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ABSTRACT BOOK

2026 EAS Abstracts

This volume contains the final abstracts for the oral and poster presentations which take place Monday, November 16, through Wednesday, November 18, 2026. 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
Life of Joseph Touchstone, First President of the CFDV
William Champion, SLED, 1016 Tweedbook Rd., Wilmington, DE 19810

Professor Joseph C. Touchstone was one of the founding members of the Chromatography Forum of Delaware Valley. Joe was Professor Emeritus of Obstetrics and Gynecology in the University of Pennsylvania School of Medicine. As a scientist, he made contributions in chromatography and in physiology and biochemistry using chromatography. He published several books on Thin Layer Chromatography. We will discuss some of his person life, some of his contributions to the Chromatography Forum and his contributions to chromatography and to the life sciences.

2
Particles, Teaching, and a Little Detour to FFF. The Extraordinary Career of Jack Kirkland
Joseph Glajch, JLG AP Consulting LLC, 25 Bicentennial Dr. Nashua, NH, 03062

Jack Kirkland received degrees at Emory University (AB, MS) and the University of Virgina (PhD) and began his career in 1953 at DuPont. In the late 1950s he began to use Gas Chromatography with Steve Dal Nogare to analyze agricultural products. In the early 1960s, he started working on liquid chromatography and was inspired by the work of Joseph (JFK) Hiber in Vienna. He also realized that being in an area of industrial chemists, there needed to be a way to share knowledge broadly so he and a few others formed the Delaware Valley Chromatography Forum in 1966. His technical work in HPLC focused on particles and method development, but in 1971 he also formed a collaboration with Lloyd Snyder to begin teaching and writing books on HPLC. Together, they taught more than 5000 students in 25 years and published “the Bible” of HPLC, Introduction to Modern Liquid Chromatography. Another book, Practical HPLC Method Development, followed and another set of students taught by Jack and Lloyd, along with John Dolan and Joe Glajch. Although he continued working on HPLC particles until the end of his career, he also took a side trip with Dr. Wallace Yau into the field flow fractionation. His unique contributions to three different separation techniques was almost unique during his scientific career. He was also an extraordinary mentor to many others with whom he worked.

3
Founding Fathers of the Delaware Valley Chromatography Forum: Walt Supina, co-founder of Supelco
Mary Ellen McNally, FMC Corporation, Stine Research Center, 1090 Elkton Rd., Newark, DE 19711

There were several outstanding analytical chemists that started the Chromatography Forum of the Delaware Valley, CFDV, Walt Supina was one of those individuals. A chemical engineer by training, having received his B.S. degree from Penn State, he went to work for DuPont in Victoria Texas before serving in the army, In Korea and Japan. Following his army service, Walt, N3WS, returned to Penn State and received his MS and Ph.D., also in engineering. After graduation, Dr. Supina continued to reside in State College and worked at Applied Science Laboratories. Six years later, he and his friend, Nicholas Pelick founded Supelco, Inc. in Bellefonte, Pa., in direct competition to his former employer. The Supelco corporation contributed to the prosperity of the State College area by creating jobs for hundreds of people. For this accomplishment, both Dr. Supina and Dr. Pelick were the recipients of the 2019 Pittcon Heritage award, the award will be on display at the symposium. Dr. Supina was the also the recipient of the Outstanding Engineering Alumni Award from Penn State in 1989, although in his own biography he stated, “My professional career started with a doctorate in Chemical Engineering, but I soon became an analytical chemist.”

4
"Groovy Grob" - Teacher, Mentor, Friend; Celebrating the Career of Bob Grob
Thomas Brettell, Cedar Crest College, 100 College Dr., Allentown, PA 18104

Robert (Bob) L. Grob, PhD (2-13-1927 – 10-22-2006) was Professor Emeritus of Analytical Chemistry at Villanova University and a consultant in analytical and environmental chemistry. Dr. Grob received his BS in Chemistry from the Franciscan University of Steubenville and his MS and PhD in Analytical Chemistry from the University of Virginia. Bob was a long-time active EAS Board member and an enthusiastic advocate of professional involvement in analytical chemistry. He was a founding father of the Chromatography Forum of the Delaware Valley and served as its president in 1981-1982. Among his awards are the EAS Award for Outstanding Achievement in the Field of Separations Science, the Chromatography Forum of the Delaware Valley’s Stephen Dal Nogare Award, Villanova University’s Outstanding Research Scholar Award, and the Daniel Egan Award (from his undergraduate alma mater) for Excellence in Science and Teaching. Over his career Bob taught courses at numerous venues including the Chromatography Forum of the Delaware Valley, the Eastern Analytical Symposium, and the Pittsburgh Conference. He authored or co-authored over two hundred research papers and several books including his most popular book “*Modern Practice of Gas Chromatography*” (4 editions). Bob was not only an outstanding professional and analytical chemist, but he was a dedicated teacher and mentor to his students who all fondly remember him as “*Groovy Grob*”.

5
LC-MS Versus Ligand Binding Assays: Scientific Considerations for Quantification of Challenging Protein Biomarkers
Kai Wang, GSK, 1250 South Collegeville Rd., Collegeville, PA 19425

Protein biomarkers play increasingly important roles in modern drug discovery and development, supporting target engagement assessment, pharmacodynamic evaluation, patient stratification, translational decision-making, and clinical proof-of-mechanism studies. While ligand binding assays (LBAs) remain the most widely applied platform due to their high sensitivity and operational simplicity, advances in immunocapture liquid chromatography-mass spectrometry (LC-MS) have expanded the bioanalytical toolbox for protein quantification. Selection of the optimal analytical platform is rarely driven by sensitivity alone. Scientific considerations including molecular specificity, analyte heterogeneity, endogenous interference, therapeutic drug interference, proteoform

characterization, reagent availability, and context of use must be carefully balanced when designing fit-for-purpose assays. Recent advances in both LBA and LC-MS technologies have further blurred traditional boundaries between the two platforms, making technology selection increasingly dependent on the biological question being addressed. This presentation will discuss a practical framework for selecting between LBA and LC-MS approaches for protein biomarker quantification. Two case studies will be presented. In the Case Study A, quantification of an endogenous protein biomarker in the presence of a highly homologous therapeutic protein required differentiation of structurally related molecular species, making molecular specificity the primary analytical challenge. In Case Study B, quantification of a low-abundance proteolytic fragment highlighted the complex trade-offs between assay sensitivity, selectivity, reagent performance, and robustness across multiple analytical platforms. These examples illustrate how distinct scientific challenges can lead to very different assay strategy decisions, despite similar biomarker objectives.

6

Fit-for-Purpose Non-Regulated Bioanalysis Workflow for Targeted Protein Degradation Programs

Qin Ji, Abbvie, 1 North Waukegan Rd., Bldg. AP9, Noth Chicago, IL 60064

Discovery stage studies are conducted to rapidly generate data for internal decision-making. This approach helps researchers quickly identify and eliminate compounds with suboptimal properties while selecting the best candidates for further development. By doing so, time and resources are conserved before investing in more expensive regulated studies. Non-regulated bioanalytical support is essential for generating reliable data quickly and ensuring that resources are allocated thoughtfully to meet study needs. Targeted protein degradation drug discovery programs require assays to monitor protein degradation across multiple species as well as various cell lines and tissue matrices. However, antibody reagents for ligand-binding assays such as ELISA and MSD may not be readily available and sometimes can be very difficult to develop. To address this need, a fit-for-purpose LC-MS/MS targeted protein quantification assay was rapidly developed and subsequently improved as the program progressed. The analytical innovation included the development of a two-dimensional LC-MS/MS configuration with a novel “trap-and-elute” design to improve sensitivity by enhancing analyte separation from background matrices while maintaining assay robustness at a standard analytical flow rate. The assay was also successfully bridged with a flow cytometry assay for a matrix sample type in which an antibody reagent was available. The assay was readily adapted for multiple sample types, including different cell lines and preclinical species, as program needs evolved. These assays supported the program by enabling rapid decision-making and accelerating progress for discovery-stage compounds.

7

From an Overlooked Innovation to a High Value Technology: The History and Revival of Slalom Chromatography to Support DNA/RNA Separation in Cell and Gene Therapy

Fabrice Gritti, Waters Corporation, 34 Maple St., Milford, MA 01757, Kevin Wyndham, Jamuna Vaishnav, Bala Addepalli, Matthew Lauber

Slalom chromatography (SC) is a nucleic acid separation technique originally reported in 1988 but largely abandoned due to limitations in instrumentation, column technology, and practical applications. Recent advances in bio inert UHPLC systems and column design have enabled the revitalization of SC as a fast, robust, and scalable analytical tool for the characterization of large DNA and RNA species ranging from 3 to 30 kbp. This presentation describes the development of a new SC column, clarifies its underlying separation mechanism, and demonstrates its direct relevance to plasmid DNA and mRNA manufacturing workflows. We demonstrate excellent retention reproducibility and column efficiency and introduce a practical method development framework spanning flow rate, buffer composition, and temperature to support routine implementation in regulated environments. SC performance is illustrated through high value bioanalytical use cases, including plasmid topology assessment (supercoiled versus open circular species), plasmid linearization efficiency monitoring, dsRNA impurity profiling in IVT mRNA processes, DNA restriction mapping, and preparation of large DNA amplicons for next generation sequencing. Comparative results versus agarose gel electrophoresis highlight substantial gains in speed, resolution, sensitivity, reproducibility, and accuracy. In addition, we will show that operating the SC column under vacuum (10^{-5} mPa) improves separation efficiency by 25%. Overall, this work positions SC as a rapid, high resolution, and automation-ready alternative to gel-based methods, enabling more efficient, quantitative, and scalable analysis of large nucleic acids in biopharmaceutical R&D, process development, and quality control.

8

Accelerating In-Vitro Permeability Assays: From High-Throughput Biology to High-Throughput Analytics

Wenying Jian, Johnson & Johnson, 1400 McKean Rd., Spring House, PA 19477

In vitro permeability assays are a cornerstone of drug discovery, providing insightful information on compound absorption, transporter interactions, and bioavailability. As discovery programs demand larger screening campaigns and faster decision-making, traditional permeability workflows can become limited by assay throughput, bioanalytical capacity, and data-processing efficiency. This presentation will discuss strategies to accelerate permeability assessment across the entire workflow. Different permeability models will be introduced and highlighted for how each provides information on passive diffusion, transporter-mediated transport, and cellular exposure. Advances in assay miniaturization and automation will be presented, examining high-throughput formats, and discussing approaches to maintaining assay robustness through standardization of the experiments. The presentation will then focus on overcoming the bioanalytical bottleneck through approaches including automated MS optimization, predicted MRM, HRMS-based data acquisition, multiplexed analysis, trap-and-elute solution, and emerging ultra-high-throughput platforms such as RapidFire and Echo MS. Finally, future directions, including AI-enabled data processing, *in silico* permeability prediction, and integrated predictive workflows, will be explored. Together, these advances demonstrate how innovation in both biology and analytics can deliver faster, high-quality, decision-enabling permeability data for modern drug discovery.

9
From Peaks to Predictions: High-Throughput Mass Spec & PTM Risk Modeling for Biologics
Richard Huang, J&J Innovative Medicine, 1400 McKean Rd., Spring House, PA 19002

High-throughput mass spectrometry (HTP MS) is increasingly enabling rapid, scalable characterization of biologics, supporting early decision-making in discovery and developability assessment. Modern HTP intact protein LC/MS platforms enable sub-minute analysis times and large-scale screening capacity, allowing hundreds of molecules to be evaluated per week with fast turnaround, thereby supporting dynamic biologics pipelines. Complementing intact MS, high-throughput peptide mapping provides site-specific resolution of PTMs. Advances in automation, including liquid-handling platforms and streamlined data processing, have significantly increased throughput and reproducibility, transforming peptide mapping from a bottleneck to a scalable platform for PTM liability assessment. These datasets enable the correlation of sequence and structural features with modification hotspots, supporting predictive frameworks for stability and manufacturability. By shifting analysis from descriptive characterization to data-driven prediction, HTP MS enables early identification of liabilities, prioritization of candidates, and more informed engineering strategies, which has been served as a foundational technology for next-generation, model-enabled biologics discovery.

10
Accelerating Enzyme Assay Development using In-Silico Bayesian Optimization
Rose Yin, Merck & Co., Inc., 33 Avenue Louis Pasteur, Boston, MA 02115, Ophelia Ukaegbu, Jonathan Fine, Christopher Wang, Arthur Arcinas, Holst Halsey, Sasidhar Nirudodhi, Erik Regalado, Pankaj Aggarwal

Ensuring consistent enzyme performance is crucial in regulated drug process development. Traditional methods of assay optimization, such as one-factor-at-a-time (OFAT) and design of experiments (DoE), can be resource-intensive and prone to bias, particularly when dealing with multiple optimization objectives and a large number of parameters. The objective of this study was to use Bayesian Optimization (BO) to develop and optimize an analytical assay for a ligase enzyme used in process development. The key goals included enhancing assay robustness, enzyme range linearity, rate linearity, and productivity to ensure reliable quality control in process development and manufacturing. Using and customizing obsidian, a BO package for multi-objective optimization primarily used for Algorithmic Process Optimization (APO), we optimized six experimental parameters over two rounds with only 10 unique designs. BO rapidly identified settings that matched or exceeded OFAT-derived performance while reducing experimental workload by approximately 85% versus OFAT and 65% versus DoE. The optimized assay achieved improved enzyme linearity, rate linearity, and productivity, and an in-silico robustness analysis produced predictions closely aligned with experimental outcomes. Beyond performance gains, BO delivered interpretable insights into parameter interactions and sensitivities, informing mechanistic understanding and enabling more efficient method transfer. This work demonstrates that multi-objective BO can substantially cut time and cost in analytical assay development while enhancing reliability.

11
In-Line Raman Spectroscopy for Low-Dose Content Uniformity Monitoring
Vladimir Villanueva-Lopez, Pfizer Inc., Worldwide Research and Development, Eastern Point Rd., Groton, CT 06340, David Wilsdon

At Pfizer, Near-Infrared (NIR) spectroscopy is used for in-process API quantification via a fiber-optic probe installed within the tablet press feed frame, enabling measurement of powder blends immediately before compaction in both batch and continuous manufacturing. However, the weak overtone and combination bands measured by NIR can limit analytical performance for low-dose formulations, defined by the European Medicines Agency (EMA) as containing less than 2% w/w API. Raman spectroscopy offers a sensitive alternative due to its sharp fundamental vibrational bands and chemical specificity even at low API concentrations. To enable systematic deployment of Raman-based in-process monitoring, a two-stage implementation framework was developed. The first stage employs an in-silico feasibility assessment to predict API detectability prior to experimental method development. Pure-component Raman spectra are acquired using automated exposure optimization to achieve approximately 95% detector saturation. These spectra and acquisition parameters are then used to simulate theoretical blend spectra at the target formulation composition, enabling prediction of API signal-to-noise ratio and measurement feasibility. Following feasibility confirmation, calibration models are developed using a feed-frame simulator. Calibration materials incorporate excipient lots with varying fluorescence characteristics to represent realistic raw-material variability. Raman spectra of moving blends are collected using short integration times with long exposure averaging. This approach produced robust quantitative models across a range of fluorescence conditions, demonstrating a risk-based strategy for evaluating Raman feasibility and supporting future GMP implementation of process analytical technology for low-dose drug products.

12
In Silico and Modeling Approaches for Understanding Synthetic Molecule Pharmaceutical Stability
Jessica Hoskins, AbbVie, 1 N Waukegan Rd., North Chicago, IL 60064

Understanding of chemical degradation pathways of active pharmaceutical ingredients (APIs) is key to assessing development and stability risks associated with new pharmaceutical drug substances and drug products. Accelerated Predictive Stability (APS) approaches can be applied early in development to characterize degradation pathways, assess critical quality attributes, estimate potential shelf life, and enhance overall scientific understanding of a program. By integrating *in silico* tools, experimental data, and modeling approaches, stability knowledge can be generated in a relatively short timeframe compared with the time required to obtain long-term stability results. These insights can inform key development decisions, including excipient selection, packaging configuration, and storage conditions. This presentation will highlight case studies demonstrating the application of these approaches to synthetic molecules, including traditional small molecules and synthetic peptides.

13
Detecting, Identifying and Counting Sub-visible Particles in Heterogeneous Mixtures with Total Holographic Characterization
Laura Philips, Spheryx, Inc., 330 E 38th St., #48J, New York, NY 10016, Juliana Lumer, Valentina Flores-Montes, David Ruffner

Total Holographic Characterization (THC) is a robust and reliable method of detecting contaminants in complex biologic formulations over the size range of 500 nm – 10 um. This sub-10 um regime is important because issues affecting product stability and safety often begin with the formation of particles in this size range. Early detection at these scales enables intervention before visible or clinically significant problems emerge. THC differs from other particle-imaging techniques because it is based on quantitative analysis of holographic microscopy data. Holograms inherently contain more information about particles than direct microscopic images. By fitting recorded holograms using multiparameter optimization based on Lorenz-Mie theory, THC directly measures particle size and refractive index. Because the refractive index is determined by particle composition, this approach reliably identifies particle type as well as accurate concentrations of each species present, even when they are the same size and shape. For example, silicone oil has a characteristic refractive index of 1.41, and consists of spherical particles. Other emulsion droplets will have the identical particle shape – spheres – but can be distinguished by their different refractive indexes (e.g. oleic acid at n=1.46). In contrast, protein aggregates exhibit a broader and more variable refractive index because they are heterogeneous mixtures of protein and solvent. Silicone oil can mask the presence of more dangerous contaminants and can promote the formation of protein aggregates, both of which pose risks to product safety and efficacy. THC detects, distinguishes and quantifies protein aggregates even in the presences of silicone oil.

14
Characterizing Biopharmaceutical Particles in a High-Throughput Unlabeled Fashion with AI-Based Image Analysis
Christopher Calderon, University of Colorado, 3609 Osceola St., Boulder, CO 80212

Subvisible particles in biopharmaceuticals have historically been characterized using devices that aim to measure count and particle size distribution present in a sample. Recent advances in imaging and AI/ML-driven image analysis have made it possible to leverage particle “morphology” as an additional feature which can be used as a quantitative tool to aid in the analysis of a wide variety of applications ranging from formulation development to quality control. This talk will introduce supervised and unsupervised AI/ML techniques which have proven useful in analyzing biologic products ranging from protein therapeutics to cell therapies. In the former, case studies demonstrating the benefits of utilizing morphology to characterize and understand the sources of protein aggregation will be discussed; in the latter case, the cells are the “product”, and we discuss how non-invasive image based analysis systems have the potential to improve the quality and reduce the manufacturing cost of cell therapy products.

15
No abstract submitted by the author.

16
Particle Reference Material Development and Characterization
Srivalli Telikepalli, National Institute of Standards and Technology, Bioprocess Measurements Group, 100 Bureau Dr., MS 6457, Gaithersburg, MD 20899

Reference materials play a critical role in ensuring the reliability of particle measurements by supporting instrument qualification, method validation, calibration, quality control, analyst training, standardized reporting, and measurement uncertainty estimation. This presentation highlights NIST’s development of two unique particle reference materials designed to improve the detection, characterization, and monitoring of particles in biotherapeutic products. The presentation will briefly discuss the significance of subvisible and visible particles in biotherapeutics before focusing on the development, characterization, and applications of Reference Material (RM) 8634 and Standard Reference Material (SRM) 1989. RM 8634 consists of irregular, translucent ethylene tetrafluoroethylene (ETFE) particles that closely mimic proteinaceous particles in morphology and optical properties. It provides reference values for particle size distribution (1–30 μm) and particle morphology, established through multiple orthogonal measurement techniques. SRM 1989 is a photolithographically produced particle standard that provides a certified particle size value for an irregularly shaped visible particle and is intended for instrument qualification and training of visual inspection analysts. Together, these reference materials provide the biopharmaceutical industry with standardized tools for evaluating particle measurement performance, improving comparability across analytical methods and laboratories, and supporting the development of more robust particle characterization strategies. As the first reference materials of their kind, RM 8634 and SRM 1989 are expected to facilitate greater industry collaboration and advance measurement science for particulate matter in biotherapeutics.

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Vibrational Spectroscopy for Questioned Document Analysis
Marisia Fikiet, University of New Haven, 300 Boston Post Rd Dodds 403, Southington, CT 06516

The forensic discipline of questioned document (QD) examination includes many sub-disciplines like Ink and paper analysis. Often the first step of analysis is characterization and classification of the materials used with priority given to techniques that are non-destructive due to the samples being irreplaceable evidentiary materials. Therefore, techniques like vibrational spectroscopy are key. Raman and ATR-FT-IR analysis can be essential due to the amount and quality of information. This is not a novel realization, but with writing technology changing through the years new types of samples are continuously becoming available. Gel-pen ink which uses pigments instead of dyes and which has only been in use in the US since the 1980’s has not been studied as extensively. Additionally, mediums colored pencils which are not often encountered in QD analysis have not been extensively studied either. This research aims to close gaps and address newer and uncommon QD relevant materials.

18
A Universal Approach to the Forensic Identification of Biological Stains based on Raman Spectroscopy: Recent Advancements Toward Commercialization
Igor Lednev, University at Albany-SUNY, 1400 Washington Ave., Albany, NY 12222

This presentation will provide an overview of a 20-year research effort that led to the development of the first universal Raman spectroscopic method for the nondestructive, rapid, and confirmatory identification of all major body fluids encountered in forensic investigations, including blood, semen, saliva, vaginal fluid, sweat, and urine. The methodology combines Raman microspectroscopy with machine learning algorithms to achieve automated analysis and classification while preserving biological evidence for subsequent DNA testing. Extensive validation studies have demonstrated the robustness of the approach for environmentally challenged samples, body fluid mixtures, stains deposited on common substrates, and trace amounts of evidence. The sensitivity of the method enables the identification of blood from a single red blood cell and supports the analysis of highly contaminated crime-scene samples. Beyond body fluid identification, the developed platform provides information-rich forensic intelligence. Research from our laboratory has demonstrated the ability to differentiate human and animal blood, distinguish peripheral and menstrual blood, estimate the time since deposition of bloodstains over extended periods, and perform donor phenotypic profiling. Using Raman spectroscopy and advanced statistical analysis, donor characteristics including sex, race, and age can be predicted directly from biological traces. These capabilities have the potential to transform body-fluid evidence from a simple source of DNA into an independent source of investigative leads. The presentation will conclude with a discussion of recent efforts to commercialize this patented technology through *SupreMEtric LLC* and the prospects for deploying portable Raman-based systems for routine forensic use at crime scenes and in forensic laboratories.

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Rapid Authenticity Screening of Suspect Pharmaceuticals Using Portable NIR Technology Coupled with Rapid Cloud-Based Data Analysis
Lyndsay Kissell, United States Food & Drug Administration, National Forensic Chemistry Center, 6751 Steger Drive, Cincinnati, OH 45237, Hervé Rais, Olivier Delémont, Pierre Esseiva

Pharmaceutical counterfeiting is global public health issue. In particular, counterfeit tablets that resemble authentic medicines continue to be identified in the United States. Some of these counterfeits have been found to contain harmful drugs or incorrect active pharmaceutical ingredients (APIs), including fentanyl. To combat this problem, investigators and field scientists require rapid and accurate methods for screening tablets in the field. In this work, an application of portable Near-infrared (NIR) spectroscopy to develop cloud-based authenticity models and screen suspect tablets that are visually consistent with alprazolam or oxycodone-containing pharmaceuticals is presented. The use of NIR spectroscopy, combined with a cloud-based model for comparison of measurements to those of authentic medicines, returned results that were 100% consistent with traditional lab-based testing. These results demonstrate a promising application of rapid portable NIR measurements as first-line screening tools for large seizures of suspect pharmaceuticals.

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Seeing Over the Peaks: Contributions of Microscopy to Vibrational Spectroscopy
Christopher S. Palenik, Microtrace, 790 Fletcher Dr., Elgin, IL 60123

Infrared and Raman spectroscopy are two of the most commonly applied spectroscopic methods in practical problem solving, often within the context of forensic investigations, whether conducted in crime laboratories or in related areas of industrial problem solving. With the ease and accessibility of accessories such as ATR, a great deal of analytical work is performed without ever critically observing the sample. This talk aims to illustrate the many advantages that microscopic examination can offer over an otherwise blind analysis. Among other benefits, (a) microscopy can aid in evaluating a sample prior to infrared spectroscopic analysis, helping to assess its homogeneity or heterogeneity; (b) stereomicroscopy can be used to separate components of a physical mixture on a microscopic scale, effectively improving the detection limits of vibrational spectroscopy; and (c) characteristics observed during microscopic examination can provide critical contextual information that strengthens spectral interpretation. For example, microscopic examination can reveal features of a cellulose-containing material that may be difficult to classify more specifically by infrared spectroscopy alone. Such observations can help distinguish among wood, other plant tissues, rayon, cotton, and various forms of microcrystalline cellulose. Microscopy, in conjunction with spectroscopy, can also be used to handle, prepare, and target particles far smaller than those readily accessible by spectroscopy alone. This talk will demonstrate these concepts through practical examples drawn from forensic science, art, and industry.

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SFC of Short Chain PFAS
Luis Colón, University at Buffalo, Dpt. of Chemistry, 120 Founders Promenade, Buffalo, NY 14260, Karla Rios-Bonilla, Dulan Dulan H. Edirisinghe, Damalka Balasuriya, Raúl Díaz-Santiago

One of the most persistent challenges in analyzing ultrashort- (C1–C3) and short-chain (C4–C6) PFAS is their poor chromatographic retention when using reverse-phase (RP) HPLC). Traditional RP columns provide insufficient retention for these compounds; trifluoroacetic acid and perfluoropropionic acid are two examples. These compounds often elute near the void volume, resulting in weak retention, poor separation, and low sensitivity. Despite these limitations, a substantial proportion of studies still rely on RP-HPLC, even though it frequently fails to retain key analytes adequately. Hydrophilic interaction liquid chromatography (HILIC) and mixed-mode anion-exchange (AEX) chromatography are potential alternatives, but their use remains inconsistent and lacks standardization across laboratories. To address these challenges, we are investigating supercritical fluid chromatography (SFC) as an alternative approach for ultrashort- and short-chain PFAS analysis. We have evaluated commercially available chromatographic columns for the separation of these compounds using SFC and synthesized new stationary phases via click-chemistry modification of organo-silica

particles containing reactive azide groups. In this presentation, we describe our findings that led to the selection of an amide-based column for ultrashort- and short-chain PFAS analysis by SFC and highlight our efforts to synthesize azide–organo-silica hybrid adsorbents to anchor stationary phases via “click chemistry” for both dispersive SPE (d-SPE) and SFC, with an emphasis on PFAS analysis.

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Using Comprehensive Separations to Inform Scent Detection Canine Training for Detecting American Foulbrood Disease in Honey Bees

Breanna E. Christensen, William & Mary, Nontargeted Separations Laboratory, Chemistry Department, 540 Landrum Dr., Integrated Science Center 1053, Williamsburg, VA 23185, Meghan O. Milbrath, Peter D. Fowler, Katelynn A. Perrault Uptmor

Bee health is often assessed through disease outcomes, as climatic and nutritional stressors can increase bees' susceptibility to disease. American foulbrood (AFB) is one serious disease targeting *Apis mellifera* honey bees that are depended on as livestock and pollinators. Because the *Paenibacillus larvae* bacterial spores causing brood infection are very persistent, early identification is necessary to prevent spread to other hives. Diagnosis is generally done through visual inspection that is disruptive to bees, requiring the removal of frames from hives. In states where AFB is reportable, identification requires euthanasia of the colony and destruction of the entire hive. Even when there is no destruction order, best practice for infection involves burning all frames, a costly process. Such devastation has inspired an expedited detection process for AFB using scent detection dogs. Dogs would allow beekeepers to quickly screen hives, identifying infected colonies before the disease has spread, and minimizing the equipment and livestock that would be destroyed. To understand the odors causing the dogs to alert for AFB, a volatile profile of infected hive material was produced with a nontargeted analysis using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. This profile describes the volatile compounds specific to *P. larvae* compared with healthy samples. Furthermore, the success of a proposed treatment method using gamma-irradiation was evaluated by inspecting whether the volatile organic compounds associated with *P. larvae* were eradicated in treated samples. This research used solid-phase microextraction arrow, quad-jet dual-stage cryogenic modulation, and analysis with alignment software to characterize AFB-associated volatiles.

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Revisiting Peak Capacity Production at Ultra-Short Analysis Time as a Lever to Improve the Speed and Peak Capacity of 2D-LC Separations

Dwight Stoll, Gustavus Adolphus College, 800 West College Ave., Saint Peter, MN 56082, John Biebighauser, Callum Harmes, Tina Dahlseid, Konstantin Shoykhet

Over the past two-and-a-half decades numerous strides have been made in both the theory and practice of multi-dimensional liquid phase separations. Very powerful and robust commercial instrumentation has been introduced that enables a wider range of users to effectively deploy the technology on a routine basis for analysis of both complex and difficult-to-resolve samples in fields ranging from polymer characterization to analysis of foods and beverages. However, in spite of these advances, and theoretical predictions that suggest 2D separations should be superior to 1D ones for analyses longer than about 5 minutes, in practice most comprehensive 2D-LC separations are still more than 1 hour long. In this presentation we will discuss our recent efforts to fill this gap between 5 minutes and 1 hour by re-visiting the performance of ultra-fast second dimension separations that are needed in this space. Under conditions where first dimension peaks are severely undersampled, theory predicts that 2D-LC peak capacity is directly proportional to the productivity of the second dimension separation – i.e., peak capacity per unit of cycle time ($^2n_c/^2t_g$). Currently we are leveraging a confluence of improvements in instrument and column technology to reassess the productivity of second dimension separations in the range of 3 to 30 seconds. We will show that highly productive separations can be obtained at times much shorter than 20 seconds, where the previous optima were observed by Carr and coworkers, and discuss how these new capabilities could be leveraged in the context of actual 2D-LC separations

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Multidimensional Chromatography for Chemical Analysis – from Small Molecules to Synthetic Polymers

Peilin Yang, Kingsley (Hengyu) Lin, Dow Chemical, 230 Abner Jackson Pkwy, Lake Jackson, TX 77566

As society increasingly demands materials with safer and more sustainable profiles, the detailed characterization of complex chemical mixtures becomes crucial in new product development, material life-cycle analysis, and evaluating their environmental impact. Multidimensional chromatography has proven to be a highly effective technique for chemical analysis. When coupled with universal and information-rich detectors such as high-resolution mass spectrometers, it provides more comprehensive chemical composition information, leading to new insights in material design and process development. This presentation will discuss several industrial applications that demonstrate how practical solutions utilizing GC×GC and LC×LC can address challenges in the analysis of recycled materials and complex polymer mixtures, spanning a wide range of molecular weights from small molecules to macromolecules.

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Understanding Neuronal Excitability Using Mass Spectrometry Imaging of SCN2A-Deficient Organoids

Alyssa Moore, Purdue University, 60 Oval Dr., West Lafayette, IN 47906, Xiaoling Chen, Yang Yang, Julia Laskin

Human induced pluripotent stem cell (hiPSC)-derived organoids are powerful models for studying disease states without the use of animal models and can be grown to mimic different biological systems. In this study, we use nano-DESI MSI to understand alterations in molecular signaling in *SCN2A*-deficient iPSC-derived organoids developed as models of neuronal excitability in neurodevelopmental disorders. We used nano-DESI MSI to examine molecular differences between wild type (WT) and *SCN2A*$^{+/-}$ (HET) iPSC-derived cortical organoids. Imaging experiments were performed on a Q-Exactive HF-X Orbitrap mass spectrometer in

both positive and negative ionization modes. Lipid alterations were determined through statistical analysis. Through comparison between WT and HET cortical organoids we identified multiple lipids that were altered in the *SCN2A*-deficient systems. In the fresh frozen HET cortical organoids multiple PS lipids and gangliosides were downregulated when compared to WT organoids. These trends may be due to hindered neuronal development in the HET organoids caused by *SCN2A* deficiency. This will be confirmed with immunofluorescence imaging of the different neuronal layers. Distinct spatial localizations of lipids were also observed within the organoids which indicate location-dependent cell differentiation. Our results highlight the capabilities of nano-DESI for imaging iPSC-derived organoids of different disease states, providing insights into cell differentiation within organoids.

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Investigation of Radical-Driven Peptide Fragmentation in the Solution-Cathode Glow Discharge Ion Source for Mass Spectrometry

Jared Viggers, Rensselaer Polytechnic Institute, 110 Eighth St, Troy, NY 12180, Jacob Shelley, Josefin Hufgard

The analysis of peptides and proteins are essential in many fields such as proteomics, for understanding molecular behavior and determining sequence information. Typically, these biomolecules are ionized through electrospray ionization, while sequence information is obtained through fragmentation within the instrument by selectively depositing energy to the ions through e.g., collisions. However, collision-induced dissociation predominantly forms only b- and y-type fragment ions. Meanwhile, a-, x-, c-, and z-ions can be formed through photon- or electron-driven dissociation. However, we have shown that all these peptide-fragment types can be produced within an atmospheric-pressure solution-cathode glow discharge (SCGD) ionization source, leading to enhanced primary sequence coverage. However, the mechanisms that lead to peptide fragmentation within the SCGD are not well understood. Here, we investigate the role of plasma-produced radical in fragmentation along the peptide backbone to assess how different fragment-ion types are formed. Radical-induced dissociation (RID) within the plasma was studied through the addition of radical modulators into the sample solutions to alter the radical density of the plasma environment. The abundance of a-, x-, c-, and z-type fragments decreased substantially with the addition of radical scavengers to the sample solution, whereas b- and y-type ions were only minimally impacted. Conversely, addition of radical promoters, like hydrogen peroxide, led to an increase in a-, x-, c-, and z-type fragment ion signals. The use of optical emission spectroscopy to determine the specific radicals impacted by these modulators will also be discussed. This study helps clarify the role of plasma-generated radical species in SCGD peptide dissociation.

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3D Confocal Raman Imaging of PFOA Bioaccumulation in Trehalose-Immobilized DOPC Giant Unilamellar Vesicles

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This study investigates the bioaccumulation of perfluorooctanoic acid (PFOA) within model cellular membranes using confocal Raman microscopy. Giant unilamellar vesicles (GUVs) composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were utilized to simulate biological boundaries and evaluate the partitioning behavior of perfluorinated compounds. To ensure structural stability during high-resolution scanning, the liposomes were immobilized in a trehalose matrix to prevent laser and vibration induced movement disrupting the scan. Using a Jasco NRS-4500 confocal Raman spectrometer, we performed 3D layer-by-layer imaging to map the volumetric distribution of PFOA. GUVs ranging in size from 1-20 µm were created via electroformation with stainless steel electrodes. The PFOA concentration was maintained below the critical micelle concentration to ensure that the detected signals originated from molecular partitioning rather than the formation of independent free-floating PFOA micelles. Spectroscopic mapping reveals a significant localization of PFOA within both the DOPC lipid bilayer and the interior of the GUVs. The results demonstrate that PFOA effectively permeates into the DOPC membranes at sub-micellar concentrations. This distribution pattern suggests a strong affinity for the lipid environment and underscores the compound's ability to infiltrate encapsulated biological compartments. By providing direct spectroscopic evidence of PFOA localization, this research highlights the efficacy of confocal Raman imaging for the label-free study of persistent organic pollutants and their bioaccumulation pathways at the membrane level.

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Quantification of Argireline in Commercial Skin Aging Serums by MALDI MS^2 Approach Using Digital Ion Trap

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Argireline, Arg is a bioactive peptide derived from N-terminus SNAP (Synaptosome-associated 25 kDa protein) that has shown to reduce fine lines and wrinkling. LC-MS/MS has been the preferred method of choice for Arg quantification due to its sensitivity and specificity. We introduce Matrix Assisted Laser Desorption Ionization, MALDI for Arg quantification against its Arg ($^{13}C_6$,$^{15}N_4$) isotopomer in a cosmetic serum. Quantification in MALDI is performed in MS^2 mode without prior purification or extraction. The method shows good linearity ($R^2 > 0.99$) and reproducibility, good recovery, low limit of detection < 10 fmol/µL and negligible matrix effect in a blank serum. Method cross validation with LC-MS demonstrate that both MALDI and LC-MS show similar Arg levels in serum and therefore our method could pave the way for quick, sensitive, cost-effective and accurate analysis of Arg for quantification and quality control purposes.

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Systemic Overview and Validation of Fibrinogen Alpha as a Biomarker for Early-Stage Breast Cancer Analysis

Pathea Bruno, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Sarah Abbott, Shaelee Torres, Costel Darie

Breast cancer (BC) is one of the top causes of death for women. Early stages of breast cancer may cause dysregulation of proteins and peptides, which can be exploited as biomarkers for early identification, particularly in younger women. Peptidomics is the study of a sample's peptidome, which typically consists of polypeptides with molecular weights of less than 20 kDa. Mass spectrometry is

useful in the research of peptidomics due to its high sensitivity and capacity to detect low-abundant and smaller peptides. Particularly, Matrix assisted Laser Desorption Ionization (MALDI-MS) is vital for evaluation of the serum peptidome. In this investigation, serum samples from 100 serum samples, 50 from invasive ductal carcinoma (IDC) BC and 50 from control groups, were analyzed. The majority of the tumor types were a maximum of 50mm in dimension, present in a maximum of 3 axillary lymph nodes with no metastasis. Samples were previously fractionated using filters with molecular cut-offs of 30 kDa, 10 kDa, and 3 kDa to provide 3 sets of serum for analysis. Here, we present an overview of the 3 sets using MALDI-MS as a discovery and validation of the presence of thrombin-derived fibrinogen alpha (FGA) in the serum peptidome, where higher spectral counts were identified in samples with lymph node involvement. FGA has shown involvement in preventing metastasis, where its presence causes cancer cell apoptosis. Further analysis can allow for this peptide to be used as a progression marker for BC and a possible treatment option for BC.

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Why Establishing a Robust Method Operable Design Region (MODR) is Superior to a Single-Method Solution

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

In the enhanced approach to AQbD method development, the experimental region is a multidimensional region consisting of the combined ranges of the instrument and chemistry parameters included in the study. A robust MODR is a subset region within the full experimental region in which all methods repeatably perform to requirements for all critical method performance characteristics. A fully characterized MODR also establishes the subset region or regions containing the methods which fail to perform consistently to requirements and the demarcating boundaries, or edges of failure, between these two sub-regions. This presentation will describe five critical advantages of establishing a robust MODR listed below which are not available from a single-method solution: (1) identifies the optimal method for both a mean (average) performance and method repeatability (robustness), (2) Defines the ICH Established Conditions – the robust operating ranges for all included critical method parameters (CMPs), (3) specifies the two critical elements of the preliminary Analytical Control Strategy to transfer to APLM Stages 2 and 3, (4) minimizes the serious risk associated with the traditional validation approach to method robustness studies, and (5) supports successful method transfer and fast re-capture of method performance with minimal performance re-verification.

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Addressing Method Transfer and Robustness Challenges Using Design Space Modeling

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In high-performance liquid chromatography (HPLC), robust and easily transferable analytical methods are essential to ensure consistent performance throughout the entire lifecycle of an analytical procedure, including unforeseen future applications. Nevertheless, robustness–, and ruggedness–related issues are frequently reported in industrial practice, particularly during method transfer between laboratories. These challenges often arise from incomplete method understanding and insufficient consideration of HPLC system-related effects, such as dwell volume, extra-column contributions and other, at first less evident, instrument-to-instrument variations in solvent delivery, mixing behavior and column thermal control. In this context, modeling approaches aligned with Analytical Quality by Design (AQbD) principles provide valuable support for both proactive method development and reactive troubleshooting. In the current work, we used DryLab modeling Design Spaces to create digitized modeling fingerprints of various instruments, enabling extensive *in silico* investigations, including in-depth assessment of method transferability and evaluation of subtle, instrument-dependent robustness characteristics.

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Optimizing LC Method Development Through Intelligent Column Selection and Implementation of a Structured Screening Protocol

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

LC method development activities are often performed on an ad hoc basis and heavily rely on the expertise of the analyst when it comes to the approach used. While acceptable, this process is not ideal as it may require experts to perform routine method development tasks. Utilizing a structured protocol for method development allows less experienced analysts to perform these routine tasks, freeing up the experts to handle the more complex samples that require special attention or optimization. Using a structured protocol is a good first step, but it must be coupled with appropriate column selection to maximize its effectiveness. Intelligently selecting columns to screen for method development requires an understanding of the chromatographic characteristics of different stationary phases in order to choose columns covering a range of selectivities. By choosing the columns based on stationary phase selectivity as well as information about the analytes, where available, method development activities have a higher chance of success. In this presentation, a quick guide to column selection will be shown along with an example of employing a structured method development protocol for the analysis of a mixture of twelve steroids containing three pairs of isobars. The resulting method provides baseline separation of all twelve analytes with a 5-minute run time.

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Silencing RNAs, Guide RNAs, Messenger RNAs and Beyond. A Guide to Tackling Ion Pair/Reversed Phase LCMS Method Development

Joshua McBee, Advanced Materials Technology, 3521 Silverside Rd., Ste. 1-K, Quillen, Wilmington, DE 19810, Peter Pellegrinelli, Barry Boyes

Approaching ion pair/reversed phase LCMS methodology can be a daunting task due to the added complexity of the method development, and the many variables available for modification. Frequently, finding a suitable method for each oligonucleotide requires optimization of multiple parameters including ion pair reagent, counterion, pH, column temperature, silica pore size, and

more. This presentation provides an introduction to IP/RP oligonucleotide separations, the underlying chemistry principles, options for selecting reagents, preparation of mobile phases buffers and HPLC column choice. We will show examples of how various method parameters have an impact on the separation and LC/MS results using our HALO OLIGO C18 columns in the 120 and 1000 angstrom pore sizes. Finally we will discuss how to optimize your separation using mass spectrometry as a detection system and how to minimize complications such as cation adduction when performing your analysis. The presentation aims to assist those who are either new to LCMS or are approaching oligo analysis for the first time.

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Improved HPLC Method Development Using Wide pH Range

Conner McHale, Advanced Materials Technology, 3521 Silverside Rd., Quillen Bldg., Ste. 1K, Wilmington, DE 19810

Many chromatographers are familiar with the difficulties of separating basic compounds on silica-based columns due to the increased peak broadening that may occur when operating at neutral or low pH. There are several ways to improve the peak shape of basic compounds such as increasing the ionic strength of the mobile phase (adding a salt/buffer), use of an ion pair agent, increasing the pH of the mobile phase, using a non-silica-based column, or even using an alternative stationary phase such as a charged surface material. The HALO® ELEVATE column line (C8, C18, Phenyl-Hexyl) is developed to create a silica-based support that is stable under alkaline conditions to obtain improved peak shape, loading capacity and retention for basic analytes when using high pH mobile phase conditions. HALO® ELEVATE incorporates a wide pH compatibility from 2 to 12 which allows not only chromatographic separations to be performed under high pH conditions, but also at low pH. This versatility allows for easier method development along with achieving selectivity differences that may benefit the separation. Whether the goal is to separate acids, bases, or neutrals, HALO® ELEVATE enables users to achieve excellent peak shape and column stability under a wide pH range due to its alkaline resistant properties. Demonstrations of how adjusting the pH of the mobile phase to modify retention, improve peak shape, and change selectivity will be demonstrated along with showing the advantages of superficially porous particle sizes.

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Are Your LC Method and Associated Sample Preparation Method Ready for Transfer?

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

The Analytical Target Profile (ATP) defines the precision and accuracy requirements of the final method for each Critical Quality Attribute (CQA) at a specified probability and level of confidence. For any given drug substance or drug product, the Upper Acceptance Limit minus the Lower Acceptance Limit (UAL – LAL) equals the Total Acceptance Width (TAW). However, not all of the TAW is available to the analytical procedure. Some of this width must accommodate production output variation – the variation about the output target due to the combination of output bias, within-batch variation, and between-batch variation. In addition, a measurement result at an acceptance limit has an equal likelihood of being either a passing or failing result, and therefore has an unacceptable level of decision risk. This presentation connects the dots to clarify the relationships between the five critical elements which drive method performance requirements: (1) the Analytical Target Profile (ATP), (2) the Total Acceptance Width (TAW), (3) the Production Tolerance Width (PTW), (4) the Total Analytical Error (TAE), and (5) the Reportable Values. It will also describe how implementing simple guard bands can dramatically reduce the risk of Type I and Type II errors associated with batch pass/fail decisions.

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Fabric Differentiation Using Reflectance Spectroscopy and Multivariate Statistical Analysis

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

Textile waste represents a major environmental challenge, and improved recycling strategies are essential for developing a more sustainable and circular textile economy. Because many recycling approaches are specific to particular fiber compositions, accurate identification of textile materials is a critical first step. In this project, textile fiber composition was investigated using fiber optics reflectance spectroscopy (FORS) combined with chemometric analysis. A dataset containing eight textile fiber types (wool, linen, cotton, polyester, nylon, polyamide, acrylic, and rayon) was collected from multiple sources and analyzed using reflectance spectroscopy. Spectral preprocessing was evaluated to improve class discrimination, with first-derivative preprocessing providing enhanced separation between textile classes by emphasizing subtle spectral differences and reducing baseline variation. Processed spectra were subsequently analyzed using Partial Least Squares Discriminant Analysis (PLS-DA). Preliminary results demonstrated reliable separation of broad categories such as natural and synthetic fibers. However, chemically related cellulose-based materials, such as cotton and linen, remained difficult to distinguish due to their similar compositions. Overall, the model achieved good classification performance, with a Cohen's Kappa of 0.7. Importantly, the use of an interpretable chemometric model such as PLS-DA enabled the identification of chemically meaningful spectral features associated with specific fiber classes, including bands related to protein-containing fibers and cellulose-based materials. These results highlight the potential of portable reflectance spectroscopy and chemometric analysis as a rapid, non-destructive, and high-throughput approach for textile identification and classification.

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Multivariate Authentication of Lavender, Peppermint, and Helichrysum Essential Oils Using Chemometrically Enhanced Spider Plot Analysis

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Conventional quality control of essential oils often evaluates individual constituents against predefined specification ranges. While this approach can identify major compositional abnormalities, it may overlook subtle adulteration, botanical substitution, processing effects, or coordinated changes among several compounds. Because essential oil composition is inherently multivariate, authenticity is often better understood through the relationships among constituents rather than through isolated values alone.

This poster presents a chemometrically supported spider plot approach for evaluating essential oil composition, using lavender, peppermint, and helichrysum essential oils as representative examples. Samples are analyzed by gas chromatography with mass spectrometric and flame ionization detection. Selected major constituents, minor constituents, marker compounds, and diagnostically useful ratios are normalized against authenticated reference datasets and displayed as multidimensional chemical fingerprints. Chemometric tools, including principal component analysis, hierarchical clustering, standardized deviation analysis, and multivariate distance measurements, are used to support interpretation of the spider plots. Lavender illustrates cultivar, geographic, and lavender versus lavandin variation. Peppermint demonstrates the importance of biosynthetically related constituent patterns and the recognition of compositional imbalance caused by substitution or isolated compound addition. Helichrysum highlights the challenges of natural variability, chemotype differentiation, low abundance markers, and adulteration. Across these examples, spider plots provide an immediate visual summary of complex chromatographic data and reveal abnormal relationships that may not be apparent through conventional single component assessment. When combined with validated chemometric models and authenticated reference populations, this approach offers a more comprehensive framework for evaluating essential oil identity, authenticity, and compositional integrity.

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Data Dense Dissolution Testing Based on Low Temperatures

Ishai Nir, Distek, Inc., 121 North Center Dr., North Brunswick, NJ 08902, Jeff Seely, Gary Dromgoole

Conventional dissolution testing at 37 °C provides reproducible release data under broadly physiological conditions, but it can mask meaningful differences among rapidly dissolving or highly optimized formulations. This poster discusses the option of low temperature dissolution testing, e.g. at 5 °C, as a deliberate, non-physiological stress condition designed to improve method discrimination. Lowering temperature can reduce apparent solubility and molecular diffusivity, increase medium viscosity, and alter interfacial wetting, surfactant behavior, polymer hydration, particle deagglomeration, and hydrodynamic mass transfer. These changes may expand compressed release profiles and reveal formulation, particle-size, processing, or lot-to-lot differences that are not resolved using standard conditions. Low temperature method can also demonstrate improved signal-to-noise, reproducible rank-ordering of relevant variants, and a mechanistic link to critical quality attributes or control strategy. Data demonstrating how low temperature dissolution testing and the attendant slowed dissolution rate permit the generation of a more data-dense profile which can be better suited for use with physiological pharmacokinetic modeling and correlations such as f2 will be included.

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ICP-OES Analysis of Copper Recovered from Li-Ion Batteries for Foil Manufacturing

Ana Garcia Gonzalez, Agilent Technologies, 2850 Centerville Rd., Wilmington, DE 19808

Copper is a critical element recovered during lithium battery recycling, representing 10–15% of a battery's total weight. It is reused in the production of copper foil for current collectors in new batteries. In the remanufacturing process, recycled copper is dissolved in sulfuric acid to produce a copper sulfate electrolyte, which is then electroplated into high-purity foil. This electrolyte also contains valuable metals such as lithium, nickel, and cobalt that can be further recovered. Accurate elemental analysis of this solution is essential for quality control and further recovery of valuable metals. In this study, an Agilent 5800 ICP-OES, coupled with the Advanced Dilution System 2 (ADS 2), was used to quantify 23 elements in three copper electrolyte samples. Copper was the dominant element, measured at ~1.4% in two samples and nearly 2% in the third. Other major elements included Ca, Fe, Na, and Ni in the hundreds of ppm, with additional elements such as Co, Mg, Si, and Zn present at lower concentrations. The method demonstrated excellent matrix tolerance and long-term stability, despite the challenges of analyzing dense, corrosive sulfuric acid solutions. This workflow provides reliable, high-throughput elemental analysis to support quality control and material recovery in copper foil remanufacturing for battery recycling.

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USP 467 on PerkinElmer Clarus® Nova GC HS FID

Thomas Mancuso, PerkinElmerAES, 710 Bridgeport Ave, Shelton, CT 06484, Miles Snow

USP 467 is for testing the testing of organic residual solvents which are commonly used in the production of active pharmaceutical ingredients (API) and pharmaceutical products. The solvents are used for varying purposes including as synthesis reaction media, in the separation and purification of API during synthesis, to enhance yield, to impart desired characteristics such as crystal form and solubility, and other uses. This poster reports the results from the analysis of residual solvents according to USP 467 requirements performed with the PerkinElmer Clarus® Nova GC System and the PerkinElmer HS 2400™ Headspace Sampler, showing improved productivity and lab time optimization.

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Effect of Column Dimensions and Pressure Distribution on Low-Pressure Gas Chromatography Performance

Nazanin Keyhani, Seton Hall University, 400 South Orange Ave., South Orange, NJ 07079, Nicholas Snow

Effect of Connected Column Dimensions and Pressure Distribution on Low-Pressure Gas Chromatography PerformanceLow-pressure gas chromatography (LPGC) uses a narrow restrictor connected to a wider-bore analytical column to increase flow resistance and produce a strong pressure gradient. This study investigated how differences in column length and internal diameter affect pressure distribution, carrier gas flow, analyte transfer, retention time, and signal intensity. Conventional GC and several LPGC column configurations were compared using GC–MS and GC–FID. The results showed that most of the pressure drop occurred in the narrow, high-resistance column section. When the carrier gas entered the wider column section, the pressure and gas flow behavior changed because of gas expansion and the difference in column diameter. LPGC produced faster analyte elution and larger signals. These

studies show that LPGC performance is strongly controlled by the column dimensions and inputs into the system, as the pressure distribution, gas expansion, and effective analyte loading are all affected.

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Enhanced Risk Assessment for a Traditional LC Separation Method Using ICH Q14 Regulatory Principles and a Simplified HPLC System

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

USP <1220> and the ICH Q-series guidelines establish a lifecycle-based, science-driven framework for developing, validating, transferring, and maintaining analytical methods. The enhanced (modern) ICH Q14 approach acknowledges a risk-based, data-rich method development to provide better predictions for long-term method performance, compared with traditional (legacy) method development. This presentation describes the use of DOE based, QbD software to enhance understanding of method performance and provide method-transfer risk-assessment information when modernizing legacy separation methods.

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Mitigating the Effects of Organic Solvents in Sample Diluents for Ion Chromatography: A Comprehensive Study and Guidelines for Optimization

Mingwan Leng, MRL, 126 E Lincoln Ave., Rahway, NJ 07065, Tianyu Yuan, Frank Bernardoni, Heather Wang, Erik Regalado, Christopher Pohl

Ion chromatography (IC), the gold standard for the separation and analysis of ionic species, has been extensively used for detection of anions/cations in industrial applications. Pharmaceutical samples with limited water solubility often require the use of organic solvents as diluents, which can pose significant challenges on IC analysis. Herein, the effects of various organic solvents as sample diluents in IC are systematically investigated. Electrochemically stable solvents such as 2-propanol (IPA), acetone, and dimethyl sulfoxide (DMSO) were found to have the least impact on the IC baseline. For solvents with a greater impact, a specialized dilution device was designed to minimize their effects. The device effectively diluted the organic solvents before they reached the suppressor electrode, reducing the generation of oxidized products. The results showed that the baseline stability and separation efficiency of IC can be maintained when using a wide range of organic solvents as diluents. Guidelines are proposed to minimize the impact of organic solvents in IC sample preparation and analysis. The study also investigates the origin of impurity peaks introduced by the organic solvents and demonstrated the effectiveness of an ion-exchange resin treatment for their removal. Overall, this study provides insights for the use of organic solvents as IC diluents and offers practical solutions to overcome their challenges in pharmaceutical applications.

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Decoding ADC Spectra for DAR Measurements: Deconvolution of Drug-Linker UV/Vis Absorbance Spectra for ADC Characterization

Kevin Lance, Unchained Labs, 4747 Willow Rd., Pleasanton, CA 94588, Tine De Ryck, Tine Duthoit, Tom Boonefaes

UV/Vis absorbance spectroscopy is a fast and scalable approach for antibody-drug conjugate (ADC) quantification, allowing determination of antibody concentration, drug-linker concentration, and drug-antibody ratio (DAR). However, conventional two-wavelength UV/Vis methods require too much advanced knowledge of molar extinction coefficients to be generally applicable for early-stage or novel drug-linkers. Additional problems can get in the way of method development if drug-linker spectra change upon conjugation or in different solvent environments, and when protein and drug-linker absorbance spectra overlap significantly. Here we demonstrate Stunner's ADC Decode application, which uses full-spectrum Unmix deconvolution to extract protein and drug-linker absorbance spectra directly from ADC absorbance data — without any prior drug-linker spectral knowledge. Using three trastuzumab-derived ADCs (trastuzumab emtansine, trastuzumab deruxtecan, and trastuzumab vedotin), ADC Decode determined drug-linker spectra and DAR values with excellent reproducibility (drug CV ≤ 0.6%; DAR CV ≤ 0.5%) from 2 µL samples in under one minute per well on a microfluidic 96-well plate. Results were benchmarked against certified DAR values. Dynamic light scattering (DLS) measurements performed simultaneously confirmed aggregate-free profiles in two molecules and identified a potential aggregation signal in the third. ADC Decode’s performance was equivalent to the classic two-wavelength approach when extinction coefficients were available, while offering the critical advantage of operating without them.

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Characterization and Optimization of the Electrochemiluminescent Response of a Bipolar Electrode-Based Sensor

Kieran Cross, Middlebury College, 14 Old Chapel Rd., Middlebury, VT 05753, Kira Rahn

Relative to conventional laboratory-based methodology, point-of-care (POC) testing is often performed with inexpensive devices that are easy to operate and interpret. POC sensors have previously been used to test for diseases such as COVID-19 and HIV. Additionally, it may be possible to apply this form of sensing to detect multiple cancer biomarkers in human blood samples using microfluidic devices. Whereas conventional 3-electrode cells require multiple leads to drive an electrochemical event, bipolar electrodes (BPEs) can be multiplexed within microfluidic devices using only two driving electrodes. These devices can output colorimetric or chemiluminescent signals that are correlated to the concentration of one or more analytes. Moreover, these electrodes can be generated by efficient techniques such as screen-printing and stencil-printing. However, multiplexed analysis has not been extensively studied on stencil-printed carbon ink electrodes that use luminol and hydrogen peroxide to generate electrochemiluminescence (ECL). Here, we validate BPEs in inexpensive laser-cut microfluidic devices. By pairing the reduction of ferricyanide and the oxidation of luminol, ECL is induced on one BPE pole using linear sweep voltammetry and analyzed using a Python script. To characterize these devices, the following are assessed: the reproducibility of distinct batches of electrodes and solutions generated during the same day, the reproducibility of devices and solutions across days, and the reusability of devices

between scans. It is found that devices are reproducible across different days, while ECL intensity differs significantly across days. Additionally, we determine that the relative position of individual BPEs in an array does not impact ECL intensity.

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Photostability Assessment for Low Melting Point APIs: Decoupling Thermal and Photochemical Degradation Under ICH Q1B Conditions

Matthew Gunsch, Merck & Co., Inc., 126 East Lincoln Ave., Rahway, NJ 07065, Eryn Nelson, Ying Wang, Weidong Tong

Low melting point active pharmaceutical ingredients present unique challenges during ICH Q1B photostability testing, where radiant energy can elevate sample surface temperatures beyond the compound's thermal stability threshold. Aligned with ICH Q1B the typical approach is to include a dark control during photostability testing, however this may not be sufficient for low melting point compounds. Without appropriate controls, thermal degradation may be misattributed to photodegradation and lead to unnecessary packaging changes, manufacturing controls, and delayed development timelines. This work presents a systematic approach to designing photostability studies for low-melting-point compounds that decouple thermal and photochemical contributions based on experimental observations of a low-melting point compound under development. Thermal imaging during open-dish photostability studies revealed that sample surface temperatures reached >60°C despite chamber temperatures remaining lower, resulting in localized thermal degradation. A dark control did not experience the elevated surface temperature and degradation did not occur. Controlled-temperature studies at 25°C and 5°C markedly reduced degradation while still meeting ICH Q1B total exposure requirements. Additionally, experiments varying UV lamp power demonstrated that lower intensity over longer durations minimized localized hot spots and associated thermal degradation. These findings establish practical photostability strategies for low-melting point APIs, including active temperature control and monitoring of true sample surface temperature during ICH Q1B testing, modulation of lamp intensity to reduce radiant heating while maintaining compliant total exposure, and thermal imaging as a diagnostic tool to identify localized degradation risks. This framework enables accurate photostability assessment and prevents unnecessary risk mitigation in early development.

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Integrated Extraction and Cleanup for Rapid Multi-Class Analysis of Food Contaminants in Honey

Alicia Stell, CEM Corporation, 3100 Smith Farm Rd., Matthews, NC 28106, Brianna Warren, Maria Swasy

Food sources need to be tested for possible contaminates such as pesticides, veterinary drug residues, and mycotoxins. Each of these classes of contaminates cover a variety of different compounds. Due to the complexity and variety of food matrices, combined with the abundant number of possible contaminates, testing can be very challenging. To address these challenges, multi-class methods with the ability to quantify many analytes in a single run for different food samples are needed. Prior to analysis, however, a solvent extraction step must first be completed. Having an efficient solvent extraction method that is multi-class is just as important as the analysis. In this study, a fully automated process was tested using pressurized fluid extraction with in-cell cleanup to extract key pesticides, veterinary drug residues and mycotoxins in a single run. Honey was chosen as a relevant matrix for these contaminates and spiked with pesticides, veterinary drug residues, and mycotoxins covering different chemistries. The extraction, cleanup, and filtration of honey was achieved in under fifteen minutes, demonstrating automated preparation, which was followed directly by LC-MS/MS analysis. Good recoveries and reproducibility for the chosen pesticides, veterinary drug residues, and mycotoxins was achieved. These results suggest that this methodology enables a fast, reliable, and scalable workflow suitable for multi-class analysis.

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Charge-Transfer and SERS Coupling on TiO2 Nanoparticles

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We report the SERS enhancements of Raman forbidden surface modes of TiO_2 in different sized TiO_2 crystals. This current study utilizes the relationship between the vibronic coupling and the degree of charge-transfer to explain the differences of Surface Enhanced Raman Scattering (SERS) enhancements. Our study shows a direct correlation between the degree of charge-transfer and vibronic coupling. This relationship suggests that charge-transfer between the N-719 dye and TiO_2 due to vibronic coupling plays a fundamental role in SERS enhancements. Furthermore, this study shows a strong dependence of the enhancements of the N-719 dye molecular modes to that of the surface modes. This indicates that the mechanism that governs the enhancements of the surface modes in TiO_2 crystals most likely also dictates the enhancements of the N-719 dyes.

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Evaluation of Plasma and Serum Controls for Peptidomic Serum Analysis for Breast Cancer Biomarker Discovery

Sarah Abbott, Clarkson University, Department of Chemistry, Potsdam, NY 13699

Breast cancer (BC) is one of the top causes of death for women. Early stages of breast cancer may cause dysregulation of proteins and peptides, which can be exploited as biomarkers for early identification, particularly in younger women. Peptidomics is the study of a sample's peptidome, which typically consists of polypeptides with molecular weights of less than 20 kDa. Particularly, Matrix assisted Laser Desorption Ionization (MALDI-MS) is vital for evaluation of the serum peptidome. In this peptidomic investigation, serum samples from 48 women with breast cancer were compared to 48 age-matched control subjects. Peptides less than 3 KDa that had not previously been examined were extracted using C4 top tips supplied by Glygen, followed by MALDI-MS to identify peptides and small proteins that are dysregulated between the matched pairs. The preliminary data from this project has shown a dysregulation of the protein Fibrinogen Alpha (FGA) which is produced via proteolysis using thrombin. This protein is involved with numerous cancers and has shown to be involved in cancer cell death., the peptide is cleaved from the whole protein fibrinogen which is involved in cancer metastasis as well as naturally through the clotting cascade., The same procedure was applied to plasma and serum samples unrelated to the BC serum project to determine if it was BC specific. The plasma control is expected to show the presence of the full

fibrinogen protein or other peptide representative of FGA while the serum samples are not expected to show any presence of the FGA peptide.

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Profiling Quorum Sensing Mutants of *Pseudomonas aeruginosa* Using GC×GC-TOFMS
Nicolas Zimmermann, William & Mary, Department of Chemistry, 25 Hedgerow Ln., Williamsburg, VA 19460, Isabelle Taylor, Katelynn A. Perrault Uptmor

Pseudomonas aeruginosa is a gram-negative multi-drug resistant opportunistic human pathogen responsible for serious nosocomial infections and respiratory infections in immunocompromised hosts. For example, in cystic fibrosis patients, *P. aeruginosa* is a predominant cause of morbidity and mortality. *P. aeruginosa* uses a cell-to-cell communication system called quorum sensing (QS) to regulate many of its pathogenic behaviors. QS component genetic mutations are often found in hyper-virulent clinical isolates of *P. aeruginosa*. This project analyzed the effects QS component deletions have on the volatile organic compound (VOC) profile of *P. aeruginosa*. QS deletions were completed using a bacterial conjugation method. VOCs were extracted from bacterial culture fluids using solid-phase microextraction (SPME) and analyzed using GC×GC-TOFMS with thermal modulation. Resulting chromatographic and mass spectral data were processed to identify compounds of interest. Analyses identified unique VOC profiles linked to the components of *P. aeruginosa* QS systems. These results showed that QS components differentially regulate VOCs released by P. *aeruginosa*. The chemical profiles generated can be used in comparison to those of clinical isolates to identify mutations that the isolate strains have undergone over the course of an infection. Additionally, compounds connected to microbial pathogenesis, in *P. aeruginosa*, can be used in the future as means of predicting the virulence of isolated strains, such as through exhaled breath analysis.

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Design-of-Experiment-Enabled Exploration of Cu_2O/Cu Structures for Glucose Electrochemical Detection
Thomas Dick, University of Delaware, 62 Redmond Ln., Newark, DE 19934, Minh Dang, Rachel Davidson

Non-enzymatic copper-based electrochemical glucose sensors offer a stable, low-cost, alternative to enzymatic glucose sensors. Under alkaline oxidative conditions, Cu_2O converts to CuO, facilitating glucose oxidation through the Cu(II)/Cu(III) redox couple. However, the relationship between catalyst structure and sensor performance remains poorly understood. This work systematically investigates the synthesis-structure-activity relationships of Cu_2O glucose sensors using a Design-of-Experiments (DOE) approach. Cu_2O particles were electrochemically synthesized on indium tin oxide (ITO) using copper nitrate, sulfate, and acetate precursor systems while varying synthetic parameters including precursor concentration, pH, temperature, applied potential, and deposition time. The resulting structures were characterized using scanning electron microscopy, energy-dispersive X-ray spectroscopy, X-ray diffraction, and optical profilometry. Distinct morphologies were obtained depending on the precursor system, with nitrate, sulfate, and acetate producing predominantly cubic, star-shaped, and octahedral structures, respectively. Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was used to identify and quantify oxidation products. The best-performing sensors from each precursor system exhibited rapid response times under one second, strong repeatability, stable responses over 13 days, and glucose recovery measurements of 91-103%. Ex-situ characterization revealed morphology-dependent surface oxidation toward CuO while retaining the bulk Cu_2O phase.

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Influences of Iron and pH on Phenolic Antioxidant Reactivity with Free Chlorine in Plastic Pipes
Julianne Chan, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218, Casten Prasse, Howard Fairbrother

Plastic pipes are increasingly used in drinking water infrastructure to replace metal pipes due to concerns about rust and leaching toxic metals. However, despite their durability and low cost, there are increasing concerns about the potential leaching of additives that are embedded in the plastics. Because most additives are not chemically bound to the polymer, they can leach out over time and react with the free chlorine present in drinking water, forming disinfection byproducts (DBPs). One group of widely used chemical additives is phenolic antioxidants, used to prevent oxidative degradation in plastic pipes by scavenging free radicals. This study uses High-Performance Liquid Chromatography coupled with a Diode Array Detector (HPLC-DAD) to assess the degradation of phenolic antioxidants by chlorine and the effect of drinking water factors such as pH and dissolved and particulate iron, which often co-occur. Preliminary results show that eugenol, a naturally occurring phenolic antioxidant and a potential “green chemistry” alternative to synthetic compounds, exposed to a 10-fold molar excess of chlorine at pH 7, degraded by over 88% in just one hour ($t_{1/2}$ = 19.40±1.36 min) and completely in under 15 minutes at pH 10 ($t_{1/2}$ = 1.97±0.06 min). The presence of equal moles of dissolved iron at pH 7 completely degraded eugenol in under 30 minutes ($t_{1/2}$ = 2.65±0.07 min). The poster will also discuss the identification of DBPs formed from the chlorination of eugenol using high-resolution mass spectrometry and the fate of other phenolic antioxidants, such as 2,4-di-tert-butylphenol and butylated hydroxytoluene.

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Multistage Air Sampling and Complementary LC–MS/MS and GC–MS/MS for Phase-Resolved Atmospheric PFAS Analysis
David Bell, ASKkprime LLC, 135 Lincoln Ln., Bellefonte, PA 16823, Kenichi Suzuki, Hiroshi Hayashida

Per- and polyfluoroalkyl substances (PFAS) can be transported through the atmosphere in particulate, aerosol-associated, and vapor phases, yet practical methods capable of simultaneously collecting these different forms remain limited. This study evaluated a novel multistage air-sampling device coupled with LC–MS/MS and GC–MS/MS analysis for atmospheric PFAS monitoring. The device incorporates quartz fiber filters (QFFs), polyurethane foam (PUF), and activated carbon fiber discs (GAIAC) to collect particulate-bound, semi-volatile/aerosol-associated, and vapor-phase PFAS, respectively. Field sampling was conducted in Tsukuba, Japan, under different meteorological conditions. Samples were extracted and analyzed for 54 PFAS analytes spanning ionic and neutral

compounds. The method achieved low method quantification limits in the pg/m³ range, with most analytes showing recoveries of 70–120%. Distinct phase-dependent distributions were observed across the sampling media. Neutral PFAS such as 8:2 FTOH were detected predominantly in the vapor-phase medium, whereas ionic PFCAs showed chain-length-dependent distributions. PFAS profiles also differed substantially between sampling events conducted under different wind directions. These results demonstrate that multistage sampling combined with complementary LC–MS/MS and GC–MS/MS measurements enables characterization of multiple atmospheric PFAS phases and may support source apportionment and environmental air monitoring.

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Extending Amino Acid Analysis from Essential Amino Acids to Complex Commercial Samples

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Amino acid analysis is important across pharmaceutical, food, nutritional, and other applications, where characterization of amino acid composition and stereochemistry can provide valuable information about sample identity and quality. However, the structural similarity of amino acids and the potential presence of multiple stereoisomers can complicate their simultaneous chromatographic analysis. Previous work demonstrated the separation of nine essential L-amino acids using a derivatization-based chromatographic approach and its application to a commercially available essential amino acid supplement. The present study extends this approach to evaluate its quantitative performance and applicability to samples containing a broader and more complex amino acid composition. Calibration and replicate analyses of the nine essential amino acids are used to assess quantitative response, linearity, and reproducibility. The method is subsequently applied to a commercially available liquid amino acid product containing a wider range of amino acids, with chromatographic conditions evaluated and further optimized to improve resolution within the more complex amino acid profile. Additional amino acids present in the expanded sample set are also evaluated as individual D- and L-enantiomer pairs to characterize stereochemical selectivity and assess how increasing sample complexity influences simultaneous amino acid and enantiomeric resolution. This work examines the progression of a unified amino acid analysis approach from a defined essential amino acid mixture to more compositionally complex commercial samples, with emphasis on quantitative performance, chromatographic resolution, and the stereochemical information that can be obtained from the same analytical platform.

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Performance of Commercial Portable Raman Spectrometers for Body Fluid Identification

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Currently, presumptive tests are used at crime scenes to indicate if body fluids are present, but these tests are destructive, produce false positives and negatives, require further confirmatory testing, and each only indicate one type of body fluid. There is a need for a nondestructive, molecularly specific, universal, rapid, and confirmatory method for body fluid identification at crime scenes, all of which are characteristics that Raman spectroscopy possesses. Benchtop Raman spectroscopy has demonstrated its ability to differentiate and identify body fluids (e.g., blood, semen, saliva, urine, and vaginal fluid), causing portable Raman instruments to be suggested as alternatives to presumptive tests for body fluid identification in the field. This research evaluates three commercially available portable Raman spectrometers for their suitability for body fluid differentiation and identification at crime scenes by (1) creating and optimizing a method for different fluid types for each instrument, and (2) creating and testing a spectral library of body fluids on each instrument. This work is intended to enable realization of the potential of body fluid identification using portable Raman instruments, as well as lend support for their use as better alternatives to current presumptive tests used in the field.

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Coupling SEC-MALS-RI with Pyro-GCMS to Analyze Polymer Blends

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Size-exclusion chromatography with multi-angle light scattering and refractive index detection (SEC-MALS-RI) was coupled to pyrolysis gas chromatography with mass spectrometry detection (Pyro-GCMS) utilizing fraction collection in order to analyze a polymer blend. The intended application of this technique is to determine the composition of individual peaks in a mixed sample, which is necessary to determine molar mass of the polymer by light scattering. The method was validated by analyzing fractions of a known mixture of PMMA of various molar mass. Fractions were collected corresponding to peaks in the SEC and, after the solvent was evaporated, analyzed by Pyro-GCMS. The ability to identify monomer composition was confirmed. Good SEC separation was confirmed by re-injecting the collected fraction. The method was then applied to a blended polymer. Due to a lack of baseline resolution with SEC, it was necessary to determine if there was any overlap of the peaks. This was accomplished by looking for trace amounts of the monomers not expected in each peak. Based on the Pyro-GCMS results, the chemical identities for each peak corresponded only to the expected polymer, so peak overlap was determined to be negligible for the purposes of dn/dc determination for molar mass calculation. Overall, this method is useful in determining the molar mass and chemical composition of multi-component polymer mixtures.

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Developing a Headspace GC/MS Method for Quantifying Volatile Trace Residue in Complex Matrices

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Quantifying highly volatile compounds at trace levels can be challenging, particularly in complex matrices. This study describes the optimization and application of a static headspace gas chromatography–mass spectrometry (HS-GC/MS) method for quantifying a process-related volatile residue in complex matrices. An isotopically labeled internal standard was used to support quantitation. Given the high volatility of the target analyte, method optimization focused on minimizing analyte loss while improving recovery, precision,

and overall robustness. Critical parameters related to standard preparation and headspace conditions were evaluated to establish a practical and reproducible analytical workflow. The optimized method provided fit-for-purpose quantitation of low-level volatile residues in representative samples. Analysis of multiple samples showed differences in residual levels, demonstrating the sensitivity of the method and the potential impact of manufacturing processes on residual levels. The study also underscored the importance of carefully controlled sample preparation procedures when analyzing volatile compounds at low concentrations. The resulting HS-GC/MS workflow provides a practical approach for measuring volatile process-related residues in complex matrices. Beyond the application described here, the workflow may be adapted for analyzing other volatile compounds in similarly challenging sample types.

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Determination of Fatty Acid Composition in Polysorbate 80 Using High-Performance Liquid Chromatography (HPLC) with Charged Aerosol Detection

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Polysorbates are nonionic surfactants widely used in pharmaceutical, food, and cosmetic products to improve solubility, emulsion stability, formulation performance, and texture. The complex heterogeneous nature of polysorbate 80, combined with absence of a strong chromophore, presents challenges for quality analysis. The U.S. Pharmacopeia (USP) relies on a complex reflux reaction and GC-FID analysis, which can limit throughput and efficiency in quality control laboratories. This study demonstrates an HPLC method with Charged Aerosol Detection for the analysis of fatty acids in polysorbate 80. Separation was achieved using an XBridge™ BEH™ C18 Column (4.6 × 100 mm, 3.5 µm particle size). Key detector parameters, including evaporator temperature, filter time constant, and power function value, were optimized to enhance sensitivity and reproducibility. Method performance was evaluated using system suitability, quantification limits, linearity, and range. The method demonstrated excellent repeatability, with peak area relative standard deviation (RSD) ≤ 0.48%. Quantification limits for the fatty acids ranged from 0.9 to 3.5 ng on column. A linear response was achieved over a concentration range of 0.05 to 25 µg/mL, with correlation coefficients (R2) ≥ 0.99 using a linear fit with 1/x weighting. Multiple batches of polysorbate 80 were analyzed. The free fatty acid composition was calculated as the percentage of each fatty acid following the USP monograph for Polysorbate 80 (USP–NF 2021 Issue 1). The developed method enabled reliable high-throughput quality testing of fatty acid composition in polysorbate 80 raw materials. XBridge and BEH are trademarks of Waters Corporation or its affiliates.

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Advanced Ion-Exchange Approaches for AOC Characterization Using SAX and Tandem SCX–SAX

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Antibody-oligonucleotide conjugates (AOCs) present unique analytical challenges because conjugation introduces substantial charge heterogeneity associated with differences in oligonucleotide-to-antibody ratio (OAR). Each negatively charged oligonucleotide payload alters the overall charge of the conjugate, providing a strong basis for ion-exchange chromatographic separation. In this study, Sepax Proteomix® strong anion-exchange (SAX) chromatography was evaluated as a direct approach for AOC characterization. The SAX method provided strong charge-based selectivity, enabling separation of unconjugated antibody, AOC species with different degrees of oligonucleotide conjugation, and free oligonucleotide. Increasing oligonucleotide loading resulted in progressively stronger retention, allowing conjugation variants to be differentiated according to their overall negative charge. The method therefore provides a straightforward platform for monitoring AOC heterogeneity and OAR distribution using a single chromatographic mode. To further expand the accessible selectivity and operating window, a tandem strong cation-exchange (SCX)-SAX configuration was developed. By integrating cation- and anion-exchange interactions within a single workflow, the tandem strategy provides additional flexibility for resolving antibody- and oligonucleotide-associated charge differences. Importantly, this configuration enables effective AOC profiling under milder, near-neutral conditions, reducing reliance on highly alkaline mobile phases that may adversely affect sensitive oligonucleotide payloads, linkers, or conjugates. Together, the SAX and tandem SCX-SAX approaches provide complementary and versatile IEX strategies for AOC characterization and method development.

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MALDI-MS Peptidomics of Human Breast Milk Identifies Tumor-Associated and Contralateral Molecular Signatures in Breast Cancer

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Breast cancer remains the most frequently diagnosed cancer among women worldwide and a leading cause of cancer-related mortality, with over 2.3 million new cases and 660,000 deaths reported in 2022. While mammography and MRI enable early detection, both carry limitations including high cost, reduced sensitivity in dense breast tissue, and cumulative radiation exposure. Human breast milk, a non-invasive and biologically relevant fluid, offers a promising alternative source for biomarker discovery, as its peptide composition reflects mammary gland activity and may capture early molecular changes associated with tumor development. This study applied MALDI-MS to profile the peptidome of human milk from women with unilateral breast cancer and healthy controls. Twenty milk samples were analyzed, including paired samples from tumor-affected and contralateral unaffected breasts of five breast cancer patients, and paired samples from five healthy donors. Following lactoferrin depletion, tryptic digestion, and peptide purification, samples were analyzed on a Bruker Ultraflex MALDI-TOF/TOF instrument. Principal Component Analysis and Linear Discriminant Analysis revealed three biologically distinct clusters: healthy controls, unaffected contralateral breast samples, and tumor-affected samples, with the unaffected breast occupying an intermediate profile suggestive of systemic or contralateral disease influence. Hierarchical clustering identified several discriminative m/z features including 2282.1, 1864.9, and 1829.2 that were consistently elevated in tumor-affected samples, alongside distinct control-associated peptides such as m/z 1538.9 and 973.5. These findings demonstrate that milk peptidomic profiling can distinguish tumor-associated and contralateral molecular signatures, supporting its potential as a non-invasive breast cancer biomarker platform.

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Biophysical Analysis of the Mechanisms Underlying the Extended Half-Life of the GLP-1 Peptide Semaglutide
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Glucagon-like peptide-1 (GLP-1) receptor agonists are widely used to treat type 2 diabetes and obesity. Native GLP-1 has an in vivo half-life of only 1.5-2 minutes because of rapid renal clearance and degradation by dipeptidyl peptidase-4 (DPP-4). In contrast, the modified GLP-1 analogs liraglutide have half-lives of approximately 16-18 hours. To investigate the molecular basis of this difference, we applied biophysical and functional analyses to wild-type and modified liraglutides including the wild-type, a C18-acylated analog, an analog containing an alanine-to-α-aminoisobutyric acid (Aib) substitution, and an analog containing both modifications. Dynamic light scattering was used to assess potential differences in peptide association, while circular dichroism spectroscopy evaluated secondary structure. Neither method revealed significant differences among the variants, suggesting that altered oligomerization does not account for the longer half-life. A cell-based GLP-1 receptor activation assay was then used to evaluate peptide function following incubation with albumin, DPP-4, or both. Albumin enhanced activity across all variants but did not differentiate the C16- and C18-acylated peptides. After 48 hours of DPP-4 exposure, variants lacking Aib lost most of their activity, whereas Aib-containing variants retained full activity. Albumin protected all variants from DPP-4-mediated degradation, independent of acyl-chain length. This integrated analytical approach identifies Aib-mediated DPP-4 resistance as a major differentiating factor between liraglutide and semaglutide and demonstrates the value of combining biophysical characterization with cell-based functional analysis to investigate structure–activity relationships in long-acting peptide therapeutics.

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ProjDIA: Improving Peptide Identification and Quantification in Direct-Infusion DIAProteomics
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Liquid chromatography–mass spectrometry (LC-MS) is a central platform for bottom-up proteomics because chromatographic separation reduces sample complexity and enables sensitive and reliable peptide identification. However, LC separations increase analysis time, limiting throughput for large sample series. Direct-infusion shotgun proteome analysis (DI-SPA) removes chromatographic separation and offers substantially faster analysis, but simultaneous ionization and fragmentation of many peptides produce ion suppression and highly chimeric MS/MS spectra. In addition, many conventional DIA analysis tools rely on chromatographic co-elution information that is absent in direct-infusion data. To address these challenges, we developed ProjDIA, a library-driven computational workflow that combines fragment-bin projection scoring, confidence-guided two-pass mass-error recalibration, monotone target-decoy q-value estimation, and guarded semisupervised rescoring. Projection scoring maps experimental intensities onto the complete expected fragment pattern, allowing missing expected fragments to directly penalize candidate matches. ProjDIA was benchmarked across public and experimental DI-SPA datasets spanning MS2 resolution, sample load, repeated acquisitions, and quantitative studies. At 240,000 MS2 resolution, ProjDIA identified 6,641 peptides, compared with 5,057 using CHIMERYS and 4,687 using CsoDIAq at $q \leq 0.01$. Species-entrapment experiments showed that increased identification yield was not accompanied by increased false identifications. ProjDIA also supported label-free quantification and improved recovery of biologically meaningful protein-response patterns. These results demonstrate that improved spectral scoring and confidence calibration can increase the analytical depth and reliability of chromatography-free, high-throughput proteomics.

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MALDI MS-based Peptidome Analysis from Human Breast Milk Reveals Distinct Molecular Profiles Associated with Cancer
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Cancer remains a major health challenge, highlighting the need for improved approaches for early detection and molecular characterization. Low-molecular-weight molecules present in biological samples may provide valuable molecular signatures associated with disease. In this study, we applied a matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)-based workflow to characterize molecular profiles in the peptidome (3 kDa retentate fractions) derived from human breast milk samples collected from cancer patients and healthy controls. The study included a time-course collection of human breast milk samples from 12 healthy individuals and 12 cancer patients. For the preliminary MALDI analysis, samples from two cancer patients and two healthy controls were evaluated, with six time-course breast milk samples collected from each individual. Comparative analysis of the MALDI mass spectral profiles revealed clear differences in peak patterns and signal intensities between the cancer and healthy control breast milk samples. Several distinct molecular features were observed in the 3 kDa retentate fraction of human breast milk, demonstrating differences between the two groups and suggesting the presence of disease-associated molecular signatures. The time-course sampling further provides an opportunity to evaluate the consistency and temporal behavior of these spectral features within individual patients. Overall, these preliminary findings demonstrate the potential of MALDI-MS-based analysis of the human breast milk peptidome for identifying molecular differences associated with cancer and support further investigation as a potential platform for biomarker discovery. Ongoing analyses using the expanded cohort will help validate the observed spectral features and determine their potential utility for cancer detection and monitoring.

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The Wonders of Water Droplets in Air and Air Bubbles in Water
Richard Zare, Stanford University, Department of Chemistry, Stanford University, Stanford, CA 94305, Jinheng Xu

Although bulk water is generally regarded as chemically benign, water droplets in air and air bubbles in water possess highly reactive interfaces that exhibit properties strikingly different from those of the bulk liquid. This lecture describes how strong electric fields and

charge separation at aqueous interfaces create environments that promote spontaneous formation of radicals, hydrogen peroxide, and solvated electrons, leading to chemical transformations under ambient conditions without added catalysts. Recent experiments reveal that interfacial reactivity is not simply proportional to surface area but depends strongly on curvature, with smaller droplets and bubbles exhibiting enhanced electric fields and altered reaction pathways. Droplet-on-demand mass spectrometry provides direct evidence that the interfacial activity scales approximately with the reciprocal radius, leading to optimum droplet sizes for many reactions. These interfacial effects enable unusual oxidation and reduction chemistry and have opened new routes for synthesizing materials such as boron nitride quantum dots and for promoting transformations relevant to biology and the environment. Examples include spontaneous hydrogen peroxide formation on leaf surfaces and the induction of flowering by dewdrops. I will also discuss recent observations of charge separation and microlightning associated with droplet fragmentation, which may help explain reports of atmospheric nitrogen fixation and observations of will-o'-the-wisps. Together, these studies suggest that aqueous interfaces constitute a distinct chemical environment whose behavior challenges conventional views of solution chemistry and offers new opportunities for sustainable synthesis, environmental transformation, and understanding natural phenomena.

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65 Years of Mass Spectrometry

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Parallels are drawn between the 65 years of EAS and MS research over this period. Purdue mass spectrometry in particular saw a series of innovations: from MS/MS (1975) to hybrid instruments (1978) to surface-induced dissociation and ion soft landing (1995) to ambient ionization; first DESI imaging (2004) then derivatization using reactive DESI (2000's) and finally the realization DESI reactions represented an extreme case of reaction acceleration (2011). This key finding was followed by chemical synthesis in microdroplets (2012). The topic of Accelerated Chemistry was first reviewed in 2016. The main subject of the presentation is the use of reaction acceleration in microdroplets as the basis for a single platform for rapid automated experiments covering the early stages of drug discovery. With funding from DARPA and then NCATS ASPIRE we built two versions of HT-DESI-MS systems and used them to screen thousands of reactions per hour, collect products of the most favorable reactions, subject them to bioassays and then scale up the most promising compounds for characterization by NMR. The high analysis speed depends on two factors (i) that DESI-MS is applicable to complex biological samples, so sample purification is unnecessary to get YES/NO answers re new drug candidates and (ii) that reaction acceleration in microdroplets allows product formation in the millisecond droplet flight time. Acknowledging the contributions of Nicolás Morato, Yunfei Feng, Mahdiyeh Shari, Kit Chen, Alexis Owen, Manish Jana and Mousumi Saha and the financial support of the NCATS ASPIRE program and AFOSR MURI programs.

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Tandem Mass Spectrometry of the Deamination of Asparagine: A Key Step in Aging?

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Deamidation of asparagine (Asn) residues is a major spontaneous post-translational modification in proteins and is believed to play a significant role in aging and the onset of neuro-degenerative diseases. Notably, the C-terminal residue adjacent to Asn has been found to alter the lifetime for deamidation over about four orders of magnitude (from 1 day to 30 years). Using an analytical technique called guided ion beam tandem mass spectrometry, we have examined the kinetic energy dependence for collision-induced dissociation (CID) of a series of protonated AsnXxx dipeptides where Xxx = Gly, Ala, Val, Ser, and Thr. For the protonated AsnXxx dipeptides, deamidation is found to be a major decomposition channel (along with dehydration) with a threshold energy sensitive to Xxx. These experimental results have been analyzed to measure the thermodynamics of these decompositions. Complementary infrared ion spectroscopy has been used to identify the structure of the deamidation product, with both succinimide (the biologically relevant species) and furanone products being observed dependent on Xxx. The threshold energies for deamidation to form the succinimide product correlate quantitatively with the lifetimes observed under physiological conditions. Theoretical calculations on these systems are used to identify the detailed reaction pathways, which are validated by matching the experimental thermochemistry. The mechanisms clearly show a steric effect associated with larger side chains and that side chains with heteroatoms stabilize the transition state for deamidation to form the succinimide (but not the furanone).

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65 Years of Separation Science: The Founders and Builders of the Chromatography We Use in the Laboratory Today

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Chromatography as one of the separation sciences is a common denominator for gathering data in the chemical, environmental, forensics, biological, clinical and pharmaceutical sciences research areas. Historically, it has changed in terms of mechanisms of separation and its modes of development. Mechanisms changed as developments traveled through the 20th century where in the early 1900's adsorption chromatography was predominant to the 1940's when partition and ion exchange chromatography were presented and were the norm. In the 1950's, gas solid chromatography, GSC, was introduced and then a decade later the partitioning mechanisms of paper, PC, thin layer, TLC, high performance liquid, HPLC, size exclusion, SEC, and affinity (AC) chromatography were all introduced. Modes of development that have been used include extrusion, frontal analysis, displacement and elution analysis.[1] To summarize this huge field, an overview of the founders of these major chromatographic techniques will be presented, however, since these techniques have continued to grow and be established builders of these techniques will also be highlighted. [1] Gehrke, C.W.; Wixom, R.L. and Bayer, E., Chromatography A Century of Discovery 1900-2000. The Bridge to the Sciences/Technology, Journal of Chromatography Library, Volume 64, 2001, pages 1-38. ISBN: 9780080476506

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Integrating Taylor Dispersion Analysis with Capillary Electrophoresis for Separations with Structural Insights
Christopher Baker, New Mexico State University, MSC 3C, PO Box 30001, Las Cruces, NM 88003

Capillary electrophoresis (CE) enables rapid, solution phase separations from nL sample volumes, making it a powerful tool for microscale bioanalysis. Electrophoretic resolution results from structurally descriptive properties (i.e. ionic charge and hydrodynamic radius), but we rarely leverage this structural information as descriptive metrics for chemical analyses. The key limitation is that electrophoretic mobility confounds these structural descriptors into a single empirical metric, making independent determination of ether structural descriptor by CE a mathematically ill posed problem. Taylor dispersion analysis (TDA) is a flow based technique for the calibration-free direct determination of hydrodynamic radius, with capillary TDA utilizing instrumentation highly analogous or identical to CE instrumentation. Integrating CE online with TDA uncontrains the independent determination of ionic charge and hydrodynamic radius from a single CE-TDA measurement, providing structurally descriptive metrics with the same analytical advantages afforded by CE. In this talk we will discuss a new technology for the continuous online integration of CE and TDA that leverages electroosmotic pumping to drive TDA analysis. We will show key instrumental design considerations for successfully coupling CE with TDA in a continuous, online fashion, and validate the instrumental method by mapping size and charge dynamics of proteins as a function of pH. Developments towards high precision molecular sizing using new TDA methodology will also be discussed.

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Crossing the Cutoff: Extending LC Detection into the VUV
Dale Harrison, VUV Analytics, 1500 Arrow Point Dr., Ste. 805, Cedar Park, TX 78613, Anthony Hayes, Alex Hodgson, Annika Dombrowski, Garrett Hellinghausen

Ultraviolet (UV) absorbance detection has been a cornerstone of liquid chromatography (LC) for decades because of its simplicity, robustness, and broad applicability. However, its practical operating range is constrained not only by the optical properties of chromatographic solvents, but also by detector designs not optimized for operation below 200 nm. The vacuum ultraviolet (VUV) region is rich in high-energy electronic transitions that produce stronger, more universal, and often more information-rich absorbance than is available at longer UV wavelengths, creating new opportunities for LC analysis. A novel VUV absorbance detector extends the practical spectral range of LC absorbance detection through innovations in optical, fluidic, and detector design. These advances enable routine operation at shorter wavelengths while maintaining chromatographic performance, sensitivity, stability, and ease of use. Application examples illustrate the analytical advantages of extending absorbance measurements into the VUV. Particular emphasis is placed on compound classes that exhibit little or no response with conventional UV detection but display strong absorbance at shorter wavelengths. Chromatographic data for amino acids and carbohydrates demonstrate how access to the VUV region can improve sensitivity and expand the range of analytes amenable to absorbance detection without derivatization.
Rather than replacing conventional UV detection, VUV absorbance extends a familiar and trusted analytical technique into a previously inaccessible spectral region. The result is a complementary detection approach that broadens the applicability of LC absorbance detection while improving sensitivity and chemical selectivity for many challenging analytes.

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Coupling Separation Modules with Microwave Rotational Resonance Spectroscopy: Challenges and Prospects
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Molecular rotational resonance (MRR) spectroscopy probes dipolar molecules based on their three-dimensional mass distribution, yielding rotational transitions that discriminate among isomers, isotopologues, and isotopomers, many of which cannot be easily distinguished by mass spectrometry. Hyphenation of rotational spectroscopy with gas chromatography (GC-MRR) couples high-efficiency separation with the most structurally specific gas-phase detector available, potentially enabling identification without reference standards and without the ionization-suppression artifacts that affect MS during coelution. This talk outlines the key obstacles to interfacing separation modules with MRR and the strategies to overcome them. The first-generation millimeter-wave (75-110 GHz) instrument, run as a room-temperature flow cell, was confined to molecules below approximately 120 Da and exhibited low sensitivity. Adding a supersonic jet and a Fabry-Pérot cavity cooled analytes to approximately 2 K and extended the range to 240 Da, with GC-MRR detection limits rivaling those of a thermal conductivity detector (ng to sub-ng). Carrier/makeup gas pairing proved unexpectedly interesting: nitrogen as the column carrier gas, with Ne as the makeup gas, enhanced the signal two- to threefold. A dominant data-processing barrier is data throughput; MRR digitization can easily generate a million data points per nozzle pulse, making the per-spectrum Fourier transform the rate-limiting step. Gram-Schmidt orthogonalization of free-induction-decay curves against a blank-derived orthonormal basis reconstructs chromatograms up to 10 times faster than FFT processing, with equal or superior sensitivity, peak shape, and signal-to-noise ratio, permitting near-real-time display. Prospects for Hadamard-transform and new nonlinear denoisers for GC-MRR will also be discussed.

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No abstract submitted by the author.

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Emerging Analytical Approaches for Estimating Postmortem Interval
Marlene Muhammad, New Jersey Institute of Technology, Department of Chemistry and Environmental Science, 1 Washington Ave, Clifton, NJ 07011, Sara C. Zapico

Accurate estimation of the postmortem interval (PMI) remains one of the most challenging and consequential problems in forensic science. Traditional methods, largely reliant on morphological and environmental observations, often lack precision across extended timeframes and variable conditions. In response, a new generation of analytical approaches is reshaping PMI estimation through

objective, quantifiable, and reproducible measurements. This presentation explores emerging methodologies developed in our lab that leverage advances in spectroscopy to refine PMI estimation, X-ray diffraction, Fourier Transform Infrared Spectroscopy, and UV–Vis–NIR spectroscopy. Through two different experimental models (teeth and bones), this work highlights the strengths, limitations, and forensic applicability of these techniques across diverse postmortem scenarios. Particular emphasis is placed on data analysis to enhance accuracy and extend the temporal resolution of PMI estimates. By bridging traditional forensic practice with cutting-edge analytical science, these approaches offer a pathway toward more robust, standardized, and defensible PMI determinations, advancing both casework reliability and the scientific foundation of forensic investigations.

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Bayesian Network Modeling for Assessment of Drug-Related Deaths: A Forensic Toxicology Approach

Kenny K. Khatri, Rutgers University, Department of Chemistry, 220 Cooper St., Camden, NJ 08102, Priscilla T. Merlino, Anders L. Madsen, Alan M. Langford, Michelle G. Carlin

Post-mortem toxicology is essential in establishing whether drugs contributed to an individual's death. However, interpretation is complicated by numerous factors that are not captured in standard toxicological reference tables. These include the choice of biological samples and sampling sites, post-mortem drug redistribution, chronic or previous drug use, and underlying pathological conditions. Such variables can significantly influence toxicological outcomes and must be considered in context. In this study, a Bayesian network was developed and tested using a dataset of over 10,000 cases, each including a recorded cause of death and, in most instances, an associated toxicology report. The initial phase involved data cleaning and the construction of a baseline model using only three variables: cause of death (as determined by a pathologist or coroner), age, and sex however, this was expanded to include multiple variables including alcohol, opioids, stimulants and sedative drug classes. This model was trained on the full dataset and used to assess the likelihood of a death being drug-related. Results were compared to official classifications on death certificates, and a receiver operating characteristic (ROC) curve was generated. The area under the curve (AUC) was calculated to evaluate performance, with values closer to 1 indicating stronger predictive ability, and 0.5 indicating performance equivalent to random chance. The baseline network gave an AUC value of 0.54 and current network tested producing an AUC of 0.74. Work continues to improve this value and to establish its use in interpretation of toxicological cases.

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Quantitative LC-MS/MS Analysis of Psilocybin and Psilocin in Microdosing Capsules

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Psilocybin and psilocin are tryptamine alkaloids, found in Psilocybe mushrooms, that have seen increasing interest in their analysis at concentrations associated with microdosing. This study developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the identification and quantitation of psilocybin and psilocin in mushroom material processed into microdosing capsules. This method demonstrated acceptable selectivity, negligible matrix effects, accuracy, sensitivity, linearity, and robustness, across a validated concentration range of 5 - 200 ng/mL. Application of this method to unknown capsule samples demonstrated its ability to identify and quantitatively determine psilocybin and psilocin, providing a simple analytical approach dedicated to the analysis of microdosed samples.

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Fluorescence Microscopy in a Sequential Analytical Scheme for the Discrimination of Black Electrical Tape

Grace Patzer, Cedar Crest College, 100 College Dr., Allentown, PA 18104, Larry Quarino

Electrical tape is a common, nondistinctive adhesive medium, typically manufactured in a standard black color. This uniform appearance introduces an additional layer of complexity when tape is recovered as evidence. While many methods of discriminating electrical tapes have been proposed, fluorescence microscopy remains underexplored. This study evaluates fluorescence microscopy alongside established instrumental methods in a sequential scheme for discriminating black electrical tape by pairwise comparison. Fifty electrical tapes were examined, giving 1,225 possible pairs. Physical and elemental (SEM-EDX) measurements were assessed by Tukey-Kramer HSD with Bonferroni correction across measurement dimensions. Spectral comparison used a Pearson correlation cutoff ($r < 0.90$). Fluorescence microscopy proved to be the largest single contributor to discrimination, partitioning the samples into nine groups and separating 905 cross-group pairs, or 73.9% of all comparisons. Physical measurements, infrared (IR) spectroscopy, and SEM-EDX were credited with discriminating the pairs remaining after fluorescence, yielding a total discrimination rate of 99.8% (1,223 of 1,225 pairs). Both unresolved pairs fell within the non-fluorescent group. Ten blind samples drawn from the reference set were analyzed by the same protocol, with candidate sources assigned by exclusion. Validation results will be presented. These findings demonstrate that a rapid, non-destructive fluorescence screen resolves a vast majority of pairwise comparisons before instrumental analysis and supports the inclusion of a fluorescence implementation in tape examination protocols.

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Experimental and Digitally Blended Infrared Spectral Data for Detection of Calibrated and Uncalibrated Adulterants in Edible Oils Near the Limit of Determination

Elizabeth Donkor, Oklahoma State University, 107 Physical Science, Department of Chemistry, Stillwater, OK 74078, Isio Sota-Uba, Thomas Hancewicz, Karl Booksh, Barry Lavine

Adulteration of extra virgin olive oil (EVOO) by canola oil and corn oil has been investigated using FTIR spectroscopy and pattern recognition methods. EVOO has been selected for this study because it is a frequent target for adulteration. EVOO is often mixed with less expensive edible oils with similar fatty acid and sterol profiles such as corn oil and canola oil, whose composition and infrared (IR) spectra are comparable to EVOO. To discriminate chemically similar edible oils and to model mixtures of these oils, wavelength selection is necessary to identify spectral regions that can discriminate between different varieties of edible oils. For this reason, a

genetic algorithm for pattern recognition which identifies wavelengths in the IR spectra of edible oils that conveys information about differences between classes in a plot of the two or three largest principal components of the data was employed. A novel and unique aspect of this study is the multilayered nature of the research design to authenticate EVOO using IR spectral data near the limit of determination for calibrated and uncalibrated adulterants. As part of this study, authentication of EVOO (using experimental and digitally mixed data) was investigated as a function of spectral resolution and baseline correction.

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Excitation-Emission Matrix Fluorescence for Monitoring Progress in Bioreactors

Karl Booksh, University of Delaware, Dept. Chemistry and Biochemistry, Newark, DE 19716, Conner Balickie, Fernanda Delgado, Joseph Smith

We report on our efforts to employ Excitation-Emission Matrix Fluorescence (EEMF) as an on-line and in-situ tool for monitoring and prediction of bioreactor progress. Bioreactors are complex, multivariate living systems. Growth of these cultures involve dynamic interactions among cells, media, metabolites, and product quality attributes (glycosylation, charge variants, aggregates). We will show relative performances of on-line and in-line sampling strategies to quantify select Critical Process Parameters (CPPs) and Critical Quality Attributes (CQAs). We will also demonstrate the capability of parallel factor analysis to extract CPP and CQA relevant from the intense scattering and fluorescent background of cell cultures and growth media.

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Integration of Spectral Libraries with Image Maps Using Modified Alternating Least Squares to Develop Soft Machine Learning Models

Barry Lavine, Oklahoma State University, 107 Physical Science, Stillwater, OK 74078, Thomas Hancewicz, Haoran Zhong, Elizabeth Donkor

Multivariate self-modeling curve resolution with alternating least squares (ALS) is routinely used to extract and analyze chemical information from IR and Raman spectral image data. Although ALS is reasonably fast and often produces reliable results for large datasets, the method has drawbacks (e.g., convergence problems and sensitivity to highly correlated data) that can degrade its performance for many types of imaging datasets. When constraints such as non-negativity or unimodality are applied, ALS may be slow to converge even though each iteration is fast. Modified alternating least squares (MALS) is a solution to the constrained non-negative least squares optimization problem for IR and Raman imaging. MALS offers superior stability due to the incorporation of ridge regression into ALS and has a substantial speed advantage as the algorithm can be written as a kernel. MALS excels in resolving basis vectors, even in low signal-to-noise, nearly collinear data, whereas ALS often fails. Crucial to best practice is the application of an adaptive approach to background (baseline) correction which enables the extraction of components at the limit of determination. This adaptive approach to background correction in MALS can be implemented by direct integration of baseline correction and library searching in the extraction of chemical components from the spectral image map of a sample. Studies in forensic automotive paint analysis, pharmaceutical analysis, and global impact events demonstrate the advantages of linking a spectral library to an infrared or Raman sample image map when extracting and analyzing chemical information present in imaging data using MALS.

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Pigment Clustering and Classification of Reflectance Hyperspectral Datasets for Roman Egyptian Mummy Portraits using a Gaussian Mixture Model-Based Approach

Roxanne Radpour, University of Delaware, 18 E. Main St., Newark, DE 19716, Helder Carneiro, Caelin Celani, Karl Booksh

In the continuously developing space of imaging spectroscopy applications for cultural heritage, the number of institutions adopting hyperspectral cameras and the amount of data collected are exponentially growing. However, within the data processing pipeline a bottleneck persists in quickly and robustly creating meaningful material classification maps as manual expert supervision is generally required. The cultural heritage science field requires more exploration of advanced yet accessible statistical analysis workflows that can cluster high-volume data sets without supervision and produce classification visualizations that help interpret artworks. For example, when looking at paintings, such visualizations would entail maps for key materials such as pigments, binding media, and conservation interventions. This research presents a novel application of Gaussian mixture models coupled with an integrated complete log-likelihood optimization criterion; this framework facilitates unsupervised chemical image segmentation with impressive spectral clustering results for material identification and subsequent image classification, which is demonstrated on a set of Roman Egyptian funerary mummy portraits to understand their artistic production, history of conservation and phases of repainting.

80

New Signatures of Molecular Chirality in Spectroscopy and Microscopy of Molecularly Ordered Assemblies

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Molecular chirality defines structure/function relationships in biological assemblies, novel materials, and chemical function through chirality-induced spin selectivity. However, established optical methods notoriously exhibit poor sensitivity to subtle perturbations due to molecular chirality. We describe instrumentation and theoretical foundations for a suite of chiral-specific spectroscopy and microscopy measurements. Fluorescence optical rotary dispersion (F-ORD) and dark-field absorbance circular dichroism (DF-CD) are developed as new experimental methods with exquisite sensitivity to molecular chirality (dissymmetry parameters greater than unity in magnitude). Nonlinear optical Stokes ellipsometry (NOSE) is also shown to support ultra-sensitive chiral second harmonic generation imaging through a similar mathematical framework. These new experimental methods lay the foundations for the emergence of ultrasensitve chiral-specific imaging methods to inform on the development of novel materials and on structure/function properties in biological assemblies.

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Chiral-Selective SFG Spectroscopy Probes Biomolecular First Hydration-Shell Structure
Elsa Yan, Yale University, Dpt. of Chemistry, New Haven, CT 06520

Elucidating how water influences biological function remains a major challenge in the life sciences. The organization of water in the immediate hydration layers around biomolecules is difficult to probe directly because signals from these thin shells are masked by the large background from bulk water. Interfaces add further complexity: the hydrogen-bond network of water is disrupted at boundaries, so hydration near a surface can differ substantially from hydration in solution. Here, we combine experiments and simulations using chiral vibrational sum frequency generation spectroscopy (chiral SFG) to study proteins and DNA. We demonstrate that the chiral SFG response from the O–H stretch of water arises primarily from the first hydration shell surrounding these biomolecules, and that the biomolecules can transfer their chirality to nearby water. Consequently, a complete description of chirality of biomacromolecules must include their hydration shells. Using chiral SFG, we probe the molecular basis of drug–DNA binding and protein denaturation. Overall, our results highlight chiral SFG as a powerful tool for tackling both fundamental and applied challenges in biology, including biomolecular folding at aqueous interfaces and the rational design of biointerfaces.

82
Anomalous Circular Birefringence in Mixed Crystals
Bart Kahr, New York University, 29 Washington Pl., Dept. of Chemistry, New York, NY 10003

The anomalous *linear* birefringence of mixed crystals, inconsistencies between optical and morphological symmetries, have been studied more extensively for mixed crystals of isomorphic cubic nitrates of strontium, barium and lead than for any other family of laboratory grown crystals. Anomalous circular birefringence, an unanticipated dissymmetry leading to different refractivities for circularly polarized rays, on the other hand, is harder to detect and has not been described in two centuries during which the etiologies of optical anomalies were debated. We quantify *anomalous circular birefringence* in $Ba_xPb_{1-x}(NO_3)_2$ and $Sr_xPb_{1-x}(NO_3)_2$ mixed crystals using polarimetric imaging. This circular birefringence is not natural optical activity but arises from the non-commutativity of successive linearly anisotropic sub-volumes that intersect the incident wave vector. The signal is best described as an emergent optical activity generated by orientation gradients across linearly birefringent domains. The effect was simulated by treating each growth sector as a uniaxial crystal misaligned with respect to the adjacent sector oriented along an adjacent cube body diagonal. The magnitude and spatial distribution of the experimentally observed linear and circular retardance are reproduced with fidelity.

83
Probing Biopolymer Assemblies with Polarization-Resolved Second Harmonic Generation Microscopy
MacAulay Harvey, Saint Mary's University, 923 Robie St., Halifax, Nova Scotia B3H 3C3, Canada, Richard Cisek, Danielle Tokarz

Understanding how biopolymer assemblies organize across multiple length scales is essential for explaining their mechanical, optical, and biological function. Yet quantitative methods capable of measuring this hierarchical organization *in situ* remain limited because many conventional analytical techniques require disruptive labeling, extensive sample preparation, or provide only static structural information. Second harmonic generation microscopy (SHG) addresses these limitations by exploiting the intrinsic nonlinear optical response of ordered biological structures to quantify molecular organization without exogenous labels and with submicron spatial resolution. While conventional SHG provides sensitive structural contrast, image intensity alone cannot uniquely resolve the molecular organization responsible for the observed signal. By measuring the polarization dependence of the SHG response, polarization-resolved SHG (PSHG) enables quantitative determination of the second-order nonlinear optical susceptibility ($\chi^{(2)}$) tensor, transforming SHG into a quantitative analytical probe of hierarchical structure. This presentation will highlight recent advances in PSHG for characterizing hierarchical biopolymer assemblies. $\chi^{(2)}$ tensor elements will be related directly to molecular orientation, supramolecular organization, and ultrastructural order. Applications spanning collagen-rich tissues and biomineralized systems will demonstrate how $\chi^{(2)}$ tensor-derived observables reveal structural heterogeneity, monitor chemically and mechanically induced remodeling, and distinguish changes in molecular architecture that are not apparent from SHG image intensity alone. Together, these advances establish nonlinear optical measurements as powerful analytical tools for investigating the assembly, remodeling, and functional evolution of hierarchical biopolymer systems.

84
Developing an AI Based FT-IR Spectral Analytic Platform for Industrial R&I Analytical Applications
Peter Larkin, Syensqo, 1937 West Main St., Stamford, CT 06902, JD Cho, Francis Bruey

Traditionally, use of FT-IR spectroscopy to support research and innovation efforts in an industrial environment are optimized when resourced with an experienced subject matter expert. Typically, such work is non-routine and requires an extensive skill set and experience. Critical skills include expert-driven experimental design, sampling selection, instrumentation and software knowledge, as well as method development and data analysis expertise. One of the fundamental organization challenges is the retention and transfer of this institutional technical knowledge to less experienced scientists in the organization. GPT-based Artificial intelligence (AI) based large language models (LLMs) have been growing in capabilities and adoption in the scientific community for the past several years and offer the potential to address the knowledge gap resulting from the inevitable loss of technical talent. In the work we present here, we use a chatbot as a scientific copilot to guide less experienced analysts using FT-IR spectroscopy to solve industrial problems. The workflows include both IR spectral identification and identify verifications. The scientific GPT IR copilot extracts information and provides guidance in a fast and reliable manner. We will summarize the development process of this AI based GPT IR copilot, the validation by less experienced analyst and provide several industrial examples of its application.

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No abstract submitted by the author.

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Mid-IR, Near-IR, or Raman Spectroscopy: Which One Should I Use for My Problem?
Xiaoyun (Shawn) Chen, Dow Chemical, 5417 Vandemere Dr., Midland, MI 48642

Mid-infrared (Mid-IR), near-infrared (NIR), and Raman spectroscopy are among the most widely used analytical techniques for molecular characterization, quality control, and process monitoring. Because all three techniques probe molecular vibrations, they often appear interchangeable to non-specialists. In practice, however, each method has unique strengths, limitations, and optimal application spaces. This presentation provides a practical framework for selecting the most appropriate vibrational spectroscopy technique based on the analytical question, sample characteristics, and measurement environment. Fundamental differences among Mid-IR absorption, NIR overtone and combination-band absorption, and Raman scattering will be reviewed. Topics include sensitivity to molecular functionality, qualitative and quantitative analysis, sampling depth, spatial resolution, effects of water and fluorescence, suitability for solids, liquids, powders, and slurries, and considerations for laboratory versus process measurements. Using representative non-proprietary examples, the presentation will illustrate situations in which one technique clearly outperforms the others, as well as cases where multiple spectroscopic methods provide complementary information. Rather than promoting a single "best" technique, the talk emphasizes a problem-driven approach to method selection. Attendees will leave with practical guidelines that can help accelerate analytical decision-making, reduce trial-and-error experimentation, and maximize the value obtained from spectroscopic measurements.

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Tunable Diode Laser Absorption Spectroscopy: From Fundamental Spectroscopy to Industrial Process Analytics
Mark Druy, Galvanic Applied Sciences USA, 101 Billerica Ave., Ste. 104, North Billerica, MA 01862,

Tunable Diode Laser Absorption Spectroscopy (TDLAS) has evolved from a specialized laboratory technique into an important analytical technology for real-time industrial process monitoring. This presentation will provide an overview of the spectroscopic principles that underpin TDLAS and illustrates how these principles have been translated into highly selective and sensitive process analyzers. Beginning with the interaction of laser radiation and molecular species the discussion will review absorption spectra, spectral line shapes, and the Beer–Lambert law that enables quantitative analysis. Particular emphasis will be placed on the exceptional molecular selectivity of TDLAS, arising from the unique near-infrared and mid-infrared absorption features of target compounds. The presentation will examine the evolution of TDLAS instrumentation, including tunable semiconductor lasers, wavelength modulation spectroscopy, optical path enhancement techniques that enable rapid measurements with sub-ppm sensitivity. Recent advancements in the field will be highlighted, including quantum cascade and interband cascade lasers for expanded mid-infrared coverage, multi-species and multi-wavelength measurements, and increasingly rugged sensor architectures suitable for harsh industrial environments. Applications from the chemical, petrochemical, environmental, and emerging hydrogen sectors will be presented, including moisture measurement, leak detection, and detection of critical species such as NH_3, CH_4, H_2O, H_2S, and CO_2.

88
Advancing Ultra-Trace Chemical Analysis through Microextraction and Laminar Flow Tandem Mass Spectrometry
Emanuela Gionfriddo, University at Buffalo, 710 Natural Sciences Complex, Buffalo, NY 14260

Per- and polyfluoroalkyl substances (PFAS) present persistent analytical challenges due to their occurrence at trace and ultra-trace concentrations, the diversity of their physicochemical properties, and the complexity of environmental and biological matrices. Reliable determination therefore requires analytical workflows that provide high sensitivity and selectivity while effectively controlling matrix effects and contamination. Microextraction offers a powerful strategy to address these challenges by integrating analyte isolation and enrichment into miniaturized sample preparation workflows. This presentation will discuss the development and application of solid-phase microextraction (SPME) approaches coupled with liquid chromatography–tandem mass spectrometry (LC–MS/MS) for targeted PFAS analysis. The combination of selective extraction phases with sensitive tandem mass spectrometric detection enables enrichment of PFAS from complex samples while minimizing matrix components introduced into the analytical system. Particular emphasis will be placed on the role of extraction-phase chemistry and extraction conditions in controlling analyte recovery, selectivity, and method sensitivity across PFAS with different structural and physicochemical properties. The use of laminar-flow tandem mass spectrometry further supports ultra-trace analysis by enhancing ion transmission and providing sensitive and selective detection of PFAS following chromatographic separation. Together, microextraction and advanced LC–MS/MS instrumentation provide complementary mechanisms for improving analytical performance: sample preparation controls what reaches the instrument, while sensitive mass spectrometric detection expands the concentrations that can be reliably measured. These studies demonstrate how strategic integration of extraction chemistry and instrumental analysis can advance PFAS determination in complex matrices and support increasingly demanding applications in environmental and exposure analysis.

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Solid-State NMR and DNP Investigation of PFAS Binding in Zr-Based MOFs
Kunyu Wang, University of Pennsylvania, Dept. of Chemistry, Philadelphia, PA 19104, Duhan Zhang, Yaojun Zeng, Ashley Beers, Jochem Struppe, Daniel Banks, James Kempf, Shu Yang, Yifan Quan

Metal-organic frameworks (MOFs) are crystalline porous materials with tunable pore environments for molecular adsorption and separation. We have developed a Zr-based MOF with accessible binding sites for per- and polyfluoroalkyl substances (PFAS), persistent contaminants widely found in drinking water. Owing to its hierarchical porosity, the MOF removes over 90% of perfluorooctanoic acid (PFOA) from water within one minute. Strong coordination between Zr nodes and carboxylate groups also provides excellent chemical stability, enabling the framework to withstand strong acid for 24 hours. To elucidate the host-guest interactions, we performed solid-state NMR experiments at 500 MHz using a 1.3 mm HFX MAS probe. 1H MAS and 13C CP-MAS spectra of PFOA-loaded MOF reveal deprotonation of PFOA and formation of coordinated carboxylate species, with additional

stabilization through hydrogen bonding. Two-dimensional 19F-1H HETCOR spectra show direct spatial correlations between MOF hydroxyl protons and both CF2 and CF3 groups. Together with density functional theory (DFT) calculations, these results support a binding model in which the PFOA carboxylate headgroup coordinates to Zr6 clusters while the fluorinated chain extends into the pore and interacts with the framework. We further investigated dynamic nuclear polarization (DNP) at 400 MHz using AMUPOL as the polarizing agent. A 1H enhancement of ε ≈ 32 was achieved, whereas the 19F enhancement remained limited (ε ≈ 3), highlighting the need for improved HFX probe technology to enable more sensitive 19F-detected DNP studies of PFAS adsorption

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No abstract submitted by the author.

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Detecting the Presence of PFAS in Chicken Eggs: Assessing PFAS Contamination in Complex Matrices Using UHPLC-MS/MS Technology
Marisa Vatteroni, University of Connecticut, 3107 Horsebarn Hill Rd., Storrs, CT 06269, Austin Pelletier, Akshay Jayachandran, Anthony Provatas

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. 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. Using analytical methodology developed for the extraction and analysis of 28 PFAS, this study aims to determine PFAS concentrations in chicken products, mainly focused on various types of eggs. The extraction process was tested with and without the use of cleanup methods and through the use of the QSight500 UHPLC-MS/MS technology focusing on a robust triple quad mass spec system, the two extraction methods show minimal differences. Thus, resulting in a more efficient high-throughput workflow, which allows for further investigation into PFAS in consumer products and eventually leading to regulations that are necessary to protect public health.

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Advantages of Superficially Porous Particle Columns for HPLC and LC-MS
Stephanie Schuster, Advanced Materials Technology, Inc., 3521 Silverside Road; Suite 1-K, Quillen Building, Wilmington, DE 19810, Joshua McBee, Conner McHale, Peter Pellegrinelli

Superficially porous particle (SPP) columns have existed in one form or another since the late 1960s. However, the benefits of the particle with the solid silica core surrounded by the porous silica shell were not realized for small molecules until 2006 when the first sub-3-µm superficially porous particle columns were introduced. This particle technology enabled efficiencies similar to sub-2-µm fully porous particles (FPPs) without the need for ultra-high pressure capable instrumentation. As a result of the high efficiencies and lower back pressure, the columns may be run at faster flow rates to enable high throughput separations. Additionally, the SPP columns are rugged and offer long column lifetimes. This presentation will highlight comparisons between SPP columns and FPP columns for separations of pharmaceuticals, pesticides, and oligonucleotides.

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Monodisperse vs. Polydisperse Packing Materials in Liquid Chromatography: Implications for Performance and Practice
Fabrice Gritti, Waters Corporation, 34 Maple St., Milford, MA 01757

Since the foundational work of Horváth, Giddings, and Knox in the 1960s, the relationship between column performance (separation speed and resolution) and the macrostructure of randomly packed beds of spherical particles has been a recurring theme in liquid chromatography. The relentless push toward ever faster and more efficient separations has driven research, from elevated-temperature LC and ordered packing concepts to highly permeable monoliths, and ultimately to the transition from conventional HPLC (400 bar) to UHPLC (1000-1500 bar) enabled by sub-2 µm particles. Yet, despite these advances and the widespread availability of modern particle technologies, including sub-3 µm core–shell particles with relatively narrow particle size distributions (PSDs) and, more recently, quasi-monodisperse materials, the true impact of PSD on the fundamental speed-resolution trade-off of LC columns remains surprisingly unclear. Claims regarding the superiority of monodisperse packings are often repeated, but are frequently supported by limited experimental datasets, selective comparisons, or qualitative arguments rather than by rigorous, systematic, and physically grounded validation. In this context, PSD has arguably become a proxy for performance expectations rather than a well-quantified design parameter. Here, we critically re-examine 60 years of key simulation and experimental studies to disentangle established facts from persistent myths and opinions regarding the role of PSD in column permeability and efficiency. We further identify the critical physicochemical, structural, and packing-related parameters that ultimately determine performance, and discuss what truly limits the achievable speed-resolution envelope of randomly packed LC columns composed of spherical particles.

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Hardware-Driven Interactions in Oligonucleotide HPLC: The Role of Ion-Pairing and Oligonucleotide Chemistry
Peter Pellegrinelli, AMT, 3521 Silverside Rd., Wilmington, DE 19810, Stephanie Schuster, Barry Boyes

The influence of inert hardware on oligonucleotide separations by HPLC remains an underexplored but critical factor in achieving accurate recovery and robust performance. This study systematically compares inert and stainless steel (SST) column technology to evaluate the impact on analyte recovery, peak shape, and method reproducibility under ion-pairing conditions. Preliminary results demonstrate a strong dependence on ion-pair concentration. Using a single-stranded DNA ladder with 25mM hexylammonium acetate, SST systems exhibited severe oligonucleotide loss and poor peak shape relative to inert hardware. Although partial improvement in

recovery was observed over repeated injections, substantial discrepancies persisted even after multiple runs. In contrast, inert columns consistently provided high recovery and symmetric peak profiles. When the ion-pairing system was switched to 100mM triethylammonium acetate (TEAA), both SST and inert configurations yielded comparable performance, suggesting suppression of deleterious surface interactions at elevated ion-pair concentrations. Reducing TEAA to 25mM reinstated the pronounced loss of recovery on SST, confirming that ion-pair concentration, rather than ion-pair identity alone, is a dominant factor. Further investigation using a fully phosphorothioated antisense oligonucleotide (Gem-91, Trecovirsen) revealed persistent recovery deficits and peak distortion on SST even at 100mM TEAA, while inert hardware maintained high signal and favorable peak shape. These findings support a working hypothesis that oligonucleotide-metal interactions are both concentration- and chemistry-dependent. Ongoing studies are evaluating additional variables, including ion-pair type, counter-ion, nucleic acid structure (DNA vs RNA, single- vs double-stranded), and the potential for column passivation. This work highlights the importance of hardware selection in developing reliable oligonucleotide HPLC methods.

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Fast Mobile Phase pH Screen: Application in Pharmaceutical Analytical LC Method Development

Lynn Krushinski, Briston Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901, Yiyang Zhou, Ashley Liu, Kelci Schilly

Mobile phase pH is a crucial LC method attribute in pharmaceutical method development as it can affect analyte retention, on-column stability, and overall method robustness. Traditionally, if mobile phase pH optimization is performed, it relies on a series of trial-and-error experiments at different pH values or the software prediction of the pKa values for molecules of interest. These approaches can be labor intensive and may not give a complete or accurate picture of analyte behavior across a broad pH range under experimental conditions. Here we present a facile mobile phase pH screening workflow which enables fast screening across a broad pH range (1.5-12) via on-line mixing of phosphate or acetate buffer mobile phase on a RP-UPLC system. The utility of the MP pH screening system is demonstrated through multiple pharmaceutical LC method development case studies. These examples illustrate how the platform workflow enables 1) rapid assessment of retention time robustness across a defined pH range, 2) early detection of pH-dependent on-column stability issues, and 3) fine-tuning of MP pH for optimal chromatographic separation. These results highlight the broad applicability of this approach in expediting the development of robust, pH-optimized methods for complex, pH-sensitive separation challenges.

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Analytical Resolution of Bispecific Antibody Complexity: A Comprehensive Chromatographic Strategy

Yinan Zhang, Sepax Technologies, Inc., 5 Innovation Way, Ste. 100, Newark, DE 19711, Linting Li, Xuyang Zhang, Ke Yang

Bispecific antibodies (BsAbs) represent a major advance in therapeutic design, but their structural diversity creates significant analytical challenges. Unlike traditional monoclonal antibodies, BsAbs are prone to chain-pairing heterogeneity, including mispaired light and heavy chains and unwanted homodimer species such as knob-knob or hole-hole forms. Their structural asymmetry further introduces variability in size, charge, and hydrophobicity, while multiple variant pathways may coexist, including aggregates, fragments, and post-translationally modified species. As a result, no single chromatographic technique can provide sufficient resolution to fully assess the molecular integrity and stability of these complex biologics. To address this challenge, the Sepax platform offers an integrated suite of high-resolution chromatographic solutions that together provide a comprehensive analytical strategy for BsAbs characterization. The platform combines four orthogonal separation modes to deliver a broader and more informative view of BsAbs heterogeneity across development. Affinity chromatography enables targeted capture and enrichment of selected BsAbs populations, improving selectivity in complex samples. Size exclusion chromatography (SEC) monitors size heterogeneity by resolving monomers, aggregates, and fragments. Ion exchange chromatography (IEX) maps charge variants associated with post-translational modifications, structural asymmetry, and compositional heterogeneity. Hydrophobic interaction chromatography (HIC) complements these methods by resolving hydrophobicity-related variants and revealing subtle conformational or compositional differences. Together, these orthogonal methods establish a flexible and scalable platform for comprehensive BsAbs analysis, accelerating method development, strengthening critical quality attribute monitoring, and improving analytical confidence in modern biopharmaceutical development.

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Comprehensive Ion-Pair Reversed-Phase Workflow for Accelerated Oligonucleotide Separation in Pharmaceutical Development

Mohamed Hemida, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Erik L. Regalado, Rodell Barrientos, Emmanuel Appiah-Amponsah, Davy Guillarme, Jonathan Maurer

The pharmaceutical industry is rapidly advancing toward new drug modalities to address unmet needs, requiring the development of advanced strategies. Ion-pair reversed-phase liquid chromatography (IP-RPLC) is widely regarded as a reference technique for therapeutic oligonucleotides; however, separation remains challenging because retention and selectivity are highly sensitive to the nature of the ion-pairing reagent, organic modifier, and stationary phase. Here, we present a high-throughput IP-RPLC screening platform to systematically evaluate these parameters for the separation of challenging oligonucleotide impurities. The setup combined wide range of columns chemistries with multiple mobile phase compositions containing alkylamines of increasing hydrophobicity with either methanol or acetonitrile as organic modifier. Three representative commercially available oligonucleotide sample sets were investigated: shortmers and longmers from a synthetic antisense oligonucleotide, phosphodiester/phosphorothioate (PO/PS) oxidation variants related to 21-mer fomivirsen, and low-purity 18-mer synthetic ASO samples containing a broad range of process-related impurities. For the PO/PS impurities, the screening highlighted the strong influence of backbone chemistry on retention and demonstrated that PS content differences could be effectively discriminated using 25 mM triethylamine with methanol on a phenyl column. Finally, impurity profiling of low-purity synthetic ASO showed that 1 mM hexylamine with acetonitrile on a BEH C18 shield column provided the most informative chromatographic fingerprint, revealing increasingly complex impurity patterns as sample purity

decreased from > 95% to 30%. Overall, the proposed automated screening strategy enabled rapid identification of suitable IP-RPLC conditions for distinct oligonucleotide impurity classes and appears particularly well suited for accelerating method development for emerging therapeutic oligonucleotides.

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The Case for Accuracy and Quality in the Age of Superhuman Measurement and Intelligence: Lessons Learned from the NIH Common Fund
Christopher Kinsinger, National Institute of Health, NIH Common Fund, Bethesda, MD 20892

Throughout history, data sets, painstakingly collected and aggregated, have enabled transformative scientific breakthroughs. From early examples that laid the foundation for scientific empiricism to more recent collections that have catalyzed precision medicine, high-quality data sets based on accurate measurements have been fundamental to improving society through science. In this current era of Artificial Intelligence (AI), when analytical instruments exceed the limits of the naked eye and calculations detect patterns imperceptible to the human mind, science increasingly relies on accurate measurement and high-quality data. And yet, accuracy and quality, while necessary, are not sufficient. AI ready data for biomedical research require computability, sustainability, ethical standards, provenance, and explainability. To that end, the Common Fund of the National Institutes of Health (NIH) serves as an innovation hub to transform emerging technologies into established methods for advancing health sciences. From single-cell sequencing to the spatio-temporal architecture of the nucleome, the Common Fund has funded the advancement of next-generation technologies for the age of genomic medicine. Beyond technology development, the Common Fund also funds projects to catalyze research through data. Bridge2AI aims to define AI-ready data, and the Common Fund Data Ecosystem seeks to integrate data across Common Fund programs and beyond through consistently high quality, common standards, and user-friendly data infrastructure. With strategic resource allocation from funders such as the NIH Common Fund and continued care toward measurement and data quality by unsung heroes from labs across the country, the future of health science holds tremendous promise.

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Portable Spectroscopy – Quo Vadis?
Richard Crocombe, Crocombe Spectroscopic Consulting, 30 Thornberry Rd., Winchester, MA 01890

In the past thirty years spectrometers have shrunk dramatically in size, giving us successively laboratory-portable, toaster-sized instruments; cordless-drill-sized portable instruments for use in the field; and onto spectrometers the size of a computer mouse or deck of cards, and the latest sensors the size of a grain of sand with the possibility of a spectroscopic ‘ingestible’. We can take these smaller instruments to the sample,instead of vice versa. This size reduction has been achieved with acceptable performance reductions, for instance, in sampling versatility, spectral range, spectral resolution, and signal-to-noise ratio (SNR) that are balanced by the benefits of their portability. This talk will cover the latest technological advances, including low-cost multispectral sensors, sparse and computational spectrometers, metamaterials and flat optics, single photon avalanche photodiodes, and photonic integrated circuits. Because of their widespread applications, the technologies will improve rapidly, and their prices will drop, leading to a variety of portable and wearable spectroscopic applications. It’s estimated that around 400 million wearable devices (smart watches, rings) will be sold in 2026, and that one in four Americans has a wearable device. Miniature spectrometers and spectroscopic sensors will soon be widespread in the home, turning up in the most unlikely places, including toilets!

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Community Engagement with Portable Spectroscopy
Mary Kate Donais, Saint Anselm College, 100 Saint Anselm Dr., Manchester, NH 03102

Portable instrumentation provides opportunities to characterize samples that are challenging or even impossible to relocate to a laboratory. These field campaigns also connect researchers from varying areas of expertise (eg chemistry, archaeology, anthropology, art history) to join forces in pursuing research questions through multi-disciplinary research design. This talk will feature recent applications of portable spectroscopy to aid community partners with questions regarding objects in their collections.

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A Portable Revolution: Over-the-Horizon Spectroscopy
Pauline Leary, Noble, 1934 Bulls Head Rd., Stanfordville, NY 12581

The quick and reliable detection and identification of hazardous threats in the field is a critical capability dependent upon the use of portable spectrometers. These instruments have advanced significantly since 9/11. They are based upon many different technologies and can detect many different types of threats. They are optimized to meet aggressive size, weight, and power requirements, and remain functional when exposed to environmental stresses including extremes in temperature, vibration during storage and operation, and water ingress. The next natural step in their evolution is integration into unmanned vehicles. Primary benefits of this type of integrated system are (1) safety, i.e., threats can be detected remotely, and (2) improved situational awareness. Although the concept of over-the-horizon spectroscopy is straightforward, developing and deploying these systems locally, regionally, and globally is complex. For these systems to succeed, five key elements are required: spectrometers must withstand the unmanned vehicle's operating environment, hardware and software integrations must be seamless, data must be in an easily digestible format at the command center, there must be alignment of the unmanned system within the concept of operations (CONOPS) of the deploying organization, and reliable end-to-end communications must be established and maintained. In this presentation, a brief review of the history of these integrated systems will be described. In addition, challenges to the successful development and deployment of unmanned ground and air vehicles integrated with portable spectrometers for the remote detection of hazardous threats in local, regional, and global areas of operation will be detailed.

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Networked Hyperspectral Raman Spectroscopy for Real-Time Detection of Drugs and Pharmaceuticals
Ari Forman, Scatr, 331 Jevlan Dr., Unit 2, Woodbridge, ON L4L 8C1, Canada

The analysis of drug materials is increasingly challenging due to their chemical complexity, variability, and heterogeneous composition. Illicit drug samples are often mixtures containing multiple active substances, fillers, cutting agents, and trace adulterants that may be unevenly distributed throughout the material. Legal pharmaceutical products can present similar analytical difficulties, as the active pharmaceutical ingredient may constitute only a small fraction of a tablet or capsule, surrounded by a much larger proportion of excipients and other formulation components. In both cases, accurately identifying and characterizing the chemical composition of a sample requires analytical approaches capable of detecting and distinguishing compounds present across a wide range of concentrations. This presentation will discuss networked portable hyperspectral Raman spectroscopy as a field-based approach for analyzing complex drug samples. By collecting spatially resolved information across a sample, hyperspectral Raman mapping can help reveal chemical differences within the material and improve detection of mixtures. Networked instruments can also create a powerful real-time intelligence network across field sites, enabling rapid sharing of results, revealing emerging drug trends as they appear, and supporting applications ranging from monitoring a constantly changing unregulated drug supply to quality control of pharmaceutical formulations.

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No abstract submitted by the author.

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Data Analytics in Batch Release: Leveraging AI for Faster and Smarter Decision Making
Nikolai Makaranka, Daikon, 19 Dutch St #13 I, New York, NY 10038

Deviations are a leading cause of batch release delays and can contribute to drug shortages that directly affect patients. According to the FDA, more than half of drug shortages are associated with quality issues. Investigating deviations is often a lengthy and complex process, requiring laboratory studies, root cause analysis, and cross-functional collaboration. While these investigations generate valuable insights, the learnings often remain fragmented across reports and are rarely transformed into institutional knowledge that can be systematically reused. This presentation explores how AI can help organizations convert historical investigations into a continuously growing knowledge base that accelerates future investigations, improves problem solving, and supports proactive quality management. By synthesizing prior investigations, procedures, and supporting documentation, AI enables faster access to relevant precedents while promoting greater consistency across investigations. The presentation will also discuss how reasoning models can improve adherence to standard operating procedures (SOPs) and reinforce best investigation practices. Advanced optimization techniques, including genetic pareto optimization, can be used to refine AI reasoning and better capture how procedures are applied in real-world GxP environments. Finally, I will briefly discuss practical considerations for implementing AI in regulated GxP settings, including validation, governance, and human oversight.

105
Digital Twin—Enabling the Future of Development and Manufacturing of Pharmaceutical and Biopharmaceutical Products with Standardization and Consistency
Desmond Hunt, USP, 12601 Twinbrook Pkwy., Rockville, MD 20852

Digital twin technology is emerging as a transformative capability for pharmaceutical and biopharmaceutical development and manufacturing. By creating dynamic virtual representations of products, processes, equipment, and facilities, digital twins integrate real-time data with predictive modeling, simulation, artificial intelligence (AI), and machine learning (ML) to improve process understanding, optimize operations, and enable proactive, data-driven decision making. Potential applications span the product lifecycle, including formulation development, process scale-up, manufacturing process control, predictive maintenance, real-time quality assurance, supply chain optimization, and continuous process improvement. Although the technology has advanced rapidly, broader adoption remains constrained by challenges related to data integration, model credibility and validation, interoperability, workforce readiness, cybersecurity, and the lack of harmonized standards and regulatory expectations. As interest continues to grow, establishing a common scientific and regulatory framework will be essential to enable consistent, reliable, and compliant implementation across the pharmaceutical industry. This presentation provides an overview of digital twin concepts, architectures, and emerging pharmaceutical applications while examining the key scientific, technical, and regulatory considerations that will shape future adoption.

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No abstract submitted by the author.

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High-Throughput Small-Angle X-Ray Scattering for Biologics Developability Screening
Pin-Kuang Lai, Stevens Institute of Technology, 1 Castle Point Ter., Hoboken, NJ 07030

High-concentration monoclonal antibody (mAb) formulations are often limited by elevated viscosity arising from protein–protein interactions, yet early-stage developability assessment lacks high-throughput experimental screening methods. We developed a high-throughput small-angle X-ray scattering (SAXS) workflow that detects antibody self-association at dilute concentrations. Across 21 mAbs formulated in histidine buffer (pH 6.0), low-q structure factor transitions observed below 10 mg/mL accurately predicted viscosity at 150 mg/mL, correctly classifying all antibodies in the study. This scalable, sample-efficient approach enables rapid early-stage developability screening while providing experimental data for computational model validation. In this talk, I will demonstrate how this

SAXS platform can be broadly applied across multiple therapeutic antibody modalities, including conventional mAbs, T-cell engagers, and antibody–drug conjugates.

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Enhanced Rational Protein Engineering to Reduce Viscosity in High-Concentration IgG1 Antibody Solutions

Tyler Lefevre, AstraZeneca, 1 Medimmune Way, Gaithersburg, MD 20878, Jenna Caldwell, Austin Gallegos, Qun Du, Erin Williams, Gilad Kaplan, Reza Esfandiary

Subcutaneous (SC) delivery of therapeutic antibodies can offer multiple benefits to patients and healthcare providers, including convenience, time savings, and cost reduction. To improve the SC injection experience, drug developers may seek a low injection volume (1-2 mL), which for some antibody drugs necessitates a high concentration solution (≥100 mg/mL) to meet dosage requirements. Several molecular-level challenges hinder the development of high concentration antibody drug products, including high viscosity caused by reversible self-association (RSA). Here, we take an enhanced rational design approach to reduce RSA via protein engineering. Using hydrogen-deuterium exchange mass spectrometry (HDX-MS) and *in silico* modeling in Molecular Operating Environment (MOE), we identified potential self-interaction hotspots on the surface of an in-house IgG1 which has known viscosity issues at high concentration. With this information, several variants were rationally designed which vastly improved on the parent antibody's viscosity issues and offer insight into the mechanism of self-association. This workflow and strategy for developability improvement will be discussed alongside results and implications for future molecules.

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Biophysical Characterization as a Decision-Enabling Tool in Biologics Development

Ivan Budyak, Eli Lilly and Company, Lilly Corporate Ctr., 893 S Delaware St., Indianapolis, IN 46285

Biophysical characterization underpins critical decisions across the biologics development lifecycle, from candidate selection through process and product development to regulatory submission. As therapeutic modalities grow more complex, biophysical tools such as spectroscopy, calorimetry, light scattering, analytical ultracentrifugation, and electron microscopy have become increasingly important for assessing structural integrity, stability, purity, and other key product attributes. This talk highlights characterization strategies and practical case studies illustrating the essential role biophysical characterization plays in biologics development.

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Comprehensive Biophysical Characterization of Targeted Lipid Nanoparticles (tLNP) to Access and Enhance Physical Stability

Aaron Liao, AbbVie, 381 Plantation St, Worcester, MA 01605, Parvez Alam, Ronan Baynes, Shipra Malik, Christopher Uchechukwu, Dave Chu

Comprehensive analytical characterization is fundamental to understanding the critical quality attributes of tLNPs, thereby enabling innovation and risk mitigation throughout the development process. In vivo CAR T-cell therapy using tLNPs offers substantial advantages over conventional ex vivo approaches, including improved stability, enhanced delivery efficiency, reduced immunogenicity, and shorter manufacturing timelines at lower cost. The chemical stability of the multicomponent formulation of tLNP, as well as the physical properties of the overall system, remains critical to achieving appropriate delivery, efficacy, and manufacturability. While size and encapsulation efficiency are the most assessed tLNP attributes, these critical quality attributes alone do not adequately capture results from cell-based or animal-based assays. Techniques such as SAXS and cryo-EM have been highlighted for their ability to provide high-resolution structural information on tLNPs. However, these methods are costly and require substantial maintenance. Moreover, distinct formulations and cationic lipids may produce similar structural profiles despite markedly different physical and chemical properties. Accordingly, additional biophysical and particle characterization methods are needed to deepen understanding of tLNP behavior. Bio-layer interferometry (BLI) can be used to evaluate particle binding kinetics to antigens. Structural changes may also be reflected in distinct heat uptake profiles during melting or crystallization, which can be assessed using differential scanning calorimetry (DSC). In addition, FlowCam can be used to measure aggregate formation following cryogenic stress. Collectively, these advanced analytical approaches are essential for defining the physical and chemical stability of tLNPs and for supporting their quality, safety, and efficacy in clinical applications.

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SFC Separation and Purification of Hemp Extracts from Analytical to Prep and Beyond.

Jeff Caldwell, Princeton Chromatography, 259 Prospect Plains Rd., Bldg. L Cranbury, NJ 08512

Supercritical Fluid Chromatography (SFC) has proven successful for analysis and purification of natural products dating back decades. SFC is particularly useful for separation of cannabinoids in hemp extracts. We have developed a series of novel stationary phases for the specific purpose of SFC purification and isolation of various cannabinoids, with the ability to scale to pilot/process size SFC instrumentation. This work will show several real world projects where SFC was employed to purify a range of hemp derived wide spectrum extracts, including for THC remediation, minor cannabinoid isolation, and Delta8/Delta9 separation. The focus across all projects is on optimizing low modifier percentage, cycle time, and efficiency. We will show analytical and lab scale preparative SFC chromatography, as well as examples of where our stationary phases have scaled to larger industrial SFC units and processes.

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The Use of Supercritical Fluids in the extraction and purification of Phytochemical Analytical Standards

René de Vaumas, Extrasynthese, Impasse Jacquard. Genay 69730, France

Phytochemical Analytical Standards are samples of small molecules issued from plants, highly purified and precisely quantified to be used for calibration of quality control or research experiments. Since 1986, Extrasynthese (France) has developed a large catalog of these particular standards. They are generally issued from complex natural mixtures, hence extraction and purification process are usually quite challenging to get materials. Among many chromatographic technics (HPLC, CPC, SEC, …), supercritical-CO2 chromatography (SFC) can provide unique selectivity or performance. Case studies will be presented.

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From Production to Extraction & Analysis: A Chemical Perspective on Citrus
Jeff Povelones, International Flavors & Fragrances, 4330 Polytechnic Circle, Lakeland, FL 33805

Citrus flavors are shaped by a complex interplay of raw material selection, extraction technology, chemical composition, and sensory perception. This presentation explores how analytical chemistry provides critical insights into the factors that define citrus flavor quality, authenticity, and performance. Beginning with an overview of citrus processing and extraction methods, to include traditional approaches used to obtain essential oils and flavor materials, the discussion highlights how processing conditions influence the resulting chemical profile. The presentation then examines the major classes of compounds that contribute to citrus flavor, including terpenes, oxygenated compounds, sesquiterpenes, and other trace constituents that drive characteristic aroma and taste attributes. Through the application of chromatographic techniques, relationships between chemical composition and sensory experience are explored, demonstrating how subtle compositional differences can significantly impact flavor perception. Finally, the role of analytical tools in authenticity assessment and product characterization is discussed, with examples illustrating how chemical profiling can be used to differentiate origins, processing methods, and commercial products. By connecting extraction practices, compositional analysis, and sensory outcomes, this presentation provides a comprehensive overview of how chemistry can be leveraged to better understand, evaluate, and innovate within the world of citrus flavors.

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How to Detect Adulteration in Supercritical Fluid Extracts: Special Focus on Ginger CO_2
Prabodh Satyal, Aromatic Plant Research Center, 230N 1200E, Ste. 100, Lehi, UT 84043

Supercritical fluid extraction is increasingly used for flavors, fragrances, nutraceuticals, cosmetics, and botanical ingredients because CO_2 extraction can preserve both volatile aroma compounds and heavier, less volatile plant constituents. This broader chemical range is valuable, but it also creates new authenticity challenges. Unlike traditional essential oils, CO_2 extracts may contain terpenes, pungent markers, lipids, and other extractable compounds, so routine volatile profiling alone may not be sufficient to detect adulteration. This presentation will discuss practical analytical approaches for detecting adulteration in supercritical fluid extracts, with a special focus on ginger CO_2 extract. Ginger CO_2 is an excellent example because its authenticity depends on both aromatic terpenes and pungent compounds such as gingerols and shogaols. GCMS is used to evaluate volatile constituents as well as gingerols and shogaols, while chiral GCMS helps detect the addition of isolated or synthetic terpenes by comparing enantiomeric patterns with authentic ginger CO_2 references. Potential adulteration includes the addition of steam-distilled ginger oil, the blending of different extract types, the dilution with vegetable oils, and the mislabeling of solvent-assisted extracts as pure CO_2 extracts. The addition of vegetable oil is evaluated using derivatization and FAME analysis to detect nonvolatile lipid adulterants.

115
Molecular Simulation of Hydrophilic Interaction Chromatography (HILIC): How HILIC works
Mark Schure, Kroungold Analytical, 1299 Butler Pike, Blue Bell, PA 19422, Hsiao-Feng Liu, Chung-Kai Chang, J. Ilja Siepmann, Stephanie Schuster, Peter Pellegrinelli, William Miles

In this talk we probe deeper into HILIC using Monte Carlo (MC) simulations of atom-based models. Specifically, we examine the distribution of the solvent components within stationary phases of silica (both crystalline and amorphous models), and a diol phase. Solutes include a number of small molecular weight alcohols and diols. These simulations are verified using experimental data of the same solute and solvent system. We emphasize the role of stationary phase-bound water in this study as previous work from other laboratories has suggested a tightly-bound layer of surface water as the effective stationary phase. These findings were obtained by Molecular Dynamics (MD) simulations which are time-marching simulation techniques limited to roughly a microsecond of physical time. Conversely, MC simulations march through phase-space and provide a direct handle to thermodynamics information, which is lacking in MD simulations. We find no evidence of tightly-bound surface water in these simulations. However, we note that analysis of the hydrogen bond network shows that additional water near the surface provides additional retention points for alcohol solutes. This spatial arrangement will be described in detail. In addition, the thermodynamic analysis of the model HILIC system, through free energy analysis, provides a complete description of the retention process. This focus in this talk includes mixed-mode behavior of solutes where both stationary phase and solvent contribute to the dominant retention mode at high-water solvent concentrations, as compared with the “standard” HILIC experiment at high organic solvent concentrations.

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Insights into Hydrophilic Interaction Chromatography by Quantitative Assessment of Retention Mechanisms
Yong Guo, Fairleigh Dickinson University, 230 Park Ave., Florham Park, NJ 07932

Hydrophilic interaction chromatography (HILIC) has been increasingly applied to the separation of both small and large polar compounds. However, polar compounds exhibit complex retention behaviors and selectivity in HILIC driven by different retention mechanisms (hydrophilic partitioning, surface adsorption, and electrostatic interactions). Many observed retention behaviors are difficult to explain based on simple qualitative understanding of the retention mechanisms and the selectivity is not linked to the retention mechanisms. To address this knowledge gap, we have developed a quantitative assessment methodology to quantify relative contributions of various retentive forces involved in the retention of polar compounds. Quantitative assessment of retention

mechanisms allows us to identify the main retention mechanism for specific compounds in a chromatographic system consisting of the stationary and mobile phases. In addition, we have applied the quantitative assessment methodology to evaluate the retention behavior of polar compounds at different water levels, the effect of organic solvent modifiers (e.g., methanol and isopropanol), and the use of polar stationary phases based on Type C silica. Quantitative assessment of retention mechanisms also provides a means to better understand the selectivity for polar compounds, especially isomers in HILIC and identify the driving force for selectivity. In summary, quantitative understanding of the retention mechanisms not only helps fill the knowledge gap in HILIC, but also provides meaningful guidance to difficult separations in HILIC.

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From Retention Mechanisms to Method Development: HILIC for Nucleic Acids

Jonathan Maurer, University of Geneva, School of Pharmaceutical Sciences, CMU - Rue Michel Servet, 1, Geneva, 1211 Switzerland, Agathe Bousquier, Camille Malburet, Jean-François Cotte, Megane Aebischer, Agathe Depraz-Depland, Davy Guillarme

As oligonucleotide- and mRNA-based therapeutics continue to transform modern medicine, HILIC is increasingly recognized as a powerful analytical tool for the characterization of these complex biomolecules. However, despite its growing adoption, the molecular interactions governing nucleic acid retention remain incompletely understood, often limiting the development of predictive analytical methods. This presentation will explore how advances in the understanding of HILIC retention mechanisms can directly support analytical method development for therapeutic nucleic acids. Recent investigations into oligonucleotide retention will be discussed, highlighting the respective contributions of partitioning, adsorption, hydrogen bonding, and solvophobic interactions. These mechanistic insights provide a rational framework for understanding selectivity and optimizing separations. The second part of the lecture will illustrate how these fundamental concepts have been translated into practical applications for the characterization of therapeutic oligonucleotides and large messenger RNA molecules. Recent developments from our laboratory will be presented, including HILIC-based approaches for the assessment of mRNA integrity and encapsulation efficiency. The presentation will also highlight how HILIC complements existing chromatographic techniques and addresses emerging analytical challenges associated with increasingly large and structurally complex RNA therapeutics. By connecting chromatographic fundamentals with real-world pharmaceutical applications, this lecture aims to demonstrate that a deeper understanding of retention mechanisms is not only of academic interest but also a key driver for the development of innovative and predictive analytical methods for the next generation of nucleic acid therapeutics.

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Examples of Using Hydrophilic Interaction Liquid Chromatography for Small Molecule Analyses: A Case Study of Radiopharmaceutical Precursors

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

Radiopharmaceutical development requires the creation of a linker-ligand conjugate, whether it be small molecule in design or a monoclonal antibody, and bonding a metal chelator, which would deliver the radionuclide to the cancerous site. While the linker-ligand conjugate may have considerable hydrophobicity, the chelating group is, by definition, highly polar with multiple electron rich sites ideal for chelating to a electron deficient metal. In this presentation, background information into radiopharmaceuticals is given including the importance of vial selection. Next, examples of analyzing the precursors of PSMA-617, an unbound radiopharmaceutical, using reversed-phase and HILIC chromatography will be shown. The use of HILIC chromatography shows improved retention for the chelating group and alternate selectivity for the other components due to the unique selectivity that HILIC provides.

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Expanding Nontargeted Analysis with Hydrogen Carrier Gas for High Resolution Separations in Forensic Science

Kira M. Fisher, William & Mary, Nontargeted Separations Laboratory, Chemistry Department, 540 Landrum Dr., Integrated Science Center 1053, Williamsburg, VA 23185, Emma L. Macturk, Katelynn A. Perrault Uptmor

The selection and control of carrier gas are critical factors influencing the performance of both one-dimensional gas chromatography (1D GC) and comprehensive two-dimensional gas chromatography (GC×GC). Helium is widely used due to its inertness, but increasing cost, limited supply, and reliance on fossil fuel extraction raise sustainability concerns. Hydrogen offers a viable alternative, with faster optimal linear velocity to reach comparable efficiency, and more sustainable production through generators relying on water electrolysis. While tools exist to translate 1D GC methods from helium to hydrogen, their application to GC×GC has not been well established, particularly for parameters unique to multidimensional separations. This work presents the translation of helium to hydrogen carrier gas for cryogenically-modulated GC×GC-MS. Three translated methods derived from 1D tools were evaluated alongside modulation period using standard mixtures, followed by validation with authentic fingermark residue samples in a forensic application. The translate option, using a combination of flow and temperature adjustments that matched column efficiency between helium and hydrogen, delivered comparable peak capacity, as well as acceptable S/N, tailing factors, and resolution values between key peak pairs. Modulation period could be reduced by 50%, providing a practical guideline for method translation. Hydrogen reduced overall run time by up to 60%, increasing sample throughput and lowering per-sample energy consumption. Although sustainability comparisons are nuanced, hydrogen showed improved green chemistry alignment overall, supported by higher sustainability metric scores and gains in efficiency and resource use.

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Green Extraction Methodologies for Drugs of Abuse

Emanuela Gionfriddo, University at Buffalo, 710 Natural Sciences Complex, Buffalo, NY 14260-1660

The rapid evolution of the illicit drug market and the increasing complexity of biological matrices create significant challenges for analytical toxicology, requiring methods that combine sensitivity, selectivity, speed, and sustainability. Sample preparation remains a critical component of these workflows and often represents a major source of solvent consumption, waste generation, and analytical time. Green extraction methodologies provide opportunities to reduce this footprint while maintaining the analytical performance required for reliable drug screening and quantification. This presentation will discuss the development and application of microextraction strategies for the determination of drugs of abuse in complex matrices, with emphasis on solid-phase microextraction (SPME) and thin-film microextraction (TFME). These approaches integrate sampling, analyte isolation, and preconcentration while substantially reducing or eliminating organic solvent consumption and minimizing sample requirements. Applications to the analysis of illicit drugs in beverages and biological fluids demonstrate how microextraction can be combined with chromatographic and direct mass spectrometric detection for rapid analysis. More recently, biocompatible SPME has enabled quantitative determination of cannabinoids directly from plasma, illustrating the potential of matrix-compatible extraction phases to simplify sample preparation while addressing challenges associated with complex biological matrices. Beyond solvent reduction, the sustainability of analytical workflows requires consideration of sample volume, materials, energy consumption, analysis time, automation, and waste generation. These examples illustrate how advances in extraction materials, device geometry, and coupling with mass spectrometry can support more efficient and sustainable analytical strategies for drug analysis without compromising analytical performance.

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Innovations in Forensic Applications of Portable Spectroscopy

Brooke Kammrath, University of New Haven, Henry C. Lee Institute of Forensic Science, 300 Boston Post Rd., West Haven, CT 06516

Portable spectroscopy has moved well beyond single-purpose field screening to a diversified toolkit capable of confronting increasingly complex forensic samples. For example, recent innovations in portable Raman spectroscopy, including surface-enhanced (SERS), spatially offset (SORS), standoff, and hyperspectral imaging modalities, now allow examiners to address longstanding challenges such as fluorescence interference, subsurface penetration, trace-level detection within heterogeneous mixtures, and safe analysis from a distance. Emerging systems integrate AI/ML-based algorithms for real-time classification, complete a full threat survey in under ten seconds, and perform reliably in direct sunlight, with select instruments deployable on drones and robotic platforms for standoff detection of chemical warfare agents, explosives, and toxic industrial chemicals from a distance of up to 15 meters. These advances arrive as the illicit drug supply grows more complex and potent, with fentanyl analogs, novel psychoactive substances, and poly-drug mixtures outpacing traditional reference libraries and presumptive colorimetric tests, the reliability of which is now being restricted by law in several states. This presentation will highlight innovations in portable spectroscopy and spectrometry and include case studies demonstrating how these tools are strengthening the delivery of rapid, reliable, and legally defensible results in criminal justice.

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Field and Lab Portable LC Instrumentation for Drugs of Abuse

Ira Lurie, The George Washington University, 2100 Foxhall Rd. NW, Washington DC, 20007

Liquid chromatography (LC) is a highly versatile technique for the separation of seized drugs due to its ability to analyze target compounds that are thermally degradable, polar and non-volatile, analytes that are problematic by gas chromatography. However, LC employing conventional and microbore columns (1.5- 4.6 mm ID) uses a large amount of highly toxic solvents such as acetonitrile and methanol and generates a significant amount of waste. The use of capillary LC columns (150-500 µm ID) provides for substantial lower flow rates which result in significantly lower solvent consumption and much less generated waste. In this vein the use of portable LC instrumentation weighing as little as ~ 18 lbs. employing capillary columns for the analysis of seized drugs will be discussed. This instrumentation not only allows onsite analysis, which determines whether the seizure should be sent for further analysis, and provides for probable cause for a defendant. In addition, a portable LC provides a viable alternative for bulky relatively expensive LC instrumentation in a laboratory setting. The use of single and dual capillary columns with fixed UV or diode array detection for the analysis of seized drugs will be discussed. Employing a C8 and biphenyl column in series with on column dual LED UV detection provides for relatively uncorrelated retention times, and somewhat unique absorbance ratios. An analytical eco-scale will be used for assessing the greenness of the portable instrumentation (1).

(1) Agnieszka Gałuszka et. al., Analytical Eco-Scale for assessing the greenness of analytical procedures, TRAC 37:61 (2012) http://dx.doi.org/10.1016/j.trac.2012.03.013

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Molecular-Level Understanding of the Interactions Between Model Nanoplastics and Phospholipid Vesicles (Liposomes)

Kushani Mendis, University of Maryland Baltimore, Department of Chemistry and Biochemistry, 1000 Hilltop Circle, Baltimore, MD 21250, Laura Kesner, Zeke Piskulich, Qiang Cui, Zeev Rosenzweig

Nanoparticles induce rearrangements of cell membranes that can affect membrane function. Our previous work uncovered a unique mechanism underlying interactions between polystyrene nanoplastics (PSNPs) and phospholipid vesicles (liposomes). The study used laurdan-labeled liposomes and PSNPs as a model for nanoplastics. Laurdan is a phase-sensitive fluorophore. Results showed that interactions between PSNPs and laurdan-labeled liposomes induced a membrane phase change. Computational studies showed that non-cross-linked carboxylated PSNPs are electrostatically attracted to positively charged domains of the liposome membrane. Hydrophobic interactions between dangling polystyrene chains and phospholipids facilitate the intercalation of the nanoparticles into the membrane. Our ongoing study builds on this previous work. It aims to understand the parameters that affect the strength of interactions between nanoplastics and phospholipid membranes, as well as the general applicability of the discovered mechanism. For example, is the mechanism specific to PSNPs, or is it also applicable to other types of nanoplastics? We employ small-angle X-ray scattering (SAXS) and fluorescence anisotropy to understand the interactions between nanoplastics and liposome membranes at the molecular level. SAXS provided insights into the interactions between nanoplastics and liposomes by analyzing X-ray scattering

patterns. SAXS detects structural changes in liposomes upon interaction. Fluorescence anisotropy is a sensitive analytical technique that provides information on membrane order/disorder and membrane phase transitions due to interactions with nanoplastics. The ongoing experiments utilize Isothermal Titration Calorimetry (ITC) as a complementary technique to assess the binding dynamics of these interactions. This research aims to increase awareness of the adverse effects of nanoplastics on environmental organisms.

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Quadrant-Based Large-Area SEM–EDS Mapping for Elemental and Morphological Characterization of PM2.5

Motunrayo Oladele, University of Kentucky, 506 Library Dr., Lexington, KY 40503, Marcelo Guzman

Fine particulate matter consists of particles with heterogeneous morphologies and elemental compositions, making representative characterization difficult when electron microscopy is restricted to manually selected particles or a limited number of fields of view. This presentation examines a spatially distributed scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS) approach for characterizing PM2.5 filter samples collected in Tucuman, Argentina. A quadrant-based large-area mapping strategy was applied to PM2.5 samples collected on fiberglass and nitrocellulose filters during different periods of 2023. Elemental maps were interpreted alongside particle morphology to assess spatial heterogeneity and identify distinct particle populations. Large-area mapping results were evaluated against manual single-particle analysis for representative higher- and lower-loading fiberglass filters. The two methods showed strong agreement in elemental composition for the representative burning and non-burning-period samples. Microscopy revealed soot-like aggregates, tar-ball-like particles with mixed elemental compositions, while elemental analysis showed enhanced Si and K signals and a shift toward Si-rich compositions during the active burning period. Differences between sampling periods were consistent with changing source contributions, including agricultural burning, rather than a single exclusive source. These results demonstrate how quadrant-based LAM SEM-EDS can provide a spatially representative, semi-quantitative approach for characterizing heterogeneous atmospheric particles and comparing particle composition across sampling periods.

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Preparation of Standards and Spectroscopic Analysis of Wooden Furniture Finishes for Determining Period of Manufacture

Ethan Radcliffe, West Chester University, 1230 Harper Ave, West Chester, PA 19094

In the field of art conservation, spectroscopy is the ideal method for non-invasive analysis of art and artefacts. Although there are compendiums of spectral data for natural and synthetic organic compounds, they are mostly limited to pure substances and so the art conservation community lacks a database of spectra for complex mixtures. Because traditional wood finishes are often mixtures of several resins or gums in a solvent, their spectral analysis cannot be easily compared to the existing literature for pure substances. This study seeks to characterize various ingredients common in 18th century wooden furniture finishes using Raman and FTIR. Prior research of period recipes informed preparation of an array of standards of about twelve different resins/gums that are dissolved in two different solvents (ethanol and turpentine), and then binary and ternary combinations of each solvent system made prior to analysis. Standards, applied to a wood substrate and scanned, will be compared against scans of an unknown wood finish and an attempt will be made to identify the make-up of the unknown.

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Comparative MALDI-ToF/ToF Analysis of Digested and Non-Digested <10 kDa SerumPeptidomes for Early Detection of Breast Cancer

Shaelee Torres, Clarkson University, Chemistry Department, Potsdam, NY 13699, Sarah Abbott, Pathea Bruno, Costel Darie

Breast cancer (BC) remains a leading cause of cancer-related mortality among women worldwide. Early-stage disease is often associated with dysregulation of circulating proteins and peptides, offering inimally invasive biomarkers, particularly for younger women with reduced screening sensitivity to mammograms. Mass spectrometry (MS)–based proteomics and peptidomics are well suited for detecting low-abundance peptides with high sensitivity and specificity and serum analysis provides a window into tumor-derived peptides and host responses, In this study, serum samples from women with invasive ductal carcinoma and no or limited lymph node involvement, alongside age-matched controls (50 vs. 50), were filtered using 10 kDa molecular weight cutoff (MWCO) filters where the eluted flow through is analyzed (>10 kDa). Previous peptidomics datasets revealed upregulation of fibrinogen alpha chain (FGA) peptides, including the presence of m/z 1465 peak within BC samples representative of the sequence DSGEGDFLAEGGGVR. To further validate the identification of FGA in serum, the current study evaluated the >10 set using MALDI-ToF-ToF analysis. By comparing both digested and non-digested peptides we aim to identify more peptides or even proteins to perform LC-MS/MS and validation multiple reaction monitoring (MRM) analysis. Current validation using quantitation software mascot distiller supports these results, and extending MALDI-MS analysis to the <10 kDa fraction is anticipated to further reinforce the consistency of FGA-derived peptides as candidate serum biomarkers for early-stage BC.

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Understanding Mead Production via Comprehensive Two-Dimensional Gas Chromatography, Fermentation Kinetics, and Chemometric Analysis

Sarah C. Foster, William & Mary, Integrated Science Center 1, 540 Landrum Dr., Williamsburg, VA, 23185, Samuel W. Straight, Katelynn A. Perrault Uptmor

Mead is a fermented beverage made from honey, water, and yeast. Factors such as honey concentration and yeast strain can impact the volatile organic compounds (VOCs) produced from the metabolism of sugar during fermentation. Despite the increasing consumer popularity of mead, the relation between its production variables and final aroma profile remains an under-examined topic. The mixture of VOCs from mead 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 examine mead produced from five strains of yeast and brewed with three different starting honey concentration using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry and flame ionization detection (GC×GC-TOFMS/FID) as well as assess kinetic data collected during the fermentation process. Chemometric analysis included Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), Hierarchical Cluster Analysis (HCA), and Partial Least Squares (PLS) regression. Preliminary results demonstrated a relationship between honey concentration and VOC abundance. Understanding the relationship between production variables and the final VOC profile of mead will contribute to consumer understanding and allow manufacturers to produce specific flavors in a more efficient manner.

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Fluorescence Sensing of Lamotrigine Enabled by Nitrogen-Doped Carbon Dots

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In the pharmaceutical industry, the detection of analytes with high selectivity and sensitivity is a common goal. In this study, we demonstrate the feasibility of achieving this goal using carbon dots (CDs): quasi-spherical nanoparticles that exhibit tunable fluorescence based on choice of precursor materials and dopants. They are recognized for their ease of synthesis and versatility as fluorescent probes. Previous studies have shown enhanced fluorescence emission of nitrogen-doped CDs in the presence of atrazine, a herbicide and common water contaminant. However, our current study focuses on lamotrigine, a wastewater-persistent pharmaceutical that – like atrazine - possesses a triazine core, and due to this structural similarity, we hypothesized that N-doped CDs synthesized from o-phenylenediamine in ethanol (oPD-CDs) could prove effective as fluorescence sensors for lamotrigine. A bottom-up, solvothermal synthesis method for oPD-CDs was conducted. Maximum emission was observed at 570 nm (following excitation at 420 nm), with intensities linearly correlated to lamotrigine concentrations up to 300 μM. For samples initially prepared with 0.30 mg/mL of oPD-CDs, the limits of detection for lamotrigine and atrazine were determined to be 50 nM. A series of interference panels will be analyzed to determine oPD-CD selectivity. Futhermore, capillary electrophoresis with laser-induced fluorescence detection (CE-LIF) will be used to characterize oPD-CD properties in free solution by comparing unbound vs. analyte-associated CDs. By coupling the resolving power of CE with the sensitivity of LIF detection towards oPD-emission, we aim to develop uniquely selective, sensitive, and rapid assays for lamotrigine and atrazine.

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Conformal Predictions of Synthetic Data for Pharmaceutical Analysis

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A recent development in data science is synthetic data. “Synthetic data” are vectors of data which have been built from linear combinations of real data, or data that has been made artificially noisy in some way. Many methods aim to generate supplemental data that is unbiased and represents the noise patterns of real instrumental measurements. Synthetic data can be used to “mix” data to represent possible spectra for analyte combinations that do not exist in the dataset. Via a dataset of Raman spectra of various pharmaceutical ingredients, I investigate the utility of synthetic data in building a more robust dataset that can treat analyte mixtures that have not yet been measured. Conformal predictions are frameworks for quantifying uncertainty in the predictions. Fundamentally, they do so by converting an algorithm’s predictions into prediction sets. As opposed to giving an output with a certain related probability, conformal predictions give confidence intervals that contain all class identities that sum to a total x probability. This gives more specific information than just the most probable class. For a mixture with the character of multiple classes, any singular class would be a less probable match for the data. However, the conformal prediction confidence interval would (ideally) contain the class identities that together represent the mixture. Using the Raman dataset, I build upon prior work in uses of conformal prediction, to help address some of the struggles in classification found in quality control or process analytical technology.

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Detecting Micro- and Nanoplastic Uptake in Human Cancer Cells with Spatially Resolved 3D Raman Spectroscopy

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The continued use of plastic in manufacturing has led to a proliferation of microplastics (MPs) and nanoplastics (NPs) in food, water, and the air. Though research is ongoing, current literature suggests that MPs and NPs have a toxic effect on a variety of human cell lines. Detecting and quantifying particle uptake is a key component of research aiming to investigate cell response to MP and NP particles. 3D spatially resolved Raman spectroscopy is a non-destructive method of MP and NP detection that provides important data on particle localization within the cell as well as chemical identity of the particles. While polystyrene is heavily studied in uptake literature, other polymers like polyvinyl acetate and polyurethane remain understudied. In this study, human cancer cells incubated with MPs and NPs were scanned using 3D Raman spectroscopy, and spectra collected were used to generate spatially resolved images of polymer particles within cells. Data collected was analyzed using a MATLAB algorithm constructed to identify the presence and type of polymer particles in the cell. From this analysis, limits of detection for the 3D Raman scanning technique for various polymers were determined.

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Synthesis, Characterization and NMR Spectral Deconvolution Assignment of Ir(III) Cyclometalated Complexes Using 2,3-diphenylquinoxaline Derivatives

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Organic Light Emitting Diodes (OLEDs) are thin-film electronic devices that have become central to modern display technology, being widely implemented in cell phones, televisions, and computer screens. Their appeal stems from their ability to produce brighter light emission while enabling thinner, lighter, and more flexible devices compared to conventional inorganic light-emitting technologies. The emission properties of OLEDs are determined by the choice of emissive materials, often including transition metal complexes. Iridium-based complexes are of particular interest due to their favorable photophysical characteristics, such as high radiative quantum yields, short excited-state lifetimes, and overall chemical stability. These attributes make iridium complexes promising for efficient and durable OLED performance. This work focuses on the design, synthesis, and characterization of new heteroleptic iridium complexes of the general formula $[Ir(C^N)_2(N^N)]^+$. In this structure, C^N denotes cyclometalating ligands such as 2,3-diphenylquinoxaline or 2,3-*bis*(4-fluorophenylquinoxaline) while N^N represents bidentate nitrogen-donor ligands including 4-methyl-1,10-phenanthroline. Comparing the non-fluorinated complex to the fluorinated complex will give insight to how the structural modifications influence electronic properties as well as the compounds behavior through nuclear magnetic resonance spectroscopy. Here we report the synthesis and characterization of a series of iridium complexes following the $[Ir(C^N)_2(N^N)]^+$ pattern. Characterization was performed using 1D and 2D NMR spectroscopy, including: ^1H, ^{13}C, COSY, HSQC, HMBC, inverted direct response HSQC-TOCSY, and ROESY.

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Multidimensional NMR Assignment of Cyclometalated Iridium(III) Complexes Enabled by a Fluorinated Analogue

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Cyclometalated iridium(III) complexes present significant challenges for structural characterization due to extensive spectral congestion arising from ligand frameworks and reduced symmetry upon coordination. In this work, we report a systematic NMR based strategy for the complete structural assignment of a homologous series of complexes of the form $[Ir(C^N)_2(N^N)]^+$, where C^N = 2,3-diphenylquinoxaline derivatives and N^N = 1,10-phenanthroline. Central to this approach is the initial synthesis and analysis of a fluorinated derivative, [Ir(2,3-*bis*(4-fluorophenylquinoxaline))$_2$ 1,10-phenanthroline]PF_6, which served as a foundational reference for spectral assignment. The incorporation of fluorine introduced ^{13}C-^{19}F heteronuclear coupling, facilitating the unambiguous assignment of carbon environments, including cyclometalated and quaternary positions *via* $^nJ_{CF}$ heteronuclear coupling. These features, combined with enhanced spectral dispersion, provided a robust starting point for assignment. A combination of 1D and 2D NMR techniques, including COSY, HSQC, HMBC, inverted direct response HSQC-TOCSY, and ROESY, was employed in a stepwise and integrated manner to establish proton-proton connectivity, proton-carbon correlations, long-range heteronuclear interactions, and relative ligand orientation. The assignments derived from the fluorinated complex were systematically extended to the remaining analogues through comparative analysis of chemical shifts and coupling patterns. This work demonstrated that strategic incorporation of a fluorinated moiety enables resolution of highly congested spectra and provides a transferable framework for complete structural assignment in cyclometalated iridium(III) complexes.

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Overcoming Helium Supply Issues by Adopting Hydrogen as a Carrier Gas for GC/MS and GC/MS/MS Techniques in Environmental Laboratories

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In response to helium shortages, tools and best practices now enable a predictable transition of GC/MS methods to hydrogen carrier gas. Hydrogen is the most practical alternative to helium for many GC/MS applications, but successful conversion requires deliberate evaluation of both hardware and method parameters. This presentation summarizes proven hydrogen implementations across pesticides, volatile organics (VOAs), semivolatiles (SVOCs), and polycyclic aromatic hydrocarbons (PAHs). Key conversion elements include sample introduction/injection technique, column dimensions and phase ratio, and EI source selection. Method translation, combined with retention time locking, can preserve elution order and closely match helium retention times. The HydroInert EI source, optimized for hydrogen carrier gas, helps mitigate in-source reactions observed for certain analytes. When recommended conditions are followed, excellent library match, strong peak shape, and sensitivity approaching helium performance can be achieved. Because some compounds are more reactive in hydrogen, this talk also highlights practical guardrails and examples where hydrogen performance has been demonstrated: 1. VOAs by headspace-GC/MS: accurate quantitation from 0.05–25 ppb, sub-ppb MDLs, and an average NIST 2020 library match score of 94. 2. SVOCs: linearity maintained across a wide calibration range (0.1–100 ppm). 3. PAHs in soil: hydrogen method improved peak shape and internal standard stability, with linearity from 0.1–1,000 ppb and detection limits as low as 0.09 ppb by GC/MS/MS. Overall, these guidelines have enabled successful conversion of multiple GC/MS methods to hydrogen across diverse applications.

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Improved Baseline Stability and Sensitivity in GC–MS Using a Novel Column with an Uncoated Interface Section

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A novel gas chromatography–mass spectrometry (GC–MS) column design has been developed to enhance analytical performance by addressing persistent challenges in baseline stability and sensitivity. This innovation integrates a unique interface configuration that minimizes stationary phase degradation and thermal stress effects, particularly under elevated transfer line temperatures. Comparative evaluations using a diverse panel of pesticide analytes demonstrated significantly faster baseline stabilization and reduced background noise relative to conventional columns. The new column architecture consistently delivered improved signal-to-noise ratios across full-scan and SIM modes, with pronounced benefits observed for high-boiling-point and late-eluting compounds. These enhancements suggest a substantial reduction in analyte loss and column bleed, enabling more reliable detection of thermally labile substances.

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Ultra-Low Bleed and High Inertness in the Next Generation of 5%-Phenyl Gc Columns: Improving Trace-Level Quantification in GC/MS

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Continued advances in mass spectrometry have significantly improved detection limits, placing increased performance demands on gas chromatography (GC) columns. As ion sources become more sensitive, chromatographic background—particularly stationary-phase bleed—has a greater impact on baseline noise, spectral interference, and confidence in trace-level identification. Bleed-derived siloxane ions can complicate mass-spectral interpretation and reduce quantitative accuracy, making column thermal stability and inertness critical to modern GC/MS workflows. Recent developments in 5%-phenyl polysiloxane column chemistries have delivered enhanced oxidative and thermal robustness, supporting extended high-temperature operation with substantially reduced bleed. Improved baseline stability enhances signal-to-noise ratios and minimizes spectral artifacts, especially in trace-level analyses requiring deconvolution or reliable library matching. High column inertness further supports accurate analysis of chemically active analytes by reducing adsorption-related peak distortion. When paired with high-performance GC/MS platforms, these column characteristics enable improved method sensitivity, tighter retention-time reproducibility, and enhanced data quality across multi-residue, environmental, industrial, and regulatory applications. This presentation will highlight how reduced bleed, increased inertness, and improved thermal stability translate into cleaner mass spectra, stable retention behavior, and extended operational reliability, underscoring the essential role of modern 5%-phenyl GC column technology in next-generation GC/MS analysis.

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Towards the Development of Advanced Identification Methodologies of Counterfeited Perfumes: Programmable Temperature Vaporization (PTV) Inlet-GC-MS/MS of Fragrances

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Between rising costs, changes in the ease of procurement of raw materials, and social media marketing allowing for direct-to-consumer sales the perfume/fragrance industry has seen an influx of what are called dupe perfumes and counterfeit perfumes. Dupe and counterfeit perfumes are different, dupes are legal, affordable alternatives that mimic popular scents without copying branding, whereas counterfeits are illegal, deceptive copies that replicate packaging to mislead consumers. Ultimately, the raw materials are the same: water, ethanol and a specific formula of essential oils and synthetic aroma chemicals. Dupes and counterfeits are continuing to improve and to a regular consumer becoming less distinguishable from the real. This study leverages the sensitivity of the GC-MS/MS Multiple Reaction Monitoring (MRM) mode and the cold trapping and controlled sample introduction of Programmable Temperature Vaporization (PTV) Inlet to better identify and distinguish the minor difference in ratio, purity, and source. Six common fragrances from a commercially available perfume were selected to optimize the PTV-MRM methodology, and then applied to the commercially available perfume and its respective dupe perfume.

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Monograph Testing for Compressed Gases in the Pharmaceutical Industry - What is Required?

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Presentation will describe the need for testing of compressed gases (oxygen, nitrogen, carbon dioxide, etc.) commonly used in cGMP processing and packaging. A brief comparison of the existing USP and EP monographs will be provided along with the differing analytical techniques listed in each Pharmacopoeia. Best practices for compressed gas applications will be discussed along with specific references outlining exactly when monograph testing is required. Examples of previous / known issues and OOS findings will serve as examples for why accurate testing and reporting is needed.

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Mass Spectrometric Investigation of Sera from Women with Breast Cancer for Identification of Potential Early-Stage Protein Biomarkers

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Breast cancer (BC) is the second leading cause of cancer-related death among women in the United States and remains the most frequently diagnosed malignancy. Current screening guidelines recommend biennial mammograms for women ages 40–74, but new approaches are required for early detection in women of all ages. Dysregulated serum proteins represent a promising source of biomarkers, as they may play definable roles in tumor development. This study investigated serum protein alterations in BC using mass spectrometry–based proteomics. Human blood samples from 48 invasive ductal carcinoma (IDC) cases and 48 controls (96 total) were analyzed using a NanoAcquity UPLC coupled to a QTOF Xevo G2 XS mass spectrometer with nanoLC-MS/MS. Prior to analysis, samples underwent albumin depletion by affinity chromatography and in-solution tryptic digestion. Following data acquisition and conversion, Mascot Daemon was used to search spectra against the NCBI human database. Protein identification and quantification was performed using Scaffold software. Identifying proteins that differ between BC and control sera in a large cohort may support earlier detection and diagnosis across age groups. To date, dysregulation has been observed in several protein families, including anti-trypsin proteins (Alpha-1-antichymotrypsin, Alpha-1-antitrypsin), Complement proteins, and Apolipoproteins. Both Apolipoproteins and Complement proteins are heavily involved in tumor progression and proliferation. Additional upregulated proteins include glycoproteins such as alpha-1-acid glycoprotein, alpha-2-HS glycoprotein, and zinc-alpha-2 glycoprotein, all of which are associated with cancer development, cell growth, and regulation of proliferation and apoptosis.

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Membrane Proteomics of Small Extracellular Vesicles Unveils the Role of the Aged Host Microenvironment in Ovarian Cancer Progression
Reihaneh Safavisohi, Seton Hall University, 400 South Orange Ave., South Orange, NJ 07079

Epithelial Ovarian Cancer (EOC) is the most fatal gynecological malignancy. Because early-stage symptoms are vague, EOC is primarily diagnosed at an advanced stage (III/IV) after dissemination throughout the peritoneal cavity. Notably, EOC is a disease of aging, with most patients diagnosed over age 60. Within the peritoneal microenvironment, small extracellular vesicles (EVs) act as critical regulators of inter-cellular communication, driving tumor progression via molecular cargo transfer. Because EV-mediated adhesion and invasion are governed by surface interactions, profiling the vesicular membrane proteome is vital for understanding these pathways. Here, we investigated the impact of peritoneal-derived EVs, specifically from aged and tumor-bearing hosts, on EOC and mesothelial cell behavior. EVs were isolated from mouse model peritoneal lavage using a microfluidic device and characterized via Nanoparticle Tracking Analysis and Western blotting (TSG101, CD9, CD63). To interrogate proteins driving metastatic success, we optimized robust bottom-up proteomics workflows tailored to the highly hydrophobic EV membrane proteome. EV samples were lysed and analyzed utilizing high-resolution mass spectrometry. Label-Free Quantification (LFQ) and gene ontology (GO) analyses revealed distinct phenotypic differences between EVs from tumor-bearing/aged mice versus healthy controls. Optimized membrane profiling highlighted significant overexpression of specific surface proteins in metastatic and aged-host samples that promote EOC adhesion and invasion. Ultimately, this analytical pipeline provides deep insights into vesicular communication networks and identifies critical, age-associated host membrane proteins serving as key drivers of EOC progression.

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Untargeted, Quantitative Metabolomics Analysis Using ZT Scan Data Independent Analysis
David Colquhoun, SCIEX, 250 Forest St., Marlborough, MA 01752, Paul Baker

Untargeted metabolomics has traditionally relied on data-dependent acquisition (DDA) scan modes on high-resolution mass spectrometers (HRMS) to provide a metabolic “snapshot” of biological samples. However, the stochastic selection of precursor ions and its reliance on MS-level quantitation limit reproducibility and accuracy. In complex biological matrices, data-independent acquisition (DIA; SWATH) provides comprehensive data sampling by generating MS/MS data of all precursor ions within a specified mass range that enables quantitation at the MS/MS level. However, precursor selection is governed by relatively large windows (>3 Da). Consequently, DIA-derived MS/MS data are frequently affected by chimeric signal contributions and co-isolated interferences, thereby reducing confidence in compound identification and quantitation, particularly for small-molecule omics analysis. Recently, a novel sliding-window DIA approach—ZT Scan DIA —was introduced for the ZenoTOF 8600 system that enables post-acquisition deconvolution to generate a narrower effective precursor ion selection window, greatly improving precursor ion specificity over traditional DIA, and yielding better coverage with accurate MS/MS-based quantitation. Here, NIST SRM 1950 plasma was analyzed using multiple untargeted metabolomics workflows, including DDA and ZT Scan DIA, on the ZenoTOF 8600 system. The data were processed with MS-DIAL to assess how coverage indices for the NIST 1950 plasma metabolome vary across analytical workflows. The results show that ZT Scan DIA delivers both broad coverage of the plasma metabolome and quantitative MS/MS data.

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Metabolic Characterization of Common Bacterial Growth Media
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Research into the metabolic outputs of bacterial communities is a growing field that relies on liquid culture models using common media types for microbial growth. The majority of these common media are deficiently characterized, which can lead to barriers in evaluating data extracted from these metabolic studies. To remedy this limitation and better aid in media selection for metabolic studies, we conducted metabolic profiling of common bacterial media using both targeted and untargeted metabolomics, including Brain Heart Infusion, Heart Infusion, Phenol Red, Nutrient Broth, and Luria-Bertani Broth. Samples were run using both C18 and HILIC methods in positive and negative ionization mode and analyzed with MS-Dial to identify and quantify metabolites. Identification of untargeted data utilized publicly-available libraries and in-house libraries including sodiated mono- and di-saccharides of interest and dueterated internal standards for normalization. Targeted analyses were conducted using an Agilent QQQ, and untargeted using a Waters Q-TOF. We were able to detect and tentatively identify over 450 metabolites with untargeted methods in addition to the 200+ in our targeted pipeline. These data highlight stark differences in the metabolite abundances of the different media samples, including non-disclosed carbohydrate sources. Additionally, longitudinal studies of the same media over time, or from different batches, have indicated the possibility of variability within media composition over time. This study of bacterial media aims to provide a background for media selection for future microbial metabolite studies.

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Mass Spectrometry-Based Proteomic Analysis of Breast Milk for Early Breast Cancer Detection
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Breast cancer (BC) is a leading cause of cancer-related mortality among women worldwide, and early detection remains essential for improving survival outcomes. Invasive ductal carcinoma (IDC), the most common subtype, accounts for approximately 85% of cases and originates in the milk ducts before invading surrounding tissue. Human breast milk, enriched with secreted proteins, immune factors, and exfoliated epithelial cells, represents a promising non-invasive medium for detecting molecular alterations associated with breast cancer. In this study, we applied mass spectrometry (MS)-based proteomics to identify differentially expressed proteins in breast milk. Proteins were separated by SDS-PAGE, digested, and analyzed by nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS). Two studies were employed. In the first cohort (10v10), paired left and right breast milk samples were obtained

from five individuals in whom one breast was affected by cancer, while the contralateral breast served as an internal control, enabling within-subject comparisons. In the second cohort (12v12), longitudinal repeated sampling was performed in four individuals (two with breast cancer and two healthy controls). Multiple milk samples were collected from each participant over time, reflecting the availability of stored milk samples provided by participants. This repeated sampling enabled evaluation of temporal variation in the breast milk proteome in both healthy controls and breast cancer participants. Across both cohorts, several proteins were consistently dysregulated between cancerous and non-cancerous samples and across time points. Preliminary findings identified bile salt-activated lipase, xanthine dehydrogenase, and β-casein as differentially expressed proteins, suggesting their potential involvement in breast cancer-associated molecular pathways.

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Modified Nanomaterials Engaging in Halogen Bonding Toward Explosive Detection Interfaces for Sensor Development

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The continued use of explosives by terrorists for improvised explosive devices (IEDs), calls for the development of fast, on-site sensing techniques. A promising approach utilizes halogen bonding (XB), a highly directional noncovalent interaction formed between a region of positive electrostatic potential on a halogen atom receptor site or XB donor ligand and a Lewis base or XB acceptor target molecule. Explosive-related compounds such as 2,3-dimethyl-2,3-dinitro butane taggant or cyclohexanone by-product, contain electron-rich peripheral oxygens allowing them to function as XB acceptors. Halogens with fluorination allow for a highly tunable sigma-hole on ligands acting as the XB donor counterpart for the XB interaction. The strength that interaction is indicated by short X-B bond distances and more linear angles of interaction which facilitate stronger and more selective binding, as leveraged by density functional theory (DFT) analysis. This study presents a nanomaterial-based scheme, featuring various modified gold substrates, including both self-assembled monolayer modified electrodes as well as alkanethiolate-stabilized gold nanoparticles, each modified with specific perfluorinated ligands to equip them with XB donor capability. The findings show that augmented single-walled carbon nanotube conductivity sensors markedly outperform previously developed schemes of similar materials. The enhanced performance of the sensing scheme is attributed to reduced bond angle constraints that allow XB acceptors or targeted molecules to engage in more optimal XB interactions both in solution as well as in the gas-phase. Such presented methodology demonstrates the potential of XB systems as rapid and selective sensing schemes for explosive by-products in field settings.

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Differentiating Pepper Spray Products using GC×GC and Tile-Based Fisher Ratio Metrics

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Pepper spray is a common personal protection agent often analyzed as evidence in forensic cases. Pepper spray is a complex matrix consisting of oleoresin capsicum (OC), a carrier, and a propellent. Various brands of pepper spray differ in composition. The ability to identify and characterize the components of assorted pepper spray products is a valuable tool in forensic investigations. Traditionally, the volatile organic compounds (VOCs) associated with pepper spray products are separated and analyzed by gas chromatography-mass spectrometry (GC-MS). GC-MS separates analytes within a capillary column coated with a stationary phase, allowing analytes to separate based on their chemical affinity for the column coating. Complex samples like pepper spray 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 differentiate brands of pepper spray using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry and flame ionization detection (GC×GC-TOFMS/FID) and tile-based Fisher ratio data processing methods. Preliminary results show that VOCs can be used to differentiate between pepper spray brands, with some brands containing more VOCs or VOCs in differing abundances. Additionally, while there are five common capsaicinoids found in pepper spray, using GC×GC, an additional seven compounds were detected having a *m/z* of 137. This study is significant in its determination of VOCs that can act as differentiators between pepper spray brands, ultimately aiding the identification of brands in a forensic setting.

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Investigation of Altered Glycosylation in Endometriosis: Toward Improved Biomarker Discovery

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Endometriosis is a gynecologic condition in which endometrial tissue is detected outside of the uterus, contributing to chronic pelvic pain and infertility. Currently, no effective non-invasive diagnostic tools exist, leading to a barrier in diagnosis. Aberrant adhesion of endometrial cells to the mesothelium is largely mediated by cell surface proteins, many of which are glycosylated. Glycosylation can influence the structure, function, and interaction of a protein with other biomolecules, and we hypothesize that dysregulated glycosylation plays an important role in the cell adhesion and immune evasion associated with endometriosis. To explore this further, we performed glycoproteomic analyses on forty serum samples, twenty from patients diagnosed with endometriosis and twenty from patients without the disease. We enriched the samples for O-glycopeptides using a method developed in our lab, GlycoFASP, which takes advantage of the selective digestion enabled by O-glycoproteases. Samples were analyzed using liquid chromatography-mass spectrometry, and files were searched against the human proteome followed by label-free quantification. In total, we identified 589 proteins across the two conditions. In the endometriosis samples, we identified 128 unique proteins, and in the control samples, 72 proteins. Gene ontology analysis further identified the upregulation of various immune system responses in endometriosis. Additional upregulated pathways include the negative regulation of fibrinolysis and keratinization. Downregulated processes of note include fibrin clot formation and positive regulation of opsonization. This proteomic analysis reveals insights into glycoproteins and associated pathways that could serve as potential diagnostic biomarkers for endometriosis.

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Scaling Up Real-Time Precision Opto-Control
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Being able to control cellular behavior and monitor cellular responses is critical for fundamental biological research and for understanding disease progression. Real-time precision opto-control (RPOC) is a technology that integrates laser scanning, chemical imaging, and closed-loop decision-making to regulate biochemical processes at targeted subcellular sites. The key advantage of RPOC is its precision: it can deliver a calibrated optical perturbation to a chemically defined structure while simultaneously monitoring cell responses. To become a viable analytical technology, however, RPOC must move beyond single-cell case studies. Individual cells can establish that a perturbation has an effect, but they provide limited population-level context for interpreting that effect and for distinguishing dose dependence from cell-to-cell variability. Here, we present our efforts to scale RPOC to high-throughput imaging and investigate the long-term effects of optical perturbation across cell populations under different conditions. We describe a home-built Python-based acquisition and control platform with a backend designed to facilitate large-language-model-assisted software development, allowing users without programming expertise to create customized workflows using natural language. We demonstrate diverse optical perturbation and longitudinal imaging workflows in which multiple fields of view are automatically selected, treated, and revisited for high-throughput analysis. Finally, we discuss extensions of RPOC toward broader biological and chemical applications, where precise, adaptive perturbation and quantitative response monitoring can provide new capabilities for studying cellular processes.

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Analysis of Membrane Protein Mixtures Using Capillary Electrophoresis-Mass Spectrometry
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Membrane proteins are key components of biological systems, playing important roles in signaling pathways, intra- and extracellular transport, and other cellular functions. The unique location of membrane proteins, serving as the bridge between the intracellular and extracellular environments of the cell, also makes them ideal therapeutic targets. Traditionally, analysis of membrane proteins is complicated by the unique conditions required to maintain membrane protein solubility and stability, including high concentrations of salts, co-factors, and membrane mimetics, which make mass spectrometry-based analysis challenging. In this work, we expand upon our previous methods for the analysis of membrane proteins, under near-native conditions, via capillary electrophoresis-mass spectrometry (CE-MS) and apply the same workflows to mixtures of membrane proteins and cell isolates. Individual protein analysis of bacteriorhodopsin (*H. salinarum)* and aquaporin z (*E. coli*)under near native conditions were performed using linear-polyacrylamide (LPA)-coated capillaries, (Sciex), with intact masses observed of 26,417 Da and 98,250 Da, respectively. We have also expanded this membrane protein CE-MS analysis to more complicated samples, including cell lysate samples that have been enriched in membrane proteins. Membrane protein-enriched samples from cell lysate samples were analyzed utilizing a Q-Exactive UHMR mass spectrometer in order to maximize the detectable range of protein molecular masses. The resultant electropherograms showed separation of multiple intact protein species ranging from 10 kDa to over 150 kDa in a single run. The developed workflow enables the coupling of high resolution analytical separation techniques with mass spectrometry to enable more in-depth membrane protein identification and characterization.

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Understanding Neuronal Excitability Using Mass Spectrometry Imaging of SCN2A-Deficient Organoid and Assembloid Systems
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Human induced pluripotent stem cell (hiPSC)-derived organoids are powerful models for studying disease states without the use of animal models. In this study, we use nano-DESI MSI to understand alterations in molecular signaling in *SCN2A*-deficient iPSC-derived organoids developed as models of neuronal excitability in neurodevelopmental disorders. We used nano-DESI MSI to examine molecular differences between wild type (WT) and *SCN2A*$^{+/-}$ (heterozygous knockout; HET) iPSC-derived cortical organoids. We examined both fresh frozen and paraffin embedded (FFPE) organoids. Imaging experiments were performed on a Q-Exactive HF-X Orbitrap mass spectrometer in negative ionization mode. For FFPE organoid tissue sections, we developed a sample preparation protocol to remove paraffin while preserving lipid signals on tissue. Using this protocol, we analyzed WT and HET cortex brain organoids. Through comparison between WT and HET cortex brain organoids we identified multiple lipids that were altered in the *SCN2A*-deficient systems. In the HET organoids, multiple polyunsaturated phosphatidylserine (PS) and phosphatidylethanolamine (PE) lipids were observed to be upregulated when compared to WT organoids, for example PS 40:7, and PE 40:4. These trends mirror prior observations in mouse brain models and consistent with *LPCAT3*-associated remodeling. Distinct spatial localizations of lipids were also observed within the organoids which indicate location-dependent cell differentiation. We also tested methods for analyzing fresh-frozen organoid sections, which enhanced the sensitivity of nano-DESI MSI experiments by generating higher lipid signals than FFPE sections. Our results highlight the capabilities of nano-DESI for imaging iPSC-derived organoids of different disease states, providing insights into cell differentiation within organoids.

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Towards a Bioinorganic Programming Language: X-Ray Crystallography of Metalated DNA
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Metal-mediated DNA (mmDNA) replaces canonical hydrogen bonds with coordinate bonds to place metal ions within a programmable DNA duplex, offering routes to electronic, catalytic, and responsive nanomaterials. Progress is limited by the lack of routine, high-

resolution structural information, leaving coordination chemistry partially understood and rational design of extended metalated architectures inaccessible. We present a rapid crystallographic screening platform embedding metal-responsive base pairs within self-assembling, macroscopic DNA crystals where anomalous scattering determines metal identity, position, and occupancy. This workflow yields 3-4 Å structures in two weeks, enabling screening of 2,000+ mmDNA combinations, resolution of 50+ novel base pairs, and capture of four transmetalation reactions. Beyond structural characterization, this research seeks to develop a bioinorganic programming language for molecular electronics. DNA nanotechnology relies on semantomorphic self-assembly, encoding molecular shape, topology, and connectivity within sequence. However, typically employed promiscuous pyrimidine mismatches introduce functional chemistry without preserving language. To restore informational orthogonality, I am developing a metalated alphabet modeled after the natural four-letter genetic code: minimal complexity, strict orthogonality, directional asymmetry, and predictable geometry. Screening revealed a fully occupied, gold-selective 2,6-diaminopurine:2-thiothymine pair serving as a metalated A:T analog; candidate gold-mediated G:C analogs, and pH-dependent mercury, gold, and silver coordination within a single mmDNA system. Sulfur-rich nucleobases further produced square-planar copper- and palladium-mediated pairs and inverse-sandwich complexes of gold and palladium with preserved native duplex dimensions. Together, these results establish a high-throughput engine for developing an orthogonal bioinorganic programming language for semantomorphic molecular electronics, catalytic nanomaterials, and programmable plasmonic and photonic architectures.

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Rapid Determination of Drug-to-Antibody Ratios in Antibody–Drug Conjugates Using Ultrafast Microdroplet Digestion Technology

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Accurate determination of drug-to-antibody ratios (DARs) is essential for the development, quality control, and performance evaluation of antibody–drug conjugates (ADCs); yet conventional analytical approaches often require extensive sample preparation, long analysis time, and substantial sample consumption. The peak distribution of intact ADCs is highly complex due to inherent glycosylation heterogeneity and variable drug conjugation. By applying enzymatic digestion, ADC can be converted into smaller subunits or deglycosylated species, thereby significantly simplifying the mass spectral profile. This reduction in structural heterogeneity facilitates clearer peak assignment and enables more accurate and reliable DAR quantification. Herein, we report an ultrafast microdroplet digestion–mass spectrometry strategy for rapid DAR characterization of ADCs. Microdroplet enzymatic digestion of antibodies and ADCs occurs within microsecond-time scales during spray ionization, enabling direct online subunit analysis with minimal sample preparation. The method was validated using NIST monoclonal antibody (mAb) conjugated to ADC mimics spanning low to high DAR (0-14) ranges, Cetuximab-derived ADC mimics (DAR~5) with complex glycosylation, and the commercial ADC Kadcyla (DAR~3.5). Consistent DAR values were obtained across multiple enzymatic workflows (IdeS, EndoS2, and EndoF3) with good reproducibility (%CV typically <5%). This approach substantially reduces analysis time while maintaining analytical accuracy and structural specificity, providing a rapid, sensitive platform for high-throughput ADC characterization and process monitoring.

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Scaling Electrochemiluminescence with Electroactive Surface Area in a Multiplexed Electrochemical Biosensing Platform

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Multiplexed point-of-care sensors offer the potential to detect multiple biomarkers rapidly and inexpensively using a single device. Bipolar electrode (BPE) arrays are promising platforms for multiplexed sensing because analyte-specific electron-transfer reactions can be coupled to electrochemiluminescence (ECL), producing an optical signal without requiring a wired connection to each electrode. Our laboratory has developed four-electrode BPE arrays containing either identically sized electrodes or electrodes with varied cathode sizes. For reliable results, identically sized BPEs should produce consistent ECL intensities, whereas differently sized BPEs should produce distinguishable intensities that scale predictably with electrode size and thus, electroactive surface area (EASA). Here, we evaluate how ECL response scales with EASA. First, the EASA of differently sized cathodes is determined from three-electrode cyclic voltammetry experiments. Then, we compare these results to the ECL response of corresponding BPE cathodes. Establishing this relationship is an important step toward validating multi-BPE platforms in which electrodes generate distinguishable signals for the simultaneous detection of multiple analytes.

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Facilitating Science-Based Extraction Solvent Selection Through Solvent Compatibility Assessment in Medical Device E&L Studies

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Selection of extraction solvents is a critical aspect of extractables and leachables (E&L) study design. While solvent selection is commonly guided by applicable ISO standards and historical experience, additional evaluation of material-solvent compatibility may provide valuable information regarding device integrity and extraction suitability. To support solvent selection with additional scientific rationale, a systematic solvent compatibility evaluation strategy was incorporated into E&L study planning.

Candidate extraction conditions spanning a range of solvent polarities were evaluated. Extracts were characterized using gravimetric nonvolatile residue (NVR) measurements, visual assessments of material integrity and extract appearance, and orthogonal analytical techniques including nuclear magnetic resonance (NMR) spectroscopy and gel permeation chromatography (GPC). These assessments provided insight into both the quantity and characteristics of extracted materials, including high molecular weight polymeric species that may interfere with mass spectrometric characterization of extractables. Two medical device case studies will be discussed. In one study, nonpolar extraction conditions resulted in elevated NVR levels, material degradation, and increased extraction of polymeric species, indicating limited suitability for extractables characterization. Alternative solvents maintained device

integrity while providing more representative extraction conditions. A second case study demonstrated differences in extract composition and polymer extraction behavior among candidate solvents, enabling selection of conditions compatible with downstream analytical characterization. These case studies demonstrate how solvent compatibility assessments can strengthen E&L study design by improving understanding of material-solvent interactions and providing a scientifically justified basis for solvent selection. Incorporation of this approach may improve outcomes and support justification of extraction strategies during study planning.

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Peptide Adsorption Profiling: Evaluation of Commercially Available Peptides for Enhance Filtration Efficiency

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Ultrafiltration is commonly used for peptide sample preparation, yet recovery cannot always be predicted from the nominal molecular weight cutoff defined by the filter. Substantial loss may occur even when the peptide mass is well below the stated membrane cutoff. We therefore investigated whether molecular conformation contributes to filtration behavior. Met Enkephalin (574 Da), Octreotide (1019 Da), Argipressin (1084 Da), and Somatostatin (1638 Da) were evaluated using Amicon Ultra regenerated cellulose filters and Sartorius Vivacon 500 hydrophilic (HY) and polyethersulfone (PES) filters. Membrane cutoffs of 2, 3 and 10kDa were tested over peptide concentrations from 31.25 to 1000 µg/mL. Radius of gyration and maximum intramolecular distance determined by conformational modeling were used as structural descriptors of peptide compactness and flexibility. Recovery varied with membrane chemistry and peptide concentration and correlated with conformational rigidity. Flexible peptides capable of adopting compact conformations showed greater membrane passage. In contrast, structurally constrained peptides with larger radius of gyration exhibited lower recovery despite having molecular masses below the nominal cutoff. These observations support that molecular mass alone is insufficient to explain peptide filtration. Conformation may influence effective molecular dimensions, while peptide and membrane chemical interactions may further reduce recovery through adsorption. Filter selection should therefore consider peptide flexibility, structural compactness, membrane chemistry, and concentration rather than molecular weight cutoff alone. Applying these factors during sample preparation may reduce empirical screening, identify otherwise unrecognized sample loss, and improve the accuracy and reproducibility of peptide quantification.

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Analysis of Fentanyl in Illicit Drug Mixtures Using the Scatr, a Portable Raman Hyperspectral Imaging Instrument

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Over the past few years, use and abuse of synthetic opioids have dramatically increased across the United States. According to the June 2026 NIST RaDAR report, the median weight percentage of fentanyl-containing submissions analyzed was 6.2%, with a large range including samples less <1% (1). Although portable Raman spectroscopy has become an important tool for rapid field analysis of illicit drugs, a well-known gap is its inability to detect fentanyl at low concentrations consistently. The Scatr Series One is a portable Raman hyperspectral imaging instrument intentionally designed to analyze drug samples in real time, providing rapid, reliable, and non-destructive identification of substances and their relative concentrations (2). The instrument rasters a 1064nm laser across multiple points on the sample, creating a spatial map of its chemical composition. A small fraction of the laser light is inelastically scattered by the sample, producing a wavelength shift that is detected and converted into a Raman spectrum characteristic of each substance. In this evaluation of the Scatr Series One, drug mixtures containing fentanyl with common cutting agents (acetaminophen, lactose, and mannitol) were tested from 50% to 0.1% w/w. Results show that the instrument is capable of identifying fentanyl in both bulk and low-concentration mixtures. The results demonstrated the value of hyperspectral imaging for the detection of a range of both bulk and trace fentanyl samples, thus making it a powerful tool for use by first responders and law enforcement when combating the current illicit drug landscape.

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Method Development for the Determination of Fentanyl, its Cutting Agents and Selected Metabolites in Whole Blood Using LC-MS/MS

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The presence of xylazine and medetomidine, both veterinary sedatives, in fentanyl cases as “cutting agents”, has seen a dramatic rise in recent years, driving a polydrug overdose epidemic. The aim of this study is to develop a rapid liquid chromatography tandem mass spectrometry (LC-MS/MS) method, for the simultaneous determination of fentanyl, xylazine, medetomidine and selected metabolites in whole blood. Forensic laboratories may take advantage of the high throughput analysis of LC-MS/MS, instead of using GC-MS, assisting the reduction of backlog cases, at comparable cost, and providing shorter time of analysis. Target precursor and product ions (MRM ion transitions) are optimized by injecting standard reference materials and analyzing them via Flow Injection Analysis (FIA). The detection is achieved using an Agilent Jet Spray Ionization source operating in positive mode. The separation is optimized on a Poroshell (2.7µm, 100 x 2.1mm) analytical column, by examining various isocratic and gradient programs, using ammonium formate 10 mM/formic acid 0.1% (solvent A), and acetonitrile or methanol with 0.1% formic acid (solvent B), and different temperatures of the analytical column. In addition, three different solid phase extraction (SPE) cartridges (Clean Screen DAU, Clean Screen Xcel, Bond Elut Certify, OASIS HLB) have been evaluated for the recovery of the three analytes. The method demonstrates short run time (less than 10 minutes), sufficient separation, and specificity of the analytes which makes it suitable for further development. In this presentation, the method’s optimized parameters and representative figures and data are reported.

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Development of Blinking Environmental Sensors for Single Molecule Fluorescence Imaging Using Novel Derivatives of Nile Red

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This study aims to advance single-molecule spectroscopy techniques by developing an environmental sensing method based on fluorescence blinking. Nile Red (NR) is a solvatochromic dye whose emission color and intensity varies with its environment due to intramolecular charge transfer (ICT). We hypothesize that the fluorescence blinking of NR molecules may contain information about their local environment. Here, we examine the blinking dynamics of NR derivatives in various solvents. Blinking and machine learning analyses show that several blinking metrics vary with environmental polarity and can be used to differentiate a moderately polar environment from a non-polar environment with high accuracy. These results show promise for blink-based environmental sensing at the single-molecule level.

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An Integrated Chromatographic Platform for Comprehensive Analytical Characterization of Bispecific Antibodies

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Bispecific antibodies (BsAbs) pose substantial analytical challenges because of their structural complexity, molecular heterogeneity, and diverse degradation and misassembly pathways. To address these challenges, we present an integrated HPLC-based approach combining size exclusion chromatography (SEC), ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), and affinity chromatography to assess multiple critical quality attributes. Two structurally distinct BsAbs were selected to assess the applicability of the analytical framework across different molecular architectures and physicochemical profiles. Affinity chromatography enhanced analytical specificity through the selective capture and enrichment of target populations, facilitating the analysis of complex or low-abundance species. Conventional SEC characterized size-related heterogeneity, including high-molecular-weight aggregates and low-molecular-weight fragments, while IEX resolved acidic and basic variants associated with post-translational modifications, sequence heterogeneity, and structural asymmetry. HIC complemented these methods by detecting variations in surface hydrophobicity linked to conformational and compositional changes. In addition, mixed-mode SEC combined size exclusion with controlled hydrophobic interactions, providing enhanced selectivity for differences in surface characteristics and higher-order structure. This dual-retention mechanism differentiated correctly assembled BsAbs from mispaired variants in a single chromatographic method, substantially simplifying workflows that would otherwise require multiple orthogonal separations.
This work establishes a coordinated analytical framework that leverages the complementary selectivity of multiple chromatographic modes. The combined strategy facilitates rapid method development, enhances the resolution of heterogeneous species, and enables broader evaluation of BsAb quality attributes.

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Quantification of Inorganic Counterions using Hydrophilic Liquid Chromatography Coupled with Charged Aerosol Detection

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Pharmaceutical drug substances are frequently formulated as salts to enhance solubility, physicochemical stability, dissolution rate, impurity profile, and polymorphic behavior. Accurate identification and quantification of counterions are essential for confirming salt stoichiometry, supporting drug substance purity, and demonstrating mass balance. In this study, a hydrophilic liquid chromatography (HILIC) method coupled with charged aerosol detection (CAD) was developed for the analysis of inorganic pharmaceutical counterions in drug substances. Anionic and cationic counterions, including chloride, nitrate, sodium, phosphate, potassium, calcium, and magnesium, were separated using an Atlantis™ Premier BEH™ Z-HILIC Column. Key CAD parameters, including evaporator temperature, ion trap voltage, and power function value, were systematically evaluated for their effects on the method performance and linearity. All counterions demonstrated a linear response using a linear fit with 1/x weighting, with correlation coefficients ≥ 0.998. Inter-day performance was assessed by replicate injections (n=10) of a standard mixture across multiple days, yielding peak area relative standard deviations (RSDs) ≤ 1.15%. Counterion recoveries from drug substances ranged from 99 to 103%, with excellent repeatability of RSD ≤ 0.80% (n=6). The HILIC-CAD method delivered robust, accurate, and reproducible workflow for inorganic counterion analysis, enabling confident salt characterization and reliable quality testing of pharmaceutical drug substances. Integration with Empower™ Software supported efficient, compliance-ready data processing, reporting, and management across pharmaceutical development and manufacturing. Atlantis, BEH, and Empower are trademarks of Waters Corporation or its affiliates.

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Expanding Biotherapeutic Characterization Across Modalities: Rapid LC-UV-MS Analysis of Peptide Biologics and LNP Lipids Using Next-Generation Wide-Pore RP 230Å Columns

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The growing diversity of biotherapeutics, including peptide drugs and lipid nanoparticle (LNP)-based gene therapies, requires analytical methods capable of rapid, high-resolution characterization across multiple modalities. This work demonstrates the use of next-generation BioResolve™ and GTxResolve™ Reversed-Phase (RP) Columns with 1.6 µm, 230 Å superficially porous charged-surface particle technology for LC-UV-MS analysis of peptides and lipids. BioResolve RP 230 Å Columns were evaluated for the separation of insulin, its deamidation impurity, cagrilintide, semaglutide, liraglutide, and tirzepatide. Using a 2.1 × 50 mm format and MS-compatible mobile phases, baseline resolution of all analytes, including the critical separation of insulin from its deamidated impurity, was achieved. Coupling with a BioAccord™ LC-MS System and usage of waters_connect™ Software based Intact Mass Application enabled accurate mass measurements for confident analyte identification in an automated fashion. GTxResolve RP 230 Å Columns were used for reversed-phase separation of representative lipids from the major LNP lipid classes, including ionizable lipids, phospholipids, cholesterol, and PEG-lipids. A rapid 10-minute gradient with UV and MS detection provided efficient baseline

separation, reliable peak shape, and identification of lipid components and related impurities. These results demonstrate a versatile LC-UV-MS workflow for rapid characterization of biotherapeutics including peptides, impurities, and LNP-associated lipids during development. Apart from MS-based analysis, these robust UV-based detection methods can facilitate rapid testing by QC laboratories supporting quality control and deployment.

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Automated Benzoyl Chloride Derivatization of Amines Using an OT-2 Liquid Handling Robot Integrated with a Custom-Built Sonicator

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Opentrons is an open-source platform for automating laboratory experiments, enabling accurate handling of small liquid volumes and supporting a wide range of chemical and biochemical reactions. Its compatibility with standard labware, programmability through Python-based protocols, and adaptability via customizable 3D-printed accessories make it a versatile tool for improving precision, reproducibility, and efficiency in laboratory workflows. However, efficient mixing of reaction components during automated processes remains a key limitation. To address this challenge, a custom in-house sonicator module was developed and integrated with the Opentrons OT-2 system. The sonicator is controlled using an Arduino microcontroller, enabling seamless communication with the robotic platform and programmable incorporation of sonication into automated workflows. The primary objective of this work was to improve mixing efficiency and reduce sample preparation time. Mixing performance was evaluated by comparing repetitive pipette mixing (15 cycles) with sonication (15 seconds), demonstrating that sonication provides superior mixing efficiency. The system was further validated through automated dilution and derivatization, with performance compared to manual protocols. Prepared samples were analyzed using liquid chromatography–triple quadrupole mass spectrometry (LC-QqQ-MS). Results indicate that automated sample preparation provides improved accuracy and precision relative to manual methods. Additionally, integration of the sonicator significantly reduced overall sample preparation time. Overall, this work demonstrates that incorporating a low-cost, Arduino-controlled sonicator into an automated liquid handling platform enhances reaction mixing, improves analytical performance, and streamlines chemical sample preparation workflows.

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Bioanalytical Challenges, Practical Solutions, and Establishment of Reliable PK Profiles for a Volatile 6F-NDEA Impurity in a Mouse Pharmacokinetic Study

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Nitrosamines as unintended impurities in some drug substances and drug products, have prompted broad regulatory, manufacturing, and analytical efforts to ensure the drug's safety and quality. We used 6-fluoro-nitrosodiethylamine (6F-NDEA), a non-carcinogenic API nitrosamine-related impurity, as a model compound to investigate its preclinical pharmacokinetic profile and associated bioanalytical assay development. There is limited pharmacokinetic information in literature regarding nitrosamine drug substance related impurities including methods to accurately quantify them in biological matrices. After conducting a carefully designed set of experiments with 6F-NDEA, it was discovered that the unique physical and chemical properties of 6F-NDEA presented multiple challenges for conventional or high-throughput bioanalytical approaches. These challenges include rapid evaporation from plasma and whole blood, limited solubility in aqueous solution, and low sensitivity when using LC-MS/MS with ESI or APCI detection modes. We are reporting the method development of a linear, accurate and precise bioanalytical assay for the quantitation of 6F-NDEA in mouse whole blood for the first time in industry using GC-ECD with peak verification by GC-MS. Additional assay recovery and stability parameters were also evaluated to ensure assay integrity. We report the first pharmacokinetic data for 6F-NDEA, analyzed using the qualified method with incurred sample reanalysis to confirm the accuracy of results. This methodology will also serve to establish a blueprint for the bioanalysis of volatile nitrosamine impurities.

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Development of an LC-MS/MS Method to Detect and Quantify Free Oligonucleotides in Antibody-Oligo Conjugate (AOC) Serum Samples

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Antibody-Oligonucleotide Conjugation (AOC) for targeted delivery is rapidly expanding, but bioanalytical quantification and characterization of each component is quite challenging due to the structural differences between proteins and oligonucleotides. In this poster, we present two LC–MS-based methods for comprehensive AOC bioanalytical characterization. First, an LC–MS/MS method that enables quantification of free sense or antisense oligonucleotides in serum below 12 ng/mL with a linear range of 12–1000 ng/mL. Second, an isocratic SEC–MS method for oligonucleotide-to-antibody ratio (OAR) determination in AOCs with a sensitivity of up to 100 µg/mL in formulation buffers, overcoming limitations of conventional gel-based methods. An immunocapture-based SEC–MS workflow using AOC-specific magnetic beads, along with digestion studies, is under development to facilitate OAR monitoring in serum and assess conjugate stability over time. These methods would allow a comprehensive characterization of AOCs to understand stability and OAR distribution.

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Optimization and Validation of a Robust rCE-SDS Method for Drug Substance Purity Assessment of Protein Nanoparticles in Potassium-Based Formulations

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Bristow, Igoris Cajevskis, Ray McClain, Sarah Aubert

The assessment of critical quality attributes (CQAs), particularly Drug Substance (DS) purity, via reduced Capillary Electrophoresis-Sodium Dodecyl Sulfate (rCE-SDS) is essential for developing complex biomacromolecules like protein nanoparticles. However, this application is severely constrained by formulation matrix incompatibilities. Specifically, the current potassium-based formulation induces SDS precipitation, causing target proteins to co-precipitate and form artifactual aggregates. Additionally, the inherently low antigen concentration (1-2 mg/mL) makes the requisite electrokinetic injection highly sensitive to minor matrix salt fluctuations, further compromising assay robustness. To overcome these limitations, a systematic optimization of sample preparation and instrumental parameters was conducted. A multi-step buffer exchange process was implemented to mitigate potassium-induced interferences and normalize the salt matrix. Sample reduction conditions and denaturation temperature were refined, and the SDS concentration was increased to effectively redissolve transient microaggregates, thereby preventing injection bias and ensuring consistent peak areas. Furthermore, electrokinetic injection settings, including voltage and time, were systematically tuned to optimize sample introduction. The optimized rCE-SDS platform was comprehensively evaluated using forced degradation samples, demonstrating robust stability-indicating power and the capability to resolve product-related impurities. During validation in a GxP environment, the developed method exhibited excellent linearity, accuracy, precision, and Limit of Quantitation (LoQ). Following successful validation, the method was formally transferred to routine testing laboratories. Ultimately, this robust analytical tool ensures reliable DS purity characterization, enabling informed decision-making and meeting rigorous regulatory expectations for challenging protein nanoparticle formulations.

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Efficient and Simplified REE Analysis with Combined ICP-OES and ICP-QQQ-MS

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Rare earth elements (REEs), comprising the lanthanides along with scandium and yttrium, are widely used in advanced technologies due to their unique magnetic, optical, and catalytic properties. However, their similar chemical behavior makes separation and accurate quantification challenging. In plasma-based analytical techniques, REEs readily form oxide species, leading to interferences, particularly from low-mass REE oxides overlapping higher-mass isotopes. In addition, partially filled 4f orbitals produce numerous closely spaced emission lines across the UV–visible spectrum, increasing spectral complexity and limiting interference-free analytical line selection in REE-rich matrices. In this work, a combined ICP-OES and ICP-QQQ-MS approach is employed to streamline REE analysis. ICP-OES enables robust quantification of major elements, while ICP-QQQ-MS improves interference control and enhances trace-level detection. Together, these complementary techniques provide efficient and reliable REE characterization across complex matrices.

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Ensuring High-Quality Trace Element Data for Environmental Samples Using Microwave-Assisted Acid Digestion

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Trace element content in both aqueous and soil samples is heavily regulated in the United States. These environmental samples can be difficult to work with, particularly solid samples and semi-solid such as soils and sludges. Ensuring data that is accurate and reproducible is essential to meet these regulations. This study outlines the use of microwave-assisted acid digestion using either U.S. EPA Method 3015A, 3051A or 3052 to achieve quality results. The use of microwave-assisted acid digestion is a rapid, efficient, and robust approach that meets the low detection limits required for these environmental samples. Standard reference materials as well as a variety of complex soil samples were analyzed via ICP-OES following microwave-assisted acid digestion. These results demonstrate reliable data, as excellent recovery and reproducibility was achieved for all samples analyzed. The findings confirm that EPA Methods 3015A, 3051A and 3052 are suitable and robust methodologies for generating high-quality analytical data.

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Extractables and Leachables Screening Standard for GC by Perkin Elmer Clarus NOVA

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Throughout manufacturing, storage, and administration, pharmaceutical products come into contact with a variety of packaging systems composed of different materials. Comprehensive compatibility studies may be necessary to ensure that these materials do not compromise product quality or introduce safety risks resulting from product–material interactions. Among these evaluations, extractables and leachables (E&L) studies constitute a significant component of analytical characterization. These studies are designed to identify and assess substances that may migrate from packaging materials into the pharmaceutical product. One of the most valuable analytical instruments to use in these studies is a GCMS. This poster shows the use of the Perkin Emler Clarus GCMS to obtain results for a specially designed CRM for the qualification of a GCMS to do these studies.

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Fan-Free Micro Slurry Direct Air Capture with Ion-Exchange Sorbents and Seawater-Enabled Regeneration

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Direct air capture (DAC) is limited not only by sorbent performance but also by energy-intensive air contacting and regeneration. We developed a fan-free micro-slurry DAC architecture in which ~100 μm DeCarbonHIX ion-exchange particles are suspended in water and recirculated as a thin falling film over an inclined surface exposed directly to ambient air (~420 ppm CO_2). Under a condition of 15 wt% solids and 90 mL s^{-1} slurry flow, CO_2 uptake reached ~0.33 mol kg^{-1} in 1 h and 0.75–0.80 mol kg^{-1} in 5 h, while extended operation reached ~1.20–1.27 mol kg^{-1} after 30 h. Parametric experiments identified a practical operating window governed by trade-offs among particle size, solids loading, aqueous ionic strength, and pumpability. After capture, the particles were retained in a sand-bed column and regenerated using seawater followed by dilute alkaline conditioning, enabling reuse of the sorbent and carrier water

without high-temperature desorption. System-level energy analysis showed that the direct operational energy burden is shifted away from forced air movement and concentrated primarily in slurry circulation, identifying hydraulic optimization as the main scale-up target. This work demonstrates a water-based, low-temperature, resource-efficient DAC architecture that integrates capture, separation, and regeneration using simple unit operations.

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Navigating Analytical Challenges in the Characterization of a Reactive Raw Material used in the Manufacture of an ADC Drug Linker

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A raw material used in the synthesis of an antibody-drug conjugate (ADC) drug linker required thorough characterization to support process development and finalization of the commercial control strategy. However, the high reactivity of the compound created analytical challenges for method development. The first challenge was assessing the root cause of an elevated hydrolysis impurity observed by gas chromatography (GC). Initially hypothesized to be related to either the sample preparation or exposure to elevated humidity levels, further investigation traced the discrepancy to the use of an incompatible inlet liner. The second challenge was related to the quantitation of a trimer impurity. As the trimer impurity is nonvolatile and can readily degrade at high temperatures, a secondary method to GC was required. The raw material and trimer are unstable under acidic, basic, and aqueous conditions, which made reverse-phase high-performance liquid chromatography (HPLC) not suitable. A normal-phase HPLC method with charged aerosol detection (CAD) was therefore developed to specifically detect and quantify the trimer impurity. Resolution of the GC discrepancy and implementation of the complementary trimer method enabled a robust analytical control strategy and successful method transfer for commercial manufacturing of the drug linker.

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Effect of Pore Size on Plate Height for 23-mer Oligonucleotides Separated Using Fully Porous Particles and Ion-Pairing Reversed-Phase Liquid Chromatography

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Identification and quantification of impurities in therapeutic oligonucleotides (ONs) by liquid chromatography is a critical component of control strategies used in pharmaceutical manufacturing. While ion-pairing reversed-phase liquid chromatography (IPRP-LC) remains the gold standard for these separations, the fundamental influence of stationary phase physical parameters on the kinetic performance of ON separations remains under-characterized. We have investigated the effect of stationary phase pore size and particle size on plate height for a 23-mer surrogate therapeutic oligonucleotide under IPRP conditions. Isocratic plate heights were determined for eight fully porous stationary phases, including polymeric and silica-based materials, with pore sizes ranging from 80 to 4,000 Å and particle sizes from 1.8 to 5.0 µm. The results show that intraparticle pore size is a stronger determinant of plate height than particle size for the ON studied. For silica-based materials, increasing the pore size from 80 Å to 300 Å resulted in significantly lower plate heights and a weaker dependence of efficiency on mobile phase velocity. Moreover, we found that the observed trends in isocratic plate heights translated well to solvent gradient elution conditions that are more relevant to practical separations of ON mixtures. Finally, results from a brief set of pore penetration experiments using poly-thymidine standards suggest that diffusion of ONs larger than five bases through the intraparticle pores of conventional stationary phase materials (80–120 Å) is probably highly hindered. These findings underscore the importance of using wider-pore (e.g. >200 Å) stationary phases to maximize resolution in the analysis of therapeutic ONs.

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Evaluation of Portable Mass Spectrometry Platforms for Field-Based Detection of Fentanyl Analogues and Novel Psychoactive Substances

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The proliferation of illicit fentanyl, its analogues, and novel psychoactive substances (NPS) has worsened the opioid crisis. These potent drugs often appear at low concentrations in street samples mixed with cutting agents, complicating field detection. Portable spectrometers typically lack sample preparation to isolate the target analyte from its matrix, allowing interferents to obscure signals. Given the risks to drug users, first responders, and the public, rapid, sensitive, field-deployable methods capable of reliable detection are needed regardless of sample composition. Portable mass spectrometry (MS) is among the most sensitive and selective field techniques for identifying synthetic opioids and NPS, though performance depends on several factors. Mass analyzer type, quadrupole versus ion trap, can substantially influence library search and matching accuracy. Ion trap systems are especially prone to ion interactions within the trap, and factors like ionization method, operating pressure, sample amount, and matrix composition can further alter spectra. Thorough characterization of target compounds under platform-specific conditions is essential for reliable field identification. This study evaluated approximately 300 synthetic opioids and NPS using three commercial portable MS platforms: a toroidal ion-trap GC-MS, a quadrupole GC-MS, and a linear ion trap MS. While two platforms use gas chromatography for separation, only MS performance was assessed. Evaluation criteria included detection/identification capability and spectral fidelity, emphasizing library matching against NIST, Cayman Chemical, and vendor-specific libraries and search algorithms.

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Nanoparticle-Assisted MALDI-TOF Mass Spectrometry for Peptide Analysis
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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is widely used for the rapid analysis of peptides and protein digests. Although MALDI-TOF MS offers high sensitivity, simple sample preparation, and rapid data acquisition, peptide detection can be influenced by factors such as analyte abundance, ionization efficiency, and interference from the organic matrix. In particular, conventional MALDI matrices can generate background signals in the low-mass region, which may complicate the detection of low-molecular-weight analytes. The incorporation of nanoparticles into MALDI-TOF MS provides an alternative strategy for improving sample ionization and enhancing analytical performance. Gold nanoparticles (AuNPs) are particularly attractive because of their optical and surface properties, as well as the ability to modify their size and surface chemistry. AuNPs can be incorporated into MALDI workflows either as supporting materials for laser desorption/ionization or in combination with conventional organic matrices. In this study, different sample preparation conditions were evaluated for peptide analysis using MALDI-TOF MS. Nanoparticles were incorporated onto the MALDI target before sample analysis, and the resulting mass spectra were evaluated based on spectral quality and background interference. The results demonstrate that nanoparticle incorporation can influence peptide detection and MALDI-TOF MS performance. Optimization of nanoparticle properties and sample preparation conditions may provide a useful approach for improving MALDI-based peptide analysis and expanding its application to more complex samples.

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Using Analytical Modeling to Rapidly Develop Robust Chromatography Methods with Fewer Experiments: A Model-First Approach
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Analytical chemists are increasingly challenged to deliver robust chromatographic methods rapidly, often with limited material and an evolving understanding of the synthetic process. A model-first strategy addresses this challenge by leveraging predictive chromatography software to identify fit-for-purpose separation conditions with fewer experiments, reducing trial-and-error while conserving sample, time, and laboratory resources. This poster highlights chromatography modeling case studies across multiple separation techniques for small molecule drug substance compounds. A reversed-phase liquid chromatography (RPLC) case study demonstrated how modeling can rapidly establish methods capable of resolving known process impurities. Predictive modeling accelerated evaluation of new solvent impurities on existing gas chromatography platform methods while minimizing experimental effort. Challenging chiral normal phase separations, where ternary solvent mixtures were needed, and chiral supercritical fluid chromatography separations, leveraging pressure along with flow rate, temperature and mobile phase composition, illustrated the applicability of using modeling to solve complex separations. For high-throughput studies which often require shorter analytical methods, an RPLC separation demonstrated how modeling can optimize methods for speed while preserving critical impurity resolution and maintaining existing column chemistries and mobile phase conditions, simplifying method bridging. Finally, an RPLC late-stage robustness example highlights how thousands of chromatographic conditions can be evaluated computationally in seconds, providing deeper insight into parameter sensitivity and potential separation failure modes. Together, these examples demonstrate how analytical modeling enables faster, more efficient, and more transferable chromatography method development while providing valuable insight into the most critical method parameters, enhancing process understanding, and reducing consumable and solvent use along with experimental burden.

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cVSSI Enhances Microflow UPLC–MS Metabolomics in Negative-ion Mode
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Capillary vibrating sharp-edge spray ionization (cVSSI) was compared with electrospray ionization (ESI) in negative-ion mode for microflow UPLC–MS metabolomics. A 22-component oxylipin standard mixture was used to compare analytical response and sensitivity. A urine sample was also analyzed to evaluate metabolomic coverage in a complex biological matrix. Result showed that cVSSI produced approximately 2.7–5.6-fold greater sensitivity than ESI. The LODs ranged from 0.004–0.266 ng/mL for cVSSI and 0.009–0.428 ng/mL for ESI. The corresponding LOQs were 0.013–0.806 and 0.028–1.30 ng/mL, respectively. Signal enhancement showed a positive correlation with MolLogP ($r = 0.611$) and pKa ($r = 0.434$), but a negative correlation with TPSA ($r = -0.573$). This suggests that cVSSI provides greater enhancement for relatively hydrophobic compounds with lower polar surface area. Overall, the results show that cVSSI provides better sensitivity and broader metabolomic coverage than ESI under the conditions evaluated.

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Proteomics Approach to Investigate Age-Related Macular Degeneration
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Age-related macular degeneration (AMD) affects a large proportion of the elderly population, and the leading cause of vision loss. No effective therapies exist for the most frequent form of disease, dry AMD as the mechanisms that lead to dry AMD are not well understood. Among the many strategies used to accomplish this unmet need, valuable insights were provided by proteomics studies of biological fluids and eye tissues affected by the disease. In this study, we utilized proteomics analysis of plasma from donors who were *diagnosed with AMD and with no AMD.* A pair of 50 plasma samples were depleted of highly-abundant proteins including albumin, transferrin, haptoglobin, and α-1-antitrypsin using Proteospin™ Serum depletion-kit (Norgenbiotek). These abundant proteins have

acidic isoelectric points, preventing their interaction with the negatively-charged resin at near-neutral pH. The depleted samples were subjected to filtration using a 10-kDa molecular weight cutoff membrane, followed by in-solution trypsin digestion of the retentate, enabling comprehensive protein identification. The retentate was analyzed using LC-MS/MS using nanoAcquityUPLC coupled to XEVO G2-XS QToF-MS (Waters Corporation) to investigate differences in protein expression. The flowthrough was saved for peptidomics study using both LC-MS/MS and MALDI-ToF. Mascot Daemon from Matrix Science was used for database searches on the MS-data against Swiss-Prot database for protein identification. Scaffold Proteome (scaffold_4.3.4) was used for protein quantitation. The preliminary data identified several biologically relevant proteins, including complement, fibrinogen, apolipoprotein, and SERPIN families.

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Invention, Development and Application of Affinity Selection-Mass Spectrometry

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Affinity selection-mass spectrometry (AS-MS) was invented during the 1990s in our laboratory and others as a high-throughput approach to address the need for rapid screening of mixtures of compounds including pooled combinatorial libraries and botanical extracts. Based on binding interactions between macromolecular receptors and ligands of low molecular mass, AS-MS facilitates the isolation of pharmacologically active small molecules from complex mixtures for MS characterization and identification. AS-MS requires no radiolabels or chromophores, and is compatible with all receptors, enzymes, buffers, cofactors, and ligands. Variations of AS-MS differ primarily in how the ligand-receptor complexes are separated from non-binding compounds. The most popular approaches include the solution-phase methods of ultrafiltration AS-MS and size exclusion chromatography AS-MS, and the solid-phase approach of magnetic microbead affinity selection screening (MagMASS). Based on accurate mass and MS/MS measurements, affinity-selected compounds are identified by comparison with known compounds in a combinatorial library, or if natural products, through dereplication by searching on-line databases of known compounds. For novel natural products, AS-MS provides retention times of active compounds for chromatographic isolation and structure determination using a combination of spectroscopic techniques. Advantages of AS-MS over conventional high-throughput screening are speed, reduced consumption of reagents, compatibility with any receptor and ligand, and the ability to discover allosteric as well as orthosteric ligands. Due to these advantages, AS-MS has been incorporated into many drug discovery programs in the pharmaceutical industry. Examples of AS-MS from our laboratory will focus on natural products screening using our inventions of ultrafiltration AS-MS and MagMASS.

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From Systems to Structure: How Mass Spectrometry Drives Biological Discovery

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In this presentation I will describe how modern mass spectrometry (MS) technology is accelerating many aspects of biological discovery. First, I will describe how the latest generation of high resolution and accurate mass MS systems enable very rapid and deep proteome analysis in just tens of minutes. These technologies are now enabling systems-type applications of MS and here I will describe examples of using MS to discover protein function en masse. Next, I will describe a new technology for rapid peptide mapping of therapeutic antibodies using direct infusion MS. With this technology several hundred to 1,000 peptide maps can be acquired in a single day with results comparable to traditional LC-MS-based experiments. Finally, I will present new technology that couples mass spectrometry with cryo-electron microscopy. By using mass spectrometry to prepare cryo-EM samples we have the potential to eliminate many major sample preparation barriers including: air-water interface, ice thickness control, preferred particle orientation, and size limitations.

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Fundamental Retention Mechanisms in HILIC: A Foundation for Robust Method Development

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Hydrophilic-interaction liquid chromatography (HILIC) is characterized by a complex interplay of partitioning, polar, and ionic interactions, with their relative contributions governed by both analyte properties and stationary-phase chemistry. Consequently, understanding the molecular interactions responsible for retention, together with the specific interaction profile of a stationary phase, provides an important foundation for rational HILIC method development. This presentation will examine approaches for evaluating the relative contributions of partitioning and ion-exchange mechanisms across diverse HILIC stationary phases and demonstrate how these differences influence retention and selectivity. Comparative studies of silica, pentahydroxyl, pentafluorophenyl, amide, zwitterionic, and related phases illustrate that nominally similar HILIC classifications can encompass substantially different interaction profiles. In particular, evaluation of retention as a function of mobile-phase ionic strength provides a practical means of assessing ion-exchange contributions and distinguishing phases according to their predominant retention mechanisms. These fundamental considerations provide a framework for selecting stationary phases based on the physicochemical characteristics of the analytes and the interactions most likely to generate useful selectivity. Rather than relying primarily on empirical column screening, this approach focuses method development on stationary phases capable of exploiting the most significant differences among the analytes. Application to nucleoside separations demonstrates how this strategy can identify effective starting conditions and guide subsequent optimization. A mechanistic understanding of HILIC retention does not eliminate experimentation, but makes it more purposeful, enabling the development of efficient, reproducible, and robust separations.

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Solving Problems with Vibrational Spectroscopy

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Solving a scientific or forensic problem begins with selecting the right tool for the question at hand, and few analytical approaches offer as broad a toolkit as vibrational spectroscopy. Infrared and Raman spectroscopy are available across a wide range of instrumentation: handheld and portable devices for rapid, non-destructive investigations in the field; benchtop systems for confirmatory, high-resolution laboratory analysis; and infrared and Raman microscopes that couple chemical identification with spatial resolution down to the micron scale, enabling analysis of the smallest and most degraded traces. Each instrument class carries its own strengths and limitations in sensitivity, selectivity, spatial resolution, sample size requirements, and speed, and no single configuration can address every analytical challenge. Effective problem solving therefore depends less on any one instrument than on understanding these trade-offs well enough to match the right technique, at the right scale, to the specific sample being interrogated. This presentation will use case examples to demonstrate how vibrational spectroscopy can be used to solve a range of forensic problems, which ultimately rests on having, and knowing how to choose from, the full instrument arsenal.

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Remembering Rob Schurko's Myriad Contributions to NMR Crystallography

Sean Holmes, Florida State University, 1800 E Paul Dirac Dr, Tallahassee, FL 32310

NMR crystallography (NMRX), which integrates solid-state (SSNMR) spectroscopy, X-ray diffraction, and density functional theory calculations, provides deeper insight into crystal structures than any of these methods individually. In this lecture, I will highlight some of Rob Schurko's most notable achievements in this field, for which he is honored with the 2026 EAS Award for Outstanding Achievements in Magnetic Resonance. Rob's work in this area applied quadrupolar SSNMR spectroscopy as an important means of analyzing molecular-level structure and dynamics in active pharmaceutical ingredients (APIs). In particular, his interest in 35Cl SSNMR was spurred by the ubiquity of chloride anions in pharmaceutical salts, as well as its ability to provide distinct spectral fingerprints for HCl APIs and their polymorphs, solvates, hydrates, and cocrystals because of the strong dependence of the 35Cl electric field gradient (EFG) on the local structural environments of chloride ions. Along with other nuclides like 2H, 14N, 17O, and 81Br, quadrupolar SSNMR techniques provide key insights into molecular-level structure, bonding, and crystal packing, and are therefore relevant in all types of NMRX studies. Several illustrations are provided in which quadrupolar SSNMR techniques are indispensable, with applications ranging from novel solid form discovery to crystal structure prediction and NMR-guided Rietveld refinement.

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Precision Lipidomics by Mass Spectrometry: From Lipid Isomeromics to Lipid–Protein Interactions

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Lipids comprise one of the most structurally diverse classes of biomolecules, yet this complexity is often reduced to molecular compositions in conventional lipidomics, overlooking structural features and molecular interactions that govern biological function. To address this challenge, my research group seeks to establish precision lipidomics through the development of innovative mass spectrometry technologies that integrate structural characterization, quantitative analysis, spatial mapping, and lipid–protein interaction profiling. This talk will focus on the development of aziridination-enabled mass spectrometry, a versatile chemical platform that transforms carbon–carbon double bonds into information-rich functional groups. The resulting platform enables comprehensive lipid isomeromics through the identification of double-bond positional, sn-positional, and cis/trans geometric isomers, providing insights into lipid metabolic pathway remodeling that are inaccessible using conventional compositional analyses. Building upon this chemistry, we have developed isotope-encoded and isobaric labeling strategies for accurate isomer-specific quantification, enabling both global and spatially resolved analyses of lipid isomers in biological tissues. More recently, we have extended aziridination chemistry to lipid–protein cross-linking mass spectrometry, providing a general approach for identifying lipid-binding proteins and mapping lipid–protein interaction sites. Applications of these technologies to cancer metabolism demonstrate how precision lipidomics reveals molecular mechanisms that remain hidden by conventional lipidomics. These advances establish a unified analytical framework that connects lipid molecular structure, abundance, spatial distribution, and biomolecular interactions, providing a more comprehensive understanding of lipid biology and new opportunities for biomarker discovery and therapeutic development.

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Characterization of an Unattributed T. Jefferson Declaration Desk Facsimile

Zachary Voras, West Chester University, Chemistry Dpt., 750 S. Church St., West Chester, PA 19383, Ethan Radcliffe

Spectroscopy is the ideal method for non-invasive analysis, of art and artefacts, by conservators. This study seeks to use a multi-modal spectroscopic approach to determine the attribution of an unattributed facsimile of the T. Jefferson 'Declaration Desk' that is currently in the possession of a private collector. Digital stereomicroscopy with ultraviolet fluorescence, micro-X-Ray fluorescence spectroscopy (μXRF), reflectance Fourier-transform infrared spectroscopy (rFTIR), and Raman spectroscopy were employed for comparison against known attributes of the original 'Declaration Desk' (manufactured c. 1775 by Benjamin Randolph) and facsimiles manufactured c. 1880 by the Department of the Treasury. Preliminary results shown will be relevant for determining manufacturing consistency with the two noted time periods.

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Reviving a Collection: Technical Insights into the MFA Boston's Tibetan Thangkas

Marcie Wiggins, Museum of Fine Arts-Boston, Dept. of Conservation & Scientific Research, 465 Huntington Ave., Boston, MA 02115, Hsin-Chen Tsai, Jacki Elgar

The Museum of Fine Arts, Boston houses an extensive collection of Asian artworks, many of which have remained in storage and unstudied since they entered the collection over a century ago. This presentation focuses on a set of three 16^{th} – 17^{th} century Tibetan thangkas depicting Tara, a Buddhist deity. As a part of their ongoing conservation, technical analysis of their materials has been a key

component to understanding their creation. Thangkas of this type would have been a part of large sets produced in workshops by a team of artists. Consistent with other Asian workshops at this time, they utilized color notations- small, coded symbols or inscriptions that guided pigment selection and application. Multiband imaging was used to locate color notations and representative painted regions for targeted, non-invasive analysis by in situ X-ray fluorescence (XRF) and fiber optic reflectance spectroscopies (FORS). Subsequently, microsamples were analyzed by Raman and Fourier transform infrared (FTIR) spectroscopies, as well as scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS), to confirm pigment identifications. Thus far vermilion, lead white, white clay, red lead, iron oxides, azurite, malachite, and orpiment were identified in this set. Beyond establishing the materials used, this study seeks to correlate Tibetan color notations with specific pigments, providing new insight into workshop practices and production. These findings contribute to the study of Tibetan painting and complement broader research on similar works within the MFA's Asian art collection.

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Using the Method of Continuous Variation to Elucidate the Effects of pH and Ionic Strength on the Metal:Dye Stoichiometry of Lake Pigments

Jessica Heimann, Salisbury University, Department of Chemistry, 1101 Camden Ave., Salisbury, MD 21801

Organic scaffolds such as anthraquinones and flavonoids have been used to make pigments and dye textiles since ancient times. These chromophores are historically extracted from the roots or flowers of plants or from scaled insects. To use these soluble organic dyes as pigments, they must be precipitated with a mordant, usually a metal salt such as hydrated potassium aluminum sulfate, to form a lake pigment. In this process, the dye molecules act as Lewis bases and donate electrons to make coordinate bonds with the metal ions supplied by the mordant, which results in the formation of insoluble metal-dye coordination complexes. There is a significant body of evidence showing that the conditions during synthesis can influence the appearance, stability, and even the structure of a lake pigment. The present study uses the method of continuous variation or Job's method to first probe how the pH during complexation – and consequently the protonation state of the dye – impacts the binding stoichiometry between various metal cations and carminic acid, a pH-dependent dye extracted from *Dactylopius coccus* (cochineal). A preliminary exploration of the effect of ionic strength on the binding stoichiometry is also presented. Based on the mole fractions identified with Job's method, this study then aims to provide insight into the binding modes (*e.g.* O1/O9 vs. O1/O2) likely formed in these carmine lake pigment models.

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Technical Investigation of Piet Mondrian's Transatlantic Paintings: Materials, Chronology, and Revision

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Over the course of his career, Piet Mondrian moved between the Netherlands, Paris, London, and finally New York, with each location shaping his materials and methods of working. However, the limited documentation of works in progress from that period makes it challenging to determine how he revised his compositions. This study focuses on the application of large-area micro-X-ray fluorescence (XRF) scanning to investigate a group of paintings dating from 1921 to 1942, with particular attention to works Mondrian changed in New York City, complemented by limited sampling and vibrational spectroscopy. XRF scanning proved to be a powerful tool for mapping the compositional and material changes Mondrian made at different times over his career and movement. The findings illustrate the progression of Mondrian's selection of primary colors, his development of black lines, his comprehensive revision process, and elucidate Mondrian's dating system for works revised in New York. XRF scanning proved to be a powerful tool for mapping the compositional and material changes Mondrian made and for elucidating compositional changes that are not apparent in more traditional imaging techniques, such as X-ray radiography and UV fluorescence. Taken together, these results demonstrate how scientific analysis provides insight into Mondrian's materials, techniques, and revisions across locations, especially where documentary evidence is limited or lost.

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3D Printed Sample Processing and Handling Platforms for Scaling up Mass Spectrometry Experiments

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Sample preparation and handling are critical determinants of both analytical performance and throughput in mass spectrometry (MS). In this presentation, we will discuss our recent efforts to develop 3D-printed platforms that enable parallelized sample preparation and rapid sequential sample introduction for MS analysis. In the first example, we developed a platform for parallel single-cell sample processing coupled with liquid chromatography–mass spectrometry (LC-MS)–based proteomics. The platform integrates 3D-printed channels and wells to enable parallel fluid and sample manipulation. Using a moving magnet to control magnetic beads, up to 10 individual cells can be processed simultaneously through the complete proteomic sample preparation workflow, providing a scalable approach for parallel single-cell analysis. In the second example, we developed a modular, 3D-printed platform for rapid sequential sample injection. The platform adopts a well plate–like format and enables direct loading of discrete sample droplets using either manual micropipetting or automated liquid handling. During transport, individual droplets are separated by air gaps and introduced directly into the mass spectrometer through capillary vibrating sharp-edge spray ionization (cVSSI). A sampling throughput of approximately 20 s per sample was achieved at a flow rate of 50 µL/min. Quantitative analysis of lysine demonstrated excellent linearity (R^2 = 0.9988), and sample carryover was also evaluated using chemically distinct small molecules. Together, these studies demonstrate the versatility of 3D printing for creating customizable and scalable platforms that streamline sample preparation and sample introduction, with the potential to substantially increase the throughput of mass spectrometry–based analyses.

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Innovating Excipient Analysis Through End-to-End Automation on an Integrated Robotic–HPLC ELSD System

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Excipients, inactive components of biopharmaceutical formulations, are critical for maintaining drug stability, solubility, and overall product performance. While many are not designated Critical Quality Attributes (CQAs), their accurate quantification and degradation product monitoring can influence product quality and provide valuable information regarding process readiness and lifecycle management. This presentation describes the development and evaluation of an end-to-end automated analytical platform integrating robotic sample handling with High-Performance Liquid Chromatography coupled to Evaporative Light Scattering Detection (HPLC–ELSD) for excipient analysis. The system was designed to streamline sample preparation, improve analytical consistency, and reduce manual intervention while supporting release and stability testing. The platform consists of the Waters Andrew+ liquid handling robot and associated Devices+, including Shaker+, Sealer+, Extraction+, Portal+, and POD+, for automated sample preparation. Integration with a Waters ACQUITY Premier System enabled chromatographic analysis of excipients and related degradation products. A key application evaluated was the analysis of polysorbate 80 (PS80), a widely used surfactant that protects biotherapeutics from aggregation and surface-induced degradation. Because PS80 lacks a strong UV chromophore, ELSD remains the industry-standard detection technique for its quantification. The integrated system demonstrated the capability to support a fully automated, hands-off workflow, enabling end-to-end sample preparation and chromatographic analysis with minimal analyst intervention. Evaluation of the platform demonstrated improved operational efficiency, enhanced data quality, reduced analyst hands-on time, and improved assay reproducibility. This work highlights the feasibility of end-to-end automation for excipient testing and its potential to increase laboratory throughput and modernize analytical testing operations.

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A Modular and Affordable Electrochemical Self-Driving Lab Enables Fully Autonomous Electrode Modification and Characterization

Michael Pence, University of Utah, 315 1400 E, Salt Lake City, UT 84112, Griffyn Sgro, Bingxin Yang, Long Luo

Electrode modifications are key to many functional electrochemical devices, such as electrolyzers, batteries, and sensors. Successful modification that achieves the desired functionality often requires tuning many experimental handles, making comprehensive manual exploration time and resource prohibitive. To this end, an autonomous and high-throughput way to modify and characterize electrodes is needed to enable efficient data-driven exploration. However, a one-size-fits-all approach to automating electrode modification remains a challenge as the automated platform must allow for refreshed pristine surfaces, incorporation of downstream characterization, and storage of electrodeposited samples for offline characterization. Here, we introduce a modular and affordable electrochemical self-driving laboratory designed to accelerate the discovery and optimization of functionalized electrodes. We demonstrate the platform using metal electroplating as a testbed for fully autonomous electrode modification and characterization. A vision-guided Bayesian optimization workflow identifies electrodeposition conditions that produce targeted film properties. We use interpretable machine-learning methods to identify highly impactful experimental parameters and experimentally validate their effects. Finally, we demonstrate the broader applicability of the platform to additional materials, including electropolymers and nanoparticles.

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High Throughput Optical Characterization of Single Plasmonic Nanostructures

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Colloidal nanocrystal syntheses achieve incredible range in the shapes and sizes of metallic nanoparticles that can be prepared, from simple geometric structures like spheres and cubes to more complex structures such as octahedra and octopods. Specific shapes and sizes are targeted by utilizing a variety of metal salt precursors, chemical reducing agents and shape-directing surfactants under optimized reaction conditions. However, even the best syntheses yield unavoidable heterogeneity in the reaction products, resulting in both intra- and inter-sample differences in nanocrystal shapes and sizes. Characterizing this structural heterogeneity requires single particle techniques, with electron microscopy being the gold standard for achieving high resolution morphological information. Unfortunately, electron microscopy typically operates under high vacuum, can induce damage to the nanostructures, and requires expensive instrumentation. Optical microscopy, on the other hand, overcomes many of the challenges associated with electron microscopy, but suffers from a fundamental spatial resolution based on the diffraction limit of light, resulting in a complete loss of structural information about the nanocrystals of interest. To overcome this limit, the Willets lab has developed calcite-assisted localization and kinetics microscopy (CLocK), an optical imaging strategy that encodes structural information into optical images while remaining compatible with real time, *in situ* imaging under ambient conditions. Extracting structural information from CLocK images is achieved by using tools from the data science and machine learning communities, accelerating the throughput of our approach and moving us closer to automated analysis of heterogeneity in colloidal nanoparticle populations.

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SERS Imaging and ICP-MS Quantification of the Biological Translocation of Nanoplastics Using a Dual-Detectable Model Nanomaterial

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Nanoplastics (nP) pose significant concerns to human health due to their wide environmental presence and high potential for biological uptake, transport, and accumulation. Conventional analytical methods for studying nP-biota interactions suffer from low accuracy and precision due to limited reliability and quantitative capability. To address these challenges, a dual-detectable nP material was developed that allows for *in-situ* imaging by surface-enhanced Raman spectroscopy (SERS) and *ex-situ* quantification by inductively coupled plasma-mass spectrometry (ICP-MS). Here, a model nP that has a core-shell structure with Raman reporter-functionalized gold nanoparticles as the core and a layer of plastic as the shell was synthesized. The model polystyrene (PS) nP demonstrated stability in structure, morphology, size, and surface charge over one year, enabling stable signals and reliable long-term monitoring in complex biological systems. A single nP was successfully detected by SERS, indicating the single-particle detection capability of this

approach. We used the dual-detectable nP to examine the intestinal barrier-crossing behavior of nP in an aquatic animal model (*Daphnia magna*) and revealed the major mechanisms underlying the barrier-crossing process. The nP were further tested in a plant model (*Allium sativum*). They enabled both semi-quantitative nP visualization via SERS imaging and accurate quantification analysis through ICP-MS, serving as a powerful tool for studying the biological uptake, transport, and removal of nP. This dual-detectable nP model can significantly improve our understanding of their fate and effects on biota in the future.

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Lifecyle of Micro- and Nanoplastics in the Body and Their Contribution to Alzheimer's Disease

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Micro- and nanoplastics (MNPs) are emerging environmental contaminants of increasing concern, yet their biodistribution, persistence, and contribution to human disease remain poorly understood. Our laboratory has investigated the neurological consequences of MNP exposure using multiple mouse models and complementary cellular systems. We previously demonstrated that acute (3-week) exposure to 0.1 and 2 μm pristine polystyrene (PS) MNPs via drinking water induces significant neurobehavioral deficits in both young and aged C57BL/6J mice. MNPs accumulated within intracellular compartments of all major organs, including brain parenchyma, and were associated with altered inflammatory responses, including a marked reduction in glial fibrillary acidic protein (GFAP) expression. In vitro studies further revealed that PS-MNPs readily enter cells, localize around the nuclear envelope, and disrupt cellular homeostasis and viability. To determine whether genetic susceptibility modifies MNP-induced neurotoxicity, we exposed humanized APOE3 and APOE4 knock-in mice to PS-MNPs. Acute exposure impaired locomotor activity and recognition memory in a sex-dependent manner in APOE4, but not APOE3, mice and reduced both GFAP and the microglial marker IBA1, suggesting altered neuroimmune responses. Extending these findings, chronic (3-month) exposure of APP/PSEN1 mice resulted in worsened cognitive deficits, increased amyloid-β plaque burden, and additional neuropathological changes, indicating that MNPs may accelerate Alzheimer's disease progression. New findings on the bioaccumulation, tissue retention, and elimination of MNPs using our newly developed quantitative workflow for measuring particles across biological tissues will be shared. Together, these studies provide new insights into the lifecycle of MNPs in the body and their role in promoting neurodegeneration and age-related disease.

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Female Reproductive Impacts of Real-World Endocrine Disruptor (EDC) Mixtures

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Rivers are increasingly contaminated with complex mixtures of endocrine-disrupting chemicals (EDCs) that may threaten human reproduction. However, most toxicological studies focus on individual EDC. Here, we integrated high-resolution mass spectrometry (HRMS), environmental chemistry, and mechanistic toxicology to investigate the reproductive effects of river water EDC mixtures from major New Jersey (NJ) rivers. Surface water samples were collected from eight sites across the Passaic, Raritan, and Delaware Rivers, and organic contaminants were concentrated by solid-phase extraction. Targeted HRMS identified 72 established EDCs, including alkylphenols, per- and polyfluoroalkyl substances (PFAS), phthalates, and pharmaceuticals. The Passaic River, particularly the Elmwood Park site, exhibited the highest EDC burden. Using a 3D in vitro mouse ovarian follicle culture model, we found that organic extracts from multiple Passaic River sites impaired follicle growth, disrupted steroidogenesis, and inhibited ovulation. RNA sequencing identified activation of aryl hydrocarbon receptor (AhR) signaling, oxidative stress, and mitochondrial dysfunction. We next reconstructed representative mixtures of PFAS, phthalates, and legacy persistent organic pollutants (POPs), including dioxins, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). POP mixtures, but not PFAS or phthalate mixtures, reproduced the ovarian toxicity of river water extracts, and these effects were attenuated by pharmacological inhibition of AhR. Chronic drinking water exposure of female mice to Passaic River EDC mixtures further verified EDC mixture-induced irregular estrous cycles, anovulation, and molecular alterations. Together, these findings identify AhR-activating POPs as major drivers of river water-induced female reproductive toxicity and demonstrate the value of integrating analytical chemistry with mechanistic toxicology for environmental health research.

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Phthalates and Nanoplastics Disrupt the Mouse Placenta

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Exposure to micro- and nanoplastics is unavoidable. Foods and beverages contain plastic particles from environmental contamination and processing and packaging materials, which are frequently made of polyethylene terephthalate (PET). Recent studies found plastic particles can cross the placental barrier and reach the fetus, but little is known about the effects nanoplastics have on development. We hypothesized that combined exposure to nanoplastics and plasticizers would disrupt placental growth and function. We orally exposed pregnant CD-1 mice to vehicle control, 2 mg/kg of 240 nm secondary PET particles, 200 mg/kg of a mixture of seven phthalates based on biomonitoring in pregnant women, or both PET and phthalates from gestation day 7 to day 15. In male placentas exposed to both treatments, the placenta:fetus ratio was borderline significantly decreased. Placenta RNA was subjected to RNA sequencing, which identified differentially expressed genes in the nanoplastic-exposed male placentas only related to syncytium formation, translation, and bioenergetics. Deconvolution of the bulk RNA-seq using a single cell atlas of the mouse placenta at gestational day 15 revealed altered major cell type proportion and identified parietal trophoblast giant cells as targets in the males and sinusoid trophoblast giant cells as targets in the females. Follow up gene expression analysis and morphology measurements of placenta layer area are underway. Overall, these results suggest that nanoplastics disrupt placenta structure and signaling in a sex-dependent manner and that combined exposure to nanoplastics and chemicals can lead to increased toxicity.

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Artificial Neural Networks for Discrimination of Automotive Clear Coats by Motor Vehicle Manufacturer
Barry Lavine, Oklahoma State University, 107 Physical Science, Department of Chemistry, Stillwater, OK 74078, Collin White, Douglas Heisterkamp

Modern automotive paints have a thin undercoat and color coat layer protected by a thick clear coat layer. All too often, only the clear coat layer of the automotive paint is recovered at the crime scene of a vehicle related fatality. Searches of motor vehicle paint databases of clear coats using commercial software typically generate large hit-lists that are difficult for a forensic paint examiner to work though unless additional information is provided for the search. To address this problem, deep learning has been applied to the infrared spectra of automotive clear coats to identify patterns in their spectra indicative of the motor vehicle manufacturer. An in-house automotive paint library of 2796 clear coat infrared spectra from six automotive manufacturers and 100 assembly plants was partitioned into training, validation and prediction sets. Each spectrum has 1880 measurements over the spectral range of 4000 cm^{-1} to 376 cm^{-1}. Several multilayer perceptron neural network models, each with three hidden layers, were developed that achieved high classification success rates for the training, validation, and prediction sets. The addition of convolutional layers to the deep learning neural network models did not improve the performance of these models.

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Conformal Predictions: More than just a Confidence Interval Machine
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Conformal Predictions (CP) are an adaptable tool for placing confidence intervals on classification and regression predictions. For classification applications, a prediction set is determined that is guaranteed to contain the correct label with a predetermined confidence level. This prediction set size scales with the difficulty the model has in classifying the sample; areas of greater uncertainty will be associated with larger prediction sets. This presentation will show how CP prediction set size statistics can be used to compare and contrast models of nominally comparable performance. That is, two models may have similar accuracies for classifying the training and validation sets, but have very different distributions of prediction set sizes. The utility of CP will be demonstrated on two different classification applications. Infrared spectra were collected from clear coats of 6 different automobile manufacturers The goal is to identify car manufacturer following forensic analysis of paint smudges following hit and run accidents. Laser induced breakdown spectroscopy spectra were collected from 19 different species of exotic hardwood timber. The goal is to identify timber species in order to combat the illegal timber trade.

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Extracting Chemical Information from Infrared Images of Polymers to Detect Contaminants and Other Defects Essential for Quality Control
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Polymers are found in a wide range of industrial products. Unfortunately, polymers sometimes contain contaminants that make their way into the final product. Infrared mapping and imaging techniques are routinely employed to detect these defects. Due to the irregular nature of these defects and how the contaminants are integrated into the polymers, obtaining mapping spectra without baseline distortion and/or noise is challenging. Spectra that suffer from baseline distortions can prevent the detection of contaminants in an infrared spectral image or map of the polymer when applying the ALS software provided by the instrument manufacturer, and the software used for baseline correction is limited to a linear or quadratic fit, which does not account for component concentrations across the sample. Improvements in multivariate curve resolution and spectral preprocessing are crucial to ensure the detection of these defects in these polymers. For this reason, a modified alternating least squares (MALS) algorithm has been developed. An adaptive approach for baseline correction is coupled to MALS utilizing three well known baseline correction algorithms (rubber band, penalized least squares, and asymmetrically reweighted penalized least squares) to produce an accurate snapshot of the background and to minimize error in the infrared spectra. Utilizing an adaptive approach for baseline correction which requires the direct integration of baseline correction and library searching into MALS, background corrected spectra were obtained as well as a background corrected spectra of the library that allowed for the detection of defects in our sample from the infrared spectral image of the polymer.

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Accelerating Pharmaceutical Development via Machine Learning-Enabled Process Analytical Technology
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Across microcarrier-based cell culture processes for pharmaceutical manufacturing, a deep understanding of a variety of process phenomena, spanning cell surface adhesion, cell aggregation, microcarrier aggregation, cell lysis, cell death, surface detachment and accumulation of cellular debris, may be needed to help assess process performance and robustness. However, characterizing these critical process phenomena often remains a challenging task, especially with respect to real time analysis methods that directly interface with the process itself. Process analytical technology (PAT) offers unique advantages to potentially overcome the challenges of traditional analytical methodologies, especially when coupled with machine learning approaches. In this work, we propose the utilization of *in situ* microscopy with machine learning analysis as a real-time, in-line PAT to directly visualize and characterize both cell and microcarrier behavior simultaneously. To the best of our knowledge, this is the *first* report of *in situ* microscopy applied to an upstream live virus vaccine cell culture process, providing direct visualization and characterization of process phenomena in real-time. Moreover, we herein also offer spectroscopic-based PAT tools coupled with machine learning to elucidate real time information with applications across pharmaceutical research and development efforts. Specifically, cell growth, cell death, surface detachment, accumulation of cellular debris, and aggregation of microcarriers are directly elucidated via our proposed technology. As such, we offer several PAT methodologies, including optimal data collection and data analysis strategies, to enable pharmaceutical development

in a manner that significantly advances process understanding beyond what can be achieved using traditional offline analytical characterization methods.

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Utilizing ^{19}F Solid-State NMR to Understand Form Changes in Pharmaceutical Solids

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Characterization of the active ingredient within solid dosage forms represents an ever-challenging problem facing pharmaceutical scientists. Detailed solid-state analysis of a drug product can include active ingredients as well as excipients, interactions among them, and potential changes that could occur during processing or storage. Solid-state NMR excels in cases of relatively simple solid form determination in drug products due to its exquisite specificity and ability to distinguish between specific components in complex mixtures. This presentation will focus on modern applications of solid-state NMR spectroscopy to gain insights into changes in active substances within formulated products as a function of unit operations such as compression, and stability under storage at varying degrees of humidity. ^{13}C remains the most widely studied nucleus in pharmaceuticals due to its presence in virtually all drug molecules and most other pharmaceutical ingredients. High resolution spectra can be obtained with facile differentiation among various components present in mixtures. However, due to its low natural abundance and sensitivity its usage can be somewhat hindered for formulations, where the active ingredient of interest may be diluted significantly among the inactive ingredients. For both sensitivity and selectivity reasons, ^{19}F is a more attractive nucleus from an NMR standpoint, and fluorine continues to become more widespread in pharmaceutical materials. We will examine two cases of how it can be used to monitor hydration changes in a formulated API, as well as help identify the crystal structure of a minor phase only observed following tablet compression.

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Solid-State NMR Investigation of Co-Crystals and Solid Solutions in Structurally Related Model Compounds

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Understanding how structurally related molecules organize in the solid state is essential for the rational design of multicomponent materials in pharmaceutical science. In this study, model compounds including benzoic acid, 2-thenoic acid, toluic acid, and 2,5-dimethylpyrazine were investigated to determine whether they form co-crystals or solid solutions. To probe these solid-state arrangements, 1H–13C HETCOR solid-state NMR was combined with DSC-TGA and single-crystal X-ray crystallography. HETCOR experiments provided direct insight into local proton environments around specific carbon sites, enabling discrimination between co-crystal and solid-solution behavior and assessment of how components are distributed within the lattice. These measurements also probed molecular-level mixing beyond overall phase composition and helped evaluate whether the components share a common proton reservoir, thereby supporting structural models of the solid forms. Thermal analysis complemented the spectroscopic findings by revealing phase transitions and thermal stability, while single-crystal X-ray crystallography supported the proposed structures. Collectively, these results demonstrate that subtle molecular differences can lead to distinct solid-state outcomes. This work highlights the value of solid-state NMR, particularly HETCOR, as a powerful approach for characterizing complex solid forms and advancing pharmaceutical solid-state design.

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New Insights into Quantitation and Detection in Drug Product Formulations Using Solid-State NMR Spectroscopy

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Solid-state NMR spectroscopy is a powerful tool for the characterization of pharmaceutical drug products and allows for the detection and quantification of different solid forms of a drug substance within a given drug product sample at low levels of detection. Different solid forms of a particular drug substance will have different spin lattice relaxation times and spin lattice in the rotating frame relaxation times. These differences in relaxation times can be used to differentiate between different solid forms that have similar chemical shifts in order to resolve a specific solid form allowing for its detection and quantification. Here we highlight how different filtering experiments that exploiting the differences in the relaxation times can be utilized to detect and or quantify a given solid form of a drug substance within a drug product sample. This allows for the development of a solid-state NMR of a drug product sample that can be used for quality control for different lots.

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Non-Destructive Evaluation of Lyophilized Products Using Solid-State NMR Spectroscopy

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While various non-destructive techniques can measure water content or water activity in lyophilized products, these methods often fail to serve as reliable indicators of long-term stability. Traditionally, product quality is assessed via visual inspection of the solid matrix. However, physical appearance does not always correlate with critical stability attributes. To bridge this gap, we introduce an *in-situ*, non-destructive ^{1}H NMR-based approach for the direct assessment of lyophilized vial contents to ensure manufacturing processes preserve product quality. This technique provides an analytical complement to visual inspection and conventional moisture testing by probing the molecule environment of the cake. In this presentation, we demonstrate that our NMR approach can: 1) monitor water uptake over time, 2) differentiate mobility between vials within the same batch, and 3) correlate monomer content with the ^{1}H NMR signal. Our findings reveal substantial vial-to-vial variability across multiple batches and demonstrate the impact of collapse on increased sample mobility. These results suggest that ^{1}H NMR offers a sensitive, stability-indicating tool for characterizing lyophilized products to ensure manufacturing consistency.

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Rapid Characterization of Antibodies, Mutated Antibodies and Antibody–Drug Conjugates Using Ultrafast Microdroplet Digestion Technology

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Accurate determination of drug-to-antibody ratios (DARs) is essential for the development, quality control, and performance evaluation of antibody–drug conjugates (ADCs); yet conventional analytical approaches often require extensive sample preparation, long analysis time, and substantial sample consumption. The peak distribution of intact ADCs is highly complex due to inherent glycosylation heterogeneity and variable drug conjugation. By applying enzymatic digestion, ADC can be converted into smaller subunits or deglycosylated species, thereby significantly simplifying the mass spectral profile. This reduction in structural heterogeneity facilitates clearer peak assignment and enables more accurate and reliable DAR quantification. Herein, we report an ultrafast microdroplet digestion–mass spectrometry strategy for rapid DAR characterization of ADCs. Microdroplet enzymatic digestion of antibodies and ADCs occurs within microsecond-time scales during spray ionization, enabling direct online subunit analysis with minimal sample preparation. The method was validated using NIST monoclonal antibody (mAb) conjugated to ADC mimics spanning low to high DAR (0-14) ranges, Cetuximab-derived ADC mimics (DAR~5) with complex glycosylation, and the commercial ADC Kadcyla (DAR~3.5). Consistent DAR values were obtained across multiple enzymatic workflows (IdeS, EndoS2, and EndoF3) with good reproducibility (%CV typically <5%). This approach substantially reduces analysis time while maintaining analytical accuracy and structural specificity, providing a rapid, sensitive platform for high-throughput ADC characterization and process monitoring.

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Myths and Half-Truths in HPLC and Pharmaceutical Analysis

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Despite significant advances in instrumentation and column technology, many long-standing practices in HPLC persist as “rules” that may no longer hold true in modern laboratories. This presentation examines several widely circulated myths and half-truths in reversed-phase HPLC and evaluates their relevance to today’s testing for small-molecule drugs. Using experimental evidence and case studies, the presentation will clarify when these practices add value and when they should be abandoned. Topics include: 1. Whether mobile-phase filtration is still required; 2.Buffers are essential to reduce peak tailing of basic analytes; 3. The caution against operating mobile phase A near an analyte’s pKa; 4. The standard 4.6 mm ID column remains the best choice for regulated methods; 5. The expectation that AI, QbD, automated software, and ICH Q14 will expedite HPLC method development. This presentation aims to help analysts and method developers to streamline workflows, improve robustness, and make better-informed decisions in pharmaceutical analysis.

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Advancing Small-Molecule Identification: Standards-Free Structural Assignment Using Molecular Rotational Resonance (MRR) Spectroscopy

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Accurate structural identification of small molecules remains a persistent challenge in analytical chemistry. This is particularly true for closely related regioisomers and stereoisomers with similar mass spectrometric and chromatographic behavior. In many applied settings, including pharmaceutical impurity analysis and complex mixture characterization, definitive assignments often require reference standards, increasing time and cost. We present molecular rotational resonance (MRR) spectroscopy as a practical technique for unambiguous, standards-free structural identification based on comparison to quantum chemical predictions. Advances in instrument sensitivity, automated sample introduction, and integrated software workflows now enable routine MRR analysis of both pure compounds and complex mixtures. MRR directly measures molecular moments of inertia, enabling structure-specific identification through comparison of experimental spectra to predicted candidates. The approach was evaluated using complex mixtures of isomeric compounds, including oxygenated sesquiterpenoid alcohols with identical molecular formulas but differing stereochemistry. Automated comparison of experimental spectra to predicted candidates enabled accurate identification of both major components and minor impurities without reference standards. Relative quantitation was also achieved using calculated response factors. These results demonstrate that MRR provides an orthogonal, structure-specific capability that increases confidence in analytical workflows while reducing reliance on reference materials. The method is particularly well-suited for applications in pharmaceutical impurity identification, natural products, and other industrial settings where rapid and definitive structural assignment is required.

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In-Situ Investigation of Monoclonal Antibody Crystallization Kinetics for Designing High-Concentration Protein Drug Products

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High (≥100 mg/mL) and ultra-high (≥250 mg/mL) protein formulations enable patient-centric drug delivery for subcutaneous administration of high-dose therapeutics, but they often present technical challenges due to elevated viscosity, which can limit syringability. Crystalline suspensions provide a promising avenue of relief towards these ends. Compared to their soluble counterparts, crystalline suspensions demonstrate reduced viscosity. Furthermore, the crystallization of biologics may present an attractive platform strategy for purification as it markedly reduces costs classically associated with chromatography, streamlining workflow, cost, and resources. Critically, there is a dearth of knowledge surrounding the components of crystallization formulations that tune particle size, shape, and other attributes that would allow for the rationale optimization of the pharmaceutical attributes of protein crystals. Moreover, knowledge of the crystal habit at single crystal resolution has yet to be fully elucidated. Herein we report the *in-situ* crystallization

kinetics at single crystal resolution of a therapeutically relevant mAb. Utilizing Second-ordered nonlinear imaging of chiral crystals (SONICC) and advanced AI enabled processing analysis, we elucidate the kinetics and habit at single crystal resolution of mAb crystallization across formulation space. Further, investigating the impact of agitation and temperature on the crystal attributes. Demonstrating there is a direct link between components of the formulation, the manufacturing environment, and the resulting crystal profiles. Elucidating the critical formulation parameters that can facilitate the rationale design of ultra-high concentration protein crystalline suspensions.

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Effects of Zinc (II) Ion on Structure, Stability, and Activity of Semaglutide in Prefilled Syringe Formulations

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Glucagon-like peptide-1 agonists (GLP-1As) are widely used peptide therapeutics for diabetes and obesity. Injectable semaglutide, a leading GLP-1A, is commonly supplied in prefilled syringes that require refrigeration to maintain stability. At elevated temperatures, metal ions, particularly zinc, can leach from syringe components such as the inner needle shield, potentially promoting peptide aggregation and device clogging. Here, we model temperature-induced zinc exposure and evaluate its impact on semaglutide structure and function. Circular dichroism, dynamic light scattering, and nuclear magnetic resonance were used to assess structural changes and aggregation, while cell-based fluorescence and luminescence assays evaluated cytotoxicity and GLP-1 receptor activity. Zinc exposure caused a transient, concentration-dependent increase in α-helicity, followed by a gradual decrease in ellipticity at 37 °C over several days. Notably, the presence of phosphate buffer mitigated these effects, likely due to preferential zinc–phosphate interactions that reduced peptide destabilization. Importantly, zinc-induced structural changes were fully reversible upon dilution, and no significant differences in cytotoxicity or receptor activation were observed. These findings highlight the resilience of semaglutide to zinc stress under relevant conditions and underscore the importance of device–formulation interactions in the development and bioanalytical evaluation of combination drug products.

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Methyl Proton Spin Relaxation (R2/R1): A Sensitive NMR Method for pH-Dependent Oligomerization of GLP-1 Analogs, Complementing DLS and DOSY-NMR

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Liquid buffer pH influences peptide higher order structure (HOS), including oligomerization, with important implications for peptide drug formulation development and stability control. Although dynamic light scattering (DLS) and diffusion-ordered spectroscopy (DOSY) NMR are commonly used to assess particle size (e.g., radius r) through measurement of translational diffusion coefficients (Dt), their sensitivity to subtle pH-dependent oligomerization changes can be limited. Here, using glucagon-like peptide-1 (GLP-1) analogs liraglutide and semaglutide as model peptides, we introduce the methyl proton spin relaxation rate ratio (R2/R1) as a sensitive NMR metric for detecting pH-dependent changes in peptide oligomerization. Diffusion coefficients measured using DLS and DOSY exhibited an overall increasing trend from pH 6.6 to 8.5, consistent with a shift toward smaller oligomers in basic solution; however, insignificant differences (p value > 0.05) were observed between pH 7.1 and 8.5 for liraglutide and between pH 6.6 and 8.2 for semaglutide. In contrast, the methyl proton R2/R1 decreased significantly with increasing pH, even within the narrow pH range of 7.1–7.7 (p value < 0.05), demonstrating smaller oligomer formation and less exchange at basic pH. The improved sensitivity arises because R2/R1 depends on both rotational diffusion ($Dr \propto r^{-3}$) and exchange kinetics, whereas DLS and DOSY depend on Dt ($\propto r^{-1}$). Consequently, the R2/R1 metric offers enhanced discriminatory power for resolving subtle pH-dependent peptide oligomerization in solution and serves as a practical analytical approach for peptide formulation development and stability control.

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THz-TDS Evaluation of Water Content in Flax-Based Natural Fiber-Reinforced Composites for Aircraft Applications

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Natural fiber-reinforced composites (NFRCs) offer many promising advantages over other fiber-based composites, including lower density, potential reduced manufacturing costs, and improved vibrational dampening. However, their reliability and overall performance need to be explored and better understood to determine potential implementation in aircraft applications. The Convergent Aeronautics Solutions (CAS) project consisted of various short-term activities that tested the feasibility of high-impact, high-risk innovations for the aviation industry. One activity explored the feasibility of NFRCs for aviation applications, with significant focus placed on understanding the degradation kinetics and mechanisms of these materials, especially since many degrade with exposure to moisture. To support adoption of these materials, nondestructive evaluation (NDE) methods can probe the degradation, identify damage, and characterize potential fatigue. One such method is terahertz time-domain spectroscopic (THz-TDS) imaging, which is sensitive to changes in density and composition of samples. Prior work showed that effective refractive index values derived from THz-TDS imaging can be related to density in plastic samples, with calibration curves enabling density determination in similar samples [I. Catapano *et al.*, "Foam density mapping via THz imaging," *Scientific Reports,* 14, 1, 15560, 2024]. In this study, we show that THz-TDS imaging can be used to determine water content in NFRCs, particularly flax fiber-based composites. The results indicate that the method's sensitivity decreases as water is absorbed into the material due to attenuation of THz signals. Despite this limitation, the method demonstrates practical utility for assessing moisture levels in these materials.

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Improving Sensitivity of a Microfluidic Electrochemiluminescence Biosensor
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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 biological targets 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. However, less toxic and inexpensive ECL reagents, which would be advantageous in a POC setting, require optimization to report relevant current levels from the sensing reaction. Here, we investigate several ways to improve the sensitivity of the ECL reporting reaction for sensing applications in a microfluidic device containing a wireless electrode array. As a proof-of-concept, we utilize this device to perform quantitative measurements of a biological target using in-device electrochemical immunoassays.

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Unified T-Ray Volumetric Metrology and Its Necessity: Advancing Beyond SEM, TEM, AFM, and SIMS Methodologies
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Recent semiconductor architectures and complex nanostructures have exposed a critical metrological gap: current characterization standards—SEM, TEM, AFM, and SIMS—are fundamentally limited to surface-only or thin-section snapshots. This paper presents a scientific validation of unified Terahertz (T-ray) volumetric metrology as the inevitable successor to these fragmented arts. By integrating the spatial resolution of electron microscopy with the chemical specificity of mass spectrometry within a non-destructive framework, T-ray technology addresses the demands of the AI and quantum computing eras. Six experimental case studies demonstrate its multi-capability nature, including: sub-nanometer nanoparticle metrology validated against TEM; sub-surface semiconductor lattice damage detection; 3D tomographic reconstruction of composite defects; pathogen sensing via molecular eigen frequencies; non-destructive epitaxial carrier concentration profiling; and universal complex refractive index measurement over a 30 THz bandwidth. These results establish unified T-ray metrology as the primary platform for deep materials characterization, capable of reclaiming lost yields in manufacturing and enabling the discovery of new material phenomena.

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The Batch Nature of Continuous Flow Production via Natural-Abundance Stable Isotopes
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By the name of the process of production, Continuous Flow (CF) production indicates a certain continuity in the products; however, virtually all CF products derive from batched reactants. With that, virtually all aliquots of CF production will have certain batch characteristics. Wokovich *et al*. (2005, *J. Pharma. Biomed Anal.*, 38:781-784 and many investigators thereafter) have demonstrated the highly-specific nature of natural-abundance stable isotopes in individual batches of pharma-chemical materials. We employ Elemental Analysis/Isotope Ratio Mass Spectrometry (EA-IRMS) to quantify the natural-abundance stable-isotopic variations in CF production of the product. Here we show that the batch nature of reactants A (bromonapthol) and B (methyiodide) is transmitted to the product C (bromonapthylmethyether). Instead of maintaining a smooth, continuous isotopic composition in CF production, the episodically batch-varying isotopic values ($d^{13}C$, dD, $d^{18}O$) of the reactants are transmitted downstream to the products C. Beyond demonstrating the batch nature of CF products, there are at least two other utilities in measuring isotopic compositions of CF production: (i) Product Authentication (batch/aliquot auditing), and (ii) Process Understanding (understanding from which reactant batch that individual batches/aliquots derive).

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Analysis of 3D Printing Resins via Thermal Analysis Methods
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Analytical instrumentation plays a central role in research laboratories by enabling the development of advanced measurement methodologies. These instruments also influence many aspects of daily life in ways that are not always immediately apparent. One such example is the analysis of 3D printing resins used in both industrial and household settings. In 3D printing, heat sources are used to process resins, followed by UV light to cure liquid photopolymers into solid, highly detailed objects layer by layer. During the heating process, resins undergo physical and thermal changes prior to resolidifying. Thermal analysis techniques enable the characterization of these changes. In this study, Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) were employed to analyze a range of common 3D printing resins. TGA measures changes in mass as a function of temperature, providing information on decomposition and moisture content, while DSC measures heat flow associated with thermal transitions such as melting, glass transitions, and curing reactions. The resins analyzed in this study include Nylon, Nylon Carbon Fiber, Polylactic Acid (PLA) Transparent, PLA Rapid, PLA Carbon Fiber, Polyethylene Terephthalate Glycol (PETG), Thermoplastic Polyurethane (TPU), and Acrylonitrile Butadiene Styrene (ABS). This presentation provides an overview of the fundamental principles of thermal analysis and discusses the results of TGA and DSC measurements performed on 3D printing resins. These results are used to better

understand the physical and thermal changes that occur as samples are heated from ambient conditions to the resin's post-processing temperatures, varying between 400–600 °C depending on the material.

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Vibrational Spectroscopic Detection of Bodily Fluids in Fingermarks for Forensic Applications

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The ability to detect bodily fluids within fingermark impressions offers a powerful dual source of forensic evidence, combining both biometric and biological information. In our research, we utilized attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy to identify oral fluid (OF) traces in fingermark impressions. Since oral fluid is usually invisible and lacks distinctive visual features in fingermark residue, we incorporated machine learning algorithms with ATR-FTIR, enabling reliable detection of trace OF contamination. In a complementary study, we applied Raman hyperspectroscopy to detect blood within fingermarks across a sequence of four successive impressions. We observed that the characteristic Raman bands of blood remained clear up to the third impression, whereas the fourth impression showed reduced blood signals and more pronounced fingermark features. Importantly, environmental interferents produced unique spectral profiles, allowing us to distinguish them from blood-containing fingermark samples. We also evaluated the effects of substrate interference and successfully detected blood-contaminated fingermarks on both paper and glass. These findings highlight the potential of vibrational spectroscopy as a rapid and non-destructive tool for forensic analysis of bodily fluid in fingermarks, advancing the capabilities of evidence detection in forensic investigations.

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A Study in Spectra: Non-Destructive Estimation of Late Postmortem Interval Using Portable UV–Vis–NIR Spectroscopy

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Estimating the postmortem interval (PMI)—the elapsed time between death and examination—remains a fundamental challenge in forensic science, particularly for late PMI (LPMI) assessment in skeletonized remains. While analytical methods such as proteomics, X-ray diffraction (XRD), and Fourier-transform infrared (FT-IR) spectroscopy provide valuable molecular insights, they often require destructive sampling, specialized infrastructure, and laboratory-based instrumentation. This study evaluated a non-destructive, portable alternative using UV–Vis–NIR spectroscopy acquired with the NaturaSpec™ Ultra Spectroradiometer. Femoral and cranial elements from fifty skeletons in the Mercyhurst University collection, spanning PMIs of 1–20 years, were spectrally analyzed to capture optical fingerprints associated with postmortem change. Distinct spectral profiles correlated strongly with PMI, enabling differentiation among temporal categories and demonstrating analytical consistency across anatomical regions. The observed trends reflect cumulative physicochemical alterations in bone matrix composition, supporting UV–Vis–NIR spectroscopy as a sensitive proxy for postmortem change. Together, these findings highlight the utility of UV–Vis–NIR spectroscopy as a rapid, non-invasive tool for LPMI estimation. By translating subtle spectral signatures in bone into meaningful temporal indicators, this work bridges advanced spectroscopic chemistry with applied forensic science, offering a scalable pathway toward field-ready PMI assessment.

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The Effect of Water Temperature and Type on the Evolution of Decomposition Volatile Organic Compounds from Mammalian Tissue

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When humans and animals decompose, they emit odors that can be detectable by scent detection canines. The odor compounds, known as volatile organic compounds, result in an odor profile which can be analyzed for its composition. VOCs can be collected and are typically analyzed using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GC×GC-TOFMS). GC×GC separates analytes based on their affinity for two column phases connected in series and separated by a modulator. GC×GC-TOFMS increases peak capacity for complex samples, such as those arising from decomposition odors. Decomposition under submerged conditions is less well researched than in soil-based conditions. The purpose of this study was to determine the effect of temperature conditions (5˚C, 22˚C, 32˚C) and water type (tap, river, lake) on the resulting VOC profile from submerged decomposing tissue. The samples in this research were pork belly placed in Mason Jars filled with water and left to decompose for 12 days. Samples were extracted and introduced using solid phase microextraction (SPME) arrow. The identified VOCs were then compared with existing studies to determine similarities and the new VOCs not previously reported. Key components not previously identified include isocaproic acid, octyl propanoate, dipropyl trisulfide and more. These compounds were consistently found amongst the results. New information on VOC profiles under unique circumstances would support training techniques for scent-detection dogs, which are widely used to search for and locate remains associated with bodies of water.

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Ultra-Sensitive Trap Headspace GC-MS/MS for Trace Analysis of Leachable NDBA and Other Small Molecule Nitrosamines in Infusion Bags

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US FDA issued new guidance on leachable N-nitroso-Dibutylamine (NDBA) and other small molecule nitrosamines in infusion bags in August 2025. NDBA has been detected as an impurity in drug products packaged in infusion bags with printed pouches, raising concerns that nitrosamine impurities may leach from the packaging into the drug products. Thus, the FDA is requesting drug

manufacturers to provide risk assessments or testing results on this matter. Due to their classification as probable human carcinogens and extremely low acceptable intake (AI) limits, nitrosamines present significant safety concerns. For example, the AI limit of NDBA is 26.5 ng/day. Given that large volume parenteral (LVP) products packaged in infusion bags typically have high maximum daily doses (≥100 mL/day), analytical methods with sub-ppb sensitivity are required to analyze nitrosamines with their low AI limits. An ultra-sensitive method is needed for detection and quantitation of small molecule nitrosamines to assess the concentration of nitrosamine impurities in drug products. In this presentation, we report an ultra-sensitive trap headspace GC-MS/MS method for the detection and quantification of NDBA. The method achieves an LOQ of 0.1 ppb and a linearity range of 0.1 to 10 ppb. Results showed improved sensitivity compared to static headspace GC-MS/MS and direct injection GC-MS/MS approaches. The method was further applied to analyze the neat material and solvent extracts of infusion bags with printed pouches. The root causes of the NDBA from infusion bags with printed pouches were investigated and will be presented in this talk.

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Using Copilot to Support Unknown Identification in Extractables and Leachables Studies

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Unknown identification is a critical yet challenging component of extractables and leachables (E&L) studies, particularly when interpretation relies heavily on high-resolution mass spectrometry (HRMS) and HRMS/MS data. This presentation demonstrates how Microsoft Copilot can be integrated into an experienced analyst's workflow as a scientific decision-support tool, enhancing interpretation efficiency while preserving analytical rigor and regulatory alignment. Three real-world examples will be presented. First, Copilot was used to support moiety identification by correlating neutral losses and relevant fragment ions with plausible functional groups or substructures, helping focus expert-driven interpretation. Second, Copilot assisted in evaluating and distinguishing structural isomers by assessing chemically reasonable fragmentation pathways and proposing key MS/MS features to look for, which were then evaluated against the analyte's HRMS/MS data. Finally, examples are shown where HRMS/MS data provided limited structural information. In these cases, Copilot facilitated scientifically sound proposals of partial structures, functional groups, or chemical classes, or, when appropriate, a putative identity, while avoiding over-interpretation—an approach that aligns well with established E&L identification conventions and regulatory expectations. Together, these examples illustrate how Copilot can enhance efficiency, consistency, and confidence in unknown identification workflows for E&L studies, while maintaining the chemist's central role in critical assessment and final identification decisions.

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Managing Extractables & Leachables in ADC Development and Manufacturing

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Antibody–drug conjugates (ADCs) represent a rapidly growing class of targeted therapeutics that combine the specificity of monoclonal antibodies with the potency of cytotoxic small molecules. This structural complexity introduces unique challenges in assessing extractables and leachables (E&L), as ADCs sit at the intersection of biologics and highly potent small molecules, often involving diverse materials across manufacturing, storage, and delivery systems. This presentation will provide a comprehensive overview of E&L considerations specific to ADC applications, including risk-based assessment strategies across drug substance and drug product stages. Key topics will include material compatibility, the impact of organic solvents and formulation conditions, and the role of single-use systems in upstream and downstream processing. In addition, the presentation will address analytical challenges associated with detecting and identifying leachables in the presence of high-value biologics and cytotoxic payloads, highlighting advances in orthogonal analytical techniques and safety thresholds. Regulatory expectations and industry best practices will also be discussed, with emphasis on leveraging existing guidance (e.g., USP <1663>/<1664>, ICH Q3E concepts) in the context of ADC-specific risks. Case studies and practical examples will illustrate how E&L programs can be designed to ensure product quality, patient safety, and compliance while enabling efficient development timelines. By integrating scientific, analytical, and regulatory perspectives, this talk aims to equip professionals with practical strategies to proactively manage E&L risks in ADC development and lifecycle management.

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Chemometric Evaluation of Plasma N-Glycomes for Time-Sensitive Drug Administration in High-Fat Diet Mice

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Metabolism of a high fat diet (HFD) may fluctuate during different times in the light dark cycle (day and night) resulting in changes of metabolic processes. Certain drugs can be used to help intervene and treat these metabolic changes, however specification on the time of an administered drug are not emphasized. Biomarker expression in plasma can indicate the time dependency of the drug administration. This study highlights the variation in time dependent drug/placebo administration and the expression of N-glycans in HFD mice plasma. The N-glycome was analyzed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The identified glycans and their pre-processed spectral data acquired from MALDI-TOF MS were evaluated using chemometrics data modeling. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) were employed to assess the differentiation of the sample groups based on N-glycome content. This approach assessed the potential of MALDI-TOF N-glycome profiles combined with chemometric analysis identifying potential time-of-day drug administration dependent patterns in HFD mouse plasma.

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Enhanced Electrocatalytic Performance of Metal-Doped Boron Nitride for Nitrate Reduction

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Conventional catalysts for wastewater treatment and CO_2 conversion are often expensive, inefficient, or limited in reusability. This study investigates metal-doped boron nitride (BN) as an electrocatalyst for nitrate reduction, with potential applications in wastewater treatment and CO_2 conversion. Metal acetylacetonate precursors, melamine, and boric acid were dissolved in 250 mL of deionized water, followed by pyrolysis and calcination at 900 °C for 4 h under a continuous N_2 flow of 30 sccm. Catalyst inks were prepared by dispersing 50 mg of catalyst in 10 mL of ethanol containing 0.12 mL of Nafion, followed by sonication for 15 min. Subsequently, 0.06 mL of the catalyst ink was drop-cast onto a 1 × 2 × 0.5 cm glassy carbon electrode and dried under ambient conditions. The electrodes were evaluated using linear sweep voltammetry (LSV), cyclic voltammetry (CV), and chronoamperometry in 0.1 M $NaNO_3$ electrolyte. Compared with samples synthesized using the previous method, which employed urea and a lower calcination temperature, the new samples demonstrated substantial improvements in electrochemical performance. CuBN exhibited a higher current density than previously reported, although its productivity yield was lower. CoBN showed an approximately tenfold increase in current density, while FeBN demonstrated an approximately fourfold increase. Overall, the new synthesis method significantly enhanced current density, with a productivity yield of 9.17 mg h^{-1} mg_cat^{-1} achieved.

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FeBN Electrocatalyst for High-Performance Nitrate Reduction and Sustainable Ammonia Synthesis

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The Haber–Bosch process, the conventional method for ammonia production, is highly energy-intensive and environmentally burdensome, motivating the development of more sustainable alternatives. Electrochemical nitrate reduction to ammonia (NO_3RR) offers a promising decentralized route for ammonia production with potentially lower energy consumption and environmental impact. However, practical implementation remains challenging due to limited catalytic activity and selectivity. This study investigates porous boron nitride (BN_x)-supported transition-metal catalysts synthesized by pyrolysis with approximately 1 wt% metal loading. The catalysts were designed to promote synergistic interactions between the metal species and BN support while increasing the electrochemically active surface area for efficient nitrate reduction. Among the catalysts evaluated, FeBN exhibited promising NO_3RR performance, achieving an ammonia productivity of approximately 9.17 mg h^{-1} mg_cat^{-1} and a Faradaic efficiency of approximately 87% at −0.7 V vs. RHE. At more negative potentials, both Faradaic efficiency and nitrate conversion decreased, primarily due to the competing hydrogen evolution reaction (HER). These results demonstrate that FeBN is a promising electrocatalyst for selective nitrate-to-ammonia conversion and highlight the potential of porous BN-supported transition-metal catalysts for sustainable electrochemical ammonia production.

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Revealing What Thermalgravimetric Analysis Misses: TG-IR-GC/MS

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Combining complementary analytical techniques, often referred to as hyphenated analysis, provides a deeper understanding of materials than can be achieved with a single method alone. A common example is the integration of Thermogravimetric Analysis (TGA) with Fourier Transform Infrared (FTIR) spectroscopy and Gas Chromatography/Mass Spectrometry (GC/MS), known as TG-IR-GC/MS. Each technique contributes unique information: TGA measures mass changes associated with thermal events, FTIR identifies gases as they evolve during heating, and GC/MS enables highly sensitive separation and identification of volatile compounds. By integrating these capabilities into a single experiment, researchers can simultaneously monitor thermal behavior and characterize decomposition products. The result is a powerful, information-rich dataset that provides greater insight into material composition, degradation pathways, reaction mechanisms, and overall performance under changing temperature conditions.

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Development of a HILIC-MS Method for Quantification of Residual EPPS in Pharmaceutical Process Organic Layer

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4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS) was introduced as a process buffer during the enzyme cascade reaction. Following a process change from phosphate to EPPS buffer, interference with Diamine crystallization, a drug substance intermediate, was observed, leading to concerns that residual EPPS may be present after workup and contributing to crystallization challenges. Accurate determination of residual EPPS in the Diamine organic layer was therefore required to support process development and root-cause investigations. Analysis of EPPS presented significant analytical challenges because EPPS is highly polar with poor UV absorptivity, whereas Diamine, a macrocyclic peptide, is highly hydrophobic, limiting the applicability of conventional chromatographic approaches. Several separation strategies were evaluated, including reversed-phase C18, mixed-mode, and ion chromatography methods. However, a hydrophilic interaction liquid chromatography (HILIC) method coupled with a single quadrupole mass detector operated in selected ion recording (SIR) mode provided optimal retention, peak shape, sensitivity, linearity, and robustness for EPPS quantitation. Application of the optimized HILIC-MS method demonstrated very low levels of residual EPPS in multiple Diamine organic layer lots, with concentrations ranging from approximately 0.6 to 1.8 µg/mL. These results enabled the process team to eliminate residual EPPS as the primary cause of crystallization interference and focus subsequent efforts on

crystallization process optimization. The rapid development of this selective and sensitive analytical method provided critical process understanding and accelerated readiness activities for laboratory scale-up and manufacturing support.

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Analysis of Biopharmaceutical Critical Quality Attributes Using Capillary Scale Liquid Chromatography

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Large biologic-based therapeutics including monoclonal antibodies (mAb's), oligonucleotides, and antibody-drug conjugates (ADCs) have seen significant interest within the pharmaceutical industry in recent years. The size and structure of these analytes present significant analytical challenges, often requiring multiple characterization methods to ensure structural similarity. Multiple modes of liquid chromatography (LC) are used in the analysis of these compounds including reverse phase for mAb mass and fragmentation and oligonucleotide separation, ion exchange for charge variance, hydrophobic interaction liquid interaction for glycosylation profiling, hydrophobic interaction chromatography for determining the drug to antibody ratio of ADCs, and size exclusion chromatography for the analysis of antibody aggregation. This poster will demonstrate the development of each separation mode on the capillary scale, allowing for considerable reductions to both solvent and sample consumption.

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Robust Size-Exclusion Analysis of Lipid Nanoparticles Using Polymer-Based 3000 Å SEC

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The analytical characterization of nanoscale assemblies requires chromatographic media capable of accommodating large particles while minimizing surface-induced artifacts. This is particularly important for lipid nanoparticles (LNPs), nucleic acid therapeutics, virus-like particles, and related biomolecular assemblies, whose heterogeneous composition and susceptibility to hydrophobic and electrostatic interactions can compromise recovery and distort size-based separation. In this study, an SEC-MALS method for the analysis of full and empty LNPs was developed using a Sepax MonoMix MC10-SEC 3000 Å column. Buffer systems, salt concentrations, and selected mobile-phase additives were systematically screened. Chromatographic recovery, peak symmetry, retention behavior, baseline quality, and MALS response were evaluated to identify conditions suitable for reproducible LNP analysis. Under the optimized mobile-phase conditions, MonoMix MC10-SEC 3000 Å provided effective separation with improved recovery, peak symmetry, baseline quality, and light-scattering response. These results are attributed to the combination of its 3000 Å pore architecture, which accommodates intact LNPs and other high-molecular-weight assemblies, and its hydrophilic PMA matrix with a specialized surface coating, which minimizes hydrophobic and electrostatic interactions with the stationary phase. The resulting SEC-MALS method provides a reproducible platform for characterizing full and empty mRNA-LNPs. Its combination of particle accessibility, reduced nonspecific interactions, and consistent MALS performance supports applications in formulation screening, stability assessment, process development, and batch comparability.

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Evaluation of System Performance Parameters for Quality Control in GC×GC-TOFMS Systems

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Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS) is a high-resolution separation technique that results in the production of peaks on chromatograms that result in large and complex datasets. Differences in instrumentation, column configuration, and carrier gas can affect separation and identification quality of these peaks, making proper quality control (QC) practices essential. There are 4 key parameters that can be monitored to examine analytical performance: retention time reproducibility, peak resolution, signal-to-noise ratio (S/N), and peak shape. This study aimed to compare these factors across instruments and their configurations for QC purposes using the Century Mix, a mixture of approximately 100 unique compounds from multiple chemical classes. Samples were analyzed by GC×GC-TOFMS. System evaluation was performed comparing (1) flow and thermal modulation and (2) hydrogen and helium carrier gas. To measure retention time reproducibility, each sample was run 10 times using each configuration. The analytes using hydrogen as a mobile phase showed mixed trends for retention times, demonstrating an increase in the secondary retention of alkane compounds but a decrease in secondary retention times for lactone compounds. By using reference mixtures, we were able to contextualize our findings across instruments, configurations, and carrier gases. This allowed us to compare the reproducibility of retention times, resolution of key peak pairs, S/N ratios, and tailing factors of peaks. These results provide insight into the importance of QC procedures as it pertains to evaluating different GC×GC-TOFMS systems.

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Analysis of Extracellular Vesicles Using Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

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Cancer is a leading cause of death in the United States, and early detection can significantly improve patient outcomes. Liquid biopsy, which involves the analysis of bodily fluids for disease-associated biomarkers, has gained increasing interest as a minimally invasive approach for cancer detection and monitoring. Extracellular vesicles (EVs) are nanosized, membrane-bound particles released by cells into extracellular environments, including bodily fluids. EVs contain a variety of biomolecular components, including proteins and peptides, and their stability in biological fluids makes them promising targets for liquid biopsy research. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid and high-throughput analytical technique that can be applied to the molecular analysis of EV samples. However, sample preparation can strongly influence spectral quality, reproducibility, and the number and intensity of detected signals. In this study, we optimized sample preparation steps for the analysis of EVs using MALDI-TOF MS. EVs obtained from multiple cancer cell lines were used to evaluate different sample preparation

conditions and MALDI analysis parameters. The resulting mass spectra were processed and assessed to determine conditions that provide consistent and informative spectral data. This work establishes a practical MALDI-TOF MS workflow for the analysis of EV-associated molecules and supports the broader application of MALDI-TOF MS in EV research.

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Guiding the Molnupiravir Tablet Formulation Using Physiologically Based Biopharmaceutics Modeling and Successfully Establishing Dissolution Safe Space

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Physiologically Based Biopharmaceutics Models (PBBMs) are evolving tools which can be used to aid in drug product quality specifications. PBBMs have gained increased regulatory interest and acceptance over the past 5 years. For molnupiravir, PBBM has been used for: a) calculation of a bioequivalence safe space for capsule formulation and tablets, b) informing of the definitive bioequivalence study (P011) with a target tablet formulation selection designed to be bioequivalent to the capsule formulation, c) selection of a deliberately slower release tablet formulation (beyond *f2* similarity criteria) designed to widen the dissolution knowledge space, d) help select the clinically relevant specification and the dissolution bioequivalence safe space. PBBM data inputs, model development, validation, and application using data from several independent clinical studies are described. Predictive errors for Cmax and AUC were < 25%. The z-factor for dissolution data modeling is described for QC (Quality Control) method(s). The molnupiravir bioequivalence safe space or knowledge space widening is described. The value of the wider dissolution space includes increased potential flexibility with scale-up and post approval changes (SUPAC), including adding or changing a site for a high-volume drug product such as molnupiravir. PBBM is not only important in the development of drug products aiding formulation selection but can also be a key component toward regulatory flexibility and approval of clinically relevant specifications, such as when the clinical knowledge space has been widened through inclusion of a slow-release variant, as was done for the relative bioavailability study for molnupiravir.

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Investigating the Effects of Overexpression and Downregulation of Human Jumping Translocation Breakpoint Protein (hJTB) Using In-Gel and In-Solution Digestion-Based Proteomics in HEK293 Cells

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Human jumping translocation breakpoint protein (hJTB) is encoded by a gene located on human chromosome 1q21 and has been implicated in unbalanced chromosomal translocations associated with various cancers. JTB is ubiquitously expressed in normal cells and has been reported to be overexpressed in several types of cancer, including prostate and breast cancer. In this study, HEK293 cells were transfected with JTB cDNA in HA-, His-, and FLAG-tagged CMV expression vectors to induce JTB overexpression, as well as with shRNA plasmids to induce JTB downregulation. We analyzed proteins extracted from transient and stably transfected cells using in-gel and in-solution digestion-based proteomic approaches. JTB expression was confirmed by Western blotting. The resulting peptides were analyzed using a NanoAcquity UPLC system coupled to a Xevo G2 QTOF mass spectrometer. Mass spectrometry data were processed using Mascot 2.4 and Scaffold 4.1 software. Proteomic analysis identified several proteins, including heat shock proteins (HSPs), actins, and tubulins, whose expression was associated with altered JTB levels. Furthermore, gene set enrichment analysis (GSEA) was performed to identify biological processes and pathways associated with JTB expression. These findings may improve understanding of the molecular mechanisms by which JTB influences cellular proliferation and may help evaluate JTB as a potential therapeutic target in malignancies characterized by JTB overexpression.

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Breast Milk Proteome Alterations Associated with Breast Cancer Revealed by a Longitudinal Mass Spectrometry-Based Time-Course Proteomics Study

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Breast cancer (BC) remains a major cause of cancer-related mortality among women worldwide, emphasizing the need for improved strategies for early detection and disease monitoring. Human breast milk is a biologically rich and non-invasive source of proteins, immune components, and epithelial-derived material that may reflect disease-associated molecular changes. In this study, we characterized longitudinal protein expression patterns in human breast milk from individuals with breast cancer and healthy controls using mass spectrometry (MS)-based proteomics to identify differentially expressed proteins in breast milk. Proteins were separated by SDS-PAGE, digested, and subjected to MS analysis followed by nano-liquid chromatography-tandem mass spectrometry (nanoLC-MS/MS). A total of 24 breast milk samples were analyzed, including 12 samples from two individuals with breast cancer and 12 samples from two healthy individuals, with six time points collected from each individual. SDS-PAGE was initially performed to visualize overall protein profiles and assess differences in protein banding patterns across disease groups and longitudinal time points. MS-based proteomics was used to compare protein abundance and temporal expression patterns between breast cancer and healthy controls. Longitudinal analysis identified disease-associated changes while accounting for individual and time-dependent variation. Preliminary findings revealed differentially expressed proteins, including bile salt-activated lipase, xanthine dehydrogenase, and β-casein, which may be involved in breast cancer-associated pathways. These proteins represent potential candidates for further investigation as non-invasive breast cancer biomarkers. Overall, combining SDS-PAGE, MS-based proteomics, and longitudinal breast milk sampling may help identify molecular changes useful for breast cancer detection and monitoring.

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Mass Spectrometry–Based Proteomic Analysis of Combined Left- and Right-Breast Milk (9v9) for Breast Cancer Biomarker Discovery

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Breast cancer (BC) continues to be a major cause of cancer-related deaths among women globally, highlighting the need for improved strategies for early detection. Invasive ductal carcinoma (IDC), which constitutes nearly 85% of breast cancer cases, originates in the milk ducts and progressively invades surrounding breast tissue. Human breast milk is a biologically rich fluid containing proteins, immune components, and shed epithelial cells, making it a valuable and non-invasive source for identifying disease-associated molecular changes. In this study, we have used a mass spectrometry (MS)-based proteomics workflow to investigate protein expression patterns in breast milk. Samples underwent separation by SDS-PAGE, followed by in-gel digestion and analysis using nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS). A total of 18 combined left and right breast milk samples were included in a 9v9 study design, consisting of nine breast milk cancer samples and nine healthy controls. The comparative analysis revealed distinct differences in protein abundance between cancer and control groups. Among the proteins showing altered expression were bile salt-activated lipase, xanthine dehydrogenase, β-casein, osteopontin, and perilipin, indicating their potential association with breast cancer-related biological processes. Overall, these findings demonstrate consistent alterations in the breast milk proteome linked to breast cancer and emphasize its promise as a non-invasive platform for biomarker discovery. Ongoing studies with expanded sample sizes aim to validate these observations and support the development of diagnostic tools based on breast milk analysis.

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Using Density Functional Theory Methods to Probe the Energetics of the Quinone Intermediates of the Photosynthetic Reaction Center, Photosystem I*

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Photosystem I (PSI) is a photosynthetic reaction center, which upon light-induced charge separation extends the lifetime of the charge-separated state and facilitates trans-membrane electron transfer by transferring the electron across a series of cofactors, including the A_{1A} and A_{1B} phylloquinone intermediates. The energetics of the electron transfer steps depends on the A_{1A} and A_{1B} intermediates exhibiting specific midpoint potentials, which are exquisitely tuned by both, the structure of the quinone and the its interaction with the surrounding protein environment. Assessing the properties of the A_{1A} and A_{1B} sites through replacement of the native phylloquinones by a variety of quinone derivatives is enabled by the use of photosystem I (PSI) reaction centers from the *menB* deletion variant (□*menB*) in the cyanobacterium *Synechocystis* sp. PCC 6803. In this study, we probe the energetics of electron transfer in wild-type (WT) and □*menB* PSI by estimating the reduction potentials of the quinone molecules bound in the A_1 sites. We perform molecular dynamics (MD) simulations on the X-ray and cryo-electron microscopy structures of trimeric WT PSI and Δ*menB* PSI, with 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ) bound in the A_1 sites, instead of the native phylloquinones. We use the optimized structures to construct computational models for the density functional theory (DFT) calculations. We validate the models by comparing the hyperfine coupling parameters predicted by DFT with the experimentally determined couplings and employ numerical frequency calculations to estimate the reduction potentials of the quinone cofactors. This work was supported by the U.S. DOE under the contracts DE-FG02-07ER15903 (KVL) and DE-SC0010575 (JHG). PL was supported by the NSF GRFP.

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Copper-Porphyrin Aluminum Metal-Organic Framework, Sorption of Diethyl Sulfide from Air, Kinetics, Mechanism, and Stoichiometric Adsorption Complex

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Metal-organic frameworks (MOFs) are 3D coordination polymers with metals and organic linkers. They are of strong interest for separation, pollution remediation, chemosensing and catalysis. MOFs of complex and controlled composition, structure and functional properties are highly desired. We report aluminum MOF with copper-porphyrin ring (Al-MOF-TCPPCu), mechanistic and kinetic studies of adsorption of volatile organic sulfur compound diethyl sulfide (DES) in air by it, and studies by vibrational spectroscopy and complementary methods. Its analysis was conducted by FTIR spectroscopy, XRD with Rietveld lattice refinement and electron microscopy. The comprehensive study of mechanism and kinetics of DES sorption is presented, under dynamic conditions in the flow of air. This is achieved by using novel method of in-situ time-dependent ATR-FTIR spectroscopy in the controlled environment. The binding sites of DES include the μ(O–H), the COO−, phenyl and pyrrole groups in the MOF and they are ranked by strengths of bonding based on ATR-FTIR spectra. Kinetics of sorption obeys Langmuir model of the pseudo-first order rate law with effective rate constant keff = 0.95 ± 0.05 min− 1. Further, linear dependence of kinetic rate constant on DES concentration in air suggests that sorption is diffusion limited. Next, sorption of DES under static equilibrium conditions results in the binary stochiometric adsorption complex $(Al\text{-}MOF\text{-}TCPPCu)_2(DES)_7$. Finally, after DES sorption, facile regeneration of spent sorbent (adsorption complex) is achieved by sustainable protocol without using chemicals. Transition metal containing porphyrin aluminum MOFs are promising for removal of volatile organic sulfur compounds from air.

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Stoichiometric Binary Complexes of Pharmacological Terpenes with Metal-Organic Framework: Synthesis, Bonding, Delayed Release, and Nanocolloids

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Metal-organic frameworks (MOFs) are coordination polymers with significant promise for encapsulation and controlled release of drugs. Terpenes are natural substances with antibacterial, anticancer, antiviral, anti-inflammatory properties. We test a new concept of pharmaceutical stoichiometric binary solid-state complexes of MOFs with terpenes prepared by sustainable mechanochemistry

methods, study their composition, structure, delayed release of terpenes, and conversion to nanocolloidal form. The solid-state stoichiometric binary complex lag(CYCU-3)$_1$(CIT)$_2$ was prepared from aluminum MOF and citronellol (CIT). It features functional groups and crystal structure of CYCU-3 host and contains hydrogen bonds at H-O(Al) group of CYCU-3 and O-H group of CIT as found by ATR-FTIR spectra. Significant photoluminescence quenching and distinct vibronic structure of CYCU-3 host indicate its strong π-π interactions with C=C bond of CIT guest. Further, stoichiometric binary complex lag(CYCU-3)$_1$(GER)$_2$ was synthesized from pharmaceutical terpene geraniol (GER). The mode of bonding GER and CIT is similar, yet it differs in the strength of host-guest interactions in complexes. This correlation is consistent with significant differences of complexes under in vitro release of terpenes to phosphate buffered saline (PBS) at 37 ℃, in timescale and amounts of released compounds based on HPLC analysis. Facile and "green" mechanochemical colloidization of complexes is demonstrated using high-efficiency planetary ball mill. TEM and dynamic light scattering data show bimodal size distribution of obtained colloids. Facile synthesis and processing of sustainable functional solids to stable nanocolloids is promising for delayed release and delivery of natural pharmaceutics.

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Application of Raman Spectroscopy for the Development of High Crystallinity and Oriented Polyethylene Films

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Raman spectroscopy was used to support the development of high crystallinity and oriented polyethylene films. Raman spectra of polyethylene films prepared before and after drawing were acquired under both polarized and depolarized excitations. A Python program was written to calculate spectral correlations for quick computational comparison of the polyethylene developed under different conditions. The spectra were then calculated to quantify polyethylene crystallinity and the orientation factor R based on specific peak area ratios. Overlapping Raman peaks were deconvoluted for more accurate integrated peak area measurements. The methods for calculating polyethylene crystallinity and the polymer orientation factor, i.e. polarization ratio R = I(Parallel)/I(Perpendicular), will be presented. The results showed that polyethylene crystallinity and orientation factor increased significantly after the polymer was stretched. Furthermore, under the same drawing ratio and temperature, single layer polyethylene films exhibited higher orientation than multilayer (1024 layers) polyethylene films. The study has helped to develop and understand the complex variables for the development of high strength polyethylene films.

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Enabling Purification of Challenging Samples Through Advanced Chromatographic Strategies

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The increasing structural and chemical complexity of modern therapeutic modalities continues to challenge the capabilities of conventional reversed-phase HPLC methods. To overcome these constraints, a range of non-traditional separation strategies have been implemented, including mixed-mode stationary phases, non-linear gradient profiles, and two-dimensional liquid chromatography (2D-LC) workflows. While these approaches have demonstrated utility at the analytical scale, their translation to preparative chromatography introduces additional challenges related to scalability, system constraints, and method robustness. This work presents selected case studies in chromatographic purification that examine these challenges and demonstrate strategies for method adaptation and scale-up. Particular emphasis is placed on practical considerations for implementing complex separations in preparative environments, including stationary phase selection, gradient design, and system configuration (e.g., 2D-LC). These examples illustrate how expanded chromatographic toolsets can enable purification of challenging compounds that are not readily resolved using conventional approaches.

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Recent Advances in the Use of the Charged Aerosol Detector at the Discovery Stage of Pharmaceutical Research

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The charged aerosol detector (CAD) has been utilized for the quantitation of purified products resulting from a workflow involving mass-directed, high-throughput purification of samples at microgram levels (μgHTPP). While enhancements to the overall μgHTPP processes have been made to improve efficiency, there have been significant improvements in the understanding of factors that influence CAD signal response and increase accuracy. In this presentation, the impact of chromatographic behavior on CAD signal will be discussed, demonstrating how peak shape can clearly impact results. In addition, a new equation will be presented that shows how to improve the accuracy of CAD results when dealing with non-ideal peak shapes by reducing the overall error (e.g., from a maximum error of 55% to 28% for a validation dataset).

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Ensuring Quality Data for Organochlorine Pesticide Analysis by Using an Optimized Dual Bed Sample Extract Cleanup Cartridge

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One of the most common challenges in analyzing soil samples for organochlorine pesticides is instrument downtime and shortened calibration periods, both caused by the deleterious effects of co-extracted matrix components introduced during sample injection. In addition, chromatographic interferences further complicate analyte identification and quantification, adding to the difficulties faced by environmental analysts. Ensuring high-quality data depends on maintaining instruments in optimal condition so accurate results can be collected without interference. To achieve this, effective extract cleanup procedures are essential. While traditional sorbents such as Florisil, silica gel, and alumina are widely used, these normal-phase options often fail to adequately remove less polar and high

molecular weight compounds that compromise instrument performance and sample-path inertness. In this study, a dual-bed cartridge cleanup containing Florisil and Graphitized Carbon Black (GCB) was formulated and designed to be compatible with standard Florisil cleanup workflows. Using this approach, recoveries of all 23 organochlorine pesticides ranged from 89-105%, while trichlorophenol was held below 1%, in compliance with EPA Method 3620C. The relative standard deviation (RSD) was less than 10% for all analytes. This optimized dual-bed cartridge demonstrates clear advantages when processing highly pigmented and inlet-degrading samples. GCB was found to enhance the cleanup properties of Florisil while minimizing the breakdown of labile analytes such as endrin and 4,4'-DDT. For analysts, this translates into greater instrument reliability and consistently higher quality data.

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Capillary Electrophoresis Based Separation of Virus-Like Particles and Nano-Emulsions for Vaccine Development

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Immunogenic nano-emulsions can be added to vaccines as adjuvants to strengthen vaccine-induced immune responses. However, analytical characterization of vaccine antigens such as virus-like particles (VLPs) alongside nano-emulsion adjuvants is difficult because both are heterogeneous nanoscale entities with comparable sizes but markedly different physicochemical properties. As a result, commonly used nanoparticle separation techniques, such as size exclusion chromatography and field-flow fractionation often struggle to separate these components due to their similar size distributions. In this work, a capillary electrophoresis method that separates analytes based on charge-to-size ratio is developed. The approach enabled quantification of human papillomavirus VLPs in mixtures containing a squalene nano-emulsion adjuvant, while simultaneously measuring buffer components such as chloride and histidine. The method was evaluated per ICH Q2 criteria and demonstrated acceptable performance for linearity and range, accuracy, precision, and limits of detection and quantitation. This study demonstrates that CE can feasibly characterize prospective vaccine formulations using dilution as the only sample preparation step. The same overall approach could be applied to other vaccine–adjuvant pairs and to other emulsion- or nanoparticle-based systems.

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Simultaneous SIM/Scan P&T-GC/MSD: High-Confidence, High-Sensitivity VOC Quantitation

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Environmental VOC analysis requires sub-ppb sensitivity without compromising spectral data for confirmation and unknown review. This work demonstrates a simultaneous SIM/Scan purge-and-trap GC/MSD method that delivers robust performance across the full EPA 8260 target list, from early-eluting light gases (e.g., vinyl chloride) to late-eluting aromatics (e.g., hexachlorobutadiene). The method uses a 30 m DB-624 UI column and Tekmar #9 trap on an Atomx XYZ–GC/MSD system with a ≤15-minute total cycle time. Samples and standards were prepared in 5 mL deionized water with 100 µL methanol compensation. Time-segmented SIM maximized low-level sensitivity while concurrent full-scan acquisition preserved complete EI spectra for confirmation and retrospective analysis. Calibration ranges covered 0.2–1000 ppb for vinyl chloride, 10–1000 ppb for gas compounds, and 1–100 ppb for standard VOCs, with expanded low-range calibration for challenging late eluters (e.g.1,4-Dichlorobenzene 0.1-100 ppb; Hexachlorobutadiene 0.5-100 ppb). Sub-ppb MDLs were achieved for most analytes, along with improved peak shape and retention stability for early-eluting gases. All production MDL criteria were met except DBCP, for which alternate quantifier evaluation is ongoing. This approach provides a practical, production-ready balance of SIM sensitivity and full-scan spectral context in routine P&T workflows.

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The Role of Surface Deactivation for the Analysis of Low Level Acidic, Basic, and Neutral Compounds for EPA 8270E

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Environmental laboratories are working towards implementing low-level detection methods using highly sensitive gas chromatography instrumentation such as gas chromatography tandem triple quadrupole mass spectrometry (GC-MS/MS). Reactive surfaces in the flow-path of the GC-MS/MS contribute to problems in analyte peak shapes, complicating instrument calibration and consistency. Reagents used in GC column production vary between manufacturers, imparting different types of activity from that surface that becomes more apparent at low levels. Residual silanols at the fused silica surface impart imperfections to the surface of the column, affecting the performance of acids, bases, and neutrals in different ways, depending on the chemistry used for deactivation. More information is needed to understand the impact of surface deactivation on low level analyses where residual activity is exacerbated and more likely to affect productivity. In this study, we evaluated activity of acidic, basic, and neutral compounds associated with the Environmental Protection Agency's method 8270E, comparing performance at low-levels (0.1-10 pg on column). Multiple 5ms columns were evaluated and the results show how column selection affects analytical performance of acidic, basic, and neutral analytes at low-levels.

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In-Process Analytical Support for Conjugation Process Development of Antibody Drug Conjugates

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Antibody–drug conjugates (ADCs) represent a rapidly expanding class of targeted therapeutics that integrate the specificity of monoclonal antibodies (mAbs) with the potency of small-molecule payloads. The structural complexity and heterogeneity of ADCs present unique analytical challenges, particularly for monitoring and controlling the conjugation process. To address these challenges, a suite of complementary analytical methods has been developed to support in-process control (IPC) and process understanding during ADC conjugation. Key critical quality attributes (CQAs) include drug-to-antibody ratio (DAR), aggregation, residual process-

related impurities, and protein concentration. This presentation highlights analytical strategies used to monitor these CQAs throughout conjugation development. Reduction steps are tracked to ensure thiol generation as expected, while DAR is determined using orthogonal techniques including LC–MS, reversed-phase LC (RPLC-UV), hydrophobic interaction chromatography (HIC), and native size-exclusion chromatography (SEC). Aggregation of mAbs and ADCs is assessed by SEC, and protein concentration is measured using SoloVPE. In addition, residual payload-linker, reducing reagents, and organic solvents are quantified using LC-UV and LC–MS methods. The integration of these analytical tools enables real-time process insights and supports the development of robust conjugation processes suitable for GMP manufacturing. Case studies will be presented to demonstrate how in-process analytics inform process optimization and control strategy development for ADC programs.

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Control of Free Drug and Related Impurities: A Case Study to Address Challenges of Complex Mixtures

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Free drug and related impurities (FDRI) represent high-concern process- and storage-related impurities in antibody-drug conjugate (ADC) manufacturing and storage, as residual payload-linker species drive off-target systemic toxicity and must be purged to justified low levels to ensure patient safety, product consistency, and regulatory compliance. A unique FDRI, the Payload-linker Reductant Adduct (PL-RA), was identified as a new species arising from the conjugation process. Its strong non-covalent interaction with the ADC presented significant challenges for both purification and accurate quantitation. Tangential flow filtration (TFF) buffer optimization was critical to disrupt these interactions and achieve sufficient FDRI clearance. For analytical method development, two sample preparation approaches were evaluated: neat injection and ADC precipitation. However, hydrolysis of PL caused by the mobile phase during neat injection generated artificial results, while results from the ADC precipitation method were sometimes non-reproducible. A root cause investigation revealed that high sugar concentrations in the formulation buffer and high salt concentrations from the reductant reagent induced phase separation in precipitated samples, causing inhomogeneous FDRI distribution and non-reproducible results. This issue was systematically resolved by reducing reductant and sugar concentrations and redesigning the mixing order, yielding homogeneous solutions with robust on-board stability for up to 48 hours. The optimized method demonstrated good accuracy, precision, specificity, and recovery across IPC, release, and stability testing, and successfully supported GMP campaigns both internally and externally.

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From Stability to Control Strategy: Analytical Lessons Learned for Complex ADC Drug-Linkers.

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As antibody-drug conjugates (ADCs) continue to expand across the pharmaceutical industry, increasingly diverse drug-linker and payload chemistries are being explored to enhance potency and therapeutic differentiation. This increasing molecular complexity presents analytical challenges, particularly for highly potent, structurally complex, and stability-sensitive drug-linkers. Because the drug-linker and payload moiety are closely associated with ADC potency and quality, a stage-appropriate analytical control strategy for the drug-linker is critical to support development and rapid entry into the clinic, while ensuring appropriate control of free drug and related impurities that may be carried forward into the drug substance. For complex drug-linkers, analytical control strategy development requires an integrated understanding of specification setting, stability behavior, impurity formation, and free drug method development for both drug-linker and ADC. Purity and assay specifications should be supported by stability studies evaluating key stress conditions, including thermal, pH, moisture, and storage-related risks. These studies inform packaging, handling, and storage strategies. In parallel, free drug and impurity methods should be designed to provide meaningful information on impurity origin, upstream process control, and potential downstream impact during bioconjugation. In this presentation, we will discuss practical considerations and lessons learned from the development of analytical control strategies for complex payload-derived drug-linkers. The discussion will highlight how early stability understanding, phase-appropriate method development, and impurity profiling can support robust and scientifically justified control of complex ADC intermediates.

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Comprehensive LC-MS & LC-MALS Assays for Antibody-Drug Conjugated (ADC) and Antibody-Oligonucleotide Conjugate (AOC) Characterization

Samantha Ippoliti, Waters Corporation, 5 Technology Dr., Bldg. A, Milford, MA 01757, Ying Qing Yu

Antibody-oligo conjugates (AOCs) are a similar class of biotherapeutics which builds upon the fundamentals of antibody-drug conjugates (ADCs). The purpose of both AOCs and ADCs is the targeted delivery of a payload (oligonucleotide moiety or cytotoxic drug, respectively) using a full-length monoclonal antibody (mAb) or fragment thereof. AOCs will likely provide opportunities for targets thought to be inaccessible by other therapies. mAbs themselves are large and complex, requiring extensive characterization and monitoring. Conjugated mAbs require the same characterization as mAbs, plus analysis of oligo-to-antibody ratio (OAR), characterization of linker-payload building blocks, as well as in-process monitoring. This study builds upon previous analysis with light scattering techniques to incorporate high resolution LC-MS using the BioAccord™ System and Xevo™ G3 QToF Mass Spectrometer into the toolbox of approaches for AOC characterization. Samples of unconjugated “free” mAb and two AOCs were used for this case study. The AOC samples require a non-denaturing, non-reducing approach, for intact analysis as the AOC may dissociate under typical denaturing RPLC-MS conditions. For intact AOC characterization, we employed MS-compatible size-exclusion chromatography (SEC)-MS and strong cation exchange (SCX)-MS methods. SEC-MS and SCX-MS were also employed for partial conjugation site localization at subunit level. Complete conjugation site localization at peptide level was made possible via an enzymatic digestion workaround prior to tryptic peptide mapping analysis.

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Quantitative Benchtop Nuclear Magnetic Resonance for Vaccine Development
Adam Sutton, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486, Richard Rustandi

Vaccines and biologic therapeutics are regularly examined in the pharmaceutical industry. Vaccines can contain large molecules such as proteins and polysaccharides or larger particles such as attenuated viruses, lipid nanoparticles or virus like particles. All these therapeutic types contain a complex mixture of small and macromolecules, therefore several analytical tools often need to be assessed in order to assist in the various stages of vaccine and biologics process development. Benchtop NMR is an advancing technology that can be a versatile analytical tool that provides quantitative information about the multiple components, using a single internal standard, that arise in the development of a therapeutic. Benchtop NMR often involves minimal sample preparation since macromolecular species are usually not detectable or can be removed by T_2 filters. The software for benchtop NMR is easy to use and provides automatic data processing further making benchtop NMR an attractive analytical tool that can be quickly assessed when comparing different analytical approaches. Herin, the use benchtop NMR as an alternative to chromatographic methods will be discussed. Its use for real-time monitoring and fast method development will be demonstrated. Examples of using benchtop NMR to quickly determine solvent compositions, to confirm excipients and study alum sedimentation in vaccine-related samples are some of the examples that will be covered, with some comparison to chromatography-based methods. Furthermore, we will discuss how benchtop NMR method development is possible even from those with no NMR experience, which are clear advantages for the implementation of benchtop NMR in the pharmaceutical industry.

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Benchtop NMR as a PAT Sensor: Enabling Process Understanding and Real-Time Control
Anh Le-McClain, Magritek Inc., 103 Great Valley Pkway, Malvern, PA 19355

Benchtop NMR spectrometers, with compact size and versatile capabilities, have become recognized as a powerful and practical Process Analytical Technology (PAT) sensor both laboratory developments and manufacturing environments. Recent advances in high-resolution benchtop NMR enable rapid, non-destructive, and information-rich chemical analysis directly at the point of need. This presentation will discuss how the benchtop NMR can be integrated as a PAT sensor for direct and continuous monitoring of chemical processes through the highly selective solvent suppression for non-deuterated solvents, multinuclear analysis, and precise sample temperature control. Benchtop NMR systems can be configured for at-line, on-line, or in-line sampling of the processes to provide continuous quantitative information on reactants, intermediates, products, and impurities to support process understanding and informed decision-making. Real-world application examples will demonstrate how the benchtop NMR is been used to monitor heterogeneous chemical reactions, optimize flow chemistry processes, and analyze complex pharmaceutical formulations. These examples highlight how real-time analytical insights improve process understanding, accelerate optimization, and enable more effective process control.

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Development of Online Low-Field Benchtop NMR Coupled to High-Performance Liquid Chromatography for Pharmaceutical Analysis
Noah Menard, The University of Texas at Arlington, Dpt. of Chemistry and Biochemistry, 700 Planetarium Pl., Arlington, TX 76019, Bita Firoozbakht, Jonathan Ferguson, Masayuki Nishimura, Scott Kuzdza, John Frost, John Price, Kevin Schug

While high-performance liquid chromatography (HPLC) is a staple of modern science, it cannot determine the structure of an analyte alone. Nuclear magnetic resonance spectroscopy (NMR) can elucidate the structure, but complex mixtures can present significant identification issues. LC-NMR attempts to combine the two into a single test, while removing the requirement of evaporating significant amounts of solvent from the analyte before testing in a standard NMR fashion. Historically, only high-field NMRs have been used, but as benchtop NMRs have seen significant technological improvements and widespread application, hyphenation of a benchtop instrument for LC-NMR has become an option. The use of a low-field 125 MHz benchtop NMR for online LC-NMR on an analytical scale will be discussed. Utilization of this system has shown the successful separation of an analyte mixture containing acetaminophen, caffeine, warfarin, and ibuprofen, in gradient separations of both protonated methanol and acetonitrile. These two most common HPLC organic solvents provide different benefits in terms of separation as well as solvent peak locations observed in the NMR and can be altered depending on analyte requirements. The separation and analysis of all analytes have been shown to be successful with a SNR ≥ 3 in both solvents, demonstrating that this is a method that can be adopted and expanded. The potential for solvent suppression is also demonstrated.

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3M Benchtop NMR: Applications and Capabilities
Travis Gregar, 3M, Building 201-BS-03, Maplewood, 55144

Nuclear magnetic resonance (NMR) spectroscopy is a well-established analytical technique used to obtain both qualitative and quantitative information. Traditional high-field NMR systems rely on large superconducting magnets cooled with cryogens and can analyze very small sample quantities, down to the low-microgram range. At 3M, these instruments routinely support research and development efforts and expand the boundaries of analytical science. Over the past decade, benchtop NMR systems have advanced significantly. These systems do not require cryogens and are increasingly accessible to users without specialized NMR expertise. The 3M Corporate Research Analytical Laboratory (CRAL) has deployed multiple benchtop NMR instruments in manufacturing environments, where they have served as a disruptive quality control technology for monitoring coating solutions, polymer composition, raw material verification, and solvent water content. Benchtop NMR has become an important addition to the analytical toolbox, enabling analysts to maintain quality assurance objectives while broadening the range of practical in-process and at-line measurements. This presentation will highlight several examples of benchtop NMR applications in factory settings.

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High-Throughput Small Angle X-Ray Scattering Reveals Structure Factor Transitions Linked to High-Concentration Antibody Viscosity
Pin-Kuang Lai, Stevens Institute of Technology, 1 Castle Point Terrance, Hoboken, NJ 07030

High-concentration monoclonal antibody (mAb) formulations are often constrained by elevated viscosity, largely driven by protein–protein interactions, which complicates manufacturing and limits subcutaneous delivery. Early viscosity risk assessment is essential during discovery, yet traditional measurements require large sample volumes, and lack high-throughput capability. Here, we develop a high-throughput small-angle X-ray scattering (SAXS) protocol to detect mAb self-association at dilute concentrations, enabling early predictive insights into high-concentration viscosity. Synchrotron SAXS measurements were conducted for 21 mAbs formulated in a histidine buffer at pH 6.0. An initial subset of 10 mAbs analyzed across 1–150 mg/mL revealed that effective structure factor transitions in the low-q region, indicative of interparticle interactions, consistently emerged below 25 mg/mL. Subsequently, 11 additional mAbs were analyzed at 1–25 mg/mL using automated liquid handling and flow cells to enable high-throughput screening. High-viscosity mAbs exhibited detectable low-q upturns at concentrations ≤10 mg/mL, whereas low-viscosity mAbs showed downturns. A classification criterion based on effective structure factor transitions accurately classified all high- and low-viscosity mAbs at 150 mg/mL, offering a scalable, sample-efficient alternative to conventional methods. These results extend recent findings on the concentration-dependent sensitivity of SAXS to short-range attractions, demonstrating that they can emerge at lower concentrations than previously reported. This study presents the most comprehensive and diverse SAXS dataset for mAbs reported to date, providing a valuable resource for developing coarse-grained models that can more accurately capture intermolecular interactions governing high-concentration solution behavior, enabling rational antibody engineering and improved developability.

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No abstract submitted by the author.

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***w*NMR Relaxometry for the *In Situ* Assessment of High-Concentration Biologics and Product Security**
Marc Taraban, University of Maryland, School of Pharmacy, 9600 Gudelsky Dr., Rockville, MD 20850

Water presence is often regarded as a nuisance in NMR spectroscopy affecting the quantification of critical process parameters and quality attributes in pharmaceutical products. However, as the most abundant component in many drug products, water dynamics analysed by benchtop time-domain (TD) NMR instruments can be a sensor for quantitative characterization of aqueous drug formulations. In this presentation, we introduce water proton nuclear magnetic resonance (*w*NMR) relaxometry as a powerful, noninvasive tool that could address many critical priorities in situ. Operating directly within primary containers like prefilled syringes, vials, injection pens, *w*NMR enables real-time stability monitoring of high-concentration formulations without sample dilution or destruction. *w*NMR could be also provide precise estimates of the empty/full ratio of the viral capsids containing therapeutic gene material, and drastically enhance pharmaceutical safety and security by generating distinct molecular fingerprints that detects even sophisticated falsifications and sub-potent batches. By combining advanced formulation analytics with rapid authentication, in situ wNMR provides an essential dual-defense mechanism to ensure drug quality, secure the supply chain, and guarantee patient safety.

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Predicting High Concentration Viscosity in Multi-Specific Antibody Solution
Sudeep Adhikari, Stevens Institute of Technology, 2427 Strawberry Ct., Edison, NJ 08817, Pin Kuang Lai

Predicting viscosity is essential to developing high-concentration antibody formulations, especially for subcutaneous delivery, where high viscosity can compromise injectability and clinical performance. Monoclonal antibodies (mAbs) have received considerable study, but multi-specific antibodies (msAbs), spanning bi-, tri-, and tetra-specific formats, add structural complexity that makes viscosity prediction more difficult. We describe an integrated computational workflow that pairs an automated, msAb-specific structure-generation pipeline with all-atom molecular dynamics (MD) simulation and a one-bead-per-domain coarse-grained model for predicting viscosity. Intramolecular interaction parameters extracted from the MD simulations feed directly into the coarse-grained framework. Preliminary results show close agreement between predicted relative viscosity and experimental trends, pointing to this multiscale approach as a way to gain mechanistic insight and assess viscosity risk early for both monoclonal and multi-specific antibody therapeutics.

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Fluorescence from Natural Resource Cannabinoids for Biomedical Imaging
Gregory Sotzing, University of Connecticut, 25 King Hill Rd., Storrs, CT 06269, Pragati Rout

The development of sustainable fluorescent compounds is limited by a continued reliance on petroleum-derived aromatics, rare metals, and environmentally persistent dyes. Here, we identify oxidized phytocannabinoids, such as cannabinol (CBN) as an underexplored class of renewable, metal-free organic fluorescent compounds derived from hemp plant natural resource. CBN is a model scaffold for fluorescence via dimerization to enable tunable fluorescence spanning the near-UV to visible blue region, with quantum yields comparable to benchmark petroleum based organic dyes. Systematic structure–property analysis reveals that linker chemistry and parity govern emission efficiency through modulation of conformation, intermolecular packing by way of aromatic slip stacks. Single-crystal X-ray diffraction, solution- and solid-state NMR spectroscopy, and concentration-dependent fluorescence measurements collectively show that odd-numbered alkyl linkers promote adaptive packing and enhanced radiative decay, whereas the even-numbered alkyl linkers do not. CBN within the dimers can undergo aromatic sulfonation chemistry to render the dyes water soluble, with enhanced quantum efficiencies. These findings establish oxidized phytocannabinoids as a versatile, bio-derived platform for sustainable organic luminophores and provide transferable design principles for renewable fluorescent materials.

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Intraarticular Sustained Release of Dicannabidiol for Prolonged Osteoarthritic Pain Relief in Monoiodoacetate Induced Osteoarthritis in Rat Model

Erick Orozco Morato, University of Connecticut Health Center, 263 Farmington Ave., Farmington, CT 06030, Pragati Rout, Joseph Walker, Gregory Sotzing, Lakshmi Nair

Osteoarthritis (OA) is a degenerative joint disease associated with persistent pain, synovial inflammation, and progressive cartilage loss, yet therapies with sustained analgesic efficacy remain limited. Here, we report an injectable platform based on dicannabidiol adipate (diCBD), a dimeric cannabidiol prodrug, encapsulated in poly(lactide-co-glycolide) (PLGA) microspheres for managing OA pain . In vitro studies showed that diCBD exhibited minimal cytotoxicity towards F-11 cells and suppressed pro-inflammatory gene expression, including IL-6, IL-1β, and Nos2 in lipopolysaccharide-stimulated bone marrow-derived macrophages. Microspheres prepared using a blend (3:1 w/w) of 75:25 and 50:50 PLGA polymers achieved diCBD loading up to ~27 wt% with ~50% encapsulation efficiency and spherical morphology The microsphere carriers provided sustained in vitro release of diCBD over time. In a monoiodoacetate-induced rat OA model, a single intra-articular injection of diCBD-loaded microspheres provided significantly reduced mechanical allodynia from day 2 through day 21 compared to the control group. Coadministration with ropivacaine in hyaluronic acid caused early hypoalgesia while providing reduced mechanical allodynia for a period of 21 days post injection. making diCBD-loaded PLGA microspheres as a promising biomaterial-based depot strategy for prolonged management of OA pain.

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Cannabinoids and Addiction

Steven Kinsey, University of Connecticut, 231 Glenbrook Rd., U-4026, Storrs, CT 06269

The rapid commercialization of *minor* cannabinoids has outpaced the scientific evidence needed to evaluate their addiction and withdrawal potential. For example, although delta-9-THC (Δ^9-THC) remains the primary driver of Cannabis Use Disorder (CUD), emerging products enriched in delta-8-THC (Δ^8-THC), cannabinol (CBN), or cannabidiol (CBD) are increasingly marketed as “milder,” quasi-legal alternatives. Human case reports and epidemiological data already suggest that these compounds may carry risk for misuse, dependence, and withdrawal, echoing the historical trajectory of gabapentinoids, which entered the market as low-risk therapeutics but later demonstrated clear abuse liability. We determined the dependence-related effects of the major cannabinoids Δ^9-THC and CBD, and the minor cannabinoids Δ^8-THC and CBN, using validated behavioral assays of cannabinoid withdrawal in mice. Repeated Δ^9-THC exposure produced robust somatic withdrawal signs (paw tremors, head twitches), elevated corticosterone, and altered stress-related behaviors including increased struggling in the tail-suspension test and suppressed marble-burying. Spontaneous withdrawal also emerged within 24-48 hours of abstinence, demonstrating that antagonist-precipitated paradigms underestimate the breadth of THC-related withdrawal behavioral morphology. Similarly, the minor cannabinoids Δ^8-THC and CBN (marketed as an herbal sleep aid) produced classic CB_1-mediated tetrad effects, tolerance, cross-tolerance to the synthetic cannabinoid WIN55,212-2, and antagonist-precipitated withdrawal. These data challenge the notion that the minor cannabinoids Δ^8-THC and CBN are “safe” or less dependence-forming cannabinoids.

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Cannabinoid Therapeutics

Mark Scialdone, St. Michael’s College, Dept. of Chemistry, School of Natural and Health Sciences, Colchester, VT 05439

Phytocannabinoids, a key class of secondary metabolites from Cannabis sativa L., have diverse biological activities and have long been investigated for their potential to treat diseases and medical conditions in humans and animals. The purified compounds themselves can also provide starting materials for new structural analogs and derivatives. Modifying their reactive functional groups can introduce small structural changes while largely preserving the natural molecular scaffolds. These changes in structure may enhance biological activity and target selectivity, generating new therapeutic leads for drug discovery. Such compounds can act on the endocannabinoid signaling system, which is present in all mammals and regulates multiple physiological functions, including inflammation, to maintain homeostasis. This talk will examine the development of new therapeutics based on known and novel phytocannabinoid derivatives.

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Sustainable Method Evaluation throughout the Lifetime of an Agricultural Product

Mary Grace Guardiean, FMC Corporation, Stine Research Center, 1090 Elkton Rd., Newark, DE 19711, Austin Whittington, Anna Grifo, Xiaoyan Wang, Mary Ellen McNally

In efforts to incorporate a greenness score throughout analytical method development, we have evaluated not only final product, but all routinely used analytical methods for a subset of our agricultural active ingredients.[1] This evaluation included methods developed in R&D as well as those developed at manufacturing facilities and contract research organizations whose thought process throughout development might not have included the long-term greenness of a method. The effort was undertaken to ascertain where the greatest improvements could be made and the common denominators which were causing excess solvent use and waste generation, as well as the greatest energy consumption methodologies. A presentation of the results of our evaluation will be given. [1] F. Power, P. Ferguson, A. Herbert, S. Ryan, M. Osborne, and L.Trezise, Green Chem., 2025, 27, 14088-14100.

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Green Chemistry in Forensics: Utilizing AGREEprep Scoring for Method Comparisons and Development

Kira Bochard, NMS Labs, 200 Welsh Rd. Horsham, PA, 19044, Shayna Kasher

As laboratories seek to reduce their environmental impact, there is a growing need for tools that assess the sustainability of analytical practices. Developed by Wojnowski, Tobiszewski, and Pena-Pereira, the AGREEprep scoring method is a free open-source software used to evaluate extraction methods based on the 12 Principles of Green Analytical Chemistry. The qualitative and quantitative assessment allows for simple, comprehensive method comparisons while prioritizing environmental responsibility. While originally designed for general analytical and environmental chemistry laboratories, we believe that there is a growing necessity to adapt this software for the forensic space. Both forensic toxicology and forensic chemistry present an inherent set of challenges for incorporating green chemistry as complicated extractions tend to require larger sample volumes and regular use of hazardous materials. Therefore, we adapted the AGREEprep scoring criteria to better reflect the priorities and constraints of forensic workflows. Our modified version incorporates toxicology-specific considerations while maintaining the structure of the original AGREEprep model. This presentation will explain how the AGREEprep software works, explain how the criteria was specifically tailored for forensic applications, and expand on our pilot study of method comparisons highlighting method improvements. We will also demonstrate how this adapted version of criteria can be used to evaluate and compare a variety of high-volume tests. The modified AGREEprep scoring method allows for the analysis of multiple types of methods. Our goal is to provide the scientific community with a standardized method for integrating green chemistry while maintaining the high standards required for forensic analysis.

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

Ettigounder Ponnusamy, MilliporeSigma, 2909 Laclede Ave, St. Louis, 63103

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.

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Green Analytical Chemistry: Importance and Guidance for Industry and Academia

Isamir Martinez, American Chemical Society, 1155 16th Street, NW, Washington, DC 20036, Keith Gaylan, Matthew Osborne, Mark Zell, Paul Ferguson, Michael Hicks

Analytical chemistry is essential for ensuring the quality and purity of pharmaceutical products, yet it contributes an estimated 5–10% of industry waste through solvent use, water consumption, single-use materials, and energy-intensive instrumentation. As sustainability commitments grow, integrating Green Analytical Chemistry (GAC) principles into analytical development is both an environmental necessity and a scientific opportunity. This presentation outlines a practical framework based on the “4Rs” (Remove, Replace, Reduce, Recycle) to systematically improve method sustainability. Strategies include replacing chromatographic methods with spectroscopic techniques where appropriate, substituting hazardous reagents with safer alternatives, and leveraging advanced separations such as UHPLC and supercritical fluid chromatography to significantly reduce solvent consumption and analysis time. Central to this approach is the Analytical Method Greenness Score (AMGS), developed by the ACS Green Chemistry Institute Pharmaceutical Roundtable. AMGS provides a quantitative, standardized assessment of method environmental impact, enabling data-driven comparisons and informed optimization. Case studies demonstrate that targeted modifications can deliver substantial reductions in waste, solvent use, and energy demand without compromising analytical performance, offering a clear pathway to more sustainable analytical practices across industry and academia.

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3D Confocal Raman Imaging of PFOA Bioaccumulation in Trehalose-Immobilized DOPC Giant Unilamellar Vesicles

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This study investigates the bioaccumulation of perfluorooctanoic acid (PFOA) within model cellular membranes using confocal Raman microscopy. Giant unilamellar vesicles (GUVs) composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were utilized to simulate biological boundaries and evaluate the partitioning behavior of perfluorinated compounds. To ensure structural stability during high-resolution scanning, the liposomes were immobilized in a trehalose matrix to prevent laser and vibration induced movement disrupting the scan. Using a Jasco NRS-4500 confocal Raman spectrometer, we performed 3D layer-by-layer imaging to map the volumetric distribution of PFOA. GUVs ranging in size from 1-20 μm were created via electroformation with stainless steel electrodes. The PFOA concentration was maintained below the critical micelle concentration to ensure that the detected signals originated from molecular partitioning rather than the formation of independent free-floating PFOA micelles. Spectroscopic mapping reveals a significant localization of PFOA within both the DOPC lipid bilayer and the interior of the GUVs. The results demonstrate that PFOA effectively permeates into the DOPC membranes at sub-micellar concentrations. This distribution pattern suggests a strong affinity for the lipid environment and underscores the compound's ability to infiltrate encapsulated biological compartments. By providing direct spectroscopic evidence of PFOA localization, this research highlights the efficacy of confocal Raman imaging for the label-free study of persistent organic pollutants and their bioaccumulation pathways at the membrane level.

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Towards Super Resolution Two-Dimensional Infrared (2DIR) Spectroscopy
Olivia Tancredi, University of Delaware, 163 The Green, Newark, DE 19716, Audrey Carson, Saul Tonalli Rodriguez Vazquez, Ariel Alperstein

Two-dimensional infrared (2DIR) spectroscopy is a pump-probe ultrafast vibrational technique that measures molecular vibrations and their couplings on relevant timescales to provide bond-specific information on molecular structure and system dynamics. Compared to other standard linear infrared spectroscopies such as Fourier transform infrared (FTIR) spectroscopy, 2DIR offers enhanced sensitivity to vibrational coupling, as its nonlinear signal scales differently with the transition dipole moment, making secondary structure easier to resolve. However, conventional vibrational techniques, including 2DIR, are constrained by how small the laser light can be focused in a given optical setup, otherwise known as the diffraction limit. Overcoming this barrier is essential for studying biological and material systems whose relevant structural features fall below this threshold. We are developing a super-resolution 2DIR setup designed to push spatial resolution beyond the diffraction limit. Progress toward this goal and current development of our laser setup will be presented.

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Revealing the Fugitive Palette in Art Using Surface-Enhanced or Single-Molecule Spectroscopy
Kristin Wustholz, William & Mary, Department of Chemistry, 540 Landrum Dr., Williamsburg, VA 23185, Alisha Khodabocus, Chloe Autry

Identifying “fugitive” organic colorants in art is important and challenging. This talk presents and compares two state-of-the-art approaches, surface-enhanced Raman scattering (SERS) and blink-based multiplexing (BBM), and how these studies inform artists’ and conservation choices. Recent SERS studies of 18th-c. oil paintings at the Art Museums of Colonial Williamsburg made by C.W. Peale, Joshua Reynolds, and Joshua Johnson are discussed. We also recently developed BBM, which harnesses the intrinsic fluctuations in fluorescence over time of a single molecule (blinking) as a barcode for identification. Our studies demonstrate BBM can identify individual molecules of modern artists’ dyes and inks.

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Direct, Selective Analysis of Ethylene Oxide and Acetaldehyde in Complex Food Matrices by Molecular Rotational Resonance Spectroscopy
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Food safety testing frequently requires the analysis of small, highly volatile analytes in aqueous and injection-unfriendly matrices (e.g., viscous or non-volatile systems). Ethylene oxide and acetaldehyde are important targets due to their regulatory significance and analytical challenges, arising from their identical molecular formula and mass, as well as their high volatility and reactivity, which often necessitate derivatization or chromatographic separation in conventional methods. Together, these constraints increase complexity and limit throughput for routine screening of food and related products. We present a direct MRR-based approach for the selective identification and quantitation of ethylene oxide and acetaldehyde from challenging matrices relevant to food safety. MRR provides intrinsic molecular specificity by resolving rotational transitions unique to each molecule, enabling unambiguous differentiation of structural isomers. Two workflows were evaluated. First, the headspace autosampler enabled direct quantitation from a viscous, injection-unfriendly matrix (PEG-3350). Secondly, for aqueous samples, a compact preconcentration front end with a short packed column was used to separate volatile analytes from water prior to MRR detection. Across both workflows, representative run times of 3–13 minutes per sample were achieved. Limits of detection reached 0.2 µg/mL for ethylene oxide and 2 µg/mL for acetaldehyde, with excellent linearity (R^2 >0.999). This approach provides a rapid, selective, and high-throughput solution for routine screening of volatile contaminants in food and related products. By simplifying workflows while maintaining high specificity, MRR provides a powerful complementary approach for food safety testing.

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Quantitative Screening of Isomeric Impurities in Small Molecule Raw Materials Using Targeted MRR
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Reliable detection and quantitation of isomeric impurities in raw materials is critical for developing sound analytical strategies that ensure the quality and safety of final pharmaceutical and chemical products. This is because similar physicochemical properties of these impurities and intended starting materials often result in propagation of unwanted by-products through multiple synthetic reaction steps to form inactive or toxic isomers of desired compounds. The commonly used techniques for isomeric impurity analyses are chromatography coupled with chemically selective detectors, such as mass spectrometry. Yet, because of identical masses and highly similar fragmentation patterns, the analysis of isomeric impurities often relies mainly on effective chromatographic separation of these impurities from the desired compounds. In turn, the development of effective separation methods for each raw material and each possible isomeric impurity may quickly become a notoriously cumbersome task. Molecular Rotational Resonance (MRR) spectroscopy is an inherently structure-specific technique capable of unambiguous, spectral-overlap-free discrimination between all classes of isomers, including constitutional, stereo, and isotopic isomers, directly in mixtures, without requiring chemical separation. This presentation provides several examples of fast, direct, and highly selective quantitative screening of challenging isomeric impurities in different raw materials, including diastereomers, regioisomers, and enantiomers. These data suggest that MRR can improve and streamline impurity screening in raw materials for chemical synthesis and contribute to developing next-generation QbD-based quality control strategies.

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Uncovering Hidden Trends in Analytical Data Using PCA
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We present a comprehensive analysis of spectral datasets across various techniques (IR. Raman, GC-MS, LC-MS, NMR, UV-Vis) using Principal Component Analysis (PCA) that reveals underlying patterns and correlations that remain invisible when examining individual spectral measurements in isolation. This study demonstrates how PCA can enhance scientific discovery by extracting insights from high-dimensional data. Our findings show that PCA successfully identifies spectral clusters and feature correlations, demonstrating its value for improving analytical workflows.

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Optimizing Solvent Selection and Dimensionality Reduction Workflows For GC×GC Fire Debris Analysis
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Comprehensive two-dimensional gas chromatography (GC×GC) resolves complex ignitable liquid residue (ILR) mixtures with unmatched chemical detail, yet its high-dimensional output creates interpretive bottlenecks that hinder application to large forensic datasets. This study evaluated how five extraction solvents influence ILR profiles and demonstrated how chemometrics can streamline interpretation of GC×GC data. Chromatographic features from 100 ILR samples were analyzed using Principal Component Analysis (PCA), a linear variance-based method, alongside Uniform Manifold Approximation and Projection (UMAP), a stochastic manifold learning approach, to assess solvent-dependent clustering among ILR-classes (gasoline, kerosene, and diesel). UMAP provided clearer ILR-class separation and reduced intra-class variance compared to PCA, which was prone to outlier-driven distortion, obscuring meaningful cluster structure, revealing that solvent choice shapes the profile of extracted analytes. Hexane produced the clearest cluster separation, while diethyl ether did not separate the classes; methanol performed comparably to non-polar solvents despite its polarity. These results highlight UMAP as an effective diagnostic tool for evaluating extraction performance and improving pattern recognition in large GC×GC datasets. By clarifying both intra- and inter-class similarities and differences, this approach supports a systematic and reproducible workflow for ILR data processing. Overall, this work contributes to the development of data driven method workflows for forensic fire debris analysis and informs solvent selection strategies that reduce hazardous exposure and align with federal regulatory guidance governing solvent use. Coupling GC×GC with UMAP-based chemometric visualization establishes a reproducible, scalable framework for evidence-driven ILR classification that enables broader adoption in forensic labs.

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Integrating Conformal Prediction with Laser-Induced Breakdown Spectroscopy for Hardwood Species Identification
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The illegal timber trade is a global challenge, contributing to deforestation and billions of dollars in economic losses. This issue is particularly relevant in regions such as Kentucky, where active and proposed logging projects in the Daniel Boone National Forest require methods to verify that harvested timber aligns with approved management plans. Current approaches for timber identification, including direct analysis in real time (DART) mass spectrometry, DNA analysis, and stable isotope ratio analysis, often require laboratory-based workflows and long turnaround times. These limitations restrict field-based and rapid decision-making use. Laser-induced breakdown spectroscopy (LIBS), coupled with chemometric analysis, is a promising alternative due to minimal sample preparation, rapid acquisition, and field deployment potential. A dataset of seven Kentucky hardwood species (e.g., oak, maple, walnut) was collected to develop classification models. Principal component analysis showed minimal species-level separation, highlighting the need for supervised models. Supervised multivariate models, including partial least squares discriminant analysis (PLS-DA), are being developed using LIBS spectral features to classify timber samples. Classification is performed using a hierarchical approach, first distinguishing genus and then species to improve discrimination between closely related species. To further improve model reliability, conformal prediction is incorporated to provide statistically valid prediction sets for each classification, identifying samples with low prediction confidence. This work establishes a spectroscopic framework for timber identification that combines hierarchical classification with conformal prediction. By quantifying prediction uncertainty, this approach supports more reliable field-deployable timber identification tools. This framework has applications in law enforcement, environmental monitoring, and sustainable forest management.

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Artificial Intelligence–Assisted LC–MS Method Development for Screening Triglycerides in Vegetable Oils and Animal Fats
Mark Wang, Bristol-Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903, Scott Huffman, Ravi Kalyanaraman

We developed a liquid chromatography–mass spectrometry (LC-MS) screening method for triglycerides in vegetable oils and animal fats. Artificial intelligence (AI) tools (Anthropic Claude), together with accurate-mass data acquired by quadrupole time-of-flight mass spectrometry (QTOF-MS), accelerated method development and helped resolve issues encountered during the process.

270
Evaluation of Generative AI to Assist with NMR Pulse Programming
Ryan Cohen, Merck & Co., Inc., 126 East Lincoln Ave., Rahway, NJ 07065

Generative AI has started to attract attention as a tool for scientific coding, but its usefulness in NMR pulse programming is still not clear. In this work, several GenAI programs were tested for their ability to generate, modify, and interpret NMR pulse sequences using a set of increasingly difficult challenges. These included writing a simple gradient COSY pulse program for Bruker TopSpin 4, adding presaturation and excitation sculpting water suppression to an HSQC experiment, translating a Varian pulse sequence into Bruker

format, analyzing pulse sequence timing and product operator behavior, and proposing a new homodecoupled selective INADEQUATE experiment. The results were mixed. For simpler tasks, GenAI often gave a reasonable starting point and helped speed up early development. For more difficult problems, however, the output usually needed significant revision, especially for syntax, timing, phase cycling, and coherence transfer elements. In some cases, the AI was more useful for brainstorming or troubleshooting than for producing a final working sequence. All pulse programs were tested experimentally on a 600 MHz Bruker AVANCE NEO spectrometer. Overall, these results suggest that GenAI can be a useful aid in NMR pulse programming, particularly for drafting and idea generation, but expert review is still essential before implementation and experimental use.

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