EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION

# Across The Analytical Spectrum: Diversity of Scientific Ideas

**November 17-19, 2025**

Crown Plaza Princeton - Conference Center
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# ABSTRACT BOOK

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# 2025 EAS Abstracts

This volume contains the final abstracts for the oral and poster presentations which take place Monday, November 17, through Wednesday, November 19, 2025. If an abstract is not provided in this volume, 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.

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**1** **Demystifying AUC: A Multi-Attribute Platform Technology for Biologics.**

Lake N. Paul, PhD., BioAnalysis, LLC, 3401 I St., Philadelphia, PA 19134

Analytical Ultracentrifugation (AUC) will mark its 100th anniversary in 2026. As one of the oldest classical separation techniques, AUC enables the characterization of macromolecules based on their molecular weight, shape, and diffusion properties. Although widely utilized through the mid-20th century, the adoption of chromatography-based methods such as size-exclusion chromatography (SEC) and high-performance liquid chromatography (HPLC) led to a decline in AUC usage and expertise. However, since approximately 2018, AUC has experienced renewed interest, driven largely by applications in cell and gene therapy development. AUC is a first-principles analytical method that requires no immobilization, labeling, or calibration against empirical standards. Sedimentation behavior is monitored using ultraviolet (UV) absorbance or Rayleigh interference optics. Unlike chromatographic methods, AUC avoids issues such as matrix interactions, buffer-dependent variability, and column stability concerns. This presentation will examine the advantages and limitations of AUC across discovery and current good manufacturing practice (cGMP) environments. Case studies will illustrate how AUC can function as a multi-attribute analytical tool for biologics characterization and in the QC environment.

**2** **AF4 Method Development for Vaccine Characterization**

Troy Halseth, Merck & Co., Inc., Vaccine Analytical Research and Development, 770 Sumneytown Pike, West Point, PA 19486

Vaccines include a variety of complex modalities such as protein-polysaccharide conjugates, virus-like particles, and lipid nanoparticles, giving rise to challenges in product characterization. Controlling for aggregation, an important attribute for vaccine quality and efficacy, requires analytical methods capable of evaluating the analyte in its native state. Size exclusion chromatography (SEC) is typically used for this analysis due to its widespread availability, robustness, and ease of use. However, interactions with the column stationary phase as well as high shear forces experienced when moving through narrow pores in the column can potentially alter the sample, leading to differences in parameters such as weight-averaged molecular weight and degree of aggregation. Asymmetric Flow Field-Flow Fractionation (AF4) is a size-based separation technique with no stationary phase, wherein an analyte's diffusion against a cross-flow determines its retention time, leading to a reversed elution order compared to SEC. The gentler approach of differentiating molecules provided by AF4 is an ideal method for characterizing large, complex particles that may form loosely associated aggregates or be prone to secondary actions with a column matrix. This presentation will cover basic AF4 theory, practical considerations for optimizing an AF4 separation, and case studies applied to different vaccine modalities.

**3** **A Tale of Tails: Using the Power of Capillary Electrophoresis for the Analytical Characterization of mRNA**

Deanna Di Grandi, Regeneron, 777 Old Saw Mill River Road, Tarrytown, NY 10591

The 3´poly(A) tail is an important component of messenger RNA (mRNA). The length of the poly(A) tail has a direct impact on the stability and translation efficiency of the mRNA molecule and is therefore considered a critical quality attribute (CQA) of mRNA-based therapeutics and vaccines. Various analytical methods have been developed to monitor this CQA. Methods like ion-pair reversed-phase liquid chromatography (IPRP-LC) can be used to quantify the percentage of mRNA with a poly(A) tail but cannot provide the resolution needed for longer lengths to offer information on the actual lengths and degree of heterogeneity. High-resolution methods, such as liquid chromatography coupled with mass spectrometry (LC-MS) or next-generation sequencing (NGS), can separate poly(A) tail lengths by one nucleotide (n/n + 1 resolution). However, these methods are complicated to implement for release testing of manufactured mRNA. In this study, an assay utilizing capillary gel electrophoresis (CGE) for characterizing the poly(A) tail length of mRNA was developed. The CGE method demonstrated resolution comparable to the LC-MS method. With UV detection and the use of an optimized set of size markers, this method can provide poly(A) tail length information and quantitation of each poly(A) length, making it a suitable release method to monitor the CQA of poly(A) tail length.

**4** **RNA on the Run: Sprinting to Separation with Ion Mobility**

Marcelino Varona Ortiz, Genentech, 1 DNA Way, South San Francisco, CA 94080, Chris Crittenden

Single guide RNA (sgRNA) is a critical component of CRISPR-based therapeutics. Extensive characterization of sgRNA is necessary to ensure efficacy and patient safety. One important characterization test includes full sequencing of the sgRNA. Our group has previously developed several different sgRNA sequencing approaches. In this presentation, we demonstrate sequencing and characterization of sgRNA using traveling wave ion mobility spectrometry (TWIMS) enabled by structures for lossless ion manipulation (SLIM) coupled with high resolution mass spectrometry (HRMS). A bottom up approach was employed which involved using 3 different ribonucleases to generate unique fragments. Digests generated by each ribonuclease were analyzed by SLIM-HRMS and provided structural and sequence information. The developed method enabled short run times (<5min) and full sequence coverage.

**5** **Understanding Olefin Content in Complex Samples via Multidimensional Separations**

Petr Vozka, California State University, 5151 State University Dr., Los Angeles, CA 90032, Genesis Barzallo, Hung Gieng, Ananya Sharma, Jyotika Patel

While the focus in fuel and chemical production has long centered on petroleum-based systems, the role of olefins has often been underappreciated. With the rise of chemical recycling of plastic waste, however, the need for accurate olefin characterization has become increasingly clear. This presentation revisits how olefin content has historically been determined, highlights the strengths and shortcomings of current analytical methods, and proposes future strategies for improved analysis. We developed a robust technique for quantifying olefins in fuels and products derived from plastic depolymerization by employing (only) comprehensive two-dimensional gas chromatography with flame ionization detection (GC×GC-FID) in combination with derivatization using disulfide reagents. This approach enables the selective relocation of olefins in the chromatographic space, allowing for their indirect yet precise quantification within complex mixtures.

**6** **Monitoring Time-Course Trends of food Spoilage in Tomato Samples with GCxGC and ChromaTOF Sync Alignment Software**

Elizabeth Humston-Fulmer, LECO Corporation, 3000 Lakeview Ave Saint Joseph, MI, 49085, Joseph Binkley

Non-targeted characterizations of complex food and beverage samples can be part of quality control or other problem-solving tasks and also have broad applicability for a range of discovery objectives for these sample types. In this work, the chemical profile and time-course trends related to food spoilage were investigated which can have relevance for shelf stability and food safety. A tomato sample was pureed and analyzed every day over the course of a week. These are complex samples with many chemical changes and analytes of potential interest, and powerful hardware and software tools were crucial for this work. Comprehensive two-dimensional gas chromatography (GCxGC), time-of-flight mass spectrometry (TOFMS), and advanced data analysis software tools were used. GC is a well-established technique for the separation of volatile and semi-volatile components of a sample. Extending the separation to two dimensions with GCxGC, improved the sample characterization and analyte coverage by increasing the peak capacity, allowing more individual analytes to be determined. TOFMS provided full mass range, sensitive detection to successfully identify the analytes. The ChromaTOF Sync 2D software facilitated the analysis of this detailed data by processing the sample set together to compile analyte information across the multiple samples. This helped to reveal important analytes, trends, and information about the spoilage process. This presentation highlights results for the tomato sample and also demonstrates this flexible and versatile workflow that could be applied to other time-course processes or for other non-targeted investigations of complex sample sets.

**7** **The Full Transcript: Comprehensive mRNA Profiling with an Online Multiplexed Multidimensional LC Platform**

Daniel Nguyen, Genentech, 1 DNA Way, South San Francisco, CA 94080, Kelly Zhang, Peter Yehl, Alexandre Goyon

Messenger RNA (mRNA) has emerged as a pivotal therapeutic modality in modern medicine, highlighted by its role in COVID-19 vaccines and its increasing applications in cell and gene therapies. Despite its promise, mRNA presents significant analytical challenges due to its high MW, polarity, dynamic size, and complex set of critical quality attributes (CQAs). Comprehensive characterization remains a demanding and resource-intensive task. To accelerate drug development, there is an urgent need for platform methods that can deliver multi-attribute analysis rapidly while minimizing sample consumption. This presentation introduces an online multiplexed multi-dimensional LC platform to simultaneously obtain information on mRNA sequence, concentration, aggregation, and poly(A) tail length and content. The platform's capabilities are enabled by evaluating the latest wide-pore size-exclusion chromatography (SEC) technologies and developing a novel online nucleotide mapping approach using hydrophilic interaction liquid chromatography (HILIC). Finally, we outline the potential expansion of this platform to assess additional CQAs, further supporting the development and quality control of mRNA therapeutics.

**8** **Implementing Multivariate Statistics for Analyzing Multidimensional Kombucha Data**

Sarah C. Foster, William & Mary, 540 Landrum Dr, Williamsburg, VA 23185, Ian Buhner, Laura Tipton, Katelynn A. Perrault Uptmor

Kombucha is a fermented beverage produced through the addition of a symbiotic community of bacteria and yeast (SCOBY) to a sugary tea. Despite the odors associated with kombucha, studies lack in understanding its chemical aroma profile. The mixture of volatile organic compounds (VOCs) from kombucha and its fermentation are examined through analytical techniques like gas chromatography (GC). Traditional GC separates analytes within a capillary column coated with a stationary phase, allowing analytes to separate based on their chemical affinity for the column coating. More complex samples benefit from the higher resolution and separation capacity of comprehensive two-dimensional gas chromatography (GC×GC) which

separates analytes based on their affinity for two distinct stationary phases. This study aimed to identify the aroma profile of Virginia kombucha products using comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry with flame ionization detection (GC×GC-TOFMS/FID). Samples were taken from 12 sources including two SCOBY, one starter tea, and eight kombucha teas. Samples were extracted via headspace solid phase microextraction (SPME) arrow and analyzed using GC×GC-TOFMS/FID. Data analysis resulted in a comprehensive view of the VOCs present in various kombucha products. It was possible to distinguish products from one another as well as differentiate the tea matrix from the microbial matrix. Preliminary analysis suggests detected compounds can be attributed to SCOBY microorganisms described in literature. This work builds on the overall aim of linking bacterial genetics, metabolism, and flavor profile in fermented foods as examined by advanced analytical techniques.

**9** **The Application of ATR-FTIR Spectroscopy for the Identification of Cosmetic Foundations on Cloth**

Jaden Force, Towson University, 8000 York Rd., Towson, MD 21252, Kelly Elkins

Cosmetics may be encountered as trace evidence in criminal investigations due to their ability to be transferred and the frequency with which they are worn. While several papers have been published on lipsticks as forensic evidence, a relatively limited number have been published on cosmetic foundations. Previous articles published on the forensic analysis of cosmetic foundations found Fourier transform infrared (FTIR) spectroscopy to be a fairly successful technique for the differentiation of various cosmetic foundations. However, an item of evidence such as clothing containing trace amounts of cosmetic foundation may look visually similar to an item that has been discolored after coming into contact with other commonly encountered sources such as food, beverages, and dirt. This project seeks to examine whether or not attenuated total reflectance (ATR) FTIR spectroscopy can differentiate between white cotton cloth discolored by cosmetic foundations and cloth discolored by other potential sources of staining, including other types of cosmetic products.

**10** **Decomposition Analysis Using Differing Data Processing Methods to Identify Volatile Organic Compounds and Provide New Ways to Use Water as Evidence in Casework**

Virginia Weina, William & Mary, 540 Landrum Dr., Williamsburg, VA 23185, Katelynn Perrault Uptmor

When a body decomposes, it emits volatile organic compounds (VOCs). Decomposition odor has been studied; however, more research is needed to understand decomposition VOCs coming from submerged remains. VOCs representing the decomposition process can be captured by water. Comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS) can be used to profile VOCs. Gas chromatography (GC) separates analytes based on their affinity for a column phase. GC×GC separates analytes based on their affinity for two column phases separated by a modulator to increase peak capacity for complex samples. Nine mason jars filled with pork belly and tap water were placed in three different temperature conditions (hot, cold, room) in replicates of three, including controls that contained no pork. The time trial lasted 12 days with 5 mL of water sampled from the jars each day until day 3, then every third day until day 12. Samples were analyzed using headspace solid phase microextraction (SPME) arrow and GC×GC-TOFMS with dual-stage cryogenic modulation. Data was processed using peak table-based software, batch alignment software, and tile-based software. The compound lists generated by each software were compared to determine which program is best suited for forensic investigation. The batch alignment software showed promise for forensic research for longitudinal data to show trends in VOCs that emerge over time. The tile-based class differentiation software was best suited to compare samples from submerged remains to water containing none. This type of comparison can provide forensic scientists with a method to locate submerged remains.

**11** **Illicit Pill Profiling: Chemical Trends in Fentanyl Tablets from the Southwest Border and Beyond**

Macenzie Powell, CFSRE, 206 Welsh Rd, Horsham, PA 19044, Mandi Mohr, Barry Logan, MJ Menendez, Mia Borrelli, Alexandra Kuchinos, Madison Schackmuth

Over the past five years, just over 2,500 suspected illicit tablets collected from the Southwest Border have been qualitatively and quantitatively analyzed to monitor evolving trends in fentanyl tablet composition. The primary objective of this ongoing research is to better understand the variability and potential public health effects of these tablets, by identifying adulterants and precursors used in the creation of these tablets. Quantitative testing revealed that the average fentanyl content per tablet is approximately 1.8 mg which is just under the Drug Enforcement Administration's estimated lethal dose of 2mg. Notably, several tablets contained more than 6 mg of fentanyl, demonstrating the unpredictable and dangerous nature of the illicit drug supply. Additional components were identified and quantified to provide a more complete profile of each tablet. Acetaminophen emerged as a major excipient, present at an average of 55mg per tablet and accounting for over half the tablet weight in many cases. Other frequently detected substances included metamizole and fentanyl precursors such as 4-ANPP. This research highlights the significance of detailed chemical profiling to understand the ever-changing illicit pill market. The variability in potency and formulation emphasizes the ongoing public health risk and reinforces the need for continued monitoring and harm reduction efforts.

**12** **Direct Analysis in Real Time – Mass Spectrometry as a Technique for Rapid Drug Analysis and Near Real-Time Surveillance of the Illicit Drug Landscape**

Elise Pyfrom, National Institute of Standards and Technology, 100 Bureau Dr., Gaithersburg, MD 20899, Edward Sisco, Meghan Appley, Elizabeth Robinson

Rapid insight into the illicit drug landscape is critical for law enforcement, public health, and forensic science entities to quickly and effectively assist in overdose prevention efforts, addiction treatment approaches, investigations, and community awareness. To accomplish this task, Rapid Drug Analysis and Research (RaDAR) program uses Direct Analysis in Real Time – Mass Spectrometry (DART-MS) as a rapid screening technique to identify drug compounds, adulterants, and cutting agents found in complex drug mixtures. Using minimal sample preparation and trace amounts of drug material, samples are received, extracted, and analyzed via DART-MS. Compounds are then identified using the NIST Data Interpretation Tool (NIST DIT), and results are disseminated within 24 hours. Despite having a robust library for compound identification using the NIST DIT, drug mixtures are becoming more complex, resulting in difficulty identifying unknown drugs and adulterants and requiring additional testing. To combat these difficulties, confirmatory analysis via Trapped Ion Mobility Spectrometry – Time of Flight (timsTOF) and Gas Chromatography – Mass Spectrometry (GC-MS) are used in tandem with DART-MS when an unknown compound is seen in a DART-MS sample spectrum. To date, several unknown compounds have been identified using these mass spectral techniques to provide rapid, near real-time surveillance of the illicit drug landscape to law enforcement, public health, and forensic science entities. In this talk, the presentation will discuss our method for sample extraction, analysis by DART-MS, data interpretation using the NIST DIT, and provide examples of how timsTOF and GC-MS assist with identification of unknown compounds in a complex drug mixture.

**13** **Assessing Drug Product Structure After Induced Stresses by Raman Spectroscopy and cIEF/MS**

Diana Novo, Johnson & Johnson, 1400 McKean Rd., Spring House, PA 19477, Colin Fitzpatrick, Riley Schaefer, Kristen Nields, Karin Balss

Large molecule pharmaceuticals undergo significant purification and formulation from first being expressed by a cell to their patient-suitable final state. Changes in concentration, buffer matrix, pH, temperature, and mechanical stressors inherent in this process as well as environmental stressors (e.g. UV exposure) all pose the risk of degrading product quality. The integration of smart sensors in the drug production stream offers the opportunity to continuously monitor biotherapeutics through various steps of the manufacturing process and ensure that product quality is maintained at each point. Raman spectroscopy is explored as a process analytical technology to analyze monoclonal antibody samples which have been subjected to environmental stressors. A multivariate analysis technique is used to build a surrogate quality model to distinguish between nominal and degraded protein and identify the potential stressors. A comparison of the model and complimentary analytical methods (HPLC), capillary isoelectric focusing (cIEF), and mass spectrometry is discussed.

**14** **Monitoring the Imidization of Poly(amide acid) Films by TGA-IR**

Michael Hall, SABIC, 1 Noryl Ave., Selkirk, NY 12158

Polyimide films provide desirable attributes for use in flexible electronics applications including: thermal stability, chemical resistance and dielectric properties. In this investigation, polyamide acid films were cured in a Thermal Gravimetric Analyzer (TGA) equipped with an IR detector. The rate of conversion to the polyimide was found to be sensitive to the temperature program employed. In addition, the liberated volatiles were quantified via external calibration approaches and validated by Thermal desorption GC/MS.

**15** **Regenerative Electroactive Self-Assembled Layers: A Spectroscopic and Mechanistic Investigation on Reversible Non-Covalent Interactions**

Nico Maldonado, University of Chicago, 929 E. 57th St., Chicago, IL 60637, Caroline Hou, Anna Wuttig

Electrochemical transformations central to energy conversion, storage, and molecular sensing occur at electrified interfaces. Molecular-level control over these interfaces is traditionally achieved through electrode-specific covalent tethering of molecules, which inherently limits the scope and potential stability window of molecularly tunable electrodes. To overcome the limitations of such linkers, the Wuttig Group recently reported an approach that leverages non-covalent electrostatic and

van der Waals interactions of amphiphiles to effectively immobilize a tethered redox-active probe molecule (ferrocene) at electrode interfaces. Since these layers are not permanent, i.e., they are easily removed upon rinsing—we hypothesized that effective immobilization arises from reversible and dynamic exchange of amphiphile monomers. In this talk, I will disclose our recent results that demonstrate that the dynamics of reversible self-assembly can be harnessed to achieve regenerative electrode modifications. We quantify the kinetics of monomer assembly and disassembly at electrode surfaces using voltammetry, isotope-labeling studies, and in situ surface-enhanced infrared absorption spectroscopy. We intentionally degrade the self-assembled layers and find that shorter tail lengths increase monomer lability. Amphiphile structures with slow dynamics lead to electrode fouling, while those with fast dynamics enable the non-covalent layer to regenerate and recover from redox-triggered degradation. Together, this work presents a new molecular-level design concept to enhance durability in electrochemical applications.

**16** **Capturing Transformations in Soft-Matter via Mashups of Rheology, Raman Spectroscopy and Optical Microscopy**

Kalman Migler, NIST, 100 Bureau Dr., Gaithersburg, MD 20899

The quantification of kinetic transformations in material systems frequently requires the use of multiple measurement methods. However, when measurements are carried out on independent analytical instruments, the measurement particulars may influence the kinetics thus casting uncertainties on the results. We have thus developed a hybrid instrument, a rheometer-Raman-microscope (rheo-Raman). It is particularly advantageous in situations where viscosity or moduli vary due to changes in chemical or conformational changes in molecular structure, such as in crystallization, melting, gelation, or curing processes. Here, we demonstrate how we extract fundamental insights into kinetic transformations on two important polymeric systems: chemical crosslinking in epoxies and conformational changes in thermoplastics via flow/temperature fields. Future enhancements will be discussed.

**17** **Instrumentation and Algorithms for Photothermal Infrared Hyperspectral Microscopy**

Garth Simpson, Purdue University, Department of Chemistry, 560 Oval Dr., West Lafayette, IN 47907

Photothermal infrared microscopy enables determination of IR absorption with spatial resolution dictated by a visible-light probe beam. Different modalities and sources present unique opportunities and challenges for integration of sparse-sampling image reconstruction approaches supporting hyperspectral microscopy. Recent advances will be reviewed, along with a critical assessment of the current state of the art and future growth opportunities.

**18** **Machine Learning and Hyperspectral Imaging to Accelerate Pharmaceutical Process Development**

Joseph Smith, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486

Developing manufacturing processes to globally supply medicines and vaccines is essential to advancing and improving human health. Maximizing the knowledge gained during each experiment conducted during pharmaceutical process development is critical for successfully delivering an innovative, robust commercial process. In this work, we share a novel technology using machine learning and hyperspectral imaging for analysis of human papillomaviruses (HPV) vaccine self-healing particles (SHPs) to enable pharmaceutical process and product development. HPV is a highly prevalent virus and is known to cause a variety of diseases, including cervical cancer. Because of the risks associated with HPV, Gardasil® and Gardasil®9 were developed by Merck & Co., Inc., Rahway, NJ, USA and approved by the Food and Drug Administration, providing significant protection against HPV. The HPV vaccine may be given as 2- or 3- doses; however, vaccine administration as a single dose with a sustained release mechanism may potentially offer benefits to meet emerging health needs. To explore this, HPV vaccines were formulated within microporous SHPs to enable potential controlled release of HPV virus-like particle (VLP) antigen. Machine learning, in the form of multivariate curve resolution-alternating least squares, with Raman hyperspectral imaging was used to determine the molecular identity and spatial distribution of all relevant species within this HPV vaccine formulation. Results indicate machine learning with Raman hyperspectral imaging was able to spatially resolve HPV VLP antigens within SHP vaccines for the *first* time, providing crucial information necessary for vaccine development.

**19** **Clustering Hyperspectral Spectra via Subspace Clustering**

David Hong, University of Delaware, 314 Evans Hall, Newark, DE 19716, Suhas Cristy Mathey, Roxanne Radpour

Hyperspectral imaging (HSI) plays an important role in the analysis of paintings in conservation science. An important task in analyzing these artworks is to create a map of where different pigments were used in the painting. Since HSI obtains a spectrum across many wavelengths at each pixel, it provides a wealth of information for this task. However, most conventional workflows are currently only semi-automatic and require an extensive amount of expert intervention, making it challenging for analysts to keep pace with the ever increasing amounts of data being collected. This talk describes recent work on applying methods from subspace clustering to accelerate these analyses by more robustly automating the mapping of pixels to pigments. The key idea is that pixels with the same pigment tend to have spectra with similar characteristic shapes and consequently lie in a corresponding low-dimensional subspace. Subspace clustering methods seek to divide the spectra into groups with common low-dimensional structure. Subspace clustering is an unsupervised machine learning approach and does not rely on pre-existing libraries or catalogues of characteristic spectra, which may not always be representative of a given artwork. Instead, we obtain both mappings of pixels to pigments together with characteristic spectra for each pigment. Preliminary results suggest this is a promising approach for automatic pigment mapping for art conservation.

**20** **Mapping Strain Effects in 2D $Ga_2Se_2$ via Hyperspectral Imaging**

Lottie Murray, University of Delaware, 201 Dupont Hall, Newark, DE19716, Helder Carneiro, Caelin Celani, Eric Herrmann, Karl Booksh, Xi Wang, Matthew Doty

Quantum photonic technologies require atomic-like quantum emitters to serve two crucial functions: providing a deterministic source of single photons and hosting a matter-based qubit able to be manipulated via light. To integrate quantum emitters into existing devices and applications, there is a need for a platform that produces deterministic and reliable quantum emitters. In this work, we investigate gallium selenide ($Ga_2Se_2$) due to its strain-tunable bandgap, which holds promise for integration into next-generation quantum devices. Once thin $Ga_2Se_2$ flakes are transferred onto patterned substrates, spatially resolved photoluminescence (PL) mapping is employed to characterize the effects of strain on the material's bandgap. This approach enables the collection of robust, high-resolution datasets. To further understand the relationship between induced strain and PL emission peak shifts, we combine machine learning techniques with physics-based simulations. Additionally, predictive modeling informed by experimental data is used to reduce the reliance on computationally expensive simulations and to estimate strain distributions across the sample.

**21** **Mitigating Kidney Toxicity Risk and Prioritizing Drug Candidates Using MALDI Mass Spectrometry Imaging**

Bingming Chen, Merck & Co., Inc., 126 E. Lincoln Ave, Rahway, NJ 07065

Nephrotoxicity caused by drugs presents a significant obstacle in the fields of drug discovery and development, contributing to nearly 25% of serious adverse effects in current pharmacological treatments. The use of antimicrobials is particularly linked to this issue, with approximately one-third of nephrotoxic cases associated with these medications. During the lead optimization phase of our antibacterial programs, we observed nephrotoxicity characterized by degeneration of renal tubules and the presence of tubular granular casts. To investigate the mechanisms behind nephrotoxicity and prioritize compounds, we employed matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to analyze the distribution of compounds in rat kidney sections. MALDI-MSI has proven to be an effective method for spatially localizing drugs and their metabolites directly from tissue surfaces without requiring labels. Our comparison of the renal distribution patterns of toxic versus non-toxic compounds revealed a significant correlation between distribution in the renal cortex and outer medulla and the occurrence of nephrotoxicity in vivo for most tested drug candidates. This correlation has enabled us to rank compounds, thereby enhancing the lead optimization process in antimicrobial drug discovery. We believe that MALDI-MSI can serve as a valuable tool for mitigating the risk of drug-induced nephrotoxicity during drug discovery and development, particularly when a link between tissue distribution and nephrotoxicity is established.

**22** **Targeted High Spatial Resolution Metabolite Imaging using DESI on Tandem Quadrupole MS**

Bindesh Shrestha, Waters Corporation, 28 King St, Swampscott, MA 01907

Desorption electrospray ionization (DESI) mass spectrometry imaging (MSI) complements MALDI by easily enabling mapping of metabolites and drugs that are not often detected by MALDI. As interest grows in cellular-level imaging, there is increasing demand for imaging TCA cycle metabolites critical to cellular metabolism. However, reducing pixel-size decreases the amount of analyte, making it more challenging to detect many metabolites. As pixel-size decreases, the total number of pixels also increases exponentially, significantly extending imaging time. Tandem quadrupole (TQ) mass spectrometers (MS) are renowned for sensitivity and speed. Here, we present a higher spatial resolution DESI-TQ-MS (e.g., Xevo™ TQ Absolute XR with DESI XS) for imaging metabolites at cellular-level. To evaluate the high-resolution capabilities, targeted DESI images of lactate, glutamine, glutamate, arachidonic acid, and PI (38:4) were acquired from liver tissue at pixel sizes of 50 µm, 10 µm, and 5 µm. Image fidelity and resolution were manually assessed by observing improvements in image quality. Targeted list of TCA cycle metabolites (pyruvate, lactate, fumarate, succinate, malate, α-ketoglutarate, glutamine, glutamate, cis-aconitate, citrate) were imaged in mouse brain at 20 µm. Using the same approach metabolites

were imaged at 5 µm in mouse retina. To address long imaging runs, a timesaving data-driven reacquisition strategy was implemented - a lower resolution acquisition followed by high-resolution imaging of regions of interest on the same tissue or a serial section. The combination of low-flow DESI and the high sensitivity of tandem quadrupole MS enables improved spatial resolution for detecting low-abundance metabolites, advancing toward cellular-level imaging.

**23** **Implementation of DESI-MSI as a shared resource for untargeted and targeted small molecule analysis**

Christina Ferreira, Purdue University, 3229 Edgerton St, West Lafayette, IN 47906

Over the past fifteen years, desorption electrospray ionization mass spectrometry imaging (DESI-MSI) has evolved from a proof-of-concept technique into a powerful analytical platform for spatially resolved metabolomics and lipidomics. At Purdue University, sustained method development has enabled both untargeted and targeted small molecule imaging across a range of biological and material specimens. This presentation will highlight key technical advances in DESI-MSI, including improvements in spatial resolution, acquisition speed, and annotation workflows, as well as integration with tandem mass spectrometry and ion mobility for enhanced chemical specificity. A central focus will be the transition of DESI-MSI from a specialized technique to a broadly accessible shared resource at Purdue. This includes the establishment of standard operating procedures, quality control protocols, and tailored user support to facilitate robust data acquisition and interpretation. Training materials developed for new users include hands-on workshops, annotated datasets, and structured modules covering experimental design, sample preparation, instrument operation, and data analysis. The presentation will also feature selected interdisciplinary projects enabled by DESI-MSI, including spatial lipidomics of brain tissue in neurodegeneration models, neurotransmitter mapping, and nanoparticle toxicity. Together, these efforts exemplify the versatility of DESI-MSI and its value as a shared resource supporting hypothesis-driven research and translational discovery.

**24** **Investigation of Localised Tissue Immune Response to Intramuscular Drug Treatment with a Mass Spectrometry Imaging-Based Spatial Multi-Omics Approach**

William Hardesty, GSK, 1250 S Collegeville Rd., Collegeville, PA 19426, Fang Xie, Heath Patterson, Wanqiu Zhang, Maria Mantas, Eliot McKinley, Alice Ly, Nico Verbeeck, Marc Claesen, Reid Groseclose

Measuring the quantitative distribution of an intramuscular injected drug and the local immune response can improve our understanding of immunogenicity, temporal progression, and overall safety profile. A strength of mass spectrometry imaging (MSI) is the ability to quantitatively track the distribution of drug compounds within tissues over time. Highly multiplexed protein imaging approaches such as imaging mass cytometry (IMC), can measure the spatial distribution of different cell populations and phenotypes. In this study, these two complementary spatial omics methods, MALDI MSI and IMC, were used to quantify the distribution of drug components and resulting immune response over time at the site of intramuscular injection and local draining and non-draining lymph nodes. As the IMC and MALDI- MSI data were acquired on different platforms, we built a bioinformatics pipeline and accompanying user interfaces that allowed integration and simultaneous visualization of the MALDI-MSI and IMC data as well as the digitized histological images, accommodating the different spatial resolution of each modality. The co-registration of IMC and MALDI-MSI data from serial sections enabled us to spatially correlate the distribution of the drug metabolites to the immune microenvironment responses for the first time. When combining the MALDI-MSI and IMC datasets with the histology, patterns between the distribution of the drug compounds and different cell populations could be observed. These results give insights into the mechanism of action and localized immune response to drug treatment.

**25** **Resolving Conflict and Building Positive Lab Relationships**

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

Addressing conflict around the lab can be difficult and frustrating. Most lab managers have little or no training around conflict resolution and need to grow this skill to be effective leaders in their organizations. It is a big change for most people moving from being a participant or an observer of lab conflict to having the responsibility to resolving conflict the skills to mediate conflict through a win/win lens helps build stronger relationships across the lab. Effective conflict management requires a variety of skills. This presentation will highlight several skills that will be beneficial to both new and experienced lab managers: Surfacing conflict; Investigation and root cause analysis; Exploring hurt feelings; Exploring win/win outcomes; Engaging in positive, healthy debate to find the best options for the lab.

**26** **The Changing Workforce: New Facets of Old Problems**

Catherine Smith, Arkema, 900 First Ave., King of Prussia, PA 19406

Some of the problems that managers face are eternal – how to recruit the best talent, how to retain good employees, and how to best utilize the available resources, for example. But as technology advances and the world changes, those same old problems show new facets – Are you offering the same WFH flexibility as competitors? What does work/life balance look like in a world with increased connectivity? When is it appropriate to use AI? Laboratory managers need to understand how the world is changing, how these changes impact operations, and how they impact people. This lecture will discuss some of the ideological shifts we are seeing in the workforce, and ways we can use that knowledge to better support our teams.

**27** **Dealing with Difficult People**

Dwayne Henry, Montgomery College, 7600 Takoma Ave., LB108A, Takoma Park, MD 20912

Dealing with difficult people in the workplace can be one of the most frustrating aspects of management. Not only can it stifle productivity, but it can negatively affect the atmosphere and culture of the work environment. The true question is: Is it the person that is being difficult, is it the person's behavior that is creating a difficult situation, or am I the difficult person? This session will include (but not be limited to) discussions of strategies for dealing with difficult people, the difference between difficult people and difficult situations created by people (as well as how to deal with those situations), and ways to recognize our own behaviors that may be contributing to the difficulty. Lastly, as part of these interactive discussions, we will also examine changes that can be made to help create successful outcomes.

**28** **Keeping Up Morale in the Lab**

Scott Hanton, Lab Manager, 26458 Harrow Ct., South Lyon, MI 48178

Lab work can be challenging and difficult. Lab managers can take specific actions to help staff remain motivated and engaged when the work is hard and the solutions are slow to develop. The more lab managers can help staff make progress, have some control over their work, and address their basic needs, the more staff will retain their motivation and morale in the lab. It is also important for lab managers to avoid actions that will demotivate staff. This presentation will explore the different ways to improve morale, including the following: 1) Enable daily progress; 2) Identifying and avoiding demotivators; 3) Helping staff exert control over their work; 4) Developing staff skills to increase mastery; 5) Ensuring a clear understanding of purpose; 6) Delivering key staff needs to improve employee engagement.

**29** **Don't Ignore These Warning Signs of Potential Phase 3 Analytical Validation Failures**

Michael Spangler, Spangler Consulting LLC, 6 Martin Ct., Martinsville, NJ 08836

Early analytical methods (especially HPLC methods) for pharmaceutical analysis of drug substances and drug products are often developed quickly by small Sponsors at a vendor laboratory (CDMO). Phase appropriate first generation methods are developed and qualified during API discovery synthesis, early drug product formulation development, and preparing for toxicological batch release. There are sound reasons not to challenge these methods too hard or change them too much during Phase 1 and Phase 2. Conversely there is great business value to ensure that the Phase 3 analytical method validations succeed on the first attempt, with the first validation protocols. Phase 3 validations should not begin without a thoughtful assessment of risk. This presentation identifies specific risk factors that warn of potential validation failures during the more rigorous Phase 3 validation exercise. Warning Signs include historic qualification or validation failures. If the method robustness has not been previously systematically challenged. Or if the method has not performed well in Phase 1 and Phase 2 real-world use. A procedure finicky to run or requiring specialized manual analyst techniques presents risk. If validation materials are limited or if the placebo is complex, these are risks. Has the method has ever been Tech Transferred into a new laboratory? Sometime the stability of stressed API and drug product have not been scrutinized with an optimized analytical method. Objectively assessing your confidence in the analytical laboratory validating the method is also informative in managing validation risks. Strategies to mitigate these Phase 3 validation risks are emphasized.

**30** Withdrawn by the author.

**31** **Mapping Key Elements in the Current ICH and USP Guidances to an Enhanced Workflow for Analytical Procedure Development**

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

The new USP guidances, including <621> and <1220>, and ICH guidances, including Q2(R2) and Q14, 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 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 with production, 2) correct integration of robustness simulation into method optimization to

efficiently establish a true, multi-dimensional, robust Method Operable Design Region (MODR), 3) the critical integration of Replication Strategy optimization, which includes USP <1210> interval metrics, to identify the most efficient strategy for generating Reportable Results which meet the method precision requirements defined in the ATP, and 4) using the established MODR, the optimized replication strategy, and bi-directional experiment automation in method transfer. These presentation topics are described in the context of the development, validation, and transfer of a liquid chromatography method.

## 32 Simultaneous Targeted, Non-Targeted Per- and polyfluoroalkyl Substances (PFAS) Screening as Part of Extractables Screening for Pharmaceutical Packaging, Manufacturing Components, and Medical Device by LC-HRAM-MS

Rajesh Chennam Shetti, SGS Pharma, 75 Passaic Ave, Fairfield, NJ 07004, Sven Hackbusch, Chongming Liu, Dujuan Lu, Mark Rogers, Sebastien Morin, Jon Bardsley

Per- and polyfluoroalkyl substances (PFAS) are known for their persistence in the environment and human body, leading to potential health issues. Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals characterized by strong carbon–fluorine bonds and polar head groups. Their long-term stability and resistance to biological and environmental degradation have led to their classification as emerging contaminants of concern, due to their association with adverse health effects. However, there is currently no regulatory guidance on acceptable PFAS levels in pharmaceutical products and medical devices, potentially compromising the safety and efficacy of drug products. Therefore, the ability to detect and quantify PFAS in pharmaceutically relevant test materials is essential. In this study, we present an LC-MS–based analytical strategy for both targeted and non-targeted PFAS analysis, aimed at detecting compounds extractable from manufacturing components and container systems as part of extractables screening. To demonstrate the utility of this method, its sensitivity and linearity were established, and it was applied to evaluate extracts from two fluorine-containing polymer components.

## 33 Optimizing Trace Metals Analysis: Advanced Sample Preparation and Microwave Digestion Techniques for Food and Environmental Samples

Alicia Stell, CEM Corporation, 3100 Smith Farm Rd., Matthews, NC 28106, Elaine Hasty, Macy Harris

Accurate trace metals analysis is essential for producing reliable analytical results. This requires not only proper sample preparation but also the selection of appropriate equipment and methodology. Trace metal impurities, commonly found in food and environmental samples, pose potential health and safety risks. The "big four" heavy metals—arsenic, cadmium, lead, and mercury—are particularly concerning and are subject to strict regulatory limits. Therefore, achieving precise trace metal measurements is crucial for ensuring compliance. This presentation explores recent advancements in digestion technology, focusing on flexible, purpose-driven solutions designed to quickly optimize conditions across a wide variety of sample types, from routine to more complex, challenging matrices. By establishing ideal digestion parameters rapidly, laboratories can enhance workflow efficiency, enabling high-throughput batch processing or sequential workflows tailored to diverse sample requirements.

## 34 Batch Process Understanding (BPU) Refines a Method for Process Patent Protection (PPP) via Natural-Abundance Stable Isotopes

John Jasper, Molecular Isotope Technologies, LLC, Niantic, CT 06357, Anthony Sabatelli, Ann Pearson

Serendipitous discovery of what became known as "Batch Process Understanding (BPU)" (Jasper *et al., Pharma* Mfg. April 11, 2024:1-4) combined with our earlier patented work on Process Authentication (Sabatelli *et al*., 2017, *Org. Proc. Res. Dev*, 21(7):956-965) reveals a refined method of Process Patent Protection (PPP). In hindsight of a successful $1.2 billion process patent infringement case, we isotopically determined that the batch records provided for the case had been compromised by batch-record shifting and batch mixing (viz., ~30% mass carryover). With that, we assert that initial stable-isotopic analysis of key synthetic components would allow an objective determination of the actual reactant-to-product batch relationships, thereby testing the validity of written batch records. Clear reactant-to-product relationships would provide a record that could confidently be assessed for process patent infringement. With the insight of BPU, we can now more accurately determine the validity of batch records, thereby focusing cases of process patent protection which may have previously been overlooked, compromising billions of dollars in potential patent protection. These isotopic analyses are relatively fast, inexpensive, and highly informative. We will give a prospectus of natural-abundance isotopes in (bio) pharma manufacturing processes in context.

## 35 A New Form of Protein Aggregates: An Early Warning Sign for Polysorbate Degradation in Biologic Pharmaceuticals

Laura Philips, Spheryx, Inc., 330 E. 38th St., #48J, New York, NY 10016, Juliana Lumer, Valentina Flores-Montes

Aging surfactants produce free fatty acids that can induce formation of dangerous protein aggregates. Protein aggregates, however, can form as a result of other conditions, including handling and thermal or pH changes. Total Holographic Characterization® (THC) detects a unique peak at the index of refraction characteristic of protein aggregates in the size range of 1-3 mm only when polysorbate degradation products are present. The distribution of protein aggregates exhibits standard aggregation behavior when other stresses such as shaking, heating or freezing are applied. This unique feature is induced only in the presence of degradation products of PS80 or PS20. THC is used to distinguish individual sub-visible contaminants in model biologic pharmaceuticals, ranging in size from 0.5 µm – 10 µm. All measurements are performed on xSight. In biologic formulations different contaminants are distinguished by their different refractive indexes. THC uses Lorenz-Mie Theory to analyze holograms of individual sub-visible particles. The analysis yields the size distribution of particles and simultaneously determines the composition from their refractive index. Hologram symmetry provides information about the morphology of the particles. Spherical particles, such as those of emulsion droplets or air bubbles are distinguished from irregularly shaped particles, by the symmetry of their holograms. Particles that are identical in shape, such as innocuous silicone oil emulsion droplets and dangerous degradation products of surfactant degradation, can be easily distinguished by their refractive indexes. In addition, xSight meets all of the requirements for 21 CFR Part 11.

## 36 Single-Sided NMR as a Tool for Non-invasive Oil Classification and Quality Control

Katelyn Blair, University of Delaware, 210 South College Ave., Newark, DE 19711, Levi Bielewicz, M. Fernanda Delgado, Jocelyn Alcantara-Garcia

Accurately tracking the freshness and quality of edible oils is key for industrial and commercial success as well as food safety. However, quality control relies on destructive sampling, often accompanied by time-consuming analyses. This work investigates the use of portable single-sided nuclear magnetic resonance as a non-destructive alternative. The mobile universal surface explorer (MOUSE) helped to track oil freshness by measuring the longitudinal (T1) and transverse (T2) relaxation times through the bottle. The MOUSE was able to differentiate across some of the most common edible oils (e.g., avocado, corn, olive, etc.), by showing their characteristic relaxation times. Since measurements can be done in situ and without opening the bottle, the methodology can potentially save time and reduce costs. With the technique being portable, testing can happen in warehouses. Ongoing research focuses on olive oil, as it is heavily regulated under established parameters. The best-quality product is significantly more expensive, and a spoiled batch of high-end oil can have drastic consequences, especially for smaller producers. By enabling producers to track the quality and freshness of the product through sealed containers, they can potentially streamline their quality control. Our results demonstrate that NMR-MOUSE relaxation measurements provide a sensitive, accurate, portable, and non-destructive tool for oil classification with broad applications in analytical chemistry and food science.

## 37 Raman-Based Identification and Quantification of Microplastics in Food and Agricultural Wastes

Guangyu Zhu, NJIT, Colton Hall, Ste 200. University Heights, NJ 07102, Sowmya Atukuri, Jiahui Hu, Hui Mu, Wen Zhang

The pervasive presence of microplastics in industrial and agricultural waste streams raises concern for environmental contamination. In this study, Raman spectroscopy and mapping were employed to detect and identify microplastics in real-world samples, including manure, food waste, yard waste, wood materials, and silage. Microplastics were separated via digestion, flotation, and filtration, enabling initial visual screening under optical and fluorescence microscopy. Samples (e.g., nylon filters with suspected particles) were analyzed by point-mode Raman to generate spectra, which were compared with a spectral library to confirm polymer identity. A total of 53 solid waste samples were analyzed, revealing ethylene-vinyl acetate (EVA) copolymer as the most frequently identified polymer, particularly dominant in manure, food waste, and yard materials. Other polymers included ethylene-acrylic acid (EAA) copolymer, polyethylene (PE), vinyl chloride-vinyl acetate (VC/VAc) copolymer, polystyrene (PS), and polyamide resin. EVA in manure and food waste likely originated from feed and packaging films, while PE and polyamide were traced to sawdust and kitchen residues. Raman mapping showed particle concentrations ranging from 0.09 to 4.44 particles·$g^{-1}$ dry solid, with the highest in household leaf waste and food scraps. Yard and food-related waste generally showed higher concentrations than manure or hay. Density ranged from 0.34 to 1.03 particles·$cm^{-2}$, allowing comparative assessment of surface-level prevalence. Cost analysis, including materials, instrumentation (~$50/h for Raman mapping), and labor, estimated $300–$1500 per solid sample. These findings provide evidence of microplastics in

food- and agriculturally relevant waste and offer new insight into the costs of managing such contamination.

**38** Withdrawn by the author.

**39** **Monitoring Oxidative Degradation of Desmosines by MALDI MS2 Ion Trap Approach with Chemical Derivatization**

Muhammad Ali, CUNY York College, 94-20 Guy R. Brewer Blvd., Jamaica, NY 11451, Emmanuel Chang

Desmosine and isodesmosine (collectively called 'desmosines') are crosslinking amino acids unique to mature elastin. They have been suggested as possible biomarkers for several diseases that include COPD, Marfan syndrome, cystic fibrosis, aortic aneurysm, etc. Our work involves subjecting desmosines to copper-type fenton reaction and monitoring its oxidative degradation with time. The quantification of desmosines is performed with a novel MALDI MS2 method that involves chemical derivatization of desmosines, fragmentation of analyte and internal standard and quantifying by using fragment intensities and plotting normalized response, NR as a function of time. MALDI MS2 quantification involves preparing internal standard in-house by incubating desmosines with labeled acetic anhydride-d6 and leaving to room temperature for 1 hour to acetylate amino groups. A time course measurement is performed whereby desmosines are diluted with water, hydrogen peroxide and CuSO4. MALDI assay was validated by an LC-MS assay that involved utilizing peak areas for quantification. Peak areas were taken at each timepoint in LC-MS and normalized relative to the initial peak area. Both MALDI and LC-MS show consistent degradation of desmosines under oxidative conditions with time. Our method offers fast, accurate quantification of desmosines with a simple step to synthesize internal standard in-house without the use of complex and long organic steps for desmosine internal standard synthesis.

**40** **An In-Depth Analysis of Mass Spectrometry Data: Decoding the Noise**

Zuliana Loaiza, Clarkson University, 17 Gary Lane, Orangeburg, NY 10962, Ulfet Erdorgan, Taniya Jayaweera, Pathea Bruno, Costel Darie

Proteomics uses mass spectrometry (MS), a powerful tool that analyzes compounds using either positive ionization through protonation of their amino groups or negative ionization through deprotonation of their carboxyl groups. In addition, a compound (or a peptide) can be protonated by one, two, three, or more hydrogen ions, or by Na ions, or by K ions, or by a combination of them. Here, we describe how to interpret the MS data using Glufibrinopeptide as an example. Both MS and MSMS data are discussed.

**41** **Re-Development and Optimization of a Liquid Chromatographic Method for Purity Impurity and Assay of a Small Molecule Pharmaceutical Intermediate with Enhanced Robustness**

Xiaomei Zhou, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901, Yan Zha, Peter Tattersall

To enhance analytical method robustness for a small molecule in the BMS portfolio, we re-developed and optimized a liquid chromatography (LC) method for assessing purity, impurities, and assay of its pre-penultimate intermediate. The previous method, designed for multiple intermediates, was not tailored for optimal impurity profiling at this stage; it used a high organic solvent content (35% methanol, 9% acetonitrile), which caused poor retention and peak shape for early-eluting impurities. Additionally, reproducibility and column robustness issues prompted us to refine the method. Our redevelopment focused on optimizing buffer concentration, pH, and gradient, as well as addressing method specificity, which was found to be sensitive to pH. We also challenged the method with additional impurities to evaluate its comprehensiveness. Using DryLab simulation, we modeled three-dimensional operating ranges (pH, gradient and temperature) for key LC parameters, guiding effective optimization. The improved method enabled the detection of a previously unobserved impurity (0.05%-0.30%), which had co-eluted with residual toluene on the old method, thus revealing enhanced sensitivity and specificity. A confirmatory robustness evaluation was conducted, examining chromatographic conditions such as gradient, instrument type, column selection, wavelength, and solution stability—now including the newly detected impurity. This demonstrated the new method's robustness, enabling its successful validation for the Process Performance Qualification (PPQ) campaign and suitability for specification setting for this chemical intermediate.

**42** **Optimization of Universal Detection Methods for Lipid Nanoparticles**

Andrew Steere, Waters Corporation, 34 Maple St., Milford, MA 01757, Norris Wong, Jennifer Simeone, Paula Hong

Lipid nanoparticles (LNPs) are a drug delivery mechanism that has gained popularity in recent years due to use in mRNA vaccines. LNPs are comprised of four components that encapsulate RNA for delivery into cells. These components must be present in specific ratios to properly control potency and efficacy. Traditional UV based methods of quantitation are not suitable for LNPs because many of the lipid components lack the necessary chromophores for UV detection. For this reason, universal detection is commonly used for LNP analysis. The two primary forms of universal detectors for LNP analysis are evaporative light scattering detection (ELSD) and charged aerosol detection (CAD). Due to the differing mechanism of detection, CAD is typically more sensitive at low analyte concentration and has tools to improve linearity. In this study, a formulation of cholesterol, DSPE-PEG 2000, and HSPC is used as a representative LNP. The ASTM International method will be used and compared across HPLC and UHPLC platforms using CAD and ELSD detection. The application was originally developed for use with an HPLC system with CAD detection and recommends that CAD parameters should be re-optimized when migrating to UHPLC. As the application does not originally include UHPLC or ELSD, method optimizations were performed to include these systems and modules in the evaluation. The results are compared across detectors to examine the differences in sensitivity (limit of detection), accuracy, and linearity.

**43** **Impact of Impurities in Raw Materials on the Impurity Control Strategy for Drug Substance: A Case Study from Nemtabrutinib Synthetic Route**

Hanlin Luo, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065

Nemtabrutinib is a Bruton's tyrosine kinase inhibitor for treating multiple B-cell malignancies like chronic lymphocytic leukemia. Merck developed a new synthetic route to achieve higher yield and process simplification in support of PPQ. Diisopropylethylamine (DIPEA ) is introduced into the amidation step, which was soon proven responsible for API impurities: hydroxylamine impurity and ethylisopropylamine impurity. Because hydroxylamine impurity cannot be purged from EOR to dry cake and is structurally labeled as a potential Class 3 mutagenic impurity, it is paramount that the DIPEA raw material quality is critical to ensure the Nemtabrutinib quality. The root cause of the ethylisopropylamine impurity was determined to be ethylisopropylamine in the DIPEA. However, the source of the hydroxylamine impurity was not readily discernible. By screening DIPEA batches and ruling out known intrinsic impurities, a new unknown impurity: Nitrone was discovered and identified by GC-FID and GC-MS. The nitrone level in DIPEA was confirmed to be positively correlated to the hydroxylamine impurity level in API. Additionally, Nitrone is a low-level impurity (< 0.06%area) within DIPEA, and its level varies among different vendors and storage. A series of DIPEA stability studies at cold, 25 °C, 40 °C, and 60 °C with varying headspace were conducted to facilitate setting the nitrone specification, release, and storage strategy of DIPEA in commercial space. In summary, the DIPEA raw material was carefully evaluated to investigate the two new impurities in the API after process updates. An appropriate control strategy was established, enabling a successful Nemtabrutinib PPQ campaign.

**44** **A Proteomic Investigation to Identify Potential Protein Biomarkers for Breast Cancer Detection Using Sera from Control Donors and Women with Triple-Negative Breast Cancer**

Nicholas Versaci, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Danielle Whitham, Isabelle Sulivan, Norman Haaker, Brain Pentacost, Costel Darie

Breast cancer (BC), one of the most common cancers, is the leading cause of death for women in the United States. Triple-negative breast cancer (TNBC) is defined by low levels of estrogen and progesterone receptors and HER2. It is the most aggressive kind of breast cancer, making early detection and treatment crucial. Mass spectrometry was employed to profile serum proteomes from women with breast cancer, matched controls, and biological replicates to identify differential protein expression. Serum, reflecting the physiological state at the time of collection, is a suitable medium for cancer biomarker discovery. Additional protein biomarkers may provide new early detection tools if significant dysregulations are discovered in the sera. In this work, we looked for protein variations in serum from women with breast cancer, in matched controls, and in biological duplicates. Samples from women with TNBC along with matched controls (8 vs. 8, 16 total) were analyzed using SDS-PAGE separation, in-gel and in-solution digestion, followed by nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS) using a NanoAcquity UPLC and a QTOF Xevo G2 XS MS or QTOF Xevo G2 MS. The raw data were processed with ProteoWizard MSConvert (v. 3.0), Mascot Daemon server (v. 2.5), and Scaffold 4.3 software. The dysregulated proteins are now being explored as potential breast cancer markers and will be compared to a larger BC data set currently under analysis. To date, we have found a number of proteins associated with BC or other malignancies from the antitrypsin family, complement family, and apolipoproteins.

**45** **Analytical Development from HTE Screening to Late-Stage Process Development, a Case Study on 2-(Piperazin-2-yl)acetonitrile**

Dake Mao, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901, Brian He, Adrian Doggett, Catherine Mihalec, Robert Menger

Liquid chromatography (LC) is a critical tool for generating knowledge during chemical process development. This study focuses on LC method development strategies for 2-(Piperazin-2-yl)acetonitrile , a challenging analyte due to its unique structure. The challenges include detecting non-UV active products and impurities, effectively separating structurally distinct piperazine derivatives, and improving the peak shape

of highly retained analytes. A fit-for-purpose method was rapidly developed for high-throughput catalyst screening and subsequently optimized for process development and impurity characterization. To support high-throughput catalyst screening, various chromatographic techniques, including reversed-phase (RP), hydrophilic interaction (HILIC), and mixed-mode chromatography, were systematically evaluated. Additionally, detection methods such as UV (with or without derivatization), mass spectrometry (MS), and charged aerosol detection (CAD) were investigated to ensure reliable detection of the target compounds and impurities. Ultimately, a targeted 6-minute UPLC-HILIC-MS method was selected, utilizing MS-SIR (single ion recording)-based area percent data analysis. As the research transitioned to process development, the analytical methods required a holistic understanding of the compound's impurity profile. Although multiple CAD-based LC methods were developed, these methods did not prove suitably robust for utilization in quality control laboratories. Consequently, a sample derivatization method was introduced and evaluated, which enhanced both separation in RP-mode and simplified quantitation with UV detection. Column screening, mobile phase study, and dry-lab modeling were employed to improve separation and quantitation, accommodating new process-related impurities. Overall, this research provides fit-for-purpose strategies to support high-throughput process screening, process development, and impurity tracking, and further enhances knowledge gathering during chemical process development.

## 46 Investigation of a Gel Formation Issue and Identification of the Gel Complex in a Dianion Addition Synthesis Step of a Small Molecule Drug Substance Using SEC-MS and IR Spectroscopy

Van Truong, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065

During the scale up of a Dianion Addition reaction for pilot plant readiness, the flow reactor was clogged due to gel formation between Diethyl oxalate and dilithiated starting material (SM). This posed multiple analytical challenges to identify the root cause and chemical information of the gel. The investigation focused on potential oligomers formation as the root cause and its impact on the mass balance. Since the gel was not stable in solution, identification of the composition through traditional liquid chromatography (LC) methods was challenging. Size exclusion chromatography (SEC) coupled with mass spectrometry (MS) detector enabled identification of Li+ adducts of [Li(OTFA)]n and [Li(OFA)]n clusters generating on-column from interaction of methyl-lithium reagent and LC mobile phases (MP) using trifluoroacetic acid (TFA) or formic acid (FA) additive, respectively. The observation led to hypothesis that Li in the reaction mixture readily formed oligomer clusters with any carboxylic acid. Since the SM's and the product from this process were carboxylic acids, the gel was likely formed through the same mechanism. To test this hypothesis, Li salt cluster formation mechanism was mimicked by adding SM or product to the LC-MS aqueous mobile phase as MP modifier. The results confirmed clusters of Lithium-SM or Lithium-product complexes that were formed on column in SEC-MS. Infra-red (IR) spectroscopy data also supported the finding. Although MS data showed a similar repeating unit of oligomerization, the gel was actually not stable oligomers and therefore should not carry through the process to impact product quality.

## 47 Development of Control Strategy for Peroxide in Tetrahydrofuran (THF) Based on a Simple Method to Mitigate the Risk of Oxidation Product Formation for TNG462

Zhengyang (Allen) Xin, Tango Therapeutics, 201 Brookline Ave., Ste. 901, Boston, MA 02215, Yong Liu, Colin Liang, John Zhang, Michael Palmieri

Trace amount of THF peroxide can exist in THF, which can oxidize other compounds to form oxidation products. A sensitive method for ppm levels quantitation of organic peroxide through derivatization and LC-UV analysis was published recently. However, this method is complex and lengthy, and isn't suitable to be used at manufacturing sites. Commercially available test strips for hydrogen peroxide are quick and economic, but semiquantitative. TNG462 is a small molecule for oncology and has a risk to form oxidation product in THF, a solvent used in the final crystallization step. Our study goal is to examine if a correlation between the two methods can be established. If yes, we can implement test strips for quick semiquantitative determination of peroxide in THF to guide the crystallization of TNG462. Our data showed that there is a linear regression correlation between the two methods from analysis peroxide in pure THF. Furthermore, crystallization of TNG462 using THF spiked in with different levels of THF peroxide was carried out. The results showed that there is a linear regression correlation between levels of THF peroxide (measured by test strips) and the amount of TNG462 oxidation product. Based on that we established a specification for peroxide level in THF and use it to guide the TNG462 crystallization. In conclusion, a control strategy based on the simple test strip method with quantitative measurement (≤1 ppm) for THF peroxide is established which can mitigate the risk of TNG462 oxidation product formation.

## 48 Analysis of Nitrite in Excipients as a Function of Particle Size Using Headspace Gas Chromatography

Elizabeth Morisseau, Pfizer, 445 Eastern Point Rd, Groton, CT 06340, Ingrid Pomaquiza, Paul Gerst, Denise Grimon

Control of nitrite in excipients has become an important strategy for mitigation of nitrosamine formation in pharmaceuticals. Surface level nitrite is likely to be more available to react than nitrite within the crystal matrix of an excipient. Therefore, excipient particle size may be a predictor of nitrite availability. Since smaller particle size excipients have a higher ratio of surface area to volume, they may have more readily available nitrite than their larger particle size counterparts. In this experiment, several grades of microcrystalline cellulose were sieved into various particle size ranges. The nitrite content at each particle size was measured using cyclamate derivatization and headspace gas chromatography. A relationship was observed between particle size and nitrite content.

## 49 Harmonizing Resource Ambiguity and Assay Expectation: A Multivariate Approach to Enhance Specificity and Accuracy of Acylcarnitine Profile in Plasma and Serum by High Performance Liquid Chromatography – Tandem Mass Spectrometry (HPLC-MS/MS)

Dahai Shao, Cleveland Clinic, Case Western Reserve University, 9500 Euclid Ave. LL3, Cleveland, OH 44106

Plasma acylcarnitine profiling is an essential diagnostic tool for numerous inherited metabolic disorders, particularly mitochondrial fatty acid oxidation disorders (FAODs) and organic acidemias. It is routinely applied in follow-up testing after abnormal newborn screening results and in the evaluation of suspected metabolic disease in children and adults. Triple-quadrupole tandem mass spectrometry remains the most widely used platform for this analysis. However, its relatively low resolution can limit differentiation of isobaric and isomeric species. In addition, the nonselective fragmentation pattern of most acylcarnitines—dominated by m/z 85 and 141 ions—provides little structural information on the acyl side chain, which is critical for species identification. To address these challenges, this study reexamined fragmentation patterns and identified lesser-known ion fragments that can improve assay specificity. Chromatographic separation of odd-chain acylcarnitines (C15, C17, C19, etc.) also clarified previously unexplained peaks from hydroxylated species such as C14:1-OH, C16:1-OH, and C18:1-OH. Furthermore, high-resolution mass spectrometry was applied to resolve interferences, including the C18:1 crossover in C18 transitions. In summary, these strategies enhance the specificity and reliability of acylcarnitine analysis, offering improved diagnostic performance for metabolic disorder screening and evaluation.

## 50 Creating an Analytical Network Community Across Pharmaceutical Development R&D at Bristol Myers Squibb

Peter Tattersall, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903, Anthony Leone, Brent Donovan

Analytical needs are extensive and critical throughout the development stages of pharmaceutical products. Specialized analytical chemists are essential across various disciplines to support pharmaceutical development. The development, utilization, and communication of testing methods, as well as the collation and leveraging of data to support a portfolio of projects, is a complex process. As analytical methods are required across a number of functional areas organizational structures vary across pharmaceutical companies. This variation can lead to challenges, with analytical chemists isolated within specific functional areas or large consolidated analytical departments that have complex working arrangements to meet development testing needs. These business models may function well enough but do they fully harness the potential of analytical chemists and create the right environment for career growth? To overcome some of these shortfalls we propose an analytical community that transcends functional areas to creates opportunities for analytical chemists to connect beyond project requirements. With a network of over 450 scientists, from diverse backgrounds and skills, united by a common goal of supporting of biologics and small molecule drug substance and formulation pharmaceutical development, we can encourage an environment to share experiences, learn, grow, and collaborate with like-minded professionals. In turn this would benefit all projects and functional areas with enterprise mindsets and with analytical synergies through collaboration.

## 51 Proteomic Analysis of Contents in Varying Plant Milk Types Using Gel-Based Mass Spectrometry Methods

Celeste A. Darie, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Alivia Sochia, Angiolina Hukovic, Niyogushima Nuru, Taniya Jayaweera, Pathea S. Bruno, Costel C. Darie

Milk is widely recognized as a nutritionally complete food, as it provides a balanced composition of macronutrients (lipids, proteins, and carbohydrates) along with essential micronutrients such as calcium, selenium, riboflavin, and various vitamins. As consumer interest in healthier dietary options grows, there has been a notable shift toward plant-based or blended milk alternatives. This trend is driven by per-

sonal health considerations and dietary restrictions, leading to a significant rise in the consumption of plant-based milk products. In response to this growing demand, we initiated a study to compare the protein composition of various plant-based milk alternatives. These milk substitutes are valued for their bioactive components, which are associated with health benefits and disease prevention. However, the protein content of such products cannot be accurately assessed through nutritional labels alone. To address this gap, we conducted proteomic analyses on four plant-based milk varieties. The objective was to identify and compare the specific proteins present in each sample across different dilutions. Initially, a one-dimensional SDS-PAGE was performed using varying concentrations of each milk type. Protein bands were excised, digested with trypsin, and prepared for mass spectrometry analysis. The resulting peptides are being analyzed using an XEVO G2 QTOF mass spectrometer, with protein identification carried out via our Mascot Daemon server. Protein levels across the samples are being quantified based on identification scores, and further measurements will follow subsequent data analysis. Additionally, we are expanding this investigation to include other plant-based milk alternatives to deepen our understanding of their proteomic profiles.

## 52 Development of a High-Throughput Proteostat-Based Fluorescence Assay for Detection of Aggregates in Vaccine Modalities

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The ability to detect and characterize aggregation in vaccines and other pharmaceuticals is critical as aggregates can impact product efficacy and safety, and is particularly challenging for large-molecule modalities due to the stochastic nature and complexity of aggregate formation. Analyses used for the quantitation of aggregates include separation-based techniques that provide size-distribution profiles such as size-exclusion chromatography (SEC) and flow cytometry. However, method development and sample analysis can be time-consuming depending on the method or technique, and this can hinder analysis of large sample sets during formulation or process development, or when fast turn-around of data is required. Another, more rapid approach is to incubate samples with rotor-based dyes that fluoresce when restricted in hydrophobic pockets of aggregated sample and measure fluorescence emission, where the intensity is proportional to the amount of aggregation present. Herein, we present an evaluation of vaccine modalities such as live attenuated virus (LAV), virus-like particles (VLP), protein-conjugates, and proteins CRM197 and IgG, where we correlate relative levels of aggregates in control and stressed samples incubated with PROTEOSTAT® dye via fluorescence emission outputs using a plate reader. These higher-throughput results are compared against those obtained with orthogonal techniques such as intrinsic/extrinsic protein fluorescence spectroscopy and dynamic light scattering to understand method performance. Additionally, an LAV and CRM197 were evaluated with flow cytometry and SEC coupled with UV/FLR and UV-MALS-RI detection, respectively, to examine the potential use of this plate reader-based method for rapid and semi-quantitative analysis of aggregates when linked with a separations-based method.

## 53 Probing Water Content in Active Pharmaceutical Ingredients Using Quantitative NMR

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Quantitative Nuclear Magnetic Resonance (qNMR) has emerged as a valuable analytical tool for determining water content in active pharmaceutical ingredients (APIs). This study examines qNMR as an alternative to traditional methods such as Karl Fischer (KF) titration, highlighting its capacity to reduce hazardous waste generation and enhance laboratory efficiency. While KF titration relies on chemicals like sulfur dioxide and iodine, which present safety and environmental concerns, qNMR offers a reagent-free, non-destructive solution that facilitates routine analysis and minimizes associated risks. By directly integrating the water proton signal, qNMR provides accurate quantification and is particularly suited for APIs that are insoluble or reactive with KF reagents. Despite certain limitations—such as reduced sensitivity compared to KF in the low ppm range, the necessity for deuterated solvents, and potential interference from exchangeable protons—the method demonstrates significant applicability across diverse sample matrices, including complex or heterogeneous materials unsuitable for KF analysis. Importantly, advancements in benchtop NMR instrumentation have improved accessibility streamlining the process for routine pharmaceutical quality control while reducing operational complexity and environmental impact. Our comparative analysis demonstrates that qNMR offers an effective and environmentally sustainable alternative for routine water content analysis, enabling reliable quantitation. This approach holds promise for integration into future compendial applications, especially in contexts where reagent management or intricate sample preparation pose practical challenges.

## 54 Towards a Universal HPLC Method for the Quantitation of Residual Protein Levels in Products Isolated from Biocatalyzed Reactions

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Biocatalysis involving enzymes engineered through directed evolution is vital for synthesizing complex pharmaceuticals with excellent selectivity and sustainability. As enzymatic biocatalysis gains prominence in commercial and early-phase synthesis, accurately measuring and controlling product residual enzyme levels is crucial. However, this poses challenges due to interference from cellular debris, host proteins, reaction products, and solvents. Enzyme degradation during reaction workup can lead to peptide fragments of varying lengths, complicating analyte analysis. Unlike colorimetric methods such as BCA or Bradford assays, HPLC-UV methods offer a streamlined analysis applicable in laboratories focused on small molecule drug development. HPLC-based techniques could provide greater robustness and precision than traditional bioassays. Our initial attempt at a universal HPLC method for residual proteins relied on SEC with bovine serum albumin as a standard but faced high variability due to enzyme degradation during reactions. To enhance method applicability, we are evaluating a new reversed-phase HPLC-UV approach to quantify residual peptide and protein levels in the dry cake of a Merck therapeutic currently in clinical development. Results will be compared with orthogonal methods like BCA, Bradford assays, and SDS-PAGE, confirming the HPLC method's comprehensiveness. Future testing of this HPLC method across various biocatalyzed synthesis steps aims to establish its utility as a generic platform to replace fit-for-purpose methods, thereby streamlining enzyme measurement and control strategies, expediting process development, and ultimately delivering therapeutics to patients more swiftly.

## 55 Ultrafast Microdroplet Digestion of Antibodies with Fc-Silencing Mutations

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Many novel therapeutic monoclonal antibody (mAb) modalities contain mutations that not only silence unwanted binding to Fc-gamma receptors but also could bring resistance to IdeS enzymatic cleavage into mAb subunits, limiting the antibody middledown analysis by mass spectrometry (MS). Herein we showed, for the first time, the efficient, reproducible, and ultrafast microdroplet digestion (less than 1 ms) of mAbs carrying a series of mutations "LALA", "LAGA", and "LFLE" (e.g., tool antibody LALA-DS, Nivolumab, Pembrolizumab, Vedolizumab, and PD-L1), by a new enzyme: FabRICATOR Xtra (Xtra). The digestion took place during the spray ionization process using an Agilent jet stream (AJS) ion source with a digestion efficiency close to or more than 80%, leading to subunits with high ion abundances for identification and characterization. Increased enzyme/antibody ratio or partial reduction of disulfide bonds increased the digestion efficiency. "One-pot" disulfide reduction and digestion in microdroplets could occur simultaneously by spraying the antibody and enzyme along with the reductant. Notably, for the highly digestion-resistant PD-L1 antibody, the Xtra-based microdroplet digestion process was found to be 9 million times faster than in-solution digestion. Furthermore, a workflow was developed, using a script that can automatically choose a preferred enzyme (Xtra or IdeS) for digestion, based on a target antibody input sequence, which allowed quick analysis of 94 antibody samples within 104 min. Our method is a fully automated microdroplet protein digestion technique that integrates flow injection (FI) and online MS analysis, providing a rapid and robust method for the structural characterization of mAbs with mutations.

## 56 Targeted Identification of Extracellular Vesicle Protein Biomarkers Using StageTip Fractionation and MALDI-TOF

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There is interest among bioanalytical chemists to use extracellular vesicle (EV) proteins as biomarkers due to their various roles in the body including the development and progression of cancer. Matrix assisted laser desorption ionization-time of flight (MALDI-TOF) is a fast, cost-effective, and green method for targeted identification of EV protein biomarkers. A limitation of MALDI-TOF is lack of analyte separation prior to mass spectrometry analysis which is especially detrimental to peptide/protein identification in complex samples like EV protein digests. Stop-and-go-extraction tip (StageTip) fractionation is a fast, cheap, and versatile method for peptide fractionation that could address MALDI-TOF's limitations. In StageTip fractionation a functionalized Empore disk is inserted into a micropipette tip acting as a microcolumn where peptides are loaded, desalted, and fractionated. Preliminary results indicate that more unique peptides from EV protein biomarkers were identified in fractionated samples over unfractionated samples resulting in more confident protein identifications. Empore disks with different functionalities are available including reversed-phase, cation exchange, and anion exchange allowing for a variety of peptide fractionations based on different peptide affinities. This research compares

the effectiveness of different StageTip fractionation methods, using different Empore disks, in their ability to identify EV protein biomarkers. When comparing StageTip fractionation and MALDI-TOF to the more commonly used proteomics platform, LC-MS/MS, the former is potentially less time consuming, more cost-effective, and greener. To the best of our knowledge this research represents the first time StageTip fractionation and MALDI-TOF were used for the application of targeted identification of EV protein biomarkers.

## 57 The Effects of Hair Bleaching on Fentanyl Concentrations and its Implications in Drug Testing

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Hair analysis is an important tool in forensic toxicology, providing a long detection window for drug use compared to urine testing. However, cosmetic treatments, such as bleaching, may alter drug concentrations within the hair. It may also complicate extraction, analysis and interpretation. Extensions were spiked with known concentrations of fentanyl and bleaching procedures were applied to hair samples. Quantitative analysis was conducted using gas chromatography–mass spectrometry (GC–MS). Results demonstrated a reduction in detectable fentanyl concentrations following bleaching, with most samples falling below standard detection thresholds, or resulting in abnormal peak shapes resulting in complicated interpretation. These findings highlight the importance of considering cosmetic hair treatments when interpreting toxicological results. In forensic and clinical settings, bleaching may lead to false negatives or underestimation of fentanyl exposure. This has implications in criminal investigations, workplace drug testing, and postmortem toxicology, especially in cases involving opioids where accurate detection is essential. Future work should investigate other hair treatments and their effects on different drug classes to improve the reliability of hair as a toxicological matrix.

## 58 Investigation on Irregular Light Degradant Peak Fronting and Recovery in Small Molecule API Method Development

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The development of the commercial route for a BMS drug substance created a new impurity profile in the active pharmaceutical ingredient (API) material. This necessitated the redevelopment of the API's achiral liquid chromatography (LC) purity/impurity analysis method. We employed column screening and DryLab modeling to identify optimal parameters and establish a robust method operable region. This approach enabled us to quickly optimize conditions and develop a robust method, addressing key variables such as temperature, gradient, flow rate, and column batch-to-batch variability. However, after finalizing conditions, we observed peak fronting in a light degradation impurity containing an aldehyde functional group. A sharper gradient and increased temperature were found to improve the peak shape. Additionally, during this development, we observed the loss of this impurity's peak area over time in the originally selected diluent. Through comprehensive diluent screening, we discovered that this loss was exacerbated by increased aqueous content and the presence of acid. Consequently, we selected a diluent that prevented peak area loss. Further investigations suggested that the loss might be attributed to the impurity binding to the vial, a common issue for impurities with aldehyde functional groups. Ultimately, this method development ensured enhanced accuracy and reliability in the analysis of API impurities.

## 59 HYDRA: Introducing Vacuum Ultraviolet Detection to the LC World

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This poster introduces HYDRA's spectral detection capability for LC workflows. Key features include 120–240 nm wavelength coverage, unique absorbance fingerprints, and compatibility with existing LC hardware/software. Example data from amino acids and pharmaceutical mixtures are shown, highlighting HYDRA's potential to transform LC detection.

## 60 Membrane Protein Analysis: Integrating Metallic Nanoparticles with MALDI-TOF-MS

Nichole Donofrio, Seton Hall University, 400 S. Orange Ave., South Orange, NJ 07079, Alexandria Gallagher, Kenneth Tomkovich, Reihaneh Safavi-Sohi

Bottom-up proteomics is a method of protein analysis where protein identification occurs after enzymatic digestion by matching peptide fragment masses to theoretical databases, with high sequence coverage being crucial for identification. Membrane proteins are of interest as cancer biomarkers due to their adhesive functions that promote cancer metastasis. Challenges in identifying membrane proteins include their relatively low abundance, and their hydrophobicity makes their solubilization and digestion more difficult than other proteins. These challenges result in low amounts of tryptic peptides from membrane proteins, which necessitate a sensitive mass spectrometry method. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is widely used for peptide analysis. Typical matrix solutions used, like α–cyano-4-hydroxycinnamic acid (CHCA), can contribute to background noise within the resulting mass spectra, especially when salt is in the sample. Metallic nanoparticles can be used in place of matrix to produce cleaner mass spectra by minimizing the interference of background signals from the matrix, or salts and detergents in the sample. This study investigates the effects of adding a layer of metallic nanoparticles to membrane protein digested sample on the MALDI plate instead of using traditional matrix. The objective was to enhance the signal-to-noise ratio and allow for clearer identification of peptide peaks even in low peptide abundance. The ability to identify more peptide mass peaks from the MALDI-TOF-MS results allows for higher sequence coverage identification of the digested protein. By optimizing mass spectrum data, we can more easily identify proteins by peptide mass fingerprinting from complex samples.

## 61 Per- and Polyfluoroalkyl Substances (PFAS) in Food Waste: Detection and Cost Analysis

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The entry of per- and polyfluoroalkyl substances (PFAS) into food waste recycling streams presents a significant threat to environmental health by compromising the safety of end products like compost and animal feed. This study investigated PFAS levels in food waste from various sources (restaurants, schools, and landfills) and commercial fish fillet. Twenty-five target PFAS compounds were digested and extracted from food samples following the U.S. EPA Method 1633A using solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). The analysis confirmed the presence of PFAS across all samples, with concentrations varying significantly by sources. Notably, a school sample yielded the highest concentration of 6:2 fluorotelomer sulfonate (6:2 FTS) at 22.04 ± 3.23 $ng \cdot g^{-1}$, while a restaurant sample showed the highest level of perfluorooctanoic acid (PFOA) at 3.04 ± 0.45 $ng \cdot g^{-1}$. Critically, the analysis of fish tissue confirmed a significant risk of bioaccumulation and re-entry into the human food chain, with detected concentrations of key regulated compounds like PFOA (up to 2.65 $ng \cdot g^{-1}$) and PFOS (up to 1.15 $ng \cdot g^{-1}$) suggest potential PFAS contamination in the fishery pond water or fish feed. Ensuring viability of the food waste recycling industry requires a multi-faceted strategy of source control, robust monitoring, and science-based regulatory standards. To support these strategies, we analyzed costs for PFAS detection in solid samples, ranging from approximately $500 to $1300 per sample, which is crucial for food waste treatment or recycling facilities to manage liability, ensure regulatory compliance, and secure market access for their recycled products.

## 62 Proteomic Profiling of Breast Milk for Early Breast Cancer Detection: In-Solution Digestion Mass Spectrometry Analysis

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Breast cancer (BC) is the most frequently diagnosed cancer in women worldwide, and early detection remains essential for improving outcomes. Invasive ductal carcinoma (IDC), the most prevalent subtype of BC, arises in the milk ducts and invades surrounding breast tissue, accounting for approximately 85% of all cases. Human breast milk is a rich, non-invasively collected biofluid that contains diverse proteins, immune factors, and epithelial cells, making it a promising source for biomarker discovery. In this study, we analyzed breast milk samples from lactating women diagnosed with IDC and from healthy controls. To investigate the effect of protein depletion on biomarker detection, we prepared two sets of samples (10 IDC vs. 10 controls) using both undepleted and lactoferrin-depleted breast milk. Proteins were digested in-solution and analyzed using nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS). This approach enabled the identification and quantification of differentially expressed proteins between cancer and control groups. Notably, osteopontin (OPN), transferrin, and butyrophilin showed consistent dysregulation, supporting their potential role as early detection biomarkers. These findings highlight the value of breast milk proteomics, particularly with optimized depletion strategies, as a promising non-invasive approach for breast cancer detection. These preliminary results lay the groundwork for validation in larger cohorts.

## 63 Identification of Fibrinogen Alpha in Peptidomic Serum Samples from Breast Cancer Patients and Matched Healthy Controls for Progression Biomarker

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Breast cancer (BC) remains one of the leading causes of mortality among women. Dysregulated proteins and peptides detected during the early stages of BC have the potential to serve as diagnostic biomarkers, particularly for early detection in younger patients. The comprehensive study of all proteins, their interactions, and modifications—known as proteomics—is essential in this context. This study's interests lie in peptidomics, which focuses on low-molecular-weight peptides, including less commonly studied proteins and peptide molecules. Peptidomics involves the analysis of the peptidome, a subset of the proteome composed primarily of polypeptides with molecular weights under 20 kDa. This field also includes the study of

fragmented peptides and potential metabolites present in biological samples. Serum analysis is a powerful method for detecting tumor-secreted molecules that reflect disease responses, often analyzed using mass spectrometry. In this study, serum samples from 50 breast cancer patients and 50 age-matched healthy controls were processed using Glygen's C4 TopTips, targeting peptides under 3 kDa. The matched samples were then analyzed using nanoLC-MS/MS to identify dysregulated peptides and proteins. Data processing and identification were conducted using ProteinLynx Global Server (v2.4), Mascot Daemon (v2.5), Mascot Distiller Workstation, and Scaffold 4.3. To validate the findings, additional analysis was performed using MALDI-TOF, which confirmed the peptide sequences identified. Preliminary results suggest that Fibrinopeptide A, a peptide involved in blood clotting and previously implicated in other cancers, may also play a role in the progression of breast cancer.

## 64 Bifunctional Transition Metal based Boron Nitride Electrocatalysts for Nitrogen Reduction Reaction and Nitrate Reduction Reaction

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Electrochemical nitrate reduction to ammonia offers a sustainable and delocalized approach to ammonia synthesis, providing an environmentally friendly alternative to the conventional Haber–Bosch process. However, the high capital costs of centralized plants and their environmental impacts undermine the viability of this route in meeting present-day developmental needs. Therefore, the development of sustainable alternatives for ammonia synthesis has become imperative. Among the reported strategies, the nitrogen reduction reaction (NRR) and the nitrate reduction reaction ($NO_3RR$) are considered promising, though decades of study have yet to overcome the challenge of limited catalytic activity. Here, we report porous boron nitride ($BN_x$)-supported transition-metal single-atom catalysts, synthesized via pyrolysis with ~1 wt% metal loading, designed to stabilize isolated metal atoms at single-atom sites. Among the tested catalysts, $CuBN_x$-1 wt%-1:36 exhibited the best performance. For NRR, it achieved a peak ammonia productivity of ~118 μg $h^{-1}$ $mgcat^{-1}$ with a Faradaic efficiency of ~85% at –0.7 V vs. SCE and a BET surface area of 43.66 $m^2$ $g^{-1}$, markedly higher than the bare $BN_x$ substrate (~48 μg $h^{-1}$ $mgcat^{-1}$). For $NO_3RR$, the same catalyst delivered a peak $NH_3$ productivity of ~3184.9 μg $h^{-1}$ $mgcat^{-1}$ at –0.7 V vs. SCE, with Faradaic efficiency above 95% at low potentials and >90% $NH_3$ selectivity across the potential range. At more negative potentials, both productivity and efficiency decreased due to competing hydrogen evolution. Ammonia was quantified with Nessler's reagent (420 nm), nitrite by the Griess assay (548 nm), and nitrate with a HACH kit (345 nm).

## 65 Comparative Peptidomic Analysis of <10 kDa Serum Fractions from Breast Cancer Patients and Healthy Controls for Biomarker Discovery

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Breast cancer (BC) is one of the leading causes of mortality among women. Early-stage dysregulation of proteins and peptides holds promise as a source of biomarkers for early detection, particularly in younger patients. Peptidomics is a subfield of proteomics that investigates smaller and less commonly studied protein molecules and peptides. Specifically, peptidomics examines the peptidome, which consists predominantly of polypeptides with molecular weights below 20 kDa. This includes fragmented peptides and potential metabolites that may be present in biological samples. One key approach in this area is serum analysis, which enables the identification of small tumor-related molecules, for instance, proteins secreted by the liver, which can reflect systemic disease responses. These compounds are typically analyzed using advanced instrumentation such as mass spectrometry. In this study, serum samples from 50 breast cancer patients and 50 age-matched healthy controls were processed using 10 kDa molecular weight cut-off (MWCO) filters, followed by in-solution digestion. The matched samples were analyzed by nanoLC-MS/MS to identify dysregulated peptides and proteins. Data analysis was performed using ProteinLynx Global Server (v2.4), Mascot Daemon (v2.5), Mascot Distiller Workstation, and Scaffold 4.3. Preliminary results suggest that Fibrinogen Alpha, a protein known for its role in blood clotting and involvement in other cancers, may contribute to the progression of breast cancer. Further validation, including in-gel analysis, is currently underway to complement and strengthen the findings of this study.

## 66 Orthogonal Analytical Evaluation of Photoprotection in Hair: DSC, SDS-PAGE, and HPTLC

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Understanding UV damage to hair requires methods that resolve protein changes and lipid depletion along different analytical axes. We focused our study on three complementary techniques to quantify the protective effects of flavonoids and UV filters: differential scanning calorimetry (DSC), SDS-PAGE, and high-performance thin-layer chromatography (HPTLC). Human hair was treated with test formulations, containing UV filters and flavonoids, and irradiated under controlled UV exposure. DSC measured keratin denaturation temperature and enthalpy as proxies for amorphous-matrix crosslink density and relative amount of keratins. SDS-PAGE, run under reducing conditions, and in-house developed densitometric analysis tool resolved changes in bands, providing an orthogonal confirmation of protein damage/protection. In parallel, lipids were extracted using a solvent and separated by HPTLC, allowing for class-level quantification of triglycerides, free fatty acids, cholesterols, and wax esters to assess UV-induced lipid loss. Both flavonoids and sunscreen formulations significantly mitigated protein damage. In contrast, lipid endpoints diverged: among the protective agents tested, only the UVB filter yielded a significant preservation of hair lipid classes by HPTLC. Flavonoids and broad-spectrum UV filter showed limited or no lipid protection at the tested doses.

These results demonstrate the value of orthogonal methods: thermal, electrophoretic, and planar-chromatographic measurements agree on robust protein protection yet reveal spectral selectivity for lipid preservation. Future work will extend HPTLC with oxidation markers and confirm findings by LC-MS.

## 67 Using EPR and DFT Methods to Understand the Heterodimer BChlg'/Chla' Generated by Exposure of Heliomicrobium Modesticaldum Primary Donor P800 to Dioxygen

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Heliobacteria are anaerobic phototrophic bacteria with a Type I homodimeric reaction center (RC) using bacteriochlorophyll *g* (BChl *g*). *H. modesticaldum*, the model organism used for heliobacteria, has been well studied and is understood to convert BChl *g* into $8^1$-OH-chlorophyll $a_F$ (BChl $a_F$) in the presence of light and dioxygen. Conversion of BChl *g* results in the loss of the light-driven charge separation. Previous work work shows that once both of the BChl *g'* molecules of the primary donor $P_{800}$ have been converted to BChl $a_F$', the RC can no longer perform electron transfer. We show that a partially converted $P_{800}$ can exist by exposing the RC to dioxygen and demonstrate the presence of a BChl *g'*/ BChl $a_F$' heterodimer by Q-band $^1$H ENDOR, $^{14}$N HYSCORE spectroscopy and DFT methods. The DFT calculations of a BChl *g'*/ BChl *g'* homodimeric primary donor predict that the unpaired electron spin of $P_{800}^+$ will be evenly delocalized across both of the BChl *g'* molecules, which is in excellent agreement with experimental hyperfine couplings of the anaerobic samples. Exposure to dioxygen drastically changes the experimental hyperfine interactions of the RC, which displays greater localization of the unpaired electron spin in $P_{800}^+$. In agreement with the experimental hyperfine couplings, DFT calculations using a computational model of the heterodimeric primary donor $P_{800}$ obtained by replacing one of the BChl *g'* with BChl $a_F$' shows significant localization of the electron spin density on the BChl *g'* molecule in the heterodimer.

## 68 Investigation of the Dysregulated Proteins in Fishes from the Great Lakes Exposed or not to Environmental Contaminants

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A major goal of the project is to compare the proteomes of male fishes that were exposed or not exposed to estrogenic activity compounds using quantitative proteomics. The first goal is to identify the proteins that are dysregulated in the exposed fishes, as compared to unexposed ones. The second goal of the project is to identify the specific EDCs that are responsible for expression of vitellogenin in male fishes. Initially, we identified male fishes that express vitellogenin and the fishes that do not express vitellogenin, and then classified these fishes as EDC-exposed (i.e. they express vitellogenin) and not exposed (i.e. they don't express vitellogenin). We then separated fish samples by large scale SDS-PAGE, cut the gel lanes into 15-20 gel bands bands, digested them by trypsin, and then analyzed them by nanoliquid chromatography-tandem mass spectrometry using a NanoAcquity UPLC coupled with a QTOF Xevo G2 mass spectrometer (Waters Corp). Database search was performed using the in-house Mascot Daemon Server (Matrix Science) against Actinopterygii. Once the Mascot database search is finished, all proteins that are dysregulated in EDC-exposed vs not exposed fishes (i.e. they express or don't express vitellogenin) will be identified using the in-house Scaffold server (Proteome Software). The study is ongoing and will attempt to identify the series of proteins that may be dysregulated (like vitellogenin) upon exposure to EDCs, which may lead to identification of the molecular mechanisms that are dysregulated upon exposure to EDCs.

## 69 Automated Online LC Sampling for Data-Rich Reaction Monitoring in Chemical Process Development

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Innovative approaches to synthetic route development demand real-time, actionable data to guide dynamic process changes. Common tools in the analytical workbox include Process Analytical Technology (PAT) probes, however these measurements (e.g., IR/NIR/Raman) only provide general spectroscopic information. This poster will demonstrate adaptations of liquid chromatography (LC) systems for online sampling, enabling rapid separation and confirmation of species with minimal-to-no

sample handling. Both autosampler-based online derivatization and mobile direct sampling platforms will be discussed, illustrating their versatility for direct monitoring of unstable intermediates, heterogeneous reaction (e.g., slurry) mixtures, endpoint determination, and potent compound analysis. Automated online LC sampling streamlines data collection and supports comprehensive impurity profiling for real-time tracking of impurity formation and purge, rather than reliance on only starting material / product quantities for reaction completion. Proof-of-concept use cases from Bristol Myers Squibb's Chemical Process Development portfolio demonstrate impactful applications in small molecule and antibody-drug conjugate (ADC) process development, as well as analytical method optimization. This work highlights how online LC sampling advances process understanding, enhances flexibility in development, and supports robust, data-driven decision-making for modern pharmaceutical manufacturing.

## 70 The Impact of Cooking on Isothiocyanate Formation in Broccoli using Gas Chromatography-Mass Spectrometry (GC-MS)

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The interest in isothiocyanates in cruciferous vegetables have increased due to their chemopreventive properties associated with carcinogenesis suppression, modulation of metabolism and detoxification in the body. Isothiocyanates are produced when plant cells are destroyed through cutting or mastication and myrosinase is liberated as a catalyst for the hydrolysis of the glucosinolates found in these vegetables. Isothiocyanates are one of the several glucosinolate derivates which also include nitriles and epithionitriles. The production of these derivatives can vary due to the presence of specifier proteins, pH values and temperature. Cruciferous vegetables like broccoli are common in the diets of many individuals which can provide health benefits with the correct cooking methods. The introduction of the air fryer has brought a lot of attention for the health and nutrition community with its ability to prepare food with minimal amounts of oil without sacrificing taste. However, researchers have not yet included air fryer methods into isothiocyanate cooking comparisons. This study monitors isothiocyanate and nitrile production in broccoli with different preparation methods. These methods include boiling, steaming, blanching, stir frying and air frying. Cooked broccoli samples are homogenized using a hand mixer and incubated for 3 hours in water at 45 °C. Glucosinolate derivatives, specifically isothiocyanates are extracted with dichloromethane via liquid-liquid extraction and then concentrated using a rotary evaporator. Isothiocyanate samples are detected and quantified using gas chromatography- mass spectrometry. Significant differences were observed where the quantities of bioactive isothiocyanates compounds and nitriles were reduced or undetected with the introduction of heat.

## 71 Beyond Conventional Methods: Leveraging SFC for Faster, Greener and More Efficient Pharmaceutical Analysis

Michael Hicks, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Haley Herderschee, Melissa Skuriat, Sima Sakhaei, Henry Sanders, Alison McQuilken, Timothy Nowak

Supercritical fluid chromatography (SFC) serves as a powerful complement to liquid chromatography (LC), offering unique selectivity, enhanced resolution, and rapid analysis. It is particularly valuable in early drug discovery for real-time assessments of reaction endpoints and chiral purity. SFC continues to play a crucial role in early Phase I and II drug development and scale-up processes. However, its application for example in chiral analyses often diminishes in Phase III due to sensitivity challenges, leading to a preference for chiral LC methods. This discussion presents real-world examples demonstrating the effectiveness of SFC for in-process control and its applications throughout both early and late-stage development. We will emphasize SFC's sustainability as a separation method for in-process reaction control up through raw material assessment in manufacturing. Additionally, we will investigate the robustness challenges that may occur during late-stage transfers, especially those related to sensitivity, and propose strategies to improve performance by utilizing a deeper understanding of UV spectral behavior. This evaluation seeks to establish SFC, with its exceptional resolution capabilities, efficiency and sustainability, as a compelling alternative to LC for late-stage filing methods.

## 72 Development and Validation of a Supercritical Fluid Chromatographic Method for Quantitation of the Active Enantiomer in Indoxacarb End-use Product

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Approximately 43% of the 35 agrochemical products introduced to the market between 2018 and 2023 are chiral molecules containing one or more chiral centers [1]. Consequently, the development of efficient chiral separation methods remains a key focus in the agrochemical industry. Traditionally, chiral separations in this field have relied on normal-phase liquid chromatography (NPLC) using chiral columns. However, this approach is not environmentally green, as it requires large volumes of toxic hydrocarbons and other hazardous solvents, and involves lengthy equilibration times. Supercritical fluid chromatography (SFC) has emerged as a greener and more efficient alternative. Unlike NPLC, SFC uses supercritical carbon dioxide ($SF\text{-}CO_2$) as the primary mobile phase. $SF\text{-}CO_2$ offers low viscosity, high diffusivity, and strong solvating power, enabling high flow rates with low back pressure and resulting in equivalent or superior chromatographic performance. Additionally, $SF\text{-}CO_2$, typically a by-product of industrial processes, is considered environmentally benign - it is inexpensive, inert, nonflammable, nontoxic, and does not contribute to net carbon emissions. In this study, we developed an SFC method and compared it with a conventional NPLC method for the separation and quantification of the active enantiomer in three indoxacarb end-use products. The SFC method was validated for one of the products according to the EC SANCO/3030/99 rev. 5 guidelines, with all validation parameters meeting the required criteria. Furthermore, the environmental impact of both methods was assessed using the Analytical Method Volume Intensity (AMVI) and Analytical Method Greenness Score (AMGS), allowing for a comprehensive comparison of their sustainability profiles.

## 73 High Throughput Analysis Applications (using SFC/MS)

William Farrell, Virscidian, LLC, 225 Franklin St., FL 26, Boston, MA 02110

Over the past three decades, Supercritical Fluid Chromatography (SFC) has steadily gained traction in analytical and preparative separation sciences, owing to its unique combination of high efficiency, speed, and environmental sustainability. This presentation will explore key advancements in SFC technology, with a focus on its application in the analysis and purification of compounds derived from combinatorial and medicinal chemistry. Emphasis will be placed on innovations that have enhanced throughput, resolution, and scalability, making SFC an increasingly valuable tool in modern drug discovery workflows.

## 74 Multi-Target Surrogate Optimization for Supercritical Fluid Extraction – Supercritical Fluid Chromatography – Mass Spectrometry of Drug Compounds

Kevin Schug, The University of Texas at Arlington, 700 Planetarium Pl., Arlington, TX 76019, Niray Bhakta, Jaivardhan Sood, Yujing Yang, Chen Kan, Jay Rosenberger, Victoria Chen

On-line supercritical fluid extraction – supercritical fluid chromatography – mass spectrometry (SFE-SFC-MS) is a power analytical tool that can be applied in a variety of application areas. The primary challenge is method optimization, given the large number of parameters that need to be optimized and the interactions that occur between variables. A surrogate optimization approach has been developed, which is more flexible and efficient than standard multivariate optimization techniques. In an iterative explore and exploit fashion the surrogate optimization replaces expensive experiments with intermittent in silico response modeling to select subsequent analyses until an optimum is reached. A series of model pharmaceutical compounds were chosen to demonstrate the potential for multi-target optimization, guided by measures of molecular similarity. The development of a composite output function ensures that developed methods are robust and reproducible. The latest developments from our lab in surrogate optimization of SFE-SFC-MS will be presented.

## 75 Pharmaceutical Regulations: An Overview for the Analytical Chemist

Leon Doneski, Arcutis Biotherapeutics, Inc., 1407 Lipscomb St., Fort Worth, TX 76104

The pharmaceutical industry develops and manufactures life-saving medicines and is regulated by government authorities to ensure drug products' safety, efficacy, and quality before reaching patients. This presentation will provide a high-level overview of the regulatory process including pharmaceutical regulations, health authorities, public quality standards, pharmaceutical organizations, and trends. We hope to offer the analytical chemist a better understanding of underlying concepts and quality systems and show how working in a GMP environment can contribute to product quality and patient benefits.

## 76 Analytical Testing to Support the Development of Novel Combination Products

Keith Faucher, Aura Biosciences, 80 Guest St., Boston, MA 02135

Aura Biosciences is a biotechnology company developing a new class of oncology targeted combination product therapies using viral-like drug conjugates (VDCs) to treat tumors in ocular and urologic oncology. Aura's combination products consist of a drug product (belzupacap sarotalocan, or bel-sar) that is delivered to tumors using a local drug delivery device and photoactivated with a laser to achieve its intended therapeutic effect. During the development of combination products, it is important to consider the regulatory requirements for analytical testing that apply not only to the drug product or device individually, but also how testing applies to the drug-device combination based on its clinical use in the context of differing global regulatory standards. An integrated drug-device combination product analytical testing plan should be developed that evaluates characteristics such as in-use stability, long-term stability, container closure testing, extractables/leachables, chemical/physiochemical properties, and device performance testing. Consideration of the

combined device and drug characteristics early in the combination product development process can reduce the potential for delays in both product development and the regulatory review process and aid the innovation of new products.

**77** **Elevating Quality and Compliance with Transformative Software Solutions**

Tracy M. Hibbs, Waters Corporation, 34 Maple St., Milford, MA 01757

As organizations navigate the challenges of increasing efficiency and productivity without compromising quality or compliance, new software solutions are emerging that enable laboratories to reduce risk through vigilant monitoring, ensure precision, and uphold the highest standards of compliance. By streamlining processes, minimizing errors and downtime, and providing real-time insights, these innovations not only enhance operational efficiency but also support laboratories in meeting stringent regulatory requirements for quality management maturity. Join us to uncover how embracing these innovations can lead to safer, more effective operations by fostering a culture of accountability, continuous improvement, and sustainable compliance.

**78** **Navigating the Future - Some Regulatory Considerations for Implementing Innovative Analytical Technologies in Pharmaceutical Manufacturing**

Ting Wang, Amgen, One Amgen Center Dr., Thousand Oaks, CA 91320, Nina Cauchon, Paul Kirwan, Marisa Joubert, Marquerita Algorri, Brian Bell, Robert Soto, David Semin

Analytical technologies and methods play a pivotal role in attribute understanding and control which are essential to the rapidly evolving field of pharmaceutical development and manufacturing. These technologies are advancing quickly, where innovations often involve both new scientific approaches and novel applications of established techniques. In many cases, the lack of harmonized global regulatory expectations presents challenges for the adoption of advanced technologies. This presentation explores some emerging technology trends and applications, while highlighting regulatory considerations for integrating innovative analytical approaches in pharmaceutical manufacturing. We provide examples on the specific technologies such as rapid microbial testing for environmental monitoring while discussing aspects of the current regulatory landscape and desired future advancements in the regulatory framework. We hope to promote the adoption and implementation of innovative analytical technologies for enhanced patient access, while ensuring product quality and safety.

**79** **Hard & Soft Skills I Rely On But Don't Remember Being Taught in School**

Nancy Jestel, SABIC, 1 Noryl Ave. Technology T2, Selkirk, NY 12158

Looking back on my 25+ years in industry as an analytical chemist, I can point to specific skills that I wish I had been taught in school – even as I also know I received a great education and was well-prepared for my job. The reality is that there will never be time to learn everything in school that you might need in the real world, nor frankly would you want to do that! (Life would be boring!) Employers often provide professional development training to narrow the skills gaps they perceive between what an employee learned in school and what is needed to get a job done and then assume on-the-job training provides the rest. The initial focus often is on hard (technical) skills because they are much easier and less subjective to define and assess, but career performance usually is driven by mastery of soft (non-technical or human-related) skills. This talk will explore selected hard and soft skills that I use regularly but I learned or mastered after my formal education.

**80** **The Depth of the Undergraduate Lab Experience at a Large University**

Zachary Rhoden, Penn State University, 331B Whitmore Lab, 4575 Pollock Rd., State College, PA 16801

Delivering a meaningful and rigorous laboratory experience to thousands of undergraduate students each year presents unique logistical and pedagogical challenges. At large research universities where introductory chemistry courses serve as foundational experiences for students pursuing chemistry, but also serve non-chemistry and non-science majors, there is a constant tension between driving student engagement, designing accessible, scalable content, and providing educational breadth and depth. This talk explores how a large university navigates these challenges, particularly the methods used to provide depth in the undergraduate lab experience.

**81** **Preparing Undergraduates for Next Steps Beyond College: What's the Same and What's Changed after 25+ Years at a Small Liberal College**

Mary Kate Donais, Saint Anselm College, Chemistry and Forensic Science Department, 100 Saint Anselm Dr., Manchester, NH 03102

Having entered academia after a handful of years in government and industry labs, practical concepts and lab skills are very deliberately emphasized in my teaching. For all science majors there are certain concepts and skills, many of which have not changed over the years, that are included in introductory courses like General Chemistry and Quantitative Analysis. Today's students are not the same as those from 25 years ago, 10 years ago, and even 5 years ago, however. So with those changes in students come adjustments to teaching to better prepare those students for success in both their upper-level science courses and then ultimately after college. Our General Chemistry I course now has more time allotted for foundational concepts and lab skills. More group work and instructor-guided problem solving also are included to better gauge student understanding of course material. Upper-level science majors courses also have evolved over the years in parallel with industry and research trends. Through my encouragement, our department has expanded our instrument holdings. I've developed and teach a 2-credit course in chemometrics and integrate coding in courses. I have always, however, designed labs to maximize student independence and skill building. Students do as much of their own solution and sample preparation as time allows. They operate the instruments and perform calculations with just enough guidance to promote success and confidence. Lastly, the majority of our students conduct faculty-directed research. These and other aspects of student post-college preparation will be discussed in this presentation.

**82** **Education is Everyone's Responsibility, Especially the Societies**

Ellen Miseo, Miseo Consulting, 39 Blacksmith Dr., Needham, MA 02492, Stephanie Shaffer

Where is someone educated? If you ask that question of a group of scientists, you will get a variety of answers, including universities, books, scientific papers, and conferences. They might include instrument vendors. With the advent of YouTube, on-line webinars, e-newsletters, and sponsored white papers, how do you decide if the information you are using is of good quality? Many of the current practicing spectroscopists believe that there is a major disconnect between what is taught and what is needed. Even if someone learns how to **run** an instrument, they are not given the skills to appropriately **apply** that technique to solve a problem. This is made more complex because instrument vendors build instruments making it easier to acquire data but not why you want to acquire that data or what to do with it. The problem is compounded by the fact that a lot of users of spectroscopy do not even have chemistry exposure. Industry cannot expect practical application training to happen in school. To solve the problem requires commitment from a variety of stakeholders. Classes need to be readily accessible; that means on-line. Employers must recognize the need for training, be willing to pay for it, and not expect that a recipe will be provided with the instrument. And most importantly the analyst must be motivated to learn from the experts, possibly on their own time. This presentation will explore some of the approaches that are being taken to address this gap in user knowledge.

**83** **Mapping your Life and Everything Else: The Promise of High Dimensional Phenomics**

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

The human genome project is recognized as being one of the most successful big science projects in modern history. One of the primary motivational underpinnings 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 the frontiers of our knowledge expanded dramatically, we also uncovered profound biological complexity that we could not understand. This led to the current frontier in the measurement science 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 (including DNA, RNA, proteins, lipids, carbohydrates, metabolites), 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 (including minimal sample preparation, fast measurements, high concentration dynamic range, low limits of detection, and high selectivity) and computational approaches to organize data into actionable information. Ion mobility-mass spectrometry (IM-MS) provides rapid (ms) gas-phase electrophoretic separations on the basis of molecular structure and is well suited for integration with rapid (µs) mass spectrometry detection techniques to perform integrated proteomics, metabolomics, lipidomics, glycomics, among many others. This report will describe advances in IM-MS integrated omics measurement strategies in the analyses of complex biological samples of interest in systems, synthetic, and chemical biology.

**84** **Ion Mobility-Enhanced Lipidomics Reveals the Role of Diacyl-Phosphatidylcholine in Ferroptosis**

Fereshteh Zandkarimi, Columbia University, 3000 Broadway, New York, NY 10027

Ferroptosis is a regulated, iron-dependent form of non-apoptotic cell death driven by lipid peroxidation, particularly affecting phospholipids containing polyunsaturated fatty acid (PUFA) tails. While mono-PUFA phospholipids have been widely studied, the role of diacyl-PUFA phosphatidylcholines (PC-PUFA2) remains unclear. We ap-

plied LC-HDMSE-based lipidomics—combining liquid chromatography with ion mobility-enhanced high-definition mass spectrometry—to profile PC-PUFA2 species in ferroptosis-sensitive and -resistant cancer cell lines. Under basal conditions, PC-PUFA2s were significantly more abundant in sensitive cells, whereas monoacyl-PUFA phospholipid levels showed no significant differences. Supplementation with PC-PUFA2s induced ferroptosis, which was suppressed by mitochondria-targeted antioxidants, suggesting mitochondrial reactive oxygen species (ROS) as a critical driver. PC-PUFA2-treated cells also showed elevated mitochondrial superoxide and membrane depolarization. To assess broader biological relevance, we analyzed caudate nucleus tissue from Huntington's disease (HD) patients and age-matched controls. Targeted lipidomics revealed a significant depletion of PC-PUFA2s in HD brains, correlating with oxidative stress markers—supporting their role in neurodegenerative disease. These findings identify PC-PUFA2s as key lipid mediators of ferroptosis through mitochondrial ROS generation and oxidative lipid damage. The use of ion mobility-enabled lipidomics provided enhanced resolution of lipid isomers and structural features, allowing for confident annotation of functionally relevant species. Our study highlights a previously underexplored lipid class as a ferroptotic vulnerability and potential therapeutic target in cancer and neurodegeneration.

## 85 Advancements in Ion Mobility Spectrometry Measurements for Native Protein Mass Spectrometry

Rachel Buckley, Rutgers University, 170 Frelinghuysen Rd., Piscataway, NJ 08854, David Clemmer, Lauren Aleksunes, Brian Buckley

Ion mobility spectrometry is a measurement of the shape of an ion of interest, which can provide structural information about the ion. By coupling electrospray ionization, ion mobility separation, and mass spectrometric analysis, rough molecular structures can be quantifiably measured, using shape, mass, and charge. Similarities exist between the structure of a molecule as it exists normally in solution and the structure of the molecule in the gas phase, measured by a mass spectrometer. Native mass spectrometry was the term minted to describe the use of mass spectrometers, often in tandem with ion mobility measurements, to measure the structure of an ion of interest. Native mass spectrometry has evolved since its inception. Changes to ionization sources allowed for new questions to be asked regarding protein structures in solution and to push the boundaries of what types of media containing proteins are measurable. Ion mobility devices have evolved from simple linear drift field separation devices to multi-field-transient electrostatic devices with different methods to improve the resolution of mobility measurements so that more information pertaining to protein structure can be determined. Changing the internal energy of the protein ion, after it has left solution, allows investigators to probe the energetics of conformational moieties during the measurement process. This talk focuses on the advancements made in native mass spectrometry, specifically protein measurement, by modifications to the electrospray processes, advancements in mobility instrumentation, and the development of strategies and techniques to modify protein structures within the instrument.

## 86 Protein Charge State Confirmational Studies on LC-ESI-MS and Ion Mobility Mass Spectrometry-A Comparative Study

Srinivas Chakravartula, Rutgers, The State University of New Jersey, New Brunswick, NJ 08903

Liquid chromatography interfaced to electrospray ionization mass spectrometry (LC-ESI-MS) and ion mobility mass spectrometry (IM-MS) are valuable techniques for studying protein charge states and conformations. Understanding the structure of biomolecules is important as different conformations may affect biological activity, an important factor for the biopharmaceutical industry and it can also provide clues to protein related pathologies for clinical diagnostics. By looking into charge state distributions (CSD's) patterns of proteins under varying conditions LC-ESI-MS and IMS can provide insights to uncover protein conformational changes. In this study, protein standards: cytochrome c, myoglobin and lysozyme were used to evaluate the structural proteomics applications using LC-ESI-MS and IMS under native and non-native conditions which will be presented.

## 87 Interfacial Phenomena in Lipid Nanoparticle (LNP) Systems

Yulia Eygeris, Oregon State University, College of Pharmacy, 2730 S. Moody Ave, Portland, OR 97201, Gaurav Sahay

Nucleic acid therapeutics have emerged as a powerful therapeutic modality for cancer, genetic diseases, and infectious diseases. To enter the cells, nucleic acids rely on delivery platforms such as lipid nanoparticles (LNP). Design of efficient LNPs is a complex task involving considerations in the nucleic acid cargo, the chemistry of individual lipid components, and the processes involved. The fine structure of LNPs, characterized by parameters such as shape, lipid membrane density, and lipid distribution through the particle, is a topic that is gathering a lot of attention since the structure of the LNP interface correlates with stability, transfection capability, and immune response to the delivery vector. Our research group has established that LNPs go through significant morphological changes in their lifetime, as demonstrated by cryo-TEM. These changes can be inflicted by virtually every aspect of the LNP formulation process, including 1) chemical structures of lipids used in formulation, 2) manufacturing process and 3) neutralization or storage buffer. Notably, the LNP structure is also heavily influenced by the nature of nucleic acid cargo. The irregular architecture of LNP constructs – e.g., presence of aqueous pockets, multilamellar structure, and polymorphic shape - seems to have the most positive impact on nucleic acid delivery in cell and animal models. These observations seemingly contradict the narrative that LNPs must present as homogenous, spherical constructs fully containing nucleic acids within the body of LNPs. Understanding the phenomena happening at the LNP interface will unravel the fundamental principles connecting the LNP design and properties to their therapeutic efficacy.

## 88 Leveraging Advanced Characterization Techniques in Drug and Device Combination Products: Enhancing Safety, Performance, and Stability

Guangli Hu, Merck & Co, Inc., 126 E. Lincoln Ave., Rahway, NJ 07065

The integration of advanced characterization techniques is vital for the development of drug and device combination products, significantly enhancing their safety, performance, and stability. This presentation will focus on three key areas of investigation. First, we will discuss the selection of robust primary container systems, specifically examining coring in elastomeric needle shields of pre-filled syringes and needle clogging issues related to high-concentration formulations. By utilizing advanced imaging techniques, material characterization, and predictive modeling, we aim to identify effective mitigation strategies that enhance product reliability. Next, we will explore the application of advanced characterization techniques in studying siliconization and protein formulation stability. These insights are essential for optimizing the performance of pre-filled syringes and ensuring consistent drug delivery. Finally, we will address the optimization of injectability through innovative approaches, analyzing high-concentration suspensions and the factors contributing to needle clogging. Our findings will provide critical insights into formulation and device design, ultimately improving injectability and ensuring accurate drug delivery. By leveraging these advanced analytical methods, we aspire to enhance the overall safety and efficacy of drug/device combination products, paving the way for more reliable therapeutic solutions. This presentation will also highlight the importance of interdisciplinary collaboration in overcoming the challenges inherent in developing these complex products.

## 89 Connecting Complex Drug Product Design Space with In Vitro and In Vivo Performance through Image-based Prediction

Shawn Zhang, digiM Solution, 500 W Cummings Park, Ste. 3650, Woburn, MA 01801, Josh Lomeo, Phil Yawman

Rapid improvement in spectroscopy and imaging analysis enable mechanistic understanding of complex drug products, thus rendering these tools essential in development, failure analysis, and regulatory filing. However, advanced imaging analysis tools characterize the product in its current formulation and manufacturing conditions, which still requires in vitro testing to correlate with, and iterations of formulation and manufacturing campaigns to implement optimizations toward a desirable performance. This talk highlights two predictive software tools that complete the complex drug product design life cycle: a). dissoLab, an image-based, microstructure informed dissolution and release prediction tool [1]; and b). sGAN, an image-based microstructure synthesis tool[2]. Leveraging the microstructure properties from spectroscopy and imaging analysis, dissoLab and sGAN connect product design space (particle size, drug loading, process conditions, material choices) to product performance (mechanical, release, stability), allowing pharmaceutical development to iterate toward a desirable therapeutic performance with substantially reduced need for material, iterations, and cost.

[1] J. Gautreau et. al., Intrinsic Particle Dissolution Prediction from Images: Method and Validation of dissoLab Platform, AAPS Open, Accepted AAPO-D-25-00070R1 (2025)

[2] T. Hornick et. al., In silico formulation optimization and particle engineering of pharmaceutical products using a generative artificial intelligence structure synthesis method, Nature Communications 15:9622 (2024) https://www.nature.com/articles/s41467-024-54011-9

## 90 Quantitative Morpho-Chemical Imaging of Subvisible Particles in Biopharmaceutical Formulations

Dan Fu, University of Washington, Department of Chemistry Box 351700, Seattle, WA 98195

The characterization of subvisible particles (SVPs) is critical for ensuring the safety and efficacy of biopharmaceutical formulations. However, existing analytical techniques, such as light obscuration and microflow imaging, focus on measuring particle size and number based on the light scattering properties of those particles. There is no chemical information from these measurements. While infrared and Raman spectroscopy can provide chemical characterization, they lack the throughput for measuring a large number of SVPs. This talk introduces a novel approach that integrates Raman spectroscopy with rapid particle imaging to overcome these limitations. By providing simultaneous morphological and chemical analysis of individ-

ual SVPs, our method enables detailed characterization of heterogeneous particle populations within formulations. This powerful combination not only offers a more complete picture of product quality but also has the potential to reveal the mechanism of SVP formation, paving the way for more robust and stable biopharmaceutical products.

**91** **Polydrug Case Examples Containing Novel Psychoactive Substances (NPS) in the United States Drug Supply**

Mia Borrelli, Center for Forensic Science and Research & Education, 206 Welsh Rd, Horsham, PA 19044, Amanda Mohr, Barry Logan

The illicit drug supply is increasingly complex and unpredictable, with many samples containing multiple substances beyond the primary drug of interest. Adulterants and novel psychoactive substances (NPS) are commonly added to enhance effects or increase profitability, posing significant risks to public health. NPS often mimic the effects of controlled substances but are frequently unregulated or unscheduled, making them particularly dangerous due to their unknown pharmacological profiles. State-funded laboratories often lack the resources, time, and advanced instrumentation needed to proactively search for unknown compounds and NPS, limiting their ability to keep pace with the rapidly evolving drug landscape. With many seized drug laboratories concentrating on the highest scheduled substance in the sample, combinations of drugs can be underreported. Additional factors that lead to underreporting of substances include analytical methodologies implemented by laboratories that are unable to encompass all substances present in the sample (i.e. shorter run times, less sensitive instrumentation, limited scope). In the examples, submitted drug materials were evaluated using our in-house testing protocols using various pieces of instrumentation, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry with quadrupole time-of-flight technology (LC-QTOF) to determine all substances present. One sample contained 32 compounds present other than the main compound which was Fentanyl. Additionally, the workflow in our laboratory for the identification of an unknown substance is described based on a real-world case example.

**92** **Applications of Chemometrics to NPS Drug Analysis**

Jennifer Bonetti, Virginia Department of Forensic Science, 830 Southampton Avenue Suite 400 Norfolk, VA 23510, Arian van Asten, Saer Samanipour

The field of forensic drug analysis has evolved substantially over the past several decades. Increases in new substances present a notable analytical challenge because typical forensic methodology (Gas Chromatography-Mass Spectrometry [GC-MS], for instance) is frequently incapable of identifying the small structural differences between similar compounds. Positional isomers, wherein the only difference is the precise location of a small moiety on an aromatic ring, often produce visually indistinguishable mass spectra. In some instances, these isomers also display similar chromatographic behavior, further complicating the analysis. The work presented in this presentation offers an alternative method of handling challenging drug exhibits by focusing on advanced data science techniques rather than improvements in instrumental hardware. The application of chemometric methods to data that is commonly generated in the regular course of forensic analysis is likely to be more accessible to laboratories than the purchase of additional instruments or by conducting supplementary time-consuming chemical analysis. The instrumental techniques surveyed in this work include GC-MS, Direct Analysis in Real Time – Time-of-Flight Mass Spectrometry (DART-TOF MS) and Gas Chromatography – Infrared Spectroscopy (GC-IR). The chemometric methods utilized include Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA), the sequential combination of the two methods (PCA-LDA), Welch's t-test, and the Random Forest algorithm. For each instrumental method, chemometrics provided increased objectivity and improved the discrimination capabilities when faced with the challenge of distinguishing between highly similar compounds.

**93** **Advancing Nontargeted Analysis with GCxGC Using a Sustainable Approach**

Kira Fisher, William & Mary, 540 Landrum Dr., Office 1052, Williamsburg, VA 23185, Katelynn Perrault Uptmor

Forensic science often deals with the analysis of true unknowns in evidence samples such as drugs and chemical residue where analyte identification comes with no pre-existing information. Nontargeted screening can therefore support the comprehensive characterization of true unknowns more successfully than targeted methods for one or several analytes. One nontargeted technique is comprehensive two-dimensional gas chromatography (GC×GC) with time-of-flight mass spectrometry (TOFMS), a separation technique that separates analytes based on two independent retention mechanisms. This study aimed to demonstrate the effectiveness, sustainability, and efficiency of hydrogen as a carrier gas for GC×GC-TOFMS by characterizing analytes from pepper spray. Using hydrogen over helium as a carrier gas leads to significantly shorter run times and can reduce carrier gas costs, both benefits that were observed in this study. Significant improvements in resolution were also seen for GC×GC-TOFMS analysis of forensic samples. Nontargeted analysis with GC×GC allowed for more peak capacity for unknown analytes instead of only targeted analytes in a specific range. With sustainable chemistry becoming more of a focus across the industry, the use of helium is less favorable, and alternative options are being sought. Acknowledging these enhancements when using hydrogen, this study demonstrated an effective and sustainable way to measure analytes in chemical agents such as pepper spray using GC×GC-TOFMS.

**94** **Forensic Applications Using GC/FTIR with Light Pipe Technology to Acquire Infrared Vapor Phase Spectra**

Lewis Smith, Cape May County Forensic Lab, Cape May County Court House, 4 Moore Road, DN 110, NJ 08210

Forensic examination of most seized drug samples involves solvent dilution followed by Gas Chromatography due to the limited quantity and/or sample impurities. The "gold label" detector of choice for gas chromatographic effluents is Mass Spectrometry which, in some cases, cannot discern the molecular ion or identify isomers. Regioisomers having identical mass spectra and similar retention times can result in mis-identification. Coupling Infrared with GC can provide an effective solution to these problems. Early GC/IR prototypes using dispersive/grating spectrophotometers could not offer spectra in the vapor phase due to slow scan rates. Column effluents were trapped and later scanned in the condensed phase. With the advent of interferometric multiplex-scanning techniques from Digilab, FTIR made it possible to obtain "on-the-fly" spectra from chromatographic peaks. Sensitivity was further enhanced with the development of capillary column/light pipe technology. Another full magnitude of sensitivity was achieved in 1986 by Tomas Hirschfeld designing the HP 5965A IRD. Current version of this unit "IRD3" is available from ASAP in Covington, Kentucky. This talk will explain differences between GC/FTIR light pipe/solid-deposit, flow/static cells. Molecules in the vapor state at elevated temperatures are free from the effects of intermolecular H-bonding, water, and polymorphism and enable rotational isomerism. This made it possible to completely categorize all Synthetic Cannabinoids into a basic structure classification for easy identification. Applications will be given for various drugs. Most importantly, principals will be outlined to illustrate that VP libraries offer the most accurate search results for solving unknowns.

**95** **Lego Blocks and Raman Spectroscopy: Evaluation of Advanced Data Collection Techniques**

Richard Crocombe, Crocombe Spectroscopic Consulting, 30 Thornberry Rd., Winchester, MA 01890, Peter Larkin, Pauline Leary, Brooke Kammrath, Mary Kate Donais

In 2024 we proposed the use of a set of Lego blocks as samples to evaluate fluorescence mitigation and avoidance techniques, and performance with dark samples, in Raman spectroscopy. In that paper we characterized their Raman spectra using laboratory instruments with a variety of exciting wavelengths. At last year's SCIX we described the performance of ten different handheld Raman instruments, employing a number of these schemes and a variety of exciting lines, using these blocks. The combination of a series of colored blocks (white, yellow, red and blue), and successively darker tone blocks (white, gray and black) did challenge these instruments, and shed light on the ways that their manufacturers have optimized these instruments in specific areas and for different purposes. The importance of fluorescence avoidance and/or mitigation for handheld Raman instruments cannot be overstated, given the challenges of scanning and identifying unknowns. The operators of these instruments are highly likely to be non-scientists, wearing personal protective equipment (PPE), who rely on the instrument giving an accurate identification of the material under test. We have now extended this work to advanced data collection techniques which are poised to appear in handheld Raman instruments: (a) deep-UV excitation; and (b) the use of high repetition rate, short pulse duration, exciting lasers coupled with single photon avalanche photon array detectors (SPAD arrays), which have very fast time resolution capabilities. To further characterize the blocks, we have also examined them using handheld X-ray fluorescence spectrometry. The results will be reported in this presentation

**96** **Analytical and NMR Studies on the Photodegradation of Plant Chromophores**

Kamrun Nahar, Brooklyn College, Department of Chemistry, 2900 Bedford Ave., Brooklyn, NY 11210, Serah Essang, Akshaya Iyer, Alexander Greer

Strategies to understand the photodegradation of organic and plant chromophores are needed. Photogenerated singlet oxygen and oxygen radicals are reactive species that play a key role in the process, however, mechanisms of tandem reactions, such as the uptake of two singlet oxygen molecules, are poorly known. A 1D and 2D NMR study provided insight to the tandem addition of singlet oxygen into the natural product diprenyl phenol; first at the inner geranyl C=C bond, and second at the outer geranyl C=C bond leading initially to dihydrofurans and later allylic hydroperoxides. Results show that photodegradation leads to dihydrobenzofurans, hydroperoxides, epoxides, and quinones. Our study of singlet oxygen quenching by NMR spectroscopy opens up opportunities to new understanding the photooxidative processing of plant chromophores.

## 97 Composition and Structure of Fluorinated Polymers in the Molten State by High Temperature Static NMR

Alexander Marchione, Chemours Discovery Hub, 201 Discovery Blvd., Newark, DE 19713, Michael Davis

High temperature nuclear magnetic resonance spectroscopy is applied to insoluble fluorinated polymers to obtain compositional and structural information in the molten state without need for magic-angle spinning. In this study, the composition of various fluoropolymers is determined by $^{19}F$ NMR at an acquisition temperature between 150 and 350 °C. In the case of partially fluorinated polymers, where quantitation is not possible by $^{19}F$ alone, high temperature $^{13}C$ experiments function to the same end. The use of two-dimensional correlation experiments, mediated by scalar coupling, can provide additional information about the structure of the polymeric analytes. These include $^{19}F$ homonuclear and $^{19}F$-$^{1}H$, $^{13}C$-$^{19}F$, and $^{13}C$-$^{1}H$ heteronuclear experiments.

## 98 Sorption and Desorption of 17alpha-ethinylestradiol and beta-estradiol on Montmorillonite Clay and Nylon Microparticles Using Fluorescence Detection

Christian Manuelli, University of Massachusetts-Dartmouth, 531 N. Front St., New Bedford, MA 02745, Shouwei Cai

There is growing concern of the adverse effects of endocrine disrupting chemicals (EDC's) on the environment, wildlife, and human beings. To better understand the transportation and fate of EDC's (such as poly-chlorinated biphenyls, perfluoroalkyl substances and hormones) in the environment, it's important to study the solid-liquid interactions of these compounds with common substrates. In this study, the sorption and desorption of estrogens 17a-ethinylestradiol (EE2) and b-estradiol (E2) to montmorillonite clay, a major mineral component in surface aquifers, and nylon microparticles, a common plastic pollutant, was investigated in aqueous solutions. The effect of pH, salinity, and background ionic strength to sorption and desorption was also studied. A novel methodology using fluorescence detection was used as a more efficient alternative to chromatographic determination. Equilibrium between the aqueous phase and clay concentrations was reached after 72 hours, compared to 24 hours with nylon concentrations. The adsorption capacity for nylon microparticles was higher for both estrogens than for montmorillonite, with EE2 displaying stronger interactions to both. Four different models were fitted to the sorption kinetics of both estrogens. Sorption of estrogens to montmorillonite fit only the Freundlich isotherm, while sorption to nylon fit both the Langmuir and Freundlich models well, with $R^2$ values both over 0.95. The proposed mechanism for sorption to nylon was hydrophobic interactions, and intraparticle diffusion for sorption to clay. Desorption of E2 from clay was slightly stronger than EE2, while desorption from nylon was insignificant for both estrogens after 24 hours.

## 99 Nitrosamines: Explaining Why Ranitidine HCl Degrades in the Solid State

Eric Munson, Purdue University, 575 Stadium Mall Dr., West Lafayette, IN 47907, Jianchao Xu

Nitrosamines are of great concern to the pharmaceutical industry. Nitrosamines can form either during the synthesis of the active pharmaceutical ingredient, or upon storage. Ranitidine HCl was one of the first drugs that was recalled due to the formation of nitrosamines. King et al. from GSK performed an analysis of the cause of ranitidine degradation, and found that there was about a 25x increase in nitrosamine (specifically NDMA) formation depending upon how the ranitidine was crystallized, but did not provide any mechanism for the root cause of the different reactivities between samples. We have been investigating the cause of ranitidine degradation, and found that it is related to solid-state reactive species (SSRS), which are crystal defects and amorphous material that can be produced by either milling or crystallization. We have identified a stable sample of ranitidine HCl that did not significantly degrade upon storage, and cryoground it for 5-30 minutes. Unground samples changed from ~1.5 to ~3 ppm of NDMA upon storage at 60 C for 24 days, whereas samples that were cryoground for 30 minutes degraded to ~300 ppm of NDMA. Moreover, tablets prepared with unground and cryoground ranitidine HCl showed similar trends, with the unground being essentially unreactive (~3 ppm after storage), whereas samples containing 30 minutes cryoground material degraded to 900 ppm NDMA. This highlights the challenges of using API that has a significant amount of SSRS, and suggests an alternative method for reducing nitrosamine formation in drugs that avoids reformulating a product.

## 100 Establishing a Robust Analytical Technique for Ultralow Nitrite Detection and Quantification in Pharmaceutical Excipients

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Pharmaceutical excipients are key components of a drug product, playing critical roles in bioperformance, stability, appearance, and manufacturability. Excipients used in drug formulation frequently contain nitrite ion impurities, increasing the risk of nitrosamine formation upon reaction of nitrite with a secondary amine source.[1] Nitrosamines are potential mutagens, and therefore detection and exclusion of nitrosamine formation agents is critical. Routinely employed GC and IC analytical techniques, although highly sensitive, rely heavily on suitability of analyte physical characteristics and commercial site availability. A nitrite derivatization method has been reported, which relied on the reaction of nitrite with an amine probe to generate an UV active derivative. [2] Here, we developed a robust analytical method that can utilize this derivatization chemistry to detect and quantify nitrites in a range of hydrophobic to hydrophilic excipients. With this approach, we have been able to make nitrite detection and quantification highly selective using HPLC-UV. Automation of the sample preparation was also developed to achieve comparable results to manual sample preparation. The universality of the sample preparation and analysis in addition to fast response, selectivity and ultra-low nitrite detection limits can lead to a wider adoption industry-wide for assaying nitrite and de-risking nitrosamine formation in formulations.

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## 101 LC-MS Screening of Nitrosamines Using Superficially Porous Particle Columns

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

Nitrosamines are a class of compounds formed from reactions between a nitrosating agent, such as nitrites or nitrates, and a nitrogenated precursor, such as a secondary or tertiary amine. They can be found in food, medical devices, industrial products, and the environment and can also be formed as drug substance related impurities in pharmaceuticals. Their presence is concerning because most nitrosamines are carcinogenic. In 2018, N-nitroso-dimethylamine (NDMA) was found in valsartran, which prompted recalls of the drug. As more investigations occurred, the presence of nitrosamines was discovered in other pharmaceuticals, such as ranitidine, nizatidine, metformin, rifampicin, rifapentine, varenicline, and sitagliptin. Both gas chromatography (GC) and liquid chromatography (LC) can be used to analyze nitrosamines, however, both have their limitations. GC inlet temperatures can cause some compounds to generate nitrosamines so pharmaceutical testing is mostly done by LC/MS. Achieving adequate retention of nitrosamines under typical reversed-phase conditions using a C18 stationary phase can be challenging. This presentation will include LC-MS screening of multiple superficially porous particle silica columns with reversed-phase stationary phases using a 12-component nitrosamine standard. Atmospheric pressure chemical ionization (APCI) will be employed since it improves detection sensitivity. Retention time and peak shape will be evaluated and an optimized method will be highlighted.

## 102

Withdrawn by the author.

## 103 Using High pH LCMS Conditions for Impurity Characterization of GLP-1 Therapeutics

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As biological-based therapeutics become increasingly complex, trace impurity characterization via LC/MS has become a critical step in the QA/QC manufacturing process. While LC/UV is still the preferred detection method for routine testing in manufacturing, many impurities can be difficult to separate and distinguish from the primary product using traditional chromatography methods such as C18 columns and trifluoroacetic acid as an ion pairing agent. An alternative to using acidic buffers for peptide separations is to use high pH tolerant silica for separation in basic conditions. At high pH, peptides become deprotonated, impacting their interactions with the bonded phase. In this presentation we demonstrate how the use of peptide separations at high pH can be used to enhance impurity characterization of GLP-1 therapeutics. We compare the impurity profile of pharmaceutical grade Tirzepatide and Semaglutide to research grades using a charged surface C18 column in acidic conditions and a high pH C18 column in basic conditions using Ammonium Hydroxide as the mobile phase modifier. We also examine the impurity profile of compounded Semaglutide and compare it to the commericial pharmaceutical grade Semaglutide (Ozempic®).

## 104 Reliable Analysis of Genotoxic Impurities Using Ultrahigh-Resolution MRR

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Small genotoxic impurities can be introduced into pharmaceutical, natural or chemical products during essentially any stage of manufacturing or storage. Due to complexity of both the analytes and sample matrices, the analysis may require

use of state-of-the-art hyphenated techniques such as high-end chromatography combined with high-resolution or tandem mass-spectrometry. Even for these combinations of high-end techniques, achieving reliable and reproducible analysis can still be a challenge. Molecular Rotational Resonance (MRR) spectroscopy combines extraordinary chemical selectivity with ultrahigh spectral resolution to enable reliable identification and accurate quantification of challenging analytes even in most complex sample matrices. MRR spectral fingerprints precisely reflect molecular geometry and are truly unique, even for structurally similar chemicals such as stereo and constitutional isomers. In addition, MRR frequencies are absolute and extremely narrow to essentially eliminate spectral overlaps and, thus, a need for chemometric calibration models. Finally, confident molecular identification can be performed without reference standards, by comparison to quantum chemistry calculations. In this presentation, we demonstrate performance metrics for MRR analysis of various genotoxic impurities that are challenging for other analytical techniques or combinations of techniques. The examples include direct and derivatization-free analysis of formaldehyde, challenging residual solvents and other important genotoxic impurities.

## 105 Taming Odor: Investigating Odor Measurement and Control

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With an ever-expanding population, the management of waste comes closer into contact with the development of residential areas. Waste handling operations (WHOs), such as landfills, must be cognizant of the odor release from their facilities and manage this release. An important part of assessing odor release is its reliable measurement. A gas chromatography – triple quadrupole – mass spectrometry method was developed to simultaneously determine 43 odor compounds of concern from multiple odor classes, including volatile fatty acids, nitrogen- and sulfur-containing molecules, and other volatile organic compounds. Separation was achieved on a 60 m RTX-1 column with a 7 µm film thickness. Samples are collected in silco-treated air canisters, and a 1.0-mL volume is taken from the canister using a pressure-lock syringe and injected into the instrument in splitless mode. Limits of detection and quantitation for most of the 43 analytes fall below their odor threshold, which makes the method well viable for monitoring odors from WHOs. Additional experiments have been performed using headspace – gas chromatography to understand the potential for odor sequestration provided by different odor control formulations.

## 106 Applications of Nanocarbons in Chromatography, Sample Preparation, and Membrane Separation

Somenath Mitra, New Jersey Institute of Technology, Dept. of Chemistry and Environmental Science, Newark, NJ 07102, Karwa Mahesh, Chutarat Saridara, Ornthida SaeKhow

This study presents the versatile applications of carbon nanotubes (CNTs) and their functionalized analogs in chromatography, sample preparation, and membrane-based separations. Using chemical vapor deposition (CVD), CNTs can be precisely self-assembled on a variety of substrates, with distinct strategies demonstrated for single- and multi-walled CNTs. These self-assembled CNT structures have been applied in fabricating open-tubular gas chromatography (GC) columns and as sorbents for micro–solid phase extraction (micro-SPE). Their unique sorption behavior enables efficient separation of compounds with widely varying volatilities and polarities. A complementary approach involves incorporating CNTs into polymeric matrices to develop membranes with improved permeation rates and selectivity. The integration of CNTs introduces multiple transport pathways for solutes and analytes, enhanced by their high aspect ratio, nanostructured morphology, and expanded active surface area. These properties increase mass transfer rates and partition coefficients, collectively contributing to enhanced permeation performance. Applications of CNT-enhanced membranes span from analytical-scale extractions to large-scale seawater desalination. In organic molecule extraction, CNTs act as nanosorbents, promoting rapid solute exchange. In membrane distillation for desalination, they enable selective sorption and transport of water vapor, improving both efficiency and selectivity. Overall, this work demonstrates how nanocarbons—through tailored functionalization, self-assembly, and hybrid membrane fabrication—offer unique opportunities for advancing separation science. Their multifunctional properties make them promising materials for high-performance chromatographic stationary phases, sample preparation sorbents, and membrane separations across environmental, analytical, and industrial applications.

## 107 Good Taste: Field-Deployable Methods and Devices for Determination of Smoke Taint in Wine Grapes

Erin Kalbaugh, Oregon State University, Dept. of Chemistry, 153 Gilbert Hall, Corvallis, OR 97331, Vincent Remcho

Smoke taint, a wine fault characterized by a smoky flavor and aroma, has led to significant agricultural and economic loss. Losses have risen in recent years owning to a marked increase in wildfire events in areas where wine grapes are primary crops – most notably the west coast of the US and parts of Australia. Conventional smoke taint analysis uses both GC-MS and LC-MS to quantify levels of smoke taint markers. These techniques offer high sensitivity, but are not generally field portable, are costly, and the number of labs offering smoke taint analysis is small. We have innovated an alternative technique for smoke taint determination that is simple, inexpensive, field portable, and rapid. Our approach is focused on the metabolic products of grapes that have been exposed to smoke: thiophenols.

We synthesized a selective fluorophore label via a simple one-step reaction: fluorescein covalently modified with two electron-withdrawing 2,4-dinitrobenzenesulfonyl (DNBS) groups to quench its fluorescence. At pH 7.3, the probe undergoes nucleophilic aromatic substitution (SNAr) with thiophenols, releasing fluorescein as a product. Fluorescence intensity is then measured using a purpose-built reader to quantify thiophenol concentration. Our approach produces a linear fluorescence response with a detection limit of 911 nM, as well as a strong selectivity for thiophenols over nonspecific thiols, with fluorescence responses 9-12 times higher. The low detection limit and fast response time of our field portable system, coupled with its strong performance in complex matrices, demonstrate the potential of this approach to smoke taint analysis as a practical tool.

## 108 Fast Direct MS Screening with Chromatography/MS Confirmation with a Single SPME Device

Wei Zhou, University of Waterloo, Department of Chemistry, Waterloo, ON N2K 1W7, Canada, Janusz Pawliszyn

Ambient mass spectrometry (AMS) offers rapid screening but faces challenges in analyzing complex samples because of high matrix effects. The absence of a separation step can also lead to false positives due to the isomers or isobars. In this study, a sequential analysis strategy which combines ambient MS and LC-MS was developed for the first time based on the non-exhaustive microdesorption in solid-phase microextraction (SPME). This strategy was demonstrated by combining coated blade spray (CBS)-MS with LC-MS. In the first step, a few microliters of solvent was used for non-exhaustive desorption with high enrichment factor for rapid screening by CBS-MS facilitating high sensitivity. For the suspicious samples, the remaining analytes on the SPME coating undergo exhaustive desorption, then followed by LC-MS confirmation. The matrix-compatible coating used in the SPME device significantly reduces matrix effects while enhancing sensitivity through analyte enrichment at the extraction step. This method is environmentally friendly, utilizing only a few microliters of organic solvents for screening. The approach was rigorously validated, both theoretically and experimentally, and successfully applied to anti-doping testing, enabling detection of 53 prohibited substances in urine samples by integrating CBS-MS with LC-MS.

## 109 Living the Light

Geraldine Richmond, University of Oregon, Department of Chemistry and Biochemistry, Eugene, OR 97403

As spectroscopists, our love of light in all its colors and behaviors provides us with remarkable insights into our world around us and the cosmos beyond. And it can take our science and inventions in directions and places we may never have imagined. Or as Dr. Suess would say, “Oh, the Places You’ll Go”! This presentation will be a personal reflection - and means of thanking so many who have been central to my being honored with this wonderful SAS Gold Medal Award.

## 110 When is it Good Enough? Rethinking Spectrometers as Fit for Purpose Analyzers and Meters

Adam J. Hopkins, Metrohm, 9250 Camden Field Parkway, Tampa, FL 33578

In the world of NIR and especially Raman spectroscopy, the definition of “good enough” varies dramatically depending on who is using the instrument and why. While academic researchers often prioritize flexibility, sensitivity, and innovation, industrial users typically require tools that are reliable, easy to operate, and tailored to specific tasks. This talk explores how spectrometers are reimagined as fit-for-purpose analyzers—tools that meet the needs of their users not by maximizing performance in every dimension, but by aligning with the practical realities of the application. We will examine how understanding the end-user’s expertise, workflow, and decision-making context is essential to delivering meaningful analytical outcomes. From production lines to field testing, spectrometers are increasingly used by non-specialists who depend on clear, actionable results rather than complex data interpretation. By reframing our expectations around usability, robustness, and relevance, we can expand both the industry and user base of vibrational spectroscopy. Presented in honor of Dr. Geraldine Richmond, this talk also reflects on how the scientific and mentorship training received in graduate school foster the skills need to translate complex spectroscopic principles into solutions that serve diverse users and real-world applications.

## 111 Under Pressure. Unraveling Liquid Monopropellant Combustion Mechanisms with Optical Emission Spectroscopy

Robert Walker, Montana State University, Chemistry and Biochemistry Department, Rom 103, Bozeman, MT 59717

Liquid monopropellants stand out as the most effective and efficient means for generating power in air-independent propulsion systems. Liquid monopropellants are substances comprised of both fuel and oxidant, meaning that these materials burn in the absence of a separate oxidizing source. Sustainable monopropellant combustion, however, typically requires high pressures (>30 bar) where collision frequencies are high enough to propagate the reactions that release energy and convert the fuel into products. Under such conditions, identifying chemical intermediates is challenging, and mechanisms often must be inferred indirectly or relay on computational predictions that have not been experimentally validated. We have recently commissioned a high-pressure combustion assembly based on a liquid propellant strand burner capable of sustaining liquid monopropellant flames for up to 60 minutes at pressures up to 80 bar. The assembly, dubbed "Dreadnought", contains three optical ports that enable real-time monitoring of flame species. Using nitromethane as a simple, model monopropellant, optical emission spectroscopy has identified a host of intermediates in nitromethane flames in both inert ($N_2$) and oxidizing (air) atmospheres. Specifically, wavenumber-resolved emission spectra show water with 4 quanta of vibrational excitation relaxing to its ground state as well as spatially resolve emission from OH, CN, and NH radicals in the flame. Spectra show unusual rotational and vibrational temperature distributions and have begun to clarify the complex reaction dynamics occurring in these high energy systems.

## 112 Structured Water and Surface Potential at Aqueous Interfaces: A New Angle on an Old Problem

Dennis Hore, University of Victoria, Department of Chemistry, Victoria, BC V8W 2Y2, Canada

The interaction of molecules with natural and synethic aqueous surfaces is a fundamental aspect of environment science, biology, and biomedical engineering. There is increasing evidence that the unique water structure and hydrogen bonding environment within the first nanometer of the surface may be largely responsible for driving those interactions. That microscopic region is also influenced by electrostatic interactions, even though the origin of the surface charge is not always clear. This presentation will illustrate how nonlinear optical spectroscopy has the ability to offer a unique probe of interfacial water structure which in turn may be used to reveal the surface charge density at a variety of interfaces. It will illustrate our newest angle-scanning sum frequency experiments in which the phase matching conditions are continuously varied. This enables us to disentangle contributions from the Stern and diffuse regions of the electrical double layer, and to determine the interfacial potential using optical means.

## 113 Evaluating Quality Indicating Assays of the Pharmaceutical and Compounding Pharmacy Industries Towards Improving Accuracy and Reliability

Michael McGinley, Sohve, 476 35th St., Manhattan Beach, CA 90266

The increase in personalized medicine and well documented drug shortages has led to an explosive increase in the use of compounded pharmaceutical products over the last few years. Compounded pharmacies provide customized formulations of approved pharmaceutical drugs where a traditional formulation or dosing is not appropriate for a specific patient or indication. The FDA and USP have developed guidelines to regulate the use and quality of such formulated drugs that is unique from quality guidelines set forth for traditionally approved small molecule therapeutic drugs. Pharma quality testing panels tend to be exhaustive with several non-critical tests; compounding tests tend to focus on sterility. Working with several experts in both fields we looked at several different assays for specific product and use cases and proposed potential right-sized approaches for improving stability indicating assays toward increasing product quality and lifetime. Often simple low-cost LC or even LC/MS assays can provide critical information on purity or stability of a specific batch of product. The impact of right sized approaches will be discussed on its potential impact for both industries.

## 114 NERDVANA - The Place Where Science, Compliance and Common Sense Co-Exist

Carla Kutz, Click Compliance, 6430 Stallion Dr., Imperial, MO 63052

A brief comparison of recommendations vs. requirements for 503A and 503B organizations. Learn the first steps to build the processes and programs required at each level of regulatory oversight. Gain an awareness of current pit-falls and the industry traps most commonly seen in 503A and 503B labs and pharmacies. Understand when to apply USP <795>, USP <797> and CFR 210/211 to be prepared for your next inspection.

## 115 Analyzing Analytical Compendial Testing Requirements: A Comparison Across Regulatory Categories for Compounded Medications

Stephen W. Hoag, University of Maryland, School of Pharmacy, 20 N Pine S., Baltimore, MD 21201

Since the enactment of the Drug Quality and Security Act (DQSA) as part of the 2012 FDA Modernization Act, the regulatory landscape for compounding pharmacies has undergone substantial change. The legislation introduced a new category, 503B outsourcing facilities, which are subject to higher levels of FDA oversight and more rigorous analytical testing requirements compared to traditional 503A pharmacies. Recently, the United States Pharmacopeia (USP) revised its two core compounding monographs, <795> Non-Sterile Preparations and <797> Sterile Compounding, to include additional guidance aimed at strengthening quality assurance (QA) processes. However, the specific requirements for quality control (QC) product testing remain somewhat vague, primarily emphasizing proper documentation and the investigation of any out-of-specification results. Typically, commercially sold medications are tested for identity, strength, purity, and performance, with methods such as identity testing, assay, and impurity profiling primarily conducted through high-performance liquid chromatography (HPLC), which isn't generally accessible to 503A organizations. This presentation will examine the detailed regulatory requirements necessary to ensure product quality, highlighting the differences between 503A and 503B pharmacies in their testing requirements. While small batch sizes in traditional pharmacies make comprehensive testing prohibitively expensive, advancements in low-cost Micro NIR spectrometers offer promising opportunities to improve testing capabilities of traditional pharmacies, enabling enhanced quality control without imposing significant financial burdens.

## 116 Harmonizing Analytical and Regulatory Considerations between Pharma and Compound Pharmacies

Teresa Spann, Fagron, 20 Dan Rd., Ste. 1, Canton, MA 02021

As medical care grows into treating individuals for their specific ailments and required treatments for successful health, compounding pharmacies are filling the gap for their role to reach the patient quicker than the medications available that are manufactured by traditional pharmaceutical companies. In doing so, there are overlaps within the industries to consider related to product testing. This presentation will provide industrial background for those looking to see how their work in the laboratory translates into industrial practice.

## 117 Environmentally Relevant PFAS and Nanoplastics Disrupt Adult Female Ovarian Function in Mice

Genoa Warner, New Jersey Institute of Technology, 161 Warren St., Newark, NJ 07103

Exposure to environmental contaminants has been proposed as a major contributing factor to reproductive disorders and declining fertility. Micro- and nanoplastics (MNPs) and per- and polyfluoroalkyl substances (PFAS) are two classes of persistent environmental pollutants to which humans are widely exposed, yet little is known about their impacts on female reproductive health. The ovary is responsible for the development of follicles, which each contain a maturing oocyte, and the production of sex steroid hormones. Disruption of these processes can alter hormone levels, decrease fertility, and lead to premature ovarian failure. The objective of these studies was to determine if environmentally relevant doses of common PFAS and MNPs disrupt follicle growth, hormone production, and gene expression using an ex vivo mouse ovary model. We focused on two common PFAS, perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), and two model nanoplastics, 200 nm pristine polystyrene spheres (PS) and lab-generated 240 nm polyethylene terephthalate (PET) particles. Ovarian follicles isolated from adult female CD-1 mice were exposed to PFOA, PFOS, PS, or PET from 0.1–100 µg/mL in culture for 5 days. All four treatments impacted follicle growth at the highest doses and altered expression of genes related to the cell cycle and/or apoptosis. PET disrupted hormone production at multiple doses. Overall, each toxicant differently impacted follicles. These findings suggest that PFAS and nanoplastic exposure at environmentally relevant concentrations may pose a risk to female reproductive health by disrupting hormonal and molecular pathways.

## 118 Efficient Strategies for Selective Preconcentration of Per- And Polyfluoroalkyl Substances in Aqueous and Gas Phase

Emanuela Gionfriddo, University at Buffalo, The State University of New York, 710 Natural Sciences Complex, Buffalo, NY 14260

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants characterized by strong carbon–fluorine bonds, which confer high chemical stability, resistance to degradation, and long-term persistence across environmental and biological matrices. These properties present significant challenges for their detection at ultra-trace levels. This work focuses on improving preconcentration strategies to enhance extraction efficiency and selectivity for PFAS prior to chromatographic analysis by GC-MS and LC-MS. Advanced solid-phase microextraction (SPME) devices, particularly in thin film microextraction (TFME) geometries, were

developed to improve mass transfer rates and increase sorptive surface area. Compared to conventional fibers, thin films enabled significantly faster equilibration and higher extraction efficiency, facilitating high-throughput workflows using multi-well plate formats. These advances are applicable to a wide range of matrices, including seawater, melted snow, and human plasma. Sorbent materials were tailored through the incorporation of electrostatic, hydrophobic, and fluorophilic functionalities. The inclusion of fluorinated groups and polyethyleneimine (PEI) moieties enhanced sorbent affinity and stability under varying pH, ionic strength, and solvent conditions. Further, the integration of polymeric ionic liquids (PILs) into TFME devices allowed for efficient ion-exchange and hydrophobic interactions, improving selectivity for anionic PFAS. Moreover, the adaptability of SPME across different extraction modes enabled the preconcentration of volatile PFAS in both gaseous (headspace-SPME) and aqueous (direct immersion-SPME) samples. This capability addresses key challenges in capturing highly volatile PFAS and contributes to the development of more effective analytical tools for environmental and regulatory monitoring.

## 119 There may be ALOT of Barbie in All of Us, Could it be Nanoplastics?

Phoebe Stapleton, Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, 170 Frelinghuysen Rd., Piscataway, NJ 08854

It is safe to say that plastic particles are ubiquitous in all environments. Micro- (less than 5mm) and nano-sized (less than 100nm) plastic particles (MNP) have been identified within the depths of the ocean, glacier ice, remote locations, urban settings, indoors, and in human tissues once thought to be safely protected by biological barriers. It has become evident that humans are exposed to MNP through ingestion, inhalation, dermal, and injection routes, leading to deposition and accumulation in systemic tissues. This bioaccumulation leads to secondary effects impacting cellular, neurological, and reproductive functions. Particle physicochemical properties can greatly affect particle uptake and cellular toxicity. This presentation will discuss the importance of thorough material characterization, current techniques utilized to identify and quantify MNP, and challenges to assess environmental, biological, and human samples. This work has been supported by the National Institute of Environmental Health Sciences (R01-ES031285), Rutgers Center for Environmental Exposures and Disease (P30-ES005022), and the Herbert W. Hoover Foundation.

## 120 Capture and Ingestion of Nano- and Micro-plastics by Bivalve Molluscs: Implication for Bioaccumulation and Toxicity

Abhishek Naik, University of Connecticut, 1084 Shennecossett Rd., Groton, CT 06340, Evan Ward

Suspension-feeding bivalves continuously process large volumes of water and particles, positioning them as key interfaces between plastic pollution and marine food webs. While microplastics (MP) and nanoplastics (NP) are pervasive in marine systems, bivalves typically retain only ~1% of available MP in digestive tissues, suggesting strong biological barriers to ingestion and accumulation. Bivalves rely on gill-mediated particle capture and post-capture selection, influenced by particle size, shape, and surface properties. In our studies, bivalves exhibited a ~1000µm size threshold for ingestion of MP spheres and fragments, whereas fibers were ingested more readily if one dimension fell within ingestible size limits. Suspended NP, however, were poorly captured or ingested unless incorporated into aggregations through marine snow formation or self-aggregation. Particle concentration is also critical: at high concentration, indiscriminate rejection of particulate matter (e.g., microalgae, silt, MP) can occur. Interspecies differences further diversify particle capture and ingestion. After ingestion, particle selection continues. Residence time in the bivalve gut is a key determinant of risk from chemical leaching; low aspect-ratio MP <500 µm are generally retained longer in the gut. Polystyrene NP ingested within aggregations exhibited longer gut residence times than polystyrene MP. Our studies also show that ingested nylon microfibers, despite carrying a microbial "plastisphere", do not alter bivalve gut microbiome composition. Whereas biodegradable plastics may have reduced persistence compared to conventional polymers, their gut residence time and resulting ecotoxicity are currently unknown. Together, these findings clarify how particle traits and bivalve biology interact to govern plastic ingestion, retention, and bioaccumulation potential.

## 121 NanoLC-MS-Based Proteomic Analysis of the Human Cytomegalovirus Virus Microenvironment (VME) Enabled by a Novel Fluorescence Labelling Platform

Isabel Matthews, Princeton University, 6526 Frist Campus Center, Princeton, NJ 08544, James C. Kostas, Ileana M. Cristea

Human cytomegalovirus (HCMV) is a ubiquitous β-herpesvirus that establishes lifelong infection in most of the global population and is the leading infectious cause of birth defects and transplant rejection. Our lab recently discovered that HCMV infection significantly remodels the cellular microenvironment surrounding the site of infection, termed the virus microenvironment (VME). By adapting a fluorescence-based cellular microenvironment labelling platform for the VME and pairing this with fluorescent-activated cell sorting (FACS), liquid chromatography (LC)-mass spectrometry (MS)-based proteomics, and functional virology, we found that, before the spread of the initial infection, uninfected cells adjacent to HCMV-infected cells in the VME are primed for secondary or co-infection, whereas distal cells are poised to slow the spread of the infection. Here, with the goal of understanding the timing and mechanisms behind these spatially-dependent changes, we build on these findings by adding a temporal dimension to the analysis of a VME for the first time. To enable earlier and more precise discrimination of cells in a VME, we first established a novel multimodal fluorescence-based labelling system. By pairing our labelling system with FACS and ultrasensitive nanoLC-MS-based proteomics using a Bruker timsTOF Ultra mass spectrometer, we generated proteome-wide datasets across multiple stages of infection. This framework allows us to analyse viral protein transfer, host immune responses, and other spatially heterogeneous features of the HCMV VME across time. Overall, by integrating high-sensitivity proteomics with spatially defined labelling, we established an analytical platform for systematically characterising how a viral infection reshapes its surrounding microenvironment.

## 122 Forensic Analysis of a Wounded Book Held in WCU Special Collections

Madelyne Salgado, West Chester University, 55 S. Valley Rd., Paoli, PA 19301, Zachary Voras, Ron McColl

This research investigates a wounded book from the War of 1812, believed to have been struck by a musket ball during a naval battle between the USS Enterprise and HMS Boxer. The book, now housed within the WCU Special Collections, displays visible physical damage and includes a handwritten note attesting to its wartime injury. However, this claim has never been scientifically tested. To examine the damages, a combination of forensic light microscopy and scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) was used to analyze key areas of interest for the detection of trace elements. Sampling was guided by high-resolution imaging under overhead, raking, and ultraviolet light. SEM-EDS analysis confirmed the prominent presence of metallic lead, supporting the hypothesis of projectile impact. Additional elements such as iron, calcium, and silicon were detected, potentially reflecting contact with marine or battlefield materials. These findings provide new physical evidence that supports the book's historical narrative. The presence of lead and other trace elements aligns with Locard's Principle of Exchange - *every contact leaves a trace - suggesting the artifact may still carry physical forensic evidence of its claimed past.*

## 123 Investigation the Effects of Overexpression and Downexpression of Human Jumping Translocation Breakpoint Protein (hJTB) in Using in Solution and Ingel Digestion Based Proteomics in MCF7 Cells

Peter Biggers, Clarkson University, Biochemistry & Proteomics Group, Department of Chemistry & Biochemistry, Box 5810, 8 Clarkson Ave., Potsdam, NY 13699, Taniya Jayaweera, Madhuri Jayathirtha, 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.MCF7 breast cancer cell lines and HEK293 normal 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 insolution digestion based Proteomics. The expression of JTB was confirmed by the western blotting technique. In-gel digested peptides 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/or tubulin proteins are closely associated with hJTB function. Furthermore, we performed a GSEA analysis to identify the biological processes and pathways associated with the JTB protein. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a potential drug target for malignancies with overexpression of the protein.

## 124 Optimizing a Point-of-Care Fluidic Electrochemical Assay for Cancer Biomarker Quantification

Athena Antippas, Middlebury College, 276 Bicentennial Way, Middlebury, VT 05753, Edith Mauch

Cancer biomarkers are biomolecules often found in the blood that can indicate cancer in the body, making them an effective tool for cancer screening and diagnosis. Current biomarker tests must be performed in a laboratory setting, resulting in a high price point and longer processing times. However, there is potential to develop an inexpensive point-of-care (POC) device that can efficiently perform multiplex cancer target screening using fluidic electrochemical assays. As a first step towards this larger goal, we seek to optimize a straight-channel electrochemical assay for biomarker testing. Key variables to consider include the device channel design, volumes and concentrations of reagents involved, the fluid velocity via strength of capillary action, and volumes of the sample liquids. Optimizing this assay technique

to obtain an accurate quantification of biomarkers at a sensitivity comparable to commercially available static assays will contribute to making accessible POC cancer biomarker testing a reality.

**125** **Enhanced Ignitable Liquid Residue Differentiation Using Chemometrics and GC×GC-TOFMS**

Madison O'Brien, College of William and Mary, 3848 Ironbound Rd., Williamsburg, VA 23188, Katelynn Perrault Uptmor

The identification of ignitable liquid residues (ILRs) in fire debris plays a critical role in arson investigations, yet analysis remains challenging due to sample complexity. This study evaluates data processing workflows for comprehensive two-dimensional gas chromatography – time of flight mass spectrometry (GC×GC-TOFMS) to distinguish among three commonly encountered ignitable liquids gasoline, kerosene, and diesel. Data was also compared when analysis was performed by one-dimensional gas chromatography – mass spectrometry (GC-MS). A total of 100 ILR samples were analyzed using LECO's ChromaTOF software, including both ChromaTOF and ChromaTOF Tile, alongside Pirouette chemometric modeling and R statistical computing. Each sample, representing a combination of ignitable liquid, solvent, and replicate, was initially processed using ChromaTOF for peak identification and grouping and peak alignment. After, Tile-based feature selection was applied to evaluate unsupervised differentiation among fuel types. Pirouette was used to assess supervised classification models such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA). R statistical compare was used to develop images such as heat maps to visualize peak area intensities. Results highlight the improved resolution of GC×GC over conventional GC, with Tile providing effective visualization of complex patterns across ILRs. Classification models developed in Pirouette using ChromaTOF Tile-derived features successfully distinguished between ILRs and outliers. This project emphasizes the value of advanced data processing in GC×GC fire debris analysis and offers new workflows for optimizing software-based procedures in forensic chemistry applications.

**126** **Should We Use Splitless or Split Injection? Analyzing Active Pharmaceutical Ingredients with GC-MS**

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

Most errors in gas chromatography come from sample preparation or the inlet. A syringe enters the inlet and injects your sample, then the inlet is heated with the goal of evaporating both the solvent and the solute. When a split injection occurs, the sample is split into two parts. The smaller part of the sample is transported onto the column by the carrier gas. The larger portion of the sample is vented through the split exits. Ideally, the amount of sample that goes onto the column is proportional to the split flow. If the split ratio is 100:1, 1% of the sample reaches the column. When a splitless injection occurs, the split vent is closed, causing almost all of the sample to reach the column. Thus, splitless injections are better suited towards lower concentration samples. Grob addresses the comparison between method through comparing the split peak area to the splitless peak area to confirm split ratio. Our approach is a bit different, by monitoring assumed on-column mass of analyte between a split injection and splitless injection a direct comparison can be made. This can be done at various split ratios and concentrations in order to build a stronger comparison. Various parameters can also be compared such as peak area, peak height, retention times, and percent differences. Would one injection method be better? This research seeks to provide an understanding between the differences between a split injection and a splitless injection.

**127** **Detecting the Presence of PFAS in Household Aerosols and Cleaning Products: Assessing Hidden Contamination Using UPLC-MS/MS Technology**

Marisa Vatteroni, Center for Environmental Sciences and Engineering, University of Connecticut, 3107 Horsebarn Hill Rd., Storrs, CT 06269, Angelica Velasquez, Christopher Perkins, Anthony Provatas, Austin Pelletier

Per- and poly-fluoroalkyl substances (PFAS) are synthetic chemicals known for their resistance to oil, water, and heat, making them prevalent in various industries and consumer products. Also known as "Forever Chemicals," the fluorinated structure of PFAS causes these compounds to be difficult to degrade, thus accumulating in the human body. Most PFAS, particularly long chain PFAS, have half-lives of several years in the body, therefore small exposures rapidly accumulate and can result in severe health complications. The main routes of PFAS exposure to humans are through ingestion and inhalation. This can occur in large through contaminated food, water, and air, but many consumer products have hidden PFAS contamination contributing to bioaccumulation. One such example is through household aerosols and cleaning products which are widely used and present a risk for PFAS exposure through inhalation. Thus, it is important to ensure these products are PFAS free to minimize exposures. Given the widespread nature of PFAS and growing health concerns, further investigation into the regulation and elimination of PFAS in consumer products is necessary to protect public health. An analytical methodology has been developed for the extraction and analysis of 28 PFAS in household aerosols and cleaning products utilizing UPLC-MS/MS and is presented in this poster.

**128** **Method Development for the Determination of Fentanyl and Its Cutting Agents in Whole Blood Using GC-MS**

Paige Ickes, West Chester University of Pennsylvania, Department of Chemistry, 750 South Church St., West Chester, PA., 19380, Sarah Heinrichs, Lisa Mundy, Constantinos Pistos

A constantly changing drug landscape remains a challenge for the public health, public safety, and forensic science communities. New and emerging compounds across multiple drug classes are continually being detected and identified, as evidenced by work from efforts like New Psychoactive Substances (NPS) Discovery and the European Monitoring Centre for Drugs and Drug Addiction. In addition to the detection of new compounds, there is a need to monitor for known compounds that may be diverted or misused. This need has become increasingly obvious with the introduction of xylazine and medetomidine, veterinary sedatives, into the fentanyl and heroin drug supply as "cutting agents". The aim of this study is to develop and validate a gas chromatography mass spectrometry (GC-MS) method, for the simultaneous determination of fentanyl, xylazine and medetomidine in whole blood to be applicable by the forensic laboratories. Selected ions are optimized by injecting standard reference materials into an Agilent GC-MS system and analyzing them in SIM mode. The separation of the analytes, which is optimized by examining various temperature programs, demonstrates simplicity, and specificity which makes it suitable for further optimization of the extraction method in whole blood, and the method validation. The method will finally be applied in authentic forensic samples. In this presentation, the method's optimized parameters and representative figures and data are reported.

**129** **Targeted Quantitative Serum Proteomics Analysis in Donors with Breast Cancer (BC) and Matched Controls**

Brian Pentecost, Clarkson University, Department of Chemistry and Biochemistry, 8 Clarkson Ave., Potsdam, NY 13699, Victor T Njoku, Kaya R Johnson, Sumona Mondal, Claudia Gaithe, Adeline Shanker, Robert Popp, Christoph H. Borchers, Costel Darie

Breast Cancer (BC) is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide. We employed targeted analysis to identify dysregulated serum proteins in women with stage IIA/B Invasive ductal carcinoma of the breast (IDC BC). We focused on 270 proteins in sera of 33 IDC BC cases and 33 controls. Pathway analysis indicated a strong component from coagulation cascades, likely reflecting a whole-body response that is surprisingly robust given the early-stage BC diagnoses.

**130** **Early Development of a Novel Electrochemical Biosensor: Leveraging Bipolar Electrochemiluminescence for inexpensive Point of Care Biosensing**

Jasper Pearcy-Kahn, Middlebury College, 2227 N. High St., Middlebury, VT 80205, Kira Rahn

Point-of-care (POC) testing, or the ability to test a human sample and quickly generate a clinical result with a portable or handheld device, is extremely promising for pre-hospital, wilderness, and low-resource medical settings. The ability to detect multiple analytes simultaneously, also known as multiplex sensing, allows for the identification of several biomarkers in one test, which compounds the promise of these kinds of POC devices. However, conventional electrochemical multiplex sensing technologies are expensive. To address this problem, we are utilizing bipolar electrodes to pair an electrochemiluminescent reporting reaction with an analyte sensing reaction on each pole of the electrode. These microfluidic devices are small and inexpensive compared to traditional multiplex biosensors. Our first goal in the development of these platforms is to ensure that the fabrication process yields reproducible devices, and that the analysis and collection infrastructure yields consistent results. Our second objective is to ensure that each electrode within the array is behaving predictably, reproducibly, and independently. In the future, these results will then be validated by using a modified electrode for sensing glucose, a biologically relevant analyte. Glucose sensing will allow us to evaluate our platform against metrics established within the biosensing industry including limits of detection and quantification, sensitivity, and linear range. These kinds of electrode modifications will be crucial for eventual integration with electrochemical analysis techniques including enzyme linked immunosorbent assay.

**131** **Understanding the Synthesis-Structure-Activity Relationship of Copper-Based Catalysts for CO2 Electrochemical Reduction with Machine Learning Toward More Sustainable Catalysis**

Nhat Minh Dang, University of Delaware, 163 The Green, Brown Lab, Newark, DE 19716, Caelin Celani, Adil Sheikh, Rachel Davidson

The powerful activity of Cu-based catalysts during CO2 electrochemical reduction (CO2ER) is readily influenced by their well-documented drastic morphological, structural, and surface evolutions under catalytic conditions. The extent and net impact of these transformations on performance are influenced by the nature of the

starting catalyst, such as morphology, size, oxidation state, support identity, surface chemistry, and CO2ER experimental conditions, namely, electrolyte, electrolysis duration, and reduction potential. This study leverages design-of-experiments (DOE) sampling techniques to systematically explore a five-dimensional synthetic space of Cu electrochemical depositions built by varying the precursor concentration, temperature, pH, deposition time, and potential. This approach has been applied to four different Cu precursors, resulting in Cu depositions possessing various morphologies ranging from cubes, octahedra, and 2D triangular plates to more complex structures resembling quatrefoils, multipods, and butterflies. Representations of particle morphology have been. Representations of particle morphology have been extracted from their 2D scanning electron microscopy (SEM) images and vectorized via image analysis to serve as input for the construction of classification machine-learning (ML) models that learn and predict the relationship between the synthesis and structure of Cu catalysts. Current efforts involve bulk-scale measurements of their CO2ER catalytic activity and selectivity by means of gas chromatography and proton nuclear magnetic resonance, which will serve as the third data block for ML analysis to establish the underlying structure-activity-selectivity correlations.

## 132 Illicit Drug Analysis in the Field with a Portable Instrument "Toolkit"

Brooke Kammrath, University of New Haven, Henry C. Lee Institute of Forensic Science, 300 Boston Post Rd, West Haven, CT 06033, Ella Galvan, Mei Yuan, Ashlyn Evans, Desmond Brown, Samuel Friday, Drew Kuroda, Alexander Klein, Jessica Persechino, Jessica Behn, John Naples, Debbie Fuller, Isabelle Radgen-Morvant, Richard Crocombe, Pauline Leary

Portable instruments offer numerous advantages for combatting the illicit drug market in the field. The real-time data provided by portable instruments, which can identify both bulk and trace components of a mixture, can be of value to first responders given the modern dynamic illicit drug landscape which contains highly dangerous and novel psychoactive substances. Still, given the diversity of commercially available portable instruments, there remains questions regarding which portable technologies are best for illicit drug identification and what are the best practices for their implementation in the field. This research evaluated 8 different field portable methods for the analysis of a range of adjudicated seized drug samples: (1) field color tests, (2) a portable FT-IR spectrometer, (3) a handheld Raman spectrometer equipped with a 1064 nm laser, (4) a handheld NIR spectrometer; (5) a portable GC-MS equipped with a quadrupole mass analyzer, (6) a portable GC-MS equipped with an ion-trap mass analyzer, (7) a portable HPMS, and (8) a portable MS. Samples were also analyzed using traditional laboratory benchtop GC-MS and FT-IR instrumentation, for comparison. This research demonstrated the reliable detection and identification of both bulk and trace components of seized illicit drugs when at least two field deployable devices are employed. Since multiple instruments can be used in combination to achieve these results, a "toolkit" approach is recommended, which provides flexibility for use by agencies with a range of resources and is consistent with recommendations by the SWGDRUG and *ASTM E2329: Standard Practice for the Identification of Seized Drugs.*

## 133 GCMS Analysis of Street Drugs Utilizing Hydrogen Carrier Gas in Combination with a HydroInert EI Source

Kirk Lokits, Agilent Technologies, 3047 Stover Shop Rd., Churchville, VA 24421

Analysis of street drugs in the forensic realm has routinely utilized capillary chromatography with mass selective detectors (MSD). The MSD provides sensitivity, selectivity, and permits structural identification of the specific compounds found in forensic street drug samples. The purpose of this research is to demonstrate that several recent advances in inert coatings on the mass spec source assembly, found in the Agilent Technologies HydroInert™ Source, can be successfully incorporated into utilizing hydrogen as an alternative carrier gas in the current screening methods involving street drug samples. This work seeks to demonstrate the improvements in source reactivity, increases in analyte response, spectral fidelity, and speed of analysis when using the HydroInert™ source in combination with hydrogen as the carrier gas. This study applied Method Translation software to convert a conventional street drug screening method without changing peak elution patterns or negatively affecting peak resolution. The advancement of the HydroInert™ Source design facilitates the GCMS solution, utilizing hydrogen as the carrier gas, and generating spectral library matches from commercial libraries and or the generation of custom libraries for targeted drug compounds.

## 134 Analysis of New and Aged Bones by X-Ray Fluorescence Spectroscopy for Potential Estimation of Post-Mortem Interval

Michael Brown, The University of Memphis, 3744 Walker Ave, J.M. Smith Chemistry Bldg., Room 007 Memphis, TN 38152, Jacob Pate, Trinity Green

Estimation of the post-mortem interval (PMI) of skeletal remains is a complex problem that has yet to be solved. Previous research has mainly focused on the use of biomarkers, small organic molecules, and physical changes; however, no suitable methods have been developed. Surprisingly, the elemental composition of bone has not been extensively studied by forensic researchers. With recent advances in X-ray Fluorescence (XRF) spectroscopy, the composition of bone can be investigated with better accuracy and precision by researchers with various skill levels. The main objective of this research is to develop and optimize an XRF-based method to compare the elemental composition of new and aged porcine bones for the potential estimation of PMI. In order to improve sample stability and decrease powder expulsion, this method initially required selecting a suitable binder compound to add to powdered bone samples. The binding effectiveness and low background impurities of each binder were evaluated. Additionally, a series of detailed optimization and calibration studies were performed to fully assess the method and to investigate the impact of analytical and environmental variables upon bone analysis. Following these studies, a complete elemental composition baseline was established using new porcine rib bone samples from a variety of sources. Selected aged porcine bone samples between 10 and >100 years old were analyzed, and the elemental composition was statistically compared to the baseline composition using discriminant analysis. The key findings from these studies and the potential impact on forensic science will be discussed.

## 135 Differentiation and Identification of Fentanyl Analogues Using Portable Mass Spectrometry

Mei Yuan, University of New Haven, Henry C. Lee Institute of Forensic Science, 300 Boston Post Rd, West Haven, CT 06516, Jeff Johnson, Seth Fisher, Richard Crocombe, Don Ostrowski, Marisia Fikiet, Pauline Leary, Brooke Kammrath

The continually evolving and complex drug landscape presents a formidable challenge for first responders who need reliable tools for their detection and identification. Fentanyl is a commonly prescribed potent synthetic opioid analgesic which recently became a significant public health threat due to the illicit drug market. In 2023, overdose deaths exceeded 100,000 in the United States, with over 70% of those involving a synthetic opioid such as fentanyl or tramadol. These drugs are being transported to the United States across borders, and thus it has become a national priority to develop tools to help stop their proliferation. New portable technologies provide the best potential for addressing this problem, with a specific emphasis on identification of fentanyl and its analogs in the field. This research involves analyzing 250 synthetic opioids and related substances, including 210 fentanyl analogs, to build a comprehensive fentanyl detection library for a portable ion trap mass spectrometer (the 1st Detect Tracer 1000) designed for rapid identification of drugs and explosives. Ion trap mass spectrometers operate by using electrostatic and radio frequency fields to confine charged particles. This offers high sensitivity, resolution, and the ability to study ion-molecule reactions. After the fentanyl spectral library is built, its detection and identification accuracy will be evaluated using mixed samples and adjudicated case samples. This project aims to evaluate and ultimately improve the Tracer 1000's capability to identify fentanyl, fentanyl analogs, and other synthetic opioids, thus supporting its use in high-security environments like airports and other border crossings.

## 136 GC-MS Analysis of Medetomidine Using S-TPC Derivatization

Harshitha Gajula, University of Baltimore, 1420 N Charles St, Baltimore, MD 21202, Ioan Marginean

Medetomidine has been recently detected in street samples along opioids like fentanyl, a combination that may increase the chance of overdose and death. The drug is an alpha-2 adrenergic agonist that acts as a sedative and analgesic by slowing down nerve signals, producing calming and muscle-relaxing effects. Among its two optical isomers: dexmedetomidine and levomedetomidine, the former is biologically active. Increasing presence in street markets has raised concerns about its potential misuse and the associated health risks. This study focuses on the derivatization of medetomidine using the chiral reagent S-TPC, followed by analysis using gas chromatography–mass spectrometry (GC-MS). Following derivatization, the products were analyzed directly without further separation. GC-MS was selected for its effectiveness in detecting medetomidine in derivatized form and its applicability in routine forensic laboratory analyses. While LC-MS has previously been used to separate and detect medetomidine enantiomers, the instrumentation is rarely available in forensic drug laboratories. The data showed distinct chromatographic peaks consistent with medetomidine and its S-TPC derivative. Knowing the isomeric composition of medetomidine in seized drug samples can provide law enforcement with tactical intelligence by providing valuable insights into the origin of the drug. This information can guide investigations and enhance decision-making during law enforcement operations, ultimately improving efforts to combat the trafficking of illicit substances. This presentation will outline the derivatization procedure, demonstrate the GC-MS results obtained from the analysis. Data from adjudicated cases will also be discussed to highlight the real-world forensic applications.

## 137 Developing a Holistic Approach to the Analysis of Green Gunshot Residues Using Scanning Electron Microscopy with Energy-Dispersive X-Ray Spectroscopy and Comprehensive Two-Dimensional Gas Chromatography

Barbara Grace Saunders, William & Mary, 540 Landrum Dr., Integrated Science Center 1053, Williamsburg, VA 23185, Katelynn Perrault Uptmor

Gunshot residue (GSR) is expelled during a firearm discharge event, dispersing and settling on surfaces within the weapon's vicinity. GSR contains organic components (OGSR) and inorganic components (IGSR), which provide evidentiary information for forensic casework. The traditional method for GSR analysis uses scanning electron microscopy with energy-dispersive x-ray spectroscopy (SEM-EDS) to search for particles of IGSR based on a morphological and elemental profile. This profile, relying heavily on the identification of lead, barium, and antimony, has lost impact as a standard for forensic evidence analysis due to increased use of green, or heavy metal-free, ammunition. This study aimed to develop a holistic approach to the analysis of GSR by using both the traditional SEM-EDS method as a targeted approach to analyzing IGSR and comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS) as a nontargeted method for analyzing OGSR. GSR samples were collected from the hands of shooters at a police firearms recertification training. SEM-EDS was used to identify IGSR particles with some analytical difficulties encountered due to particle morphology and composition that differs from expectations outlined by guidelines standardized based on heavy metal-containing ammunition. Particles readily identified as GSR had spheroidal morphologies and elemental compositions high in copper, zinc, iron, and aluminum. Sample analysis via liquid extraction, chromatographic separation on GC×GC-TOFMS, and data analysis on compatible software has confirmed positive identification of OGSR components. Future work on this project will include the optimization of sample extraction methods, focusing on SPME as an alternative approach, and further protocol development.

## 138 Improving Sustainability through Modernization of LC Methods

Geoff Faden, MAC-MOD Analytical, Inc., 103 Commons Court, Chadds Ford, PA 19317, Matt James, Tony Edge, Gemma Lo, David Dunthorne, Arianne Soliven

This presentation will demonstrate how the sustainability of existing liquid chromatographic methods can be improved through the utilization of modern LC techniques and approaches. Significant reductions in solvent consumption and waste generation without compromising data quality are possible. One of many approaches is to reduce the column internal diameter, this concept can be further extended by employing shorter length columns, packed with smaller particles, to reduce analytical run times. Additionally, the use of solid-core particles can also provide compelling advantages. These approaches also have a demonstrable impact on the electrical consumption per analysis, together with the added benefit of improved laboratory efficiency and reduced running costs. The use of these approaches is supported within updated guidance on allowable changes to pharmacopoeia methods (e.g. USP, EuPh and JP). Often, it is perceived that significant gains can only be made by upgrading to high-pressure-limit UHPLC instrumentation; however, it is often feasible to adapt established methods and better utilize existing low-pressure-limit HPLC equipment to realise substantial improvements. The use of a modified kinetic approach is introduced, which can be used to determine the optimal particle size from a sustainability perspective for both scenarios. This important aspect is considered throughout, and in one specific example a 71.9% reduction in solvent use, 57.3% reduction in electrical consumption and 60.4% reduction in analysis time was easily achieved on a 400 bar HPLC system. Finally, the benefits of utilizing novel stationary phase selectivity and ultra-short 10 mm cartridge style columns are demonstrated.

## 139 Employing Multidimensional Design Space Modeling Across Frequent Chromatographic Challenges—Use Cases from Recently Published Results

Arnold Zoeldhegyi, Molnar-Institute, Schneegloeckchenstrasse 47, Berlin, Germany 10407, Krisztian Horvath, Imre Molnar, Robert Kormany

In high-performance liquid chromatographic (HPLC) applications, the development of efficient, robust, and transferable methods is essential to ensure consistent performance across the entire lifecycle of an analytical procedure, including some unforeseen future uses. However, robustness issues are frequently reported in the industry, often stemming from poor initial method design or insufficient identification and control of critical method attributes. In this context, modeling tools built on systematic knowledge—aligned with Analytical Quality by Design (AQbD) principles—have proven highly valuable for both proactive prevention and reactive troubleshooting of such failures. Among these, DryLab 4 modeling software uniquely integrates chromatographic fundamentals with a minimal number of input experiments, enabling the construction of experimentally calibrated Design Spaces (DS) that capture all separation possibilities within the studied system. These digitized models (acting as specific system fingerprints) enable extensive *in silico* investigations, including the evaluation of parameter settings, assessment of method transferability, robustness testing, and, more recently, systematic comparisons of complete separation systems—addressing various discrepancies often encountered in industrial practice. Through several peer-reviewed examples, we present a Design Space Comparison (DSC) strategy for addressing challenges such as identifying suitable replacement columns, comparing different stationary phase chemistries, evaluating batch-to-batch inconsistencies, mitigating system-to-system differences, and managing transferability issues between volatile and non-volatile buffer systems. Together, these case studies highlight the importance of a detailed, multidimensional understanding of method parameters—and their continuous, targeted fine-tuning—for achieving and maintaining consistent analytical performance.

## 140 Greener Routine HPLC Analysis: Translation of USP Monographs to the Capillary Scale

Samuel Foster, Axcend, 3301 N. Thanksgiving Way, Ste. 175, Lehi, UT 84043, Matthew Morse, Elisabeth Gates, Garrett Hellinghausen, Cary Simpson, John Stimus, Greg Ward

High performance liquid chromatography (HPLC) is widely used for routine analysis in many industries. Analytical scale systems are commonly employed using columns with an inner diameter (i.d.) between 4.6 mm and 2.1 mm. Each of these systems can consume ~50 L of solvent per year. Often these solvents contain toxins including trifluoracetic acid (TFA), methanol, and acetonitrile. By translating existing methods to the capillary scale using columns of 0.1 mm to 0.3 mm i.d., separations can be performed that consume only ~100 mL of solvent per year per instrument. This reduction in solvent consumption can result in significantly reduced environmental impact as well as reduction in operating expenses. In this work, a USP monograph was translated to the capillary scale using USP 621 guidelines. Capillary separations were compared to analytical scale separations as described in the monograph, demonstrating comparable separations. Additional general guidance is presented for method translation of analytical scale HPLC separations to capillary scale separations including column selection, injection volume, and gradient programming. Capillary scale HPLC is essential in the pursuit of greener separations without the need for changes in mobile phase composition or additives.

## 141 Design of High Performance of Ultrawide Pore SEC Stationary Particles

Mingcheng Xu, Waters Corp, 5 Technology Dr, Milford, MA 01757, Szabolcs Fekete, Matthew Lauber

Size-exclusion chromatography (SEC) is the preferred method to profile size variants and to quantify aggregates in biotherapeutic. As the central element of a SEC column, the stationary phase particle with desired particle size, pore structure and surface chemistry play the key role in determining the resolution power [1]. The pore structure including average pore size, pore size distribution, pore shape and pore volume that differentiate the access of molecules with different hydrodynamic radius are fully investigated. Three SEC particles with different average pore diameters of 450 Å, 1000 Å and 1600 Å were developed for the best resolution of adenosine associated virus (AAV) particles, VLPs, plasmid DNA and LNPs. Significant improvement in separation performance on the same size column configuration is seen when particle pore volume increases from 0.7 cc/g to 1.15 cc/g. Pore size distribution or fractionalization range is optimized by simulation. Peak broadening and tailing of NISTmAb RM8671 at different salt concentration and acetonitrile percentage is used as the key indicator of respective ionic and hydrophobic interaction of the PEG bonded particle [2]. The optimized PEG bonding phase crosslinked with bridged ethylene shows great inertness and high pH stability. In SEC-MALS applications, MALS noise as low as 10-20 µV gives a high confidence in determining peak absolute mass.

Reference

[1] Journal of chromatography A, 1722, 10 May 2024, 464862, Valentina D'Atri et al

[2] Journal of Separation Science, J 47 (20) 2024 · Vol. 47 · No. 20 · October 2024, Kristine Joy Camacho et al

## 142 Recent Developments in Inert Columns for HPLC Separations of Small Molecules

Thomas Walter, Waters Corp., 34 Maple St., Milford, MA 01757, Kenneth Berthelette, Melissa Aiello, Jo-Ann Jablonski

Interactions of certain analytes with metal surfaces in HPLC instruments and columns have long been known to cause a range of problems, including peak tailing, low and variable peak areas and the formation of metal ion adducts. It has previously been shown that these effects can be mitigated using a surface modification technology in which a hybrid organic/inorganic barrier based on an ethylene-bridged siloxane chemistry is applied to the metal components in HPLC instruments and columns (M. DeLano et al, *Anal. Chem.* 93, 2021, 5773-5781). This technology has been demonstrated to give reduced tailing, higher peak areas and lower injection-to-injection variability for metal-adsorptive analytes such as those containing phosphate and/or carboxylate groups (T. H. Walter et al, *LCGC Supplement*, June 2022, 28-34). Recent developments in inert columns for applications in small molecule pharmaceutical analysis and metabolomics will be presented.

## 143 Chromatographic Separation and Photodiode Array (PDA) Detector Identification of Synthetic Industrial Dyes in Foods, OTC Drugs, and Cosmetics

Catharine E. Layton, Waters Corporation, 34 Maple St., Milford, MA 01747, Paul D. Rainville, Amy Woodsmall

Petroleum derived, synthetic color additives are commonly found in processed foods, OTC drugs, and cosmetics. These industrial dyes enhance visual appeal during shelf-life storage, reduce natural product variation, and increase overall product visual desirability. Numerous studies suggest a link between these chemicals and neurobehavioral issues, allergies, and development of cancer. In the EU, foods containing industrial dyes are marked with warning labels. In the United States, some regions prohibit the sale of foods containing synthetic dyes only in schools, while other regions prohibit the sale of foods containing synthetic colorants entirely. In the work presented here, we show a single chromatographic method for the separation of nine petroleum-based food dyes in a variety of consumer products. The Alliance iS HPLC System with PDA Detector is used to separate synthetic color additives from matrix constituents, while individual colorants are identified using PDA spectral library matching.

## 144 LUMA Real-World Examples of Solving Pharmaceutical Challenges

Rafael Acosta, VUV Analytics, 1500 Arrowpoint Dr., Bldg. 8 Ste. 805, Cedar Park, TX 78613

The pharmaceutical industry faces a myriad of challenges, from ensuring the purity of active pharmaceutical ingredients (APIs) to adhering to stringent regulatory requirements. As analytical methods evolve, LUMA—VUV Analytics' Multi-Channel Vacuum Ultraviolet (VUV) detector—emerges as a game-changing tool, offering exceptional sensitivity and selectivity for complex pharmaceutical analyses. In this talk, we will showcase real-world examples of how LUMA has been effectively applied to tackle critical pharmaceutical challenges. Key applications include measuring moisture content, a critical parameter in oligonucleotide synthesis, detecting nitrosamines in pharmaceutical products, phthalates used as plasticizers and analyzing Fatty Acid Methyl Esters (FAMEs) for purity testing. By providing highly accurate results with minimal sample preparation, LUMA enables pharmaceutical companies to streamline their testing workflows, increase throughput, and meet regulatory standards with confidence. We will also highlight potential applications of VUV technology in the Liquid Chromatography (LC) domain, with a teaser on the capabilities of HYDRA—our new VUV detector designed for LC, which promises to push the boundaries of sensitivity and selectivity in the liquid phase. Join us as we explore how LUMA can simplify and improve the analysis of key pharmaceutical compounds, offering new insights, enhanced efficiency, and greater regulatory compliance.

## 145 Incorporating Ultrashort-Chain Compounds into the Comprehensive Analysis of PFAS in Potable and Non-Potable Waters

Justin Steimling, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823, Shun-Hsin Liang

Ultrashort-chain (USC) per- and polyfluoroalkyl substances (PFAS) are small and very polar compounds with carbon chain lengths shorter than C4. Their ubiquitous and high levels of occurrence in environmental aquatic systems are emerging as a significant concern, rivaling the well-established issues associated with long-chain PFAS contamination. Therefore, it is important to analyze both USC and long-chain PFAS together in water samples to comprehensively assess and address the full spectrum of PFAS contamination. In this study, a simple and reliable workflow was developed for the simultaneous analysis of C1 to C14 perfluoroalkyl carboxylic and sulfonic acids, along with other groups of PFAS, in both potable and non-potable waters. A dilute-and-shoot workflow was evaluated by accuracy and precision analysis of fortified tap water, bottled water, and treated sewage wastewater. Calibration standards were prepared in reverse osmosis water ranging from 1 – 1000 ng/L. Five and eighteen mass-labeled standard mixtures were added and served as quantitative and extracted internal standards, respectively. Both standard and water samples were diluted two-fold with methanol containing 1% acetic acid for LC-MS/MS analysis. The chromatographic separation was conducted using a polar-embedded reversed-phase LC column with an inert coating on the hardware. Additional potable and non-potable waters collected from various source waters were tested to further demonstrate that the established workflow is suitable for the accurate quantification of targeted PFAS in a wide range of water matrices.

## 146 Developing an Electrochemiluminescence Biosensor in an Inexpensive Microfluidic Device

Kira Rahn, Middlebury College, 276 Bicentennial Way, Middlebury, VT 05753, Jasper Pearcy-Kahn, Athena Antippas, Kieran Cross, Edith Mauch

Detecting multiple biomarkers is necessary for the most accurate screening and monitoring for many diseases. Point-of-care (POC) sensors, or sensors that require minimal training or specialized equipment and can be operated at the source of the sample of interest, are ideal for detecting multiple biomarkers because they can be used to generate timely information for a patient. Electrochemical sensors are good POC sensors because they are sensitive, inexpensive, and portable. However, one existing challenge with current multiplexed electrochemical sensors is the need for sophisticated and expensive instrumentation to measure multiple electrodes. Wireless electrode arrays are promising alternatives to traditional multiplexed electrochemical sensors. A wireless electrode has both an anode and a cathode, and, instead of applying the voltage directly to the electrode, the voltage is applied to the electrolyte solution via driving electrodes. At sufficiently high voltages, paired and simultaneous faradaic reactions are driven on opposing poles of each wireless electrode. Because two reactions are paired on each BPE, one reaction, such as an electrochemiluminescent (ECL) reaction, can report the progress of the second reaction, which can be leveraged in sensing applications. Here, we demonstrate and validate the performance of a wireless electrochemical array in a pump-free microfluidic device with ECL readout. We utilize this device to perform triplicate measurements of a biomarker using in-device electrochemical immunoassays. The results from this work are important because they serve as a foundation for building a more complex multiplexed biosensor that can allow primary-care physicians to quickly evaluate a panel of biomarker in-office.

## 147 DOZN™3.0 - A Quantitative Green Chemistry Evaluator for Sustainable Future

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MilliporeSigma (The life science business of Merck KGaA, Darmstadt, Germany) developed and launched DOZN™2.0 in 2017, a unique web-based greener alternative scoring matrix. This quantitative green chemistry evaluator is based on the 12 principles of green chemistry for customers to evaluate their relative greenness of their processes which provide a framework for learning about green chemistry and designing or improving materials, products, processes, and systems. DOZN™2.0 scores products based on metrics for each principle and aggregates the principle scores to derive a final aggregate score. Through the system it is possible to calculate a green score for each substance based on manufacturing inputs, GHS, and SDS data. DOZN™2.0 is flexible enough to encompass a diverse portfolio of products and it has been verified and validated by a third party to ensure best practices are applied. Based on customer feedback, an upgraded version of the tool, DOZN™3.0, launched in December 2024. Through DOZN™3.0, customers now have access to calculate the green scores of their processes and products. DOZN™3.0 keeps data privacy top of mind - allowing customers to score their processes/products in a safe and secure manner. Come learn how to make your science greener using this free, web-based tool provides users with more data so that they are properly equipped to improve their sustainability.

## 148 Proteomic Analysis of Breast Milk for Early Detection of Breast Cancer: In-Gel Digestion Mass Spectrometry Approach

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Breast cancer (BC) remains one of the leading causes of cancer-related mortality among women globally, with early detection playing a critical role in improving patient survival. Invasive ductal carcinoma (IDC), the most prevalent subtype of BC, originates in the milk ducts and invades surrounding breast tissue, accounting for approximately 85% of all cases. Human breast milk is a non-invasively collected biofluid enriched with secreted proteins, immune components, and exfoliated epithelial cells, offering a promising matrix for biomarker discovery. In this study, breast milk samples were obtained from lactating women diagnosed with IDC, alongside age-matched healthy controls. We employed in-gel digestion followed by nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) to investigate protein expression differences. Two cohorts were analyzed: 10 IDC-associated breast milk samples versus 10 controls (10v10), and 9 IDC samples versus 9 controls (9v9). Proteins were separated using SDS-PAGE, digested, and analyzed via mass spectrometry. Several proteins were found to be differentially expressed between cancer-associated and control milk samples. Notably, proteins such as transferrin, osteopontin (OPN), and butyrophilin were among those dysregulated in IDC samples, highlighting their potential as early detection biomarkers. The consistency of dysregulation across both datasets suggests that breast milk proteomics may offer a non-invasive strategy for detecting molecular changes associated with early-stage breast cancer. Ongoing validation studies using larger cohorts are underway to confirm these findings and refine the candidate biomarker panel. This approach may ultimately support the development of an early diagnostic assay based on breast milk proteomics.

## 149 18O-Water Labelling Enables Protein Turnover Measurements in Embryogenesis, Martin Wühr,

Edward Cruz, Princeton University, Icahn Laboratory, Princeton, NJ 08544, Argit Marishta, Alex Johnson, Michael Neinast, Joseph Crapse, Joshua Rabinowitz, Eric Wieschaus, Martin Wühr

Embryogenesis transforms a single fertilized egg into a complex organism with diverse cell types, each possessing unique proteomes essential for lineage specification and organogenesis. Despite advances in measuring dynamic protein and mRNA levels, discrepancies between them suggest significant post-transcriptional regulation via translational efficiency and protein turnover. While translational control has been extensively studied, protein turnover's role in establishing protein abundance during development remains poorly understood. We present a novel technique combining 18O-water labeling with complement reporter ion quantification (TMTproC) to measure protein turnover in frog (*Xenopus laevis*) and fly (*Drosophila melanogaster*) embryos. Metabolomics data revealed rapid incorporation of 18O into proteins, with a half-life of approximately 10 minutes, indicating extensive protein hydrolysis and amino acid recycling. Degraded proteins were enriched for E3 ligases, microtubule-associated proteins, and cell-cycle regulators. Conserved proteins degrading in both species exhibited turnover rates matching the distinct developmental speeds of each organism, suggesting protein degradation plays a crucial role in developmental timing. However, after accounting for developmental rate differences, protein turnover exhibited markedly distinct roles: frogs displayed a predominantly stable proteome (~12% turnover), whereas flies exhibited extensive degradation (~43%), highlighting protein turnover's greater prominence in fly embryogenesis. Our findings indicate species-specific adaptations in protein abundance regulation during embryogenesis. The presented method provides a broadly applicable tool for quantifying protein half-lives, offering valuable insights into protein turnover's influence on developmental proteomic landscapes.

## 150 Mass Spectrometric Based Approach for Lysosomal Storage Diseases Diagnostic in Newborns

Brindusa-Alina Petre, Clarkson University, Department of Chemistry & Biomolecular Science, 64 Waverly, Potsdam, NY 13676, Laura Darie-Ion, Costel C. Darie

Lysosomal storage diseases (LSDs) are a group of over 50 rare, inherited disorders caused by defective lysosomal enzyme activity. This leads to intracellular accumulation of undegraded macromolecules, resulting in severe clinical manifestations and early mortality. In some LSDs, enzyme replacement therapy is available and effective, particularly when initiated early, thus making timely diagnosis essential. Current diagnostic approaches for certain LSDs, especially in newborn screening, often rely on enzymatic activity measurements using fluorimetric or mass spectrometric (MS) assays. Our study aimed to synthesize and apply novel peptide-based substrates for improved prediagnostic detection of neuronal ceroid lipofuscinoses (NCLs) and mucopolysaccharidoses (MPS). We developed a high-throughput enzymatic assay using dried blood spots (DBSs), combining fluorimetry and tandem mass spectrometry with newly designed substrate derivatives. These substrates are based on alkylated umbelliferone molecules, synthesized by substituting groups at positions 4 and 7 of the α-coumarin core (e.g., 4-ethyl-umbelliferone, 4-propyl-umbelliferone), using Pechmann condensation reactions. The new coumarin-based substrates enabled the development of duplex and triplex MS-based assays to assess enzymatic activity associated with NCL and MPS families. In conclusion, the synthesized substrates facilitate a sensitive and specific prediagnostic platform for LSDs. These compounds allow for quantification of enzymatic reaction products via both fluorimetric and mass spectrometric methods, supporting early detection and timely therapeutic intervention.

## 151 GCMS Low Energy Ionization to Differentiate Fentanyl and Nitazene Analogs

Kirk Lokits, Agilent Technologies, 3047 Stover Shop Rd., Churchville, VA 24421

Keeping up with the ever-evolving synthetic illicit drugs, precursors, and their metabolites is increasingly difficult and the need for retrospective data mining is invaluable. This work compiles high resolution mass spectrometry low energy ionization spectra, the formation of an analyte's molecular ion, and the molecular ion's elemental isotopic abundance ratios to assist in the identification of isobaric spectra generated under conventional nominal mass GCMS 70 eV ionization energy. Low energy ionization spectra were produced at 10, 12, 15, and 17 eV ionization energies to determine the optimal formation of a molecular ion enabling the measurement of the isotopic abundances based on the elemental composition of the molecular ion. Comparisons of the exact masses and ratios (calculated) to the accurate masses and ratios (measured experimentally) are illustrated in this work. Low energy spectral patterns of known of nitazene and fentanyl analog standards were compared to seized drug case samples and used to identify nitazene analogs and other controlled substances based on the creation of their respective accurate mass molecular ion and isotopic ratios and patterns. The data demonstrates the power of low energy ionization and high-resolution mass spectrometry to produce molecular ions of many illicit drugs that would normally be difficult to identify due to their similar or isobaric spectra when generated under the conventional 70 eV ionization energies.

## 152 Current LC/MS Approaches for PFAS Analysis with Ultrashort and Long Chain Mixtures

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Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals used to make fluoropolymer coatings and products that resist heat, oil, stains, grease, and water. These toxic "forever chemicals" have led to significant public health and environmental concerns and increased needs for diligence in surveillance, production, storage and mitigation. Current Environmental Protection Agency (EPA) methods will be demonstrated using superficially porous particle technology columns including EPA 533, 537.1, 8327, and 1633. Recently, methods involving significantly larger injection volumes and elimination of solid phase extraction sample preparation have been evaluated in order to speed up analysis times and throughput. Furthermore, there has been a growing concern over the ultrashort chain PFAS chemicals, including trifluoracetic acid (TFA), and PFPrA which can be challenging to analyze due to low retention and sensitivity via LC/MS/MS. Although mixed mode hydrophilic interaction liquid chromatography (HILIC) has been demonstrated to improve retention, this approach has limitations. A new reversed phase, superficially porous particle (SPP) silica with a positive charge surface chemistry and hydrophobic silane modifier (HALO® PCS) has shown to have advantages to improve short chain PFAS while allowing adequate resolution of the mid- and long-chain analytes.

## 153 Assessing the Long-Term Stability of Synthetic Cannabinoids in Human Blood by LC-QQQ-MS

Katya Beltran, Thomas Jefferson University, 206 Welsh Rd., Horsham, PA 19044, Grace Cieri, Melissa Fogarty, Alex Krotulski, Barry Logan

Synthetic cannabinoids, classified as novel psychoactive substances, have emerged as drugs of abuse, distinct from delta-9 THC in chemical structure and pharmacological effects. This research aimed to develop and validate a method for quantifying the concentrations of various synthetic cannabinoids using liquid chromatography tandem quadrupole mass spectrometry (LC-QQQ-MS) and to evaluate their stability in human whole blood preserved with sodium fluoride and potassium oxalate at refrigerated storage conditions (4°C) over a period of 60 days. An accurate and precise method was developed and validated to detect and quantify MMB-PICA, MDMB-PICA, MDMB-BINACA, and MDMB-PINACA. All but MMB-PICA were stable for 40 days at 4°C, which was unstable after just one day at 4°C. By day 60, only MDMB-BINACA remained stable and above the 80% threshold. The findings revealed a 90% decline in the concentration of MMB-PICA from day 0 to day 1. MDMB-PICA remained stable until day 40, then sharply declined by day 60. MDMB-PINACA was stable until day 40 then gradually decreased by day 60. The findings provide valuable insights into the stability of novel synthetic cannabinoids and can help guide future testing for these novel drugs.

## 154 Protein Polymorphism Induced Reactive Oxygen Species(ROS) Transients in PC-12 Cells

Uma Nudurupati, University of Vermont, 82 University Pl., Discovery Hall W321, Burlington, VT 05403, Madeline Harper, John Williams III, Margaret Trout, James Stafford, David Punihaole, Yangguang Ou

Senile plaques, a hallmark of Alzheimer's disease, primarily contain the amyloid beta (Aβ) protein in fibrillar form. These fibrillar forms can take on different structures, called polymorphs. There is evidence that different polymorphs are correlated with different severities of symptoms in patients. It is known that amyloids can produce reactive oxygen species (ROS) and generate oxidative stress, which promotes cellular cytotoxicity. A comprehensive understanding of the relationship between Aβ structural polymorphs and the corresponding induced cellular ROS released is lacking. In this poster, we will discuss studies in which we electrochemically measure ROS released from model neuronal cells (Rat Pheochromocytoma Cells, PC-12 cells) when treated with a suite of protein polymorphs across varying peptide length and evaluate their impact on the cells' viability. Currently, we observe the difference in the temporal response of ROS depending on the length of the Aβ peptide exposed to the cells. These differences suggest potential variations in cellular stress pathways as a function of polymorphism.

## 155 In-Depth Analysis of the Tear Fluid Glycoproteome Reveals Diverse Lacritin Glycosylation and Spliceoforms

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Tear fluid comprises a diverse group of extracellular glycoproteins which are critical for ocular homeostasis. Within the tear fluid glycoproteome, lacritin is highly expressed and plays a key role in immune response, tear secretion, and antimicrobial activity. Importantly, glycosylation constitutes over 50% of lactritin's molecular weight. However, despite this fact, nothing is known about the specific glycan structures on lacritin and how they influence its protein folding, function, or downstream biological

processes. Similarly, it remains completely unknown whether alterations to lacritin glycans are correlated with ocular pathologies. To address this gap in knowledge, we harnessed mass spectrometry (MS) to conduct the first O-glycoproteomic study of tear fluid. Here, we report unprecedented coverage of lacritin glycosylation, detailing 19 O-glycosites bearing a myriad of glycan structures. Further, we leveraged Alphafold 3.0 and GlycoShape to visualize the impact of these glycans on its structure, demonstrating that O-glycosylation renders the protein backbone rigid and extended. Surprisingly, we also detected protein-level evidence of two lacritin spliceoforms, representing the first observation of these isoforms by MS. Simultaneously, we describe the most comprehensive characterization of the tear fluid glycoproteome to date, elucidating the glycosylation profile of Immunoglobulin A (IgA), lactoferrin, and other glycoproteins with demonstrated clinical relevance as diagnostic biomarkers. Overall, this study lays critical groundwork for future biochemical investigation of tear fluid glycoproteins and their application as diagnostic or therapeutic tools for ocular diseases.

## 156 Interactions of Protein and Peptide Ions with Acoustic Fields at Atmospheric Pressure through Acoustic Ion Manipulation (AIM)

Julia Danischewski, Rensselaer Polytechnic Institute, 110 8th St., Cogswell Laboratory, Troy, NY 12180, Josefin Hufgard, Yi You, Jens Riedel, Jacob Shelley

Many fields, such as pharmaceuticals and consumer healthcare, prioritize the efficient and detailed analysis of biomolecular ions. However, current analytical techniques for the determination of high-order structure, primary sequence, and other properties require complex instrumentation and/or involved sample preparation procedures. Therefore, new methods for the purification, manipulation, and analysis of proteins, peptides, and nucleic acids are essential to improve these workflows. One possible solution is through the development of front-end instrumentation, which could enhance pre-existing techniques such as mass spectrometry and ion mobility spectrometry, or serve as a stand-alone analytical apparatus. Acoustic ion manipulation (AIM) is a promising candidate for such a device, as it relies on the novel and largely unexplored interactions of gas-phase ions with atmospheric-pressure acoustic fields. Here, we demonstrate the use of AIM for the separation and analysis of protein and peptide ions. The electrospray-generated ions of bradykinin, ubiquitin, myoglobin, and cytochrome C were subjected to one or more acoustic fields for the purpose of gating, focusing, and redirecting the ion beam. Charge-state-dependent deflection was observed, with more highly charged ions being impacted the most. Interestingly, ion-acoustic interactions were not linearly related to charge state, instead having multiple distributions that may reflect a relationship to macromolecular conformation instead. Additionally, the possibility to facilitate ion-ion chemistry with oppositely charged ion beams to determine higher-order structure will also be discussed. This work expands the capabilities of AIM in the study of biomolecules and reveals novel behaviors of gas-phase ions in the presence of variable pressure fields.

## 157 Spot On: A Green(Er) Approach to Dried Matrix Spots for Analysis with Liquid Microjunction – Surface Sampling Probe – Mass Spectrometry Aided by Computer Vision

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With dried matrix spots (DMS), a fixed volume of sample, generally ~10 microliters, is volumetrically absorbed into a substrate; DMS methods have been noted as clinically-relevant, cost-effective, simple, and reproducible. Regardless of the sample, DMS often rely on at least tens-of-microliter volumes, and DMS preparative/sampling methods usually involve direct transfer of the biofluid onto a sample storage medium, which is oftentimes filter paper. This transfer step has several inherent pitfalls, including variance in sample volume being applied and/or uneven lateral distribution of the sample. Furthermore, most analytical techniques cannot be directly integrated with the storage medium, i.e., the biofluid spot must be extracted offline from the substrate before analysis. In turn, we propose surface energy traps (SETs) on an optimized paper substrate as a means by which samples might be confined, dried-down, and directly analyzed with LMJ-SSP-MS. Given the market interests in such devices, we have combined a hydrophobic surface treatment with laser-micromachining to modify substrates to prepare and store dried matrix spots (DMS) for subsequent analysis with liquid microjunction – surface sampling probe – mass spectrometry (LMJ-SSP-MS). Additionally, we are creating and optimizing an object-based detection program to leverage computer vision to aid the sampling process. Taken together, we anticipate that this work will impact: 1) The manner by which DMS are prepared and then 2) the analytical process(es) through which DMS are interrogated.

## 158 Measuring the Ligand Exchange Kinetics of Metal Chalcogenide Clusters Using Mass Spectrometry

Ronald Cutler, Purdue University, James Tarpo Jr. & Margaret Tarpo, Department of Chemistry, 4318 Sterling Rd. Downers Grove IL, 60515, Bethany Phillips, Julia Laskin

Metal chalcogenide clusters are assemblies consisting of transition metals and group 16 chalcogen elements (S, Se, Te), with ligands used to stabilize the core structure. These clusters can range from atomic to microscopic scales, and have a wide range of applications in energy conversion, catalysis, and semiconducting materials. The electronic structure and reactivity of the cluster are influenced by the stabilizing ligands used, making ligand selection a crucial step toward synthesizing clusters with desired properties. However, certain ligands do not promote the formation of a desired cluster core during cluster synthesis using traditional methods. An alternate approach involves substituting a ligand from solution onto the pre-formed cluster via ligand exchange. In order to successfully perform these ligand exchanges, one needs to know how the clusters will exchange their initial ligands. The kinetics of this process must be explored to better understand and utilize the ligand exchange method for synthesizing metal chalcogenide clusters. In this study, we investigated this exchange using an over-pressure system coupled with electrospray ionization mass spectrometry (ESI-MS). The ligand exchange kinetics of $Co_6S_8(PPh_3)_6$ clusters complexing with triethylphosphine ligand ($PEt_3$) were examined. These experiments provide valuable insights into the kinetics of the ligand exchange process, facilitating future exchanges with more complex ligands.

## 159 SpyCatcher-SpyTag for Hydrogen Deuterium Exchange Mass Spectrometry in Complex Protein Matrices

Daniele Peterle, Northeastern University, Department of Chemistry and Chemical Biology, 334 Huntington Ave. #102, Boston, MA 02115, Bindu Y. Srinivasu, Zachary A. Cohen, John R. Engen, Thomas E. Wales

Hydrogen-Deuterium Exchange Mass Spectrometry (HDX MS) is a valuable technique for studying protein conformational dynamics but is typically limited to the study of purified proteins, overlooking molecular crowding effects in complex matrices such as plasma or cell lysate. To overcome this issue, we present a method based on the SpyCatcher-SpyTag system (Zakeri B. et al., 2012). SpyTagged Transthyretin (hTTR), SpyTagged KRAS, and SpyCatcher were expressed in *E. Coli* BL21 DE3 cells and purified. The coupling of the SpyTag and SpyCatcher constructs was assessed under varying experimental conditions, showing significantly faster kinetics in non-quenching conditions. Next, we immobilized SpyCatcher onto beads and investigated their ability to remove SpyTagged proteins of interest from complex protein mixtures. SpyTagged proteins spiked into plasma or cell lysate were able to be rapidly and specifically removed from the mixture on a timescale relevant to an HDX experiment. We then developed a method to apply this tag-capture system within an HDX experiment: KRAS or hTTR bearing the SpyTag were spiked into plasma or cell lysate, followed by the addition of D2O buffer. At timepoints, SpyCatcher beads were added to rapidly remove the protein of interest from the deuterated mixture, and captured protein was simultaneously eluted from the beads and digested with pepsin prior to LCMS analysis. Small molecule binders for both proteins were assessed for both purified protein as well as with the protein spiked into plasma/cell lysate, showing distinct results.

## 160 Measurements of Copper Content, Reactive Oxygen Species, and Metallothionein in Tetrahymena Thermophila

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Tetrahymena thermophila are single-celled, freshwater eukaryotes that are common model organisms in toxicological studies. Their ability to uptake and sequester metals makes T. thermophila a good candidate to study the production and removal of reactive oxygen species (ROS) as a response to oxidative stressors. High concentrations of heavy metals can cause oxidative stress, but cells have natural defenses, including metal-sequestering cysteine-rich metallothionein proteins. Our goal is to measure the cause of stress (metal uptake), the mediators (metallothioneins), and the response of the cells (oxidative stress) in the same population of cells. To do this, we expose cells to Cu2+, then use fluorogenic dyes to indicate ROS levels and immunostaining to measure metallothionein expression. Next, we use a combination of fluorescence activated cell sorting (FACS) to sort cells based on fluorescence levels, and ICP-MS to measure the copper content within each group. Thus, we obtain correlated measurements between copper concentration, ROS, and metallothionein content. We have found that there is a correlation between high metallothionein expression and high Cu2+ concentrations, with a 1.4 fold increase in fg/cell of Cu2+ between the low and high metallothionein groups. To further our investigation into the optimization of these methods, we measured systematically tested common blocking buffers used in immunostaining for contamination with biologically relevant metals in and found that these buffers vary in metal content. These results will be used to further our work coupling FACS with ICP-MS analysis of cells.

## 161 Synergistic Arg-C Ultra and Lys-C Digestion for Quantitative Proteomics

Vyas Pujari, Princeton University, Icahn Laboratory, Princeton, NJ 08540, Joseph Crapse, Connor Nisbet, Gloria Bao, Wessley Ferguson, Christopher Hosfield, Michael Rosenblatt

Shotgun proteomics hinges on complete enzymatic digestion of proteins into peptides. Incomplete digestion narrows proteome coverage and inflates variability in quantitative workflows. Sequential Lys-C/Trypsin digestions mitigate missed cleavages at lysine residues, but arginine sites remain challenging. Arg-C Ultra, a novel cysteine protease, efficiently targets arginine residues but requires reducing conditions that inactivate Lys-C activity and compromise NHS-ester labeling in multiplexed workflows. Here, we systematically characterized Arg-C Ultra and Lys-C with chromogenic substrates that mimic arginine- and lysine-containing peptides, as well as shotgun proteomics. Arg-C Ultra operates optimally at room temperature, pH 7.5–8.5, under reducing conditions, whereas Lys-C is most active at 37 °C, pH 7.5–8.5, yet rapidly loses activity when exposed to common reductants. Among tested reducing agents, 1 mM TCEP uniquely preserved TMTpro integrity while sustaining Arg-C Ultra activity. Guided by these insights, we established a sequential digestion workflow: proteins are first digested overnight with Lys-C at 37 °C (pH 8.5), then treated with 1 mM TCEP and Arg-C Ultra at room temperature (pH 8.5). The resulting peptides can be analyzed directly by label-free DIA or subjected to TMTpro labeling. Applied to HeLa cell lysates, this protocol achieved >99% arginine and 95% lysine cleavage efficiencies, boosting the number of proteins by 6% in label-free DIA and 11% in TMTproC experiments. Replicate measurements displayed reproducibility that approached the limits set by ion statistics. Thus, the introduced synergistic Lys-C/Arg-C Ultra digestion strategy enhances proteome coverage with excellent quantitative reproducibility across both label-free and multiplexed platforms.

## 160 A Comprehensive Study of Sera from Women with Breast Cancer and Age-Matched Controls to Identify Potential Protein Biomarkers

Hailey Morrissiey, Clarkson University, 8 Clarkson Ave, Potsdam, NY 13699, Danielle Whitham, Pathea Bruno, Brian Pentecost, Costel Darie

Breast cancer (BC) is the second most prevalent cause of cancer-related mortality for women in the United States, and it is also the most frequently diagnosed malignancy. Women between 40 and 74 are advised to get a mammogram every two years as part of the current BC screening program. Finding a method to screen women of any age for the development of BC is essential to detecting the cancer early and lowering death rates. The protein variations in the serum of 48 BC women and 48 age-matched controls were investigated in this study using mass spectrometry-based proteomics. Dysregulated serum proteins may provide potential biomarkers for BC and they may have definable roles in tumor-development. A NanoAcquity UPLC connected to a QTOF Xevo G2 XS mass spectrometer was used to analyze human blood samples from invasive ductal carcinoma (IDC) (48 BC and 48 controls, 96 total) utilizing in-solution digestion procedures and nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS). Prior to running on nanoLC-MS/MS, samples underwent albumin depletion by affinity chromatography and an in-solution tryptic digestion. Following data collection and conversion, Mascot Daemon was used to search the samples against an NCBI human database. Mascot Distiller and Scaffold software will be used for identification and label free quantification of proteins. Protein dysregulations, proteins implicated in tumor/cancer formation, and protein biomarkers are yet to be identified. Final analysis is still underway. Additional investigations will be carried out employing targeted quantitative proteomics using absolute quantification (AQUA) peptides and multiple reaction monitoring (MRM).

## 163 Proteomic Analysis and Comparison of Stage IIA T1N1 ER/PR Negative Serum to Controls for Identification of Potential Biomarkers for Breast Cancer (BC)

Niyogushima Nuru, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Costel C. Darie, Danielle Whitham, Brian T. Pentecost

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 serum allows for detection via breast tissue directly and analysis of intracellular and secreted proteins from the tumor. Mass spectrometry (MS) is important for the study of proteomics because of its high sensitivity and ability to detect low-abundance 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.

## 164 Analysis of Nitrite in Pharmaceutical Excipients Using HPLC with UV and Fluorescence Detection

Reyhane Shavandi, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Jinjian Zheng

Nitrosamines are a class of chemicals which are considered mutagenic and potent carcinogen to humans. These compounds can form under certain conditions when secondary/ tertiary amines react with nitrosating agents such as nitrites in excipients. Therefore, mitigating nitrite contamination in the excipients is crucial to effectively control nitrosamine formation in pharmaceutical products. Nitrite reacts with 2,3-diaminonaphtalene(DAN) under acidic conditions to produce 2,3-naphthotriazole(NAT), which is a highly fluorescent compound. In this presentation, I will share a sensitive HPLC method with both UV and fluorescence detection. We will discuss how to minimize nitrite contamination during sample preparation, a common issue for nitrite analysis. Different techniques were used including HPLC-FLR , HPLC-MS, and HPLC-UV-VIS. Method performance will be evaluated per ICH Q2R2, and testing for nitrite in common pharmaceutical excipients will be discussed.

## 165 Integration of Process Analytical Technology during the Scale-Up of Heterogeneous Dynamic Kinetic Resolution Using In-Line Mid-IR Spectroscopy for the Real-Time Monitoring of a Key Starting Material for Lenacapavir API

Roudabeh Sadat Moazeni Pourasil, M4ALL (VCU), 506 E. Jackson St., Richmond, VA 23219, Rama Krishna Sayini, Naeem Asad, Daniel W. Cook, Barrack Stubbs, Samuel Hochstetler, Justina M. Burns, Li-Mei Jin, Ryan Littich, B. Frank Gupton

Process analytical technology (PAT) has recently gained widespread adoption in both academia and industry, particularly within the pharmaceutical sector, as a powerful tool for in-line and on-line process monitoring. In this study, the consumption of the racemic key starting material during a heterogeneous chiral dynamic kinetic resolution reaction was monitored using in-line mid-infrared (Mid-IR) spectroscopy with orthogonal confirmation by traditional offline analytical analysis. The data was processed using both model-based and model-free PAT approaches. Real-time monitoring, using the PAT tool, revealed that the reaction exhibited only negligible progress beyond six days, in contrast to the routine 10-day duration, enabling a significant reduction in process time and energy consumption. The use of PAT for this reaction provided immediate real-time information while being a reliable and more time-efficient alternative to traditional chromatographic analysis by eliminating the need for sample preparation, reducing solvent use, and increasing safety through less hands-on sampling. Lenacapavir Synthesis Reference: J. Org. Chem. 2025, 90, 471-478.

## 166 Nanobubble Characterization Using Nanoparticle Tracking Analysis (NTA) and Dynamic Light Scattering

Sowmya Atukuri, New Jersey Institute of Technology, Department of Civil and Environmental Engineering, 435 William St., Harrison, NJ 07029, Wen Zhang, Shan Xue, Julie Y.Chen

Nanobubbles have attracted significant interest due to their unique physicochemical properties and diverse applications (e.g., agriculture, water treatment, drug delivery, and surface cleaning). To accurately measure the size distribution and concentration of nanobubbles in water, this work developed a systematic assessment using both Nanoparticle Tracking Analysis (NTA) and Dynamic Light Scattering (DLS) techniques. To produce reliable, matrix-specific calibration curves, deionized (DI) water or methanol suspensions of polystyrene nanoparticles of different sizes (60–200 nm) and different concentrations were first prepared and used to establish calibration curves of particle size or concentration. Our results with NTA or DLS show the agreement (R2> 0.9) of the experimentally measured particle sizes and the nominal particle sizes reported by the manufacturers. Similarly, the experimentally measured particle concentrations agreed with the levels obtained by dilution of the stock suspension with the initial concentrations of $1 \times 10^{13}$ particles·mL$^{-1}$ for the 100 nm particles and $2.5 \times 10^{12}$ particles·mL$^{-1}$ for the 200 nm particles. The method detection limit (MDL) for particle concentration was determined to be $5 \times 10^{7}$ particles·mL$^{-1}$ in water and $7 \times 10^{7}$ particles in methanol. Finally, the nanobubble suspensions in both water and methanol (e.g., 10%-70% w/w) were measured by NTA and DLS after serial dilution to examine the matrix effects on the measurement accuracy and nanobubble stability. This analytical framework lays the foundation for accurate characterization of nanobubbles in liquid matrices and supports the research or engineering applications of nanobubbles.

**167** **Conductive Polymer Nanofibers for Biological Sensing Applications**
Kimberly Liu, The College of New Jersey, 2000 Pennington Rd., Department of Chemistry, Ewing, NJ 08628, Rebecca Hunter
Biocompatible, three-dimensional, nanofibrous materials are frequently used for tissue engineering applications due to their ability to imitate the in vivo cellular microenvironment. When fabricated using intrinsically conductive materials, they provide a promising platform for direct, real-time measurement of signaling molecules with rapid diffusion times and at small physiological concentrations, as the 3-D porous structure affords close proximity between the scaffold and live cells. The primary objective of this work is to use electrospinning to create tunable nanofiber scaffolds that can be used as electrochemical sensors in biological environments. Biocompatible materials such as polyvinyl alcohol and citric acid were selected for ease of electrospinning, uniform morphology and improved aqueous stability. In addition to the intrinsically conductive polymer PEDOT:PSS, varied concentrations of reduced graphene oxide were doped directly into the nanofiber matrix. Fundamental electrochemical properties of these doped nanofibers were determined after spinning onto traditional platinum and indium tin oxide electrodes. Sensitivity and limit of detection of the nanofiber sensors towards the analyte nitrite were calculated and compared to drop-cast and unmodified electrodes.

**168** **Rose to Rose Absolute: Does QuEChERS do More Harm than Good on Trace Analysis**
James Mizvesky, Seton Hall University, 400 S Orange Ave, South Orange, NJ 07079, Alexander Bulsiewicz, Nicholas Snow
Recently, DIY projects have become much more common in recent years, including homemade cosmetics and perfumes. Roses are one of the many plants that are commonly used for those purposes to make rose water, rose oil, rose concrete and rose absolute. Rose absolute, as the result of alcoholic extraction of rose concrete at low temperatures, is an orange-yellow viscous liquid with a rose scent. As most individuals would not have access to many of the nonpolar organic solvents used, they may elect to extract just via Ethanol. Our group is using these QuEChERS determining the amount of pesticides residue on cut flowers and transferred during the transformation process. When working with such trace amounts of analyte, the QuEChERS may end up doing more harm, as they may remove analytes from the sample that is being targeted for analysis. We conducted a comparison between using QuEChERS vs. straight dilution prior to injection a regular liquid-liquid to determine effects on methods used for trace analysis to better understand the limitation on detection limits due to concentration during sample preparation for future analysis of rose to rose absolute transformation.

**169** **Copper-Directed Regioselective Hydroxylation of Acetonaphthone Substrates—Synthesis and NMR Characterization**
Annie Shen, Bryn Mawr College, 57 Gerhard Place, Bryn Mawr, PA 07960
Overcoming the inherent stability of C-H bonds is a powerful synthetic tool that allows for the functionalization of inert organic molecules. One way to activate the C-H bond is through oxidation (e.g. hydroxylation) for C-O bond formation. In this work, bidentate directing groups along with copper and an oxidant are used to explore aromatic C-H bond hydroxylation in 1'- and 2-acetonaphthone substrates and their corresponding predicted hydroxylation product. NMR spectroscopy characterization of products obtained from varying hydroxylation conditions supports the selective formation of 1-hydroxy-2-acetonaphthone irrespective of the starting substrate and imine substrate-ligand isomer orientation.

**170** **High Sensitivity mAb Titers and Dual Attribute Analysis for Application in Bioprocessing**
Beatrice Muriithi, Waters Corporation, 34 Maple St., Milford, MA 01757, Martin Gilar, Fabrice Gritti, Yeliz Sarisozen, Stephan Koza, Matthew Lauber, Kevin Wyndham
Affinity chromatography is widely used in biopharmaceutical analysis for its ability to selectively isolate and quantify target molecules. Protein A columns are a standard tool for measuring the titers of monoclonal antibodies (mAbs). However, traditional formats, typically packed with large, porous particles, often fall short in terms of sensitivity, speed, and reproducibility, especially in high-throughput settings or when working with low-titer samples. To address these challenges, we've developed a new UHPLC-format affinity column that combines small, non-porous particles with optimized surface chemistry, conjugated with a recombinant Protein A ligand, and packed in organosilica-modified hardware. This design significantly improves assay sensitivity and reproducibility while reducing analysis time. The column's low peak volume also makes it easy to integrate with other chromatographic techniques.
We'll also present a streamlined multi-attribute workflow that connects the affinity column directly to a size-exclusion chromatography (SEC) column. This setup, combined with a new and optimal method, allows simultaneous measurement of titer, aggregates, and fragments from a single injection, which is ideal for clone selection and bioreactor monitoring. Moreover, the technique delivers high recovery (>98%), minimal carryover, and more than a fivefold increase in sensitivity compared to conventional columns, with analysis times under two minutes per sample.
This work highlights how thoughtful particles, column design, and method integration can push the boundaries of speed and sensitivity in biopharmaceutical analytics.

**171** **GCxGC Inlet Pressure Programs for the investigation of Olive Oil Polyphenols Content**
Nazanin Keyhani, Seton Hall University, 400 South Orange Ave., South Orange, NJ 07079, Nicholas Snow
Polyphenols are essential bioactive constituents of olive oil, serving as both nutritional markers and indicators of authenticity. Their structural diversity and relatively low volatility, present analytical challenges that necessitate optimized chromatographic conditions. In this study, we examined the influence of inlet pressure control on the comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS) characterization of methanol-extracted polyphenols. Six inlet pressure programs were investigated: constant high pressure (15.00 psi), constant low pressure (0.002 psi), and ramped profiles spanning the range of 0.002–15.00 psi in both increasing and decreasing pressures using two programming rates (fast and slow). The objective was to assess how inlet pressure impacts retention behavior, peak capacity, and overall separation efficiency. The findings help provide mechanistic insights into the role of pressure dynamics in GC×GC separations and establish practical guidelines for pressure programming as a tool to balance resolution with analysis throughput. Ultimately, this work will serve as a comparison for future studies into low-pressure GC×GC-TOFMS.

**172** **Rapid Detection of Microplastics and Nanoplastics in Seconds by Mass Spectrometry**
Mengyuan Xiao, New Jersey Institute of Technology, Department of Chemistry & Environmental Science, Newark, NJ 07103, Yongqing Yang, Hanin Alahmadi, Allison Harbolic, Terry Yu, Alex Guo, Jerry Liu, Phoebe Stapleton, Genoa R Genoa Warner, Hao Chen
Microplastics (MPs) and nanoplastics (NPs) are pervasive pollutants and their analyses by traditional mass spectrometric methods require time-intensive sample preparation (e.g., extraction, digestion, and separation). This study presents a rapid and novel method for detecting MPs and NPs using flame ionization mass spectrometry (FI-MS) in which a dried sample (e.g., powder, soil and tissue) is directly burnt or heated with a flame in front of the MS inlet. FI-MS enables decomposition and ionization of various plastics such as polyethylene terephthalate (PET) and polystyrene (PS), allowing for analysis to be completed as fast as 10 seconds per sample. As a demonstration of application of this technique, PET contaminants in 1 L of bottled water or in 0.65 L of apple juice contained in plastic bottles were quickly detected from a filter paper after sample filtration and brief drying. A 0.89 mg soil sample spiked with 6000 ppm PET microplastics was measured to contain 4.98 µg of PET (5595 ppm, quantitation error: 6.8 %). Strikingly, PS nanoplastics (200 nm size) in mouse placentas were successfully identified and quantified, highlighting the method's ability to analyze biological tissue without tedious sample preparation. Overall, this study demonstrates the high potential of FI-MS for real-world sample analysisof MPs and NPs in environmental, biological, or consumer product samples.

**173** **Spectral Analysis of Broad-Spectrum Sunscreens Using HPLC and a Photo Diode Array Detector (PDA)**
Catharine E. Layton, Waters Corporation, 34 Maple St., Milford, MA 01747, Paul D. Rainville, Amy Woodsmall
Ultraviolet (UV) and High-Energy Visible (HEV) electromagnetic radiation is responsible for a variety of chemical reactions including photochemical smog, bleaching of paints, and decay of plastics. Conjugated bonds in organic molecules absorb UV radiation to cause damage and oxidative stress to lipids and proteins resulting in sunburn, hyperpigmentation, photoaging, wrinkles, age spots, broken capillaries, and deadly skin cancer. Regular use of broad-spectrum Sun Protection Factor (SPF) sunscreens has been shown to reduce the risk of skin damage induced by both UV and HEV sources of radiation. In this work, the Alliance iS HPLC System with PDA detector is shown to separate and identify compounds, determine spectral purity, and visualize UV absorptive regions for chemical light filters used in sunscreen lotion formulations.

**174** Withdrawn by the author.

**175** **A Proteomics Investigation of Human Sera from African American Donors with Invasive Ductal Carcinoma Breast Cancer and Matched Controls**
Angiolina Hukovic, Clarkson University, 8 Clarkson Ave, Potsdam, NY 13669, Logan Seymour, Panashe Mutsengi, Danielle Whitham, Brian Pentecost, Costel Darie
Cancer is one of the top five causes of mortality in the United States, with breast cancer (BC) being the second most commonly diagnosed malignancy. About 300,000 additional cases of BC were observed in 2024, with more to be detected in 2025. African Americans have the lowest survival rate and a 34% lifetime risk of

BC in addition to the highest mortality rates (41% chance) with BC. Invasive breast cancers are classified into two types: invasive ductal carcinoma (IDC) and invasive lobular carcinoma. Here we used proteomics to compare serum samples from three African American donors with IDC and three matched controls. The samples were separated by SDS-PAGE, and the gel lanes were cut into 10-15 gel bands and digested by trypsin. In a second experiment, the samples were in-solution digested. Samples from both experiments were analyzed using nanoliquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) on a NanoAcquity UPLC coupled with a QTOF Xevo G2-XS MS or QTOF Xevo G2 MS. The raw data was processed with ProteinLynx Global Server (v 2.4), Mascot Daemon Server (v. 2.5), and Scaffold 4.3 software. The dysregulated proteins are currently being investigated for their biological role in breast cancer. We have so far identified proteins that are involved in BC or other cancers. These include proteins from the anti-trypsin family, such as Alpha-1-antichymotrypsin and Alpha-1-antitrypsin, and various complement proteins and apolipoproteins.

## 176 MauriceFlex Fractionation of Charge Variants Accompanied by LC-MS and Digital SPR Analysis Correlates Structure to the Function of a mAb

Peter Johnson, Bio-Techne, 3001 Orchard Parkway, San Jose, CA 95134

Monoclonal antibodies (mAbs) are important biotherapeutics and are quite expensive to develop. Biosimilars are lower-cost alternatives to the original drug and share the same protein sequence as the innovator but may havedifferences in post-translational modifications (PTMs) that can affect safety and efficacy. Regulatory agencies require demonstration that biosimilars are clinically similar to innovators and are safe. This study used Benlysta (belimumab Innovator, approved for lupus and lupus nephritis) and a research grade Biosimilar to characterize charge variants by leveraging the MauriceFlex instrument which can separate charge variants, mobilize them, and provide fractions for subsequent analyses. A single Flex fractionation run was sufficient to collect fractions for subsequent LC-MS analysis with the BioAccord System. This was achieved without additional sample preparation, revealing critical differences between the innovator and biosimilar, such as light chain fragmentation at specific sites and the presence of C-terminal lysine residues. Further, Flex fractions from a single run were extended to digital SPR with the Alto system for BLyS and Fc gamma receptor binding studies.

## 177 Utilizing X-ray Fluorescence for Rapid and Cost-Effective Characterization of Soil and Water Samples in Reservoirs Potentially Impacted by Heavy Metal Contamination

Debbie Siples, Malvern Panalytical, 2400 Computer Dr., Ste. 2100, Westborough, MA 01581, Katherine Gruchot

A vital aspect of large-scale soil removal projects is accurate characterization of the removed materials including the presence of potential health or environmental hazards. This characterization is critical when working in reservoirs downstream of historic and active mining operations- particularly when those regions are known for their lead and actinide deposits. Conventional testing for water and soil samples using ICP-MS or ICP-OES can be both time consuming and expensive, leading to an additional hurdle for small municipalities with time and financial constraints for their already expensive reservoir maintenance and dredging projects. X-ray Fluorescence (XRF) analysis provides a fast, affordable, and non-destructive alternative for both soil and water samples as well as allowing for larger analysis volumes. Water and sediment samples were collected from a reservoir in Fairplay, Colorado including the inlets, outlet, and center of the area requiring future dredging. Soil samples were prepared for XRF analysis on the Malvern Panalytical (MP) Wavelength Dispersive 4kW Zetium instrument as both fused borate beads and pressed pellets for determination of major mineralogical composition and trace geochemistry respectively. Water samples were concentrated and analyzed using the MP Revontium benchtop Energy Dispersive XRF for determination of dissolved solids and trace metal content. A representative portion of each powder sample was analyzed using the MP Aeris X-ray Diffractometer (XRD) to determine mineralogy, allowing for correlation of chemical and mineralogical data for determination of potential bioavailability and provenance.

## 178 Analysis of PFAS in Tap Water Using a Pentafluorophenyl Column

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Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are known to pose health risks due to their bioaccumulative nature and environmental persistence. In April 2026, legal restrictions setting a combined limit of 50 ng/L for PFOS and PFOA in tap and mineral water will take effect in Japan. Furthermore, the European Union has already established maximum levels for four PFAS congeners in seafood, and similar regulations are anticipated in the United States. In this study, we developed a pentafluorophenyl (PFP) column consisting of an ethylene cross-linked hybrid silica gel and a PFP stationary phase. This column is capable of simultaneously analyzing 19 PFAS compounds, ranging from short-chain to long-chain substances. Its applicability to drinking water analysis was evaluated and compared with that of a conventional C18 column. The PFP column exhibited not only greater sensitivity for PFBA but also superior separation of PFHxS isomers compared to the C18 column.

## 179 Quantification of Polysorbate 20 in a Biological mAb IgG Drug Products by HPLC with ELSD and Post Column Diversion Valve Configuration

Ashok Palakurthi, Sharp Sterile Manufacturing LLC, 480 Pleasant St., Lee, MA 01238, Lars Soderstrom

Polysorbate 20 is widely used in biopharmaceutical formulations as surfactant to prevent protein aggregation and ensure long-term stability, quality, and safety of therapeutic proteins. Quantification of polysorbates in such formulations is challenging due to their non-ionic structural complexity, low % levels in the drug products and interference from proteins and excipients. In this study, a robust trap-and-elute method was developed on a Waters HPLC, utilizing evaporative light scattering (ELS) detection with a switching valve configuration. The switching valve enabled selective diversion of matrix components, improving sensitivity, minimizing detector fouling, and ensuring accurate quantification. The method was fully validated and demonstrated reliability and reproducibility for the intended application in biopharmaceutical analysis.

## 180 The Identification of Protein Biomarkers in Human Breast Milk for Early Detection of Breast Cancer: A 6v6 Study

Benjamin Priest, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Victor Njoku, Kathleen Arcaro, Costel Darie

Breast cancer (BC) arises when breast tissue cells grow and divide uncontrollably. About 30% of all the cancers diagnosed in women are BC. While 1 in 8 women are predicted to developBC during their lifetime, approximately 1 in 43 will die from the disease. Early diagnosis is critical as diagnoses at a localized stage will increase the 5-year relative survival rate to over 99%. Mammography is the most common screening tool, but it is less effective in young women with dense breast tissue and involves repeated radiation exposure. Monitoring proteins that are differentially expressed in minimally or non-invasively collected breast milk from BC patients and their matched healthy controls, thereby identifying potential biomarkers for early detection could be an alternative approach. In this study, 12 breast milk samples (6 BC and 6 controls) were analyzed by NanoAcquity UPLC, coupled to QTOF Xevo G2-XS mass spectrometryfollowing tryptic in-solution digestion of both whole milk and alactoferrin-depleted fraction (subtracted using sepharose-coupled lactoferrin antibodies). Both “within-woman” and “between-women” comparisons were performed. Data collectedwere analyzed against NCBI human database using Mascot-Distiller for protein identification and Scaffold-Proteome for quantitation. Preliminary data revealed protein dysregulations in the breast milk that may be involved in the development and progression of BC.

## 181 Microwave-Assisted Digestion and ICP-MS for Trace Element Analysis in Dietary Supplements and Mushrooms

Alicia Stell, CEM Corporation, 3100 Smith Farm Rd., Matthews, NC 28106, Samuel Heckle, Macy Harris

Elemental analysis of dietary supplements, including edible mushrooms, is critical for ensuring consumer safety and meeting regulatory standards set by agencies such as the U.S. Food and Drug Administration (FDA). Mushrooms, which are increasingly consumed for their nutritional and medicinal properties, can bioaccumulate toxic elements from their growing environments, making accurate analysis essential. To ensure better analysis results, microwave-assisted digestion was employed in the preparation of various dietary supplements and mushroom samples—including commercially available products and Standard Reference Materials (SRMs)—prior to analysis by inductively coupled plasma mass spectrometry (ICP-MS). This method allows for safe and efficient digestion at elevated temperatures and pressures, improving throughput and the accuracy of elemental quantification. The digestion protocol was developed in alignment with USP <232> and <233> guidelines, with particular focus on Class 1 elements (arsenic, cadmium, mercury, and lead) and Class 2A elements (cobalt, nickel, and vanadium). The analytical method demonstrated high recovery rates for both SRMs and spiked samples, confirming its validity and robustness.

## 182 Identification of PFAS in Surface Samples using FTIR Analysis

Austin Pelletier, University of Connecticut, 434 Bell St., Glastonbury, CT 06033

PFAS, per- and poly-fluorinated alkyl substances, are a group of molecules having at least one carbon of an alkyl chain fully fluorinated, encompassing thousands of synthetic chemicals all related by their resistance to degradation and rapid accumulation in the environment. There is a growing body of research linking PFAS to various health complications, thus it is important to understand the specific routes of PFAS exposure so that policy and practice can be created to mitigate accumulation. The typical method of detection for PFAS analysis is liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) due to its high sensitivity and specificity allowing for accurate detection at trace levels. This study aims to assess the use

of Fourier-transform Infrared Spectroscopy (FTIR) as a viable alternative to LC-MS/MS PFAS analysis for rapid detection of PFAS in surface samples. The approach to development of a methodology for this technique and screening of samples for PFOS and PFOA is presented in this poster.

## 183 Evaluating the Separation Efficiency of Two-Dimensional Gel Electrophoresis Using Rat Liver Proteins

Natalie Waterman, Biochemistry and Proteomics Laboratories, Department of Chemistry & Biochemistry, Clarkson University, 10 Clarkson Ave, Potsdam, NY 13699, Kimbery Dunn, Pathea Bruno, Costel Darie

Sodium dodecyl-sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) can separate proteins under denaturing and reducing conditions according to their molecular mass. This method is also called one-dimensional gel electrophoresis (1D-PAGE). A second SDS-PAGE-based method is called two dimensional-PAGE (2D-PAGE), in which separation of proteins happens first based on their isoelectric points (pI) in first dimension (1D) and then based on their molecular weight (Mw) in the second dimension (2D). 1D-PAGE can resolve many protein mixtures, however, a very complex biological sample require high-resolution 2D-PAGE for better protein identification. In order to separate and visualize various proteins from complex proteomic samples, 2D-PAGE approach is widely being used. Furthermore, 2D-PAGE also allows us to identify different protein isoforms that have similar pI but different mass, or to identify different proteins that have identical mass, but different pI. Following trypsin digestion of a specific band, sample cleaning and mass spectrometry were carried out. The samples will be examined using an XEVO G2 QTOF, and the data will be processed and the proteins identified using our Mascot Daemon server. Here, we are testing to see whether two different proteins are either isoforms of the same protein or are two different proteins with the same Mw.

## 184 The Role of Metal and Substituents in the Stability of Zn(II) and In(III) Phthalocyanines

Kshmeya Chopra, Seton Hall University, 400 South Orange Ave., South Orange, NJ 07079, Ciara Taggart, Marius Pelmus

Metal phthalocyanines are highly conjugated macrocycles used in photodynamic therapy (PDT), optoelectronics, photocatalysis, catalysis, etc. Their long-term aerobic stability under intense radiation is a property important for their chemical, optical and biomedical applications. Fluorination is expected to enhance the Pcs stability. We report the photodegradation resistance of six PcM ($F_{64}$PcZn, $F_{64}$PcInCl, $(tBu)_4$PcZn, $F_{16}$PcZn, $F_{16}$PcInCl, and $H_{16}$PcZn), data aimed at providing insights into the role of the metal, degree of fluorination, and modulation by axial ligands. The spectroscopic analysis of the data suggests that an increase in the metal center radius and charge destabilized the Pcs, but the replacement of H by F increased the molecular photostability independent of other structural variations. These findings will be used for the design of robust materials.

## 185 Using the Systematic Screening Protocol and Inert Surface Columns to Separate Seven Janus Kinase Inhibitors

Melissa Aiello, Waters Corporation, 34 Maple St., Milford, MA 01757, Kenneth Berthelette

Janus Kinase (JAK) is a family of intracellular non-receptor tyrosine kinases that propagate cytokine-mediated signals. Janus Kinase Inhibitors (JKI) are small molecule drugs that interfere with the phosphorylation of JAK signal transducers and activators, thereby preventing them from being transcribed. This has many downstream therapeutic effects such as the downregulation of inflammatory signals of the immune system. As of 2024, there are 10 FDA approved JKI pharmaceuticals. In the context of an analytical laboratory, analysis of these compounds by liquid chromatography (LC) can be difficult due to their structural similarities, which makes method development a challenge. In this work, an LC-MS method was created to analyze seven different JKIs using a Systematic Screening Protocol (SSP) and inert surface columns. This involves three simple steps, (1) pH scouting (2) solvent screening (3) optimization. The reasoning behind column and solvent selections, combined with the data-driven decision making, will be discussed in shaping the analytical method. The final method conditions were created in less than two days, highlighting the efficiency of the SSP in developing new methods for structurally similar compounds.

## 186 Non-Invasive Textile Blend Identification via Multivariate Analysis of FORS Data

M. Fernanda Delgado Cornelio, University of Delaware, 210 S College Ave, Newark, DE 19716, Caelin Celani, Katelyn Blair, Levi Bielewicz, Jocelyn Alcantara Garcia, Karl Booksh

Accurate fiber identification is critical in recycling, quality control, cultural heritage, and forensics, yet conventional methods often fail to resolve fiber blends and require destructive sampling. Textiles are made from polymeric materials spun into threads and woven into fabrics, often as blends of various fibers. This study presents a non-invasive approach combining Fiber Optic Reflectance Spectroscopy (FORS, 350–2500 nm) with multivariate statistical analysis for classifying textile fibers. Certified references and commercial fabrics spanning eight fiber types, natural (cotton, silk, wool), regenerated (viscose, rayon), and synthetic (polyester, polyamide, polyacrylic), were analyzed. Spectral preprocessing (Savitzky-Golay smoothing, normalization) improved data structure for modeling. Principal Component Analysis (PCA) provided insights into variance and clustering, while Partial Least Squares Discriminant Analysis (PLS-DA) successfully classified the three major textile categories. In addition, identification of 8 different textiles was obtained offering both high predictive accuracy and chemical interpretability validated with a Cohen's Kappa of 0.82. While some chemically similar fibers (e.g., cotton vs. linen; viscose vs. rayon) remain challenging to distinguish, the method proved robust across diverse coloring chemistries. Our findings demonstrate the potential of chemometric approaches to enhance textile identification workflows, outperforming more limited spectral techniques like FTIR-ATR. Future work will develop multi-label classification and regression models to quantify blend compositions, advancing automated, high-throughput textile sorting.

## 187 Optimized In-Gel Tryptic Digestion Protocols Using Bovine Serum Albumin for Enhanced Mass Spectrometry-Based Proteomic Analysis

Stephanie Andreescu, Clarkson University, Biochemistry & Proteomics Laboratories, Department of Chemistry & Biochemistry, 8 Clarkson Ave., Potsdam, NY 13699, Danielle Whitham, Norman Haaker, Hailey Morrissiey, Costel C. Darie, Brindusa Alina Petre, Clarkson University

Proteomics has become more prevalent in current applications. The present experimental methods for gel-based proteomics investigations must be both precise and efficient. Currently, the protein digestion and peptide extraction approach from SDS-PAGE is both time-consuming and labor-intensive. This method could be optimized to decrease time and effort while producing equivalent or acceptable protein identification findings as the controls. To improve the digestion technique, we used two model proteins: bovine serum albumin (BSA) and lysozyme. The proteins have distinct molecular weights and numbers of disulfide links, which can be easily identified using the Mascot Daemon search, making them appropriate proteins for our examination. The trypsin digestion variants include different digestion periods and temperatures. Shaking and sonicating at different times and steps are examples of peptide extraction variables. Using increasing concentrations to determine sensitivity, the peptide mixtures were then evaluated by nanoLC-MS/MS with a NanoAcquity UPLC and a QTOF Xevo G2 MS, and the raw data was processed using the Mascot Daemon (v. 2.5) server. The data analysis considered both the protein score and the type of protein discovered. Variations in the parameters (digestion duration, extraction time, number of steps, extraction method, etc.) for protein digestion and peptide extraction enable us to identify which method produces appropriate protein identification scores. The recommended strategy is to digest trypsin for 4 hours at 37°C, followed by two 2.5-hour extraction processes. The second favored technique involves a 2-hour digestion at 40°C, followed by a 2.5-hour extraction procedure.

## 188 I Spy With My Chromatographic Eye: Seeing More in Every Sample

Katelynn Perrault Uptmor, William & Mary, Nontargeted Separations Laboratory, Chemistry Department, 540 Landrum Dr., Williamsburg, VA 23185, Emma Macturk, Kira Fisher, Barbara Grace Saunders, Madi O'Brien, Virginia Weina, Sarah Foster

Seeking to describe the whole composition of a complex sample is not a trivial task. As analytical chemists, we approach this task with extreme care to develop robust and trustworthy methods to confidently discern the components in a sample. Nevertheless, full characterization of a sample in a nontargeted manner is a much more challenging approach than targeted analysis. In this presentation, the challenges of nontargeted analysis will be discussed and the approach of using comprehensive two-dimensional gas chromatography (GC×GC) as a means of tackling these challenges will be introduced. Several research examples in food science and forensic science will be demonstrated, including evolving software comparison strategies, to provide an accurate chemical report of sample composition for samples that contain hundreds to thousands of analytes. Sustainable analytical chemistry approaches for nontargeted analysis, including retrospective sample analysis and alternative carrier gases will be highlighted. Alignment and comparison metrics will be discussed that allow robust class comparison of samples to develop conclusions about sample similarities and differences in food and forensic applications.

## 189 The Essentiality of Solution NMR Spectroscopy in the Post-AlphaFold Era

Lewis Kay, University of Toronto, Departments of Molecular Genetics, Biochemistry, and Chemistry, 1 King's College Circle, Toronto, Ontario M5S 1A8, Canada

Protein dynamics are critical for function and many of nature's molecules are highly dynamic. In this talk I will describe applications to several important systems that are now possible using new solution NMR approaches, including studies of molecular machines, of chromatin structural dynamics and electrostatics, and of phase separation at atomic resolution.

## 190 Nature-Inspired Chemical and Biochemical Analysis: New Frontiers in Affinity Separations

David Hage, University of Nebraska-Lincoln, 704 Hamilton Hall, Lincoln, NE 68588

Chemical and biochemical interactions play a major role in modern techniques for analytical chemistry and related separations. For instance, liquid chromatography is a key component in many such methods; however, this work is usually based on general separation modes that employ adsorption, reversed-phase, normal-phase, ion-exchange, or size-exclusion media. A more specific approach, inspired by interactions found in nature, is utilized in affinity chromatography and related techniques. In this group of methods, a biologically-related binding agent is used as a stationary phase for the isolation, analysis, or study of chemicals and biochemicals in complex samples. This binding agent, or affinity ligand, can be of biological origin or mimics the interactions seen in biological systems. Examples of binding agents that are utilized in these methods span from antibodies, immunoglobulin-binding proteins, and lectins to dye-ligands, boronates and metal-ion chelates. This large set of stationary phases makes affinity chromatography the most diverse type of liquid chromatography for analytical- and preparative-scale applications. This presentation will discuss recent developments in the creation and use of analytical applications of high-performance affinity chromatography and related methods. Approaches based on both specific binding agents, such as antibodies, and more general binding agents will be considered. Areas to be examined will include chromatographic-based immunoassays, microscale affinity methods, and separation-based tools for the study of bio/chemical interactions. These developments will be illustrated through such applications as the rapid analysis of biomarkers and biopharmaceuticals, the examination of changes in drug-protein binding during disease, and studies of emerging contaminants in the environment.

## 191 Good 'Ole GC: Are We Teaching an Old Dog New Tricks?

Nicholas Snow, Seton Hall University, Department of Chemistry and Biochemistry, 400 South Orange Ave., South Orange, NJ 07079

Gas chromatography (GC) has been at the heart of analytical science for over 70 years and is still among the most widely used instrumental techniques today. While we have seen massive improvements and changes in how we perform GC over the years, the fundamentals developed by the early practitioners still apply – or do they? In the address, we will examine and question some fundamental ideas we were all taught about GC through the lens of today's instrumentation and data analysis capabilities. We all studied the classical van Deemter and Golay Equations and we still often see plots based on them in the literature. Both equations were developed when isothermal GC predominated; today nearly all GC is performed with temperature programming. Are these equations still relevant? What measures can replace them? We are all discussing the impact of artificial intelligence (AI) on teaching and research across the sciences. Have we been using some form of AI for decades, as we have used computers and software to aid in method development? Like software, is AI limited to the skill and knowledge of the programmer? Finally, GC has been at the heart of the environmental movement since its early days. We will see the prominent role that GC has played in climate change discussions and in today's green chemistry. Although many consider GC mature, there is a bright future for innovation in GC: it is much more than "good 'ole GC".

## 192 Advancements in Mass Spectrometry for Spatial Omics and Systems Biology

Ljiljana Paša-Tolić, Pacific Northwest National Laboratory, 902 Battelle Blvd, PO Box 999, Richland, WA 99352

Mass spectrometry (MS) has revolutionized proteomics, metabolomics, and systems biology, providing powerful tools to study and better understand complex biological systems. In spatial biology, high-resolution mapping of transcripts, proteins, and metabolites creates a key link between genotype and phenotype, revealing how molecular mechanisms drive cellular functions and behaviors. Despite significant progress, spatial analysis of proteins and metabolites remains a challenging task. This presentation highlights recent innovations in MS technologies and their impact on spatial omics profiling. Notable advancements include the MALDI-enabled ultra-high mass range Orbitrap (MALDI-UHMR) and hybrid MS systems, equipped with a 21T Fourier transform ion cyclotron resonance (FTICR) analyzer. These platforms support high-precision applications, such as proteoform imaging—which analyzes specific protein variations shaped by genetic differences, modifications, or other biological processes—at higher *m/z* using the MALDI-UHMR, and isotopic fine structure analysis of metabolites and lipids at lower *m/z* using the 21T FTICR MS. Further improvements, such as integrating microfluidic sample preparation technologies like nanoPOTS (nanodroplet processing in one pot for trace samples) with laser capture microdissection, enhance spatial molecular profiling across human, plant, and microbial systems. By bridging omics disciplines, these tools refine phenotype prediction, advance the modeling of biological responses, and unlock new opportunities for engineering biological functions. Together, these methodologies represent an important step forward in unraveling biological complexity at molecular, spatial, and systemic levels.

## 193 Rethinking Confidence Intervals in Multivariate Classification

Karl Booksh, University of Delaware, Dept. of Chemistry and Biochemistry, Newark, DE 19716, Helder Carneiro, Caelin Celani

The ability to quantify uncertainty is perhaps the greatest current impediment to the reliable implementation of multivariate analyses in analytical chemistry. Sophisticated machine learning methods are widely used to develop models that predict the class label of observed samples, however little effort is generally dedicated to determining the appropriate level of confidence that should be placed label assignment. This is particularly true when the uncertainties in a multivariate measurement do not follow the assumed independent and identical normal distribution. Two recent advances in quantifying uncertainty in multivariate classification will be discussed. Both of these methods are agnostic to the modeling algorithm. The first method propagates the estimated measurement error through the model, based on the error's correlated structure, to determine the boundary region between two classes where class assignment cannot be performed with a given level of certainty. Through this method it is observed that the orientation of the error's correlation matrix, relative to the decision plane, plays a large role in determining prediction uncertainty. The second method, conformal prediction transforms a measurable, heuristic notion of uncertainty into statistically valid confidence intervals such that, for a future sample, the true class prediction will be included in the conformal prediction set at a predetermined confidence. In a Bayesian perspective, common estimates of uncertainty, namely p-values, only provide the probability that the data fits the presumed class model, $P(D|M)$. Conformal predictions, on the other hand, address the more meaningful probability that a model fits the data, $P(M|D)$.

## 194 Analytical Method Development and Transfer Enabled by In Silico Chromatography Modeling

Imad Haidar Ahmad, Amgen, 2360 Solway Ct., Thousand Oaks, CA 91362, Troy Handlovic, Muhammad Qamar Farooq, Daipayan Roy

New drug substances pose challenging demands on discovery and process chemistry which requires adequate and fast advancement in analytical tools to avoid bottlenecks in drug development. Challenges encountered in analyzing new medicines go beyond the complexities encountered during the manufacturing process, especially for biopharmaceuticals and multi-combination drugs. In this work, we highlight some of the novel analytical technologies and tools used in pharmaceutical laboratories for streamlining method development, which includes systematic exploration of chromatography columns and mobile phases to allow finding the right combination of column and mobile phase. This is followed by *in silico* simulation using a minimum number of experiments to predict optimum chromatographic conditions to maximum resolution between target peaks. Herein, the need for automated screening systems and computer-assisted modeling is also emphasized, as they enable successful analytical methods transfer among various systems and laboratories.

## 195 Extending Machine Learning Based Retention Time Prediction by Modeling Multiple Chromatography Conditions

Armen Beck, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Andrew Singh, Gwenyth Jones, Jonathan Fine, Rojan Sresthra, Edward Sherer, Katharine Williams, Erik Regalado, Pankaj Aggarwal

Liquid chromatography is an essential analytical technique providing the capability to separate, and subsequently, quantify and purify molecules from mixtures. Due to its ubiquitous presence across a multitude industries and academic fields, the importance of liquid chromatography has driven the development of computational modeling of retention of analytes. Traditionally machine learning models have been limited such that separate models would be required for differing chromatography methods, unable to incorporate the specific chemistry responsible for separation. In this work, we present the first graph-based neural network, MC-Retention (multi-condition retention time modeling), capable of modeling retention time across multiple column chemistries and buffer pHs. MC-Retention is a graph isomorphism network, first pre-trained on the SMRT (METLIN) dataset, and subsequently modified to incorporate column and buffer information via transfer learning with our newly introduced dataset of ~480 analytes and 8 chromatography conditions. By being able to accurately model multiple chromatographic methods simultaneously, MC-Retention is poised to greatly reduce method screening and development. Lastly, we show the utility of MC-Retention by showcasing its ability to correctly identify suitable conditions for separating components in a peptide coupling reaction, with the additional incorporation of uncertainty estimations for predicted retention times.

## 196 Molecular Dynamics Prediction of Analyte Retention in Reversed-Phase and Mixed-Mode Liquid Chromatography: Can Artificial Intelligence Rise to the Task?

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

One challenge in liquid chromatography (LC) is predicting the retention time of an analyte solely from its molecular structure and the experimental LC conditions. This is valuable in non-targeted LC-mass spectrometry (MS) separations, where measuring the *m/z* ratio alone is often insufficient for unambiguous compound identification. Incorporating retention time significantly enhances structure elucidation, therefore,

effort is currently being dedicated to implementing machine learning and artificial intelligence tools for retention prediction in LC. We first provide physico-chemical description of the retention factor in LC by examining the complex interfacial region between the solid stationary phase and the liquid mobile phase. Molecular dynamics (MD) models are constructed, and MD simulations are performed to predict the retention factors of neutral and charged analytes in reversed-phase (RP) and anion exchange (AEX)-RP chromatography [1]. The effect of organic solvent concentration, ionic strength, and pH, on the predicted retention factors is assessed using MD and compared with experimental data. Secondly, we establish the detailed microscopic interpretation of the retention mechanism in RPLC and AEX-RPLC, considering factors such as surface heterogeneity and local analyte density across and above the solid surface. This equilibrium microscopic information ultimately determines the overall retention time observed experimentally. Finally, drawing from the latest literature, we will discuss whether hybrid models—combining the physico-chemistry of adsorption at the liquid-solid interface with machine learning tools—can be properly trained to predict retention times in RPLC and AEX-RPLC with an accuracy of within a few percent, which is desirable in non-targeted LC-MS.

## 197 Deep Transfer Learning for Elution Time Prediction in Liquid Chromatography

Haixu Tang, Indiana University, 700 N Woodlawn Ave, Bloomington, IN 47408, Yuhui Hong

Predicting elution time in liquid chromatography is vital for small molecule identification but is hindered by data scarcity across diverse experimental conditions. Our research demonstrates a multi-faceted deep learning approach, grounded in rich 3D molecular conformations, to address this challenge. We first established that molecular representations learned from related analytical domains could enhance chromatographic predictions. This was proven by showing that knowledge transferred from a model pre-trained on tandem mass spectra could improve prediction of retention time and enantioseparability by various Chiral Stationary Phases (CSPs). Specifically, our model for CSP prediction accurately classified successful separations, demonstrating that detailed structural information is key to solving difficult chromatographic problems. However, this conventional transfer learning approach often fails on chromatographically dissimilar tasks, creating "TL-difficult" scenarios where performance remains poor. To overcome this, we developed a more advanced Task-Specific Transfer Learning (TSTL) strategy, which uses a joint pre-training algorithm and integrates multiple fine-tuned models into a single robust predictor. This task-aware approach significantly outperforms standard methods on challenging datasets, achieving $R^2$ values as high as 0.940 and confirming that sophisticated transfer learning, built on chemically-aware representations, provides a powerful and data-efficient solution for chromatography.

## 198 Achieving Paramount Selectivity: Bioanalytical Method Design for LC-MS/MS Interference Elimination and Recent Case Studies

Kasie Fang, GSK, 1250 S Collegeville Rd, Collegeville, PA 19426

Achieving paramount selectivity is pivotal in bioanalysis, as it ensures assay accuracy, reproducibility, and robustness. In this presentation, two recent cases will be discussed to illustrate bioanalytical method design for LC-MS/MS interference elimination in complex biological samples. In the first case, chiral derivatization, LC-HRMS, and supercritical fluid chromatography (SFC)-MS/MS were utilized to mitigate the bioanalytical selectivity issue caused by the presence of isobaric interferences. Efforts were made to understand whether the interference was the dehydrogenated metabolite (M4) or a stereoisomer (SS, RS, or SR). Practical considerations will be elaborated upon, which balance the requirements of instrument handling, assay throughput, and outsourcing strategy. In the second case, the bioanalysis of an ADC free payload with a labile lactone group will be discussed. Major challenges arise from the need to measure trace amounts of payload amidst abundant ADC, with minor ADC degradation potentially causing substantial payload overestimation. A successful assay requires careful evaluation of payload structures and effective management of ADC-related interferences. In the talk, the analyte in two forms, lactone and carboxylate, will be discussed along with their interconversion kinetics in aqueous solutions and human serum, which leads to an advanced sample extraction methodology to quantify total payload without sample pretreatment in clinical settings. The strategy of ADC purification prior to rigorous assay assessment will also be discussed, which eliminates assay interference and greatly improves selectivity.

## 199 Enabling Separation of Large Nucleic Acids by a Novel Slalom Chromatographic Technique

Jamuna Vaishnav, Waters Corporation, 5 Technology Dr., Bldg. B, Milford, MA 01757, Kennedy Sawyer, Fabrice Gritti, Mandana Fasth, Christian Reidy, MingCheng Xu, Kevin Wyndham, Matthew Lauber, Balasubrahmanyam Addepalli

Slalom chromatography (SC) is an emerging high-resolution, size-based separation technique optimized for the analysis of large nucleic acids. Utilizing 2.5 µm particles in MaxPeak™ Premier Column hardware, SC minimizes non-specific interactions and exploits the entropic stretching and relaxation dynamics of semi-rigid double-stranded DNA (dsDNA) under ultra-high pressure liquid chromatography (UHPLC) conditions (8–12k psi). This non-equilibrium separation mode enables rapid and reproducible discrimination of nucleic acid species based on structural elasticity and rigidity. SC demonstrates superior performance in resolving linear dsDNA fragments (2–25 kbp), plasmid topoisomers, and double-stranded RNA (dsRNA) impurities in under six minutes. As a powerful alternative to traditional gel electrophoresis, SC supports accelerated development of cell and gene therapy (CGT) products by enabling efficient analysis of plasmids, restriction digests, mRNA, and dsRNA contaminants.

## 200 Quantify Electrostatic Effects in Hydrophilic Interaction Chromatography (HILIC)

Yong Guo, Fairleigh Dickinson University, 230 Park Ave., Florham Park, NJ 07932

Electrostatic interactions have been well documented in hydrophilic interaction chromatography and are critical to the separation of ionized compounds in HILIC. It is very important to understand the impact of electrostatic interactions on HILIC separation. The stoichiometric displacement model has been used to quantify the effects of electrostatic interactions in HILIC. However, this model has significant limits. First, the observed plots of retention factor vs. the inverse of salt concentration (k vs. 1/[M+]) are not linear as predicted by the model. Second, the model can only quantify the effect of electrostatic attractions, but not electrostatic repulsions. Third, the model does not combine the contributions of hydrophilic partitioning and surface adsorption. We have developed a new methodology to quantify the electrostatic interactions of ionized compounds in HILIC without relying on the stoichiometric displacement model. The new methodology is based on the quantitative assessment method previously developed for evaluating the retention mechanisms of non-ionized compounds in HILIC. Our results show that the quantitative assessment method can be successfully applied to the ionized compounds. Both attractive and repulsive effects can be quantified, and the contributions of hydrophilic partitioning and surface adsorption are also separately quantified. This new methodology overcomes the limitation of the stoichiometric displacement model and will provide a better understanding of the electrostatic effects on the separation of ionized compounds in HILIC.

## 201 Advanced Chromatographic Method Development to Enable Comprehensive Control Strategies for Complex Drug Modalities

Hongyue Guo, GSK, 86 Howard St, Waltham, MA 02451

Complex drug modalities such as ADCs, gene and cell therapies pose CMC challenges due to their structural complexity, manufacturing intricacy and regulatory uncertainty. To ensure product quality and process consistency aligning with QbD principles, advanced analytical methods and tools are required. The presentation described the development of advanced chromatographic methods designed to enable comprehensive control strategies for novel and complex modalities using case studies. The outlook for innovative trends in advanced analytical methods to support the process and product development of complex drug modalities was also discussed.

## 202 New Instrumental Strategies in Analytical Atomic Spectrometry

Steven Ray, State University of New York at Buffalo, 410 Natural Science Complex, Department of Chemistry, Buffalo, NY 14260

Solutions to emerging chemical issues often require new chemical measurement capabilities that arise through innovative analytical instrumentation. The detection of poly and per-fluorinated substances (PFAS) is a current worldwide problem whose study is complicated by the lack of instrumental approaches capable to quantification of organic-phase fluorinated molecules at ultra-trace concentrations. Moreover, assessment of PFAS distribution is further complicated difficulties with quantitative analysis by conventional liquid-chromatography/mass spectrometry and the lack of suitable standards for the over 15,000 compounds of interest. Recently, our laboratory has begun to investigate a two-channel strategy for PFAS identification and quantification. Conceptually, this strategy uses two different detection modalities for each sample or eluent emerging from a chromatographic separation. In one channel, conventional molecular ionization mass spectrometry provides identification of the PFAS molecule based on tandem MS information. In a second channel, the total fluorine content of the sample or eluent peak is determined using an atomic source or basis of quantitation. In this way knowing the total fluorine content and the probable molecular formula, a comprehensive mass quantitation can be calculated even if the ionization efficiency of PFAS differs from molecule-to-molecule. Moreover, total fluorine quantitation provides a basis for mass balance. In this presentation, several potential instrumental methods for PFAS detection and quantitation based on atomic spectrometry will be reported and critically examined.

## 203 New Advances Using LA-ICP-MS and LIBS on Environmental Medicine

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In the last years LA-ICP-MS and LIBS have positioned as a tool of analysis and imaging in medicine, measuring and quantifying element distributions in teeth, bone, hair and soft tissue. Progressively, these tools have being used to understand the development of health conditions, the effect of medical treatment and reconstruct environmental exposure timeline in large epidemiological studies. In this way, LA-ICP-MS and LIBS allow to obtain spatial distribution of heavy metal, organic compounds and lately considered halides as fluoride and chloride. In addition, the development of new standard material that recreates the optical and chemical properties from the study samples has been used to reconstruct a quantitative scan or image from the sample. This has been demonstrated in applications with teeth, hair and soft tissue analysis. These methods bring the opportunity to reconstruct the history of early life for chemical exposure through quantitative mapping of teeth, hair and soft tissue.

## 204 Understanding Ionization and Fragmentation Processes in Solution-Cathode Glow Discharge Mass Spectrometry (SCGD-MS)

Jacob Shelley, Rensselaer Polytechnic Institute, 110 8th St., Troy, NY 12180, Jared Viggers, Courtney Walton, Josefin Hufgard

Solution-electrode glow discharges (SEGDs) are a class of emerging plasmas useful for chemical analysis, synthesis, and materials processing. These water-rich plasmas are low-power, stable, and can be formed at atmospheric pressure (AP), which simplifies sample introduction. Additionally, the diversity of SEGD plasma processes enables elemental analysis as well as detection of intact molecular analytes with mass spectrometry (MS). It was recently shown that one such SEGD, the solution-cathode glow discharge (SCGD), can produce intact biomolecular ions from solution and tunably fragment them by adjustment of the discharge power. However, a better understanding of the ionization and fragmentation mechanisms of molecules available with these plasmas in essential to improve performance. Here, ionization pathways of small molecules and biopolymers with SCGD were investigated for a variety of compound classes. The SCGD consists of a stable direct-current APGD between a metal electrode and a flowing, acidified sample solution with ambient air as the discharge gas. Most analyte classes were detected in their protonated form. Use of thermometer molecules revealed distinct protonation pathways: chemical ionization and electrospray-like ionization from droplets. Negative ions were detected as proton-abstraction and nitrate-adducted ions, with poorer sensitivity than the positive-ionization mode. Peptide fragmentation with SCGD was explored by altering the solvent composition. Less-volatile support solutions yielded few peptide fragments. Addition of radical scavengers led to a decrease in c- and z-type fragments, indicating these were formed from plasma electrons or radicals. Efforts to improve ionization and fragmentation based on these findings will be presented.

## 205 A Public Health Laboratory Approach to Arsenic Speciation Analysis of Food Matrices using Isocratic Elution LC-ICP-MS/MS

Patrick Parsons, NY State Department of Health, Division of Environmental Health Sciences Wadsworth Center Empire State Plaza, Albany, NY 12237, Austin Roberts, Christopher Palmer

Public health laboratories are often asked to solve challenging environmental analytical problems. Arsenic is one the four major toxic elements that is determined in various sample matrices ranging from food products to urine and is complicated by needing to know which chemical species are present and at what concentration. Therefore, a reliable, rapid and rugged analytical method for As speciation is a priority. A simplified LC-ICP-MS/MS method for As speciation in food matrices was developed using tetrabutylammonium hydroxide ion-pairing with succinic acid and isocratic elution on a 25-cm 5µm C18 column to avoid long re-equilibration times between samples. Food products undergo a 2-hour microwave-assisted extraction step with a 50:50 mixture of methanol/water at 90 °C. Extracts are centrifuged, filtered through a 0.2-µm Nylon syringe filter then diluted with mobile phase plus $H_2O_2$ to oxidize $As^{3+}$ to $As^{5+}$. The combined $As^{3+}$ and $As^{5+}$ are reported as "inorganic As" (iAs), which is the key measurand for public health purposes. Five As species (arsenocholine, arsenobetaine, dimethylarsinic acid, monomethylarsonic acid, and iAs) are determined in <10 minutes. An internal standard ($As^{5+}$) is injected post-column *via* a 6-port switching valve with detection by ICP-MS/MS at m/z 91 using O2 gas mode. The method was validated for food matrices using NIST SRM 1568b, NRCC DORM-4 and NRCC TORT-3, with method limits of detection ranging from 9 –11 ng/g. Preliminary studies explored separating and detecting other species including dimethylated- and trimethylated-As species and several arsenosugar species. Some potential method limitations were identified.

## 206 Foundations of Chemometric Modelling: A Practical Guide to Preprocessing, Validation, and Feature Selection

Caelin Celani, University of Delaware, 104 Brown Lab, The Green, Newark, DE 19716

Designed for newcomers to chemometrics, this talk will provide both conceptual guidance and practical takeaways for building better models by starting with better data. Before any multivariate model can yield meaningful insights, the quality and structure of the input data must be critically considered. This talk will walk attendees through the foundational steps required to ensure their data is "chemometrics-ready," highlighting the importance of thoughtful data collection, preprocessing, variable selection, and validation. We begin with best practices in experimental design and data acquisition, emphasizing how even subtle choices such as sampling scheme, instrument parameters, or environmental conditions can have outsized impacts on model performance. From there, we examine preprocessing strategies including normalization, baseline correction, and scaling, with discussion on how each transforms the underlying data structure. Variable selection techniques are introduced as a way to focus models on chemically or analytically meaningful features, reduce noise, and improve interpretability. Finally, we address the role of cross-validation as a diagnostic and safeguard by discussing strategies that balance model performance with generalizability and trustworthiness.

## 207 Exploratory Data Analysis: Standard Tools and Modern Alternatives

Stephen Driscoll, Repligen, 1445 South Park St., Halifax Nova Scotia Canada, B3J 2L1

Tukey once stated that "finding the question is often more important than finding the answer" in the context of exploring data.[1] In contrast to confirmatory data analysis, which uses concepts such as statistical significance, inference, and confidence to accept or reject a predefined hypothesis, exploratory data analysis (EDA) seeks to uncover relationships and patterns that may inspire new hypotheses. A central goal of EDA is visualizing high-dimensional data in lower-dimensional spaces (typically of dimension 2 or 3) to explore relationships between objects, such as samples from an experiment. Broadly speaking, this involves projecting objects from a high-dimensional space into a lower-dimensional space, for which a variety of methods exist that optimize different objective functions to produce this mapping. This talk will review some of the standard EDA methods used for visualizing multivariate chemical data in addition to some modern alternatives that address some of the shortcomings of traditional methods. These techniques will be demonstrated using both simulated and real-world data sets to highlight their strengths and weaknesses.

[1] Tukey, J. W. We need both exploratory and confirmatory. Am. Stat. 1980, 34, 23–25.

## 208 Modeling: Calibration and Regression

Cannon Giglio, Oak Ridge National Laboratory, 1 Bethel Valley Rd, Oak Ridge, TN 37830

This presentation will give an overview of the key ideas behind multivariate calibration and regression methods used in chemometrics. The simplest calibration model is univariate linear regression in the form $y = mx + b$. This presentation will show how this linear regression can be extended to include multiple variables, which is called *multiple linear regression*. Next, the presentation will evaluate some of the drawbacks with multiple linear regression, such as matrix rank issues at small sample sizes and coefficient instability with strongly correlated predictor variables. These issues can be addressed using latent variable–based methods: principal components regression (PCR) and partial least squares (PLS) regression. The presentation will conclude with a discussion of validation principals, including data splitting, training and test set splits, and the perils of overfitting.

## 209 Modeling: Cluster Analysis and Classification

Alexis Weber, PerkinElmer, 2622 2nd Ave, Shelton, CT 12303

In an era of rapidly expanding analytical datasets, chemometric tools such as cluster analysis and classification models have become essential for interpreting complex chemical information. This presentation will introduce the foundational theory behind unsupervised and supervised classification techniques. Attendees will gain a conceptual understanding of how these methods work, when to apply them, and how to interpret their outcomes. Practical examples from analytical chemistry and forensic science will illustrate the power of these techniques in uncovering hidden patterns, assigning group membership, and enhancing decision-making processes. Whether you're new to chemometrics or looking to strengthen your modeling toolbox, this talk offers accessible guidance and applied insights into classification and cluster analysis.

## 210 Authentication of a 'Wounded' Book Aboard the USS Enterprise in the War of 1812

Zachary Voras, West Chester University of Pennsylvania, Chemistry Department, 750 South Church St., West Chester, PA 19383, Madelyne Salgado, Ron McColl

The West Chester University of Pennsylvania Special Collections Library holds a variety of rare books, documents, and objects related to the history and affiliations of the University since its inception as the West Chester Normal School in 1871. One such book is an 1809 *Laws of the United States, Vol. 8*, a 'wounded book' that was aboard the *USS Enterprise* during the capture of the *HMS Boxer* on September 5, 1813, off the coast of Maine in the War of 1812. This book received a 'wound' through the upper spine which resulted in a large deformation and loss of material within the body of the volume, leaving the book unable to be opened beyond the first page. Using forensic microscopy techniques such as stereomicroscopy, UV fluorescence microscopy and scanning electron microscopy coupled with energy dispersive X-ray spectroscopy, the book was examined for the purpose of authentication of an inscribed detail of the battle wound. Data presented will show consistency in the materials and environment of a maritime battle during the War of 1812.

## 211 Curly Crinoid: Characterization of Natural and Treatment-Induced Deterioration of a Jurassic Fossil Slab

Mariana Di Giacomo, Yale Peabody Museum, 170 Whitney Ave., New Haven, CT 06511, Anikó Bezur, Richard Hark

The Yale Peabody Museum is preparing parts of its collection for relocation into high-bay storage to improve space efficiency and reduce its carbon footprint. During packing, it was found that crinoid specimen YPM IP.203987 (Holzmaden, Germany, Early Jurassic) showed an unusual deterioration pattern in which the fossil had peeled away from its rock matrix and curled upwards. Imaging with ultraviolet-induced fluorescence revealed two distinct responses: orange fluorescence confined to the crinoid itself and blue fluorescence primarily on the upper parts of the crinoid but also extending unevenly onto the adjacent matrix. The blue fluorescence indicates the presence of a historical consolidant, likely applied to prevent deterioration caused by "pyrite disease." A range of analytical techniques was used to identify the consolidant, analyze the composition of the matrix and crinoid, and verify the presence of pyrite and its alteration products. Scanning XRF distinguished the crinoid, which has abundant calcium (as calcite), iron, and sulfur, from the clay-rich Holzmaden shale matrix. FTIR and Py-GC/MS did not detect organic compounds in the orange-fluorescing area. Py-GC/MS identified the blue-fluorescing material mainly as shellac, an insect-derived resin that was historically used as a fossil consolidant. Raman spectroscopy confirmed the presence of pyrite and sulfates, which are possible sulfide alteration products. These findings guide the planned conservation treatment of the specimen ahead of its move into high-bay. In addition, confirming the presence of pyrite will inform the appropriate packing of the specimen to prevent its further deterioration and mitigate potential impacts on nearby specimens.

## 212 Noninvasive Examination of Old Master Paintings

Erich Uffelman, Washington and Lee University, Department of Chemistry and Biochemistry, Lexington, VA 24450, Jessica Roeders, Mireille te Marvelde

Noninvasive means of examining cultural heritage objects have, in the past quarter century, transformed art conservation, technical art history, and conservation science. Techniques such as reflectance imaging spectroscopy (RIS) in the visible and near IR (VNIR) as well as the short-wave IR (SWIR), X-ray fluorescence spectroscopy (XRF, both point-based and scanning), fiber optic reflectance spectroscopy (FORS), X-ray diffraction (XRD, both point-based and scanning), Raman microscopy, etc. permit extensive noncontact examination of artworks and cultural heritage materials. In addition, modern computational methods have allowed massive data sets to be interrogated with techniques such as principal component analysis (PCA). This talk will focus on how the authors' recent work with IR imaging methods (InGaAs and Quantum Dot Sensors), RIS in the VNIR and SWIR, portable XRF (pXRF), and FORS have allowed key insights into the working methods and artistic processes of old master painters. In addition, we will consider how these methods are frequently compatible with public conservation projects in museums. Although the presentation will survey various works, paintings by Maarten van Heemskerck will be particularly featured. PCA analyses will be shown in some cases to be crucial to obtaining the most incisive results.

## 213 The Forgotten Yellow: Rediscovering Patent Yellow Pigment

Kirsten Moffitt, Colonial Williamsburg Foundation, 309 First St., Williamsburg, VA 23185, Jocelyn Alcantara-Garcia, Gabriela Farfan

Patent yellow (also known as Turner's yellow) is a valuable chronological marker for painted cultural heritage because of its narrow window of use (1781–ca 1830), with possible U.S. production as early as 1783. Despite its historical significance, this brilliant yellow lead- and chlorine-containing pigment is rarely reported in art conservation literature, likely due to analytical challenges: lead is common in historical paint, and chlorine can be difficult to detect with common techniques like XRF and SEM-EDS. Prompted by recent discoveries of Patent yellow in collection objects from The Colonial Williamsburg Foundation, this project investigated the pigment's history and the best methods for identification with instrumental techniques. The collaborative research identified the pigment's primary component as lead oxychloride ($Pb_7O_6Cl_2$), using cross-section and polarized light microscopy along with SEM-EDS, XRD, XRF, and Raman spectroscopies (in samples from two U.S.-based collections and synthesized). These findings correspond with the discredited mineral Lorettoite and represent the first reported Raman and XRD data for Patent yellow in the heritage science field. This research established polarized light microscopy as an effective and accessible method for preliminary identification. Photomicrographs of pigment dispersions from four case studies revealed unique optical and morphological features that distinguish Patent yellow from other yellow pigments under the optical microscope. This research suggests the pigment may be more widespread than previously recognized.

## 214 Real-Time, Multi-Attribute PAT Integration for Rapid mRNA Manufacturing

Hossein Hamedi, Recipharm Advanced Bio, 650 Pleasant St., Watertown, MA 02472, Edita Botonjic-Sehic

RNA therapies have proven to be highly effective over the past decade in combating diseases. A notable example was during the COVID-19 pandemic, when mRNA vaccines were developed and used to significantly reduce the spread of the virus. Though successful and effective, RNA manufacturing still faces challenges related to readiness for future pandemics, speed of development, and geographical accessibility. These challenges, in part, stem from traditional approaches to quality testing. In these conventional methods, samples are taken from various unit operations and sent for offline analytical testing—results often take hours, if not days. This approach limits the speed of manufacturing. In this talk, we present our approach to continuous mRNA manufacturing using Process Analytical Technologies (PAT). We designed a customizable PAT platform, Recimagine, to accelerate and simplify the production of high-quality biomolecules by integrating real-time, multi-attribute analytical testing with advanced orchestration software and predictive modeling. This approach enabled us to build an mRNA manufacturing platform that reduced development and production timelines to under a month and is compact enough to be portable for enhanced geographical accessibility. As a use case, we will discuss real-time monitoring of the in-vitro transcription (IVT) unit operation using this platform.

## 215

No abstract submitted by the author.

## 216 Using BlazeMetrics Imaging and Particle Size to Understand Suspension Formulation Behavior

Gregory Lane, Bristol-Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901, Georgios Pyrgiotakis, Gary McGeorge, Elaine Pu, Barton Dear, Nicole Taylor, Freddy Arce, Umesh Kestur, Yue Schuman

In oral pharmaceutical formulations, solid API (Active Pharmaceutical Ingredient) particles are critical to the performance of the drug product. In this example, the API is the main component in an oral suspension. The behavior or these particles in suspension was studied using a Blaze Metrics in-situ particle-sizing instrument. The Blaze system features a high-frequency, shallow depth-of-field camera coupled with a pulsed laser source, enabling illumination and operation within highly dense suspensions. This adaptability allows seamless integration into diverse pharmaceutical development and manufacturing processes. Blaze could characterize the relative sedimentation rates of various batches which guided our development team and the processing conditions. The Blaze was placed into the sample bottles directly, without requiring any sample removal or dilution of the mixtures. The changes in particle-size distributions and particle counts, as well as High-Dynamic Range turbidity (HDR-TU) measurements provided insights into the process and allowed for critical decisions to be made upon the performance of the formulation(s).

## 217 The Perfect Match: Coupling PAT, Sustainability and QbD in the Pharma/BioPharma Environment

John Wasylyk, Bristol Myers Squibb (Retired), 6 Fawn Ln., Hamilton, NJ 08620, Robert Wethman

The first step towards process analytical technology (PAT) can be traced back some 50 years with the introduction of personal computers and advances in analytical instrumentation. In 2004, the FDA's guidance on PAT was quickly followed by Quality by Design (QbD) and just four years earlier the 12 Principles of Green Chemistry was published. In the background, the continuing advances in spectroscopy has become the major analytical tool that couples PAT, Green Chemistry and QbD. Coupling these initiatives with a wide range of spectroscopy-based instrumentation have positively impacted the pharma/biopharma industry. Applications range from non-contact to contact-based sampling to enhance process control and knowledge. Spectroscopy is now used for reducing and ensuring quality of raw material and decrease batch losses, ensuring real-time release of product, increasing safety through the proliferation of automated manufacturing, and facilitation of continuous process improvement. Each application aligns with Green Chemistry and Sustain-

ability initiative by limiting waste, lowering or eliminating sample preparation time and exposure, and maintaining tight production control all to ensure quality without impacting profitability. This presentation will provide an overview on these initiatives in the pharmaceutical environment encompassing large and small molecules at all stages of drug development and manufacturing and amplify impact on sustainability.

## 218 Accelerating Fit-for-Purpose Development of Analytical Methods, and Adaptation of Existing Platform Methods and Monograph Methods to New Substances and Products, using Modern AQbD tools and Approaches

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

Fit-for-purpose methods need to be developed quickly in today's business environment. In addition, method developers need to respond to changes in materials, formulation, and processing during the method life cycle. Such changes, which can be common in early development, can introduce new compounds such as impurities, degradants, and/or excipients, none of which were in the sample compound mix used for analytical development at the time the changes were made. The application of platform methods can reduce method development time and effort. However, the platform approach has certain risks common to novel method development, since molecule similarity does not guarantee identical performance, and not all current CQAs may have been addressed by a prospective platform method. The application of monograph methods can also reduce the method development work required for a new drug substance or product. However, given the age of many monograph methods, it is probable that the method will need to be adjusted to meet the required performance for the CQAs. It is therefore important to quickly assess if adjustments within allowable limits can achieve all specified performance goals. This presentation will therefore describe how a current method's performance can be quickly assessed in the presence of new compounds, or a new molecule, using AQbD tools and approaches. It will also discuss how to quickly determine if there is an opportunity to adjust an existing method within allowable limits when the current method does not meet the currently specified performance requirements for the CQAs.

## 219 Comparing C18-Type Stationary Phases to Biphenyl Using an LC Virtual Method Development Tool

Melinda Urich, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Column selection is a critical component in the development of robust and effective LC-MS/MS methods. Column chemistry directly impacts sensitivity, resolution, and selectivity. The complexity of multi-class panels can make separation and quantitation challenging, but by leveraging a columns' selectivity, additional separation can be achieved for difficult analytes. Understanding column chemistry is essential for enhancing method performance and robustness. To highlight the impacts of column chemistry, a virtual method development tool was used to compare various C18-type column chemistries to the biphenyl stationary phase. Virtually developed conditions were transferred to an LC-MS/MS instrument and the empirical results were compared to the modeled data. Results show that a virtual method development tool can accurately model different column chemistries that display alternative selectivity which can aid in resolving co-eluting analytes in multi-class panels.

## 220 Evaluation of Hybrid Silica C18 End-Capped with Bidentate Silylating Reagent for HPLC

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Silica-based reversed-phase columns have been widely used since the 1970s. Silica has the advantages of high mechanical strength and the ability to withstand high pressure, but conventional silica C18 had limited use under alkaline conditions and is only durable up to a pH of 8. In the 1990s, the end cap of silica C18 was strengthened, improving its alkaline resistance and ensuring durability up to pH 10. Since 2000, a base material called hybrid silica has been developed in which ethylene chains are introduced into the silica skeleton or bonded to the silica surface, and hybrid silica C18 that can be used up to pH 12 has become available. Hybrid silica in which ethylene chains are introduced into the silica skeleton is said to have surprisingly high durability under alkaline conditions compared to hybrid silica bonded to the silica surface. In this study, 1,2-Bis(chlorodimethylsilyl)ethane, which are bidentate end-capping reagents that form siloxane bonds on the silica surface at two locations, was used as an end-capping reagent for hybrid silica in which ethylene chains are introduced into the silica skeleton, called ethylene cross-linked silica gel. A proposed hybrid C18 was tested using standard samples to evaluate its hydrogen bonding, hydrophobicity and steric selectivity as well as peak shape of metal chelating and basic compounds. The durability under basic pH conditions was also evaluated. A commercially available hybrid type C18 column was used as a reference column.

## 221 The Importance of Refractive Index Accuracy in Determining Drug Payload Using Measured Particle Size for Lipid Nanoparticles

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Particle size serves as a key indicator for estimating drug loading in various systems, including lipid nanoparticles. Dynamic light scattering (DLS) is commonly used to estimate lipid particle size, typically reported as an intensity-weighted size measurement. Often, this method assumes the refractive index (RI) of the medium as water ($H_2O$). Paradoxically, a 1% error in the RI can lead to a 2% discrepancy in the calculated particle size, potentially introducing systematic errors in drug payload calculations. Since accurate drug payload estimation relies on precise particle size measurement, a robust determination of RI is essential. A newly developed (Patent Pending) method is available that significantly enhances RI measurement accuracy, achieving a relative standard deviation of less than 0.1%, five times better than current DLS-based RI assessments. This method requires only two calibration points, offers a linear signal output correlated with RI, is applicable across a wide range, and requires just 380 µL of sample volume. This presentation will provide an in-depth explanation of the hardware and analytical approach, complemented by real-world examples of lipid nanoparticles, to illustrate the critical role of accurate refractive index measurements in drug payload determination.

## 222 Rapid Detection of Microplastics and Nanoplastics in Seconds Bymass Spectrometry

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Microplastics (MPs) and nanoplastics (NPs) are pervasive pollutants and their analyses by traditional mass spectrometric methods require time-intensive sample preparation (e.g., extraction, digestion, and separation). This study presents a rapid and novel method for detecting MPs and NPs using flame ionization mass spectrometry (FI-MS) in which a dried sample (e.g., powder, soil and tissue) is directly burnt or heated with a flame in front of the MS inlet. FI-MS enables decomposition and ionization of various plastics such as polyethylene terephthalate (PET) and polystyrene (PS), allowing for analysis to be completed as fast as 10 seconds per sample. As a demonstration of application of this technique, PET contaminants in 1 L of bottled water or in 0.65 L of apple juice contained in plastic bottles were quickly detected from a filter paper after sample filtration and brief drying. A 0.89 mg soil sample spiked with 6000 ppm PET microplastics was measured to contain 4.98 µg of PET (5595 ppm, quantitation error: 6.8 %). Strikingly, PS nanoplastics (200 nm size) in mouse placentas were successfully identified and quantified, highlighting the method's ability to analyze biological tissue without tedious sample preparation. Overall, this study demonstrates the high potential of FI-MS for real-world sample analysis of MPs and NPs in environmental, biological, or consumer product samples.

## 223 Uncovering the Tear Fluid Glycoproteome in Dry Eye Disease

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Dry eye disease (DED) affects over 16 million adults in the U.S. and encompasses over 10 different disease subtypes which can only be diagnosed based on patient symptoms. The underlying biological cause for this pathology remains largely unknown due, in part, to analytical challenges in elucidating the complex glycoproteomic landscape of tear fluid. This is evidenced by the fact that N-glycoproteomic methods currently require high sample input, while no targeted O-glycoproteomic studies exist to date. For this current study, we sought to develop the first ever O-glycoproteomics workflow for characterizing tear fluid at a single patient level. Simultaneously, we report the most comprehensive N-glycoproteomic study of tear fluid to date. By leveraging recently introduced mucinases and a filter-aided sample preparation (FASP) strategy, we uncovered new O-glycoprotein biomarkers and their specific O-glycoepitopes. Overall, we identified over 75 glycoproteins from human tear fluid, 20 of which bear densely O-glycosylated mucin domains. We further report over 60 unique N-glycopeptides and 200 unique O-glycopeptides from a single tear strip, elucidating site-specific glycoeptiopes on glycoproteins which are involved in lubrication, tear secretion, and immune signaling. Of these glycoproteins, we obtained full sequence coverage of lacritin and characterize glycosites which may participate in binding interactions important for tear secretion. Finally, compared to previous N-glycoproteomic studies on tear fluid, our method circumvents the need to pool over 10 patient samples for a single run while also identifying more glycopeptides. Overall, these results will aid in stratifying the dry eye disease continuum and improve existing glycoprotein-based therapies.

## 224 Peptidomic In-Gel and In-Solution Comparative Study of Serum Samples from Women with Invasive Ductal Carcinoma Breast Cancer

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Breast cancer (BC) has become a leading cause of death in women worldwide, with invasive ductal carcinoma (IDC) accounting for 85% of BC diagnoses. Tumors secrete proteins into the serum, in which analysis can aid in not only host immunological responses and antibody detection but can also be applied to early-stage cancer detection. Various omics can be applied to identification on g BC biomarkers, including proteomics, peptidomics and degradomics which are novel methods used to identify proteins and peptides showing early indications of BC and other diseases. Degradomics specifically, may reveal information on how the proteins are degraded as a result of the onset of BC or the transition of BC from isolated to metastatic BC. This study uses mass spectrometry-based (MS) analysis of the serum samples both via in-solution and in-gel techniques, both with trypsin digestion. The serum samples for in-solution were fractionated using 30 kDa MWCO filters. The in-gel samples were run on a 14% SDS-PAGE. The results of these two methods are expected to complement each other all while comparing the BC samples to the control samples. The obtained raw data are examined using software such as ProteinLynx Global Server (v2.4), Mascot Daemon server (v2.5), and Scaffold 4.3. Preliminary data shows identification of a peptide sequence from the protein fibrinogen alpha (FGA) and additional proteins relating to blood function and homeostasis. In addition to FGA being involved in cancer cell apoptosis, the other peptides, for example from hemoglobin beta and alpha, may be involved in cancer metastasis.

## 225 Investigation of the effects of Human Jumping Translocation Breakpoint (hJTB) Protein Using Two-Dimensional-PAGE Coupled with nLC–MS/MS-Based Proteomics

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The human jumping translocation breakpoint (hJTB) protein which is encoded by Human JTB (hJTB) is a gene located on human chromosome 1 at q21, could be used as a biomarker for treating different malignancies and serve as a drugtarget for their treatment. . In this study, we identify DEPs, carcinogenic pathways, and biological processes associated with JTB silencing, using 2D-PAGE coupled with nano-liquid chromatography–tandem mass spectrometry (nLC–MS/MS) proteomics applied to an MCF7 breast cancer cell line, for complementing and completing our previous results based on SDS-PAGE, as well as in-solution proteomics of MCF7 cells transfected for JTB downregulation. The functions of significant DEPs are analyzed using GSEA and KEGG analyses. Almost all DEPs exert pro-tumorigenic effects in the JTB down regulatory condition, sustaining the tumor-suppressive function of JTB. Thus, the identified DEPs are involved in several signaling and metabolic pathways that play pro-tumorigenic roles: EMT, ERK/MAPK, PI3K/AKT, Wnt/β-catenin, mTOR, C-MYC, NF-κB, IFN-γ and IFN-α responses, UPR, and glycolysis/gluconeogenesis. These pathways sustain cancer cell growth, adhesion, survival, proliferation, invasion, metastasis, resistance to apoptosis, tight junctions and cytoskeleton reorganization, the maintenance of stemness, metabolic reprogramming, survival in a hostile environment, and have poor clinical outcomes. In conclusion, JTB silencing might increase the neoplastic phenotype and behavior of the MCF7 BC cell line.

## 226 Effect of Solvent, pH, Temperature, Ionic Strength and Analyte Concentration on QS-21 Isomerization Measurements by Liquid Chromatography

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Vaccine adjuvants are compounds added to vaccines to increase their efficacy. AS01 is a vaccine adjuvant used in vaccines against RSV, malaria, and shingles. AS01 contains the two active components, monophosphoryl lipid A (MPLA) and the saponin, QS-21, which are formulated into a liposome. Focusing on QS-21, it exists in four naturally occurring isomeric forms, and all of them stimulate an immune response. They are distinguished by the identity of the last sugar on the linear tetrasaccharide (xylose or apiose) and the connectivity of the glycosylated fatty acyl chain to the two hydroxyl positions on the fucose ring. The latter involves a reversible isomerization that is catalyzed by a base and moves the acyl chain from the C-4 hydroxyl position (QS-21 A) to the C-3 hydroxyl position (QS-21 B) on the fucose moiety. Existing literature offers limited insight into how various conditions affect isomerization, creating a gap that we aim to address. Here we present our data on the measurements of the QS-21 A to B isomerization under different conditions. We monitored the QS-21 isomerization under acidic, basic and saline conditions as well as elevated temperatures, different solvents and QS-21 concentrations. Our data shows that QS-21 B formation is most favored under basic conditions and higher temperatures, making it a potential indicator of the molecular stress experienced. QS-21 A was most favored in acidic conditions, in higher concentrations and in different solvent compositions. Overall, these findings provide valuable insights into the chemical behavior of QS-21 under various physical stresses.

## 227 Beyond ADC Characterization: Biotherapeutics Analysis with TSKgel® HIC-ADC™ Columns

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Hydrophobic Interaction Chromatography (HIC) is a ubiquitous technique utilized within the pharmaceutical and biotechnology industries for analytical- and process-related separations. Since HIC retains biotherapeutics without denaturing them irreversibly, it serves as an ideal approach for characterizing native structural attributes. As the biotherapeutics industry therapeutics portfolio diversifies with a wide variety of target molecules (e.g., mAbs, ADCs, VLPs, AAVs, LNPs, mRNA), the subsequent demand increases for suitable native-state analytical methods tailored to a given modality. This application note aims to present examples of suitable analytical HIC methods tailored to various molecules and their respective critical quality attributes. The TSKgel HIC-ADC Butyl and Phenyl particle technology featured herein demonstrates unique method nuances and selectivity considerations associated with a variety of applications, including highly conjugated ADCs (DAR=8), mAb oxidation profiles, and isoform separations of plasmid DNA.

## 228 Absolute Quantitation of Phosphopeptides and Glycopeptides Using Coulometric Mass Spectrometry

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Timothy Yaroshuk, Hao Chen

Phosphorylation and glycosylation are two important protein post-transitional modifications (PTMs). However, quantification of these PTMs is challenging due to the lack of protein or peptide standards. In this study, we introduced a novel approach using coulometric mass spectrometry (CMS) for absolute quantitation of phosphopeptides and glycopeptides without using standards. First, phosphorylated tyrosine peptides such as TSTEPQpYQPGENL and RRLIEDAEpYAARG can be converted into electrochemically active tyrosine peptides via enzymatic phosphate removal using alkaline phosphatase prior to CMS quantitation. Accurate quantitation was obtained with small quantitation errors (0.3–6.6%). Alternatively, for electrochemically inactive phosphopeptides and glycopeptides, derivatization of their N-termini with an NHS ester reagent, 2,5-dioxo-1-pyrrolidinyl 3,4-dihydroxybenzene propanoate (DPDP), was conducted to introduce one electroactive catechol tag, allowing the DPDP-derivatized peptides to be quantified by CMS. This strategy was first validated using peptides RGD, GGYR, phosphopeptide RRApSVA, and glycopeptide NY-IVGQPSS(β-GlcNAc) TGNL–OH, and successful quantification was achieved with quantification errors less than 6%. Taking one step further, we applied this approach to quantify glycopeptides generated from tryptic digestion of the NIST monoclonal antibody (mAb). Through hydrophilic interaction liquid chromatography column separation, five N297 glycopeptides were successfully derivatized, separated, and quantified by CMS without the use of standards. Due to the biological significance of PTMs, this study for quantifying peptides carrying PTMs would have a high potential for quantitative proteomics and biological research.

## 229 Analysis of Trace Elements in Coastal Seawater by ICP-MS

Andrea Palpini, PerkinElmer, 710 Bridgeport Ave., Shelton, CT 06484

Coastal oceans are among the most diverse ecosystems on the planet, housing a wealth of marine life and serving as the primary source of global seafood production. Due to increasing population and industrialization, contamination of coastal seawater by both metals and metalloids threaten biological and human communities relying on these waters. For this reason, it is important to monitor and assess the presence of potentially toxic heavy metals and metalloids in coastal seawater. Being one of the most challenging matrices due to its high total dissolved solids (TDS) content, the analysis of seawater requires a method of analysis that can achieve low levels of detection while overcoming matrix effects. Inductively coupled plasma mass spectrometry (ICP-MS) is a suitable technique for this analysis offering multi-element capabilities, high sensitivity, low detection limits, wide linear dynamic range, and easy automation. Still, due to the complex matrix, interferences pose a challenge. Two modes of operation were used to reduce and/or remove interferences: kinetic energy discrimination (KED) and dynamic reaction cell (DRC). These approaches can reduce and/or eliminate polyatomic interferences by introducing cell gases that cause collisions and reaction chemistries to deal with these interferences. This work describes a method of analysis that is suitable for the complex matrix of coastal seawaters.

## 230 Optimizing Column Geometry for Micro-Flow LC/MS

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Electrospray ionization has long been and current remains one of the most popular interfaces to couple liquid chromatography to mass spectrometry detection and analysis. However, due to the softness of the ionization technique the efficiency of the ionization can be significantly impacted by the environment in which the molecules reside during the ionization process. It has long been established that one of the environmental factors that impacts electrospray ionization efficiency is the flow rate of the LC separation, whereby the lower the flow rate facilitates increased ionization efficiency. The flow rate dependence on ionization improvement, however, is not linear, but rather experiences exponential increases as one drops the flow rate below 15 µL per minute with substantial increases seen once the flow drops below 5 µL/min and again once the flow drops below 0.5 µL/min (i.e. nano-flow LC). Owing to the ionization improvement experienced at flow rates in the 1 – 5 µL/min range, it is common practice in micro flow LC/MS to run the chromatographic separation at these flow rates. It is, however, also common to use columns for these separations with internal diameters requiring much higher flow rates for optimum chromatographic performance, subsequently reducing resolving power and increasing peak widths. In this presentation we will demonstrate that it is possible to optimize the chromatographic column geometries to optimize chromatographic performance while maintaining improved ionization efficiency greatly increasing sensitivity in Micro LC/MS analysis.

## 231 Expanding the Toolbox for HPLC-MS/MS PFAS Profiling in Wastewater and Sludge

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Measuring PFAS in wastewater (WW) and biosolids has long been a challenge, due to the large number of PFAS compounds that can be present and the wide range of properties they possess. This study presents next-generation HPLC-MS/MS methods for PFAS analysis in complex wastewater and sludge samples that broaden the range of PFAS compounds that can be detected and streamline and improve older traditional methods. Our methods employ mixed-mode HPLC columns, which enable large volume injection (LVI) of both water samples and biosolid extracts. In some cases, the techniques eliminate the need for solid phase extraction (SPE) for concentration, making it particularly advantageous for PFAS species that are challenging to analyze using SPE. One application is the analysis of ultrashort PFAS analytes including trifluoroacetic acid (TFA), which are typically poorly retained during SPE and usually omitted from conventional PFAS methods. Another notable application targets polyfluoroalkyl phosphates (PAPs), commonly found in sludge and biosolids from wastewater treatment plants (WWTPs) but rarely measured. Finally, the Total Oxidizable Precursor (TOP) assay is frequently utilized in attempts to close the PFAS mass balance in WW and biosolid extracts. This assay is labor-intensive and costly, and achieving adequate replication for statistical robustness is often impractical. We implemented LVI methods that reduce required sample volume and eliminate the need for sample concentration. Our approach facilitates replicate analyses, thereby enabling more precise determinations of PFAS composition and oxidative efficiency.

## 232 Analysis of Whole Blood using ICP-MS Universal Cell Technology

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For many years, inductively coupled plasma mass spectrometry (ICP-MS) has been the technique of choice for the analysis of trace elements like lead (Pb), arsenic (As), mercury (Hg), and copper (Cu) in bodily fluids such as urine, blood, serum and saliva. Unlike graphite furnace atomic absorption (GFAA), ICP-MS can rapidly analyze both single and multi-element panels of toxic and nutritional elements in these matrices. Researchers have found correlations between essential element levels and diseases, metabolic disorders, environmental exposures, and nutrition making this information vital for human health. Owing to the increased popularity of orthopedic implants, elements like titanium (Ti) and cobalt (Co) have also been added to the list of commonly tested analytes. Although these elements are not classified as either essential or toxic, they can give medical providers information on the implant's degradation. Whole blood is a common biological fluid which presents challenges for trace metal analysis. Blood is a complex mixture, composed mostly of water, but also contains proteins, glucose, mineral salts, hormones, as well as red and white blood cells. This poster demonstrates the successful analysis of whole blood using the NexION 5000 ICP-MS equipped with universal cell technology for interference removal using both collision and reaction mode.

## 233 Random Forest Models for Predicting Fluorescence Absorption, Emission, and Quantum Yield

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Fluorescent materials are essential to technologies ranging from biological imaging and chemical sensing to organic light-emitting diodes (OLEDs). Yet, characterizing their optical properties—such as absorption maximum (λ_abs), emission maximum (λ_em), and quantum yield (QY)—remains time-consuming and resource-intensive. This makes computational prediction an attractive alternative. In this work, we explored machine learning models that can predict fluorescence properties directly from SMILES strings combined with solvent descriptors. Among the approaches tested, Random Forest models that integrated molecular fingerprints with physicochemical descriptors consistently performed best. Using the full Deep4Chem dataset (20,236 entries, filtered to 11,923 valid molecules), the Random Forest model achieved mean absolute errors of 17.9 nm for λ_abs ($R^2$ = 0.91) and 24.8 nm for λ_em ($R^2$ = 0.86). These levels of accuracy, on the order of experimental uncertainty, suggest the models are reliable enough for pre-screening new fluorophores. Quantum yield proved more difficult to predict, but the framework demonstrates the clear potential of machine learning to speed up the discovery and design of fluorescent compounds. The study highlights the value of combining hybrid fingerprints with solvent-aware descriptors, and points toward future improvements through ensemble methods and external validation. Ultimately, by reducing reliance on costly experimental measurements, our results show how machine learning can streamline photophysical research and accelerate the development of fluorescence-based technologies.

## 234 Simultaneous Chiral and Achiral Separation of Several THC Isomers by SFC

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

The structural similarity of tetrahydrocannabinol (THC) isomers, including $\Delta^6$-, $\Delta^8$-, $\Delta^9$-, and $\Delta^{10}$-THC, presents a significant analytical challenge. These isomers often co-exist in cannabis-derived or synthetic preparations and differ in both pharmacological activity and regulatory status. Separation and quantification are therefore critical for ensuring product safety, supporting pharmacological research, and maintaining compliance with legal requirements. Moreover, several of these isomers may be generated through chemical conversion processes, which can introduce enantiomeric mixtures and unwanted byproducts. Chiral resolution is particularly important given that enantiomers may exhibit distinct receptor affinities and biological effects. Supercritical fluid chromatography (SFC) offers distinct advantages for these separations, combining rapid analysis, reduced solvent consumption, and compatibility with both achiral and chiral stationary phases. Developing robust SFC methods capable of resolving THC isomers and their enantiomers is thus essential to advance regulatory, clinical, and industrial applications. This study is focused on establishing such a method by using Daicel polysaccharide-based chiral stationary phases (CSPs). Although most often used for the separation of optical isomers only, CSPs have also demonstrated their ability to separate a wide range of other isomers, making them a good choice for this type of application. This work builds upon previously published results using CSPs in various chromatographic modes, but represents the first examples containing all 8 possible isomers of the 4 THC analogs referenced above.

## 235 Metal-Doped Boron Nitride as a Dual-Function Electrocatalyst for Nitrate and $CO_2$ Reduction

Meisu Wang, Princeton High School, Princeton, NJ 08540, Rehan Qureshi, Xianqin Wang

Conventional catalysts for wastewater treatment and $CO_2$ conversion often come with major drawbacks: they're expensive, not very efficient, and hard to reuse, which limits their use at larger scales. To tackle these issues, this work explores metal-doped boron nitride (BN) as a multifunctional electrocatalyst capable of handling both nitrate reduction in wastewater and $CO_2$ reduction. The catalysts were synthesized by dissolving boric acid, urea, and 1 wt% of either Cu, Fe, or Co acetylacetonate in deionized water, followed by 20 hours of stirring at 45 °C. The resulting mixture was then calcined to form BN-based powders. To prepare the catalyst inks, the powders were dispersed in ethanol and Nafion and sonicated before electrochemical testing. Electrochemical performance was tested in solutions containing $NaHCO_3$, $NaNO_3$, or a combination of the two, using a rotating ring-disk electrode system. Current density responses (mA $cm^{-2}$) were compared across these electrolytes to evaluate catalytic behavior. Among the three dopants, Fe-doped BN consistently showed the weakest activity, while Co-doped BN stood out with much higher activity and selectivity. These results highlight cobalt as the most effective dopant for enhancing BN-based electrocatalysts. Importantly, the system enables simultaneous nitrate removal and $CO_2$ conversion, directly coupling environmental cleanup with greenhouse gas mitigation. This dual functionality points to a sustainable and scalable approach for tackling two pressing global challenges: improving water quality and reducing carbon emissions.

## 236 Performance of Commercial Labs in the Application of Per- and Polyfluorinated Alkyl Substance Measurement Methods for Industrial Matrices

Cher Lindelien, NCASI, 14402 SW 3rd Blvd., Neweberry, FL 32669, Waruna Kiridenia, Giffe Johnson

The utility of multiple methods for analyzing PFAS in complex aqueous and solid

matrices was evaluated across several DoD-accredited laboratories using industry-sourced samples. Replicate untreated and treated pulp and paper wastewaters were analyzed using EPA Methods 1633A, 1621 (AOF), and the TOP assay. Primary and secondary biosolids, fly ash, compost, a reference standard, and spiked samples were analyzed using Method 1633A. The study assessed lab performance and compared Method 1633A to other non-targeted PFAS methods. Variations in wastewater and solids handling significantly impacted results. Several laboratories sub-sampled or diluted wastewater samples in anticipation of TSS exceeding 50 mg (practices not recommended under Method 1633A). For solids, extraction masses varied by lab and matrix, leading to higher reporting limits, more non-detects, and greater variability. Comparisons with non-targeted methods highlight potential gaps and considerations when employing these methods as surrogate measures for PFAS. The total mass percentage of fluoride quantified using Method 1633A accounted for only 3% (untreated wastewater) and 29% (treated wastewater) of the adsorbable organic fluoride quantified using Method 1621, indicating unidentified fluorinated compounds. Additional research is needed to assess potential biases introduced by inorganic fluoride not fully removed before analysis. In the primary biosolid sample, spike recoveries for certain PFAS subgroups,particularly fluorotelomer carboxylic acids (FTCAs) and perfluoroalkyl ether sulfonic acids (PFESAs), varied widely, ranging from 25–276%, likely due to matrix effects from protein and organic carbon interference. Consistent overestimation of perfluorohexanesulfonic acid (PFHxS) in the certified reference material highlights possible inaccuracies in laboratory calibrations.

## 237 Development of Monomeric Ruthenium-based Catalysts for Water Oxidation

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In oxygenic photosynthesis, plants, algae, and bacteria convert solar energy into chemical energy. The highly efficient water-splitting reaction and release of dioxygen occurs in the photosynthetic protein complex, photosystem II (PSII). The catalytic tetranuclear manganese-calcium-oxo (Mn4Ca-oxo) cluster in the oxygen-evolving complex (OEC) of PSII provides an excellent blueprint for light-driven water oxidation in nature. The water oxidation reaction is of intense interest due to its potential as a renewable, clean, and environmentally benign source of energy. Inspired by the OEC of PSII, we are designing water oxidation catalysts based on the transition metal ruthenium (Ru). We are developing a series of monomeric ruthenium complexes with different backbones and ancillary ligands. In this poster, I will briefly discuss representative Ru-based metal catalysts for water oxidation and describe recent functional studies on these catalysts.

## 238 Preliminary Associations Between Sebum Lipid Profiles and the Scalp Microbiome

Shamish Ganpule, TRI Princeton, 601 Prospect Ave., Princeton, NJ 08540, Vanessa Castro, Ernesta Malinauskyte

Sebum, a lipid-rich secretion of the sebaceous glands, maintains scalp barrier function, hydration, and microbial ecology. Although shifts in sebum composition have been linked to dandruff, seborrheic dermatitis, and hair loss, the interplay between specific lipid fractions and scalp microbial communities remains under characterized. We conducted a preliminary study to explore associations between sebum lipid profiles and the scalp microbiome. A reliable sebum-collection method was developed to minimize sample contamination from sampling devices and enable high-performance thin-layer chromatography (HPTLC). Sebum from healthy volunteers was analyzed by HPTLC to quantify major lipid classes, including triglycerides, free fatty acids, squalene, cholesterols, and wax esters. In parallel, the microbiome sampling technique was optimized and validated by prescreening extracted DNA with agarose gel electrophoresis. Scalp microbiome composition was characterized using 16S rRNA gene (bacteria) and internal transcribed spacer (ITS) region (fungi/yeasts) amplicon sequencing. Five scalp site replicates per volunteer were compared to assess sampling variability. Although limited by a small sample size, our initial observations support a bidirectional relationship in which sebum and microbiome compositions influence one another. Future work will expand the cohort, implement daily longitudinal sampling, and evaluate the impact of external factors, such as cleansing and product use, on the lipid–microbiome interface.

## 239 Integrating Heavy Isotope Labeling and Mass Spectrometry–Based Proteomics Reveals YeaG as a Key Regulator of Protein Degradation in E.coli

Ananya Solanki, Lewis-Sigler Institute for Integrative Genomics, Princeton, NJ 08544, Wessley Ferguson, Martin Wühr

Mass spectrometry–based proteomics provides a powerful platform to quantify protein concentration and stability across the proteome. Using this approach combined with stable isotope (15N) labeling of bacterial cultures, we quantified protein degradation dynamics in *Escherichia coli*. Under nitrogen limitation, we observed highly elevated and widespread cytoplasmic protein turnover. Strikingly, this degradation persisted even in the absence of the four known ATP-dependent proteases (ClpP, HslV, Lon, and FtsH), pointing to an uncharacterized pathway. We investigated the role of YeaG, a poorly characterized protein with AAA+ ATPase and serine/threonine kinase domains that is strongly upregulated during nitrogen stress. Mass spectrometry profiling revealed that deletion of yeaG abolished the cytoplasmic protein degradation normally observed under nitrogen limitation. Complementary biochemical assays showed that YeaG does not act as a protease itself, but instead influences proteolytic activity and protein synthesis specifically under nitrogen stress. Proteome-wide analysis further demonstrated that YeaG destabilizes IhfB, a transcriptional repressor of outer membrane proteins such as OmpF, suggesting a novel regulatory mechanism. To extend these findings, we are applying pull-down assays and APEX2-mediated proximity labeling coupled with LC-MS/MS to define YeaG-associated complexes and the functional roles of its AAA+ ATPase and kinase domains. Together, these mass spectrometry–driven approaches reveal unexpected patterns of protein turnover during nutrient limitation and establish YeaG as a key regulator of proteolysis. This work highlights how advanced isotopic labeling and proteomic workflows can expose new degradative mechanisms and regulatory networks essential for bacterial adaptation.

## 240 Automated Sample Preparation of Solid Dosage Forms: A Comparative Evaluation of Manual and JetXTM Technology

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Efficient and reproducible sample preparation is a critical pre-requisite for high quality analytical measurements, particularly in high throughput environments. Sample preparation of solid oral dosage forms for assay, content uniformity (CU) and degradation products testing requires the traditional glassware, while widely used, are inherently labor-intensive and susceptible to human error. Automated sample preparation can minimize the resource burden and analyst-to-analyst variability. JetXTM is an automated sample preparation instruments which performs complete analytical extraction of solid dosage forms. JetXTM operations include solvent addition, tablet extraction, homogenization, filtering, dilution and transferring the sample to HPLC vials. JetXTM has parallel extraction mode, enabling it to handle 30 samples in about 2 hours. In this work, we evaluated the implementation of the JetXTM automated sample preparation platform and compared its performance to the conventional manual technique. This study compared the performance of JetXTM and manual techniques based on preparation time, reproducibility, throughput, and consistency for solid oral dosage forms. Automated results were equivalent to the manual results with similar %RSD. Automation offers a faster sample preparation workflow compared to the conventional method. By integrating automation into the sample preparation pipeline, we aim to reduce human error, improve data reliability, and enhance laboratory efficiency, while assessing whether automated method can meet or exceed the performance standards of established manual workflows.

## 241 Quantitative Assessment on the Impact of Music on Athletic Performance: A Correlation between Music and Performance Confidence in Squash

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Physical improvement has been the primary focus of athletic studies. Among ergogenic resources, music is an important yet underappreciated psychological aid. Research suggests that physical performance and perceived improvement are influenced by mood, which music can affect through cardiovascular responses such as heart rate and blood flow. However, the relationship between music and athletic performance remains controversial. The effects of music may depend on genre, sport type, or stage of competition. This study explores the perceived impact of music on squash athletes, particularly through emotions like anxiety and confidence. It examines correlations between music and perceived performance confidence. An anonymous survey collected self-reported data on years played, confidence levels, and music listening habits, including preferences for integrating music into workouts. The survey was sent to 24 squash players across various genders, age groups, and at different squash clubs. yielding 18 responses. The 18 received responses revealed that 75% of respondents rated their perceived confidence at 5 or above on a scale of 1 to 10. Additionally, 60% reported actively listening to music while working out at a level of 5 or above. Statistical analysis using R Studio revealed a strong positive correlation between active music listening and perceived performance confidence. Further research into the psychological aspects of athletic performance could bridge knowledge gaps and provide valuable tools for enhancing athlete confidence and performance. Understanding the role of music in sports psychology may offer practical applications for training and competition strategies.

## 242 High-Throughput Quantitation of Plasma Trimethylamine N-oxide Using Desorption Electrospray Ionization Mass Spectrometry for Rapid Cardiovascular Disease Screening

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Trimethylamine N-oxide (TMAO) is an emerging biomarker of cardiovascular dis-

ease (CVD) risk, but current detection methods are limited by low throughput and lengthy workflows. To address this, we developed a high-throughput Desorption Electrospray Ionization–Mass Spectrometry (DESI-MS) platform for rapid and accurate quantitation of TMAO in plasma. The method involves protein removal, spot deposition, and DESI-MS analysis using isotope-labeled internal standards for calibration. Validation showed strong linearity ($R^2 > 0.97$), precision (CV < 20%), minimal matrix effects, and low carry-over (<5%). In a cohort of 197 patients from National Taiwan University Hospital, DESI-MS demonstrated high correlation with LC-MS/MS (R = 0.96), 92.9% concordance in risk classification, and a ten-fold reduction in processing time. Risk stratification revealed a 1.55-fold higher prevalence of coronary stenosis in the high-risk group. Capable of processing up to 2,000 samples per day, this DESI-MS platform shows strong potential for large-scale clinical screening and personalized cardiovascular risk assessment.

## 243 Spectroscopic Investigation of the Energetics of Electron Transfer in the MenB Variant of Photosystem I

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Photosynthetic electron transport involves quinone cofactors which serve as important photochemical intermediates. Phylloquinone (PhQ) is a naphthoquinone derivative that binds at the $A_{1A}$ and $A_{1B}$ sites of Photosystem I (PSI). Previous work showed that the disruption of PhQ biosynthesis in the Δ*menB* variant of the cyanobacterium *Synechocystis* sp. PCC 6803 results in the binding of the benzoquinone derivative plastoquinone-9 (PQ-9) in the $A_{1A}$ and $A_{1B}$ sites instead of PhQ. We recently determined the cryo-electron microscopy structure of PSI from a recent strain of the original D*menB* deletion variant (referred to as Δ*menB*(2023)), which revealed an unusual quinone in the $A_{1A}$ and $A_{1B}$ sites. Through Mass spectrometry, we found that the unusual quinone contains a benzoquinone head group similar to PQ-9 and a phytyl tail similar to PhQ. We performed whole genome and Sanger sequencing combined with transient optical and time-resolved electron paramagnetic resonance (EPR) spectroscopy to investigate the kinetics and thermodynamics of electron transfer in PSI from the Δ*menB*(2023) variant. We found that secondary mutations in the original D*menB* variant resulted in the binding of the alternative quinone, 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ), in Δ*menB*(2023). Moreover, transient optical and time-resolved EPR spectroscopy reveal that the incorporation of DMPBQ leads to markedly different energetics of electron transfer in D*menB* PSI. This work is supported by the U.S. Department of Energy, Photosynthetic Systems Program under the contract DE-SC0025359 (DJV), DE-FG02-07ER15903 (KVL), DE-SC0010575 (JHG), Discovery Grant No. 2015-04021 from the NSERC (AvdE) and the NSF GRFP (PL).

## 244 Infrared Sampling Tricks of the Trade: Selecting Optimal Classical Sampling Techniques when Diamond ATR is Insufficient.

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The wide variety of sample preparation techniques and the required technical skill set to use them is a well-known challenge for the field of infrared spectroscopy. In response, the simple to use single-reflection diamond ATR sampling is now the dominant technique employed. However, there are a wide range of sample types and analytical questions which are not well suited for diamond ATR. In these cases, an experienced spectroscopist can select the optimal sampling technique for the analytical question to be solved. In this presentation we will review the existing classical IR sampling techniques for solid samples, the criteria for photometrically accurate (Class II) spectral measurements and demonstrate the preferred sampling methodology to solve specific chemical questions.

## 245 "Just Stick it in the Instrument and Scan it!" - Guiding Engineers (and others) Through the Art of Analytical Spectroscopy

Jay Powell, Analytical Answers, Inc., 4 Arrow Dr., Ste. 1, Woburn, MA 01801

Perhaps the most rewarding aspect of a practicing analytical chemist and spectroscopist is helping those outside the field of chemistry understand and answer their questions and problems. In particular, typical problems presented are "what is this contaminant", "why is this adhesive failing", "how has my competitor built their product", "has my supplier changed materials", and others. As such questions are very open-ended, and engineers (and others) expect modern analytical instrumentation to generate specific and meaningful answers with near-infinite precision and sensitivity requiring little operator interaction, guiding clients through this interactive process presents interesting challenges. Starting off, you seldom have as much control or 'say' in how materials of interest are sampled and provided. Overcoming misunderstandings and limitations in sampling present some creative opportunities. Often, visitors who may have some or 'Google' knowledge in infrared analysis expect sampling to consist of slapping the sample on an ATR accessory and pressing "Scan". While a useful sampling technique for some samples, not every sample is suitable for macro- or micro-ATR sampling. Likewise, specular and grazing angle reflectance techniques can be useful when trying to identify thin films, coatings, and organic contaminants on smooth, flat surfaces, but such surfaces are the exception. Finally, interpretation of acquired spectra is very much an art, and often careful explanations as to the strengths and limitations in computer-assisted identification is necessary. Here, I will present multiple 'real-world' examples of challenges presented in infrared sampling, analysis, and interpretation, with suggestions on techniques illustrating the art of analytical infrared spectroscopy.

## 246 Method Optimization and Data Processing for Thermogravimetric Analysis Coupled with Fourier Transform Infrared Spectroscopy (TGA-FTIR)

Justin Lang, PerkinElmer, 2651 Warrenville Rd., Ste. 100, Shelton, CT 60515, Justin Linehan

The implementation of Thermogravimetric Analysis coupled to Fourier Transform Infrared (TGA-FTIR) in both research and quality control labs is rapidly increasing. With this increased demand, the need for data analysis guidance and techniques have also grown. During this session we will discuss best practices for sample preparation and method execution so that labs can easily and properly characterize the behavior of their samples. This includes detailed discussion on how to analyze TGA-FTIR data effectively. The utilization of advanced software applications to characterize the evolved gasses in the FTIR will be demonstrated. The advanced applications take advantage of principle component analysis (PCA) and multivariate curve resolution (MCR) to resolve overlapping FTIR spectra with the TGA mass loss thermograms.

## 247 Diamond ATR: the Good, The Bad and the Ugly

Ellen Miseo, Miseo Consulting, 39 Blacksmith Dr., Needham, MA 02492, Peter Larkin

Diamond ATR accessories are promoted as the solution to all sample challenges in an FTIR analysis. The accessories do have a number of benefits. Since the element is diamond there is less fear about damage or breakage. It is so hard that very aggressive methods can be used to remove an old sample. It is chemically inert so it can be used with strong acids and bases unlike other materials. Diamond is useful but there is no such thing as a universal sampling solution. ATR relies on the difference in refractive index between the sample and the ATR element as well as the angle of incidence. For a commercial accessory the angle of incidence is typically fixed at 45°. So the refractive ndex of the sample has a large impact on whether the techniques will provide useful data. This talk will describe the refractive index constraints and what the spectra look like when the constraints are violated. We will also show comparison data for the most appropriate sampling options for the more difficult samples.

## 248 Developments in Safer Solvent Selections for the Removal and Application of Synthetic Resins

Rosie Grayburn, Winterthur Museum, Garden & Library, 5105 Kennett Pike, Winterthur, DE 19735

Cultural heritage conservation commonly employs solvents for the application and removal of polymeric resins across diverse object disciplines. These polymeric resins serve various treatment functions: consolidants and fixatives, coatings and varnishes, adhesives, binding media for restoration paints and fillers, and barrier layers on porous surfaces. Conservators prioritize solvents with minimal health and environmental impacts, typically those with low or no odor, making the identification and selection of safer alternatives with appropriate solvation and film properties critically important to the field. This research develops a comprehensive repository of solvents meeting specific GHS-defined safety criteria while effectively solvating resins of conservation interest. A unique partnership comprising academia, chemical industry, non-profit organizations, and private practice was curated to advance safer solvent identification and education. Two specialized computer-assisted systems from Dow facilitated the identification process: CHEMCOMP™ Service and a custom CAS Sci-FinderN portal, built upon an internally developed database supplemented with materials commonly used in cultural heritage conservation. Using these tools we identified solvent blends meeting rigorous safety criteria and demonstrating solvency ranges for Laropal A81 and PARALOID™ B72. Current research advances to include sophisticated solvent blend considerations, characterization of polymeric film properties from select blends, and partner feedback on test evaluations. Solubility predictions continue validation through bench testing and computational methods. This collaborative approach aims to bring safer solvent alternatives into conservation practice through ongoing dialogue with the professional community.

## 249 Solvent Use in Conservation: Global Trends and the Path Toward Greener Practices

Ka Yee (Christy) Ching, University of Delaware, Department of Art Conservation, 5105 Kennett Pike, Newark, DE 19735, Rosie Grayburn

The 2024 Greener Solvents Survey, part of Sustainability in Conservation's (SiC) ongoing initiative, was launched to better understand current solvent use and safety practices in conservation. Conducted with the University of Delaware and supported

by the Royal Society of Chemistry, this global effort addresses a critical gap: the last major survey on solvent use in conservation was in 1998. The survey, translated into eight languages, received 878 responses from around the world, with 585 valid for analysis. It investigated the types of solvents used, workplace safety practices, health concerns, and disposal methods. The most commonly used solvents were ethanol, isopropanol, and acetone, while the use of highly toxic solvents like benzene and carbon tetrachloride has declined. However, toluene and xylene remain in use and were linked to reported health symptoms such as headaches and respiratory irritation. Although 98% of respondents use personal protective equipment, fewer had access to adequate ventilation (75%) or fume hoods (61%). Only half had received recent safety training, and just half were familiar with the Globally Harmonized System (GHS) for chemical labeling. Disposal practices varied, with 53% reporting solvent evaporation as their main method, raising concerns about compliance with safety guidelines. This presentation will share key findings from the survey, identifying trends and gaps in practice, and proposing next steps toward safer, more sustainable solvent use in conservation. It highlights the importance of continued education, better infrastructure, and collaborative research to protect both people and objects.

## 250 Varnish, Vanish, Regs, Needs- Solvents in Fine Art

Ulysses Jackson, Golden Artist Colors, 188 Bell Rd., New Berlin, NY 13411

In this talk we will offer an introduction to what a fine art varnish is and why they are important. This topic is of increasing importance as museum settings are being used as sites for protest and public art competes with street art in urban locations. The key factors affecting varnish development will be addressed such as the changing landscape of regulations, available raw materials, solvents, and requirements by artists.

## 251 Heritage Sector and Life Cycle Assessment for Sustainable Choices

Sarah Nunberg, 126 Winthrop St., Brooklyn, NY 11225

For many sectors sustainable, low impact choices are often excluded from project scopes. The heritage sector is no exception with time pressures and restricted budgets for many projects. Life cycle assessment (LCA) is a tool that provides quantitative analysis of the environmental impacts from materials and processes and is accessible to cultural heritage preservation professionals through carbon calculators and journal publications. With LCA the heritage sector can make informed decisions towards lower impact actions. This presentation will explore the intersections between life cycle assessment (LCA) and cultural heritage preservation, demonstrating how LCA can support carbon fluency and inform both professional practices and pedagogical approaches. It will cover methods for selecting lower-impact purchasing options and materials, as well as strategies for researching material impacts to support informed decision-making. The common assumption that natural materials are inherently low impact will be examined, alongside tools for evaluating actual environmental impacts. Specifically, LCA will be presented as a model for the quantitative analysis of the environmental effects of materials and processes used in preservation work.

## 252 A Scientific Inquiry into Jean-Michel Basquiat's Lost Subway Portfolio

Nick Petraco, Petraco Consulting, 524 W. 59th St., New York, NY 10019

This is a scientific inquiry into the origins of Tracey Finch's collection of postcards, drawings, and sketches she obtained from Kevin Doyle, an acquaintance of Basquiat in the 1980s who claims he retrieved Basquiat's portfolio, which Jean-Michel forgot while exiting a subway car. The previously unknown details, obtained from the recently available Basquiat Estate publications, were compared to various questioned works in Finch's collection to establish the origin of her collection. In this investigation, the question is whether Doyle is a forger of Basquiat's works. Two hypotheses were studied. The first is that Doyle may be a forger of Basquiat's artwork. The second is that Doyle is telling the truth. The empirical data collected in this inquiry is unmistakable; examples that support Basquiat made and kept images and sketches of his designs are evident. Many of the same images appear in the apartment he lived in on 12th Street in Manhattan, on the neighborhood's concrete walls, in the images he created in his early life at home, published in "King Pleasure," and in his Notebooks. Therefore, the author rejects the first hypothesis that Doyle is a forger of Basquiat's early works. The evidence presented in this paper proves, within a reasonable degree of scientific certainty, that Kevin Doyle is not a forger of Basquiat's work and that the images purchased by Tracey Finch from Doyle are early works by Jean-Michel Basquiat before he became BASQUIAT. Therefore, hypothesis number 2, that Doyle is telling the truth, is actual.

## 253 A Series of Case Studies: Microscopy in Material Analysis

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Industrial microscopy has a long history. In 1929, Dr. Lindsley from William & Mary College included a variety of topics in his text entitled *Industrial Microscopy:* crystals, chemical microscopy, pharmaceuticals, wood and paper, foods, rocks, etc. The same could be said for Burrells' book *Industrial Microscopy in Practice* in 1961. Although the term *Industrial Microscopy* is not in vogue, the concept of applying a microscope to solve a problem in industry (research to manufacturing to construction and retail application, and even re-use or disposal) still appears today in such works as the 2024 DOE's *Suspect-Counterfeit Items Resource* Handbook and Reffner's 2023 book on *Solving Problems with Microscopy*. As part of *Industrial Microscopy*, this talk will delve into materials analysis via the microscope using case studies on piping corrosion, waterproofing membrane failure, soot impact from a fire, and an adhesive failure. Various types of microscopy were applied to characterize and investigate the materials in these cases - polarized light microscopy, fluorescence microscopy, scanning electron microscopy with energy dispersive x-ray analysis, transmission electron microscopy, and hotstage microscopy. As with many industrial applications of the microscope, its use is not the only instrument applied, but certainly an incredibly critical one.

## 254 Overcoming Challenging Sampling: How Stereomicroscopy Improved GC/MS Analysis

Heather Harris, Arcadia University, 450 S. Easton Rd., Glenside, PA 19038

Microscopy is not generally an aspect of sample preparation for analysis by Gas Chromatography/Mass Spectrometry (GC/MS). Usually, a small amount of solid material is dissolved in a liquid, which is then injected directly to the GC. In the present work, the GC injection port has been supplemented with a Pyroprobe (CDS Analytical, Oxford PA) device that allows analysis of solid material directly. The Pyroprobe pyrolyzes small amounts of solid material that is contained within a slender quartz tube inserted into a small quartz chamber. However, the usual "small amount" of solid material sufficient for a liquid injection produces significant device contamination and carry-over when used in the Pyroprobe. As a result, the sample size must be greatly reduced. The stereoscope provides the ability to select solid particles with weights in the microgram range. It also allows for proper loading of this microscopic amount of material into the quartz tubes. The stereoscope is a simple microscope to use, so laboratory chemists are easily trained for this application. By using the stereoscope to prepare samples, the primary source of device contamination in the present work was eliminated.

## 255 Illuminating the Invisible: Microscopy at the Intersection of Science and Art

Danielle Parsons, Wonder Science, 140 58th St., Bldg A, Brooklyn, NY 11220

By reimagining microscopes as tools for art as well as analysis, it is possible to engage broader audiences and foster a deeper sense of wonder within scientific communities. This talk explores how video microscopy and photography can be used creatively to bridge science and culture. Danielle Parsons will highlight selected works as a science artist and founder of Wonder Science (2014), a media platform that produces science-based works that are deeply researched yet designed to be relaxing and visually appealing. Her current artist residency at BioBAT Art Space—a science-art hub at the Brooklyn Army Terminal—will be discussed alongside her history of exhibiting in non-traditional outlets beyond the usual scope of science media. The session will also examine the techniques and equipment that enable her to reveal unseen lifeforms and forces that shape the world at a fundamental level. As case studies, the talk will feature Danielle's current installation at the Brooklyn Army Terminal, Andante Micromatic, as well as a work in progress, The Living Screen, a public art installation with activations planned for Earth Day, World Oceans Day, Climate Week NYC, and the United Nations Water Conference, 2026.

## 256 Advanced Analytical Workflows for Accelerating the Pace of Drug Development

Mohamed Hemida, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Rodell Barrientos, Gioacchino Luca Losacco, Heather Wang, Ophelia Ukaegbu, Andrew Singh, Caleb Kinsey, Eli Larson, Emmanuel Appiah-Amponsah, Davy Guillarme, Erik L. Regalado

The biopharmaceutical industry is rapidly advancing towards intricate and diverse drug modalities necessitating sophisticated analytical technologies that can be implemented efficiently at a fast pace. Liquid chromatography (LC) is regarded as the gold standard analytical technique within the biopharmaceutical industry. Conventional LC method development often involves excessive time, solvent usage, and manual intervention which remain major challenges and constraints towards a more rapid and efficient implementation of robust and meaningful analytical workflows across emerging biopharmaceutical modalities. It becomes crucial to streamline chromatographic method development in order to address the unprecedented analytical challenges faced in drug development. This work will present the application of various LC techniques, incorporating automated screening and computer-assisted strategies for the generic, streamlined, and rapid downstream characterization of biopharmaceuticals. It will encompass a range of chromatographic techniques, including reversed-phase liquid chromatography (RPLC), hydrophilic interaction liquid chromatography (HILIC), and size exclusion chromatography (SEC), with spectroscopic and spectrometric detection system to effectively resolve complex mixtures at various stages of drug development, from early drug discovery to late-stage development.

## 257 Analytical Characterization of Solid Oral Protein Therapeutics

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The oral delivery of protein therapeutics offers tremendous advantages in patient health care, however, developing oral protein therapeutic products with efficacy similar to injectable forms presents significant challenges. Currently, there is limited knowledge regarding all aspects of the CMC (Chemistry Manufacturing Control) development of oral biologics, including analytical methods, formulation, stability and shelf-life. To address this problem, we have developed various advanced analytical technologies for characterizing the solid dosage form protein therapeutics. These innovative analytical approaches enhance our understanding of the structure and function of the oral dosage protein and help the team to mitigate various risks identified in the CMC development of the oral protein formulations.

## 258 Extensive Characterization of Circular RNA Impurities: Analytical Characterization

Yvonne Shieh, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486, Ravikiran Yerabolu, William Cantara, Zhongfeng (Frank) Zuo, Matthew Schombs

Circular RNA (circRNA) vaccines have emerged as a promising alternative to traditional linear mRNA technologies. Compared to linear mRNA, their unique closed-loop structure offers enhanced stability by protecting against degradation from exonucleases. However, the process of cyclization introduces different impurities that require comprehensive characterization. Therefore, developing methods to isolate and characterize these impurities would enhance understanding and quality control of circRNA vaccines. In this study, we focused on two main objectives: 1) developing a scalable ion-pair reverse phase (IP RP) chromatographic method to isolate and generate pure circular RNA and various impurities, including pre-main peak and post-main peak species; and 2) characterizing these purified samples using various advanced analytical techniques. We successfully established a scalable IP RP chromatographic method that isolated pure circular RNA and its associated impurities into distinct fractions. The purified fractions were characterized using IP RP, native E-Gel, size-exclusion chromatography coupled with multi-angle light scattering (SEC-MALS), mass photometry, and other analytical techniques. Our methodology not only highlighted the limitations of various analytical techniques in detecting certain impurities compared to IP RP but also provided valuable insights into circRNA impurities.

## 259 Challenges on Process Equipment Related Leachables Analysis for Biologics Manufacturing

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In the production of biological therapeutics such as Self-Amplifying RNA-LNPs, monoclonal antibody (mAb) and etc, process equipment related leachables analysis plays a big role in safety risk assessment. Process equipment related leachables generated from downstream processing steps are considered to pose higher risk, comparing that from upstream of the UF/DF step. The Extractables and Leachables study presented in this work demonstrated the how process equipment related leachables were analyzed. We also demonstrated how Tangential flow filtration help mitigate the risk of leachables from upstream. This study described and addressed the safety concerns of extractables and leachables in the single use technologies application, which is one of the important aspects in the development and design of single use technologies in biopharmaceutical and bioprocess industries. This study demonstrated that UF/DF is an effective Self-Amplifying RNA-LNPs downstream process with robust PERLs clearance capability. The major process related leachables are indeed well-cleared after TFF step and thus present a lower overall safety risk.

## 260 Advancing Nucleic Acid Analysis: Large-Pore Superficially Porous Particles for Enhanced Separations

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Recent advances in oligonucleotide synthesis have expanded their roles from research tools to key components in diagnostics and therapeutics. With the growing diversity of chemical modifications and conjugates of ribo- and deoxyribonucleotides, there is increased demand for high-performance separation and analytical techniques. We previously demonstrated that small-diameter superficially porous particles (SPP) with enlarged pore sizes improve reversed-phase HPLC (RP-HPLC) resolution of large biomolecules such as polypeptides and protein conjugates. The development of elevated pH-tolerant hybrid SPP materials has now enabled their use under oligonucleotide separation conditions requiring high pH and temperature. In this study, we evaluated oligonucleotide separations using typical high-pH ion pair RP conditions with the 120 Å pore size hybrid silica HALO® Oligo C18 and a novel 1000 Å pore size hybrid SPP material. We present the retention and separation of both single- and double-stranded nucleic acid mixtures using various ion pair reagents, including those compatible with LC/MS. The new larger pore SPP materials demonstrate excellent separation performance under diverse conditions. Notably, the enhanced mass transfer properties of the 1000 Å pore particles provide high-resolution separations for longer single-stranded oligonucleotides (100+ nucleotides), surpassing current commercial materials. Efficient separation of homopolymeric sequences such as polyadenylated (polyA) tails is also demonstrated, highlighting the materials' utility for biologically relevant sequence motifs. Similar improvements were observed for double-stranded DNAs, single-stranded RNAs, and modified RNAs. These advances are particularly relevant as synthetic methods continue to produce longer sequences for emerging molecular biology applications, underscoring the importance of high-resolution chromatographic tools.

## 261 Stability Impact from a Titanium Dioxide-Free Film-Coated Tablet: An Analytical Investigation into Photo-, Physical, and Chemical Stability of Compressed Tablets Made with Alternative Film-Coating Materials

Matthew Gunsch, Merck & Co., Inc, 126 East Lincoln Ave, Rahway, NJ 07065, Sara Koynov, Devan McCoy, Mario Gutierrez, Trent Eastman, Yun Chen, Jiaying Liu, Plamen Grigorov, Pankaj Aggarwal, Jonathan Fine, Leonardo Allain, Pavithra Sundararajan, Sanjaykumar Patel, Andre Hermans

Titanium Dioxide (E171 Grade, $TiO_2$) is a widely used component of film coats for pharmaceutical drug products for its opacifying and coloring properties. However, recent concerns by regulatory agencies over long-term safety have prompted the search for alternative film coats. The stability impacts of replacing $TiO_2$ in film coating of a fixed-dose combination product containing two co-formulated active pharmaceutical ingredients were investigated and three potential replacements were evaluated: a calcium carbonate ($CaCO_3$)-based coating and two rice starch-based coatings. The photostability, physical stability, and chemical stability of the tablets were assessed during various stability experiments. Photostability experimentation revealed that while $CaCO_3$ provided acceptable protection against UV-induced degradation of one of the APIs, it required a higher weight gain compared to $TiO_2$ to achieve the same level of opacity and UV protection. Rice starch coatings showed insufficient opacifying properties and led to color changes under UV exposure. Physical stability experiments indicated that all $TiO_2$-free coatings increased the propensity for API crystallization due to moisture absorption, with rice starch coatings being the most severe. Chemical stability highlighted potential degradation risks with $CaCO_3$ coatings from base-induced degradation pathways on a base-labile API. However, the result indicates that this degradation was limited to the film coat/core interface. Overall, while compared to rice starch, $CaCO_3$ was a more suitable replacement for $TiO_2$ as an opacifier in the film coat, it may still lead to stability risks not seen with $TiO_2$. These findings underscore the need for careful consideration of $TiO_2$ alternatives in pharmaceutical formulations.

## 262 Automated Iterative Targeted Detection in Hyperspectral Imaging – Fast, Accurate Detection of Minor Target Signal in a Swamp

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Hyperspectral imaging (HSI) is uniquely suited for detection of minor sources of signal in highly variable and spatially complex scenarios. As a result, HSI is an important analytical tool in process forensics, defect detection in surface analysis, quality control, and chemical and pharmaceutical process monitoring. The utility of HSI for detection of known target spectral signatures can be attributed to the following properties: 1) target signal of interest may make only a minor contribution to the overall signal, but it can dominate signal in single pixels, 2) HSI measures many pixels relevant for characterizing, and accounting for, interference (clutter) signal, and 3) Clutter suppression algorithms (e.g., extended least squares and generalized least squares) can be easily identified and adapted on an image-to-image basis resulting in highly sensitive, image-specific target detection.1 To improve detection sensitivity, local windowing approaches typically employ guard pixels to characterize clutter. However, windowed approaches are often very time-consuming and often model clutter signal not relevant for the pixel under test. An alternative approach discussed here uses an automated iterative target detection algorithm that has been demonstrated to be fast and accurate for stand-off applications.2,3 The algorithm partitions an image into target signal, near target, non-target (clutter), and no-calls (anomalous signal). The concepts are demonstrated with an application using infrared HSI for the detection of plastic particles in sand1 and detection of minerals in a standoff scenario.3

1. Gallagher, et al., 10.1016/j.chemolab.2023.105032.

2. Myers, et al., 10.1117/1.JRS.13.034527.

3. Forland, B.M., et al., 10.1117/1.JRS.19.016509.

## 263 Understanding Regional Molecular Alterations in Scn2a Mutated Mouse Brains using Nanospray Desorption Electrospray Ionization Mass Spectrometry Imaging

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Mass spectrometry imaging (MSI) is a powerful tool for mapping the spatial distribution of biomolecules. Our group has developed nanospray desorption electrospray ionization (nano-DESI), an ambient, liquid extraction-based technique for MSI that enables imaging of a wide range of biomolecules. In this study, we use nano-DESI MSI to investigate the effect of the *Scn2a* gene knockout in the mouse brain. *Scn2a*, which encodes the voltage gated sodium channel NaV1.2, is critical to neuronal excitability, and its dysfunction is linked to epilepsy and neurodevelopmental disorders. Despite its importance, the molecular alterations associated with *Scn2a* dysfunction are still poorly understood. Herein, we present the first comprehensive study of regional lipid alterations associated with *Scn2a* knockout, achieved by comparing brain tissues from wild type and *Scn2a* homozygous knockout mice. Nano-DESI MSI experiments were performed on an Orbitrap mass spectrometer using three biological replicates per group to ensure reproducible detection. Region of interest (ROI) analysis revealed multiple species with altered abundance in the *Scn2a* homozygous knockout mouse brain. Notably, several phosphatidylethanolamine (PE) lipids were observed at higher abundance in different regions of the brain. For example, PE 40:4 in the cortex, and PE O-36:5 in the hippocampus of the *Scn2a* knockout brains. Meanwhile, several lipid species including phosphatidylglycerol (PG 38:4) were observed in lower abundance in the cortex. In contrast, abundant structural lipids including phosphatidylserine (PS 40:6) and phosphatidylcholine (PC 34:1) showed no significant differences between wild-type and knockout brains. Our findings offer new insights into the lipid alterations caused by *Scn2a* deficiency.

## 264 High-Speed IR Microplastic Analysis with <300 nm Spatial Resolution

Jay Anderson, Photothermal Spectroscopy Corp, 325 Chapala St., Santa Barbara, CA 93101, Eoghan Dillon, Mustafa Kansiz

Vibrational spectroscopic techniques such as Infrared (IR) and Raman spectroscopy are widely used for chemical characterization across diverse fields. However, both face significant limitations when analyzing microplastics smaller than 20 µm. Traditional IR methods (e.g., FTIR and QCL-based systems) suffer from poor spatial resolution, suboptimal reflection mode performance, and interference from scattering artifacts. While Raman spectroscopy offers improved spatial resolution, it is frequently hindered by autofluorescence and reduced sensitivity, particularly as microplastics decrease in size. Optical Photothermal Infrared (O-PTIR) spectroscopy addresses many of these limitations. It delivers submicron IR spatial resolution and high-quality spectra in reflection mode without the scattering and saturation artifacts associated with conventional IR systems. Furthermore, when integrated with Raman spectroscopy, O-PTIR enables simultaneous IR and Raman measurements from the same micro- or nanoplastic particles, offering rich, complementary chemical information from a single point. A recent innovation—counter-propagating O-PTIR—utilizes high numerical aperture (NA) refractive objectives to enhance spatial resolution to below 300 nm, while also improving sensitivity, imaging quality, and compatibility with immersion studies. Coupled with high-speed O-PTIR imaging, users can now collect full spectral datasets up to 30× faster, significantly increasing throughput. These advancements enable automated particle segmentation and classification workflows, dramatically improving the detection and analysis of the smallest, most hazardous micro- and nanoplastics. This technology is now unlocking critical insights into how these particles are absorbed, inhaled, or ingested by plants, animals, and humans—shedding light on their potential health and environmental impacts.

## 265 Sub-Micron Multimodal IR (O-PTIR) Micro-Spectroscopy to Improve Pharmaceutical Product Knowledge and Process Understanding

Mustafa Kansiz, Photothermal Spectroscopy Corp, 325 Chapala St, Santa Barbara, CA 93101, Kevin Dahl

Optical Photothermal Infrared (O-PTIR) overcomes the traditional spatial resolution limitations of IR spectroscopy and enables multimodal capabilities for spectroscopy, imaging and microscopy. Utilizing a mid-IR pulsed tunable quantum cascade laser (QCL) and a visible laser beam, O-PTIR allows for simultaneous acquisition of both IR and Raman spectra with submicron spatial resolution (<500 nm) from the same spot at the same time, with the same resolution. This presentation will demonstrate how multi-modal O-PTIR extends robust characterization of particulate matter and protein aggregates in biologics modalities from the visible, through the sub-visible, to below the single micron range. Proteinaceous aggregates smaller than 10 microns, long a challenging sample type, can be screened and characterized in a matter of seconds by leveraging the flexibility of the QCL to produce high signal-to-noise IR spectra. Excellent spectral quality allows for assessment of secondary structural changes within single aggregates relative to the bulk monomer. O-PTIR facilitates improved particulate control strategies that enhance product knowledge, container/closure selection, product stability, and process understanding.

## 266 Identification of Key Sensory-Active Compounds in Cannabis by Aroma Dilution Analysis

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Cannabis is an exponentially growing market as legalization continues worldwide. To meet consumer demands, selective breeding creates strains with various scents, a key factor in consumer preference. The scents can be attributed to the volatile secondary metabolites often present at very low concentrations. High-capacity extraction techniques coupled with gas chromatography-olfactometry/mass spectrometry (GC-O/MS) allow for the separation and identification of low-concentration, sensory-active compounds in complex sample matrices with minimal sample preparation time. In this study, the GC-O method, aroma dilution analysis (ADA), a solvent-free approach to aroma extract dilution analysis (AEDA), is used to determine the odor potency of sensory-active compounds in cannabis.

## 267 Fast Analysis of 140 Pesticides, PAHs, and PCBs by GC/MS/MS

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Pesticides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) are persistent environmental pollutants with the potential for bioaccumulation. Previously these compound classes have been analyzed using gas chromatography with an electron capture detector (GC-ECD), but this requires full chromatographic separation of all analytes for confident chemical identification. With a triple quadrupole detector (QQQ), analysis time significantly drops due to mass identification eliminating the need to chromatographically separate all components. Further, multiple reaction monitoring (MRM) mode on the QQQ provides a higher confidence in peak identification than analysis by scan or selected ion monitoring (SIM) mode. The analysis time for 140 pesticides, PAHs, and PCBs has been sped up from 3 injections over multiple hours to a single injection under 21 minutes. Initial analysis by GC-ECD took 165 minutes for PCBs, 165 minutes for pesticides, and 24 minutes for PAHs. Standards were prepared for each of the three compound classes (pesticide, PAH, and PCB), final concentrations of the samples ranged from 3 to 675 ppb. Samples were run in dynamic MRM (dMRM) mode with at least 1 quantifier ion and 1 qualifier ion transition per analyte. This new QQQ method creates a significant time savings and allows full calibrations to now be completed in hours instead of days. Using a single unified acquisition method, 24 PAHs, 24 pesticides, and 92 PCBs have been identified from environmental samples at low ppb levels.

## 268 Octahydroacridine Artifact Generated in Gas Chromatography Injector during Extractable & Leachable Study of a Medical Device

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Gas chromatography coupled with mass spectrometry (GC/MS) is a modern, essential technique, especially for the analysis of volatile, semi-volatile, and thermally stable compounds. However, increases of artifacts have been reported in literature, and artifact(s) formation has become a major concern when using GC. This case study discusses analytical strategies employed to confirm octahydroacridine as an artifact through targeted analysis using multiple detectors during an extractable and leachable (E&L) study of a medical device. The methodologies presented here may also be applied to identify and confirm other compounds that may be misidentified as extractables or leachables.

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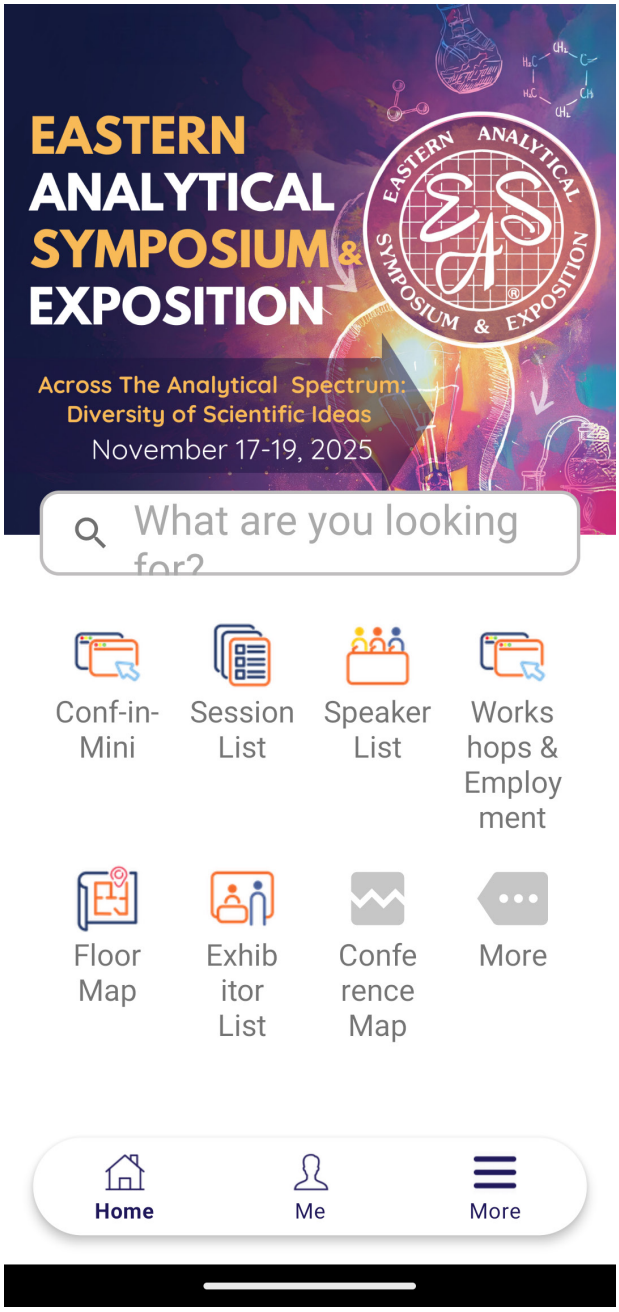


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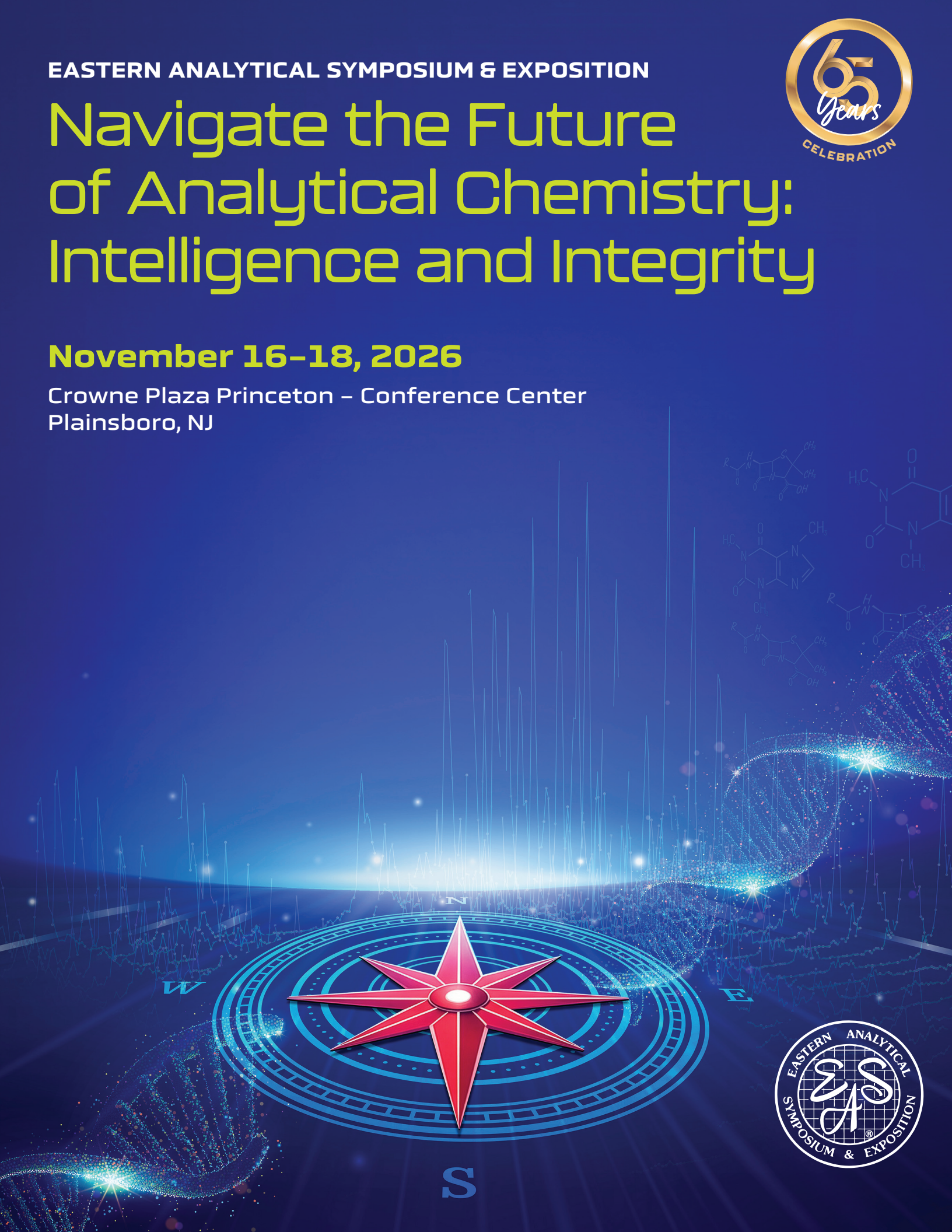


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