other nuclei have obvious chemical interest, e.g., 43Ca or 35Cl, but suffer from challenges in sensitivity, acquisition, and interpretation. As part of the Network for Advanced NMR (NAN), the National Magnetic Resonance Facility at Madison (NMRFAM) is commissioning a 1.1 GHz NMR spectrometer dedicated to the study of solids. The NAN mission is to provide access to state-of-the-art NMR instrumentation, facilitate data stewardship that meets findable, accessible, interoperable and reusable (FAIR) data standards, and develop NMR Knowledgebases in the areas of solution structural biology, metabolomics, biological solid-state NMR, and materials solid-state NMR. Quadrupolar nuclei in particular benefit from ultra-high magnetic fields, and use pulse sequences which may be unfamiliar to spectroscopists working with spin ½ nuclei. This presentation discusses various ways to leverage quadrupolar nuclei using solid-state NMR, including methods for direct detection, indirect detection, sensitivity enhancement, and how to choose the most appropriate magnetic field strength for a given experiment.

141 Highly Standardized NMR-Metabolite Biomarker Discovery Using Clinical Samples

Alessia Trimigno, Olaris, Inc., 175 Crossing Blvd., Framingham, MA 01702, Kari Boardman, Chen Dong, Jifang Zhao, Keri Sheehan, Elizabeth O'Day

Metabolites provide energy and biomass for life to exist, and altered metabolism has been linked to nearly all diseases. Thus, metabolite-based biomarkers have the potential to transform how diseases are diagnosed and treated. However, metabolites are highly sensitive to non-disease-related factors (diet, ethnicity, physical activity, bias from analytical measurement, sample handling, processing and more), posing significant challenges to discovering and developing robust metabolite-based clinical diagnostics. Consistent with these challenges, recent meta-analyses have demonstrated low concordance from metabolomic studies in diseases such as pancreatic cancer and kidney transplant. We have pioneered a multi-dimensional non-uniform sampling (NUS) nuclear magnetic resonance (NMR)-based metabolomics discovery platform for detection and relative quantification of a broad range of metabolites (μM to mM) with high reproducibility (%CV<10%) and a turnaround time amenable to clinical testing (~1 hour/sample). This platform is enabling diagnostic development and aiding in biopharma drug development. Ensuring high-quality clinical samples is a prerequisite for this discovery. Here, we report the development of "Navigators," a proprietary combination of molecule(s) to monitor sample processing, to estimate the pH and the specific gravity (SG) of the sample, and to ensure "batch" consistency for longitudinal studies. These molecule(s) do not influence sample pH, nor do they interfere with the chemical shift or intensity of biofluid metabolite resonances, but rather the chemical shift position and intensity levels of the Navigators themselves serve as read out for quality control and quality assurance (QA/QC).

142 Painting a Portrait of a Protein that Won't Sit Still

Alexandra Pozhidaeva, UConn Health, 263 Farmington Ave., Farmington, CT 06030, Yulia Pustovalova, Irina Bezsonova, Oksana Gorbatyuk, Jeffrey Hoch

Over 500,000 people die from malaria every year. Recent emergence of drug-resistant *Plasmodium falciparum* underscores the urgency of developing new treatments to complement the existing therapies. Protein methyltransferases (PMTs) represent a class of enzymes that use S-adenosyl methionine (SAM) to methylate protein substrates. PfPMT found in malaria parasites is crucial for synthesizing phosphatidylcholine (PC), a major component of plasmodium membranes. A PC synthesis pathway in which PfPMT converts phosphoethanolamine to phosphocholine, is absent in mammals making the enzyme an attractive therapeutic target. Crystal structures of a single SAM-dependent catalytic domain of PfPMT with its substrates/inhibitors have been determined. Interestingly, residues of the enzyme involved in direct interactions with the substrates in these structures are not solvent-exposed, indicating that conformational changes are necessary for substrate recognition. In addition, the structure of the apo protein has never been crystalized further suggesting conformational dynamics. To address this, we characterized apo PfPMT enzyme using solution NMR methods in combination with molecular dynamics simulations. Our findings revealed distinct conformational differences between the apo and the substrate-bound states. We also gained insight into the mechanism of PfPMT inhibition by amodiaquine (AQ). AQ binds away from the active site of the enzyme and the mechanism of its action remained unclear from the crystal structure. We found that chemical shift perturbations of PfPMT residues upon addition of AQ propagate from the binding site to the N-terminal helices and the active site. This work provides a basis for future efforts to develop new potent anti-malarial compounds.

143 Nanoparticle Enhanced Laser Induced Breakdown Spectroscopy (NELIBS) for the Analysis of Gunshot Residue

Alyssa Marsico, University of New Haven, 300 Boston Post Rd., West Haven, CT 06516

Gunshot residue (GSR) is a plume of organic and inorganic particles produced after discharging a firearm and can be important in forensic cases. The inorganic components include lead, barium, and antimony, which are critical in its detection. Laser induced breakdown spectroscopy (LIBS) provides elemental information about samples, and can be used in conjunction with nanoparticles, a technique known as nanoparticle enhanced LIBS (NELIBS). NELIBS has been used to investigate a possible method for the detection of GSR in forensic cases. Samples of gunpowder were burned in a controlled environment in the laboratory. A 9mm firearm was discharged at a shooting range and GSR stubs were used to collect material from the shooter's hands, which were washed between firings and collection. All samples collected were analyzed using conventional LIBS and NELIBS with a variety of nanoparticles. The resulting spectra were then analyzed for the presence of lead, barium, and antimony, and the signal intensities were compared. The limit of detection (LOD) for the analysis of burnt gunpowder improved by 10^4 when using magnetic oxide nanoparticles and NELIBS (100 fg LOD) instead of LIBS (1 ng LOD). In GSR samples, gold nanoparticles resulted in barium signals that were 20x higher, lead signals that were 50x higher, and antimony signals that were 1.2x higher than LIBS. This data suggests that NELIBS could be a viable method of GSR detection in forensic analyses and provides both low limits of detection and large signal enhancements for the signature elements in GSR compared to LIBS.

144 Isotopic Profiling of Explosives Using Gas Chromatography Triple Stage Quadrupole Isotope Ratio Mass Spectrometry

Stephan Hlohowskyj, Federal Bureau of Investigation, 4940 Fowler Rd., Huntsville, AL 35898

Explosives used for criminal and terrorist activities are a continuous threat to public safety. Forensic examinations of explosive chemistry produce actionable intelligence which improves efficiency of law enforcement (LE) investigations. Additionally, through studying explosive related incidents or post-blast crime scenes, LE can trace the origins, determine the relationships, or cross reference explosive evidence.

Thus, LE agencies may try to link explosive evidence to a common source or precursor chemicals. Isotope ratio mass spectrometry (IRMS) is specifically designed to measure the isotopic signatures of materials that are chemically identical by converting a material to primary component gases, such as carbon dioxide, nitrogen, oxygen, or hydrogen. To convert an explosive sample to component gasses, different sample preparation and introduction apparatus are used for either combustion or high temperature conversion. Specifically, IRMS can leverage a gas chromatography (GC) 'front-end' to isolate constituents from a complex chemical mixture before converting each to component gases which are measured to produce stable isotopic signatures. In this research we coupled a triple stage quadrupole (TSQ) mass spectrometer GC system to the IRMS. This setup allowed a sample to be aliquoted between the TSQ and IRMS via GC separation, permitting simultaneous collection of molecular mass data and stable isotope ratios from component gases. Using this instrument setup, we tested the potential to isolate explosive components, extracted materials, and solvent soluble explosives. Our data demonstrate a potential improvement of explosives forensic analyses by using sample aliquots from traditional forensic exam processing to generate actionable source attribution intelligence.

145 Capabilities and Limitations of Canine Training Across an Explosive Odor Spectrum

Melissa Singletary, Auburn University, College of Veterinary Medicine, 116 Greene Hall, Auburn, AL 36849

Detection canines represent a unique biological sensor platform that is mobile and rapidly programmable. With selective pressures the dog has evolved to have a highly specialized and sensitive olfactory system. Importantly this detection platform is adaptable with optimization training to expand their pre-programmed library of the target odor spectrum for a given explosive. This combination of high olfactory acuity and natural intelligence result in the ability to predict and modulate their odor libraries that may include odors that represent variations of the original odor across its odor spectrum. However, this process of odor learning and generalization, is not uniformly applied with all target odors or across all dogs. These attributes can be either an asset or a liability dependent on the operational demands and use-case for the detection canine. These capabilities and limitations of canine training across an explosive odor spectrum are important considerations in the application of odor presentation in the field of detection canine sciences.

146 Development of Strategic Analytical Methods to Support the Modernization of Gunshot Residue (GSR) Practice in Forensic Science.

Tatiana Trejos, West Virginia University, 1600 University Ave., Oglebay Hall, #208, Department of Forensic and Investigative Science, Morgantown, WV 26506, Luis Arroyo, Kourtney Dalzell, Thomas Ledergerber, Leah Thomas, Madison Lindung

Despite the scientific validity of the GSR discipline, there are persisting challenges regarding the speed of analysis, preservation of the evidence, and interpretation of results. This study presents a comprehensive approach to overcome these concerns and enhance current capabilities. This project developed and validated fast and reliable tests, using laser-induced breakdown spectroscopy (LIBS) and electrochemical (EC) sensors for GSR detection at the laboratory and at the crime scene. The screening tools can generate results in under 5 minutes as opposed to over 4 hours per sample needed with the standard method. The performance rates are evaluated with an extensive one-of-a-kind database of organic and inorganic gunshot residues using a multi-technique approach on various populations of legitimate shooters and background non-shooters. The sample sets included complex scenarios such as those individuals that may represent a high risk for occupational/environmental false positives, individuals who have conducted post-shooting activities, and shooters who have used mixed leaded and lead-free ammunition. The combination of LIBS and EC data permitted the accurate identification of organic and inorganic residues on authentic specimens (OGSR & IGSR, accuracy ranging from 92-99% depending on the subpopulation). Moreover, the technique's utility and versatility were demonstrated for several substrates commonly found in firearm-related cases, such as hands and other skin areas of individuals of interest, clothing, and large non-movable objects. These research findings are anticipated to increase current knowledge in this field and assist in the modernization of protocols for the analysis and interpretation of gunshot residue evidence.

147 Sorption and Desorption of 17Alpha-Ethinylestradiol (EE2) and Beta-Estradiol (E2) on Montmorillonite Clay

Christian Manuelli, University of Massachusetts Dartmouth, 531 N Front St., Apt #1W, New Bedford, MA 02745, Yuegang Zuo

There is growing concern of the adverse effects of endocrine disrupting chemicals (EDC's) on the environment, wildlife, and human beings. EDC's have been detected in freshwater and marine sediments as well as soils around the planet. Thus, it is important to understand the transportation and fate of EDC's, and their interactions with soil, sediment, and suspended particles in aquatic ecosystems. In this study, the sorption of the EDC's 17a-ethinylestradiol (EE2) and b-estradiol (E2) to montmorillonite (a major mineral component in surface aquifers) was investigated in aqueous solutions at 25°C. Time-based batch sorption experiments of 0.50-4.0 mg/L EE2/E2 were conducted for 30 minutes for up to 4 days. Fluorescent measurement of aqueous EE2 and E2 displayed significant sorption on montmorillonite, with an equilibration time of at least 36 hours. Mass balance isotherms of the solute with the aqueous and solid phase displayed 50-70% of EE2 associated with the clay, depending on the initial concentration. The linearized Freundlich isotherm model was used for 48-hour sorption data, and yielded a KF value of 0.116, and an n value of 0.72. Sorption experiments revealed a slower equilibrium time, but a strong affinity for the high surface area powdered montmorillonite clay.

148 PFAS Dark Matter, Slippery Cannabis and Catechin Epimers: Disparate Problems with a Similar Path to a Solution

Frederick Strathmann, MOBILion Systems, Inc., 4 Hillman Dr., Ste. 130, Chadds Ford, PA 19317, Thomas Lubinsky, Rachel Harris, Julie Wushensky

Per- and polyfluoroalkyl substances (PFAS) compounds have a long and diverse history of applications yet only recently has sufficient attention been focused on the environmental and toxicological impacts of their use. The number of PFAS compounds has expanded rapidly, and it is estimated that over 5,000 PFAS compounds exist. Despite the number of PFAS compounds in existence, a relatively small number have been studied in depth and are commercially tested routinely. The term "PFAS Dark Matter" has emerged to signify the recognized gap between total organic fluorine, total oxidizable precursors, and targeted methods using mass spectrometry for PFAS concentration assessment. Similarly multifarious, cannabis has a complex chemical composition that includes terpenes, sugars, hydrocarbons, steroids, flavonoids, amino acids, and other compounds of potential interest. More than 700 natural constituents have been identified and more than 100 are classified as cannabinoids. The toxicological community has been challenged with the appearance of isomers of various cannabinoids causing numerous analytical challenges with limited solutions beyond chromatographic run time extension. In food and natural extracts, similar challenges exist with catechin epimers a well-known example highlighting the same analytical challenges in widely different contexts. Herein we provide a similar path to a solution by incorporating high resolution ion mobility to go faster, see more features, and focus on the right stuff.

149 Your GC/MS Knows What You're Doing at Home...Sort of: Looking at VOC Makeup of New and Occupied Homes Using Pyrolysis Gas Chromatography.

Khadiza Mom, Quantum Analytics, 8301 New Trails Dr., Ste 100, The Woodlands, TX 77304

There's no doubt that volatile organic compounds (VOCs) of indoor environments are shaped by what's in them. On a more personal level, VOC's inside of a home is shaped by home and homeowner. This blind study employed Pyrolysis-gas chromatography mass spectrometry (GC-MS) to observe differences in volatiles found in newly built homes before and after occupation. This was done by placing solid phase extraction elements (SPEE) in living spaces to collect the volatiles in the air. The SPEE's were then analyzed by thermal desorption in the pyrolyzer-GC-MS. Paint and flooring were also tested by direct thermal desorption in the same system to observe if they were sources of any VOC's. Beyond experimentation, we are intimately familiar with the conspicuous "new house smell". Over time, the smell ebbs, homeowners move in, and add their own scents. However, though analysis, we also find that within the terpenes from air fresheners are inodorous compounds that suggest the walls and floors leave their trace in time.

150 Analytical Chemistry is Essential for Gaining Understanding of the Earth's Climate, Past Present and Future

Roland Hirsch, Retired, 13830 Metcalf Avenue, Apt 15218, Overland Park, KS 66223

Many uncertainties exist about the past climate of the Earth. The drivers of the current climate are not yet understood and thus models of future climate are poor (even just for temperature). Analytical chemistry is playing a major role in improving the knowledge of the key aspects of climate. Many analytical technologies are used for this, including spectroscopy from microwave to x-ray, gas and liquid chromatography, mass spectrometry, and electrochemistry. This presentation seeks to explain how these technologies are leading to better understanding of climate.

151 Photooxidation and Phenol Decomposition Processes on Hydrophobic Nanoparticles

Alexander Greer, Brooklyn College, Graduate Center of the City University of New York, 2900 Bedford Ave. Brooklyn, NY 11210, Serah Essang, Lloyd Lapoot, Britney Singh

Although silica nanoparticles have been used in oxidations for the production of peroxides, studies of airborne singlet oxygen ($^1O_2$) at nanoparticle interfaces are very limited. Here, with prenyl phenol-coated silica nanoparticles and delivery of singlet oxygen through the gas phase, we uncover the formation of primary products dihyd-

robenzofuran and allylic hydroperoxides. The hydrophobic nanoparticles lead to the secondary production of hydrogen peroxide and methane, respectively. Analytical results are discussed on the secondary formation of hydrogen peroxide and methane, for mechanistic insight to the photooxidation and prenyl phenol decomposition process at the air/nanoparticle interface.

152 Rapid, Efficient and Safe Microwave-Assisted Digestion of Li Battery Components for Trace Metals Analysis

Alicia Stell, CEM Corporation, 3100 Smith Farm Rd, Matthews, NC 28104, Samuel Heckle, Macy Harris

Currently there is a drive for research, development and implementation of the next generation of Li battery high purity raw materials for improved performance of the basic battery components. Research initiatives are focused on the elemental composition, specifically aimed at replacing Co with the more abundant and easier to extract Mn. Meanwhile, testing the basic battery components including cathode, separator and even the electrolyte materials for elements down to ppb level to improve battery performance in now the norm. The new automated CEM BLADE microwave-assisted acid digestion system can safely operate at temperatures and pressures required to provide a more rapid and efficient extraction or total digestion of these material, thus providing a more accurate analysis via inductively coupled plasma optical emission spectrometry mass spectrometry (ICP-OES) and ICP-mass spectrometry (MS). Different sample types, including digested and analyzed lithium ores, salts, cathode materials and anode materials were digested and analyzed. Samples were digested in triplicate and selected reaction monitoring (SRM) and spiked samples were used to validate both the microwave-assisted digestion method as well as digest analysis. Excellent recoveries were achieved for the various sample types, proving the CEM BLADE is a valuable tool for Li Batter research and development.

153 Applied Chemistry in the Clinical Microbiology Laboratory

Melissa Gitman, Icahn School of Medicine at Mount Sinai, 1425 Madison Ave., Rm 9-52C, New York, NY 10029

This session reviews the overall workflow and function of a clinical microbiology laboratory, and the role-played in patients care. The session explores all the steps from sample collection to identification of an infection. Various applications of chemistry in the clinical microbiology laboratory are discussed including an overview of the traditional use of biochemicals in organism identification. Particular emphasis is placed on the utilization of the following diagnostic technologies: matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry, magnetic resonance imaging and multiplex PCR. Finally, a look at future integration of novel technologies into the clinical laboratory is included.

154 CRISPR-Powered Microfluidic Biosensing Systems for Point-of-care Detection of Infectious Diseases

Changchun Liu, UConn Health, 263 Farmington Ave., Farmington, CT 06030

Simple, rapid, and sensitive molecular detection plays a crucial role in early diagnosis and treatment of infectious diseases. CRISPR technology has recently emerged as a powerful biosensing tool for point of care molecular diagnostics when coupled with microfluidics technology. In this talk, I will present our recent development of combining CRISPR technology with microfluidic technology for detection of HIV virus and SARS-CoV-2 virus. I introduce our effort in developing low-cost CRISPR-based microfluidic biosensing systems, including bioinspired CRISPR-mediated cascade reaction biosensor, instrument-free, self-contained microfluidic CRISPR biosensing system et al. These simple, sensitive, and affordable diagnostic tools exhibit potential for rapid detection of infectious diseases at the point of care.

155 Advances in Sterilization and Disinfection in Healthcare Settings

Scott Roberts, Yale School of Medicine, 20 York S., Hunter 527, New Haven, CT 06510

This presentation reviews up to date guidance and techniques for achieving appropriate sterilization and disinfection in healthcare settings. Managing contaminated surfaces and medical equipment used in various patient care settings are discussed.

156 The Treatment and Prevention of Clostridioides Difficile Infection

David Banach, University of Connecticut School of Medicine, 263 Farmington Ave., Farmington, CT 06030

Clostridioides difficile (C. difficile) infection is the most common healthcare-associated infection in the United States. Infection prevention strategies have primarily focused on preventing exposure to C. difficile organisms and antibiotic stewardship as broad spectrum, systemic antibiotics are known to disrupt the intestinal bacterial flora and predispose to C. difficile infection. Treatment of C. difficile infections has generally consisted of antibacterials that eradicate the C. difficile organisms. Advances in understanding the spread of C. difficile and the role of the intestinal microbiome have led to the development of targeted infection prevention and treatment strategies. These include targeted monoclonal antibodies and antibacterials, live bioengineered biotherapeutics and vaccination. This talk discusses these novel, emerging approaches to the prevention and treatment of C. difficile infection and provide insight into future prevention and treatment strategies.

157 Capillary Isoelectric Focusing (cIEF) Technology Bridging

Christopher Cammarata, Janssen Research and Development, 200 Great Valley Pkwy., Malvern, PA 19355

Capillary isoelectric focusing (cIEF) is an analytical technique that separates peptides/proteins and other charged analytes based on their isoelectric point. cIEF is commonly used in the pharmaceutical industry during development and product quality control to monitor critical quality attributes such as deamidation and glycation. The Protein Simple iCE3 instrument was first introduced in 2012 and has become the pharmaceutical industry's standard for cIEF. Most recently, Protein Simple discontinued the iCE3 and has replaced it with their newest model, the Maurice. With substantial changes to the instrument's layout and capillary design, a bridging strategy is required to demonstrate the interchangeability of instruments for molecules at various stages of clinical and commercial development for which iCE3 methods have been used. A representative selection of molecules with varying pIs, method conditions, and profiles was selected to assess the equivalence of the Maurice and iCE3. Statistical analysis was performed on both typical samples and stressed samples. Results indicated profiles and method system suitability criteria are highly similar showing a < 2% absolute difference in reportable results between the two instruments.

158 Controlling Process-Related Impurities in the Biopharma Setting

Hope McMahon, GSK, 709 Swedeland Rd., King of Prussia, PA 19406, Chris Gerberich, Robert Luo

Process related impurities (PRIs) can potentially affect the safety (toxicity, immunogenicity, and biological activities) of a drug product. Typical PRIs include cell substrate-derived impurities, cell culture-derived impurities, and downstream process-derived impurities. A routine testing strategy is often unrealistic and unnecessary for all PRIs. Therefore, a risk assessment approach is used to guide the testing strategy. A risk assessment approach will be described, where impurities are identified, the risk is categorized, and further calculations provide a list of impurities where demonstration of clearance must be provided. This risk assessment approach has been successfully implemented. However, additional complexity is introduced when utilizing commercially available media, where individual components are proprietary. Three case studies are provided to demonstrate the challenges encountered when authoring a risk assessment using proprietary media.

159 Large Molecule Bioassay Development: Strategies and Analytical Challenges

Julie McIntosh, Merck & Co., 2000 Galloping Hill Rd., Kenilworth, NJ 07033

Large molecule biologics have advanced from traditional monoclonal antibodies to drugs with greater complexity, including fusion proteins, multi-specifics, and antibody-drug conjugates, each containing distinct active domains. A key consideration in the development of these biologics is an in-depth understanding of the structure-function relationship of the molecule. Bioassays such as ELISA (enzyme-linked immunosorbent assay) and cell-based assays measure the potency of these compounds, which reflect the functional part of that relationship. By correlating the structural and potency changes, attributes and conditions that impact the compounds functionality can be identified, which guide process development and product quality control. The regulatory authority expectations for bioassays are to be specific to each product and to reflect the mechanism of action of the molecule. As such, there are unique considerations and challenges surrounding bioassay method development and overall potency strategy throughout the drug development lifecycle. In this presentation, a case study on the development of bioassay methods and potency strategy for a large molecule is presented.

160 The Importance of High Throughput Analytics and Automation in Vaccine Analytical Research and Development

Kristen Feibelman, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486

Rapid vaccine development will likely remain an integral component of global health strategy going forward. In order meet demand for rapid development of safe and effective vaccines in the future there is a need to streamline research and development workflows by increasing the scale and speed of analytical testing. One way to optimize analytical testing workflows is the use of automation and high throughput testing strategies. Automated liquid handlers, robotics and integrated computer technologies can be used to greatly enhance an organization's analytical capabilities by increasing sample throughput, reducing turnaround time, decreasing testing error frequency, and decreasing FTE requirement for analytical testing. Rapid generation of large data sets with these automated workflows has myriad benefits including providing a means to speed vaccine process and formulation development, enabling rapid completion of product-related investigations, and ensuring that work is focused on the strongest vaccine candidates by enabling data-driven prioritization of pipe-

line assets. Incorporation of high throughput analytics and automation into analytical testing workflows allows vaccine research and development to keep pace with the increasing need to rapidly bring vaccines to market.

161 Fifty Years of Innovations in HPLC Column Reproducibility, Efficiency, Stability and Inertness

Thomas Walter, Waters Corporation, 34 Maple St., Milford, MA 01757

One of the first commercially available high performance liquid chromatography (HPLC) columns (Waters µBondapak™ C_{18} column) was introduced 50 years ago at the 1973 Eastern Analytical Symposium. This was the first column packed with 10 µm silica particles bonded with a C_{18} silane, and established the initial benchmark for reversed-phase column performance. Over the ensuing 50 years the technologies used to synthesize stationary phases, to fabricate column hardware and to pack columns have steadily advanced. The attributes that have been the focus of these improvements include column reproducibility, efficiency, stability and inertness. This presentation highlights several of the key technical advancements made over the last 30 years, including high purity stationary phases, hybrid organic/inorganic particles, ultra-HPLC columns and hybrid surface technology hardware. The importance of these advancements for addressing some current separation challenges are demonstrated.

162 Fifty Years of Innovations in HPLC Column Selectivity

David Bell, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

To achieve resolution in high performance liquid chromatography (HPLC), a column must offer retention, selectivity and a certain level of efficiency. Retention results when an analyte interacts with a stationary phase, however selectivity only happens when the analytes interact differently with the surface. One of the most powerful means to adjust selectivity is to change the surface chemistry of the column. Although C18 phases still reign supreme, many different stationary phases have been developed over the years that have broadened the applicability of HPLC by offering alternative selectivity. In his presentation we review many of the more important developments in HPLC columns from the perspective of surface chemistry and focus on the impact the different chemical surfaces have had on selectivity. We also weave in a look at some of the more important trends, technological advances and drivers that have resulted in new abilities. Compared to retention and efficiency, selectivity is the most powerful tool to manipulate toward achieving resolution. What might a different surface chemistry enable for you?

163 Innovations in HPLC Columns: Perspectives from a Pharmaceutical Scientist

Michael Dong, MWD Consulting, 55 Cranbury Rd., Norwalk CT, 06851

High performance liquid chromatography (HPLC) debuted ~50 years ago and became an indispensable pharmaceutical analysis tool. My career path coincided roughly with the development of HPLC, with personal connections to many innovators in HPLC technology. I want to cover four important HPLC column innovations from the perspective of a pharma separation scientist who witnessed how many pioneers whose research, ideas, and applications enabled these developments: 1. Reversed-phase chromatography (RPC) to enable assay reproducibility and mass balance- Joe DeStefano (AMT), Jack Kirkland (AMT), Csaba Horvath (Yale), 2. The use of high-purity silica to reduce peak tailing of basic analytes and column batch-to-batch reproducibility - Dennis Hill (U. Conn), Kervin Harrison (Vydac/The Separations Group), Fred Regnier (Purdue), Jack Kirkland, and Uwe Neue (Waters), 3. Superficially porous particles (SPP) to increase column efficiency - Jack Kirkland, Szabolcs Fekete (Waters), 4. Hybrids and Charged Surface hybrids to enhance pH range and reduce silanophilic activities - Klaus Unger (U. Mainz), Tom Walter (Waters).

Reference.

C. H. Arnaud, 50 years of HPLC, Chem. Eng. News, 94 (24), 28-32, 2016.

164 Innovations in HPLC Column Technology for Faster and More Efficient Separations of Large Biomolecules

Szabolcs Fekete, Waters, CMU - Rue Michel Servet, 1, Geneva, 1211, Switzerland, Matthew Lauber

Macromolecules usually show a particular elution behavior which is often referred to as “on/off” chromatography. The consequence of this mechanism – in gradient elution mode – is that only a very short inlet segment of the chromatographic column retains the large solutes effectively. Therefore, much shorter columns - like only a few centimeters or even shorter can be used to analyze such macromolecules. We recently introduced an approach to estimate the “effective column length”. With this approach, the required column length for various biomolecules and elution modes can be determined. Representative examples of very fast separations are presented. This presentation also discusses the control of undesired adsorptive interactions. The separation of biomolecules is especially challenging in aqueous separation modes as there is a huge potential for both hydrophobic and ionic secondary interactions to occur with chromatographic hardware or with the stationary phase to the detriment of peak recovery and the overall sensitivity of the chromatographic analysis. To decrease non-specific adsorption and undesired secondary interactions, new column hardware and packing materials have been developed. In the form of new size exclusion columns, these technologies have shown clear benefit to obtaining higher apparent recoveries to better ensure accurate aggregate quantitation. Several examples are presented to illustrate the importance of inert surfaces for size-based separations. Finally, the theoretical possibilities of stationary phase gradients (compositional gradients and porosity gradients) are discussed. We found that for large molecules, non-uniform columns may be of particular interest as unseen selectivities are predicted to be attained.

165 Lipidomic Phenotype of Mitochondrial Acyltransferase Deficiency Revealed by Imaging Mass Spectrometry

Yu Tin Lin, University of Florida, Department of Chemistry, 214 Leigh Hall, PO Box 117200, Gainesville, FL 32611, Manal Zabalawi, Julia Bonney, Tingting Yan1, Carolyn Dirain, Lexin Chen, Lane Smith, Ramon Miranda Quintana, Peter Stacpoole, Charles McCall, Boone Prentice

In Barth Syndrome (BTHS), an X-linked mutation in the TAFAZZIN gene (TAZ) leads to deficiency in mitochondrial acyltransferase, resulting in dysregulation of cardiolipin and lyso-cardiolipin isoforms. Cardiolipin (CL), predominantly expressed in the inner mitochondrial membrane, is closely linked to mitochondrial structure and metabolism. Using a 16-week-old TAZ-knockout C3H/HeJ mouse model, which presents without obvious cardiac symptoms, we aim to unravel associations of cardiolipin dysregulation with other lipid classes and fatty acids (FAs) using matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry. This technology provides spatially resolved, label-free relative quantification of multiple lipid classes in mouse hearts. We observed distinct clusters of glycerophospholipid and FA isoforms between wildtype and TAZ-knockout mice (n = 18) visualized in uniform manifold approximation and projection (UMAP) space, which were mapped in situ for physiological associations. Near-binary downregulation of CL isoforms and upregulation of monolyso- and dilyso-CLs in TAZ-knockout mice were revealed, with localization in ventricles corresponding to mitochondrial distribution. Moreover, downregulation of long-chain FAs and upregulation of very-long-chain FAs in TAZ-knockout mice suggest a rewiring of mitochondrial metabolism. These FAs tend to either localize in the left or right ventricle. Alterations in phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidic acid (PA), and their lyso- counterparts were observed, with localizations in either the left or right ventricle. Future work will investigate ceramide and sphingomyelin isoforms, validate lipid isoform identifications using tandem mass spectrometry, and perform pathway/network analysis for a holistic view of BTHS molecular pathology.

166 An Automated High Throughput Approach for Large Scale Retention Measurement in Liquid Chromatography

Trevor Kempen, Gustavus Adolphus College, 800 W College Ave., Saint Peter, MN 56082, Tina Dahlseid, Bob Pirok, Dwight Stoll

Many contemporary challenges in liquid chromatography - such as the need for “smarter” method development tools, and deeper understanding of chromatographic phenomena - could be addressed more efficiently and effectively with larger volumes of experimental retention data than we have been accustomed to historically. The paucity of publicly accessible, high-quality measurements has been due, at least in large part, to the high cost in time and resources associated with traditional retention measurement approaches. Recently, we described an approach to improve the throughput of such measurements by using very short columns (typically 5 mm), while maintaining measurement accuracy. The automated high throughput approach processes chromatograms “on the fly” after each acquisition and creates new methods with conditions that are designed for a specific compound/stationary phase system before appending them to a dynamic work list. This approach has been used to fit hundreds of retention models, and has shown to work for a wide variety of analyte/stationary phase combinations under reversed-phase LC conditions. Additionally, it will enable the compilation of large databases (>> 100,000 measurements) of retention values over long time periods (years), which can in turn be leveraged to address some of the most important contemporary challenges in liquid chromatography.

167 Confocal-Raman Spectroscopy Investigation of the Interactions Between Porous-Silica immobilized DNA Aptamers and their Protein Targets

Aric Potter, University of Utah, Chemistry, 315 South 1400 East, Salt Lake City, UT 84112, Grant Myres, Jay Kitt, Joel Harris

Biosensors are often based on the surface-immobilization of a probe molecule that selectively binds to a target biomolecule in solution. To investigate this interfacial chemistry, it is valuable to employ a structurally-informative, label-free method to study these probe-target interactions. By immobilizing the probe molecules in high-surface-area porous silica, confocal Raman microscopy has the ability to investigate these interactions by focusing the confocal-probe volume into center of an individual particle, whose interior surface area greatly increases the local concentration of interfacial species. In this work, we apply this methodology to investi-

gate the interactions between an immobilized DNA thrombin-binding aptamer and its protein target alpha-thrombin. Confocal-Raman microscopy allows us to monitor each step of DNA surface-immobilization, which begins with reacting the silica with a thiol-silane reagent. These thiolated silica particles were then reacted with a maleimide-modified DNA, a process monitored by the disappearance of the thiol S-H mode and the appearance of a thiosuccinimide stretch along with distinct vibrations of DNA. Having immobilized the thrombin-binding aptamer, the particles are exposed to low concentrations of alpha-thrombin in solution, resulting in the appearance of Raman scattering from the bound protein and detectable changes in the DNA modes reflective of interactions with thrombin. The goal of the work is to vary the surface density of the DNA aptamer to investigate how proximity of immobilized aptamers influences its conformation and binding interactions with target proteins.

168 Kinetic Profiling of Commercially Available Capillary Scale Columns

Samuel Foster, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Elisabeth Gates, Paul Peaden, Serguei Calugaru, Raymond West, Milton Lee, James Grinias

Capillary liquid chromatography (LC) is an essential aspect of green analytical chemistry due to its low solvent consumption based on low mobile phase flow rates. Recently, several compact capillary-scale LC instruments have been developed that are compatible with commercially available columns from multiple vendors. This allows for a wide variety of column dimensions, bonded phases, particle diameters, and particle porosities to be used. This study investigates a number of commercially available columns with varying properties in order to develop a broad overview of the achievable separation performance. Additionally, the chromatographic system was fitted with a longer pathlength flow cell to increase detector sensitivity. These advances will lead to faster and more efficient separations with improved detection limits at the capillary scale.

169 Advancing Planetary Probe Based Mass Spectrometry Through Machine Learning

Nicole North, Ohio State University, 100 W 18th Ave, Columbus, OH 43210, Abigail Enders, Heather Allen

The fragmentation patterns that appear in mass spectrometry (MS) are an excellent target for machine learning methods to automate and expedite analysis of data to identify targets such as functional groups. This is particularly important for areas of MS that are limited in terms of time, resources, and/or location. All these limitations are prevalent in planetary probes. In planetary missions, power and size of instrumentation are highly limiting factors and these limitations commonly result in lower detectible mass ranges, lower mass resolutions, and descoping of additional features typical in the laboratory, such as tandem MS. We collected over 20k electron ionization mass spectra from the United States' National Institute of Science and Technology Webbook. We have had success in training logistic regression-based models in sklearn to identify individual functional groups. Beyond determining model accuracy, we have also preformed a feature analysis allowing us to determine which masses are being utilized for functional group determination. By evaluating the accuracies and the features in tandem we can establish a sense of chemical intuition into our models and ensure that the models' reasoning maintains a level of chemical consistency. These models could be employed to assist with organizing chemically interesting data for preferential data downlink to assist in adjusting mission parameters or to help sort data for further analysis on Earth after mission completion.

170 Optoelectronic Impacts of Surface Ligands in Water Dispersible Copper Selenide Nanoparticles

Riti Sen, University of Pittsburgh, 121 S Highland Ave, 604-H, Pittsburgh, PA 15206

There is significant interest in earth-abundant plasmonic nanomaterials, but whether their performance can match or even surpass their noble metal counterparts is still a topic of study. One key plasmonic feature of traditional plasmonic materials is the Mie Theory predicted, cubic relationship between particle volume and molar extinction coefficient at the maximum localized surface plasmon resonance (LSPR) frequency. In our previous work, we found that $Cu_{2-x}Se$ NPs, which are an example of such "alternate" plasmonic nanomaterials, followed this trend. Our present work now considers the impact of dielectric environment on the LSPR of these new materials. The dielectric environment of these colloidal NPs is largely determined by the surface chemistry of the particle which is often tuned to impart additional functionality to the structures. However, for $Cu_{2-x}Se$ NPs, variation in the surface chemistry can also change their carrier densities (which are malleable in these systems), and complicates the assignment of dielectric impacts on the LSPR. In this work, we seek to decouple and define the impacts of ligand chemistry on LSPR features in $Cu_{2-x}Se$ NPs. Specifically, we synthesized $Cu_{2-x}Se$ NPs of uniform sizes (50 to 25 nm) and used mass action ligand exchange to introduce 4 different surface ligands. We found that the extinction coefficient of each ligand scales with Mie Theory predictions, and that changes between ligand type scale with the electronegativity of the capping ligand. These results suggest a key role of surface chemistry in defining and expanding the suite of plasmonic properties obtained from this material class.

171 The Application of a Raman Spectroscopy Body Fluid Identification Model on Samples Exposed to Bluestar Forensic Spray

Alexis Weber, University at Albany, SUNY, 1400 Washington Ave., LSRB 1113 Albany, NY 12205, Igor K. Lednev

The identification, testing, and collection of biological evidence at crime scenes is a complex multi-step process, even when samples are apparent. When body fluid samples are not visible, crime scene investigators must use biochemical enhancement techniques to search for stains. The interaction of these reagents with the body fluids commonly causes the stained areas to luminesce. These reagents are sprayed over large areas. This means that most, if not all, of the biological material in each area will interact with the biochemical reagents. Therefore, the purpose of this work was to determine if a previously developed body fluid identification model can discriminate between body fluids exposed biochemical enhancement reagents. This goal will focus on the identification of the most common body fluid encountered at crime scenes: blood. Bluestar and luminol are primarily used by crime scene investigators for the detection and enhancement of latent bloodstains. Approximately 20 µL of the body fluids will be deposited onto the aluminum substrate. We used increasing dilutions of blood and semen test with the biochemical enhancement reagents, starting from neat samples down to 1:200 (blood: water) dilutions. The stains were left to dry overnight under ambient conditions. The following day they were sprayed with Bluestar. The samples were then be analyzed using Raman spectroscopy. After a large sample set has been obtained, we will import the spectra into the PLS Toolbox in MATLAB for analysis and model deployment. The capabilities of our current body fluid identification model and identification results are discussed.

172 Human Breastmilk as a Bio-Fluid for the Use of Protein Biomarkers for Breast Cancer

Danielle Whitham, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Pathea Bruno, Norman Haaker, Brian T. Pentecost, Kathleen F. Arcaro, Costel C. Darie

Breast cancer (BC) is a common cancer among women with an estimated 1 in 8 women developing an invasive form of BC during their lifetime. Currently, the main screening method for BC is mammography, which is not feasible for younger women and women with denser breasts. Human breastmilk is an easily accessible biofluid that gives insight into the function of the breast. Most research regarding breastmilk has previously been focused on the composition and effects in regards to infant growth and development and thus little is known about the proteins, immune cells and epithelial cells present which could be indicative of BC formation. Human breastmilk from women with BC and controls were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Multiple proteins were identified as dysregulated between BC and control samples. Both 1D-PAGE and 2D-PAGE analysis was performed and there were perturbed proteins found in BC-related samples of both studies, including zinc-alpha-2-glycoprotein, caseins, fatty acid-binding proteins, apolipoproteins, and anti-trypsin family proteins. Each milk sample set contains both within women and across women comparison groups of BC and controls to identify dysregulated proteins between the breastmilk of women with BC and without. Within women comparisons show the differences in protein levels from one breast with BC and the healthy breast from the same woman, allowing for a stronger comparison group with the least amount of genetic variation. The proteins identified consistently dysregulated in breastmilk could be used as potential biomarkers for BC in the future.

173 Reduction of Perfluoroalkyl and Polyfluoroalkyl Substances in Drinking Water Using a Standard Filter Containing Activated Carbon Plus Ion Exchange Resin, Measured Using the QSight 420 UHPLC/MS/MS

Cole Strattman, PerkinElmer, 286A Old Coach Rd Charlestown, RI 02813

Growing environmental and health concerns about per- and polyfluoroalkyl substances (PFAS) have led to stricter and more extensive regulations of these substances in drinking water, ground water, soil and food over the past decade. PFAS are man-made chemicals used in a wide variety of commercial products like nonstick cookware, food packaging, paints, clothing, fire retardants and surfactants since the 1940's. Due to their inert nature, PFAS are persistent and have been found to accumulate throughout the environment. Originally considered biologically inactive, recent research has revealed their toxicity to humans and wildlife leading to stricter global regulations restricting their levels in food, water, air, and soil. United States Environmental Protection Agency (EPA) method 537.1 is a widely used method for the determination of selected PFASs in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry. This study will discuss the reduction of PFAS compounds in drinking water by filtration through standard activated carbon plus ion exchange resin. The PerkinElmer QSight 420 LC/MS/MS was used to measure the effectiveness of filtration to remove or reduce PFAS from drinking water samples by implementing EPA Method 537.1.

174 Determination of PFAS in Drinking Water Using Automated FREESTYLE XANA-PFAS System

Fred Foster, GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090

Water quality is of the utmost importance, and recently, the importance of analyzing water for emerging contaminants has been brought to light. Among the emerging compounds being determined are per- and polyfluorinated alkyl substances (PFAS). The current and upcoming regulations in the EU and US make it necessary to test drinking water for PFAS content. All methods require solid phase extraction (SPE) prior to liquid chromatography-tandem mass spectrometry (LC-MS/MS). Modern analytical labs are looking to automation to help increase sample throughput while ensuring the resulting data is of the highest quality. In this study we were able to show the fully automated sample preparation of water samples for LC-MS/MS analysis was successfully achieved by applying SPE with the FREESTYLE XANA-PFAS robotic system according to US EPA 537.1. The resulting extracts are introduced into an Agilent 6470 LC-MS/MS instrument for detection and quantification. By the application of fully automated parallel sample preparation, multiple samples can be processed at the same time. Thus, high sample throughput with low demand for personnel resources is obtained. The FREESTYLE XANA-PFAS robotic system is especially suited for PFAS determination because it contains no fluorine-containing plastics such as PTFE in the flow path, thus solving the significant issue of high blank values present in other systems. In this study, no measurable blank values were seen from the system.

175 Applications of HPTLC for Testing Essential Oils

Gavin Brush, doTerra, 1248 W 700 S, Pleasant Grove, UT 84062

The current practice in the United States for identification of essential oils is the use of gas chromatographic (GC) profiles. The use of GC presents limitations due to throughput, expense and the nature of the chemicals being analyzed. High-performance thin-layer chromatography (HPTLC) presents an alternative technique of essential oil identification analysis. By adapting existing monographs from the European Pharmacopoeia, we successfully established a single HPTLC method for the identification of more than 30 different types of essential oils with high specificity. This allowed for a high throughput simultaneous analysis of multiple different types of essential oils. This would allow an increase of sample throughput by at least 5-6 fold compared to GC profile analysis. HPTLC also has shown promise in differentiating essential oils with very similar chemical profiles that are difficult to distinguish using traditional testing. There is also potential for this same method to be expanded to identify other type of essential oils or establish limit tests for certain compounds of interest in essential oils. Adulterant and contaminant screening may also be integrated as part of a single test method, thus eliminating the need for multiple tests. Overall, this HPTLC assay is demonstrated as an useful technique for essential oil quality control testing.

176 Automated Polysorbate 80 (PS80) Forced Degradation Screening Workflow to Accelerate Biopharmaceutical Upstream Processing

Sharon Matamoros, GSK, 1250 S. Collegeville Rd., Collegeville, PA 19426, Ashley Reeder

The introduction of automated high-throughput screening allows relevant process intermediates to be evaluated in a reduced cycle time and with limited quantities of protein. This enables rapid drug substance intermediate screening and design for accelerated medicine delivery to patients, at a lower cost. Polysorbates are an integral part of drug products in present-day pharmaceuticals. The most common, PS80, limits protein-to-surface adsorption and aggregation. Degradation of polysorbate species observed in biopharmaceutical stability studies has prompted assessment of mitigation strategies in both upstream and downstream development to minimize risk of degradation. A new high-throughput PS80 forced degradation workflow was developed using Waters Andrew+ robot and Unchained Labs Big Kahuna pre-formulation robotic platform. A panel of biopharma intermediates were tested using 1-mL vials in a 96-well plate format, allowing the incubation step and sample preparation in the same plate. The Andrew+ robot and the Big Kahuna platform were used to perform varies steps to prepare the samples. Samples were analyzed using HPLC-CAD. The screening workflow efficiently determined the impact of stressed conditions on the degradation of PS80 for material harvested from bioreactors operated with three unique media conditions and corresponding affinity capture eluates. The results of the screen were used in conjunction with other product quality attributes to inform selection of upstream media conditions to progress in development studies moving forward.

177 Method Development for PFAS and Drugs of Abuse (DoA) Compounds Using a Virtual Method Development Tool

Melinda Urich, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823, Chris Nelson, Justin Steimling, Tim Yosca, Samantha Herbick, John Garrett

Development and optimization of Liquid Chromatography (LC) separations can be time consuming and costly, often requiring many steps including literature research, column selection, method scouting, development, and optimization. To reduce cost and save time, an instrument-free software modeling tool was developed. Allowing users to select compounds from a database and instantly model separations by adjusting parameters such as instrument/system effects (dwell and extra column volume), temperature, and mobile phase additives, the modeler delivers a fast, no-cost starting point. A PFAs library containing 33 compounds and a DoA library containing 291 compounds, were used to virtually develop methods. For each compound class, method parameters were selected within the software and virtual chromatograms generated. Generated method conditions were taken to the instrument and set up for analysis. To assess accuracy of the modeler, experiments comparing compound retention time values between wet-lab and modeled data were conducted. A retention time acceptance criteria of +/- 15 seconds was chosen for the representation of a typical MRM window. Using the DoA library, a modeled chromatogram was developed using 67 compounds. Results of retention times compared from experimental to modeled values were on average +/- 9.5 seconds. For LC method developers, novice and expert, who either lack the expertise, or the time, to develop separations quickly and accurately, this tool can be used to deliver a fast, no-cost starting point for method development and optimization. This novel, virtual method development software can improve turnaround time, increase throughput to existing methods, and offer an on-demand consultative user experience.

178 The Use of Short 10 mm Columns for Rapid LC-MS Analysis

Edward Faden, MAC-MOD Analytical, 103 Commons Court, Chadds Ford, PA 19317, Matt James, Anthony Edge

The increasing number of samples that are seen in high throughput analytical laboratories, such as clinical, drug discovery and environmental labs, means that fast LC-MS analysis, using triple quadrupole and high resolution (HRMS) instrumentation, is becoming essential. Over the last two decades, many labs have dramatically increased sample throughput using UHPLC, using shorter columns packed with sub-2 micron particles. However, the performance of modern mass spectrometers has continued to evolve. Improved sensitivity and ultra-fast data acquisition capabilities allow further reductions in analytical run times, using specially designed, high throughput columns. This presentation assesses the use of 10 mm columns for the analysis of samples derived from biological or environmental sources. The theoretical considerations of short columns are discussed, starting with van Deemter theory, before moving to a kinetic plot interpretation of their application. Practical considerations of the use of such short columns will be discussed, specifically looking at the impact of extra-column dispersion, tubing and data acquisition rates. Finally, a series of applications are presented which demonstrate the benefits that 10 mm length columns can have for high throughput analysis. The applications will include a series of therapeutic drugs, samples from a hospital laboratory and high throughput PFAS analysis.

179 Method Development for Solid-Phase Extraction and Capillary LC-UV Analysis of Drugs of Abuse and Related Metabolites

John Boughton, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Sangeeta Kurre, Ama Hackman, Samuel Foster, James Grinias

The detection of compounds related to substance use disorders in clinical settings can play a crucial role in effective treatment decisions by healthcare practitioners. Typically, this analysis is conducted by LC-MS in off-site laboratories, which can delay access to results by 2-4 days. Using LC-MS in point-of-need settings is impractical due to issues of size, cost, and user expertise. Compact LC-UV instrumentation, coupled with solid-phase extraction to reduce matrix interferents and pre-concentrate species for enhanced detection limits, may provide a preferred solution to the analysis of these compounds in these settings. This study is focused on developing a solid-phase extraction and LC-UV method using compact, capillary-scale instrumentation for the analysis of a 16 compound drugs of abuse panel. Utilizing a polymeric strong cation exchange sorbent, recoveries for the 16 compounds ranged from 73.2-116.9%, with 14 of the 16 compounds falling within the standard recovery range of 90-110%. This sample preparation strategy was then applied to samples analyzed using a compact capillary LC instrument with a focus on achieving detection limits below the standard clinical screening thresholds.

180 Deciphering the Function of RNA Modifications in the Central Nervous System and Single Cells: Strategies in Sample Preparation, Separations, and Mass Spectrometry

Kevin Clark, Tufts University, 62 Talbot Ave., Medford, MA 02155

RNA modifications are increasingly recognized for their roles in controlling the biophysical and translation properties of cellular RNA biopolymers. However, the characterization of RNA modifications in the central nervous system (CNS) has been impeded by the lack of analytical methods capable of simultaneously detecting multiple modified RNAs in small-volume samples. Here, I describe how sample preparation strategies, including the development of nucleoside-selective extraction solvents, chemical labeling strategies, and optimized lysis and digestion conditions can be interfaced with liquid chromatography-mass spectrometry (LC-MS/MS) to facilitate the characterization of modified nucleosides and oligonucleotides in tissues, extracellu-

lar fluids, and single neurons. When combined with multivariate statistical analysis, these methodological improvements provide a basis for categorizing individual cells based on their RNA modification profiles. I then provide future directions that my lab will undertake involving MS-based determination of RNA modification stoichiometries in single neurons.

181 **Analysis of the Electrochemical Oxidation of Lignin using Chemometrics**

Gobind Sah, Ohio University, Department of Chemistry and Biochemistry, 133 University Ter., Athens, OH 45701, Staser John, Peter Harrington

The economics of electrochemical depolymerization of lignin are most likely unfavorable without some control over the oxidation mechanism because depolymerization generates many unwanted compounds. Therefore, it is crucial to understand whether •OH radicals mediate the oxidation process instead of a direct electrochemical route to depolymerization. Control over the depolymerization process can lead to high-yield chemical products like aromatic phenols and carboxylic acids. In this study, lignin compounds were electrochemically oxidized using a Nickel-Cobalt (Ni-Co) electrocatalyst at several electrode potentials, and the oxidation products were analyzed using headspace solid-phase micro-extraction (SPME) gas chromatography-mass spectrometry (GC-MS), and chemometric tools, including singular value decomposition (SVD), principal component analysis (PCA), and multivariate curve resolution (MCR). The results revealed that both direct electrochemical oxidation and •OH radical attack govern the electrochemical oxidation of the lignin.

182 **Geospatial Origin Differentiation of Pinus Ponderosa Ash using Multivariate Classification and Inductively Coupled Plasma Mass Spectrometry**

Maria Delgado-Cornelio, University of Delaware, 163 The Green, Newark, DE 19716, Collin White, James Jordan, Michael Ketterer, Helder Carneiro, Caelin Celani, Barry Lavine, Karl Booksh

Multivariate classification can effectively differentiate native soil form ashed plants. The analysis of tree's ashes allows to predict forests' nutrient redistribution after wildfires. In long term this leads to ecological heath monitoring and soil fertility. In this study six soil types and eight geological locations were considered to gather 140 samples of *Pinus Ponderosa*. Geolocation feasibility was attempted by multivariate analysis of hydrogen flame ashed samples followed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) measurements. Early results show that a tree-based method works more efficiently in the separations of this data set. In addition, Partial Least Squares Discriminant Analysis (PLS-DA) and Support Vector Machines (SVM) differentiate the eight locations with accuracies of 0.85 and 0.9 respectively.

183 **Innovations in Enhanced SFC: Complex Purifications Enabled by Recent Advances in Analytical and Preparative Enhanced Supercritical Fluid Chromatography (eSFC)**

Jimmy DaSilva, Merck & Co., Inc., RY800-D319, 126 East Lincoln Ave., Rahway, NJ 07065, Imad Haidar Ahmad, Erik Regalado

Chiral separation of pharmaceutical intermediates and active pharmaceutical ingredients (APIs) by supercritical fluid chromatography (SFC) have become increasingly challenging. Some of the contributing factors are higher complexity of the molecules, poor solubility in solvents commonly used for SFC and more demanding timelines in drug development. Conventional SFC methodology, albeit capable of delivering fast results, is being pushed to the limits to address these challenging separation needs. In this presentation we demonstrate the use of multi-column parallel SFC column screening for rapid resolution methods to enable ultrafast turnaround by SFC of these complex separations. The setup presented herein possesses the ability to screen, for a given mixture, up to 10 different chiral columns with 5 different organic modifiers in few hours. This enables the identification of separation and purification conditions, in some examples, within hours. In addition, we show how this screening platform allows for the rapid purification of pipeline samples at Merck.

184 **False Positive Glycopeptide Identification via in-FAIMS Glycan Fragmentation**

Valentina Rangel Angarita, Yale University, Department of Chemistry, 275 Prospect St., New Haven, CT 06511, Keira Mahoney, Catherine Kwon, Raibat Sarker, Taryn Lucas, Stacy Malaker

High-field asymmetric waveform ion mobility spectrometry (FAIMS) separates glycopeptides in the gas phase prior to mass spectrometry (MS) analysis, thus offering the potential to analyze glycopeptides without prior enrichment. Several studies have demonstrated the ability of FAIMS to enhance glycopeptide detection but have primarily focused on N-glycosylation. Here, we evaluated FAIMS for O-glycoprotein and mucin-domain glycoprotein analysis using samples of varying complexity. We demonstrated that FAIMS was useful in increasingly complex samples, as it allowed for the identification of more glycosylated species. However, during our analyses, we observed a phenomenon called "in FAIMS fragmentation" (IFF) akin to in source fragmentation but occurring during FAIMS separation. FAIMS experiments showed a 2-5-fold increase in spectral matches from IFF compared to control experiments. These results were also replicated in previously published data, indicating that this is likely a systemic occurrence when using FAIMS. Our study highlights that although there are potential benefits to using FAIMS separation, caution must be exercised in data analysis because of prevalent IFF, which may limit its applicability in the broader field of O-glycoproteomics.

185 **Accurate Moisture and Accurate Potency**

Kerri-Ann Blake, Metrohm USA, 9250 Camden Field Pkwy, Riverview, FL 33578

As the Cannabis industry grows, testing for residual solvents, contamination, terpenes, and potency is in high demand. One of the parameters of the potency calculation is moisture. This is often determined by loss on drying. Unfortunately, limit of detection (LOD) is not specific to moisture and the loss of any volatile components, such as terpenes, will be incorrectly counted as moisture. Karl Fischer titration is the only chemically specific test for moisture. It requires less than 0.5 grams of sample and produces no odor. Attend this session to learn how Karl Fischer titration can be used to determine moisture in cannabis flowers, oils and various products.

186 **Determination of Trace Level Nitrite in Pharmaceutical Excipients Using Ion Chromatography with Conductivity Detection**

Weiqing Fu, Bristol Myers Squibb, 1 Squibb Drive, New Brunswick, NJ 08903, Qinggang Wang, Yongmei Wu

N-nitrosamines are listed as a member of the 'cohort of concern' due to their high potency as mutagenic carcinogens in ICH M7 "Assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk". Many active pharmaceutical ingredients (APIs) or organic solvents and reagents used in the process contain amine moieties. Nitrite (NO2-) is a precursor that can be converted to N2O3 under acidic condition and react with amines to form a nitrosamine. The level of nitrite in commonly used pharmaceutical excipients varies between lots and vendors. Therefore, screening of excipients for trace levels of nitrite is critical as it provides knowledge of the potential for nitrosamine formation. An ion chromatography (IC) method with conductivity detection was developed to quantitatively determine trace levels of nitrite in a variety of commonly used pharmaceutical excipients. Separation of nitrite from interfering anions was achieved using an IonPac AS 15 column and a linear gradient of KOH from 25 mM to 60 mM over 25 minutes on an ICS 6000 IC instrument equipped with a KOH eluent generator cartridge. Method parameters such as injection volume, sample diluent and concentration, were optimized to improve method sensitivity. For common excipients including mannitol, pearlitol and microcrystalline cellulose (MCC), a minimum quantitation limit (MQL) of 100 ppb was achieved with the optimized method conditions. Phase appropriate validation was successfully performed. Validation results and analysis of various batches of common excipients are also discussed.

187 **FIDIG, the Bolt that Allows Agilent FID's to Light Every Time**

Matthew Monagle, AIC LLC, PO Box 1503 Salida, CO 81201

In n flame ionization detection (FID), the flows from the jet and the added air are fundamentally laminar. With a laminar flow and with the hydrogen inside a sheath of the air that is added, it is difficult for electrons emitted from the glow plug to "reach" the hydrogen to initiate the flame. This problem can be further exacerbated when the glow plug is recessed at all in the chimney installation. FIDIG is a very simple modification to an Agilent chimney that creates a turbulent flow in front of the glow plug thereby guaranteeing it will light. This poster discusses the operating concept of FIDIG, the advantages, and the cost savings afforded by knowing that the FID will light repeatedly.

188 **Application of Ultrafast Supercritical Fluid Chromatography in a High-Throughput Pharmaceutical Process Development Workflow**

Matthew Morgan, Pfizer, 445 Eastern Point Rd., Groton, CT 06340, Mark Hardink, Angel Diaz, Giselle Reyes

Solubility screening is a high-throughput (HT) pharmaceutical process development workflow that guides the selection of solvent systems throughout a synthetic route by identifying appropriate solvents/mixtures for reaction starting materials, intermediates, and products. Due to high sample volume and compressed timelines for completion, rapid quantitative analytical methods are required for each solubility screen. While reverse-phase liquid chromatography (RPLC) is generally the default analytical technique for routine quantitation of solubility samples, test slurries are often insoluble in highly aqueous mixtures or are comprised of highly polar molecules that are not retained by RPLC. Therefore, supercritical fluid chromatography (SFC) has emerged in recent years as an alternative HT analytical technique due to the advantages of supercritical CO2 (i.e., low viscosity and high diffusion coefficient) which enable ultrafast analysis times. A highly efficient, systematic screening approach to achiral SFC-ultraviolet mass spectrometry method development of rapid single-peak quantitation methods is presented, including selection of stationary phases, co-solvents, and chromatographic conditions. Instrument configurations which permit direct nanoliter-scale injections of highly concentrated reaction samples are described. Examples of ultrafast SFC methods (1 min analysis time) and chromatograms from solubility screens are shown.

189 Direct Elemental Analysis in Cell Culture Media Using ICP-MS

Brady Frill, PerkinElmer, 710 Bridgeport Ave., Shelton, CT 06484

Metal ions are important components of cell culture media as they are enzyme cofactors and their variation can influence the growth of cultured cells. The concentrations of metals in commercially available chemically-defined cell culture media can vary greatly from 1 up to approximately 25,000 ppb. This wide range of metal concentrations has a direct impact on cell growth and critical quality attributes. Therefore, source material control, trace metals determination and monitoring, as well as culture media characterization should be considered essential strategies in upstream bioprocessing. Biopharma laboratories can therefore benefit from a highly sensitive and selective analytical technique, such as inductively coupled plasma-mass spectrometry (ICP-MS). ICP-MS has the advantages of multi-element analysis capability, high sensitivity, low detection limits, wide linear dynamic range, and easy automation. However, as with all other analytical techniques, ICP-MS analysis is subject to interferences. Culture media is a complex mixture containing a substantial amount of inorganic salts and organic compounds, which can generate polyatomic and other interferences that increase the spectral background and can compromise analytical accuracy, if the appropriate interference corrections are not applied. This poster describes the analysis of a wide variety of elements in cell culture media samples with high accuracy and precision using ICP-MS.

190 Investigation of Electrochemical Degradation of PFOA Using High Surface Area Electrodes

XingZhi Chen, Haverford College, 370 Lancaster Ave., Haverford, PA 19041, Md Tanim-Al Hassan, Timothy Yaroshuk, Hao Chen, Omowunmi Sadik

Per- and poly-fluoroalkyl substances (PFAS) are industrially common chemicals that are persistent in the environment and toxic to humans. Various degradation processes such as chemical, thermal, photochemical, and electrooxidation methods have been used to degrade perfluorooctanoic acid (PFOA) with the electrooxidation process being the most simple and efficient. Anodic materials play a pivotal role in electrooxidation of PFOA due to their ability to generate reactive intermediate species like Cl•, O_2•- and HO• in presence of aqueous electrolytes. Some common, efficient electrodes include boron-doped diamond, Pt, and Ti/SnO_2. However, shortcomings like expensive production cost, low conversion yield, and short lifetime prevent large-scale applications. In this study, several commercially available high surface area electrodes (Ti fiber, Ti/Pt fiber, RVC) and electrolytes (KCl, Na_2SO_4, KNO_3) were screened to investigate the effect of surface area and nature of electrolytes on PFOA degradation. Desalting paper spray mass spectrometry (DPS-MS), a fast, online PFAS detection method, was used to monitor degradation products and better understand PFOA degradation mechanisms. DPS-MS data showed the highest estimated PFOA degradation yield using KCl while KNO_3 resulted in formation of more carboxylic acid, alcohol, and radical products. Many of the degraded products detected correspond to a chain-shortening degradation mechanism of PFOA where the carboxylic acid headgroup is oxidized, causing carbon dioxide release and subsequent HF removal. However, further work needs to be done to improve PFOA degradation yield, quantification, and product detection.

191 Extraction of CBD from Personal Care Products Followed by Liquid Chromatography Coupled with UV Detection

Rachel Murphy, University of Connecticut- Center for Environmental Sciences and Engineering, Institute of the Environment, 112 Summer St, Marshfield, MA, 02050, Jacob Esposito, Isabella McGrath

CBD containing personal care products are more popular than ever, but do these products contain CBD? And if so, what are the benefits? Since there are so many distributors of these CBD hemp products, it is easier for companies and distributors to get away with selling products that may not fully represent what they advertise. We tested seven personal care products that claim to contain hemp and CBD, including lotion, lip gloss, muscle reliever, and deodorant. Using ultra performance liquid chromatography coupled with UV detection, our goal was to detect and quantify the amount of CBD, if any, in these products to support the validity of their labeling claims. The products tested had either CBD or hemp seed oil listed in their ingredients, some even with a specific expected value of CBD, in milligrams. All products were prepared, analyzed, and compared to report which ones contained detectable levels of CBD, and which ones may not be what they seem.

192 Identifying Potentially Different Phytocannabinoids in Experimentally Grown Hemp Extracts Using High Performance Liquid Chromatography with UV PhotoDiode-Array Detection

Austin Pelletier, CESE, 3107 Horsebarn Hill Rd., Storrs, CT 06269, James Stuart, Angelica Velasquez, Cole Strattman, Anthony Provatas

To date more than one-hundred different cannabinoids have been identified in cannabis extracts of hemp plants. However, for many of those cannabinoids, their potency and potential toxicity have not been effectively evaluated. Yet, certain of those extracts may be utilized in the ever-expanding, commercial markets of edible and personal care products. This poster shows that by evaluating retention times and ultraviolet absorption spectral data of known standards to those of the unknown significant peaks in experimentally grown hemp samples, can lead to positive identification of these additional unknown phytocannabinoids.

193 Investigation of Extraction Protocols for the Analysis and Quantitation of Cannabinoids in Gummy Matrices Using Liquid Chromatography and Ultraviolet Detection

Aaron Urbas, National Institute of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899, Haley Jensen, Walter Wilson, Ira Lurie

The aim of this work was to compare different extraction methods for the purposes of quantification of cannabinoids in gummy matrices. A modified LC-PDA method previously developed was used to screen each sample for 11 cannabinoids. Extraction methods were compared across different cannabis containing gummies and a suite of cannabinoid-free gummy samples spiked with known concentrations of cannabinoids to assess recovery, repeatability, and to look for potential interferences. Sample dissociation methods investigated included enzyme digestion (alpha-amylase), cryo-grinding, and agitation/shaking with and without heating. Dissolution was generally done in aqueous solution, with optional addition of base (ammonium hydroxide), followed by addition of various organic solvents to extract cannabinoids. Liquid-liquid extraction (LLE) via the QuEchERS method was investigated for acetonitrile extractions. At the time of writing, an amylase digestion followed by addition of acetonitrile and a QuEchERS extraction performed the best across all sample types and studies for cannabinoids analyzed. This extraction method had percent RSDs that typically ranged from 2-10%. Preliminary results showed good recoveries from spiked samples and consistency with label claims on a small number of commercial samples. In comparison, other extraction methods yielded lower recoveries or exhibited issues during processing of some samples (e.g. problems filtering). Additional studies will be conducted with gummies made from scratch and spiked with known cannabinoid concentrations to quantitatively assess recovery efficiencies in realistic samples. Method comparisons and the results of these studies will be presented.

194 The Challenges to Migrating Analytical Methods between Instruments

Tony Reinhold, Waters Corporation, 34 Maple St., Milford, MA 01757, Paula Hong

In many laboratories, there is a need to transfer methods across laboratories or within a single laboratory across two different Liquid Chromatography (LC) systems. Method migration refers to the latter, specifically the process of analyzing the same method on an originator and second receiver LC system. In addition, during lifecycle management of a method, a lab may have to update instrumentation necessitating the need to move the method to modern LC systems. By applying Instrument Quality by Design (iQbD) principles1, a lab can assess and control for the risk in method migration. In the following work, two systems will be used for method migration, one a traditional UHPLC system and another designed to minimize metal-sensitive analyte loss on system. The work will include evaluating and understanding the differences across the systems as well as those conditions of the method that contribute to system-to-system variability of the chromatographic results. The testing will evaluate both metal and non-metal sensitive analyte. Controls will be applied to reduce the system-to-system variability for non-metal sensitive analytes. Those analytes that are particularly sensitive to a metal flow path will be evaluated and assessed.

195 Evaluation, Performance and Comparison of a 1.5 mm Internal Diameter LC Column for Pharmaceutical Analysis

Alan McKeown, Vertex Pharmaceuticals Ltd, Abingdon, 86-88 Jubilee Avenue Milton Park, Oxfordshire, OX14 4RW, United Kingdom, Marianna Gonzales

Reducing LC column internal diameters may offer chromatographic performance and reduced solvent advantages to the analyst. However, as column internal diameters (and therefore column volume) reduce, considerations of extra column volume, system dispersion and even column design become paramount. The recent commercial availability of a 1.5mm internal diameter LC column was of interest for impurity profiling work for increased sensitivity and reduced solvent use where larger diameter columns dominate. In this work, we determine and compare chromatographic performance (van Deemter plots, peak height, symmetry and resolution) for a neutral analyte pair under isocratic conditions for 4.6mm, 3.0mm, 2.1mm & 1.5mm columns with standard and reduced dispersion systems. Additionally, we translated an achiral impurities gradient LC method to the 1.5mm internal diameter column and examined the results. Finally, we calculated solvent consumption across the different columns. There was a small improvement in peak height, symmetry and resolution for the new 1.5mm internal diameter column compared to the other columns during the isocratic assessment. This suggests a positive contribution to performance from the different hardware used by the vendor. Reducing system dispersion improved the performance for all column formats with the 1.5mm internal column diameter benefitting the most. The gradient translated 1.5mm internal diameter column method exhibited similar performance to the original 4.6mm column. A comparison of

solvent usage for all columns with a typical separation suggest there are meaningful reductions achievable with little impact to chromatographic performance.

196 The Effect of Solvent Choice on Limit of Detection Calculations in Gas Chromatography with Flame Ionization (GC-FID) Detection

James Mizvesky, Seton Hall University, 400 S Orange Ave., South Orange, NJ 07079, Nicolas Snow

All detector types have a minimum amount of sample, that can be detected. For example, a flame ionization detector (FID) has a minimum mass of carbon that must reach the flame over time to generate a signal. In chromatography as the mobile phase passes through the detector a baseline is generated, which serves as the reference point for measuring a given peak generated by an analyte reaching the detector. Since the baseline serves as a reference, changes in the baseline can cause problems with integration and sensitivity for those analytes. Many limit of detection (LOD) calculations use the baseline standard deviation as a variable in the calculation, including IUPAC, Propagation of errors, United States Environmental Protection Agency method detection limit and instrument detection limit. This work demonstrates the effect of solvent choice on the LOD calculation in a gas chromatography-flame ionization (GC-FID) system, using nonpolar and polar solvents, hexane and ethanol, as the sample solvents. The solvent affects not only LOD calculations between solvent type, but also experimental LODs of 4 analytes dodecane, pentylbenzene, nicotine, and caffeine even though the mass of carbon in the samples are the same

197 Analysis of Common Active Pharmaceutical Ingredients (APIs) Using Gas Chromatography-Flame Ionization (GC-FID) and Gas Chromatography-Vacuum Ultraviolet (GC-VUV) Detection as a Green Alternative to HPLC

Alexander Bulsiewicz, Seton Hall University, 400 S Orange Ave., South Orange, NJ 07079, James Mizvesky, Nicholas Snow

High-performance liquid chromatography (HPLC) is the most common instrumental technique for analyzing active pharmaceutical ingredients (API). As the technology for columns in gas chromatography has advanced significantly over the past several decades, elution and analysis of these analytes is possible, even those with high boiling points. Common assays in the pharmaceutical industry include detecting the presence and the amount of an API. GC is receiving new attention as a green alternative to HPLC. For this work, GC-FID was chosen as one of the techniques since it is the most common detector and almost all common APIs contain carbon, which is detected in an FID. GC-VUV was also chosen as it is similar to the most common detector for HPLC, UV-Visible and can be an alternative to MS. GC-VUV has unique spectra for nearly all organic compounds, which makes it a useful detector for APIs. By using these two detectors, calibration curves of common APIs were created in order to perform an assay on the amount of APIs in solutions. By using gas chromatography instead of liquid chromatography, less solvents are used and power consumption may be reduced, allowing a potentially greener analysis.

198 All Carbon Stationary Phase Material for Biomolecule Separation: Design and Characterization

Michael Parente, Millennial Scientific, 25 Health Sciences Dr., Stony Brook, NY, 11790, Balaji Sitharaman

Graphitic carbon and bonded silica were liquid chromatography's earliest-studied media.[1] Early material and manufacturing innovations caused silica-based media to dominate the LC field. Despite the promise of porous graphitic carbon, there has been limited efforts to develop carbon-based media for LC use. Here recent advancements in carbon-based microbead media are described. First, an overview of a material synthesis platform is presented describing its unique capabilities. A scalable microfluidic-based platform technology, specifically designed to pump highly viscous slurries enables manufacturing outside the purview of traditional microfluidic-based processes. The viscosities of materials processed by our state-of-art setup are between 300-600 mPa.s., which is hundreds of times greater than materials processed with off-the shelf microfluidic systems. Next, using natural graphite carbon-based stationary phase media as an example, a summary of the fundamental physicochemical properties of the microbeads will be described.[2] Further, its differentiation over the current state-of-art reverse-phase chromatography media will be elucidated. Chromatography performance studies will be discussed using several biomolecules as examples. Finally, future directions will be presented.

Literature:

[1] Ross, P. The Use of Porous Graphitic Carbon in Liquid Chrotnatography Perfonnance and Polar Retention Effect PhD thesis, University of Edinburgh, (1998).

[2] Parente, M. J., Sitharaman, Balaji. Synthesis and Characterization of Carbon Microbeads. ACS Omega (2023).

199 Automated Solid Phase Extraction and Determination of Hallucinogenic Compounds in Serum and Urine Samples Using a Novel Robotic Autosampler and LC-MS/MS Platform

Fred Foster, GERSTEL, Inc., 701 Digital Drive, Suite J, Linthicum, MD 21090

There has been renewed interest in the therapeutic use of psilocin, the pharmacologically active metabolite of psilocybin, for treating various psychiatric disorders, including pathological anxiety, mood depressive disorder, and addiction. With that in mind, the forensic community could experience increased cases involving psilocin. Therefore, there is a critical need for forensic, health care, and law enforcement scientists to quickly assess and monitor hallucinogens to respond effectively to cases involving these compounds. Solid phase extraction of biological samples enables the concentration of analytes of interest as well as their separation from interfering matrix components, resulting in better chromatography and lower detection limits. As a result of this study, we were able to show that automated solid phase extraction performed by the LCTech FREESTYLE SPE sampler could successfully be used for psilocin in either serum or hydrolyzed urine samples. Using the GERSTEL MPS robotic, the SPE eluate was then automatically concentrated by evaporation. The final extracts were then determined using an Agilent Ultivo Triple Quadrupole Mass Spectrometer with Jet stream electrospray source. Accuracy data averaged 91.9% (85.1% - 100%), and precision data averaged 1.38% RSD (0.266% - 3.44%) for psilocin extracted from serum samples. Accuracy data averaged 95.9% (88.4% - 107%), and precision data averaged 0.657% RSD (0.105% - 1.44%) for psilocin extracted from urine samples.

200 Approaches for Reducing the Environmental Impact and Increasing the Throughput of LC Separations

Jessica Hussey, MAC-MOD Analytical, 103 Commons Court, Chadds Ford, PA 19317, Matt James, Anthony Edge

Many analytical laboratories are increasingly focussing on reducing their environmental footprint. This is particularly important in high-throughput environments, where the high sample numbers analysed results in significant solvent usage and waste disposal. Significant reductions in solvent consumption and waste generated are possible for many existing analytical LC methods through translation to modern column technology (e.g. narrower bore / smaller format). This also impacts electrical consumption per analysis, whilst providing the added benefit of improved laboratory efficiency. Often, such savings are considered in terms of migrating methods to UHPLC, however, substantial gains can also be achieved using existing HPLC equipment. This poster explores these possibilities and demonstrates the gains that can potentially be achieved for isocratic and gradient LC methods. When translating existing HPLC separations to UHPLC, up to 86.7% reduction in solvent use, 86.2% reduction in electrical consumption and 89.3% reduction in run time were achieved. It was also demonstrated that by selecting a column format to better utilise existing equipment, substantial improvements can be realised. For a gradient method, 71.9% reduction in solvent use and 57.3% reduction in electrical consumption and 60.4% reduction in analysis time was easily achieved on a standard 400 bar HPLC system. Additionally, a tryptic digest sample was used to demonstrate the application of this approach to bioanalytical workflows and to enhanced analytical sensitivity for sample limited, low detection level assays. Finally, practical considerations, such as the impact of extra column dead volume on data quality when moving methods to narrower bore columns, were assessed.

201 Exploring the Behavior of RNA Molecules Using NMR

Hashim Al-Hashimi, Columbia University, Hammer Health Sciences Building - Irving Medical Center BSL2 Lab, Rm 1004, 10 Fl., 701 W 168 St., New York, NY 10032

Much effort has been directed recently toward predicting the 3D structures of biomolecules. And yet we are most interested in the behavior of biomolecules and how it enables them to perform the functions they evolved to do. Here, describe how nuclear magnetic resonance (NMR) spectroscopy has illuminated the behavior of RNA molecules, especially their dynamics. We propose that RNAs and other biomolecules can be represented using a finite-state computing machine, in which conformational and chemical states undergo transitions based on a defined set of instructions in a transition table. We suggest that biomolecules employ diverse three-dimensional structures to embody a limited number of transition rules, which can be woven together to reconstruct any biochemical process. This computational perspective of molecular behavior holds tremendous potential for providing a unified framework encompassing all biomolecules and biochemical processes.

202 An NMR Journey from Method Development to Practical Applications that all Began in New Haven

John Marino, NIST, IBBR 9600 Gudelsky Dr., Rockville, MD 20855

Advances in biotechnology are often driven by new experimental methods, analysis tools and models that address critical gaps in measurement. Innovative approaches developed to address these gaps can be underpinned by standards that establish trust and accelerate broad adoption through benchmarking and harmonization.

Among analytical tools, nuclear magnetic resonance (NMR) has become one of the most versatile and broadly applied high-resolution methods for characterizing the structure and dynamics of molecules. In this talk, I will provide highlights of how we apply NMR at NIST to advance biomolecular measurement, with a focus on recent work aimed at development of NMR methods and standards that support protein therapeutic development and manufacture. Protein therapeutics are a highly successful class of drugs that are currently used to treat serious and life-threatening conditions such as cancer, autoimmune disorders, and infectious diseases. A critical requirement unique to protein therapeutics is that these drugs must adopt and retain the correct structural fold without forming unintended conformers and/or aggregates. The development of robust, precision tools for characterizing the structure of protein therapeutics has therefore emerged as a major priority for the pharmaceutical industry and regulatory agencies. To address this gap, I describe how NMR methods can be used to generate spectral 'fingerprints' that can quantitatively assess structural comparability and reveal and classify structural variations, and how these approaches are paving the way for broad use of NMR in the biopharmaceutical industry for assessment of protein therapeutics via chemometrics and machine learning that is both objective and automated.

203 NMR: A Powerful Tool to Study Nature's Switches to Develop Biotech Tools and Therapeutics

Kevin Gardner, Structural Biology Initiative, CUNY Advanced Science Research Center, 85 St. Nicholas Terrace, New York, NY 10031

Environmental cues regulate many biological processes, coordinating cellular pathways to respond to changing conditions. Such regulation is often initiated by sensory protein domains which expand their chemical repertoire by using small molecule ligands to convert environmentally-triggered changes into altered protein/protein interactions. Combining biophysics, biochemistry and synthetic chemistry, we study the mechanistic controls of such domains to understand fundamentals of biological signaling and how this might be altered in disease or artificially controlled for therapeutic or biotech purposes. Here I will discuss several examples of this principle, showing how our work – fundamentally rooted in solution nuclear magnetic resonance (NMR) spectroscopy throughout – probing light- and oxygen-regulated signaling proteins has led to novel optogenetic tools and a first in-class anti-cancer therapeutic (Merck's belzutifan HIF-2 inhibitor). Future directions stemming from this work are also discussed.

204 Glycans on Glycoproteins: What NMR Can Tell Us

James Prestegard, University of Georgia, 315 Riverbend Rd., Complex Carbohydrate Research Center, Athens, GA 30602

The role of nuclear magnetic resonance (NMR) in Structural Biology has dramatically changed over the last two years, with an emphasis on structure determination of proteins shifting to how proteins interact with ligands and how they respond to posttranslational modifications. For glycoproteins the shift has been less dramatic, as they have always been difficult to produce with the full complement of isotope labels typically used to produce NMR structures. Over the last few years, we have developed a strategy for investigation of conformational properties of glycoproteins and their attached glycans that capitalizes on an ability to isotope label just a few amino acids (sparse labeling). Rather than using sequential connectivities along a protein backbone to make resonance assignments and short-range nuclear Overhauser effect (NOEs) to derive structure, it uses assumed protein structures, along with long-range data from paramagnetic tags. The long-range data include residual dipolar couplings (RDCs), pseudo contact shifts (PCSs) and paramagnetic relaxation enhancements (PREs). Derivation of tag properties from these data provide predictive tools that can be used to identify ligand binding sites and screen ensembles for likely glycan conformations. The assumed protein structures now come from models produced by computational methods such as AlphaFold, and comparison of experimental data on labeled protein residues to predictions based on the model can be used for validation. Recent applications to glycosylated proteins such as CEACAM1 and ST6Gal1 are discussed.

205 Industrial Spectroscopy Research Leading to the Development of Novel Bioplastics

Isao Noda, University of Delaware, 201 DuPont Hall, Newark, DE 19716

In early 1980s, a group of scientists at P&G initiated an ambitious research program, combining spectroscopy and mechanical testing to develop useful plastics designed from the basic molecular level. Curtis Marcott was the leading infrared (IR) spectroscopist along with other prominent colleagues, like Tony Dowrey and Gloria Story. I was the polymer designer of the group utilizing the incoming spectroscopic information to come up with the improved molecular architecture with useful functionalities. In the process, I had to learn so much about IR to eventually become a spectroscopist myself. Out of this earlier research effort came a powerful polymer characterization technique called dynamic infrared linear dirchroism (DIRLD) spectroscopy, which later laid the foundation of the development of two-dimensional correlation spectroscopy (2D-COS). DIRLD spectroscopy and 2D-COS analysis played a key role in the materials development effort at P&G, especially in the molecular design of a bio-based and fully biodegradable plastics called poly(hydroxyalkanoates) or PHAs made by bacteria. Unfortunately, most known PHAs had major shortcomings in their properties to become a useful material in practical applications. 2D-COS provided a critical insight into a way to modify the molecular structure of PHAs to make this class of biomaterials much more useful to become a viable replacement for conventional petroleum-based plastics. This novel type of PHAs are now commercialized by Danimer Scientific, Inc. under the trade name of Nodax®. My talk covers the earlier development of this exciting new bioplastics and subsequent fundamental scientific research effort carried out on PHAs.

206 Super-Resolution Photothermal Infrared Spectroscopy for Science and Industry

Craig Prater, Photothermal Spectroscopy Corp., 325 Chapala St., Santa Barbara, CA 93101

Optical Photothermal Infrared (O-PTIR) is a rapidly emerging technique for super-resolution infrared spectroscopy. O-PTIR illuminates a sample with pulses of light from a tunable infrared laser and then uses a shorter wavelength visible probe beam to detect infrared absorption at specific molecular bonds by detecting subtle heating in IR absorbing regions of the sample. Since the probe beam can be focused much smaller than the infrared excitation beam, O-PTIR can achieve spatial resolution 10-30X better than conventional infrared spectroscopy. O-PTIR has been successfully applied to a wide range of research areas including polymer research, analysis of microplastics, contaminant and defect analysis, cultural heritage studies, and a wide variety of biomedical research, including analysis of cells and tissue with applications in cancer research and neurodegenerative disease. O-PTIR has been combined recently with fluorescence imaging to enable the use of fluorescent tags to localize IR spectroscopic measurements, to explore differences in protein secondary structure between normal and diseased tissue in connection with neurodegenerative disease. O-PTIR has also been used for spectroscopic analysis of bacteria enabling studies of spectroscopic phenotyping, assessing antimicrobial resistance, and single cell metabolic studies. O-PTIR can also be performed with simultaneous and co-located Raman spectroscopy, providing additional complementary and confirmatory analysis. This presentation overviews technology and underlying theory of operation for O-PTIR, and survey a variety of applications.

207 Nano-Chemical Imaging and Spectroscopy to Unravel (Bio)-Organic Matter towards the Single-Molecule Level

Francesco Simone Ruggeri, Wageningen University, Organic and Physical Chemistry, Wageningen, 6708WE, Netherlands

Here, we show the latest years development and application of infrared absorption nano-imaging and spectroscopy atomic force microscopy infrared (AFM-IR) as a real breakthrough for the analysis of heterogeneous (bio-)organic molecules and materials from the single molecule scale to several multiple length scales in air and liquid environment. As a major advance in the field, we demonstrate the achievement of single protein molecule detection of infrared absorption spectra and maps by introducing off-resonance, low power and short pulse ORS-nanoIR.[1] The technique enables the accurate determination of the secondary structure elements of single proteins in the amide band I region, such as a-helices and b-sheets. Then, we show the application of this single molecule sensitivity to unravel the molecular interaction fingerprint between a small molecule and its target [2]. Similarly, we demonstrate the application of the technique to probe the surface and structural properties of functional materials, such as artificial model membranes [3], functional protein self-assemblies [4-6], and perovskites [7]. Overall, our aim is to expand the capabilities of analytical nanoscience to shed light on the structure-activity relationship of biomolecules and functional materials design.

References:

[1] Ruggeri, Nature Comm., 2020.
[2] Ruggeri, Nature Comm., 2021.
[3] Marchesi, Advanced Functional Materials, 2020.
[4] Ramer*, Ruggeri*, ACS Nano, 2018.
[5] Shen, Ruggeri, Nature anotechnology, 2020.
[6] Otzen,…, Ruggeri, Small Methods, 2021.
[7] Doherty, Science, 2021.

208 Characterizing the Microstructure of Novel Bioplastics Using Photothermal Infrared Spectroscopy

Curtis Marcott, Light Light Solutions, LLC, P. O. Box 81486, Athens, GA 30608

Realization of the potential harm caused by the accumulation of plastic waste in the environment has led to a search for alternative materials. Bioplastics made from natural resources, like vegetable oils and sugars, which can biodegrade back to nature under proper conditions, are emerging as a potential replacement for conventional petroleum-based plastics. In parallel, the use of cutting-edge analytical tools to guide the characterization and improved design of useful bioplastics has become common practice. Simultaneous optical photothermal infrared (O-PTIR) and Raman spectroscopy is an emerging capability requiring no contact with the sample. It provides high-

ly spatially resolved IR and Raman hyperspectral images down to about the 500-nm level, well below the IR diffraction limit. This range of spatial resolution is well suited for the analysis of multicomponent and multiphase samples, including composites, blends, and laminates. Such systems often exhibit varying degrees of molecular level mixing and spatial segregation of constituents at their interfaces, which in turn strongly affect the end use performance of the material. Although O-PTIR spectra are obtained in reflection, high quality spectra are obtained without any dispersive scattering artifacts and the spectral profiles resemble those of traditional FT-IR spectra collected in transmission. In this study, high spatial resolution hyperspectral O-PTIR and Raman images were simultaneously obtained for a biodegradable laminate sample comprised of macroscopically immiscible polylactic acid (PLA) and a polyhydroxyalkanoate (PHA). The results shed light on the manner in which the PLA and PHA components interpenetrate.

209 Portable Capillary LC for In-Line UV Monitoring and MS Detection: Comparable Sensitivity and much Lower Solvent Consumption

Michael Hicks, Merck & Co., Inc., 126 East Lincoln Ave., Rahway, NJ 07065, Keith Mattern, Jonathan Fine, Shane Grosser, Daya Patel, Lauren Weisel, Pankaj Aggarwal

Pharmaceutical development currently relies on quality separation methods from early discovery through to line-of-site manufacturing. There have been significant advancements made regarding the column particle packing, internal diameter, length connectivity, the understanding of the impact key parameters like void volume, flow rate, temperature all that affects the resultant separation quality i.e., resolution, peak shape, peak width, run time, signal to noise. There is however a strong need to establish better alternatives to large bulky HPLC racks either for process analytical reaction monitoring or MS analysis in establishing product quality. Compact, portable high-pressure LC can be a more efficient alternative to traditional ultra-high pressure LC and traditional LC. The compact versatile instrument evaluated here, allows good separation control with either the on-board column with fixed ultra-violet wavelength cartridge or for use with a high-resolution MS. Significant space reduction results in greener lab spaces with improved energy efficiency for smaller labs with lower energy demands. In addition, this compact LC was used as a portable reaction monitoring solution to compare forced degradation kinetics and assess portable LC-MS capability for the analyses required for pharmaceutical drug product testing.

210 Development of the Analytical Control Strategy to Support a Continuous Drug Substance Process – New Thoughts on Green Chromatography

Stephen R. Groskreutz Synthetic Molecule Design and Development Senior Director, Eli Lilly and Company Indianapolis, Indiana

The use of continuous manufacturing has been highlighted as an important element in the modernization of pharmaceutical manufacturing. Eli Lilly and Company has considerable experience in development of continuous drug substance processes, and implementation of these processes in GMP facilities. An important factor in Lilly's success has been the use of process analytical technologies to evaluate process performance in real time. A case study will be presented for the development of analytical controls to monitor a multi-step continuous drug substance process, which involves simultaneous operation of multiple reactor types and unit operations (continuous stirred-tank reactors, plugged-flow reactors, etc). On-line HPLC systems are used to monitor three chemical transformations, and on-line IR spectroscopy is used to monitor distillation performance. Topics to be discussed include: the development of analytical methods and sampling techniques, the ability of analytical systems to detect processes disturbances, the determination of appropriate strategies for diversion of nonconforming material, and contextualization of approaches to minimize environmental footprint.

211 Development of Alternate QC Techniques for More Rapid Screenings within LMIC Contexts

David Jenkins, FHI 360, Product Quality and Compliance Department, Durham, NC 27713, Matthew Eady, Ed Bethea, Chris Harmon, Jonelle Caison, Melissa Growney

Monitoring product quality is important to assure the integrity of products provided in global public health programs. Traditional pharmacopoeia approaches, often based on high performance liquid chromatography (HPLC) or ultraviolet-visible (UV-Vis) techniques, are critical tools required to assess pharmaceutical quality. However, in limited resource settings, public health programs need to optimize resources, and traditional approaches can be expensive (i.e., reagents, equipment, staff time). More rapid screening approaches, while coupled with traditional methods, are important to incorporate into quality assurance programs. Various techniques are currently being explored for assessing important public health commodities, namely spectroscopy-based approaches involving benchtop diffuse reflectance (350-2500 nm), handheld NIR (900-1700nm), and portable Raman (1064nm laser). Development activities are in process for a variety of discriminant (qualitative) and quantitative assessments. Qualitative brand discrimination for medroxyprogesterone acetate (MPA) injectables has been demonstrated with handheld near infrared (NIR) (relative to benchtop DRS), with potential applications in screening for new candidate products for supply chains. Brand discrimination for isoniazid tablets has been assessed, with approaches for building discriminant methods with multiple handheld NIRs using data scanned from different countries. Quantitative MPA and isoniazid methods are also under development with all three instruments. In comparison to benchtop DRS, handheld NIR is showing potential for oral contraceptive brand discrimination, including the detection of exposure to adverse environmental conditions. Qualitative and quantitative methods for insecticide treated nets for malaria are under development. Various spectroscopic approaches are showing potential to assess important public health commodities, where the portable nature of many of these instruments will improve use in low resource settings.

212 Green Sample Preparation: It's All Green

Doug Raynie, South Dakota State University, Department of Chemistry and Biochemistry, Brookings, SD 57007

The aims of green chemistry, chemical analysis, and sample preparation are not contradictory. Each of these favors automation, reduced solvent and energy use, efficiency, and similar attributes. In fact, several rubrics for measuring the green qualities of an analysis exist, in addition to the analytical figures of merit. In this presentation, we will review these green analytical metrics. Next, the new extraction techniques developed over the past generation will be reviewed. While these are created for their performance advantages, they also address green chemistry concerns of waste, solvent use, energy efficiency, and toxicology. We will look at an overview of new and emerging sample preparation technologies, their performance in various applications, and the resulting green benefits. Finally, since solvent use is the starting point to develop green methods in most cases, we will explore what makes a solvent green. Finally, we will look at alternatives to traditional molecular solvents, including those derived from natural products (so-called biobased solvents) and deep eutectic solvents. The key physical-chemical properties, such as viscosity and diffusivity, that make these solvents attractive for analytical extractions are investigated and examples to real-world analytical extractions shown.

213 Stereoisomer Separation of Drugs and Biomarkers Using Supercritical Fluid Chromatography to Support PK/PD Studies

Fangbiao Li, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486, Bang-Lin Wan, Guanping Bi, Rena Zhang, Daniel Spellman

Mutations in the glucocerebrocidase (GBA) gene, which encodes for the enzyme GCase, have been identified as a casual risk factor for Parkinson's disease. GBA mutations reduced GCase activity resulting in the accumulation of lipid substrates glucosylceramide (GlcCer) and glucosylshingosine (GlcSph). To provide bioanalytical support for Parkinson's disease in preclinical studies, a supercritical fluid chromatography-mass spectrometry (SFC-MS/MS) method was developed to separate GlcCer from the predominant isomer found in brain tissue (galactosylceramide, GalCer). This method was based on a hydrophilic interaction liquid chromatography (HILIC) column rather than an expensive chiral column. Compared to the reported high performance liquid chromatography (HPLC) method, a short run time, sharper peak and better reproducibility were achieved. A liquid chromatography-mass spectrometry (LC-MS/MS) method based on normal phase chromatography was developed to separate GlcSph from its isomer found in brain tissue (galactosylsphingosine, GalSph). The method is sensitive, robust and highly reproducible. The qualitative and quantitative assessment of GlcCer and GalSph levels in mouse plasma and tissue samples are presented.

214 A Novel Hybridization LC-MS/MS Methodology for Quantification of siRNA in Plasma, CSF and Tissue Samples

Long Yuan, Biogen, 225 Binney St., Cambridge, MA 02142

Therapeutic oligonucleotides, such as antisense oligonucleotide (ASO) and small interfering RNA (siRNA), are a new class of therapeutics rapidly growing in drug discovery and development. A sensitive and reliable method to quantify oligonucleotides in biological samples is critical to study their pharmacokinetic and pharmacodynamic properties. Hybridization liquid chromatography mass spectrometry (LC-MS/MS) was recently established as a highly sensitive and specific methodology for the quantification of single-stranded oligonucleotides, e.g., ASOs, in various biological matrices. However, there is no report of this methodology for the bioanalysis of double-stranded oligonucleotides (e.g., siRNA). In this work, we investigated hybridization LC-MS/MS methodology for the quantification of double-stranded oligonucleotides in biological samples using an siRNA compound, siRNA-01, as the test compound. The commonly used DNA capture probe and a new peptide nucleic acid (PNA) probe were compared for the hybridization extraction of siRNA-01 under different conditions. The PNA probe achieved better extraction recovery than the DNA probe, especially for high concentration samples, which may be due to its stronger hybridization affinity. The optimized hybridization method using the PNA probe was successfully qualified for the quantitation of siRNA-01 in monkey plasma, cerebrospinal fluid (CSF), and tissue homogenates over the range of 2.00 - 1000 ng/mL. This work is the first report of the hybridization LC-MS/MS methodology for the quantification of double-stranded oligonucleotides. The developed methodolo-

gy will be applied to pharmacokinetic and toxicokinetic studies of siRNA-01. This novel methodology can also be used for the quantitative bioanalysis of other double-stranded oligonucleotides.

215 Identification of Riboflavin as Novel BCRP Biomarker in Animal Models

Linna Wang, Bristol Myers Squibb, Route 206 & Province Line Rd., Princeton, NJ 08543, Yueping Zhang, Petia Shipkova, Bethanne Warrack, David Nelson, Runlan Huo, Jian Chen, Erika Panfen, Xue-Qing Chen, R.Marcus Fancher, Qian Ruan, Lisa Christopher, Yongjun Xue, Michael Sinz, Hong Shen

Breast cancer resistance protein (BCRP) is a member of ATP-binding cassette drug transporter superfamily. Efflux via intestinal BCRP can have a significant clinical impact on drug oral bioavailability and drug-drug interaction (DDI) risk. The United States Food & Drug Administration (FDA) and European Medicines Agency (EMA) recommend an assessment of drug–drug interactions (DDIs) risk for a NME as either a BCRP substrate or inhibitor based on in vitro studies. The use of endogenous biomarkers to assess transporter-mediated DDI is investigated to develop methodologies to detect and predict transporter DDIs in early clinical trials and regulatory demands. In mice, we observed that riboflavin was significantly elevated in the plasma of Bcrp single- and Bcrp/P-gp double- but not P-gp single-knockout mice. Dual BCRP/P-gp inhibitor caused a dose dependent increase of the area under plasma concentration-time curve (AUC) of riboflavin in mice (1.51- and 1.93-fold increases by 30 and 150 mg/kg elacridar, respectively). In cynomolgus monkeys, we observed riboflavin plasma concentrations increased 1.7-fold caused by BCRP inhibitor (ML753286 (10 mg/kg)), which correlated well with the increase of sulfasalazine (a known BCRP probe). Additionally, clinical studies on healthy volunteers indicated low intrasubject and intermeal variability of plasma riboflavin concentrations. Our results identified riboflavin as an informative BCRP plasma biomarker in animal models. Its selectivity, sensitivity, and predictivity regarding BCRP inhibition have been explored. The utility of this biomarker requires further validation by evaluating the effects of BCRP inhibitors on riboflavin plasma concentrations in humans. Ultimately, riboflavin has the potential as a biomarker for BCRP DDIs risk assessment in early clinical trials.

216 Hybrid LBA-LC-MS/MS Method for Glycan-Resolved PK Monitoring of a Therapeutic Fusion Protein

Ines Santos, Bristol Myers Squibb, Route 206 & Province Line Rd., Princeton, NJ 08543, Brian Melo, Linna Wang, Yury Chaly, Bonnie Wang, Y-J Xue, Jim Shen

Understanding the impact of glycosylation and respective metabolism-induced changes in the pharmacokinetics (PK) and pharmacodynamics (PD) properties of therapeutic proteins is critical to determine the exposure and efficacy of such drug candidates. Comparative PK studies from different manufacture lots normally rely on the overall PK parameters of the proteins; however, these vary with the type/extent of glycans present. Therefore, glycan-resolved PK monitoring would help understand differential *in-vivo* clearance for individual glycoforms and their contributions to the overall PK/PD of each lot. While ligand binding assays (LBA) are more suitable for the quantitation of total therapeutic glycoprotein, liquid chromatography tandem mass spectrometry (LC-MS/MS) is well equipped for the quantitation of individual glycoforms as explored in this work. Here, a bottom-up approach was used for the quantitation of major glycopeptides and glycan-resolved PK analysis of a therapeutic fusion protein with 6 unique glycosylation sites. The low dosing (8 µg/kg) animal study, the low ionization efficiency of glycopeptides, and the presence of multiple glycoforms pose significant challenges in the quantification of glycopeptides thus, to further improve the sensitivity of the method, a micro-LC in trap and elute mode was employed for high loading capacity as well as better detection of numerous hydrophilic/hydrophobic glycopeptides. LLOQs of 0.2 - 32 ng/mL were achieved for more than 16 individual glycopeptides, which enabled us to monitor major glycoforms at each glycosylation site for the four manufacturing lots of the therapeutic protein and assess their respective PK properties in this animal study.

217 The Fatal Bullet – Was it a Ricochet or Not?

Pete Diaczuk, John Jay College, 524 W 59th St., New York, NY 10019

This was a homicide case where the victim was hit by two bullets in his upper body. There were several spent cases from a .22 Magnum caliber firearm at the scene, but the only recovered firearm was a .25 automatic pistol. The bullets from these two calibers are similar in size and weight. There were two defects in a wall adjacent to where the victim was standing before he was shot. The two horizontal marks were at a height that closely aligned with the two bullet entry holes in the victim. A close look at the two marks on the wall suggested they were made by bullet ricochets. A careful microscopical examination of the bullets removed at the autopsy revealed whether the fatal bullets were from ricochets or direct fire.

218 Microscopy and Microanalysis of Aluminum Powders Used in Improvised Explosive Devices (IEDs)

JoAnn Buscaglia, FBI Laboratory, Research and Support Unit, 2501 Investigation Pwy, Quantico, VA 22135, JenaMarie Baldaino, Kayla Moquin, Jack Hietpas

Improvised explosive devices (IEDs) are often composed of commercial or readily available materials. Aluminum (Al) powder, a common metallic fuel, is one such material that can be obtained from multiple sources, as it has many legitimate uses and applications. Online sharing of videos and instructional manuals inform amateur bomb-makers of the easily accessible materials and methods for making Al powder for IEDs. These include ball-milling Al foil or grinding it in a coffee grinder; extracting Al flake from spray paints; melting Al cans and then lathing or filing followed by milling; purchasing Al powder as a component of binary exploding targets; and extracting Al powder from pyrotechnics such as sparklers and firecrackers. This presentation discusses the differences in Al particle surface characteristics and elemental compositions of amateurly vs industrially produced Al powders using scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM-EDS). Methods of manufacturing Al powders (i.e., industrial vs. homemade) compared using SEM-EDS show that morphology and surface characteristics can differentiate some methods of Al powder production. The results obtained from SEM micrographs demonstrate that Al powder manufactured by ball milling could be confidently differentiated from those extracted from an Al flake-containing spray paint. Furthermore, SEM-EDS analysis of the Al flake-containing spray paints provided additional information that could differentiate between brands and among products within brands. SEM-EDS analyses such as these can provide additional investigative and intelligence value beyond mere identification of Al powder as a component of an IED.

219

No abstract Submitted by the author.

220 A Review of Dispersion Staining in Forensic Casework

Nicholas Petraco, Petraco Forensic Art Investigation, 240 Abbey St., Massapequa Park, NY 11762

Dispersion staining (DS) is a powerful microscope technique for studying the refractive indices and dispersion properties of all types of transparent materials. Thanks to the extensive work and teaching of Dr. Walter McCrone, forensic scientists have used DS to study, identify and compare materials such as minerals, pigments, glass fragments, explosives, pollens, fibers, and dust specimens in their casework since the mid-twentieth Century. However, since the development of the Glass Refractive Index Measurement System (GRIM) for glass refractive index determination, the use of DS for identifying and comparing most other types of materials has declined within the forensic trace evidence community. The purpose of this review is to encourage and facilitate a resurgence of the use of DS methods within the forensic trace evidence community. An array of different dispersion staining objectives and illuminations techniques used by the author in his casework over four decades will be presented The advantages and disadvantages of utilization of the various size central and annular stops as well as different microscope contrast methods such as PLM, Hoffman Modulation, Phase Contrast and so on are presented, illustrated, and discussed in detail. Finally, the importance for a resurgence of trace evidence analysis and the use of DS as well as other microscopic contrast methods in forensic casework is conferred.

221 Mapping Key Elements in the USP <1220> and ICH Q14 Guidances to an Enhanced Quantitative Framework and Workflow for Analytical Procedure Development

Richard Verseput, S-Matrix Corporation, 1594 Myrtle Ave, Eureka, CA 95501

The new United States Pharmacopeia (USP) <1220> and ICH Q14 guidances have affirmed the expectation of incorporating statistical quantitation into analytical procedure development. This expectation is reflected in key guidance topics including the analytical target profile (ATP), method robustness estimation, and replication strategy optimization. This presentation will therefore map key elements in these guidances to an enhanced quantitative framework and workflow for analytical procedure development. Topics will include 1) defining the ATP in terms of analytical procedure variation allowances as a negotiated specification which includes production considerations, 2) the correct integration of robustness simulation into method optimization to efficiently establish a multi-dimensional, robust method operable design region (MODR), 3) the critical integration of Replication Strategy optimization, which defines the most efficient strategy for generating Reportable Results which meet the analytical method precision requirements defined in the ATP, and 4) the risk-based business case for the enhanced framework and workflow. This presentation describes these topics within the context of the development, validation, and transfer of a liquid chromatography method.

222 Faster, Cheaper, Greener! Joining HT Plate Readers and Chemometrics to Enable Enzyme Evolution

Umme Ayesa, Merck & Co., Inc., 126 E Lincoln Ave, Rahway, NJ 07065, Zachary Dance

High-throughput analytical methods are a critical component of the enzyme evolution cycle. During a single enzyme evolution campaign, tens of thousands of samples are analyzed and used to evaluate and design subsequent rounds of development. Typically, short chromatographic methods are the primary analytical methodology for this purpose because the platform is generally accurate, widely applicable, and mature. However, even with short methods, screening samples in a single round of evolution can take days which becomes a bottleneck for fast turnaround during drug development. Considering the challenges with current high throughput approaches such as chromatography, we have developed a high-throughput chemometrics (HTC) capability. This technique yields comparable information while being faster, greener, and cheaper than current liquid chromatography (LC) technologies. For systems with distinct component UV spectra, it combines relatively inexpensive plate-reader systems with well-understood chemometric analyses to deliver the same results in 5-10% of the time while using less solvent than the comparable LC approach. Furthermore, automated analyses can be implemented using the data from high-throughput plate-based method to generate results automatically into a user-friendly format. Herein, we present the HTC methodology and demonstrate its utility for evolution of cytidine deaminase used for an enzymatic hydroxyamination. This example provides evidence on the broad applicability of HTC to additional spectroscopic techniques.

223 Chemometrics Best Practices and the Impact on Quality Management

Brian Rohrback, Infometrix, Inc., 11807 North Creek Parkway, S Ste. B-111, Bothell, WA 98011

There is a lot of confusion on what constitutes best practices in the application of multivariate statistics to laboratory, process, and field analytics. The terminology pushed by literature references does not always clarify and often a technique touted in the literature is not compared to any other technology that could be applied to the same problem. The result is an incomplete education for those charged with putting technology to work in a quality control setting. Tools from chemometrics and machine learning categories certainly benefit from some user experience, but there are myriad details that need to be considered to have a system that truly functions (and justifies its cost) for the company. A case study of the ongoing work United States Pharmacopeia is spearheading on guidelines for a systematic approach to building, maintaining, and benefiting from an application-specific spectral library, will be discussed. Chemometrics provides the routine computation, but also has an impact on instrument evaluation, set-up, method development, and maintenance. The point is that to be successful, we need to deploy commonly-available chemometrics tools to simplify the handoff from R&D to the factory floor.

224 Combining Analytical Data with Contextual Metadata through CDS Platform, Suite of Applications, and Spotfire Dashboards

Henry Tat, Merck & Co., Inc., 126 E. Lincoln Ave, Rahway, NJ 07065, Jonathan Fine, Pankaj Aggarwal

The pharmaceutical field generates large amounts of data during the development of drugs, especially in stability studies. Unfortunately, many times, this data is stored in different locations and in unstructured formats. To compound this issue, associated metadata is often incomplete and inconsistently recorded during data generation. By combining CDS querying, custom low code solutions, and visualization software, a low maintenance, quick, and flexible solution creates structured metadata that is linked to chromatographic data. This presentation discusses the development of tools that visualizes stability studies to easily see data holistically, including trends and outliers.

225 Successful Replacement of Two Problematic HPLC Methods – One for API and One for Related Substances – with a Robust Single UHPLC Method Using the Enhanced QbD Approach

Marina Mavrinac, J.G.L. Pharma, Rijeka, Croatia, Gordan Dinter, Richard Verseput

An enhanced quality-by-design (QbD) approach succeeded in replacing two slow and underperforming high-pressure liquid chromatography (HPLC) methods with a single robust UHPLC method. The nature of the sample compounds posed several significant challenges to project success: the API was extremely difficult to retain, the related substances consisted of acidic, neutral, and basic compounds, and most compounds were insoluble in acidic pH solutions. Additionally, the related substances method was a multi-segment gradient method with poor repeatability in the quality control environment, and both methods had long run times – 40 minutes for the active pharmaceutical ingredient (API) method and 90 minutes for the related substances method. Project work included an initial screening experiment to assess the viability of developing a single method followed by a full chemistry system screening experiment to identify the column, strong solvent, and effective ranges of pH and gradient slope to promote to optimization. An optimization experiment was then carried out to fully characterize the method operable design region (MODR) and the robust final method. Lastly, a formal Replication Strategy experiment was carried out to determine the most efficient combination of preparations and injections to generate reportable results which would meet all method repeatability requirements defined in the analytical target profile. All experiments were carried out using the Fusion QbD method development software. The project work successfully replaced two slow, complex, and non-robust methods with a robust single-gradient method. The final method run time of 30 minutes represents a 3-fold reduction in run time relative to the original related substances method.

226 High-Performance Thin-Layer Chromatography PRO as a Quality Control Tool in Routine Analysis

Wilmer Perera, CAMAG Scientific, Inc., 515 Cornelius Harnett Dr., Wilmington, NC 28401, Shaune Liendo, Diana Catalan

With the new fully automated High-Performance Thin-Layer Chromatography (HPTLC) PRO system, multiple samples can be analyzed in sequence, overcoming the environmental effects produced by the previous open system. HPTLC PRO also adds a more rigorous control of the gas phase and is a throughput technique for the quality control of plant materials among other applications. This presentation aims to discuss important concepts such as standardization, comprehensive fingerprint and reviewing more recent ideas like the universal system suitability test, complementary developing solvents for untargeted analysis and other solvents for targeted analysis. The importance of HPTLC as a quality control tool in a dietary supplement setting by showing different case studies is also covered.

227 Is Lipid Repair in Hair a Possibility or a Pipe Dream?

Ernesta Malinauskyte, TRI Princeton, 601 Prospect Ave, Princeton, NJ 08540

Lipids are minor hair components, but their loss drastically impacts multiple hair properties. Literature suggests that in just approximately 1.5 years, European hair loses about 40% of hair lipids*. Sebum production and frequent washing practices may prevent the replenishment of lipids in longer hair fibers. High performance thin layer chromatography (HPTLC) technique is used to investigate which lipid classes are lost while fibers of various European, Asian, and African descent are aging. The findings will be related to how fiber aging and lipid loss affect fiber shine, friction, flexibility, and strength. Can adequate replenishment of lost free lipids restore degraded fiber properties, or is the damage beyond repair? *Marsh et al., J. of Cosmet. Sci. 2018.

228 High Performance Thin Layer Chromatography - Accurate Mass Spectrometry for the Rapid Identification of Unknown Compounds

James Kababick, Flora Research Laboratories, LLC, 1000 SE M St., Ste. B, Grants Pass, OR 97526, Stacy Wise, Chanze Jennings

High performance thin layer chromatography (HPTLC) is a modern, highly reproducible technique for qualitative and quantitative analysis in the analytical laboratory. It is the United States Food & Drug Administration method of choice for botanical identity testing in the dietary supplement industry. Often, unknown bands appear requiring further interrogation. We demonstrate the use of the TLC-mass spectrometry (MS) interface coupling HPTLC plates to accurate mass spectrometry to collect *in-situ* MS and MS/MS accurate mass data combined with library searching for the identification of unknowns. Case studies of real-world samples are presented.

229 USP Standards for European Elder Berry Dietary Ingredients: HPTLC Test Solutions to Address Quality and Adulteration Issues

Maria Monagas, United States Pharmacopeia Convention, Dietary Supplements and Herbal Medicines, 12601 Twinbrook Parkway, Rockville, MD 20852, Tiên Do, Eike Reich

The demand for dietary ingredients derived from European Elder berry (Sambucus nigra L.), traditionally used in some countries to alleviate cold or flu symptoms remedy, has drastically increased due to the COVID-19 pandemic. Unfortunately, this surge in demand has also led to an increase in reports of adulteration. In 2021, in response to stakeholder inquiries, United States Pharmacopeia (USP) hosted the Elderberry Standard Development Open Forum to address the need for new quality standards. Since then, USP has been actively working in the modernization of the current official monograph for European Elder berry Dry Extract and in the creation of a new monograph for European Elder berry Aqueous Dry Extract, both published in PF 49(3) (May/June 2023). The most common types of adulteration found in Elder berry products include the use of confounding materials rich in anthocyanins, such as black rice extract and purple carrot juice, undeclared synthetic dyes and species misbranding. These challenges demand the development of simple, selective, multipurpose screening methods for the detection of quality and adulteration issues. This presentation provides an overview of the collaborative work between USP and CAMAG in developing new high performance thin layer chromatography (HPTLC) methods for European Elder berry. The methods include different applications: 1) an identity test based on the detection of anthocyanins and other flavonoids, and 2) an adulterant test based on the detection of synthetic dyes. Examples of the application

of these tests to address adulteration and quality issues in different types of European Elder berry ingredients are presented.

230 Equivalency of DNA Sequencing vs. HPTLC Chromatographic Analysis vs. Botanical Microscopy Methodologies for Botanical Identity: A Statistical Evaluation

Anthony Fontana, Alkemist Labs, 12661 Hoover St., Garden Grove, CA 92841, Sidney Sudberg, Dinah Yu, Robert LaBudde, Zhengfei Lu, Yanjun Zhang, Adam Faller

A small-scale single laboratory study was carried out to illustrate methodology for establishing equivalence between different analytical methods used for identifying botanicals. Samples of different lots from different sources of known inclusion (known positive) and exclusion (known negative) species were obtained, and measurements made in replicate by DNA, high performance thin layer chromatography (HPTLC) and microscopy on each sample. Special statistical methodology for proving the claim of equivalency among methods is illustrated and the results discussed.

231 Exploration of Ultra-High-Pressure Liquid Chromatography (UHPLC) for Bioanalysis

Hayley Herderschee, University of Michigan, Department of Chemistry, 930 N. University Ave., Chemistry Building, Rm 4723, Ann Arbor, MI 48109, Noah Lancaster, Evgenia Shishkova, Austin Salome, Joshua Coon, Robert Kennedy

In the medical and pharmaceutical industries, 'omic' (i.e., proteomics, metabolomics, etc.) analyses are increasingly important in informing drug design and disease diagnosis. Yet, multi-omic analyses are inherently complex due to the wide range of structures and sizes present among different analytes and the biological nature of the analytes. Much work has been done to develop analytical techniques, methods, and sample preparation procedures to work with biological samples for multi-omic analysis via liquid chromatography mass spectrometry (LC-MS), IM-MS, and gas chromatography (GC)-MS. Separation is essential for reducing the complexity of samples as it allows for different classes of analytes to be separated based on their defining structural and mass differences. In LC, metabolites, lipids, peptides, and proteins can be separated based on polarity and hydrophobicity. Understanding the biological difference between unique phenotypic traits and identification (ID) of metabolites, lipids, peptides, and proteins is crucial. Enhancing separation efficiency can help increase the number of IDs assigned to analytes in a biological matrix. Separation efficiency can be increased by decreasing the stationary phase particles' diameter (d_p). To enhance the coverage of a multi-omic approach, we used a custom UHPLC system to compare capillary columns packed with two sizes of BEH C18 particles (1.1 and 1.7 µm) to characterize a cell lysate to understand how decreasing d_p effect peptide identification. The UHPLC system allows these multi-omic separations at up to 45 kpsi. Experimental results confirm that reducing d_p and using higher pressures increases peak capacity and metabolite IDs. Further analysis shows room for improvement in increasing peptide IDs.

232 Rapid Label-Free Cell-Based Approach Membrane Permeability Assay Using MALDI-HDX-MS for Peptides in Drug Discovery

Alexey Makaro, Merck & Co., Inc., Merck Research Laboratories, BMB-2-132, 33 Louis Pasteur Ave., Boston, MA 02115

Peptide therapeutics represent a growing drug modality that enable intracellular target engagement; therefore, requiring the establishment of cell permeability. One of the unresolved analytical challenges is the rapid measurement of target-agnostic peptide cell permeability. In this work, we demonstrate the development of a rapid high-throughput label-free methodology based on the matrix-assisted laser desorption/ionization (MALDI)-hydrogen-deuterium exchange mass spectrometry (MALDI-HDX-MS) approach to rank-order peptide cell membrane permeability using live cancer cell cultures (THP1 and AsPc1). Peptides were incubated in the presence of live cells and their permeability into the cells over time was measured by MALDI-HDX-MS. A differential hydrogen-deuterium exchange approach was used to distinguish the peptides outside of the cells from those on the inside. The peptides on the outside of the cells were labeled using sufficient short exposure to deuterium oxide, while the peptides that permeated into the cells were protected from deuterium oxide exposure and labeling. The peak area ratios of unlabeled versus deuterium-labeled peptides were compared and plotted over time. The developed methodology, cell-based approach membrane permeability assay (CAMPA), was applied to study an array of diverse peptides including cell-penetrating, stapled and macrocyclic peptides. The MALDI-MS analysis was performed in an automated manner using an internally developed Python script for MS data processing. CAMPA was demonstrated to be useful for differentiating passive and active cell transportation by using endocytosis inhibitor in cell incubation media for selected peptides. This new workflow has allowed us to assess target-agnostic cell-membrane permeability of peptides in a fast, robust, high-throughput and label-free manner.

233 Nanoparticle-Enhanced Laser Induced Breakdown Spectroscopy (NELIBS) on Lanthanide Micro Particles Tagged to Biomarker

Ali Safi, University of Massachusetts Lowell, Department of Physics and Applied Physics, Olney Science Center, One University Ave., Lowell, MA 01854, Helmar G. Adler, Joshua E. Landis, Kemal Efe Esseller, Yuri Markushin, Noureddine Melikechi

Laser-induced breakdown spectroscopy (LIBS) is an emerging analytical technique that provides rapid, simultaneous quantitative and qualitative elemental analysis with little or no sample preparation. Biomedical applications of LIBS have been demonstrated in the analysis of liquid, soft tissue, and hard tissue samples. We have recently demonstrated that Tag-LIBS allows the enhancement of the specificity of LIBS when applied to biomedical samples. In another development, nanoparticle-enhanced laser-induced breakdown spectroscopy (NELIBS) was introduced and proved to provide a significant enhancement to the sensitivity of LIBS. We will report on our study to enhance the analytical capability of LIBS by combining Tag-LIBS and NELIBS using lanthanide microparticles using glass and parafilm as substrates.

234 Identifying Size-Dependent Toxin Sorting in Bacterial Outer Membrane Vesicles

Aarshi Singh, Lehigh University, 6 East Packer Ave., Bethlehem, PA 18015, Justin Nice, Angela Brown, Nathan Wittenberg

Gram-negative bacteria secrete outer membrane vesicles (OMVs) that play a critical role in intercellular communication and virulence, and have been identified as a promising target for developing vaccines. However, OMVs exhibit considerable heterogeneity in size, biomarkers, and cargo, despite being isolated from a single bacterial population. OMV heterogeneity is difficult to capture using traditional methods like western blot and ELISA. While single particle methods like flow cytometry and electron microscopy have been developed to address this, they also have drawbacks such as sample backtracking and OMV destruction. To overcome this challenge, we developed a method using fluorescence microscopy to identify size-dependent toxin sorting in OMVs. We examined *Aggregatibacter actinomycetemcomitans* (A.a.), an oral bacterium that produces a bimodal size distribution of OMVs, and secretes leukotoxin (LtxA) in two forms as part of its virulence: soluble and surface associated on OMVs. Our objective was to determine whether LtxA was distributed based on OMV size. A size-based heterogeneity was observed in which the larger OMVs were found to contain toxins, while the smaller ones did not. Specifically, no LtxA-negative OMVs were larger than 220 nm, and no LtxA-positive OMVs were smaller than 60 nm. Among the toxin positive OMVs, no correlation between toxin density and size was found. To validate our method, we separated the OMV population using size exclusion chromatography and then performed ELISA and western blot analyses, which yielded similar results. This method of single OMV analysis provides a better understanding of OMV heterogeneity and the role of size-dependent toxin sorting.

235 Mass-Activated Droplet Sorting for Selection of Lysine-Producing Escherichia Coli

Emory Payne, University of Michigan, 930 N University Ave., Ann Arbor, MI 48109, Bridget Murray, Laura Penabad, Eirc Abbate, Robert Kennedy

Synthetic biology is a powerful tool for using engineered cells for chemical production, but engineering methods are limited by the ability to screen variant libraries for potential activity. Droplet microfluidics is a method for high-throughput sample preparation and analysis; but, has largely relied on fluorescent analysis. Here, we show the ability to select cell variants grown in 20 nL droplets using mass-activated droplet sorting, a method for selecting droplets based on signal intensity measured by electrospray ionization mass spectrometry (ESI-MS), at 0.5 droplets/s. E. coli variants producing lysine as a bioproduct were used to evaluate the applicability of MADS for synthetic biology. E. coli were shown to be effectively grown in droplets, and lysine produced by these cells was detectable using ESI-MS. Sorting of high lysine producing cells based on MS signal was shown, yielding 96-98% purity in the selected pool. Using this technique, cells were recovered after screening, enabling downstream validation via phenotyping. The presented method is translatable to whole-cell workflows where biocatalysts are developed using metabolic means to production.

236 Modernizing USP Methods According to <621> with Superficially Porous Particle Columns

Stephanie Schuster, Advanced Materials Technology, 3521 Silverside Rd., Quillen Building, Suite 1-K, Wilmington, DE 19810, Peter Pellegrinelli, Conner McHale

The United States Pharmacopeia (USP) Chapter 621 guidelines have been updated to allow changes to gradient methods in terms of particle technology, size, and column dimensions with verification instead of revalidation. This change grants laboratories the freedom to modernize what column particle sizes and dimensions they use, creating substantial time and solvent savings. Many existing methods use classic particle sizes, column dimensions, and long run times and laboratories would benefit from switching to smaller particle size, shorter column length, and reduced column dimension. Examples are reviewed for switching from fully porous

particle columns to superficially porous particle columns for various pharmaceutical compounds. Demonstration of how small changes in particle size do not impact the overall system suitability of the method is also presented.

237 **Using Ion Chromatography to Assay for Citrate and Phosphate in Pharmaceutical Formulations**

Gary He, Thermo Fisher Scientific, 1214 Oakmead parkway Sunnyvale, CA 94085, Jingli Hu, Jeff Rohrer, Carl Fisher

Citric acid is a common ingredient in many pharmaceutical formulations where it is used for its effervescent effect in antacids and dentifrices, to add flavor and stability, as a buffering agent, to assist in ingredient dispersion, and to act as an anticoagulant. With the publication of General Chapter <345> the United States Pharmacopeia replaced several different assays for citrate that include calorimetry, gravimetry, ion-exclusion chromatography, and reversed-phase liquid chromatography with an ion chromatography (IC) assay. This IC assay is efficient, rapid, and can simultaneously determine phosphate and other anions that may also be present. In contrast, the previous methods are time-consuming, labor-intensive, tedious, and can yield significant measurement errors. In this presentation we will demonstrate this IC method for the determination of phosphate and total citric acid in pharmaceutical formulations. This method used a hydroxide-selective, anion-exchange column and suppressed conductivity detection. Sample analysis is performed using a new, innovative IC system that utilizes an electrolytic eluent generator to automatically produce eluent and a more accessible, intuitive layout to simplify operation and increase consistency, reducing the amount of hands-on time needed to achieve accurate and reproducible results.

238 **Direct Quantitation of Small-Molecule Impurities Using Molecular Rotational Resonance Spectroscopy**

Alexander Mikhonin, BrightSpec, Inc., 770 Harris St., Suite # 104 B, Charlottesville, VA 22903, Ann Adele Byars, Reilly Sonstrom, Voislav Blagojevic, Justin Neill

Analytical techniques capable of direct and reliable quantitation of individual components in complex mixtures are of high demand in a wide range of manufacturing industries, including pharmaceutical, agricultural, food, natural products and fragrances. Molecular rotational resonance (MRR) spectroscopy provides unique and extraordinary-selective molecular fingerprints that are matrix-independent, and precisely reflect three-dimensional structures of the corresponding molecular species. As such, MRR can unequivocally resolve the spectral signatures of multiple compounds, including isomeric and isobaric species, and quantify them within a mixture without requiring separation or purification. In this presentation, we review MRR basic principles and analytical capabilities along with the workflows for both qualitative and quantitative analyses. Application examples will include process monitoring in pharmaceutical and chemical industries, quantitative characterization of crude mixtures of stereo- and constitutional isomers, as well as direct quantitation of gas chromatography (GC)-unfriendly volatile impurities from ethylene oxide and formaldehyde to low-volatile USP<467> residual solvents. Our ongoing developments will also be discussed to highlight future MRR applications.

239 **Towards Globally Accepted Specifications of Pharmaceutical Products: A Summary of the Current State**

Kaitlin Grinias, GSK, 1250 S. Collegeville Rd., Collegeville, PA 19426

The IQ Global Specification Harmonization Working Group consists of experienced analytical chemists from across the biopharmaceutical industry with a vision to improve the consistency of the acceptance of specifications for drug substance and drug product by major Health Authorities around the globe, focusing on those who are members of ICH. The goal is to enable sponsors of new drugs to be able to get therapies to patients faster, enable a more agile commercial supply chain, and enable the lowering of costs for production and distribution which can be passed on to patients. IQ members have expressed that they feel that push-back from Health Authorities is different, and this Working Group sought to gather data to understand these differences via an IQ Consortium Survey. A summary of key findings, including specifications with push-back, rationale, and outcomes will be presented. The results may influence analytical development and design of the quality control strategy.

240 **Analysis of Extractables and Leachables in Pharmaceutical and Medical Products Using A Novel Simultaneous UHPLC-UV-CAD-HRMS Multi-Detector Platform**

Vedha Patel, SGS Health Science, 75 Passaic Ave., Fairfield, NJ 07004, Rajesh Chennam Shetti, Dujuan Lu, Danny Hower, Chongming Lui

A novel liquid chromatography (LC) coupled with UV, charged aerosol detection (CAD) and high-resolution mass spectrometry (HRMS) multidetector platform has been recently applied to analyze extractables and leachables, from a range of sample matrices, including drug products, container closure systems, process materials and medical devices. This approach is found to be highly effective in detecting, quantifying, and identifying a broad range of extractables and leachables compounds. The inclusion of CAD detection provides complementary information to UV and HRMS, enabling more accurate quantitation non-chromophoric and non-volatile compounds. The purpose of this study is to demonstrate the effectiveness of the novel UHPLC-UV-CAD-HRMS approach for the comprehensive characterization of extractables and leachables compounds.

241 **Isotopic Batch Process Understanding for Process Validation and Quality Control**

John Jasper, Molecular Isotope Technologies, LLC, 8 Old Oak Lane, Niantic, CT 06357, Anthony Sabatelli, Ann Pearson

Lessons learned from an initial study of the natural-abundance stable isotopic relationships between reactants and products illuminate the utility of such data for quality control and regulatory compliance. As a complementary study to a larger patent-infringement study, we examined the isotopic compositions of paired reactants and products for what promised to be a straightforward one-to-one relationship between reactant-product pairs. Instead, we found that (i) the isotopic data indicated that the batch records for reactant-product pairs were offset by two batches, and (ii) the isotopic reactant-product records unexpectedly showed exponential curves consistent with batch mixing, instead of sharp square-wave results indicating clear batch differentiation. Such batch mixing can affect any tracers in the batch records—e.g., organic impurities and trace metals, as well as isotopes—thereby confounding straightforward, quantitative interpretation of those batch records. These new results are presented in the context of our earlier work on product authentication and process authentication. We suggest that natural-abundance stable isotopic analysis can be used for quality control and regulatory compliance. Such analyses are relatively fast, inexpensive, and highly informative.

242 No abstract submitted by the author.

243 **Analytical Quality by Design Based Method Development for the Analysis of Cold and Cough Formulations**

Fadi L. Alkhateeb, Waters Corporation, 34 Maple St., Milford, MA 01757, Adam Bengtson, Isabelle VuTrieu, Paul Rainville

Interest in high quality analytical methods for the analysis of pharmaceutical formulations has been increasing over the last years. One approach that has particularly gained significant attention from regulatory agencies and can help achieving high standard analytical methods is the Analytical Quality by Design (AQbD). AQbD is a systematic design-based approach to understand and control the various analytical parameters in the method development process and their impact on the quality of the data in terms of accuracy and precision. While the application of this approach has been demonstrated for the analysis of active pharmaceutical ingredients (APIs), little, if any, has been done to analyze different excipients and varying levels of excipients in pharmaceutical formulations. In this presentation, we demonstrate the use of the AQbD approach for developing a liquid chromatographic analytical method for the analysis of APIs in the presence of varying concentrations and compositions of excipients. The various AQbD steps that were followed in this study will be demonstrated. The risk assessment study and the impact of the critical method parameters on the performance of the method will also be presented and discussed.

244 **Continuous Monitoring of Method and Instrument Performance Across Various Instrument Vendors and Platforms Using a Variety of USP Monographs**

Jennifers Simeone, Waters Corporation, 34 Maple St., Milford, MA 01757

In today's global pharmaceutical quality assurance (QA) / quality control (QC) environment, laboratories are tasked with running methods on a variety of liquid chromatography systems across multiple labs. While there can be many challenges associated with transferring methods across systems and/or labs, monitoring key method performance parameters can help identify both when an unexpected result occurs and may guide troubleshooting of the problem. Unexpected results can be caused by a variety of factors, including sample/solvent preparation errors, errors in manual entries, and instrument failures. This presentation highlights how continuous monitoring of method performance for multiple established methods (USP monographs) ensured robust and reproducible performance of LC systems. When a method is first tested on a particular LC system, it can be challenging to identify and troubleshoot whether a result is unexpected or within expected method and instrument variability. Having a system in place to monitor method performance can help provide an expected range of results whereby the method is in a state of control. Additionally, investigation of unexpected results are reviewed. In this work transferring methods across many different LC systems, several interesting and unexpected results were observed, requiring a deeper dive into the data. The root cause of these incidents included human errors in sample and mobile phase preparation, improper system operation, and lack of understanding of instrument design and its potential impact on analyses. Overall, monitoring performance indicators facilitated identification of errors as well as increased understanding of both methods and instrumentation and their interplay for improved outcomes.

245 Data Integrity: How does it Impact your Laboratory

Michael Barkan, Consultant, 750 Prides Crossing, Ste. 305, Newark, DE 19713

Following 21 CFR part 11 is a United States Food & Drug Administration (FDA) expectation for companies using computerized systems with the potential to impact patient safety, product quality, or data integrity. It can be a costly, time-consuming process and companies are looking for a better understanding of the regulation and its guidance documents to comply without significant risk. With the release of the April 2016 Guidance on Data Integrity there have been many new questions generated. The FDA has also been citing companies specifically on data integrity issues. We discuss guidance and possible solutions to common concerns. In addition, for non-regulated laboratories data integrity is a best practice especially in dealing with patents and internal release of products in a non-regulated arena. We cover: The new April 2016 Guidance document on data integrity and what it means for you; Identifying activities that need to be performed; GMP vs non-GMP; How does a risk assessment reduce the effort and remain compliant?; Do vendor audits to reduce the effort? Data integrity is good for business since it can potentially eliminate rework or costly mistakes in production. Data integrity helps laboratories assure that data generated is accurate for making a data driven decision in your business. This data has a responsible person generating it and in a regulated industry a person who is responsible for approving the data. We cover the data lifecycle.

246 Employing Sustainable Solvents in Chemical Separations and Purification

Jared Anderson, Iowa State University, 1605 Gilman Hall, Ames, IA 50010

Ionic liquids (ILs) and deep eutectic solvents (DESs) are two classes of sustainable solvents that can be designed to exhibit unique properties for their use in chemical separations and purification. This talk focuses on the design and synthesis of ILs and DESs, magnetic ionic liquids (MILs), and polymeric ionic liquids (PILs). Silver-containing stationary phases will be demonstrated for use in separating olefins in comprehensive two-dimensional gas chromatography (GC×GC). In the realm of bioanalytical chemistry, major challenges faced by DNA and RNA analysis techniques in the selective extraction of particular nucleic acid sequences using rapid and sensitive methodologies are discussed. An ion-tagged oligonucleotide (ITO) strategy is introduced that can be used in conjunction with MILs to efficiently capture DNA sequences from complex samples. The ITOs can be created through thio-lene "click" chemistry and the nature of the ion tag can influence the partitioning of the ITO to the hydrophobic MIL. This novel liquid-phase approach towards sequence-selective DNA capture provides superior extraction efficiencies to conventional magnetic bead technology as well as a platform for using external fields to manipulate the liquid droplets. The presentation discusses on-going efforts to use advanced IL and DES solvent design to accelerate nucleic acid sample preparation to facilitate coupling with isothermal amplification approaches, such as loop-mediated isothermal amplification and recombinase polymerase amplification. Finally, the presentation discusses ongoing efforts to exploit the versatility of 3D printing in advancing separations methodologies.

247 Addressing the Challenge of Small Molecule Separation in Complex Samples Through Sustainable and High-Throughput Microextraction Techniques

Emanuela Gionfriddo, University of Toledo, 2801 W Bancroft St., Toledo, OH 43615

The extraction of small molecules from complex samples presents a significant challenge in analytical method development, whether for targeted or non-targeted analysis. Recent trends in the development of pre-concentration strategies of small molecules prior to instrumental analysis have shifted towards greener and faster approaches, ensuring sustainability and high throughput during the extraction process. Solid phase microextraction (SPME) is an ideal method that aligns with these features, offering simultaneous extraction and enrichment of targeted analytes. We explore novel microextraction methodologies to investigate the chemical composition of environmental and biological samples and assess the partitioning of small molecules in heterogeneous systems. Our work specifically targets various classes of environmental contaminants, such as per- and polyfluoroalkyl substances (PFAS), pesticides, and pharmaceuticals. We have developed specialized extraction technologies to ensure selective extraction and preconcentration of these compounds from complex samples before subjecting them to gas or liquid chromatography and direct introduction to mass spectrometry. These methods play a critical role in evaluating pollutants' environmental mobility and their impact on living systems. Additionally, we investigate the use of biocompatible extraction phases and alternative SPME geometries to address specific analytical needs while minimizing disturbances to partition equilibria during the extraction process. These advancements hold promise for improving the accuracy and efficiency of small molecule analysis in complex sample matrices.

248 Diverse Applications of Compact Capillary LC

James Grinias, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Samuel Foster, Benjamin Libert, Sangeeta Kurre, John Boughton, Ama Hackman

The use of portable and compact instrumentation has expanded the possibilities of integrating capillary-scale liquid chromatography (LC) techniques into realms typically dominated by analytical-scale methodology. Low-volume detector flow cells and ultraviolet-light emitting diodes light sources allow for improvements in absorbance detection for columns with internal diameters in the 0.1 – 0.3 mm range. Compact single-quad mass spectrometers with integrated vacuum systems also allow for LC-MS measurements when combined with compact capillary LC platforms. Considerations for column selection (in terms of length, internal diameter, particle size, and particle morphology) include pressure limits (both column and instrument), required efficiency for a given separation, and the balance between operating flow rate and the maximum volume that can be delivered from the pumping system in a single method. We have employed compact capillary LC instrumentation in a wide variety of application areas that will be discussed. The analysis of pharmaceutical compounds has focused on quality assurance/quality control (QA/QC) methodology (including impurity monitoring) and strategies for on-line reaction monitoring. Compact LC-MS has also been used for targeted screening of illicit drug compounds towards implementation in point-of-care settings. New instrument developments for temperature control along with the ability to connect the instrument to high resolution MS instrumentation have improve the ability to analyze antibody-based biopharmaceutical drugs. The common LC methods used in these, and other application areas have the potential to be transformed through this technology that is greener and simpler to operate while still providing efficient chromatographic separations.

249 Gas Chromatographic Separations to Extract Chemical Data from Forensic Odors: Bugs, Bacteria and Bodies

Katelynn Perrault Uptmor, William & Mary, Department of Chemistry, P.O. Box 8795, Williamsburg, VA 23187

Odors are an integral part of our everyday life and provide important cues for us to navigate the world around us. However, there is also vast chemical potential of odorous molecules to provide probative value in numerous applications. Odors are comprised of volatile organic compounds (VOCs) and they are released from both living species as well as nonliving items. They act as semiochemicals that can be experienced even in the absence of physical contact between sources. Odors are also a perfect target for gas chromatographic instrumentation since they are comprised of volatile components, typically found in mixtures, and often can be easily introduced without extensive sample workup. This talk discusses sampling and analysis of forensic odors using one-dimensional gas chromatography (1D GC) and comprehensive two-dimensional gas chromatography (GC×GC) for forensic odor profiling of bed bugs, a suite of postmortem bacteria, as well as the odors from whole pig remains. These applications will be used to demonstrate the probative value that odors from these samples provide, as well as how the results from GC separations can be used to build networks of chemical profiles to inform decision making at operational levels in forensic science.

250 Redefining the Characterization Paradigm of RNA Lipid Nanoparticles

Marshall Padilla, University of Pennsylvania, 200 South 33rd St., Philadelphia, PA 19104, Sarah Shepherd, Kushol Gupta, Michael Mitchell

Lipid nanoparticles (LNPs) are potent delivery vehicles that have accelerated the translation of RNA therapeutics and led to the United States Food & Drug Administration (FDA)-approval of mRNA COVID-19 vaccines. However, one major barrier that has prevented researchers from unlocking the full potential of LNP-based therapies is the low sensitivity of current characterization techniques, which rely on bulk analysis of LNPs. These techniques cannot evaluate RNA loading distribution, particle morphology in ambient conditions, and the percentage of unloaded LNPs, which are critical features to monitor for pharmaceutical development. In this study, we combine standard characterization techniques with multi-wavelength analytical ultracentrifugation, size exclusion chromatography-small angle X-ray scattering, and size exclusion chromatography-multi-angle light scattering to: 1) compare the resolution of each technique and 2) elucidate the advantages and disadvantages for each technique for LNP analysis. As a demonstration of the enhanced resolution of these advanced characterization techniques, we will compare clinically relevant LNP formulations when prepared by either microfluidic rapid mixing or bulk mixing, which are two common methods for small-scale LNP production. With current characterization techniques, it is difficult to assess the quality of new formulation methods; however, these advanced characterization techniques were able to provide insight into how these preparation methods affects LNP structure and RNA loading. Additionally, by combining these techniques together, we demonstrate the effect of mRNA size on RNA loading distribution. These characterization techniques can enhance our understanding of LNP structure-property-function relationships and

enable researchers to precisely define their RNA LNP products, which can improve LNP quality and potentially accelerate pharmaceutical development.

251 **The Purification of Really Big (or Small ?) Things: C-CP Fiber Isolation of Exosomes from Diverse Matrices**

R. Kenneth Marcus, Clemson University, Department of Chemistry, Clemson, SC, 29634

Exosomes are 30 - 150 nm-sized vesicles which are a subset of extracellular vesicles (EVs). Exosomes are of incredible scientific and technological interest for three reasons: 1) their role in inter-cellular communication, 2) their use in clinical diagnostics, and 3) their use as vectors in gene therapy applications. This diversity in interests is mirrored, and indeed amplified, by the diversity of matrices from which they may be harvested. In terms of fundamental biochemistry, exosomes must be extracted from any number of plant, animal, and bacterial systems. In terms of diagnostics, they can be found in all forms of body fluids, including urine, saliva, plasma/serum, cerebral spinal fluid, and breast milk. Sampling from organs and tissues is also relevant. Finally, for vector production, exosomes are targeted from cell cultures, pooled body fluids, bovine milk, and plant matter. The challenges to the high throughput and high purity isolation of exosomes are incredibly complex. Typical methods are generally only applicable on small scales and require extensity processing. We describe here the use of polyester, capillary-channeled polymer (C-CP) fibers as chromatographic stationary phases for the isolation of exosomes across the diversity of matrices presented above. FIbers are currently employed in two formats, spin-down tips applicable to benchtop centrifuges and microbore liquid chromatography columns for standard HPLC system implementation. We describe a broad cross section of the results of the diverse applications addressed to date, with comparisons to traditional methods. The low-cost, C-CP fiber processing shows impressive performance in all cases studied to date.

252 No abstract submitted by the author.

253 No abstract submitted by the author.

254 No abstract submitted by the author.

255 No abstract submitted by the author.

256 **Investigation of Tautomeric Forms of Gaseous Ions by Ion Mobility**

Athula Attygalle, Stevens Institute of Technology, Department of Chemistry and Chemical Biology, Hoboken, NJ 07030

For mass spectrometric investigations, the transformation of neutral molecules into gaseous ions is a fundamental prerequisite. Once ionized, A mass spectrum is recorded by determining mass-to-charge ratios and intensities for both precursor ions and their resulting fragments. These datasets are curated into libraries and used for compound identification through Artificial Intelligence (AI) methodologies. Despite the availability of many guidelines for interpreting fragmentation spectra and identifying compounds, nearly all approaches tend to presuppose a specific chemical structure for the precursor ion. However, recent advancements in ion-mobility and other techniques have unveiled a more intricate reality. Upon ionization, an array of tautomeric ions carrying charges at distinct locations is generated, with thermodynamically less favored tautomers frequently dominating the ion ensemble. Various models have been proposed to rationalize these observations. For example, one model pertaining to electrospray ionization suggests the repositioning of protons within the final solvation shell during the desolvation process. The presence of aprotic solvents is believed to induce kinetic trapping, impeding the transition to the stable form. Yet, an extensive survey spanning diverse ionization methods and model compounds shows that the mechanisms that govern tautomer ratios are complex. Consequently, comprehending these intricacies has become a formidable challenge. A notable complexity arises from the distinctly different fragmentation spectra exhibited by individual tautomers. This implies that recorded spectra, when isomeric mixtures are not separated, are composites. Although libraries containing extensive spectral collections do exist, it is imperative to critically assess the quality and reliability of these compilations before AI methods are implemented.

257 **Ion Mobility Mass Spectrometry in Big Pharma**

Gene S. Hall, Rutgers University, 610 Taylor Rd., Piscataway, NJ 08854, Anthony Pitts-McCoy

Ion mobility mass spectrometry (IMS) has revolutionized the community of analytical chemists. However, the use of IMS in the pharmaceutical community has been slow. Challenges include instrumentation costs and the Food and Drug Administration's (FDA) strict protocols for certifying the use of IMS in the drug regulatory space. This presentation focuses on the different applications of IMS in the pharmaceutical industry, emphasizing areas in big pharma where limited uses of IMS have been employed. In addition, a review of recent but scarce applications in the peer-reviewed space is also presented.

258 **A Proteomics Approach to Examine Brain Endothelial Cell Nuclear Protein Expression Level Changes in FTLD**

Olivia Durham, UConn Health, 263 Farmington Ave, Farmington, CT 06032, Jennifer Liddle, Amy Kimble, Evan Jellison, Jeremy Balsbaugh, Patrick Murphy

As the average age of humans continues to increase, so too does the incidence of neurodegenerative disorders such as dementia. Thus, there is a need to better understand its many forms, including frontotemporal lobe dementia (FTLD). The blood brain barrier (BBB) is a critical gateway into the brain and defects in BBB function precede cognitive decline in dementia. FTLD has been genetically linked to dysfunction of RNA-binding proteins (RBPs). While prior research focus has been on neurons, our lab has uncovered RBP dysfunction within the BBB which may be an important precipitating event in disease progression. To broadly assess changes in the levels of nuclear RBPs, we have applied a proteomic approach to isolate endothelial nuclei and assess changes in their proteome in a mouse model of FTLD. The model includes a point mutation in progranulin (Grn), one of the most commonly driver mutations in FTLD. We have successfully used both data dependent acquisition (DDA) and data-independent acquisition (DIA) approaches to assess the nuclear proteome, identifying approximately 40% and 60% of all RBPs with each approach, respectively. The number of RBPs detected is highly reproducible between samples within both DDA and DIA; so far we have identified over 400 altered RBPs. These approaches are allowing us to identify RBP alterations in FTLD – a first step towards efforts to restore their function and limit BBB dysfunction in disease progression.

259 **Integrative Single-Organoid Proteomics in Three-Dimensional Models of Ovarian Cancer Reveals Remodeled Mitochondria Bioenergetics**

Krystal Lum, Princeton University, 216 Lewis Thomas Laboratory Washington Rd., Princeton, NJ 08544, Oscar Pundel, John Muroski, Benjamin Neel, Ileana Cristea

High-grade serous tubo-ovarian cancer (HGSC) is the most lethal and prevalent subtype of epithelial ovarian cancer. Despite initial responsiveness to standard of care chemotherapies, most HGSC patients experience drug resistance and cancer recurrence. Thus, there is a significant need to identify HGSC molecular dependencies that can be targeted using new treatment strategies. A major barrier has been a lack of experimental models accurately exhibiting hallmarks of human tumorigenesis in vivo. Recently, human genomics-informed three-dimensional organoid models of HGSC were developed that recapitulate key HGSC features and pathogenesis, providing an opportunity to determine how genotype-defined tumors reprogram cell signaling pathways. Here, we investigated HGSC signatures of these organoids from genetically-defined syngeneic mouse models by integrating single-organoid proteomics, targeted mass spectrometry, thermal proximity co-aggregation profiling (TPCA-MS), metabolic flux assays, and live super-resolution microscopy. Gene ontology assessment of proteome datasets indicated that organoids bearing mutational combinations of major HGSC subtypes ($Trp53^{-/-}$;$Pten^{-/-}$;$Nf1^{-/-}$ and $Trp53^{-/-}$;$Brca1^{-/-}$) are metabolically distinct, with functional enrichments in mitochondria-associated metabolism. Follow-up targeted proteomics using parallel reaction monitoring revealed abundance changes in proteins mediating organelle-organelle contacts (e.g., mitochondria and endoplasmic reticulum, ER) and energy metabolism. Using TPCA-MS, we additionally uncovered differential thermal stabilities of protein complexes that drive mitochondrial metabolism (e.g., F1F0-ATP synthase). Live metabolic flux assays and super-resolution microscopy demonstrated that HGSC genotypes also differentially exhibited mitochondria-ER encapsulations coincident with altered calcium signaling, lipid droplet clustering, and bioenergetic respiration. Collectively, findings from our study provide an explanation for how ovarian cancer exploits the metabolic capacities of remodeled organelles to promote tumorigenesis.

260 **Proteogenomic Analysis of Pediatric Acute Myeloid Leukemia Diagnosis and Relapse Pairs**

Han Fisher, The Children's Hospital of Philadelphia, CTRB 4100, 3501 Civic Center Blvd., Philadelphia, PA 19104, Tina Glisovic-Aplenc, Lusha Cao, Jen Liddle, Kevin Nestler, Asif Chinwalla, Hossein Fazelinia, Saar Gill, Yi Xing, Mingyao Li, Kathrin Bernt, Jeremy Balsbaugh, Richard Aplenc

Pediatric acute myeloid leukemia (AML) is the second most common blood cancer in children and accounts for nearly 50% of pediatric leukemia-related mortality. While approximately 60% of pediatric AML patients are cured, about half of these eventually relapse, and relapse remains the most frequent cause of death in childhood AML. Many transcriptomic studies have identified AML subgroups, leading to improved risk stratification and targeted therapeutic approaches. However, there are few published proteomic studies examining pediatric AML, one of which by Hoff et al. encompassed 500 samples taken at diagnosis from AAML1031 and used RPPA microarrays to report on 296 proteins. A mass spectrometry-based approach can provide a more extensive characterization of patient proteomes and help identify novel therapeutic targets and underlying drivers of AML relapse. Using 182 paired diagnosis/relapse samples from 91 patients on the Children's Oncology Group AAML1031

trial, we developed a proteogenomic data analysis workflow to identify therapeutic targets. Our approach allows for the quantification of differential protein abundances between diagnosis and relapse to uncover changes in proteomic profiles. This DIA-based approach substantially extends the work by Hoff et al. and identifies more than 7000 human proteins representing multiple biological pathways and potential novel immunotherapy targets. The clinical proteomics data will be analyzed in a truly patient-centric fashion by using RNA-seq-derived, disease-specific protein sequences that vary from commonly used UniProt protein sequence databases to enable more accurate protein identification and quantitation.

261 Orbitrap Analysis of Cysteine PTMs in Signaling Proteins

Hong Li, Rutgers University - New Jersey Medical School, Center for Advanced Proteomics Research, 205 S. Orange Ave, Newark, NJ 07103, Tong Liu

At Rutgers Center for Advanced Proteomics Research, we investigate redox regulation of cysteines in proteins involved in cardiovascular diseases. However, identifying redox-regulated cysteines (Cys) and their post-translational modifications (PTMs) is a challenging task. First, some proteins contain numerous neighboring Cys within the same tryptic peptides, making it hard to map the redox changes of individual Cys precisely. Second, some Cys PTMs, e.g., nitrosation, are labile and thus need to be stabilized before LC/MS/MS analysis. Third, disulfides can render proteins resistant to protease digestion and MS/MS spectra hard to interpret, thus requiring specialized sample handling and data analysis. We've developed effective redox proteomics methods by employing novel sample preparation and bioinformatics approaches. These improved methods enable precise identification of redox-regulated Cys in signaling proteins essential for regulating blood clotting and heart ischemia. In one example, we discovered a previously unknown regulatory mechanism where ERp46, a protein disulfide isomerase, influences αIIbβ3 integrin, impacting blood clotting processes. In another example, we discovered that Trx1 transnitrosylates Atg7, leading to increased autophagy, or "cellular recycling," and ultimately protecting the heart against ischemia. These new redox proteomics methods expand our understanding of protein oxidation's role in cellular processes and reveal critical redox-mediated signaling mechanisms related to cardiovascular health.

262 Correlated Micro- and Nano-Scale Analyses of Two Particles from the Near-Earth Asteroid Ryugu

Timothy Glotch, Stony Brook University, 250 Earth and Space Sciences, Stony Brook, NY 11794

Asteroid 162173 Ryugu is a C-type asteroid with spectral properties similar to carbonaceous meteorites. It is rich in volatiles, including water and organics. Near-infrared spectra of Ryugu indicate an aqueously altered chemical composition, somewhat similar to CI chondrites. Thus, they provide a unique opportunity for the in-situ investigations of organic matter and mineral species. Ryugu particles are pristine and the only currently available samples from a primitive carbon-rich and hydrated asteroid. We conducted correlated bulk micro- Fourier transform infrared (FTIR) spectroscopy, micro-Raman imaging and spectroscopy, nano-IR imaging and spectroscopy, and synchrotron microbeam X-ray fluorescence (μXRF), X-ray diffraction (μXRD), and X-ray absorption near edge spectroscopy (μXANES) of two Ryugu particles. We were allocated two particles, A0030 and C0034 by JAXA. Portions of each sample were embedded in sulfur and cut with an ultramicrotome to produce flat surfaces for imaging and spectroscopic analyses. The microtomed samples are each ~50-100 μm across. Synchrotron XRF maps show that the samples are chemically dominated by Si. Hotspots of P and S occur throughout, with S in larger discrete grains as well as distributed finer particles. μXANES measurements indicate that sulfur occurs mostly as sulfide, with ~10% occurring as sulfate. Nano-IR spectra display substantial compositional variability at sub-micron scales. The position of the main Si-O vibrational mode varies from ~1050-1075 cm^{-1}, likely indicating variable Fe-Mg composition in the matrix phyllosilicates or differences in mineralogy (e.g., saponite vs. serpentine). Spectra also display variable band strengths related to carbonate, water, aromatic (C=C) carbon, and carbonyl (C=O) groups.

263 Recent Advances in Multimodal Optical-Photothermal Infrared Imaging and Spectroscopy

Samuel Tenney, Brookhaven National Laboratory, Center for Functional Nanomaterials, Bldg. 735, P.O. Box 5000, Upton, NY 11973, Sabine Neal

Until recently, multimodal optical photothermal infrared (O-PTIR) studies have largely been limited to observations under ambient conditions. In this talk we discuss recent advances in nanoscale multimodal O-PTIR imaging and spectroscopy under ambient and beyond ambient conditions. We will present our recent development of a modified diamond anvil cell compatible with the O-PTIR technique and the implementation of an environmental cell for analysis at cryogenic and elevated temperatures under a controlled gas environment. Some of our recent findings from applying these new capabilities towards the study of energetics, MOFs, crystals, two-dimensional (2D) materials and catalysts are illustrated alongside additional data collected from atomic force microscopy (AFM)-based PTIR.

264 Visible to Mid-IR Spectromicroscopy with Top-Down Illumination and Nanoscale (≈10 nm) Resolution

Devon Jakob, National Institute of Standards and Technology, 100 Bureau Dr., Bldg. 216/B107, Gaithersburg, MD 20899, Andrea Centrone

Measurement of light-matter interactions provides access to compositional, structural, and physical properties of materials. The global push towards device miniaturization and the advent of nanotechnology demands metrological tools capable of nanoscale spatial resolution – unattainable with conventional diffraction-limited far-field spectroscopies. Atomic force microscopy (AFM)-based nanospectroscopies, such as photothermal induced resonance (PTIR), surpass the diffraction limit by employing an AFM probe to transduce local photothermal expansion resulting from light absorption by the sample. Moreover, spectral dispersions and shifts are avoided via the photothermal effect, allowing PTIR to deliver reliable chemical identifications with IR fingerprints. However, PTIR is almost exclusively limited to the mid-IR range due to the availability of commercial systems in this range. Here, we present a new implementation of PTIR in the visible and near-IR spectral ranges which employs a top-down illumination scheme. This breakthrough instrument delivers reliable spectroscopic characterizations (i.e., absorption maps and spectra) from 434nm – 11.23μm with ≈10nm spatial resolution, while removing the sample constraints and experimental complexities imposed by our previously reported bottom-up illumination scheme. Moreover, our technique enables light polarization dependent PTIR experiments for the first time, allowing for investigations of crystalline orientation and anisotropy. We present our measurements on electronic and vibrational features of an organic dye phase separated within a poly(methyl methacrylate) film as a proof-of-concept. We envision that the broad spectral range, suburb spatial resolution, relaxed sample preparation requirements, and the ability to control light polarization will foster a broad impact in applications ranging from materials science to optoelectronics, among others.

265

No abstract submitted by the author.

266 Development of Alternate QC Techniques for More Rapid Screenings within LMIC Contexts

David Jenkins, FHI 360, Product Quality and Compliance Department, Durham, NC 27713, Matthew Eady, Ed Bethea, Chris Harmon, Jonelle Caison, Melissa Growney

Monitoring product quality is important to assure the integrity of products provided in global public health programs. Traditional pharmacopoeia approaches, often based on high performance liquid chromatography (HPLC) or ultraviolet-visible (UV-Vis) techniques, are critical tools required to assess pharmaceutical quality. However, in limited resource settings, public health programs need to optimize resources, and traditional approaches can be expensive (i.e., reagents, equipment, staff time). More rapid screening approaches, while coupled with traditional methods, are important to incorporate into quality assurance programs. Various techniques are currently being explored for assessing important public health commodities, namely spectroscopy-based approaches involving benchtop diffuse reflectance (350-2500 nm), handheld NIR (900-1700nm), and portable Raman (1064nm laser). Development activities are in process for a variety of discriminant (qualitative) and quantitative assessments. Qualitative brand discrimination for medroxyprogesterone acetate (MPA) injectables has been demonstrated with handheld near infrared (NIR) (relative to benchtop DRS), with potential applications in screening for new candidate products for supply chains. Brand discrimination for isoniazid tablets has been assessed, with approaches for building discriminant methods with multiple handheld NIRs using data scanned from different countries. Quantitative MPA and isoniazid methods are also under development with all three instruments. In comparison to benchtop DRS, handheld NIR is showing potential for oral contraceptive brand discrimination, including the detection of exposure to adverse environmental conditions. Qualitative and quantitative methods for insecticide treated nets for malaria are under development. Various spectroscopic approaches are showing potential to assess important public health commodities, where the portable nature of many of these instruments will improve use in low resource settings.

267 Analysis of FDA-Regulated Products for the Presence of Active Pharmaceutical Ingredients Using Surface Enhanced Raman Spectroscopy

Michael Thatcher, United States Food and Drug Administration, Forensic Chemistry Center, 6751 Steger Dr., Cincinnati, OH 45237, Adam Lanzarotta, Martin Kimani, Lisa Lorenz, Megan Sterling, Sara Kern, JaCinta Batson

Since its inception in 1990, the United States Food and Drug Administration's (FDA) Forensic Chemistry Center (FCC) has examined a wide variety of dietary supplements, suspect drug products and unknowns for the presence of active pharmaceutical ingredients (APIs). When the president declared the opioid epidemic as a public health emergency in 2017, the FCC responded by evaluating several portable instruments for their suitability to detect APIs in FDA-regulated products collected at international mail facilities (IMFs). Current FDA IMF satellite laboratories employ a toolkit consisting of a direct analysis in real-time ambient ionization source coupled

to a mass spectrometer (DART-MS), Fourier transform infrared (FT-IR) spectrometer and a handheld Raman spectrometer. While the handheld Raman spectrometer is excellent for detecting APIs in high-concentration formulations, sensitivity is a fundamental limitation of this technique when employed under normal operating conditions. However, sensitivity can be significantly improved using surface-enhanced Raman spectroscopy (SERS). FCC has historically utilized SERS for the analyses of numerous analytes of interest, including phosphodiesterase type-5 inhibitors (PDE-5) like sildenafil and tadalafil in tablets/capsules, lidocaine in injectables, mitragynine (Kratom) in ground plant leaves/dietary supplements, and fentanyl in suspect counterfeit tablets. Recently, SERS methods have been developed for detecting APIs in newly encountered drug products such as designer benzodiazepines and nitazenes in tablets and PDE-5 in various matrices such as honey. This presentation describes FCC's recent SERS developments and future research proposals based on recently encountered market trends.

268 The Introduction of Raman Technology into Existing Law Enforcement Strategies to Degrade the Flow of Precursor Chemicals in Myanmar for the Production of Narcotics

Michael Brown, 30 Upton Dr., Ste. 2, Wilmington, MA 01887

Rigaku, working in conjunction with a United States law enforcement agency partnered in developing a counter-narcotics, technology-based interdiction training pilot program to assist Myanmar's primary counter-drug law enforcement agency. Rigaku's focus was to enhance the operational capability of counter narcotics unit to effectively use interdiction technology to identify and degrade the illicit precursor chemical supply chain (PCSC) networks used by transnational organized criminal (TOC) groups and their proxies operating in Myanmar to mass produce narcotics with a specific focus on methamphetamine. The TOC actors operating in Myanmar and the surrounding region work in concert with PCSC criminal groups based in Asia to coordinate cross-border smuggling of multi-ton quantities of precursor chemicals required to produce methamphetamine and heroin. Myanmar's primary narcotics threat is directly linked to the explosive importation of illicit precursor chemicals required for methamphetamine production. Rigaku, supported the interdiction training strategy to target and degrade PCSC networks operating in Myanmar's eastern Shan State.

269 Street Chemistry: How Optical Spectrometry (FT-IR and Raman) are Used to Solve Crime

Pakorn Patimetha, New Jersey State Police, 1001 Fire Academy Dr., Sayreville, NJ 08872

With the advancement in modern technologies, the size of traditional table top analytical instruments such as infrared and Raman spectroscopy have decrease to a handheld portable device. These instruments are relatively affordable and can easily be used by most responders. The capability of these field instruments to rapidly and accurately identify unknown chemicals in the field is invaluable to the Hazardous Materials and Bomb Units. Despite these technologies being considered presumptive in nature, they are essential in the hazards assessment and evidence categorization for first responders. These instruments allow first responders to safely identify precursor chemicals use to synthesize narcotic substances, homemade explosive or chemical warfare agents. We explore the roles of these handheld instruments in modern criminal investigations.

270 Method Migration of Amino Acid Analysis Across Multiple Instruments to Quantify Amino Acid Content in Commercially Available Supplements

Kimberly Martin, Waters Corporation, 34 Maple St., Milford, MA 01757, Paula Hong, Jennifer Simeone

With the increase popularity of energy supplements, there is a need to monitor amino acid content in these products to ensure product safety. While the medicinal properties of amino acids in supplements is unknown, the label claim of these supplements is assumed to be accurate. To quantify amino acids in these supplements, pre-column derivatization methods are often of value to minimize the interference of other components present in the formulation that may impact quantitation. In addition, scaling of the method or adapting existing methods, column temperature and injection volume modifications may be necessary to achieve optimal separation and resolution. Whether instruments are discontinued or when instruments are updated within a lab to newer systems, the need for migration of methods is crucial. Several instruments were used to demonstrate method migration of the amino acid analysis method. To facilitate method development, existing methodologies were scaled and optimized to create a robust method for the analysis of a wide range of amino acids. Results were compared to the label claim of the products, including three powdered drink mix supplements and one amino acid supplement tablet. Any interfering peaks were identified through mass spectrometry (MS) and adjustments were made to allow for quantitation of all amino acids in the supplements. The results demonstrate the ability to quantify amino acid content in numerous energy supplements.

271 Development of a Rapid LC Method for the Determination of 3-Chloropropionic Acid and 3-Chloropropionyl Chloride Using EDC Derivatization

Yuan Ren, Bristol Myers Squibb, 556 Morris Ave., Summit, NJ 07901, John Orlet, Qian Zhang, James Chadwick, Robert Menger, Yan Zha

Acyl chlorides, including 3-chloropropionyl chloride (3-CPC), are routinely utilized in Schotten Baumann reactions to form amide-containing active pharmaceutical ingredients (API) and intermediates. Although 3-CPC is advantageous in chemical syntheses, both 3-CPC and its process impurity, 3- chloropropionic acid (3-CPA), are potential mutagenic impurities that require strict control to ppm levels in API to mitigate the potential risk of DNA damage. Furthermore, direct detection of 3-CPC/3-CPA using ultraviolet (UV) and liquid chromatography mass spectrometry (LC-MS) techniques proves to be challenging due to their poor sensitivity and solution stability. The work in this presentation will summarize two approaches to overcome these limitations. First, an LC-MS derivatization method was developed involving the reaction of 3-CPA with 2-mercaptopyridine. This fit-for-purpose derivatization method allowed for more sensitive detection of 3-CPA using mass spectrometry and enabled the rapid collection of process knowledge on 3-CPA levels in drug intermediates. However, due to long derivatization time (>4 hours) and high variability, a new derivatization method was developed by utilizing 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) chemistry, which enables the coupling of 2-Nitrophenylhydrazine hydrochloride (2-NPH·HCl) to yield amide bonds with 3-CPA/3-CPC. The optimized QC-friendly ultra-high-pressure liquid chromatography (UPLC) method enabled the derivatization to be completed within 2 hours at room temperature and converted both 3-CPA and 3-CPC into the same product while eliminating the necessity of using mass spectrometry detection. The final method offers a rapid, sensitive, and reliable method for the determination of 3-CPA/3-CPC and potentially other mutagenic impurities with similar functional groups, which is critical for ensuring the quality and safety of the drug substance.

272 The Development and Use of a Virtual Liquid Chromatography Method Development Tool

Melinda Urich, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823, Justin Steimling, Jamie York, Chris Nelson, Tim Yosca, John Garrett

The development and optimization of liquid chromatography (LC) separations can be time consuming and costly, often requiring numerous steps including literature research, column scouting, development, and optimization. To eliminate these steps, an instrument-free, software modeling tool was developed, giving users the ability to select compounds from a database and instantly model separations on different column phases. A modeled chromatogram and instrument-ready conditions are automatically generated and can be further optimized by users. Optimization of the model can be performed while maintaining critical pair separations by adjusting for instrument/system effects, mobile phase, gradient steps, and more. The initial database consisted of a Drugs of Abuse (DoA) library containing approximately 250 compounds with plans to continuously expand the utility. During the development of the software, acceptance criteria for retention time agreement between experimental and modeled values was set at +/- 15 seconds, the time of a typical multiple reaction monitoring (MRM) window. In the most complex portion of verification, 704 retention time data points were collected for the 25 compounds used throughout evaluation. Only 13 data points exceeded the +/- 15 second window, giving an overall pass rate of 98.2%. This free tool delivers a fast, no-cost starting point for method development and optimizations which enables LC method developers, novice and expert, lacking expertise, or time, to develop separations quickly and accurately. The novel software can improve turnaround time, increase throughput to existing methods, and offer an on-demand consultative user experience.

273 Quantification and Characterization of Intact Polysorbate 80, its Degradants, and its Subspecies in Biopharmaceuticals

Katie Carnes, GSK, 1250 Collegeville Road Collegeville, PA 19426, Justin Shearer, Lee Oliver, Sina Mortazavi, Timothy Brown, Mike Morris, Michelle Ward, Josh Fuller, Roberto Delgadillo

Polysorbate 80 (PS80) is the most common surfactant for stabilization of monoclonal antibody(mAb) formulations. Historically, robust analytical methods for PS80 have been challenging for several reasons. Here, we describe a platform analytical method using high-performance liquid chromatography employing a charged aerosol detector (HPLC-CAD), that quantifies intact PS80, PS80 degradants and related subspecies. The method is distinguishable from other published methods because the quantification of PS80 mono-esters is free from interference from known PS80 degradants. The method has demonstrated acceptable accuracy, precision, specificity, and sensitivity through qualification studies in both stable and degraded samples. The specificity afforded by the method allows for employment in forced degradation studies to discern kinetic information. Lastly, the method also has promising application to other polysorbates (PS60, PS40 and PS20) and shows acceptable performance with several types of biopharmaceutical proteins.

274 Deeper Understanding of the Mechanism of Water Dewetting from Hydrophobic Mesoporous Silica Particles to Improve the Design of Stationary Phases in Reversed-Phase Liquid Chromatography

Fabrice Gritti, Waters Corporation, 34 Maple St., Milford, MA 01757

A common problem in separation science by liquid chromatography (LC) is the loss of analyte retention when reversed-phase (RP) stationary phases (ex: silica-C18 particles) and fully aqueous mobile phases are used after the flow rate is stopped and resumed. Improved and novel stationary phases are then needed to cope with this serious user problem in the pharmaceutical industry (proteomics and metabolomics). In this presentation, the fundamentals of water dewetting from the surface of hydrophobic mesoporous particles (50 to 300 Angstrom average pore size) are provided including the kinetics of microbubble nucleation, their growth, and the rate at which they coalescence before they fill the entire pore volume. A chromatographic protocol is designed to measure accurately the rate at which liquid water is expelled from the mesoporous volume as a function of various experimental chromatographic parameters: they include the average mesopore size of the particle, the ionic strength of water used as the mobile phase, the presence of dissolved gases in water, the temperature, the internal tortuosity of the mesoporous path in the particle, the surface coverage of the hydrophobic ligand, and the chemistry of the hydrophobic surface. A second protocol is designed to determine the water porograms (intrusion/extrusion of water as a function of applied pressure) and determine the advancing and receding contact angles of water against the mesopore walls. Finally, it is illustrated in practice how these fundamental and experimental information on water dewetting can be utilized to properly design a novel surface chemistry in RPLC.

275 Separation of Guide RNA for CRISPR: Methods, Mechanisms and Applications

Bingchuan Wei, Genentech, 1 DNA Way, South San Francisco, CA 94080, Jenny Wang, Bifan Chen, Lulu Dai, Lance Cadang, Kelly Zhang

Guide RNA (gRNA) is a crucial component in the CRISPR/Cas9 genome editing system, providing target specificity. However, the manufacturing process of gRNAs through solid-phase synthesis often results in the presence of process-related impurities, which may raise concerns regarding specificity, efficacy, and safety for clinical applications. The large size, complex impurity profile, and secondary structure of gRNA pose challenges in its analysis. To address this, we developed liquid chromatography-based platform methods, including ion-pairing reverse-phase liquid chromatography and hydrophilic interaction liquid chromatography (HILIC), for the analysis of gRNA. Additionally, we delved into the separation mechanism, impurity profile, and mass spectrometry coupling of IP-RPLC and HILIC for gRNA analysis. Our research expands the application of liquid chromatography to the analysis of large synthetic oligonucleotides and provides valuable insights into the separation mechanisms of gRNAs.

276 Changing the Conversation for Biological Materials in Cultural Heritage: Integrating Multiple Disciplines with Multi-Faceted Scientific Approaches

Julie Arslangolu, The Metropolitan Museum of Art, 1000 Fifth Ave., New York, NY 10028

Unlocking the important information about trade, location of manufacture, and cultivation held by the biological materials used to create our cultural heritage can be challenging. Continued advances in proteomics, glycomics, metabolomics, or genomics increase our range of materials identification to be more inclusive of Latin American, African, and other understudied cultural heritage. Furthermore, targeted interrogation through proteomics can reveal molecular markers for manufacturing processes availing a wealth of information to historians, conservators, and curators. But how do we match what is of interest and value to conservators and historians to the potential of our scientific methods? And how do we maximize the impact of our work on the cultural heritage community? This presentation discusses the formation of partnerships to increase the availability of quality omics techniques such as ARCHE (https://arche.cnrs.fr/) between the University of Bordeaux and the Metropolitan Museum of Art (https://www.metmuseum.org/about-the-met/conservation-and-scientific-research/scientific-research/arche), and the need for groups that encourage cross-disciplinary discussion and debate such as Art Bio Matters (https://www.artbiomatters.org/).

277 The Current Understanding of the Organic Compositions of Chinese Export Lacquer Finishes

Catherine Matsen, Winterthur Museum, 5105 Kennett Pike, Winterthur, DE 19735

Acknowledgment of the role of Cantonese lacquerware in the economic and cultural relations between European countries, the United States, and China places this form of material culture as a primary heritage to be preserved yet, until recently, the field of black and gold Chinese export lacquerware was relatively unexplored especially with regard to their construction and materials composition. Winterthur Museum's Scientific Research and Analysis Laboratory conducted materials analysis of forty wooden objects coated with gold-decorated black lacquer whose manufacture was attributed to the area of Canton (Guangzhou), and mainly dating to the 18th and 19th centuries, using pyrolysis-gas chromatography–mass spectrometry with thermally assisted hydrolysis and methylation using tetramethyl ammonium hydroxide (THM-py-GC/MS). Interpretation of approximately 250 complex pyrograms from ground and lacquer layers was greatly aided by the Getty Conservation Institute's ESCAPE (Expert System for Characterization with AMDIS Plus Excel) protocol. Most significantly, the results indicated that Toxicodendron succedaneum was used as the lacquer source for 17th- and 18th-century export objects, but that both Toxicodendron succedaneum and Toxicodendron vernicifluum were the lacquer sources for 19th-century objects. The use of thitsi or Gluta usitata is reported for the first time in two pieces of Cantonese lacquerware. This study provided a greater understanding of the evolution of Cantonese export lacquer, the complex formulations of the finishes, and can be used to better preserve these cultural artifacts.

278 For the Culture: Collective Scientific Studies of Colonial-Era Art of the Spanish Americas

Alicia McGeachy, The Metropolitan Museum of Art, 1000 5th Ave., New York, NY 10028, Marc Vermeulen, Elena Basso, Annette Ortiz Miranda, Federica Pozzi, Diego Tamburini, Marco Leona, Marc Walton

The Center for Scientific Studies in the Arts (CSSA) at Northwestern University and the Scientific Research Partnerships (SRP) initiative at The Metropolitan Museum of Art represent increasingly valuable models for addressing questions at the interface of art history and material understanding. These diffuse research hubs provide access to scientific staff and analytical resources, free-of-charge, thereby removing the financial barrier that might otherwise prohibit science-based inquiries necessary to inform conservation and historical research and address culturally- and socially-significant questions. Through this presentation of a thematic collection of projects centered around colonial-era Puerto Rican, Mexican, Bolivian, and Colombian artists, we explore the ways that material clues can inform our understanding of the impact of global influence and the persistence of local practices. Undertaken in partnership with the National Museum of Mexican Art, El Museo de Arte de Puerto Rico, The Caryl & Marilynn Thoma Foundation, and The Hispanic Society of America, this presentation illustrates how our own global and local relationships have been critical in opening new doors to explore the evolution, transference, and endurance of artistic practices across the Americas and the Caribbean.

279 Exploring Yale's Collection: XRF Scanning at Scale

Marcie Wiggins, Yale Institute for the Preservation of Cultural Heritage, 300 Heffernan Dr., West Haven, CT 06516, Richard Hark, Aniko Bezur

The Yale Institute for the Preservation of Cultural Heritage (IPCH) employs scanning X-ray fluorescence spectroscopy (XRF) to study cultural objects housed in the Beinecke Rare Book and Manuscript Library, the Yale Center for British Art, the Yale Peabody Museum of Natural History, and the Yale University Art Gallery. This presentation aims to explore the concept and practical implementation of a "migratory" XRF instrument, catering to the diverse collections at Yale. Through two compelling case studies, this talk will showcase how the instrument has been instrumental in studying cultural heritage objects on both small and large scales while fostering cross-collection collaborations. The first case study centers around two stone reliefs from King Assurnasirpal II's Northwest Palace, where the XRF analysis provides insight into the original appearance of ancient decorations by studying both the overall structure and the particles of residual pigments. The second case study focuses on Jain medieval illuminated manuscripts. IPCH collaborates with multiple institutions in the United States and the United Kingdomw, each possessing distinct analysis capabilities. This presentation underlines the significance of the XRF instrument, showcasing its versatility and ability to explore Yale's collections and beyond.

280 Fragmentation Pathways of Monoclonal Antibodies Induced by Visible Light

Christian Schoneich, University of Kansas, Department of Pharmaceutical Chemistry, 2093 Constant Ave., Lawrence, KS 66047

There is increasing evidence that visible light exposure leads to the chemical degradation of therapeutic proteins. Mechanistically these observations are difficult to explain based on the known absorption characteristics of individual amino acids. Searching for potential rationales for the susceptibility of proteins to visible light degradation, we evaluated the role of excipients and impurities and discovered that ferric iron may be critical. Especially in combination with histidine buffer, mixed ligand complexes of ferric iron conferred a pronounced sensitivity of monoclonal antibodies to site-specific fragmentation, characterized by high-performance liquid chromatography mass spectrometry (HPLC-MS/MS) analysis and molecular dynamics (MD) calculations. The underlying mechanisms involve light-induced ligand-to-metal-charge transfer (LMCT), generating an alkoxyl radical at Thr259, which subsequently undergoes beta-fragmentation, leading to an intermediary glycyl radical. The latter either abstracts a hydrogen atom from a suitable donor, confirmed by deuterium incorporation when experiments were carried out in deuterium oxide, or adds oxygen, followed by classic peroxyl radical chemistry, leading to backbone fragmentation. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis

reveals the formation of a ca. 22 kDa fragment, which should not be mistaken for a light chain; in-gel digestion of this 22 kDa fragment confirms the chemical cleavage pathways outlined above. The addition of chelators such as EDTA (ethylene diamine tetraacetic acid) or DTPA (diethylene triamine pentaacetic acid) can prevent the site-specific cleavage processes, but only at relatively high chelator concentrations, indicating a rather high affinity of the protein for ferric iron. The latter was confirmed by MD experiments.

281 Nucleic Acid Lipid Nanoparticles: Development and Process Optimization

Jeffrey Smith, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486, Lauren Austin, Stephen Spagnol, Andrew Latham, Marian Gindy

Lipid nanoparticles (LNPs) are currently a leading delivery platform for a range of oligonucleotides used in both therapeutics and vaccines including the delivery of small interfering RNA (siRNA) and messenger RNA (mRNA). Optimization of LNPs as therapeutic products is enabled by the development of structure-activity relationships linking LNP physicochemical and macromolecular properties to bioperformance. During LNP development it is critical to control the oligonucleotide-LNP drug product assembly process to ensure reproducibility and to enable the correlation of drug product properties with efficacy and/or toxicity. Methods by which product properties can be rationally manipulated are thus critical enablers of this fundamental knowledge build. The LNPs presented herein are composed of ionizable amino lipids, neutral lipids and poly(ethylene glycol) (PEG) lipids with each component contributing to specific physiochemical properties and, therefore, bioperformance with the ionizable amino lipids being particularly impactful. Building an understanding of how product physicochemical and macromolecular properties are linked to *in-vivo* performance is necessary to develop an effective and safe LNP.Developing strong analytical and pharmacological understanding is accomplished through well controlled experimentation in order to vary particle attributes. Analytical characterization of the oligonucleotide-LNP drug product offers unique challenges and techniques which are not typically encountered in more traditional drug development. Less common techniques include ultra-high-performance liquid chromatography (UHPLC) with charged aerosol detection (CAD), ion exchange chromatography (IEX), encapsulation efficiency (EE), small angle x-ray scattering (SAXS), and cryo transmission electron microscopy (Cryo-TEM). These techniques are used for high throughput analysis for in process testing and screening as well as to monitor stability of the drug product.

282 Understanding Protein Interactions Under Hydrodynamic Stress with Multiphysics Simulation

Tonglei Li, Purdue University, Department of Industrial & Physical Pharmacy, College of Pharmacy, 575 Stadium Mall Dr., West Lafayette, IN 47907

Self-injectable protein solution has become a major delivery formulation of protein drugs due to its patient compliance and a significant reduction in healthcare costs. Maintaining the physicochemical stability of protein molecules in such formulation faces various challenges and requires a fundamental understanding of both formulation and device design, as well as local transport and absorption at the injection site. During the auto-injection process, as well as other handling and processing steps, the high-concentration protein solution experiences significant mechanical and hydrodynamic stresses, which pose potential damages to protein molecules resulting in structural denaturation and molecular aggregation. To uncover mechanistic causes of structural change of protein molecules in solution, a multiscale simulation scheme was developed by integrating our current two-phase continuum model with a coupled continuum-discrete framework. Kinetic energy obtained at the mesoscale simulation was further integrated into the atomistic simulation of molecular dynamics. Doing so allows us to study the hydrodynamic impact on individual protein molecules, including possible structural unfolding and enhanced protein-protein interactions (aggregation). The results shed light on protein formulation stability during formulation and manufacturing processes.

283 Direct Assessment of Oligomerization of Chemically Modified Peptides and Proteins in Formulations Using DLS and DOSY-NMR

Kang Chen, US Food and Drug Administration, 10903 New Hampshire Ave., WO22-1473, Silver Spring, MD 20993

Protein higher order structure (HOS) including the oligomer distribution can be critical for efficacy, safety and stability of drug products (DP). Oligomerization is particularly relevant to chemically modified protein therapeutics that have an extended pharmacokinetics profile. Therefore, the direct assessment of protein oligomerization in drug formulation is desired for quality assurance and control. Here, two non-invasive methods, dynamic light scattering (DLS) and diffusion ordered spectroscopy (DOSY) NMR, were applied to measure translational diffusion coefficients (Ddls and Dnmr) of proteins in formulated drug products. The hydrodynamic molecular weights (MWhd), similar to hydrodynamic size, of protein therapeutics were derived based on a log(Ddls) vs log(MWhd) correlation model established using protein standards. An exponent value of -0.40 ± 0.01 was established for DLS measured log(D) vs. log(MWhd) using protein standards and a theoretical exponent value of -0.6 was used for unstructured polyethylene glycol (PEG) chains. The analysis of DLS derived MWhd of the primary species showed the fatty acid linked glucagon-like peptide 1 (GLP-1) was in different oligomer states, but the fatty acid linked insulin and PEG linked proteins were in monomer states. Nevertheless, equilibrium and exchange between oligomers in formulations were universal and clearly evidenced from DOSY-NMR for all drugs except peginterferon alfa-2a. The correlation models of log(D) vs. log(MWhd) could be a quick and efficient way to predict MWhd of protein, which directly informs on the state of protein folding and oligomerization in formulation.

284

No abstract submitted by the author.

285 Development and Validation of Stability Indicating RP-HPLC Method for Estimation of Aminocaproic Acid in Tablet Dosage Form

Vrunda Patel, Conestoga College, 274 Colborne St., Brantford, ON N3T 2H5, Canada

A stability indicating reverse-phase high-pressure liquid chromatography (RP-HPLC) method was developed and validated for estimation of Aminocaproic Acid in tablet dosage form. The chromatographic separation was achieved on InertsilC8-3 (250 mm × 4.6 mm, 5µm) column using mobile phase Phosphate Buffer:Methanol (65:35 v/v) adjust buffer pH 2.2 with orthophosphoric acid solution at a flow rate of 0.7 mL/minute. The detection was carried out at 210 nm. The developed method was validated for system suitability, precision, linearity, accuracy, specificity, robustness as per ICH guidelines. Linearity of the proposed method was found in the concentration range of 250 µg/mL to 750 µg/mL with regression coefficient of 0.9990. The recovery was found to be 99.40 ± 0.75. LOD was found to be 5.83 µg/mL and LOQ was found to be 17.66 µg/mL. Stability studies were conducted by adopting the proposed method to assess the stability of standard and sample solution under acid, alkaline, peroxide, thermal and photolytic condition. From the experimental studies it can be concluded that proposed method can be applied as stability indicating for estimation of Aminocaproic acid in pharmaceutical dosage form without interference of excipients and degradation products.

286 Efficient HPLC Column and Mobile Phase Screening Protocol for Developing Stability-Indicating Methods: Diphenhydramine Case Study

Justin Mercado, FreeThink Technologies, 35 NE Industrial Rd., Branford, CT 06405, Josh Dalo, Chris Wood, Linnea Budge

Stability indicating HPLC methods must adequately quantify drug-related impurities and are required to establish small-molecule drug stability. Methods must demonstrate that all degradation products are observed (mass balance, peak purity) and have adequate resolution from each other and the main band. Current method development practices are often laborious, requiring the setting of multiple instrument parameters (e.g. column type, mobile phase, gradient) tailored toward the particular API. This study enlists automated systems and algorithms to improve the efficiency of stability indicating method development. Diphenhydramine (DPH) was used as a test case to assess the efficacy and accuracy of this screening protocol in developing an appropriate stability indicating method. DPH was stressed with only a single selected forced degradation condition based on its chemical structure, with the idea that any method developed would be assessed against other forced degradation conditions subsequently. Utilizing compatible column and solvent switching modules, the HPLC system screened six columns with four mobile phases, for a total of 24 combinations. Automated quantitative analytical filtering of these combinations eliminated twelve processes. Qualitative chromatographic factors (elution profiles and baseline quality) reduced the number of suitable methods to four. Of these four, the method with the lowest back pressure was advanced for further optimization of the method. The screening protocol was able to successfully identify a suitable stability indicating method for DPH within 72 hours (__ hours of active work).

287 Systematic Protocol Utilizing High Performance Surface Technology for the Improved Separation and Quantification of Synthetic Peptides and Associated Impurities.

Adam Bengtson, Waters Corp., 34 Maple St., Milford, MA 01747, Paul Rainville, Stephanie Harden

Antibiotics have saved millions of lives since they were discovered, but over prescription of these compounds has led to resistant strains of bacteria becoming more prevalent. In these cases, specialized peptide antibiotics have been shown to be successful in combatting specific and resistant strains. Due to this, macrocyclic antibiotics usage has increased in recent years. Given the future uncertainty that antibiotic resistant bacteria bring to our world, the availability of streamlined workflows to develop new chromatographic methods for the analysis of antibiotic synthetic peptides is important. Recently a generalized peptide workflow has been created for the rapid development of peptide separations. The workflow has thoroughly assessed the risks associated with peptide analysis; basing method development around the high-risk variables identified can help reduce time and cost of developing a quality method. In the work shown here, a single method for the analysis of oritavancin,

dalbavancin, caspofungin, daptomycin, and anidulafungin is achieved. The same method development workflow was used to develop a method capable of separating dalbavancin and its associated impurities A0, A1, B0, B1, B2. The utilization of the Arc™ Premier System incorporating MaxPeak™ High Performance Surface Technology facilitated a 5% increase in the peak area and height of dalbavancin when compared to stainless steel hardware, allowing for a lower limit of detection and quantitation.

288 Understanding Excipient-Induced Solution Instability to Enhance Drug Product Development

Margaret Brunell, Merck and Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065

Tablet formulation development of Compound A focused on excipient and film coating selection to identify optimal formulation composition for clinical trials. During prototype stability studies, film coated tablets exhibited high levels of degradation compared to tablets without film coating. To gain a fundamental understanding of solution stability, Compound A was mixed with individual excipients, including hydroxypropyl cellulose, magnesium stearate, and Opadry 266K (a commercial film coating system), as well as the individual components of the Opadry. Degradation growth was monitored at room temperature over time in solution state for each of the mixtures. Several components of the film coating system accelerated degradation of Compound A in solution during sample preparation for HPLC analysis. The degradation was successfully suppressed by modification of the diluent pH, enabling robust and reliable results across a series of prototype formulations.

289 Plasticizers in Plants - A Study on the Absorption of Plasticizers in Various Crops

Victoria Sabatelli, Lakeland Regional High School, 205 Conklintown Rd., Wanaque, NJ 07465, Elisa Uzeiri

Plastics are increasingly becoming one of the most difficult challenges that the modern world is facing. One of the most significant intakes of plasticizers and phthalates in our lives is food consumption. PVC and plastic irrigation systems play major roles in contaminating our foods and crops with phthalates and plasticizers. Plasticizer consumption has been reported to interfere with hormonal systems in the body. The objective is to measure the amount of plastics that are being absorbed from the water and the soil, ergo being ingested by consumers. To set up our experiment we construct a greenhouse in which we can grow our plants. Separated between control and experimental, we start to grow these varieties of crops. Once the germination period is over it is time to spike these plants with our plasticizer, DEHP. Twice a week the experimental plants are watered with 0.1 gram of DEHP at the root of each plant. After fully grown we use a Solid Phase Extraction Method, in which we separate each plant between the roots, stem, leaves and dirt to dehydrate. Once fully dehydrated, we dilute with acetone and out through the sonicator and centrifuge. This cycle is repeated 3 times, then the samples are run through a micro filter and put into GCMS and HPLC vials. Once the run is finished, analyze the samples from the chromatogram and write down results, findings, and conclusions.

290 Comprehensive Profiles of Fragrance, Beverage, and Building Products - A Comparison of Techniques

Megan Harper, GERSTEL, Inc., 701 Digital Dr., Ste J, Linthicum, MD 21090, Nicole Kfoury, Jackie Whitecavage, Fred Foster

This research study delves into a comprehensive examination of various extraction and introduction techniques employed for three distinct sample types: fragrance, beverage, and building products. The goal of this study is to identify, compare, and evaluate the most effective technique across each sample type, aiming to enhance workflow and user experience by minimizing sample preparation time. Overall, more sensitive techniques such as dynamic headspace and thin film solid phase microextraction (TF-SPME) devices cover the broadest range of extracted volatiles and semi-volatiles. However, results vary based on sample characteristics such as concentration, analyte composition, and matrix effects.

291 Rapid, Efficient and High-Throughput Extraction Method of PFAS from Soil

Alicia Stell, CEM Corporation, 3100 Smith Farm Rd., Matthews, NC 28106, Benedict Liu

There is increasing concern of per- and polyfluoroalkyl substances (PFAS) in our environment as a whole, due to their persistent nature. More and more regulation regarding PFAS is being implemented. Having a harmonized method to accurately determine the PFAS content in soil, as well as other solid matrices, is important to this industry. Further, the number of samples and sample types that laboratories are analyzing is rapidly growing. The extraction of PFAS can be challenging given the susceptibly to contamination and the low levels in which these compounds are present. Existing techniques are predominately manual methods that are not rapid, simple, and efficient. In this study, a high-throughput extraction system, that uses microwave technology is explored as an alternative to traditional PFAS sample preparation techniques. This method offers efficient extraction of PFAS from solid matrices in a batch format in under an hour. Acceptable extraction recovery and reproducibility of PFAS soil Certified Reference Material (CRM) is presented. This batch microwave system method offers a rapid, simple, and efficient solution for PFAS testing that can easily be integrated into any laboratories' workflow.

292 Use of a Derivatization Tag for Signal Enhancement of Organic Acids in Supercritical Fluid Chromatography-Mass Spectrometry

John Boughton, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Yih Ling Saw, Faith Wroniuk, Mahmoud Mostafa, Peter Pellegrinelli, Samantha Calvez, Alexander Kaplitz, Lark Perez, James Edwards, James Grinias

The quantitative analysis of polar metabolites, especially in complex biological mixtures, has proven to be challenging. One particular class, polar organic acids containing carboxylic acid groups, is especially difficult due to poor separation resolutions with several modes of chromatography and low MS detection sensitivity due to the use of negative ionization mode. To improve current methods for the analysis of polar organic acids, this study has focused on the utilization of a high-proton affinity labeling of carboxylic acid functional groups to enhance their detected signal. The N-(4-aminophenyl)piperidine tag contributes a tertiary amine, allowing for positive ionization mode for MS detection. The signal enhancement of these organic acids post-tagging was achieved and confirmed using SFC-MS, and by forming calibration curves to determine the sensitivity, LOD, and LOQ for each set of native and tagged organic acids. Post-derivatization detection limits were down to 0.5 ppb, with at least a 200-fold increase in sensitivity compared to the native compounds.

293 Quantitatively Determining Isoniazid in Tablets with a Handheld NIR Spectrometer

Matthew Eady, FHI360, 2810 Meridian Pkwy, Ste. #160 Durham, NC 27713, Melissa Growney, David Jenkins

Isoniazid is an essential medicine used in the treatment of tuberculosis and supplied through global public health initiatives. Confirming quality compliance of isoniazid in supply chains is a paramount concern for assuring medicines are safe and efficacious. Handheld diffuse reflectance spectrometers have entered the market at a cost-point that is more practical for wider usage and show potential as a means of screening finished pharmaceuticals. Here, we are interested in determining the percent of the active ingredient isoniazid in solid dosage tablets. Isoniazid tablets were obtained from five manufacturers at various active ingredient percentages, and combined with isoniazid tablets pressed in lab, ranging from 30 – 100% isoniazid, resulting in N = 192. Spectra were collected with a handheld spectrometer (900 – 1700 nm) in triplicate. Spectra were split into a calibration dataset (n = 134) and an independent validation dataset (n = 58) and preprocessed using a first derivative Savitzky-Golay algorithm. A partial least-squares regression model was then applied to predict the percentage of isoniazid (w/w) in the tablets. The model resulted in a calibration root mean squared error of 2.796% and a validation of 2.84%. The results show promise for a low-cost and rapid screening method that can predict the percentage of the active ingredient present in an essential medicine. Given the ultra-portable handheld nature of these spectrometers, there is future potential in using these sensors as field compliance screening tools.

294 Are You Analyzing "All" Your Extractables/Leachables? - A Case Study Involving a Simple Switch from Acetonitrile to Isopropanol for Mobile Phase B in Liquid Chromatography/Mass Spectrometry

Yunyun Yuan, Ethicon, Johnson & Johnson, 1000 Rt. 202 South, Raritan, NJ 08869, Ying Jiang, Yijun Lu

Extractables and Leachables (E&L) characterization has evolved to play an important role to ensure product quality and safety in industries such as medical devices, pharmaceuticals, and food. The International Organization for Standardization and regulatory agencies including FDA have published guidelines on how to conduct E&L studies. Some emphasize employing a greater uncertainty factor (UF) to account for variation of response factors for different types of compounds, which sometimes poses significant challenges due to instrument/method limitations. To mitigate this, Mark Jordi and colleagues proposed a multidetector approach [1]. There is no doubt that a large UF and the multidetector approach would help to cover more potential extractables and/or leachables. However, other factors, such as liquid chromatography conditions should also be assessed. A case study involving a simple switch from acetonitrile to isopropanol for mobile phase B in liquid chromatography/mass spectrometry is presented to showcase E&L profile differences upon this switch. Recommendations of mobile phase B selection are also given based on the case study.

Reference:

[1] Mark Jordi, Ted Heise, "An Analytical Strategy Based on Multiple Complementary and Orthogonal Chromatographic and Detection Methods (Multidetector Approach) to Effectively Manage the Analytical Evaluation Threshold (AET)." PDA J Pharm Sci Technol. 2021 May-Jun;75(3):289-301.

295 Method Validations of Trace Level Impurities in an Agrichemical Compound

Nicholas Chubatyi, FMC Corporation, FMC Stine Research Center, 1090 Elkton Rd., Newark, DE 19711, Michael Harrington

Method validations are required for the determination of purity of a technical active product and for the presence and abundance of significant and relevant impurities in the technical active product. Methods are validated under test country guidelines and GLP as part of product registration. Specifically, method validations for trace level impurities in a technical grade agrochemical compound have been established and developed using liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). The criteria used to establish a validated method for the trace level impurities include linearity, precision, accuracy, specificity, limit of quantitation, and confirmation of identity. By successfully meeting the criteria established for these regulatory guidelines, these trace level methods can be implemented and used to support product registration of the manufactured technical grade material.

296 Unified GC/MS Method for Detection of Alkyl and Benzyl Halides

Alex Dunleavy, Exemplify BioPharma, 1002 Eastpark Blvd., Cranbury, NJ 08512, Matt Bauerle

Alkyl and benzyl halides are compound classes that are widely used in the synthesis of active pharmaceutical ingredients and require control of the residuals in the final product due to their potential mutagenicity. Organohalides can be potent alkylating agents, making them vulnerable to nucleophilic attack by DNA bases. Separate sensitive and selective methods have been developed to detect 19 alkyl halides (BP Range 16-198 °C, 1-4 carbon chain length) and 6 benzyl halides (BP Range 130-261 °C) at parts per million (ppm) concentration in the final product. Alkyl halides are readily quantified under standard conditions by GC/FID/MS. Detection of ng/mL levels of benzyl halides by either HPLC-UV or GC-FID technique presents a challenge due to their low UV response and incompatibility with common GC diluents. Derivatization methods have been developed for a series of benzyl halides by HPLC-UV detection, but require multiple derivatization steps for benzyl chlorides. We sought to develop a single GC/MS method for direct determination of alkyl halides (BP Range 42-118 °C, 2-3 carbon chain length) and benzyl halides (BP Range 179-251 °C) with a single diluent and no derivatization. Here, we describe our selection of diluent, method optimization, and qualification data for a sensitive and selective GC/MS method for detection of halide containing compounds.

297 Optical Characterization of 2D Ga2Se2 via Molecular Beam Epitaxy and Exfoliation for Quantum Computing Applications

Lottie Murray, University of Delaware, 201 Dupont Hall, Newark, DE 19716, Mingyu Yu, Eric Herrman, Xi Wang, Stephanie Law, Matthew Doty

Harnessing the properties of quantum mechanics has allowed for developments of quantum technologies that can be used for calculations, simulations, to improve methods for secure communications, etc. However, these technologies require a more reliable and scalable way of producing two level quantum systems for a large-scale quantum device architecture. One possible approach is to develop a method for large-scale growth via Molecular Beam Epitaxy (MBE) of high-quality 2D materials in which an array of features on the substrate induces localized strain in the grown film to create an array of quantum emitters. An alternative to MBE that is commonly used when studying 2D materials is exfoliating from bulk material and transferring onto a patterned substrate. MBE grown Ga2Se2 films and exfoliated Ga2Se2 flakes were characterized using photoluminescence (PL) measurements and Raman Spectroscopy. We report a comparison of the PL intensities and Raman signal between films exfoliated from a bulk crystal and those grown by MBE, which will be used to perform studies on the oxidation process of GaSe to inform handling procedures of the MBE grown films."

298 Biophysical Characterization of Proteins: Therapeutics, Vaccines and Plant Based

Yelena Pyatski, BioTools, 17546 FL-710, Jupiter, FL 33478, Rina Dukor, Kimberly Qinn, Juanita Sanchez

Biotherapeutics is a rapidly growing fraction of the total pharmaceutical market and is very diverse with various bio-modalities. Structure and specifically Higher Order Structure (HOS), is one of many Critical Quality Attributes (CQAs) that can affect structure, purity, functionality, and stability of a potential biotherapeutic candidates. Plant-based proteins have been the subject of growing interest as dietary substitutes, because of potential health benefits, abundance, as well as positive environmental impact. At the same time there is also a need to have proper analytical methods to characterize and determine how alternative versus traditional proteins differ in terms of HOS, composition, production, and other CQAs. The most common spectroscopic techniques used for biophysical characterization in biotechnology and food industry are CD and FTIR. FTIR is cost-effective, rapid, convenient, and precise, frequently used for quantification of secondary structures of various biotherapeutics. It is also indispensable for evaluation of such gene therapy CQAs as AAV capsid genetic material payload (empty/full ratio), capsid's protein envelope assessment (Amide I & II secondary structures), proteins ratios, differentiation and aggregation. However, neither FTIR or CD provide information on secondary and tertiary structures simultaneously or have enough specificity to differentiate closely related molecules. Raman Optical Activity (ROA) provides insights on effects of local environment, including hydration, side chain conformations of aromatic residues, and disulfide bonds. Raman/ROA meets the (ICH)-Q; Q6A guidelines for ID testing of biologics. In this presentation, we showcase how the combination of FTIR, CD and Raman/ROA enhances characterization of various bio-modalities.

299 Nanoscale Orientation-Sensitive IR Spectroscopy of Crystalline Samples

Sung Park, Molecular Vista, 6840 Via Del Oro, Ste. 110, San Jose, CA 95119, Padraic O'Reilly, Derej Nowak, Patrick O'Hara

While quantitative elemental mapping at nanoscale (~1 to 10 nm) has been available for many years via energy-dispersive spectrometry in electron microscopy, the best quantitative molecular mapping has been limited to a spatial resolution of ~200 nm for ToF-SIMS (time-of-flight secondary-ion mass spectrometry), which also destroys the region of analysis in the process. In this paper, we introduce a non-destructive quantitative molecular mapping technique called infrared photo-induced force microscopy (IR PiFM), which combines infrared spectroscopy with atomic force microscopy (AFM) to provide both structural imaging and IR spectro-nanoscopy with ~ 5 nm spatial resolution. Another trait of IR PiFM, due to the 1-dimensional nature of the AFM tip and the p-polarized IR excitation, is its capability to detect more sensitively the vibrational modes that are perpendicular to the sample surface. This capability allows IR PiFM to detect molecular orientation of ultrathin molecular films, crystalline versus amorphous phases, and nanoscale crystal orientations. IR PiFM results from Poly(ε-caprolactone) (PCL) – L-ascorbic acid (AA) blend and ammonium urate crystal samples are presented.

300 Analytical Comparison of Genotoxic Impurities in Extracted Nicotine vs. Synthetic Nicotine,

Ayesha Nisathar, J-Star Research Inc., 6 Cedar Brook Drive, Cranbury, NJ 08512, Hui Chen

Nicotine is a chiral alkaloid; nitrogen containing organic compound that occurs naturally. (S)-Nicotine is extracted from Tobacco plants and used as the key addictive ingredient in many smoking products. Synthetic Nicotine has gained the interest of many smoking product manufacturers over the last few decades due to the ease and low cost of manufacturing. Another claimed advantage of synthetic Nicotine is the absence of genotoxic impurities that form during extraction process of Nicotine. These impurities are other plant alkaloids, phenolic compounds, and heavy metals. Additionally, U.S. FDA has implemented new regulations on the quality control of synthetic Nicotine. However, only a very few research articles have been published on assessing the complete impurity profile of synthetic Nicotine. Therefore, the need to know the composition difference between Tobacco extracted Nicotine vs. synthetic Nicotine is highly necessary. In this research study, the impurity profile of three different lots of synthetic Nicotine were compared with four lots of Nicotine extracted from plants using in-house developed analytical methods. First, the samples were tested for other alkaloids and phenols by reversed phase HPLC. Second, the chiral purity was analyzed by normal phase HPLC. Third, lead and arsenic content were tested by Atomic Absorption and Fluorescence Spectrometry, respectively. The reversed phase HPLC data suggested similar quantities of total impurities in both Synthetic and tobacco extracted Nicotine (0.1%). However, naturally occurring other alkaloids (Cotinine, Nornicotine) were not found in synthetic Nicotine. Additionally, the synthetic Nicotine lots used in this study have high enantiomeric purity similar to the Tobacco extracted Nicotine.

301 Identification of Potential Degradation Impurities of Moxidectin, Mechanistic Explanation on Degradation Pathway and Establishment of a Stability-indicating Analytical Method

Tyler Chen Huang, J-STAR Research, 6 Cedarbrook Dr., Cranbury Township, NJ 08512, Yao An, Frank Rinald

Moxidectin is crucially important for animals to prevent/control parasitic worms and for the treatment of river blindness of human beings, such as recently approved by USFDA for the treatment of onchocerciasis due to Onchocerca volvulus in patients aged 12 years and older. A previous study reported that the 3,4-epoxy-moxidectin is an acid degradation product of moxidectin and the epoxides are potential risk posed by mutagenic degradants in active pharmaceutical ingredients (API) and formulated products. Post marketing surveillance of any drug depends on its acceptability based on risk to benefit ratio. When risk outweighs the benefits, withdrawal of an already marketed drug is warranted. The presence of impurity is the primary cause of increased risk in a drug substance or drug product. Using the High-Resolution Mass Spectrometry (HRMS) and Nuclear Magnetic Resonance (NMR) techniques, we investigated moxidectin and its two degradants in the reported acidic conditions. The structure elucidation evidence in this study suggests that the diastereomer (23Z)-moxidectin was misidentified as 3,4-epoxy-moxidectin in the previously reported study. Furthermore, for the first time, a possible degradation pathway has been

established with mechanistic explanation. Moreover, the analytical method developed in this study will be of immense help for routine analysis of quality control and stability study samples of moxidectin in industry and research laboratories.

302 Accelerating Drug Discovery by High-Throughput Purification and Physico-Chemical Characterization by HPLC/MS

Laszlo Varady, Rilas Technologies, 400 West Cummings Park, Suite 5000, Woburn, MA 01801

Purification of target compounds from crude synthetic mixtures has always been a rate limiting step in the new molecular entity discovery process. Typical technologies employed either have limited resolving power, but simple guiding principles (flash chromatography, thin-layer chromatography (TLC)) or high resolving power but no simple universal rules (Mass-directed HPLC). Rilas Technologies' scientists have incorporated state-of-the-art technologies in their purification process resulting in dramatic increase in throughput and significant decrease in turnaround time. Incorporating into our process high-throughput physico-chemical characterizations (solubility, stability, estimated polar surface area measurement, gluthathione reactivity) not only provide pure compounds for biological screens but additional valuable insights into the compounds behavior which enrich the *specific absorption* rate (SAR) data. Our overall process draws on the years of experience of our staff and has been utilized to purify and characterize thousands of compounds. This presentation describes the steps we take from crude reaction mixtures to fully characterized molecules and the best practices to obtain a rich data set to minimize the time from idea generation to biological screening. Several case studies are presented.

303 Separation Workflows Coupled with Mass Spectrometry for Biotherapeutic Development

Nicole A. Schneck, GSK, Analytical Development, 1250 S. Collegeville Rd., Collegeville, PA 19426, Matthew D. Maust, Robert J. Schuster, Paul MacGregor, Catherine O. Brown, Mark Jennings II, Sonya Kennedy-Gabb

Monoclonal antibodies (mAbs) are a class of biotherapeutics that are growing in both numbers and complexity. Liquid chromatography coupled with mass spectrometry (LC-MS) is a critical tool that is used to support mAb product comparability, structure-function studies and analytical investigations. In this talk, a collection of case studies will be used to highlight separation methods before MS detection to achieve primary structure information of mAbs in development. One case uses a denatured SEC-MS platform for efficient mass confirmation of mAbs at the intact and reduced levels. Most mAbs have a molecular weight of ~150kDa and consist of two heavy (~50kDa) and two light (~25kDa) chains. Since SEC is based on molecular size, rapid separation and mass confirmation of various mAbs can be achieved with repeatable and predictable retention times, limiting the need for additional method optimization. For bi-specific mAbs (bsAbs) though, a reverse phase (RP) LC-MS separation approach is typically applied to accommodate the varying heavy and light chain compositions. Here novel RPLC-MS separation methods were developed to confirm molecular integrity and composition of bsAbs. Methods were fine-tuned to improve separation of 2 heavy chains and 2 light chain pairs of similar sequence and hydrophobicity and to verify the proper bsAb assembly. Lastly, separation optimization of a mAb tryptic digest using RPLC-MS is presented, along with some points to consider when coupling different separation modes with mass spectrometry for mid-to-late stage mAb development.

304 Advanced Chromatographic Tools for Accelerated Development of Nucleic Acid-Based Medicines

Balasubrahmanyam Addepalli, Waters Corporation, 34 Maple St., Milford, MA 01757, Makda Araya, Maissa Gaye, Martin Gilar, Matthew Lauber

Ion-pairing reversed phase liquid chromatography characterizes the identity, purity, and integrity of diagnostic and therapeutic oligonucleotide medicines during development and quality control. Sensitive detection and optimal retention of these analytes not only depend on the surface chemistry but also pore size of the chromatography stationary phase. Selection of an appropriate pore size enables longer oligonucleotides to interact efficiently with the stationary phase and minimize restricted diffusion effects, thereby providing improved peak capacities. In this presentation, we illustrate the utility of wide pore BEH C18 300 Å sorbent for separation of longer oligonucleotides. We also report the value of longer oligonucleotide reference materials while testing the resolution capabilities of ion-pairing reagent combinations. These advanced chromatographic tools provided superior performance in evaluating the purity and sequence of intact and digested sgRNA, and characterization of mRNA digestion products including the poly(A) tails. The ACQUITY™ Premier Oligonucleotide BEH C18 300 Å 1.7 µm Columns are compatible with online mass spectrometric (MS) detection and sequence verification. These tools have great potential for accelerated development of nucleic acid therapeutics through speedy and robust characterization.

305 Challenges and Solutions in Analyzing Variants in Antibody-Based Biologics: Some Real-Life Case Studies

Xiaodong Liu, NanoChrom Technologies, Building NE-37, 99 Jinjihu Ave., China-Singapore Suzhou Industrial Park, Jiangsu, 215000, China

Antibody-based biologics have been a fast-growing area in the bio-therapeutic market for their specificity and efficacy, for diagnosing and treating a broad range of diseases, including auto-immune, cardiovascular, infectious, cancer and inflammation. In the development and production of therapeutic antibodies, the content of impurities, structural variants and post-translational modification variants must be monitored, characterized and quantified, to prove the stability and effectiveness of final products. Antibody-based biologics are often of complex micro-heterogeneity by nature. Thus, their quality control and stability evaluation are highly challenging tasks. High performance liquid chromatography (HPLC) is an important analytical technique for characterizing and quantifying impurities and variants in antibodies and related substances. Due to the complex nature of these molecules, a variety of separation modes are employed for a thorough characterization, including size exclusion chromatography (SEC) for aggregates and fragments, ion exchange chromatography (IEC) for charged variants, hydrophobic interaction chromatography (HIC) for DAR analysis in ADC, reversed-phase chromatography (RP) for exact mass determination of antibody and subunits, hydrophilic interaction chromatography (HILIC) for glycans, and Protein A affinity chromatography for titer analysis. In the separation of antibody-based biologics, column ruggedness and batch-to-batch consistency are of common concerns/challenges. In addition, better resolution and higher throughput are often desired/required in antibody characterization and quantification. This presentation uses real-life examples, including mAb, BsAb, TsAb, ADC and fusion proteins, to illustrate the challenges as well as solutions to above challenges in determination of aggregates, fragments, charged variants in antibody-based biologics.

306 Things WE'ED Like to Avoid – Circumventing Measurement Challenges When Analyzing Complex Cannabis Matrices

Rabi Musah, State University of New York at Albany, 1400 Washington Ave., Albany, NY 12222, Benedetta Garosi, Megan Chambers

Cannabis-derived and cannabinoid-infused products have become widely available with the increasing decriminalization of recreational *Cannabis sativa* use at the state level. Their popularity has been spurred in part by the availability of multiple commodities, such as edibles, beverages, topicals, and concentrates within which cannabinoids (e.g., delta-9 tetrahydrocannabinol (Δ9-THC), cannabidiol (CBD), etc.) are infused. The diversity and complexity of the matrices make these products challenging to analyze because of the need for nuanced matrix-specific protocols for their analysis. Current chromatography-based approaches for the detection and quantification of cannabinoids can be time-consuming, resource-intensive, require heavy use of consumables, and may cause damage to instrumentation. To address some of these difficulties, this study focused on the application of direct analysis in real time – high-resolution mass spectrometry (DART-HRMS) for the analysis of THC- and CBD-containing complex matrix samples for detection and quantification purposes. In order to reveal the presence of cannabinoids, cannabis-derived products can be rapidly screened (<5 seconds) by DART-HRMS without sample pre-treatment. However, when analyzed by mass spectrometry under ambient soft ionization conditions, THC and CBD are indistinguishable isomers (MW: $C_{21}H_{30}O_2$) with a protonated monoisotopic mass of 315.2324. Therefore, universal extraction and derivatization protocols that reveal the presence of both have been developed to accommodate the analysis of a range of complex matrices available on the market. These steps are then followed by quantification experiments for determining their cannabinoid levels. Optimization and standardization of these procedures will aid in the rapid investigation of complex cannabis matrix evidence in forensic laboratory settings.

307 The Importance of Digestion Temperature on Trace Metals Analysis

Sam Heckle, CEM, 3100 Smith Farm Rd., Matthews, NC 28104

With the legalization of medical and recreational cannabis in states across the United States., many state and commercial laboratories have begun implementing protocols for testing these sample types. As labs perform elemental analysis on cannabis derived products looking for toxic elements, they may encounter a few different sources of interferences. One of these potential sources is residual carbon. Carbon can cause signal enhancement and suppression effects on a handful of elements including Arsenic, Chromium, and Selenium if not accounted for when analyzing via inductively coupled plasma mass spectrometry (ICP-MS). Tracking these interferences can be done by adding Tellurium as an internal standard and by monitoring carbon on mass 13. Through proper sample preparation, the effects of carbon on these analytes can mostly be mitigated. Achieving a digestion temperature of 210°C and using the appropriate ratios of nitric and hydrochloric acids will help to avoid these carbon-based interferences. Achieving a total digestion will remove most residual carbon, though this is not always possible. If this is the case, isopropyl alcohol can be added to the internal standard to help reduce these interferences.

308 The Characterization of Delta-9-Tetrahydrocannabinol Stereoisomers in Various Cannabis Products

Brandy Young, Certainty Analytical Labs, 260 East Main St., Ste. 6411, Rochester, NY 14604, Andrea Andreeva

Little research exists on the stereoisomeric composition of delta-9-tetrahydrocannabinol in *Cannabis Sativa* products. Some studies suggest that (-)delta-9-trans-tetrahydrocannabinol is the more psychoactive and potent enantiomer which is responsible for the majority of the characteristic effects associated with cannabis use. Conversely, (±)delta-9-cis-tetrahydrocannabinol is considered to have weaker psychoactive effects, and some studies have even suggested it might have anti-psychotic properties. This work highlights a rapid and simple method that was used to characterize the stereoisomers in various *Cannabis Sativa* products.

309 Accurate Identification and Quantitation of Contaminants – Understanding the Impact of the Cannabis Matrix

Jini Glaros, Modern Canna Labs, 4705 Old Rd. 37, Lakeland, FL 33813

Cannabis is an extremely complex matrix that has chemical and microbial properties that can make the identification and quantitation of various analytes within the product difficult to determine. As such, laboratories must develop methods that will account for matrix effects and interferences that naturally occur in everyday samples and understand the impact these have on the results being obtained. This presentation takes a closer look at the cannabis matrix and the impact that its complexity plays in laboratory testing. Cannabis laboratories must focus on producing data that is accurate, reproducible, and legally defensible to ensure consumer safety. However, to do this, laboratories must also understand the products they are working with and how to deal with matrix interferences that may arise. Throughout this presentation, various interferences are addressed, and suggestions are provided regarding how laboratories can combat these issues to ensure that they are providing the most accurate data possible.

310 Leveraging Multi-Mode Microextraction and Liquid Chromatography for Quantitative Analysis of Neurotoxic Non-Proteinogenic Amino Acids

Emanuela Gionfriddo, University of Toledo, 2801 W Bancroft St., Toledo, OH 43615, Ronald Emmons, Endri Karaj, Erasmus Cudjoe, David Bell, L. M. Viranga Tillekeratne

Cyanobacterial neurotoxins are nonproteinogenic amino acids produced by cyanobacteria in aquatic and terrestrial environments. These neurotoxins pose analytical challenges as they are structural isomers. One such neurotoxic isomer, β-N-methylamino-L-alanine (BMAA), has been found to accumulate in aquatic fauna and the human brain, with potential correlations to amyotrophic lateral sclerosis (ALS) and dementia. The literature debates the presence of these toxins in complex matrices, like the brain or crab, due to the use of different analytical methods, which include reverse-phase liquid chromatography and hydrophilic interaction chromatography (HILIC). To address this, we developed a convenient chromatographic workflow using a pentafluorophenyl (PFP) stationary phase, proving superior in terms of robustness and throughput compared to other separation methods. The method achieved efficient separation of BMAA and its structural isomers in just 8 minutes, without the need for adding salts to the mobile phase or further column equilibration. Moreover, for better isolation and preconcentration of analytes we developed a solid phase microextraction (SPME) protocol. This extraction method utilized a biocompatible mixed-mode phase, combining benzenesulfonic acid and C8 moieties, enabling multi-mode extraction and preventing biofouling. With this approach, we achieved limits of quantitation reaching 2.5 μg L^{-1} for both BMAA and its structural isomer, N-(2-aminoethyl) glycine (AEG), and 5 μg L^{-1} for 2,4-diaminobutyric acid (DABA). Overall, our innovative chromatographic separation and solid phase microextraction techniques provide efficient and sensitive tools for trace analysis of cyanobacterial neurotoxins, offering valuable insights into their presence and potential implications for human health and the environment.

311 Separation and Analysis of Oligonucleotides for Clinical Diagnostics

Jared Anderson, Iowa State University, 1605 Gilman Hall, Ames, IA 50010, Derek Eitzmann, Shashini De Silva

Nucleic acids are biopolymers that constitute important diagnostic molecules for a broad range of applications from clinical testing to forensic analysis. A major challenge faced by DNA and RNA analysis techniques is the selective extraction of particular nucleic acid sequences using rapid and sensitive methodologies. It is of particular importance that the methods used to extract and separate nucleic acids be green or environmentally friendly. This presentation discusses new approaches to pre-concentrate and separate nucleic acids for downstream diagnostic analysis. The talk discusses challenges associated with performing analysis from complex biological samples as well as the direct extraction of nucleic acids from plant tissues. Novel liquid-phase approaches developed for sequence-selective DNA capture produce superior extraction efficiencies to conventional magnetic bead technology and represent a platform for using external fields to manipulate the liquid droplets. The development of isothermal amplification approaches capable of achieving single-nucleotide resolution of nucleic acid sequences is demonstrated through the use of molecular beacons.

312 Simplifying Clinical LC-MS Development by Leveraging Unique Stationary Phase Selectivity

Samantha Herbick, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Development of liquid chromatography mass spectrometry (LC-MS) methods in any type of clinical laboratory can often pose a great challenge. Between the time it takes to develop and validate a method to the complexities that analytes and biological matrices generate, many clinical laboratories struggle when it comes to developing new methods or adding to existing methods. As mentioned, it is important to consider matrices and target analytes when setting up an LC-MS method. One thing that can simplify the method development process is choosing the best column stationary phase for the specific testing. Moving beyond traditional C18 phase chemistry is often difficult for method developers as the phase has such broad utility. While C18 columns have served as an effective starting point for method development for many years, it is important to consider novel stationary phases that have been released in recent years in order to overcome common method development issues. While C18 has strong hydrophobic retention characteristics, there are phases that offer competitive hydrophobic characteristics as well as additional selectivities such as enhanced pi-pi interactions, increased hydrogen bonding capabilities, and even ion exchange. Additionally, for extremely polar compounds, the increasing popularity of stationary phases falling under the hydrophilic interaction liquid chromatography (HILIC) umbrella cannot be overlooked. This presentation focuses on the advantages of using unique stationary phases to simplify clinical LC-MS method development.

313 Utilization of Hydrophilic Interaction Liquid Chromatography in Clinical Analyses

David Bell, Restek Corporation, 110 Benner Circle, Bellefonte, PA

Hydrophilic interaction liquid chromatography (HILIC) has established itself as a powerful solution for the analysis of polar compounds. While reversed-phase liquid chromatography (RPLC) remains the dominant mode employed in chromatographic separations, the inability to retain highly polar compounds without the introduction of ion-pair reagents limits its utility. Clinical diagnostic procedures often target the analysis of highly polar analytes and therefor often requires an alternative approach such as HILIC. In this presentation, we discuss some basics of HILIC chromatography and use this knowledge to critically review recent literature in the field of clinical and biomedical diagnostics.

314 Improved Drug Product Development and Control through Detailed Characterization of API Epimerization

Nathan Contrella, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Steven Tignor, Colin Lam, Margaret Brunell, Alexandra Andrews, Josey Topolski, Devin Swiner, Tamara Cabalu, Zhoupeng Zhang, Ryan Cohen, Brittany Kassim

An early development active pharmaceutical ingredient (API) containing a chiral center adjacent to a labile keto-amide functional group was found to readily epimerize in solution and in solid oral formulations, including suspension and tablet dosage forms. Detailed analytical characterization established that epimerization was accelerated by increasing pH, temperature, and buffer concentration, and proceeded to an equilibrium API:epimer ratio of 1.7:1. Computational modeling by density functional theory was performed to better understand this system, and calculation results were consistent with the observed equilibrium ratio. High-performance liquid chromatography (HPLC) conditions were developed to effectively separate the API and epimer peaks, and thorough investigation of epimerization kinetics was applied to ensure robust sample preparation. This was particularly important for drug product and preclinical PK samples, where stabilizing the sample solutions against further epimer growth prior to analysis was critical. In the development space, API epimerization in sample solutions prepared for film-coated tablets was found to be accelerated by several components of the film coating system and was suppressed by modification of the diluent pH, ultimately enabling robust, reliable data across a series of prototype formulations. Preclinical results established high in vivo conversion to the epimer, quantitation of which enabled refined dose projections ahead of first-in-human studies. This also allowed the epimer to be generally qualified per ICH guidelines, enabling control limits and targets for drug product development to be set at clinically appropriate levels.

315 Efficiency of Ultrafiltration / Diafiltration in Removing Organic and Elemental Process Equipment Related Leachables

Bin Sun, Cytiva, 20 Walkup Dr., Westborough, MA 01581

Production of biological therapeutics such as monoclonal antibodies (mAbs), ADCs, viral vectors for gene therapies, lipid nanoparticle (LNP)-encapsulated mRNA, and other genomic medicines incorporate various downstream processing steps including TFF (UF/DF) operations prior to final sterile filtration and/or formulation and fill.

The buffer exchange step in the DF process in theory allows clearance of any small molecule entities and is generally regarded as a leachables risk mitigation step by attenuating persistence of PERLs into the final drug product. However, clearance data available in the literature are limited to highly water-soluble organic PERLs, and the extent to which DF reduces the risk of TFF related leachables remains unclear to date. In this presentation, we will discuss the results from our studies investigating the leachables risk from TFF by comparing the leachables profiles pre- and post-TFF operations, and the clearance of representative organic and elemental PERLs spiked into a model protein-based formulation using GC/MS, LC/MS, and ICP/MS. The impact of post-TFF leachables on patient safety risk assessment as well as the effect of polarity and number of DF volumes on clearance will also be presented.

316 Application of XRF in the Pharmaceutical Industry

Sergey Mamedov, HORIBA Scientific, 20 Knightsbridge Rd, Piscataway, NJ 08854

X-ray fluorescence (XRF) spectroscopy is widely used to identify and quantify substances and confirm their identity. This technology requires little or no sample preparation. The XRF analytical microscope's new capabilities enable recording a hyperspectral image of objects. The distribution of elements extracted from a hyperspectral image is important evidence of fake or counterfeit products compared to authentic ones. It also allows one to differentiate the product from other vendors. The XGT-9000 XRF analytical microscope was used in this study. This desktop unit utilizes a portable 50W X-ray Rh X-ray tube, Silicon Drift Detector (SDD), and three programmable X-ray optics with a spot size of 10 microns, 100 UHI (Ultra High Intensity) microns, and 1.2 mm. The quantification of Mg-stearate was developed based on the XRF spectra, and a PLS model was created. It allows one to calculate the Mg-stearate concentration in unknown materials and detect impurities. The comparison of elemental distribution for Mg, Si, S, K, Ca, Ti, and Fe in aspirin from different brands may allow one to identify the vendor of the tables. The chemical images of these elements for tablets from different vendors will be presented along with the spectra comparison. Counterfeit in the pharma industry has become an important problem. The spectra and chemical images of authentic and fake pills will be shown. The presents of unexpected elements in the tablets directly prove the counterfeit product.

317 Pharmaceutical Applications Utilizing LUMA Vacuum Ultraviolet Detection: Advancements in Moisture Content, Impurity Analysis, and FAMEs Analysis

Rafael Acosta, VUV Analytics, 1500 Arrowpoint Dr., Ste. 801, Cedar Park, TX 78613, Ryan Schonert

This talk explores the cutting-edge advancements in pharmaceutical applications utilizing LUMA Vacuum Ultraviolet (VUV) Detection, an innovative technology developed by VUV Analytics. We discuss how LUMA enhances detection and analysis capabilities in pharmaceutical processes, with a particular focus on moisture content determination in solvents, pharmaceutical impurity analysis, and Fatty Acid Methyl Ester (FAMEs) analysis. LUMA, a groundbreaking gas chromatography detector, offers unparalleled sensitivity, selectivity, and sustainability, making it an ideal solution for various pharmaceutical applications. The talk provides an in-depth look at LUMA's capabilities in determining moisture content in solvents, a crucial parameter in maintaining product quality and stability. We highlight how LUMA's VUV technology simplifies the moisture determination process, providing accurate and reliable results. Additionally, we delve into LUMA's role in pharmaceutical impurity analysis, a critical aspect of ensuring product safety and efficacy. With its superior sensitivity and selectivity, LUMA can detect impurities at trace and ultra-trace levels, enabling manufacturers to adhere to stringent regulatory requirements. Finally, we discuss the application of LUMA in FAMEs analysis, a technique used to identify and quantify fatty acids in various samples. LUMA's VUV technology simplifies the FAMEs analysis process, delivering precise and consistent results. By utilizing LUMA in these pharmaceutical applications, industry professionals can benefit from enhanced analytical capabilities, ensuring product safety and quality while maintaining efficiency and sustainability. Join us to learn more about the transformative impact of LUMA on the pharmaceutical industry.

318 First-Principle-Based Investigation of Column Selectivities - Using Multidimensional Analytical Design Space Models as Tools to Find Equivalent Working Ranges Across Various Stationary Phases

Arnold Zoeldhegyi, Molnar-Institute, Scheegloeckchenstrasse 47., Berlin 10407, Germany, Róbert Kormány

Knowing and controlling chromatographic selectivities in high-performance liquid chromatography (HPLC) are indispensable for subsequent method development success. At the beginning of the development process, exploring stationary phases with distinct ("orthogonal") selectivities can facilitate the detection and control of unknown impurities in a pharmaceutical sample. At later stages however, finding backup/replacement columns with identical or similar selectivities, is essential to avoid delay of market entry, caused by problems replacing columns by substitute stationary phases. In this sense, using multidimensional model design spaces(DSs) to uniquely characterize complex LC-separation systems, has already been reported in several peer-reviewed papers. Most recent analytical chapters of ICH (Q12 and Q14, Q2(R2) drafts) also point to this direction by highlighting «enhanced» modeling approaches as key drivers for establishing holistic method understanding and knowledge management. Bearing this in mind, we used a DS-modeling software (DryLab), with the main focus on building 3-dimensional separation models of amlodipine and its related impurities to compare the measured chromatographic performance of columns in terms of separation and robustness. Based on requiring no more than 12 input experiments per column, we investigated the impact of all chromatographically relevant method parameters, such as gradient, column temperature, pH of the mobile phase and other instrumental factors. Our study comprised 25 UHPLC-columns in total, in some cases with surprising results: We found several equivalences between DSs of substantially different columns and conversely, there were also cases, where albeit the similar specifications, the model DSs revealed clear differences between the selected stationary phases.

319 Application of Self-Optimizing Support Vector Classifier-Radial Basis Function for Multivariate Classification of Maca Metabolomic Mass Spectral Profiles from China and Peru

Qudus Thanni, Ohio University, 133 University Terrace, Athens, OH 45701, Peter Harrington

Support vector classifiers are used with kernels when linear models are inadequate to define class boundaries. The most utilized kernel with support vector classifiers (SVCs) is the radial basis function (RBF). The RBF function is optimized with a width parameter, s, and the SVC with a cost parameter, λ, using a grid search of the training data. To improve the optimization of these parameters and the generalization of the model, internal bootstrapped Latin partitions (BLP) are implemented to eliminate bias by using randomly selected prediction subset objects. The average prediction errors across the bootstraps are the response variables. The response variables were fitted with a two-dimensional spline function to find the s and λ parameters with the lowest average prediction error. Maca mass spectra in the negative and positive ion modes were classified by country of origin as China and Peru. The number of maca samples in the Peruvian group exceeded the number in the Chinese group by a factor of six. Synthetic data construction was used to correct the experimental design imbalance. Synthetic and experimental spectra were used for pairwise model comparisons. The self-optimized radial basis function–support vector classifier's (SO-RBF-SVC) performance metrics were compared to other classifiers, such as super SVC (sSVC) and super partial least squares-discriminant analysis (sPLS-DA). Compared to empirical data, SO-RBF-SVC and sPLS-DA predicted more accurately from synthetic data. Generalized sensitivity analysis (GSA) was utilized to assess the nonlinearity of the SO-RBF-SVC models. All data analysis was conducted in the MATLAB® programming language environment.

320 Self-Modeling Curve Resolution of Raman Spectra from Mixed Deuterated and Protiated Phospholipid Membranes Reveals Isotopically-Segregated Lipid Domains

Jay Kitt, University of Utah, 315 S. 1400 E., Salt Lake City, UT 84124

Deuterated phospholipids are employed as "non-perturbing" probes of phospholipid membrane structure in vibrational spectroscopy, allowing resolution of frequency-shifted vibrational modes of deuterated phospholipids in mixed-phospholipid membranes. Calorimetry of deuterated-phospholipid vesicles has shown lower-temperature melting transitions and reduced cooperative melting, in deuterated lipids. A study combining infrared spectroscopy and molecular-dynamics simulations shows evidence that incorporation of deuterated phospholipids into protiated membranes induces phase-segregation, where two distinct thermal events occur in calorimetric thermograms. In the current work, phase segregation in individual, mixed deuterated and protiated vesicles is investigated by Raman microscopy. Raman spectra of mixed-lipid vesicles, collected as a function of deuterated-lipid fraction, were examined by self-modeling curve resolution allowing model-free resolution of spectral vectors and corresponding composition profiles. The resulting vectors point to microdomain formation, indicated by Raman modes in CH- and CD-stretching regions, in the membrane as a function of CH/CD composition; domain size varies linearly with deuterated lipid fraction. Deuterated and non-deuterated lipid separation allows short-range vibrational coupling within the mixed system. To study the impact of dipolar coupling on mixed-lipid structures, mixed-lipid vesicles were monitored as a function of temperature. Two distinct melting transitions are observed and the melting-transition-temperature of each lipid is shifted from that of a pure sample. Interestingly, dipolar coupling is observed in each lipid type until completion of the phase transition, suggesting that coupling between adjacent phospholipids may contribute to phase-separation during the transition. These results question the assumption that deuterated phospholipids are non-perturbing and suggest caution using deuterated phospholipids in membrane research.

321 Error Propagation-Based Optimal Threshold Determination for Classification Models: Advancing Boundary Calculation in Chemical Information Analysis

Helder V. Carneiro, University of Delaware, 063 Brown Lab, The Green, Newark, DE 19716, Caelin P. Celani, Karl S. Booksh

A new method that determines optimal threshold levels for classification models based on propagation of errors is presented. In classification model application, the threshold is optimized distinguish the target class from non-target classes. State of the art methods include Bayesian joint probabilities that assume a normal distribution of sample scores and empirical tests, such as the K-S test / ROC curve, that jointly maximize the sensitivity and selectivity of analyses. A better approach would be to determine propagation of measurement errors through the model to determine a statistical threshold. Where most statistical tests assume independent, identically distributed Gaussian errors with a diagonal covariance matrix, this strategy employs the error covariance matrix to bootstrap a statistical error distribution about the threshold value by way of Monte Carlo simulations. The idea is to build a regression using the noise extracted from each sample and set the noise score mean plus two times the standard deviation as the boundary. This approach provides us with a limit of detection in the classification with a 95% confidence level. Since this method can be applied to non-normally distributed and highly correlated error structures, it represents a significant advancement in the field. The new algorithm was tested using simulated data, inductively coupled plasma (ICP) data, and laser-induced breakdown spectroscopy (LIBS). The results were promising and can pave the way for a new approach to calculating boundaries based on chemical information. The ultimate goal is to expand this new method to hierarchical and non-parametric classification methods.

322 Transcending the Black Box: A Semi-AutoML Approach to Collaborative Model Building

Manuel A. Palacios, Eigenvector Research, Inc. 196 Hyacinth Rd., Manson, WA 98831, Sean Roginski, Barry M. Wise

Automated machine learning (AutoML) typically simplifies the model-building pipeline, often leading to a singular optimized yet opaque model. While efficient, this approach can lack transparency and customization. We propose a semi-autoML approach that actively involves the analyst in the model-building process, fostering transparency and diversity in model outcomes. Our method guides users through outlier assessment, variable selection, preprocessing, and calibration of linear models, all the way to creating less-biased non-linear models like SVM, LWR, and ANN. Users may select a single model, a series of top-ranked models, or an ensemble for further refinement. This method bridges the gap between full automation and user-led customization, yielding improved model diversity and predictive accuracy. Our research invites a rethinking of how AutoML models are constructed, evaluated, and interpreted, favoring a more collaborative, interpretable, and adaptive methodology.

323 New Technologies and Techniques for the Separation of Oligonucleotides and Polypeptides

Weston Umstead, Daicel Chiral Technologies, 1475 Dunwoody Dr., Ste. 310, West Chester, PA 19380

Oligonucleotides and polypeptides represent a major area of investigation for new therapeutic entities. Due to their complex nature, many analytical challenges exist for the accurate characterization of the entities themselves, along with their associated impurities. The extent to which these problems can be solved is limited to the existing technologies and techniques available on the market. So, in order to address these challenges, new innovations are required to provide new separations and provide orthogonality to established methods. This talk covers the innovations coming from Daicel Chiral Technologies on column technologies that are providing new and improved separations of oligonucleotides and polypeptides under several different mobile phase modes.

324 Assessing Chromatographic Systems for Use in Phosphopeptide Mapping Studies

Corey Reed, Waters Corporation, 34 Maple Street, Milford, MA, 01757, Paula Hong, Robert Birdsall, Jennifer Simeone

Peptide mapping studies used for the analysis of protein digests are complex, requiring the analyst to monitor many peaks to build a higher order structure of the original protein. This technique can be complicated by the presence of “sticky” phosphopeptides in the protein digest which, due to a negatively charged phosphate group, readily attract to positively charged metal ions throughout the flow path. This can lead to peak tailing, reduced recovery of peaks, and poor chromatographic quality due to the complex nature of the results. In this study a ultra-high-pressure liquid chromatography (UHPLC) system and column with a modified surface designed to limit off-target interactions of analytes is compared to a traditional biocompatible UHPLC system for the separation of phosphopeptides using reverse-phase (RP)LC. Peak tailing, signal/noise, and peak area are monitored using a mass detector and compared. The UHPLC system with the modified surface demonstrated lower overall peak tailing and higher signal/noise for both a phosphopeptide standard and an enolase digestion standard. Reduced peak tailing makes chromatographic analysis more reliable while an increase in signal/noise allows for lower limits of detection. Additionally, the mobile phase additive used in this study was reduced from 0.1% to 0.01% to determine if more mild conditions could be used. With the lower concentration both systems demonstrated higher signal/noise indicating mild conditions can be used to achieve lower detection limits. Overall, both systems performed well, with the modified system and column together demonstrating the highest level of benefit for use with phosphopeptides.

325 Don’t Go to Pieces on Me: Importance of Particle Architecture and Backpressure on Oligonucleotide Characterization

Cory Muraco, MilliporeSigma, 595 N. Harrison Rd., Bellefonte, PA 16823, Clinton Corman

Due primarily to the rapid success and adoption of the COVID-19 vaccines incorporating mRNA technology, oligonucleotide-based therapeutics have emerged as a promising modality to treat a myriad of diseases. However, as oligonucleotides are large, complex biomolecules, techniques to fully characterize these molecules has been an area of much research over the past several years. Any impurity in the formulation could elicit a range of side-effects, from a compromised efficacy in the drug being able to treat the disease all the way to death of the patient due to an allergic response. This paper explores different chromatographic approaches in characterizing oligonucleotides in an effort to develop accurate, reproducible, and efficient methods. It has been found that oligonucleotides can be prone to artefactual degradation at elevated pressures used in conventional ultra-high performance liquid chromatography (UHPLC) assays; therefore, this phenomenon was investigated by examining different particle architectures and assay conditions to elucidate when and how this mechanism takes place. In addition, strategies in mobile phase selection and temperature selection are discussed in order to minimize artifact formation while concomitantly improving resolution and efficiency. Finally, applications involving real oligonucleotide and modified oligonucleotide therapeutics are highlighted in order to demonstrate the advantages in selecting the appropriate column and mobile phase conditions in oligonucleotide analysis.

326 Increased Efficiency of Protein and Peptide Separations by Varying Particle Size, Column Dimension, and Pore Size of Superficially Porous Particle Columns

Peter Pellegrinelli, AMT, 3521 Silverside Road, Ste. 1-K Quillen Building, Wilmington, DE 19810, Ben Libert, Stephanie Schuster

With more complex biopharmaceutical drugs being developed each year, the testing and confirmation of these drugs becomes more important for patient safety. The ultraviolet (UV) and mass spectrometry (MS) separation demands of these drugs also has become more challenging which requires improved column technology. By decreasing particle size, column efficiency and resolution can be increased to enable better peak identification when separating complex peptide maps. Reducing the column’s internal diameter can also provide better signal allowing for better detection of peaks of interest/impurity analysis. By separating a tryptic digest and IdeS digest of trastuzumab, screening multiple HALO® Bioclass columns ranging in column ID, particle size, and pore size, an increase in sensitivity, decrease in peak widths, and marked decrease in solvent usage is demonstrated using standard liquid chromatography -MS systems which was only previously realized with specialized microflow systems.

327 Thermodynamic Stabilization of Conformations in Lewis Antigens

Darón Freedberg, CBER/FDA, 10903 New Hampshire Ave. Building 52-72, Room G438, Silver Spring, MD 20993, Jeahoo Kwon, Alessandro Ruda, Hugo Azurmendi, Jasmin Zarb, Marcos Battistel, Liang Liao, France-Isabelle Auzanneau, Göran Widmalm

The Lewis antigens are a well-known family of fucosylated glycans whose structures were thought to be conformationally inflexible, until recently. In this presentation, we present evidence for conformational flexibility between hydrogen bonded conformations and non-hydrogen bonded ones. We show here that the formation of a CH→O non-conventional hydrogen bond from the H5 of fucose III or II to the pyranose oxygen of galactose II or III, respectively, in Lewis a, Lewis b, Lewis x and Lewis y, partially stabilizes a “compact structure” of these antigens. This creates nuclear magnetic resonance (NMR) spectral conditions that allowed us to analyze Lewis antigen spectra by using the partial stabilization in NMR lineshape analysis. We analyzed temperature dependent spectra in the aggregate together with slow-exchange chemical shifts obtained from fucose monomer. These analyses led us to determine that the ΔG° values for the hydrogen bonded conformers in the Lewis Antigens studied here, range from -1.5 to -1.0 kCal/mol. Lineshape analysis also yielded rate constants which we used to determine the free energy barriers to breaking these hydrogen bonds. In Lewis Antigen analogs where a rhamnose residue replaces the fucose, the ΔG° values are comparable to those containing a fucose, suggesting that this type of non-conventional hydrogen bond is general and may be used in design of vaccines or drugs to stabilize or destabilize desired conformations.

328 i-HMBC: Unequivocal Identification of Two-Bond Heteronuclear Correlations in Natural Products at Nanomole Scale

Xiao Wang, Merck & Co. Inc., 126 E Lincoln Ave., Rahway, NJ 07065

HMBC is an essential nuclear magnetic resonance (NMR) experiment for determining multiple bond heteronuclear correlations in small to medium-sized organic molecules, including natural products, yet its major limitation is the inability to differentiate two-bond from longer-range correlations. There have been several attempts to address this issue, but all reported approaches suffer various drawbacks, such as restricted utility and poor sensitivity. Here we present a sensitive and universal methodology to identify two-bond HMBC correlations using isotope shifts, referred to as i-HMBC (isotope shift detection HMBC).[1] Experimental utility was demonstrated at the sub-milligram / nanomole scale with only a few hours of acquisition time required for structure elucidation of several complex proton-deficient natural products, which could not be fully elucidated by conventional two-dimensional NMR experiments. Because i-HMBC overcomes the key limitation of HMBC without significant reduction in sensitivity or performance, i-HMBC can be used as a complement to HMBC when unambiguous identifications of two-bond correlations are needed.

Reference:

[1] Wang, Y.; A. Fan,; Cohen, R. D.; Dal Poggetto, G.; Huang, Z.; Yang, H.; Martin, G. E.; Sherer, E. C.; Reibarkh, M.; Wang, X.; Nat. Commun. Just accepted. https://doi.org/10.1038/s41467-023-37289-z

329 Developing Benchtop NMR Spectrometer into QC and PAT

Hector Robert, Magritek, 103 Great Valley Pkwy, Malvern, PA 19355, Anh Le McClain

This presentation discusses the implementation of medium-field benchtop nuclear magnetic resonance (NMR) spectrometers in a wide range of important applications, from screening incoming materials to process understanding and optimization. Newly developed software enables automated identification and quantitative analysis of raw materials, while the efficient multi-frequency solvent suppression techniques along with the reaction monitoring set-up can help process optimization with on-line/in-line analysis of complex samples without sample preparation. Overall, the compact size, low cost and maintenance, and versatility of the benchtop NMR spectrometers makes them a powerful addition to the quality control/quality assurance (QC/QA) and process analytical technology (PAT) toolbox.

330 Nanoscale Chemical Analysis of Surfaces and Monolayers of Intentional and Unintentional Molecules

Sung Park, Molecular Vista, 6840 Via Del Oro, Ste. 110, San Jose, CA 95119, Padraic O'Reilly, Derej Nowak, Patrick O'Hara

Surface modification or functionalization is used to manage the interaction of biomolecules, bacteria, viruses, and other molecules with the surface and is utilized in many industries including biotechnology, tissue engineering, biosensors, and semiconductor. While many methods are being developed for achieving the desired modification and functionalization, there remains a critical need for an analytical characterization tool for these processes since the existing techniques lack the nanoscale spatial resolution or sensitivity that many advanced applications require. The few surface-sensitive techniques such as time-of-flight secondary ion mass spectrometry (ToF-SIMS) and X-ray photoelectron spectroscopy (XPS) are either destructive to the sample or cumbersome to use due to the requirement of vacuum in addition to falling short in spatial resolution. Out of practical necessity, many utilize a convenient technique such as water contact angle to monitor the success of the functionalization step, which can lead to a false perception of success since it is blind to the actual identity of the molecules on the modified surface. In this talk, a nanoscale analytical technique called infrared photo-induced force microscopy (IR PiFM) is introduced. IR PiFM adds IR spectroscopy to atomic force microscopy (AFM) to enable sub-5 nm spatial resolution and sub-monolayer sensitivity for precise characterization of molecular identity and chemical state of organic and inorganic surfaces via their IR signatures. IR PiFM studies of various functionalized surfaces, both intentional and unintentional, are presented.

331 Optical Imaging and Spectroscopic Analysis of Polysulfide Speciation in Li–S Battery Electrolyte

Gbenga Taiwo, University of Delaware, Mechanical Engineering, Spencer Laboratory, 130 Academy St., Newark, DE 19716, Ali Rashti, Mritunjay Mishra, Koffi Yao

To optimize electrolytes for Li-S batteries (LSB) and develop new ones, understanding the interactions and redox reactions between the electrolyte and cathode is crucial. Our study uses operando lithium-sulfur cells, in-situ Raman, and *ex-situ* UV-vis spectroscopies to investigate polysulfide speciation in fully and sparingly solvating electrolytes for LSB. Analysis of literature data reveals that the use of sparingly solvating solvents notably improves the coulombic efficiency of sulfur-cells. Our experimental optical imaging, Raman and UV-vis characterizations indicate a shift towards shorter-chain polysulfides in electrolytes with increasing lithium-salt concentration (i.e., more sparingly solvating). This shift corresponds to a reduction of polysulfide species involved in shuttling, which supports the increased coulombic efficiency in sparingly solvating electrolytes.

332 Can Magnetic Resonance Force Microscopy Detect and Image Individual Nitroxide Spins?

John Marohn, Cornell University, Dept. of Chemistry, Ithaca, NY 14850, Michael Boucher, Peter Sun, Russell Burgett, Pamela Nasr, Corinne Isaac, Lee Harrell, Roger Loring, Robert McMichael

If we could acquire the three-dimensional coordinates of nitroxide electron spins affixed to individual copies of proteins or nucleic acids, then we would have a powerful new approach for determining the assembly state of a wide range of interesting biological complexes. Magnetic resonance force microscopy has detected and imaged electron spin resonance from single dangling bonds in quartz. What about individual nitroxide spins? We have developed cantilevers having cobalt nanorod tips producing 5 mT/nm of magnetic field gradient. The estimated single-electron force is 45 aN, well above our cantilever noise floor of 5 aN (in 1 Hz) at 4.2 kelvin. We can routinely observe electron spin resonance from millions of molecules using micron-scale tips, and the observed lineshape agrees well with simulations. Yet when we replace the micron-scale tips with nanorod tips having larger gradients, the observed signal is 450 times smaller than expected and the observed lineshape agrees poorly with simulations. Why are we losing signal? Metal coating the sample reduces surface noise but, we show, damages the sample. A new, gentle, laminated-sample protocol increases signal 15-fold while reducing surface noise. Another source of signal loss is the breakdown of saturation due to large resonance offsets caused by cantilever motion. T1 reduction from stochastic magnetic field fluctuations is another concern; this is a common problem in quantum computing too. We report estimates of T1 loss from thermal conductivity fluctuations and thermomagnetic fluctuations obtained using a combination of theory, numerical calculations, and non-contact friction measurements. Supported by NIH R01GM143556.

333 Dangers in the Library: Poison Book Covers, Altered Illuminations, and Toxin-Dusted Fore Edges

Jennifer Mass, Bard Graduate Center, 38 West 86th St. New York, NY 10024, Aaron Shugar, Adam Finnefrock, Teresa Duncan, Heidi Nance

The Library of the College of Physicians, Philadelphia, houses one of the most important historical medical libraries in the United States. Its collection includes illuminated manuscripts, incunabula and printed volumes recording two millennia of medical history, with a particular emphasis on nineteenth-century medical treatises. This emphasis on nineteenth-century material raised questions of how the College's collection compared to other libraries participating in the Winterthur Museum's Poison Book Project. Of primary concern was the colorants of the hand-colored illuminations, book covers, endpapers, and edge papers. The phenomenal popularity of emerald green (copper acetoarsenite) in fashion, interior decoration, and even culinary creations is reflected in the Library's nineteenth-century collection, with book components commonly decorated with this beautiful acid-green pigment. Edge papers were of particular concern given how weakly adhered the pigments are and how often they are handled. Our study included examining the works using scanning XRF, long-wave UV imaging, and FORS. Given the broad age range of the collection, our work also included identifying arsenic, realgar, and lead pigments. We routinely identified emerald green and chrome yellow in nineteenth-century book covers, endpapers, and edge papers. We found lead white and orpiment in the earlier manuscripts and incunabula. Malachite regions contained variable levels of zinc, and blackened vermilion and lead-tin yellow were also observed. Our findings also revealed the common "strengthening" of illuminations in medieval and Renaissance volumes with later colorants such as emerald green and zinc white. Scanning XRF was critical for identifying these modifications of early illuminations.

334 Considerations and Misinterpretations: Practical Notes Regarding Authenticity from the Perspective of a Paintings Conservator

Kristin deGhetaldi, 21 Bristol Knoll Rd., Newark, DE 19711

The inclusion of science into the field of art restoration began as early as the 19th century. Since the 1950s, however, there has been a proliferation of analytical and imaging techniques that have since found their way into museums, art conservation studios, and laboratories. While some of these techniques have withstood the test of time, others have become obsolete and are no longer considered reliable. Therefore, understanding the evolution of conservation science is paramount when researching the analysis of artworks and art materials. Older case studies encountered in the literature may warrant revisiting or may even report erroneous data. As scientists and students operating outside of the art conservation field are likely unaware of some of these issues, collaboration between scientists and professional conservators is necessary in order to foster accurate and successful research endeavors, particularly when it comes to issues surrounding authentication. Case studies highlighting both successful and failed collaboration attempts involving authentication are presented.

335 Correlating Visual Changes with the Chemistry of Latex Browning in Works of Art

Teresa Duncan, Scientific Analysis of Fine Art, 843 Old SR, Berwyn, PA 19312, Satoko Tanimoto, Hannah Duggan, Esther Chao, Lena Stringari, Adam Finnefrock, Jennifer Mass

When faced with the challenge of analyzing non-traditional artists' materials, scientific literature can provide a wealth of information to help inform questions of authentication, attribution, exhibition, storage, conservation treatment, and more. However, sometimes turning to literature from other fields can misguide the heritage researcher. In this talk, we present the findings from technical analyses of the materials comprising *Expanded Expansion*, a sculpture executed in 1969 by American artist Eva Hesse (1936–1970) composed of fiberglass poles draped with latex-coated cheesecloth. Microscopy and Fourier-transform infrared spectroscopy (FTIR) were used to determine the extent of latex oxidation in correlation with the aging and associated darkening of the artwork. The scientific literature surrounding latex oxidation is predominantly focused on the degradation of the latex poly(isoprene) backbone, but careful analysis of our findings suggested other chemical reactions were involved. Specifically, the role of components other than poly(isoprene), such as polyphenols, were found to be the primary substances involved in the darkening of the latex. Findings were validated with reference material samples, which had been kept out of light and sealed from air thereby preventing degradation of the polymer backbone and yet had turned dark brown. Results showed no evidence of poly(isoprene) degradation but did show evidence of enzymatic degradation via polyphenols reacting to form brown melanin pigments. Although this research project did not involve questions of attribution and authentication, we believe our 'lessons learned' will be helpful for researchers exploring a range of questions related to non-traditional media.

336 Development, Application, and Relevance of Artificial Intelligence for Art Discovery, Authentication and Attribution

Lauryn Smith, Frick Art Museum 7227 Reynolds St., Pittsburgh, PA 15217

We live in a divisive time where artificial intelligence (AI) elicits extreme reactions from the general public; from staunch beliefs that it will transform production as steam power did during the Industrial Revolution to visions of dark Asimovian futures where humans are enslaved by AI. While several cultural heritage institutions and art historians have embraced digital methods, others have taken to both peer- and non-peer reviewed spaces (e.g., twitter) to express profound skepticism rooted in the longstanding misconceptions that the main purpose of AI for art historical research is to replace the principal researcher. This is of course due, in part, to a lack of understanding of how generative AI functions and when and how it is best employed. Drawing on my own experiences as an object-based art historian who grew to utilize computational methods for art historical research, this presentation first charts the history of AI as a technical tool for the research of art objects. It then dives into recent and ongoing projects that employ modern computational methods, namely Machine Learning, to unearth new evidence related to the creation and preservation of artworks and cultural heritage. Finally, it discusses the ramifications of these studies, potential avenues for future research, and the lacking infrastructure obstructing the Humanities from completely embracing these computational methods. This talk provides an overview of this burgeoning technology, and advocates for a cautious yet timely embracing of AI and similar computational innovations for the study and preservation of art historical objects.

337 Chromatography and Mass Spectrometry Based Approaches for Raw Materials Characterization and Finished Goods Analysis in Consumer Goods Samples

Chad Herrman, Unilever R&D, 40 Merritt Blvd., Trumbull, CT 06611

In the consumer goods industry, many personal care products have ingredient lists containing 15 or more individual components listed on their labels per INCI designations. Some of these "individual" components contain multiple molecules that often complicate chromatograms or spectrum, making it challenging to specifically measure each component within a given formulation. Technique selection and sample preparation play an important role in the analytical development for personal care products. At the outset of each development project, an overarching plan is formulated where a technique or multiple techniques are investigated as potential avenues to measure target components. Selection of the analytical technique(s) may vary depending on the chemistry of the target analytes, the chemical matrix in which they reside, the sample format (e.g., liquid washes, solid bars, etc.), and any requirements needed for limit of detection or limit of quantitation. Examples of different approaches for characterizing plant seed oils will demonstrate the how chromatography and mass spectrometry can be used to provide a best available fingerprint of lipids in plant oils. Additionally, approaches for characterizing surfactants via shotgun mass spectrometry techniques can offer insights into variation across samples or lots based on relative quantitative data. For specific targeted assays requiring absolute quantitation, a systematic approach is usually built into the development process such that a sufficient level of quality is obtained prior to any method validation. A review of targeted liquid chromatography tandem mass spectrometry (LC/MS/MS) development projects for lipids and cannabinoids highlights the various quality checks evaluated during the development process.

338 Using Biology as Inspiration for Dynamic Optical Materials

Leila Deravi, Northeastern University, 360 Huntington Ave., Boston, MA 02115

We are developing systems, structures, and machines that can perform complex tasks or exhibit intelligence in response to external stimuli all using bio-inspired materials. Inspired by systems spanning from how tissues build themselves to how animals camouflage, I will discuss our molecular-level approach to building new materials that can produce controllable transformations in response to specific chemical inputs for applications ranging from colorimetric sensors to implantable electronics.

339 Multivariate Data Analysis for Cosmetic Formulations Powered by Umetrics

Greg Casee, Sartorius, Bohemia, 207 Tulip Ln, Freehold, NJ 07728

In cosmetic formulations, the relative concentrations of active, functional, and base ingredients, along with other additives, significantly impact final product characteristics, including appearance, texture, stability, and performance. Navigating these variables and their interactions can be challenging and time-consuming without specialized tools. Multivariate data analysis (MVDA) proves invaluable in the optimization process by analyzing interactions between various formulation components and their effects on the final product's attributes. Notably, design of experiments (DOE) and multivariate regression enable formulators to systematically explore the formulation space, identifying the ideal combination of ingredients and concentrations for desired product attributes. The Umetrics MODDE software, a DOE tool, offers a user-friendly experience to generate efficient experimental designs, analyze results, and optimize formulations. This presentation will exemplify how DOE expedites cosmetic formulation optimization and highlight potential pitfalls of not adopting a holistic approach. Leveraging multivariate data analysis techniques empowers cosmetic formulators with deeper insights and data-driven decisions, ultimately enhancing formulation efficiency and effectiveness.

340 Balancing Moisture: Karl Fischer Analysis in Cosmetic Formulations

Kerri-Ann Blake, Metrohm USA, 9250 Camden Field Parkway, Riverview, FL 33578

Water content is a critical factor in cosmetic product stability and safety, especially in emulsions and creams. Karl Fischer titration is a precise method for monitoring moisture levels, helping to prevent issues like microbial growth and texture problems. This presentation will delve into the principles, applications, and the significance of Karl Fischer analysis in maintaining high-quality cosmetic formulations.

DOWNLOAD THE OFFICIAL EAS 2023 APP FOR FREE

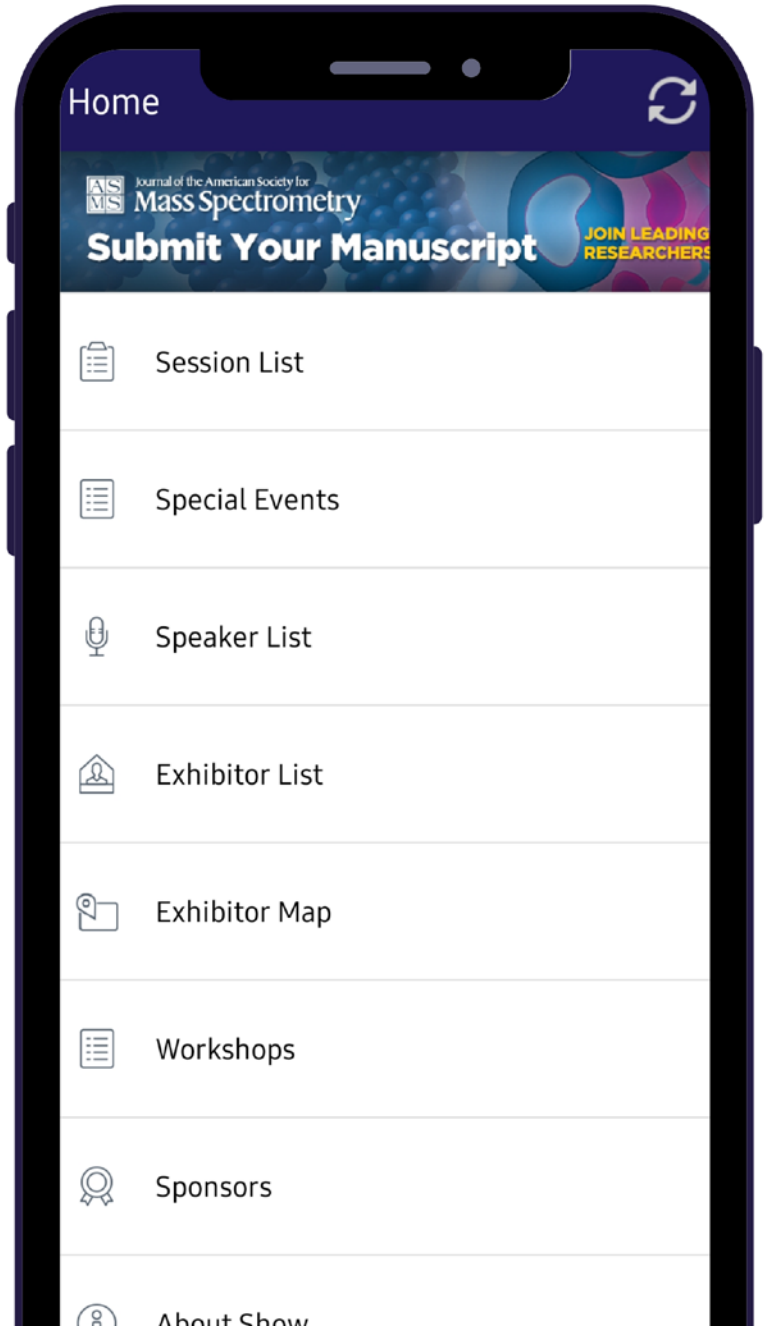
AVAILABLE ON APPLE + ANDROID

OR SEARCH:

"EASTERN ANALYTICAL SYMPOSIUM"
IN YOUR APP STORE

Android

iOS



Save Sessions

View Floorplan

Browse Speakers

Search Exhibitors

2024

November 18–20, 2024

EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION

Partners in Problem Solving

Crowne Plaza Princeton Conference Center, Plainsboro, NJ

THREE DAYS OF TECHNICAL PROGRAM
Hone your skills with technical sessions and short courses to connect you with experts from the field.

THREE DAYS OF EXPOSITION
Discover the latest advances in analytical technology and equipment.

CAREER DEVELOPMENT OPPORTUNITIES
Advance your career with workshops, employment bureau and networking events.

CALL FOR PAPERS MARCH 1, 2024
Oral & poster abstract submissions opens

eas.org