2024

November 18–20, 2024

EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION

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ABSTRACT BOOK

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EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION

Across The Analytical Spectrum: Diversity of Scientific Ideas

November 17-19, 2025

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2024 EAS Abstracts

This volume contains the final abstracts for the oral and poster presentations which take place Monday, November 18, through Wednesday, November 20, 2024. 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 Sustainability in Present-Day Forensic Science

Thomas Brettell, Cedar Crest College, 100 College Dr., Allentown, PA 18104

Green analytical chemistry (GAC) is an emerging field that emphasizes the development of environmentally sustainable analytical methods for chemical analysis. The use of sustainable and green solvents in sample preparation have been reported for various substances in the industrial laboratory and in the near future will be standard operating procedure for conducting sample preparation. A review of the recent literature indicates research trends towards greener methods in forensic science, in particular sustainable methods in forensic toxicology are being reported. This is a good trend, but these greener methods need to be implemented into practice. There are approximately 400 crime labs in the United States and although this may be a small population in comparison to the chemical industry this should not be a reason to not stress sustainability in Forensic Science. At the present time, there are many reasons for this lack of development such as workload, budget, lack of awareness, and lack of resources to name a few. The purpose of this presentation is to highlight some green analytical tools that have been used in forensic applications and to bring attention to the need for more sustainable and greener methodologies in the forensic field. Examples of sustainable analytical methods reported in forensic chemistry and toxicology will be presented. The metrics for the evaluation of the greenness for the analytical methodology of a high-performance thin-layer chromatographic (HPTLC) method used to analyze novel psychoactive substances (NPS) are discussed.

2 Driving Sustainability Goals Through GSK's Global Chromatography Technique Network

Daniel Fabry, GSK, 1250 S. Collegeville Rd., Collegeville, PA 19426

GSK, along with many other companies, understand the urgent need to slow down climate change. Leaders within these companies have set ambitious goals focused on improving lives not only through the products or services they provide, but also by investing in projects and initiatives to achieve net zero greenhouse gas emissions. As members of the global analytical community, we need to change the way we do science while remaining committed to producing quality data that is used to inform decisions and protect patients. The unfortunate truth is that analytical scientists, especially chromatographers, are large consumers of solvents and materials whose manufacturing process and mechanism of disposal increase a company's environmental footprint. The Chromatography Technique Network (CTN) is a global team of analytical scientists who are passionate about driving chromatographic innovation and technical excellence at GSK. We have taken on the challenge to act as stewards within our analytical community to reduce our environmental impact through the application of innovative tools and technology. The CTN has been successful in reducing consumption of organic solvents with the use of small-bore columns, evaluating alternative organic solvents used in reversed phase chromatography, optimizing method development with the use of Quality by Design (QbD) software like ACD Labs AutoChrom, and eliminating purification chromatography steps with the assistance of process mass intensity analysis. The CTN applied innovative thinking to reduce our environmental impact and demonstrated that implementing sustainable ways of working can be economical and used to increase operational efficiencies.

3 Continuous Manufacturing of Tirzepatide Using Online UHPLC-Based PAT: An Enabling Technology for Commercialization of a Synthetic Peptide

Bradley Campbell, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, 46285

Tirzepatide is a GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide-1) receptor agonist that has been approved for the treatment of type 2 diabetes and as an adjunct to a reduced-calorie diet and increased physical activity for chronic weight management. Commercial manufacture of a complex synthetic peptide presents significant challenges to obtaining the desired product of high quality and yield. Recently, a hybrid solid-phase peptide synthesis/liquid-phase peptide synthesis (SPPS/LPPS) approach was demonstrated at scale to leverage the benefits of continuous processing and online UHPLC-based process analytical technology (PAT). The hybrid SPPS/LPPS synthesis of tirzepatide utilizes four high purity fragments synthesized via traditional SPPS methodologies and released as GMP intermediates. Fragments are coupled in three continuous LPPS reactions performed in dedicated plug flow reactors (PFR) with two nanofiltration-based impurity purge points between chemical reactions. Each PFR is equipped with an online UHPLC and a Lilly developed process sampler to monitor reaction completion and residual starting materials. Brief case studies will be presented on how to effectively implement online UHPLC for a peptide synthesis with a PAT-directed control strategy for control of key impurities.

4 Incorporating Sustainable Practices in the Analytical Laboratory

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

Corporations are driving sustainability goals furiously to a "zero" footprint in 2050. From the December 2021 Harvard Review[1]: "Virtually all of the world's largest companies now issue a sustainability report and set goals; more than 2,000 companies have set a science-based carbon target; and about one-third of Europe's largest public companies have pledged to reach net zero by 2050." These are viable corporate objectives and certainly, the largest efforts and return for effort is from the manufacturing side of these businesses. On the other hand, R&D laboratories in pharmaceutical and the agricultural industries are the initial point of determining what transpires at the manufacturing facility including the analytical or quality control laboratories. This presentation will discuss a plan to incorporate sustainability practices in the R&D lab with the ultimate goal of incorporating them more broadly. Included are the practices of evaluating and transforming methods to be sustainable, practices to reduce sample and standard preparation, lower energy consumption, reducing waste generation as well as using analytical equipment more efficiently. These directions and practices are presented.

References:

[1] A. Winston, "Sustainable Business went Mainstream in 2021", https:/hbr.org/2021/2.

5 Charge Detection Mass Spectrometry for Viruses, Vaccines, and Particles: Mass Spectrometry in the Megadalton Regime

Martin Jarrold, Indiana University, Chemistry Department, Bloomington, IN 47405

Charge detection mass spectrometry (CD-MS) can be used to measure mass distributions for heterogeneous and high mass samples that cannot be studied by conventional mass spectrometry. CD-MS is a single ion approach where the mass to charge (m/z) ratio and charge are measured for individual ions and then multiplied to give the mass. Measurements are performed for thousands of individual ions and then binned to give a mass histogram. CD-MS is ideally suited to measuring mass distributions for viruses, virus-like particles (VLPs), gene therapies, and vaccines. It can provide information on stoichiometry (i.e., the number of capsid proteins present in a VLP) and can be used to track assembly reactions, revealing intermediates. Recent technical developments have led to a dramatic improvement in resolving power and measurement speed. CD-MS can now be performed on a chromatography timescale, facilitating online coupling to SEC and affinity chromatography. Recent results are presented for the assembly of norovirus and HPV VLPs showing how CD-MS can be used to optimize conditions to favor particular stoichiometries. There is a natural synergy between CD-MS (to determine and optimize stoichiometry) and cryo-EM (to determine structure).

6 i-HMBC and Isotope-Shift-Based NMR Structure Elucidation Strategy

Mikhail Reibarkh, Merck & Co., Inc., 126 E. Lincoln Ave., RY800-D231, Rahway, NJ 07065

Since the development of HMBC in 1986, it quickly became one of the most important NMR experiments for structure elucidation of small molecules due to its ability to detect long range proton-carbon correlations. Although HMBC was used regularly to detect ^{1}H-^{13}C correlations through 2-4 and in some cases even through 5-6 bonds, it could not differentiate 2-bond correlations from longer range correlations. This was a major issue when characterizing highly proton-deficient molecules, as ^{1}H-^{1}H correlation experiments like COSY were not informative. Although there have been a few published methods or modifications of HMBC to address this problem, they either lacked general applicability or suffered from severely reduced sensitivity. Here we present a highly sensitive i-HMBC (isotope shift detecting HMBC) methodology to identify 2-bond HMBC correlations using isotope shift, and demonstrate its effectiveness for structure elucidation of highly complex proton-deficient natural products at sub-mg/nanomole scale. In addition to characterization of proton-deficient natural products, the isotope shifts measured by i-HMBC provided an orthogonal approach, isotope-shift-based structure elucidation, to traditional coupling-based structure elucidation using a combination of COSY, HSQC and HMBC. We demonstrate the advantage of isotope-shift-based structure elucidation strategy in characterizing organic molecules with atypical coupling constants where COSY and HMBC give misleading NMR correlations.

7 Structural Characterization of Macrocyclic Peptido-Mimetic Compounds by NMR Spectroscopy

Christine Jorge, Bristol Myers Squibb, Route 206 & Province Line Rd., Princeton, NJ 08540, Luciano Mueller, Purnima Khandelwal, Alexander Brueckner, Ajay Jain, Ann Cleves, Janet Caceres-Cortes

Modalities that extend beyond traditional rule-of-5 (Ro5) small molecule drugs have gained popularity in the drug discovery landscape. These larger molecules feature more complex structures than Ro5-compliant molecules and often possess conformational heterogeneity with multiple low-energy conformers. Among these new modalities, peptide-based drugs offer an attractive means of inhibiting protein-protein interactions and constitute approximately 10% of new drug approvals in the past two years. While significant advances have been made in macrocyclic peptide design

and computational sampling methods, early biophysical data and structural characterization of solution and protein-bound states are still critical for efficiently advancing a program's structure-activity relationship. We have developed a semi-automated protocol to enable the determination of 3D-conformational ensembles using two-dimensional NOESY NMR spectra. Herein, we describe how we utilize this protocol to determine the 3D solution NMR ensemble of an initial lead and final compound for the PD-1/PD-L1 system. The lowest energy NMR structure for both compounds exhibits an all-atom RMSD of less than 1.5 Å relative to the bound X-ray structure, suggesting these successful drug candidates are preorganized in solution, thereby reducing ligand strain energy. Once a robust structural model has been established, we can use higher throughput qualitative NMR assessments of compounds related in an SAR series to ensure that the overall fold and critical correlations remain unperturbed. We present as an example a retrospective analysis where NMR derived backbone torsion angle restraints were used to computationally estimate ligand strain and rule out weak binders even in the absence of a bound protein structure.

8 **Small Molecule Crystallography to Solve Big Problems**

Amy Sarjeant, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903

Single-crystal structure determination is often referred to as the "gold standard" of characterization technique for good reason. An accurately determined crystal structure can answer myriad questions about compound identity, molecular conformation, stereochemistry, 3-D packing and intermolecular interactions. In addition to shedding light on these aspects of the solid form landscape, small molecule crystallography can help bridge the gap between a compound's structure and its bulk properties. New advances in crystallography, including Microcrystal Electron Diffraction (MicroED) help to uncover even more insights on how structure impacts everything from solubility to manufacturability. This talk provides some examples of how small molecule crystallography drives scientific development in the pharmaceutical industry and beyond.

9 **Vibrational Spectroscopy & Hyperspectral Imaging Analyses for Biomedical Related Applications**

Frank Weston, Attocube Systems Inc, Berkeley, CA 94710, Tobias Gokus, Artem Danilov

Since the 1990's researchers studying biological and biomedical samples using infrared spectroscopy and microscopy have been plagued with poor diffraction limited spatial resolutions that are wavelength dependent (~5-10um). An evolution of the attainable spatial resolution with infrared spectroscopy & imaging instrumentation and techniques will be discussed starting with millimeter size limitations to the area of interest with some traditional techniques and concluding with current spectroscopic and imaging advancements that are now limited at the nanoscale and can achieve spatial resolutions <10-20 nm for biomedical related applications. These modern near-field spectroscopic measurements allow one to identify biologically relevant materials and nano-structures, both *spatially and spectrally*, at unprecedented length scales with superb sensitivity for both living as well as fixed cells and tissues regardless of whether one is working in the visible, near-IR, mid-IR, or far-IR / THz spectral regions. Using a variety of real-world biological and biomedical samples, such as cancer cells, bacteria, viruses, and macromolecules, we demonstrate static and dynamic *nano*-spectroscopy & *nano*-imaging for living samples in a water matrix as well as those dried and fixed to a microscope slide.

10 **No abstract submitted by the author.**

11 **FTIR and Raman Spectroscopies Applied in Cosmetic and Beauty Industry**

Samuel Gourion-Arsiquaud, TRI Princeton, 601 Prospect Ave., Princeton, NJ 08540

Vibrational Spectroscopy has been a mainstay in the cosmetics and personal care industry. Indeed, these versatile techniques can be used at different levels from advance research to marketing and claims substantiation, from ex-vivo experiments to clinical evaluations. The introduction of hyperspectral imaging in both microscopic FTIR and microscopic confocal Raman imaging has created the perfect tool to investigate biological samples including skin and hair relevant for the beauty industry. Both active concentrations and molecular structural information can be obtained with spatial resolution with a myriad of applications for both cosmetics and biomedical sciences. In this presentation, we highlight the versatility of these techniques by describing different types of applications: For Skin testing: to evaluate and visualize exogenous substance penetration into the various dermal layers along with their effect on skin barrier function, hydration, lipid organization, and NMF. Evaluate and compare the efficacy and safety of different sunscreen products: micro-encapsulation technology, or the use of film formers to improve the retention of organic UV filters on the skin surface and thereby improve sunscreen efficacy and limit toxicological risks. For Hair testing: to investigate molecular and structural hair modifications through large range of insults like chemical treatments (bleaching, color), thermal styling, delipidation, and environmental stresses (UV, Ozone, chlorine). It also allows the evaluation of various treatments in terms of penetration and diffusion of products into the hair fiber.

12 **High-Dimensional Mass Spectrometry Imaging Enables Prediction of Cancer Recurrence**

Drew Jones, NYU Langone Health, 430 E 29th St., ACLS WT635, New York, NY 10016

Mass spectrometry imaging (MSI) is a rapidly evolving technique which offers spatial resolution and quantification of metabolites and lipids, peptides in snap-frozen tissue. MSI involves scanning tissues with a mass spectrometer to obtain detailed information about the molecular composition at each "pixel" within the sample. The result is a high parameter dataset where each pixel is an independent mass spectrometry analysis from which data can be reconstructed into individual ion images, or pictures of the distribution and intensity of a single unlabeled molecular structure detected by the mass spectrometer. In practice, several thousand high quality single ion images (lasso of an m/z and mobility) can be generated from a typical tissue sample making this technique analogous to hyperspectral techniques. Each image can be understood then as a separate data channel, providing highly descriptive spatial information about the molecular composition of the tissue in a much higher dimension than traditional optical images of histopathological stains represented in 3-channels (red, green, blue). We are applying a machine learning approach to the unbiased and unsupervised analyses of these datasets enabling automatic region annotation, cell type annotation, and determination of other clinical parameters. To support this ML approach, we have created an n-dimensional deep-learning CNN architecture where the thousands of individual ion images are considered as a single high-parameter image. As a test of this architecture, we have applied this model to a clinical cohort of lung cancer patients (Adenocarcinoma) to predict the likelihood of cancer recurrence at the time of tissue resection.

13 **Improving PS80 Content Analysis of Biopharmaceutical Therapeutics by Incorporation of Protein Precipitation**

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

Polysorbate 80 (PS80) is a versatile excipient in pharmaceutical formulations, acting as a stabilizer in a variety of injectable medications. In biopharmaceutical applications, it is commonly used to prevent aggregation of therapeutic monoclonal antibodies (mAbs). Degradation of PS80 in biopharmaceuticals is a prominent issue, making methods for accurately measuring PS80 content critical in drug product development. The biopharmaceutical industry standard for PS80 content measurement is performed via HPLC-ELSD using a mixed-mode column (Waters Oasis Max). In this method, mAb-containing samples are directly injected onto the column, with mAbs eluting during an initial rinse phase before elution of PS80. Unfortunately, method accuracy and column lifetime can be adversely impacted when samples contain mAbs at the high concentrations often observed in drug products. To improve analysis, the use of organic solvent to precipitate mAbs prior to injection was examined. Two methods for analyzing PS80 content, using either methanol or isopropanol as the precipitating solvent, were developed. These methods have enabled accurate and robust PS80 content analysis of samples containing mAbs at concentrations upwards of 200 mg/mL. This presentation will highlight the process of performing assay development for PS80 analysis and the transfer of these methods into a commercial manufacturing setting.

14 **Multi-Dimensional Separation for Vaccine Component Analysis and Characterization**

Arthur Arcinas, Merck & Co., Inc., 770 Sumneytown Pike, West Point, PA 19486, Rodell Barrientos, Mohamed Hamdi Said Hemida, Gunjan Dixit, Heather Wang, Andrew Singh, Emmanuel Appiah-Amponsah, Erik Regalado

Two-dimensional liquid chromatography (2D-LC) has emerged as a powerful separation technique that enables quantitation of analytes present in complex matrices where components of vastly differing size and properties exist (including virus like particles, protein, and small molecules) and pose challenges for single-dimension separation alone. Coupling similar or complementary modes of separation, such as reverse phase, ion exchange, size exclusion, and HIC, provide improved or extended separation, in conjunction with compatible various detection systems (UV, FLD, MS, CAD, and MALS) to enhanced sensitivity and selectivity for the analysis of vaccine components. The described method demonstrates a unique application for the separation and quantitation of analytes in a complex vaccine formulation, which aid to support characterization and efficient process development in the biopharmaceutical space.

15 **Directly Detecting the Degradation of mRNA in mRNA Vaccine Model System Using Deep UV Resonance Raman Spectroscopy**

Lamyaa Almehmadi, Massachusetts Institute of Technology, Department of Materials Science and Engineering, Cambridge, MA 02139, Sergei Reverdatto, Vladimir Ermolenkov, Alexander Shekhtman, Igor Lednev

The degradation of mRNA in mRNA vaccine model was directly probed using deep-UV resonance Raman (DUVRR) spectroscopy. The mRNA in the vaccine model was subjected to controlled degradation either with RNase A or by aging at room temperature. DUVRR spectroscopy showed mRNA degradation signatures of the vaccine model under both conditions. In addition to DUVRR spectroscopy, the degradation of the mRNA was confirmed by cell transfection and gel electrophoresis.

16 **Two-Dimensional SEC-SEC-UV-MALS-dRI Workflow for Streamlined Analysis and Characterization of Biopharmaceuticals**

Ophelia Ukaegbu, ARD, 126 E. Lincoln Ave., Rahway, NJ 07065, Rodell Barrientos, Andrew Singh, Mohamed Hemida, Heather Wang, Imad Haidar Ahmad, Hang Hu, Zachary Dunn, Emmanuel Appiah-Amponsah, Erik Regalado

The emergence of complex biological modalities in the biopharmaceutical industry entails a significant expansion of the current analytical toolbox to address the need to deploy meaningful and reliable assays at an unprecedented pace. Size exclusion chromatography (SEC) is an industry standard technique for protein separation and analysis. Some constraints of traditional SEC stem from its restricted ability to resolve complex mixtures and notoriously long run times while also requiring multiple offline separation conditions on different pore size columns to cover a wider molecular size distribution. Two-dimensional liquid chromatography (2D-LC) is becoming an important tool not only to increase peak capacity but also to tune selectivity in a single online method. Herein, an online 2D-LC framework in which both dimensions utilize SEC columns with different pore sizes is introduced with a goal to increase throughput for biomolecule separation and characterization. In addition to improving the separation of closely related species, this online 2D SEC-SEC approach also facilitated the rapid analysis of protein-based mixtures of a wide molecular size range in a single online experimental run bypassing time-consuming deployment of different offline SEC methods. By coupling the second dimension with multiangle light scattering (MALS) and differential refractive index (dRI) detectors, absolute molecular weights of the separated species were obtained without the use of calibration curves. As illustrated in this report for protein mixtures and vaccine processes, this workflow can be used in scenarios where rapid development and deployment of SEC assays are warranted, enabling bioprocess monitoring, purity assessment, and characterization.

17 **Enhancing Quantitative Analysis of Xenobiotics in Blood Plasma through Cross-Matrix Calibration and Bayesian Hierarchical Modeling**

Emanuela Gionfriddo, University at Buffalo, 710 Natural Science, Complex Buffalo, NY 14228, Nipunika H. Godage, Song S. Qian, Erasmus Cudjoe

This research focuses on the challenges of matrix effects and interspecies plasma protein binding (PPB) on measurement consistency during method validation across different types of plasma (human, rat, rabbit, and bovine). Accurate measurements of small molecules in plasma samples often require specific plasma types for calibration, which may not always be readily available or affordable. To address the costs associated with using human plasma for measurements, we investigate the potential of cross-matrix-matched calibration using Bayesian hierarchical modeling (BHM) to account for matrix effects related to PPB. The extent of PPB in different media can still impact the accuracy of the measurement, especially when small molecules are extracted via free concentration, as is the case with microextraction techniques. While matrix-matched calibration demonstrated high accuracy, cross-matrix calibration was found to be inadequate for precise measurements in human plasma. To address this, we used BHM to calculate correction factors for each analyte within each plasma type, successfully mitigating the measurement bias resulting from diverse calibration curve types used to quantify human plasma samples. This work contributes to the development of cost-effective, efficient calibration strategies for biofluids. Leveraging easily accessible plasma sources, such as bovine plasma, for method optimization and validation before analyzing costly plasma (e.g., human plasma) offers significant advantages for biomonitoring and pharmacokinetic studies.

18 **Effects of Radiolysis on the Stability and Chemical Fate of Pertechnetate Under Solvent Extraction Conditions for Nuclear Waste Reprocessing and Long-Term Storage**

Rachel Greenberg, Lehman College of the City University of New York, 250 Bedford Park Blvd. West, Bronx, NY 10468, Hossam Elshendidi, Benjamin Burton-Pye, Lynn Francesconi, Donna McGregor

The isotope Technetium-99 (^{99}Tc, $t_{1/2}$: 2.1 x 10^5 years; b_{max}: 293.7 keV) is a high-yield by product of uranium-235 fission (6%) and a high-yield product found in legacy waste tanks. Its long half-life and multiple oxidation states make its separation from other isotopes in spent nuclear fuel highly challenging. During the PUREX (Plutonium Uranium Reduction EXtraction) process for example, the liquid-liquid extractions of U and Pu from nitric acid using tributylphosphate (TBP) in an organic solvent often result in the co-extraction of ^{99}Tc, which complicates reprocessing efforts. Furthermore, the radiation fields present in the waste tanks can result in both changes to the oxidation state of Tc and TBP degradation products. It is known that TBP extracts pertechnetate into the organic phase, but what is unknown is how the degradation product of TBP, dibutylphosphoric acid (HDBP) behaves. In this work we explore 1) the photolytic reduction of high valent ^{99}Tc(VII) to lower oxidation states and 2) the conditions required for ^{99}Tc(VII) and ^{99}Tc(IV) extraction and subsequent stabilization using TBP and HDBP. Our work confirms that TBP extracts and stabilizes Tv(VII), but we find that HDBP extracts primarily Tc(IV) into the organic phase. The subsequent stabilization and exact speciation of the Tc(IV)-DBP construct however, depends on the acid content in the original aqueous layer.

19 **A Novel Method to Quantify and Prevent Fouling Using Superhydrophobic Surfaces**

Louis Pimpinella, The Graduate Center of the City University of New York, 365 Fifth Ave., New York, NY 10016, QianFeng Xu, Alexander Greer, Alan Lyons

Surface biofouling adversely affects a wide range of materials in contact with natural aqueous environments such as those used in medical devices, filtration systems, and marine applications. Measuring fouling easily in the field would be beneficial, especially when developing new coating materials. Here, we present a novel method to indirectly measure surface fouling using sliding angle measurements on superhydrophobic surfaces. As biomolecules such as amino acids and proteins adsorb onto a superhydrophobic surface, the surface is rendered more hydrophilic, increasing interactions with water. The angle at which water droplets can slide off the surface is especially sensitive to small changes in surface wetting properties. Eventually, water droplets become pinned to the surface and cannot slide. By measuring the water droplet sliding angle as a function of biomolecule chemistry, concentration (1 to 10,000 M) and time, we correlate the rate of sliding angle increase with the hydrophobicity of the biomolecule. Relatedly, we significantly reduced fouling by coating the superhydrophobic surface with a photosensitizer and illuminating with visible light to generate singlet oxygen ($^1O_2$) which reacts with amino acids and proteins, converting them to more hydrophilic molecules. Under visible illumination, sliding angles increased only modestly and wetting of the surface was completely prevented. The $^1O_2$ yield from superhydrophobic surfaces was quantified using an aqueous uric acid trapping solution. Peroxides generated by $^1O_2$ reactions with amino acids and proteins were quantified using FOX assay, and fluid ingress into the superhydrophobic surface was measured using optical microscopy and confocal microscopy with fluorescently-tagged proteins.

20 **NMR Detection of Primary and Secondary Products from the Photooxidation of a Phenolic Compound on Silica Particles and in Solution**

Serah Essang, Brooklyn College & Graduate Center CUNY, 2900 Bedford Ave., Brooklyn, NY 11210, Alexander Greer

While silica particles have been used as supports for oxidation reactions, studies of airborne singlet oxygen ($^1O_2$) oxidations at particle interfaces are very limited. Here, with *ortho*-prenyl phenol-coated silica particles and delivery of $^1O_2$ through the gas phase, NMR was used to detect the formation of primary products dihydrobenzofuran (a tremetone-like natural product) and allylic hydroperoxides. We used both air/particle and solution-phase techniques to detect photoproducts. After extended photolysis, additional compounds were detected by 1D and 2D NMR techniques, including dihydrobenzofurans bearing hydroperoxide, alcohol, and epoxide side-groups, as well as hydrogen peroxide and methane. The product formation appears to depend on a tandem process with an initial type II ($^1O_2$) reaction followed by type I reactions involving oxygen radicals and radical ions. The results provide mechanistic insight to primary and secondary photooxidation processes at the air/nanoparticle interface and in solution.

21 **Biomimetic Photooxidation of a Geranylated Phenol to Reach Natural Product-Like Dihydrobenzofuran and Allylic Hydroperoxides: Synthesis, Homogeneous, and Singlet Oxygen Quenching Studies**

Kamrun Nahar, Brooklyn College, 2900 Bedford Ave., Brooklyn, NY 11210, Alexander Greer

Strategies for improving singlet oxygen oxidation paths to natural product-like dihydrobenzofurans and allylic hydroperoxides are needed. Here, we describe the use of biomimetic and analytical approaches in the photooxidation of a geranylated phenol (a monoterpenoid). This photooxidation leads to the formation of a number of products that are currently being analyzed by NMR spectroscopy. Singlet oxygen oxidation of the geranyl side-group appears to enable the formation of a dihydrobenzofuran. Our previous mechanistic work on the similar prenyl side-group pointed to a novel *iso*-hydroperoxide intermediate [R(H)O$^+$–O$^-$] as preceding the

dihydrobenzofuran product. The *iso*-hydroperoxide is analogous to the *iso* species $CH_2I^+–I^-$ formed by UV photolysis of CH_2I_2, and is structurally reminiscent of carbonyl oxides ($R_2C=O^+–O^-$) formed in the reaction of carbenes and oxygen. The geranyl phenol photoproducts are also being analyzed for their cytotoxic effects, as will be discussed.

22 **Preliminary Characterization of Cultural Heritage Objects in the West Chester University Museum Collections**

Zachary Voras, West Chester University, Chemistry Dept., 750 South Church St., West Chester, PA 19383

The West Chester University (WCU) Museums include cultural heritage objects from a variety of on-campus collections, including the WCU Special Collections and the WCU Museum of Anthropology and Archaeology. The WCU Special Collections curates books, photographs, and paraphernalia related to the history of West Chester University and its faculty, staff, and administration. The WCU Museum of Anthopology and Archaeology curates objects related to archaeological and ethnographic research partaken by WCU faculty and includes objects of local and global origins, with a particular focus on indigenous peoples of North, Latin, and South America. Current work is underway to provide chemical characterization of these objects within the WCU Museums to provide insight into their state of deterioration, provenance, and preservation challenges. These characterizations utilize X-ray fluorescence spectroscopy (XRF), Fourier-transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM) for preliminary analysis. Results from a variety of research projects conducted by undergraduate students will be discussed, such as a collection of Andean headwear in the WCU Museum of Anthropology and Archaeology, an illuminated east-Asian concertina book in the WCU Special Collections, and various small course-based projects from both collections.

23 **Imaging of Fluorescent and Autofluorescent Biomaterials and Photosynthetic Microorganisms via Fluorescence Detected Widefield Photothermal Infrared Spectroscopy**

Eoghan Dillon, Photothermal Spectroscopy Corp., 325 Chapala St., Santa Barbara, CA 93101, Jay Anderson, Craig Prater, Kathleen Gough, Ting Yan

A variety of biological materials and photosynthetic organisms exhibit strong autofluorescence emission under ultraviolet excitation and the autofluorescent emission has a very strong temperature dependence, of order 1%/K. Taking advantage of this property, we have demonstrated high-speed, super-resolution photothermal infrared spectroscopy and chemical imaging of autofluorescent biomaterials and organisms using camera-based widefield autofluorescence detection. Illuminating a sample with pulses of infrared light from a tunable laser source causes periodic localized sample temperature increases that result in a significant decrease in the fluorescent or autofluorescent emission. A conventional s-CMOS camera is used to detect localized variations in autofluorescent emission over a wide area as an indicator of localized IR absorption. IR absorption image stacks can be acquired over a range of infrared wavelengths, including the fingerprint range, enabling extraction of localized IR absorption spectra. We have applied widefield FE-PTIR to analysis of autofluorescent biological materials including yeast cells, collagen, and photosynthetic organisms including diatoms and green microalgae cells. We have also demonstrated chemical imaging of live yeast and microalgae in water.

24 **A Collaborative Study on Platform 1H Quantitative NMR Method: Towards Capacity Building for Novices**

Yang Liu, US Pharmacopeia, 12601 Twinbrook Pkwy, Rockville, MD 20852

Over the past 20 years, the utilization of quantitative nuclear magnetic resonance (qNMR) technology has a significant surge. For best practice, the adoption of qNMR requires the extensive expertise, which limits its widespread adoption. In recent dialogues within the qNMR community in China (qNMR-C), the potential benefits of a universally applicable qNMR platform method have been underscored, especially for qNMR novices. To establish a qNMR platform method, it requires to demonstrate the rationale of a proposed qNMR platform method. Accordingly, the present study discussed the strategic framework of this qNMR platform method through a series of trials. These trials focused on evaluating the platform method's control strategy and design space. A collaborative effort involving ten distinct laboratories was undertaken to test and affirm the qNMR platform method's applicability to various compounds and magnetic field strengths of NMR instruments, displaying its effectiveness. The overarching goal of this research streamlines the qNMR process when qNMR novices devise their own qNMR methods in the early stages of their learning to use qNMR. It also aims to bring attentions from the global qNMR community towards a collective effort to refine and endorse this qNMR platform method. By fostering global collaboration, this study seeks to thoroughly identify potential application scenarios and assess the long-term feasibility of this proposed qNMR platform method.

25 **Automatic qNMR Data Analysis Approach: Prototype of qQMSA-Based Digital Product**

Sunil Paudel, United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, MD 20852, Yang Liu, Joo-Won Nam, Ben Shapiro, Reino Laatikainen, Pekka Laatikainen

The present study introduces a novel methodology for the automated analysis of 1H quantitative nuclear magnetic resonance (1H qNMR) data using quantitative Quantum Mechanical Spectral Analysis (qQMSA). This digital approach aims to improve data analysis efficiency beyond the capabilities of traditional manual methods in NMR. The proposed digital product pipeline consists of several key steps: (i) calculating chemical information for molecules stored in a digital spectral file (dSF), (ii) developing a validated qNMR method to acquire high-resolution NMR data, (iii) creating a database of dSFs), and (iv) implementing automated NMR data analysis. This process significantly diminishes the need for manual intervention in qNMR data analysis and exhibits potential in spectral fitting by comparing the calculated spectrum derived from a dSF with experimental NMR data in a chemometric manner. The digital product's efficacy and adaptability have been evaluated through various test samples, with case studies demonstrating its effectiveness across many sectors. The results indicate that the digital product enhanced efficiency and precision in 1H qNMR data analysis. This qQMSA approach provides trustworthy qualitative and quantitative assessments in basic 1D 1H qNMR studies and paves the way for digital solutions in future compendial applications.

26 **Measuring Therapeutic Proteins (TPs) Particulates Using Submicron IR (O-PTIR), Microscopy from >100µm to <500nm**

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

Particulates are a critical concern in pharmaceutical products due to their immunogenic potential, and impact on drug safety and efficacy. Characterization of particulates, including their chemical identity, provides valuable insight to commercial scientists and regulators, and has evolved quickly from visible particulate matter into the sub-visible range. Most current analytical techniques that provide characterization in the sub-visible are focused on particle counting, sizing, and/or morphology. Whilst there is growing appreciation of the need for chemical identification, as this ultimately aids in root-cause analysis, existing techniques are limited. Raman microscopy has proven useful, though suffers greatly from autofluorescence interference, limited SNR/slow measurements, and risk of sample damage (burning). More generally, vibrational spectroscopic techniques (FTIR, FTIR-ATR, dispersive Raman) struggle to produce any useful spectra for particulates below 20 µm. Optical photothermal infrared (O-PTIR) spectroscopy is a highly sensitive vibrational technique that has proven itself in pharmaceuticals and life sciences and is an excellent fit for sub-visible particulate analysis. It features sub-micron spatial resolution, the ability to map individual particulates, vastly improved sensitivity, zero autofluorescence interference, and low sample damage thresholds. Recent advances include the integration of complementary epi-widefield fluorescence imaging for improved particulate contrast and simultaneous Raman collection for orthogonality. O-PTIR is a versatile, easy to use multimodal microscope that facilitates the rapid and thorough characterization of otherwise difficult to measure sub-visible particulates. Example results, emphasizing sub-visible particulates, will be presented to illustrate spectral and image quality possible with O-PTIR for a variety of modern drug products.

27 **Absolute Quantitation of Peptides and Proteins by Coulometric Mass Spectrometry**

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Peptide/protein quantitation using mass spectrometry (MS) is advantageous due to its high sensitivity. Traditional absolute peptide quantitation methods rely on making calibration curves using peptide standards or isotope-labelled peptide standards, which are expensive and take time to synthesize. A method which can eliminate the need for using standards would be beneficial. In this talk, I introduce the recent development of our coulometric mass spectrometry (CMS) method which can be used to quantify peptides and proteins, without using peptide standards or calibration curves. The method is based on measurement of electrochemical current from oxidation or reductions of peptides, in combination with MS measurement of the electrochemical oxidation or reduction yield. My talk focuses on improvement in instrumentation, sample preparation protocols using selective derivatization chemistry and real-world applications of CMS.

28 **A Comprehensive Analysis of Serum from Women with Breast Cancer and Age-Matched Controls to Determine Candidate Protein Biomarkers**

Logan Seymour, Clarkson University, 21 Pineview Pl., Melrose, NY 12121, Danielle Whitham, Pathea Bruno, Brian Pentecost, Costel Darie

In the U.S., breast cancer (BC) is both the most commonly diagnosed cancer and the second leading cause of cancer-related death in women. Determining a way to

screen women of any age for BC development is crucial to catching the cancer in earlier stages and hence decreasing mortality rates. A total of 96 invasive ductal carcinoma (IDC) human serum samples (48 BC and 48 controls) will be analyzed using in-solution digestion protocols followed by nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS). Preliminary data collected on human breast milk and serum using 1D and 2D-SDS-PAGE established dysregulation of proteins that may be involved with the progression and growth of breast cancer tumors. A larger cohort needed to be analyzed to make accurate assumptions on proteins that are contributing to cancer and tumor development for BC specifically. Proteins identified previously in smaller serum studies from our lab include antitrypsin family proteins, complement proteins, zinc-alpha-2-glycoprotein, inter alpha-trypsin inhibitor and transferrin. We expect to find these proteins in our larger cohort and to confirm or deny their applicability as serum biomarkers. Some of these identified proteins in serum have been found previously to be dysregulated and linked to cancer development. Further experiments will be performed using targeted quantitative proteomics using absolute quantitation (AQUA) peptides and multiple reaction monitoring (MRM). Identifying proteins dysregulated between BC vs. controls for a large cohort could aid in earlier detection and diagnosis of tumor/cancer development in women of any age.

29 Protein Biomarkers in Human Whole and Lactoferrin-Depleted Breast Milk: Mass Spectrometry-Based In-Solution Proteomics Analysis for Facilitating Early Detection and Treatment of Breast Cancer

Tochukwu (Victor) Njoku, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Lilian Corrice, Brian Pentecost, Kathleen Arcaro, Costel Darie

Breast cancer (BC) accounts for about 30% of all the cancers diagnosed in women, with 1 in 8 women in the US predicted to be diagnosed with BC through their lifetime. The high mortality rate from BC could be linked to delayed diagnosis and presentation of malignancies, making the development of early BC detection technique crucial. Currently, mammograms are the common BC screening technique, however, it is only recommended for older women and not for young women who have denser breast tissue. Monitoring several protein dysregulations between the breast milk of healthy and BC women could be a foundation for developing protein biomarker draft for BC. This study aimed at using mass spectrometry (MS)-based proteomics analysis to identify protein dysregulations in BC milk samples against their matched healthy controls. 12-human breast milk samples (6-BC and 6-healthy controls) were analyzed with nano-liquid chromatography MS/MS using NanoAcquity UPLC coupled to a QTOF-Xevo G2-XS MS after an in-solution digestion of whole and lactoferrin-depleted (using lactoferrin antibodies coupled to CNBr-sepharose resin) milk samples. This study included both "within women" (comparing left and right breast) and "across women" comparison groups. After data collection, samples were analyzed against NCBI human-database using Mascot-Distiller for protein identification and Scaffold-Proteome for quantitation. The BC samples were compared to the control samples to identify significant protein dysregulations between the groups. Preliminary data revealed protein dysregulations in the breast milk including albumin, lactotransferrin, alpha-1-antichymotrypsin, apolipoproteins, osteopontin, and xanthine dehydrogenase/oxidase that may be involved in the development and progression of BC.

30 GC/MS Approach for Analysis of Extractables and Leachables (E&L) in Complex Matrices Using Spectral Deconvolution and Retention Indices

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Modern drug delivery systems aim to protect active drug ingredients (API's) and biologics from degradation. However, these compounds interact with polymeric components and may inadvertently introduce undesirable impurities. Identifying extractables and leachables is crucial to reduce patient risk. This presentation discusses a GC/MS workflow that employs spectral deconvolution and retention index-based time filtering along with the expanded NIST23 GC/MS library to identify GC/MS amenable E&L compounds all under a unified compliance environment. Sample extracts were obtained following USP chapter <1665> protocol (pH 3 and pH 10 water; 50:50 EtOH:H2O, and 100% EtOH) from a compound of Container Closure Systems (CCS) (saline, dextrose IV bags); several medical devices (catheters) and elastomeric syringe gasket. All the extracts were analyzed using a GC/MS system with a low-bleed GC column for enhanced compound identification. All data was collected and processed within a unified compliance environment. Operating in the OpenLab Electronic Content Management (ECM) XT configuration enabled tools that help facilitate compliance with various national and EU electronic record regulations, including audit trails, and remote data storage. The solvent extracts from the medical devices and drug delivery systems confirmed the presence of phthalate plasticizers, polymerization agents, polycyclic aromatic hydrocarbons (PAHs), and several antioxidants. The impact of extraction solvent and conditions were also compared for the GCMS amenable compounds. Preliminary findings showed that a 30 m x 0.25 mm x 0.25 µm 5% phenyl column offered sufficient chromatographic resolution and low column bleed levels for identifying low-level compounds.

31 Rapid Screening of Enzymatic Reactions via Droplet-APCI-MS and FIA-MS

Bridget Murray, University of Michigan, 930 N. University Ave., Ann Arbor, MI 48109, Moritz Pott, Jules Beekwilder, Robert Kennedy

Directed evolution is a powerful methodology that allows the researcher to alter the performance of an enzyme through random mutation and subsequent selection of high activity variants. The selection process is critical but time-consuming; more rapid analytical techniques for screening would be of use in accelerating the production of new enzymes for catalyzing chemical reactions. Droplet microfluidics coupled with mass spectrometry (MS) is a technology well-suited to address this challenge. Microfluidics reduces cost and increases analysis rate relative to traditional screening techniques because of high-throughput sample handling. Mass spectrometry offers a label-free detection method that can be performed at high rates. Previous work has shown the feasibility of using droplet-ESI-MS to screen through enzymatic reactions and its agreement with LC-MS. We expand this technology to analytes that are not easily ionized with ESI by analyzing droplets with atmospheric pressure chemical ionization (APCI). We show a droplet-APCI-MS workflow that allows for encapsulation of quenched enzymatic reactions for detection of enzymatically produced terpenes. Droplets are analyzed at up to 30 s/sample. We also show a flow injection analysis technique that provides rapid (40 s/sample) analysis of 1 µL terpene samples; an internal standard is used to improve quantification. Agreement of these methods with GC-MS across 99 enzyme reactions is shown. These workflows reduce screening time compared to traditional techniques.

32 Strategic Analytical Method Development for Trace Levels of EDC and EDU in API Material by LC-MS

Sondrica Goines, Bristol Myers Squibb, 556 Morris Ave., Summit, NJ 07901, Cong Bi, Jonathan Shackman, Robert Menger

Development of active pharmaceutical ingredients (APIs) is often dictated by C-C and C-N bonds, making amidation reactions prevalent in process development. While the use of a single reagent to facilitate amidation reactions may seem advantageous, production limitations may drive process chemistry to seek alternative reagents. Historically, 1-hydroxybenzotriazole paired with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC-HCl) has provided high purity, high yield results. Amidation within the API step of a BMS compound was updated to employ these reagents due to limited production of pentafluorophenyl diphenylphosphinate. Since EDC was previously identified as a potentially mutagenic impurity during *in vitro* studies, BMS has developed sensitive and specific analytical methods to assess EDC levels in alignment with the ICH M7 (R5) guideline. Here, we leveraged historical in-house methods to assess the conversion of EDC to EDU in-process and demonstrate that both analytes are sufficiently purged in the resulting isolated material. This method provides adequate empirical results to support control strategy options 3 and 4 in the ICH guideline, which heavily rely on fate and purge process knowledge to ensure patient safety. The method uses liquid chromatography-mass spectrometry (LC-MS) to determine levels of EDC and EDU within 4 minutes of the separation with a quantitation limit of 4.5 ppm within a sample matrix of 25 mg/mL of isolated API material. Moreover, this method simplifies the amount of analytical testing needed to support purgeability claims. In addition, we demonstrate method performance on two MS instruments commonly used in manufacturing: a Waters Acquity QDa and a SQD2.

33 New Insights into and Applications of Tandem-Column Liquid Chromatography

Joe Foley, Drexel University, Department of Chemistry 305 Disque Hall, Philadelphia, PA 19104, Megan Marrazzo, Zhiyang Liu

In conventional one-dimensional HPLC, a single column is utilized in most instances. However, two columns have occasionally been utilized in series over the years in liquid chromatography to improve resolution by increasing the plate number/peak capacity and/or by increasing the selectivity. To achieve the latter two or more columns are employed with different stationary phases in series and this is termed tandem-column liquid chromatography (TC-LC). Following a brief introduction to TC-LC, a summary of its inherent advantages [1], and some recommendations for choosing columns, we describe the separation of two classes of pharmaceutical compounds, NSAIDs and beta-blockers, that benefitted from the TC-LC approach. Finally, we report recent research conducted to answer the following question: Is there a preferred order for the tandem columns, which may differ in their overall retentivity, pore size, particle size, and/or whether they are packed with totally or superficially porous particles? Answers will be provided from the perspective of both isocratic and gradient elution.

[1] Liu and Foley, J. Chromatogr. A, 2022, DOI: 10.1016/j.chroma.2022.462890

34 Development and Application of a Selectively Tunable, Universal 1H and ^{19}F Quantitative NMR Standard

Jared Wood, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Xiao Wang, Thomas Williamson, Mikhail Reibarkh, Ryan Cohen

Quantitative NMR (qNMR) is a robust technique used in a variety of chemical industries due to its numerous analytical advantages. It exhibits a universal response, a broad linear range, diverse compound applicability, and allows for facile method development. The most accurate qNMR results are obtained with an internal standard, but no truly universal standard currently is known that is stable, non-toxic, commercially available, exhibits a unique chemical shift, is highly soluble in both aqueous and non-polar solvents, and exists as a non-hygroscopic solid for accurate weighing. In this study, we investigated several potential qNMR standards and identified 2,2-difluoroacetamide (DFA), which meets all the aforementioned criteria. This standard is soluble in a wide polarity range of both protic and aprotic solvents from D_2O to CD_2Cl_2, and it exhibits selectively tunable ^{1}H and ^{19}F chemical shifts via decoupling that resonate in sparse regions of the typical NMR spectrum. We evaluated the broad utility of this standard by assaying 13 active pharmaceutical ingredients in 12 different deuterated solvents. Our results demonstrate excellent measured accuracies and precisions of 100 ± 1% and % RSD (n=3) less than 1%, respectively. We then showcased the utility of DFA for several challenging pharmaceutical problems, such as assaying cyclic peptides and biocatalytic samples.

35 Fast and Simple Quantitation of GC-Unfriendly Impurities Using Headspace-MRR

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Faster and simpler analytical methods for resolving and quantifying impurities in complex mixtures are needed in a wide range of industries including pharmaceutical, polymer, chemical, agricultural, natural products and others. We describe the use of molecular rotational resonance (MRR) spectroscopy with automated headspace sampling that allows for fast and unambiguous detection and quantitation of volatile compounds in complex samples, while preventing nonvolatile or particulate matter from contaminating the instrument. Because of the extremely high resolution and chemical selectivity of MRR, the measurements are spectral overlap free, matrix-independent and do not require chemical separation or derivatization. Quantitation is easy and straightforward, as MRR signals of target analytes are directly proportional to their corresponding partial vapor pressures in the sample. As such, MRR can analyze compounds that are challenging to resolve or detect using conventional methods such as gas chromatography. The provided examples include but are not limited to direct quantitative analysis of fast-eluting, highly-polar, extra-low-volatile and other difficult-to-separate or difficult-to-analyze compounds such as formaldehyde, ethylene oxide, low-volatile residual solvents and complex mixtures of isomers. The method is applicable to a wide range of sample and analyte types, and can measure either single or multiple analytes in a matter of minutes.

36 Pharmaceutical Formulation Stability Studies with BeScan

Beverly Barnum, Bettersize, Inc., 3188 Airway Ave Ste K2, Costa Mesa, CA 92626, Annika Jurgilewicz

Assessment of physical stability is an essential metric for pharmaceutical drug products and cosmetic formulations. Yet despite its importance, there remain challenges in quantitative robust methods to determine shelf life. Historical assessments of important parameters such as particle size, distribution, and surface charge are typically constrained to very dilute measurement conditions, while real world formulations are typically outside the range of measurement. Formulation dilution to enable conventional measurements to alter the critical stabilizing balance among essential components such as particulates, surfactants and polymers. BeScan is a new device that quantifies the variation in the intensity of light transmitted and backscattered in a concentrated particulate formulation as a function of height. Measurements may be performed as a function of time and or temperature. Sedimentation, coalescence, flocculation, and creaming are all formulation stability phenomena that affect physical stability and can be studied with BeScan. In particular, measurements as a function of temperature are useful to assess the formulation robustness. ACH stability protocols typically require 6 months at 40 degrees centigrade. BeScan can regulate temperature up to 80 degrees and accommodate a wide variety of temperature profiles. For example, PNIPam (poly(N-isopropylacrylamide)), undergoes a structural transformation with increasing temperature, transitioning from an expanded conformation at low temperatures to a collapsed conformation at high temperatures. This structural change results in a noticeable increase in turbidity due to alterations in the material's optical properties. To illustrate the use of BeScan in formulation development, this and other formulation stability examples will be presented.

37 What Is a Scientifically Sound Method? Examples of Form FDA 483s and Warning Letters under CFR211.160(b)

Xiaohui (Sherry) Shen, United States Food and Drug Administration, Division of Compliance 10903 New Hampshire Ave., Silver Spring, MD 20993

This oral presentation provides examples of Form FDA 483 observations and Warning Letter citations under CFR 211.160(b) that have been issued over recent years. It covers violations for lack of the establishment of scientifically sound and appropriate specifications, standards, sampling plans, and test procedures including calibration of instruments designed to assure that components and drug products conform to appropriate standards of identity, strength, quality and purity. The example violations were selected from a variety of Food & Drug Administration regulated establishments, such as drug manufacturer including sterile drug manufacturer, over-the-counter drug manufacturer, homeopathic product manufacturer, biological drug and HCT/P (human cells, tissues, and cellular and tissue-based products) manufacturer, contract drug manufacturer, 503B outsourcing facility, and control testing laboratory, etc. This presentation discusses the importance of compliance with CFR211.160(b) and the adequacy of responses from the firms in selected case studies.

38 Deducing Melanin Biosynthesis and Supramolecular Organization

Dhairavi Shah, Kean University, Department of Chemistry & Physics, 1000 Morris Ave., Union, NJ 07083, Dhaara Shah, Subhasish Chatterjee

Melanin is a class of natural pigments responsible for structural coloration in animals, plants, and microorganisms, and the pigments are generated through the enzyme-catalyzed oxidation of catecholamines and amino acid-based precursors. Three forms of melanin are found in the human body: eumelanin, pheomelanin, and neuromelanin. Notably, different precursors produce different kinds of polymeric melanins. For example, the most common precursor, tyrosine, produces eumelanin, which gives a brown-to-black color pigment. On the other hand, pheomelanin is derived from a combination of tyrosine and cysteine and gives a yellow-to-red color pigment. Furthermore, biosynthetic melanins exhibit biomedical applications such as serving as a drug-delivery system, antioxidants in the skin, and cancer therapeutics. Biosynthetic melanin is also valuable in the industry, where it can be used as optically active 2D hybrid materials for biosensing, reinforcers for adhesive hydrogel materials, and as free radical scavengers for soil remediation. The exact polymeric structure of melanin has yet to be discovered because its chemically heterogeneous assembly is disordered and elusive. Our experimental project aims to study enzyme-catalyzed melanin synthesis under cell-free conditions and determine its supramolecular organization using high-resolution spectroscopic and computational methods. The current results illustrate the structural heterogeneity of pheomelanin-like polymeric materials, paving the way for comprehensive structural characterization to shed light on the macromolecular architecture of hybrid pigments.

39 Determination of Glucose in Blood by Applying Imbedded Enzyme Technology

Ben Sutter, Xylem, 1725 Brannum Ln., Yellow Springs, OH 45387, Kevin Schlueter

Glucose in human blood is a piece of data of paramount importance. Beyond the daily checks required, blood glucose is also helpful for bioprocessing and clinical trails. One way to selectively measure glucose is to utilize an enzyme that selectively lyses only glucose (Dextrose) molecules. This enzyme is embedded into a porous membrane, which allows solution to come into contact with the enzyme. When the glucose is lysed by the embedded enzyme, it cleaves the sugar while releasing a single hydronium OH- ion. This hydronium ion is measured by a Clark Electrode, and the resulting change in amperage is proportional to the amount of glucose in solution. Using this Imbedded Enzyme Technology (IET), the glucose concentration can be determined in various forms of blood, including whole blood, plasma, serum, and finger-stick capillary drawn whole blood. The enzymatic action of the membranes is optimized to minimize matrix effects and outside sources of contamination. This study demonstrates the ability of the Imbedded Enzyme Technology in the determination of glucose in blood. This information is valuable for those working to verify medical devices, for clinical testing, or for certain bioprocessing applications.

40 Probing Binding Affinities, RNA Compaction, and Complex Stoichiometry for SARS CoV-2 Nucleocapsid Protein and RNA Using Single-Molecule FRET

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The SARS-CoV-2 Nucleocapsid (N) protein is an RNA binding protein responsible for the genome packaging of the virus. However, despite its key role in the replication of the SARS-CoV-2 virus, we lack critical understanding of the mechanisms involved in the functions of this protein. Why is more than 60% of the protein disordered, and what role does the disorder play in the RNA packaging? In this project, we aim to investigate the interplay between binding and compaction of RNA induced by the N protein. Single-molecule FRET microscopy enables observing of binding affinities,

stoichiometry, and conformational changes of the RNA upon binding to the protein. By increasing the length of the RNA, we aim to understand binding site size and corresponding degree of compactions. Comparing different variants, we will identify which regions of the protein contribute to compaction. With this information we can better model the packaging of the SARS-CoV-2 virus, which may be useful in the creation of drugs to impede the genome packaging.

41 **Automation of a Capsid ELISA Method for Support of Manufacturing Process Development of AAV Therapeutics**

Monica Bond, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901, Jeanna Hill, Daniel Konovalov, Zhichao Fang, Thomas Slaney, Gloria Li, Anthony Leone

Adeno-associated viruses (AAVs) are an attractive gene therapy vehicle due to their relatively simple manufacturing and minimal cellular immune responses. Enzyme-linked immunosorbent assays (ELISAs) can selectively and precisely measure the titer of an AAV sample making them powerful analytical tools for supporting manufacturing quality control. A commercially available AAV capsid ELISA kit (Progen) was adapted to an automated liquid handler (Tecan Evo) to support high throughput testing. To validate this approach, both the manual and automated assay were used to test AAV samples. The assay was demonstrated to have sufficient sensitivity to achieve a lower limit of quantitation of 1.875E+7 gc/mL. We also verified that there was no incubator edge effect, as well as sufficient reagent stability at room temperature. This automated method can support 48 samples in 6 hours, saving 4 hours of analyst time per experiment. This advancement represents a significant step forward in the efficient and accurate analysis of AAV samples, facilitating future medical breakthroughs.

42 Withdrawn by the author.

43 **Interaction Between Fluorescent Gold Nanoclusters with Proteins and Cells**

Maima Bogar, West Chester University, West Chester, 3 Berbro Ave., Spring Lane, PA 19128, Jah'dir Cartegena-Rivers, Jingqiu Hu

Nanomaterials are increasingly used in cosmetics and personal care products due to their unique properties. However, there has been limited research on the potential health risks associated with nanomaterials. This project seeks to address this gap by investigating the interactions between fluorescent nanoclusters and proteins or cells to assess their biotoxicity. We have successfully synthesized fluorescent gold nanoclusters (AuNCs) capped with various ligands, including various amino acids, proteins, and polymers. These AuNCs emit blue, green, yellow, orange, or red light under UV irradiation, depending on their surface functional groups. The photophysical properties of AuNCs were investigated to explore their potential as fluorescent sensors for copper (II) and lead (II) ions. To evaluate their toxicity, we analyzed the interactions of AuNCs with proteins and cells using circular dichroism (CD) and UV-vis absorption spectroscopy. The introduction of histidine, proline, or tryptophan-capped AuNCs to a solution containing Bovine Serum Albumin (BSA) or lysozyme significantly altered the protein's CD signal between 210 nm and 220 nm, indicating a disturbance in the protein's α-helical structure. Additionally, several green-emitting nanoclusters demonstrated potential applications in bioimaging; for example, tryptophan-capped gold nanoclusters self-assembled onto yeast cells, causing them to fluoresce under a microscope.

44 **Ion Chromatography-Based Quantification of Tris(2-Carboxyethyl) phosphine in Antibody-Drug Conjugates**

Suji Lee, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Michael Zompa, Frank Bernardoni, Tao Liang, David Schenk, Casey Dougherty-Gunsch, Teng Peng, Neil Williams, Xinxin Han, Mirlinda Biba, Patrick Fier, Paul Bulger

Tris(2-carboxyethyl)phosphine (TCEP) is a commonly used reducing agent in both the pharmaceutical industry and cutting-edge research fields. One of the most widely-used applications of TCEP is in the antibody-drug conjugate (ADC) process, during which it reduces disulfide bonds of proteins, thereby creating sites for conjugation to drugs or to other molecules. Quantitation of TCEP is required to confirm its active level during the conjugation reaction, and also to ensure its purge from the final ADC as a measure of product quality. Herein, we introduce an ion chromatography (IC)-based method for the quantitation of TCEP based on the conversion of TCEP to its oxidized form, tris(2-carboxyethyl)phosphine oxide (TCEPO) in solution, and quantitating TCEPO as a proxy for TCEP. This IC method demonstrated high sensitivity for TCEP, and was linear in the range of 0.0001-0.01 mg/mL (0.1-10 ppm). The method was applied to analyze in-house produced ADC samples to evaluate its capability of quantitating TCEP in complex matrices, such as biological materials. Various elements of analytical method validation, including accuracy, precision, specificity, and repeatability, were carried out to demonstrate that the method is suitable for its intended purpose. An additional advantage of using this method in ADC analysis is its capability to not only detect and quantitate TCEP, but can be used to quantitate other ions as well (e.g., chloride, nitrate, phosphate), which are commonly present in buffer systems used during the conjugation process.

45 **Suppress or Not to Suppress?!... – CRAFT it! Extracting Essential Biomarker Signals Directly from the Full 1H NMR Spectrum of Serum Samples**

James Chen, Princeton University, Department of Chemistry, Princeton, NJ 08544, Ayelet Yablon, Christina Metaxas, Mateus Guedin, Joseph Hu, Kenith Conover, Krish Krishnamurthy, István Pelczer

There are sophisticated methods introduced recently (DIRE [1] and JEDI [1]) to suppress practically all the unwanted signals from the complicated 1H NMR spectra of blood serum or plasma samples retaining the signals of essential inflammatory biomarkers of the lipoproteins GlycA and GlycB [3] and a complex signal of phospho-glycoproteins (SPC) [1]. However, such experiments are technically demanding, leading to the overall decrease of sensitivity, and the suppression process may have uncontrolled influence of the signals of interest, too. As a simple alternative, we rather turned to the powerful software tool, CRAFT [4], which directly analyzes the time domain NMR data and presents the content in a spreadsheet format, including the amplitude for quantitation. Partial overlap with other signals or broad background resonances can be efficiently handled. We applied the CRAFT protocol to 20+20 horse serum samples of equal number of healthy and diseased subjects as an example. The integral ratios of the Gly and SPC signals clearly identify the diseased cohort with good validation in O-PLS-DA.

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46 **Optimizing Oligonucleotide Extraction by SPE**

Geoff Faden, MAC-MOD Analytical, 103 Commons Court, Chadds Ford, PA 19317, Colin Pipe, David Dunthorne, Tony Edge, Matt James

Therapeutic oligonucleotides and nucleic acids are becoming more widespread, due to advantages these new modalities can have over small molecule drugs. However, analysts are presented with significant challenges, as oligonucleotides are very complex. The chromatographic and extraction protocols used must therefore be optimized to ensure this level of complexity is addressed. Understanding the extraction process is critical, as is using the correct type of media. This poster examines each stage of extraction and demonstrates the use of a novel composited silica based SPE media to improve assay robustness. An LC-MS method was developed and was used to analyse a series of oligonucleotide samples. Each stage of an anion exchange SPE protocol was investigated to assess the impact on assay recovery. In particular, the use of ion pairing and organic solvents was investigated, as well as the use of multiple elutions to optimize extraction. A simple molecular modelling package was used to support experimental findings and help develop a more generic approach to method development. The use of organic solvents was shown to greatly reduced the effectiveness of extraction. Oligonucleotides were not so soluble in methanol, which led to an interesting observation that the optimal elution conditions were water, modified by an ion pairing reagent. Experimentally, the use of multiple elutions and the total elution volume were found to be important experimental parameters to optimize. Finally, a novel composite SPE media is also discussed and shown to have much better recoveries (>96%) compared loose packed media (80-95%).

47 **A Proteomics Analysis of Serum from African American Donors with Invasive Ductal Carcinoma Breast Cancer Compared to Matched Controls**

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One of the top five causes of death in the United States is cancer, with breast cancer (BC) the second most common malignancy. Of all the ethnic groups, African Americans have the greatest mortality and lowest survival rates. They have a 34% lifetime risk of getting cancer and a 41% chance of dying from breast cancer. Invasive Lobular Carcinoma (ILC) and Invasive Ductal Carcinoma (IDC) are the two primary forms of invasive BCs. The cancer cells in IDC start in the ducts, spread to various areas of the breast tissue, and have the potential to move to the axillary lymph nodes and other locations. By correlating IDC serum samples with matched controls, dysregulated proteins that may be employed as protein biomarkers may be found. In this study, sera from three African American donors with IDC and three matched healthy controls were compared using a proteomics technique. Since the IDC samples tested positive for the estrogen and progesterone receptors, the tumors were categorized as either having lymph node involvement (pN1) or not (pNX). Using a NanoAcquity UPLC paired with a QTOF Xevo G2 XS MS, the samples were processed in accordance with in-gel and in-solution digestion protocols. Nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS) was used to analyze the samples. Software such as ProteinLynx Global Server (v2.4), Mascot Daemon server (v2.5), and Scaffold 4.3 were used to examine the generated raw data. Cur-

rently, the dysregulated proteins that were found are being investigated as possible indicators of breast cancer.

48 Proteomic Study and Comparison of Sera from Controls and Stage IIA T1N1 ER/PR Cases for the Discovery of Possible Breast Cancer Biomarkers

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Breast cancer (BC) is one of the top causes of death for women. The presence ofreceptors to estrogen receptors (ER), progesterone receptors (PR), and Human Epidermal Growth factor 2 (HER2), as well as anatomic parameters (size and involvement of axillary lymph nodes), clinically characterize BC carcinomas. Protein dysregulations, which can be identified as biomarkers for early cancer identification, especially in younger women. Serum analysis may aid in the detection and study of tumor-secreted proteins, as well as whole-body reactions to the disease. Mass spectrometry is significant in proteomics research because of its great sensitivity and capacity to detect low-abundant proteins. Serum samples from five women with HR-negative breast cancer were compared to five age-matched control groups. The 1N1 tumors were less than 20 mm in size and contained tumor cells in up to three axillary lymph nodes. In-solution proteomic techniques were used to produce samples, which were then analyzed using nanoLC-MS/MS to discover proteins that are dysregulated between matched pairs. This was done in biological replicates, with a total of 15 cancer and 15 control samples digested and examined. ProteinLynx Global Server (v 2.4), Mascot Daemon server (v 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software were used to analyze the raw data. The data from this analysis will be supplemented using in-gel proteomics techniques.

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

Taniya Jayaweera, Clarkson University, Department of Chemistry & Biochemistry, 8 Clarkson Ave., Potsdam, NY 13699, Krishan Weraduwage, Bernard Crimmins, Sujan Fernando, Thomas Holsen, Costel Darie

A major goal of the current project is to monitor expression of select liver proteins in lake trout (Salvelinus namaycush) collected from the Great Lakes due to their exposure to environmental contaminants. Some of these contaminants, like polychlorinated biphenyls (PCBs), have estrogenic activity (endocrine disrupting compounds or EDCs). Two important fish proteins whose levels change upon exposure to EDCs are vitellogenin and zona radiata proteins. These are prime candidates for our study in lake trout for several reasons: a) the levels of these proteins are increased in lake trout relatives such as Atlantic salmon (Salmo salar) and rainbow trout (Oncorhyncus mykiss) upon exposure to EDCs, b) both proteins are produced in male fish only upon exposure to EDCs, c) both proteins are produced only in the liver (and secreted in the blood). In the current study we have used proteomics to analyze fish homogenates from three Great Lakes (Erie, Huron and Michigan). We have successfully identified vitellogenin in all three samples, but have not yet identified the zona radiate proteins. Moving forward, the current study will monitor vitellogenin in a larger number of fish to identify the lake trout protein expression due of the exposure to EDCs. This, in turn will allow us to monitor the influence of EDC contaminants in the lake trout from Great Lakes.

50 Rapid Machine Learning Identification of Rhodamine-B in Ballpoint Pen Ink

Alisha Khodabocus, College of William & Mary, 540 Landrum Dr., Williamsburg, VA 23185, Eden Fitsum, Walker Knapp, Sinead McWeeney, Benjamin Steinman, Kristin Wustholz

Fluorescence imaging at the single-molecule level provides an exceptional level of detail in visualizing biological systems. However, techniques rely on spectrally distinct probes, limiting accessibility due to a lack of commercially available probes and the length of experimental procedures. To address this, our lab developed blink-based multiplexing (BBM), which harnesses a rapid machine learning model based on logistic regression (LR) to classify spectrally overlapped dyes using their characteristic blinking behavior. Blinking refers to the fluctuations of a single dye molecule between emissive and non-emissive states under laser excitation. This study expands the applications of BBM for the identification of rhodamine-B (RB) dye in pen ink for cultural heritage research. A widefield fluorescence microscope with an electron-multiplying charge coupled device (EMCCD) is used to measure the blinking dynamics of rhodamine dyes and ink samples. By analyzing the blinking dynamics of RB and four other rhodamines using change-point detection and LR, we achieve minimum classification accuracies of >70% between rhodamines. LR analysis shows the blinking dynamics of single molecules from a purified BIC Cristal Pink ink sample are indistinguishable from those of RB, consistent with positive identification. We also demonstrate that RB and the purified pen ink exhibit comparable classification against the four other rhodamines, supporting the BBM's detection of RB in the ink. This study establishes BBM's efficacy for the identification of dyes at the single-molecule level, and it motivates further exploration of its use in analyzing other cultural heritage materials

51 Japanese Woodblock Prints Analysis, An Art History and Chemistry Collaboration

Kayla Geulen, Rutgers University, 307 Malaga Park Dr., Malaga, NJ 08328, Kennedy Short

At the Stedman Gallery of Rutgers University Camden Center for the Arts (RCCA), there are eighteen Ukiyo-e Japanese woodblock prints from Edo Japan. This project is a collaboration between the Chemistry and Visual, Media and Performing Arts departments, working on both art historical and chemical analysis of the Japanese woodblock prints. The chemical analysis to date has focused on two prints by Kunisada, who is also known as Toyokuni the Third. The pigments were analyzed using ALPHA Bruker FTIR attenuated total reflectance (ATR), external reflectance (ER), LUMOS FTIR microscope, and Raman microscopy. The art historical analysis clarified the artists and their lineages, identifying the content of the prints, and researching the history of the common pigments. The chemical analysis aimed to compare and identify the pigments found in the prints, in order to confirm the art history student's findings that the artists had access to the identified pigments, and to date the prints in relation to the access to specific pigments. Vibrational spectroscopy (IR and Raman) confirmed the presence of Prussian blue in both prints. Current findings match the historical records which show the accessibility and popularity of Prussian blue in the early 1800s of Japan through a small Dutch trading route. On-going analysis will focus on red pigments which could be inorganic (iron oxides or Vermillion) or organic (madder or safflower).

52 Detecting the Presence of PFAS "Forever Chemicals" in Commonly Used Infant Care Products

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PFAS, or per and poly-fluoroalkyl substances, are a group of man-made chemicals composed of fluorine atoms attached to a carbon chain that are extremely persistent in the environment and human body, earning them the nickname "forever chemicals." One area of particular concern is the exposure of PFAS to infants during critical developmental stages. Published studies have shown detectable levels of fluorine in selected products. This poster presents the results of the analysis of 28 PFAS in baby textiles, plastic containers, and oral products.

53 Testing and Validation of Elemental Impurities in Pharmaceutical Products According to ICH Q3D and USP <232>/<233> Using ICP-MS

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In 2018, the United States Pharmacopeia (USP) in General Chapters <232> and <233> updated the list of elements and maximum permissible daily exposure (PDE) values of elemental impurities in drugs, pharmaceutical substances, and raw materials. The USP is now aligned with the International Council for Harmonization (ICH) Q3D (R2) Step 5, the latest version of which was adopted in 2022. Chapter <232> specifies the list of elements and their maximum permissible daily exposure (PDE) values according to the modes of administration: oral, parenteral (intravenous injection) and inhalation drugs. Chapter <233> details the sample preparation, analytical procedures, and quality control validation protocols for the analysis of elements specified in Chapter <232>. The (ICH) Q3D (R2) Step 5 guidelines apply to the same group of pharma products and drug substances with the addition of limits for elemental impurities delivered via the cutaneous and transcutaneous route. USP <233> suggests adaptability of both ICP-OES and ICP-MS technologies for impurities analysis. However, the choice of technology depends on the permitted daily exposure (PDE), dosage forms and amount of daily dosage. While ICP-OES has the sensitivity to handle some oral drug products, ICP-MS is the ideal technique for the lowest limits of detection. This poster demonstrates the benefits of utilizing the NexION 1100 ICP-MS for the determination of a group of toxicologically relevant elements in a large volume of parenteral pharmaceutical products.

54 Navigating Challenges in Extractables & Leachables Characterization of Polyethylene Glycol-Based Medical Devices

Yunyun Yuan, Johnson & Johnson MedTech, 1000 US-202, Raritan, NJ 08869, Ying Jiang, Yijun Lu

Polyethylene glycol (PEG) is widely used in a variety of medical devices and implants because of its biocompatibility, hydrophilicity, and ability to form hydrogels. PEG-based sealants and adhesives are particularly valuable in minimally invasive surgeries and surgical procedures where traditional sutures are not ideal. Extractables and leachables (E&L) characterization has evolved to play a crucial role in ensuring product quality and safety across industries such as medical devices, pharmaceuticals, and food. However, the hydrophilicity nature of PEGs and their high or good solubility in extraction solvents such as water and alcohols present significant

challenges for E&L characterization of PEG-based medical devices. Key analytical challenges and strategies to address them using a representative medical device are discussed and reported. Most of these challenges were mitigated through orthogonal techniques, alternative approaches, and enhanced analytical procedures.

55 Novel Optimal Tryptic Digestion Methods Using Bovine Serum Albumin in-gel Sample Preparation for Mass Spectrometry-Based Proteomics

Pathea Bruno, Clarkson University, 8 Clarkson Ave., Box 5810, Clarkson, NY 13699, Niyogushima Nuru, Danielle Whitham, Norman Haaker, Hailey Morrissiey, Costel C. Darie, Brindusa Alina Petre

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

56 Proteomic Analysis of Varying Protein Contents in Different Plant Milk Types Using Gel-Based Mass Spectrometry Analysis

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

Milk is considered a healthy complete diet since it contains a balanced number of macronutrients like lipids, proteins, and carbohydrates, as well as micronutrients like calcium, selenium, riboflavin, and vitamins. Plant-based nutritional or blended milk substitutes are increasingly being researched as alternatives to normal milk for consumers seeking healthier options for a variety of reasons. The adoption of plant-based milk products is growing, whether due to dietary restrictions or personal health concerns. Therefore, we chose to investigate the varying levels of proteins of differing plant-based milk products. These milk alternatives are regarded for their functionally active components, which have been related to improved health and illness prevention. The nutrition label alone is insufficient to determine the protein content of these various goods. As a result, we conducted proteomics-based analyses on four types of plant milk: almond milk, oat milk, coconut milk, and soy milk, to determine the presence or absence of specific proteins in each sample at various dilutions. The first step was to do a 1D-SDS-PAGE with each type of milk at varied concentrations. Each band was then digested with trypsin, the sample was cleaned, and mass spectrometry was performed. The samples were evaluated using an XEVO G2 QTOF, with data processing and protein identification handled by our Mascot Daemon Server. Protein identification scores were compared to determine the protein levels in each milk sample.

57 Utilizing Monodisperse Fully Porous Particles (MFPP) for improvements in LC-MS based Metabolomics for Disease Detection

Edward Faden, MAC-MOD Analytical, Chadds Ford, PA 19317, Tim Garrett, Mark Woodruff

Metabolomics refers to the comprehensive measurement of small molecules in biofluids by either mass spectrometry (MS) or nuclear magnetic resonance (NMR) with the aim of covering multiple KEGG pathways, exposome products, and chemical reactions to provide new insights into disease etiologies. This poster shows how utilizing monodisperse fully porous particle technology (MFPP) generates significantly higher resolution as compared to polydisperse particle technology, due to improved regioisomer separation capacity of a novel bonded phase chemistry, as well as improved efficiency of the LC column due to the monodisperse particle design. The methodology was to design a very simple mobile phase that could scout a method via an untargeted approach so as to identify key biomarkers in the patient sample. Implementing this column into the workflow drastically improved resolution and gave significant advantages via the LC separation which will be elaborated on in the poster and is key to metabolomic workflows that cannot rely solely on the MS spectra to identify peaks.

58 Removal and Recovery of Lead and Cadmium Ions with Biowaste Adsorbent from Aqueous Solutions

Liang Feng, St. John's University, 8000 Utopia Pkwy., Jamaica, NY 11439, Enju Wang

The widespread presence of contaminants in the environment, especially those with toxic and hazardous properties, necessitates remediation of the environment. The challenge currently is to find the most eco-friendly path that leads to the decontamination of the environment while minimizing the financial burden. This study demonstrates the use of environmentally friendly, low-cost waste adsorbents, such as luffa peels and chamomile tea residues in isolating lead and cadmium ions from an aqueous solution. The biowaste sorbents were treated with acid (HCl) or base (NaOH). Acetic acid buffer (pH 5.5) solution of known lead or cadmium concentration was introduced into the biowaste sorbent, equilibrium isotherm and kinetics were investigated. The known concentration mixture of cadmium and lead solution was also introduced to the biowaste sorbents to obtain the selectivity of metal ions. The biowaste sorbents were packed into columns to monitor the removing and recovering abilities of both biowaste sorbents for cadmium or lead ions. The final concentration after adsorption for the metal ions was measured with inductively coupled plasma-optical emission spectroscopy (ICP-OES). The adsorption characteristics of the biowaste were also evaluated with infrared and Raman spectroscopy as well as differential scanning colorimetry. Preliminary results of this research show promising outcomes using NaOH base treated biosorbents for the removal and recovery of lead and cadmium from an aqueous solution within five minutes of contact.

59 Detection of "Forever Chemicals" per and poly-fluoroalkyl Substances (PFAS) in Water Using Surface Enhanced Raman Spectroscopy (SERS)

Syed Islam, Eastern Connecticut State University, 83 Windham St., Willimantic, CT 06226

Recently, the Environmental Protection Agency (EPA) warned the public about the presence of a class of harmful man-made chemicals called—per- and poly-fluoroalkyl substances (PFAS) which have been found in public drinking water systems across the U.S. These PFAS are also known as "forever chemicals" because they do not break down in the environment over time and have been linked to health conditions and diseases such as cancer, weakened immunity, diabetes, high cholesterol, fertility problems, and decreased birth weight. The EPA has so far learned that these PFAS are found in many different consumer, commercial, and industrial products including eggs, meat, milk, nonstick cookware, waterproof clothing, food packaging, and firefighting foams. This study reports the development of a sensitive and easy to use technique known as surface enhanced Raman spectroscopy (SERS) to detect the presence of these PFAS in water. Our preliminary investigation successfully identified ten different toxic PFAS found in the drinking water system using SERS. This study further shows that the PFAS primarily use the carboxylic and the sulfonic groups to attach to silver nanoparticles contributing to SERS enhancements. Our study detected the presence of these PFAS in water as low as femtomolar concentrations using SERS. These low concentrations are comparable to those of the concentrations of these chemicals found in drinking water. The results obtained in this study show that the analytical technique (SERS) developed using this project can be applied in real-life detection of toxic PFAS chemicals in public drinking water systems.

60 Liquid Chromatography-Tandem Mass Spectrometry for Determination of 40 PFAS Compounds

Dongmei Alvi, Occoquan Lab, 9408 Prince William St., Manassas, VA 20110, Elizabeth Smiley, Joan Wirt

Mass spectrometry (MS) is a powerful analytical tool used to analyze a wide range of substances and matrices. Since the National Primary Drinking Water Regulation (NPDWR) established the maximum contaminant levels (MCLs) for six per- and polyfluoroalkyl substances (PFAS), liquid chromatography-tandem MS (LC-MS/MS) is increasingly being utilized in environmental samples to meet these regulations. This study provides a method accreditation roadmap based on EPA draft 1633 for assessing wastewater matrices. Aqueous samples underwent spiking with isotopically labeled standards, followed by extraction via solid phase extraction (SPE) stacked cartridges with polymeric weak anion exchange (WAX), and graphitized carbon black (GCB) sorbents for sample cleansing. Initial instrument calibration involved extracted internal standard (EIS), non-extracted internal standard (NIS), native standards solution (NSS), calibration standard solutions (CSS), instrument sensitivity check (ISC), mass calibration, and mass calibration verification, bile salt interference check. To comply with accreditation requirements, further quality controls were conducted, such as initial demonstration of capability, continuing calibration verification, ongoing precision and recovery (OPR) spiked at low (LLOPR) and mid-level, and analysis of blanks (field, laboratory, and method blank). A comprehensive validation process included the assessment of the method detection limit (MDL), limit of quantitation (LOQ), mass calibration, retention time calibration, instrument linearity, response ratios (RR), and response factors (RF). To be accredited, the laboratory must pass two rounds of proficiency testing (PT) samples from PT providers and

submitted to the accreditation body (AB). The AB reviews the laboratory's quality manual and standard operating procedures before granting accreditation.

61 **New Capillary C18, Carbon, and HILIC HPLC Columns for Bio-Analysis**

Breanne Smith, MilliporeSigma, 595 North Harrison Rd., Bellefonte, PA 16823, William Maule, Michael Ye, Olga Shimelis, Cory Muraco

Capillary HPLC has drawn great attention recently due to its high sensitivity and low sample and solvent consumption. These columns employ internal diameters of 0.1, 0.3, 0.5 and 1.0 mm. The flow rates used for these columns are typically from few to tens of microliters, comparing to several hundred microliters to milliliters in conventional HPLC. The reduced column diameter results in narrower peaks, leading to better resolution and enhanced sensitivity. The lower sample consumption makes it suitable for analyzing limited or precious samples, including those in proteomics, glycomics and other bio-analysis areas. The lower solvent consumption will result in cost savings for the users and make methods more friendly for the environment. However, this miniaturization from conventional to capillary HPLC columns does come with challenges. In this poster, we present new capillary C18, Carbon, and HILIC HPLC columns in 0.3 and 1.0 mm I.D. format. The columns are proven to be reproducible from column to column and lot to lot. They will also be able to hold for high back pressure to meet today's UHPLC requirements. We will present the scalability of methods with these columns as well as the kinetic performance of the columns. While the capillary C18 column would be suitable for any convention applications, it is especially useful for bio-analysis, such as peptide analysis and peptide mapping. The capillary carbon HPLC column has been used for glycan analysis, and the results are discussed.

62 **Method Development Made Easy for GC×GC Users**

Kira Fisher, William & Mary, 524 Fallen Leaf Lane, Chesapeake, VA 23320, Katelynn Perrault Uptmor

Comprehensive two-dimensional gas chromatography (GC×GC) is a separation technique that utilizes two distinct columns with different retention properties. This allows for greater chromatographic resolution than traditional one-dimensional gas chromatography (GC). The goal of this project was to first develop a GC×GC method on a sample of interest using helium carrier gas and then translate the developed method to use hydrogen carrier gas, while preserving the gained chromatographic resolution. Using a step-by-step workflow, six parameters were first tested and optimized to make a GC×GC method. To choose values for certain parameters, an open-source peak modeler was used to develop an optimized 1D method. Parameters included modulation period, hold time at the start and end of each run, oven ramp rate, hot pulse time, and secondary oven offset. For each parameter, there were three possible options to choose based on which had the best resolution. After choosing an option for each parameter, a sample was run with the chosen option for each parameter, leading to the best possible resolution in this final method. Finally, an open-source method translation tool was run to convert the method to hydrogen carrier gas. Method development in 2D GC×GC has been seen as a very difficult and timely task; however, this project demonstrated that with an easy workflow, method development is possible within a few days. The future use of hydrogen carrier gas has the ability to reduce run times and cost to accomplish the use of 2D GC×GC in routine laboratories.

63 **Comparison Between Plasma and Serum Matrices for Perfluoroalkyl and Polyfluoroalkyl Substance Detection**

Diana Mathes, New Jersey Department of Health, 3 Schwarzkopf Dr., Ewing, NJ 08628

Per-and polyfluoroalkyl substances (PFAS) are emerging contaminants. Testing has become widespread due to their persistence in the environment and potential health effects. At the New Jersey Public Health Environmental & Agricultural Laboratories (PHEAL), we have many years of experience detecting PFAS in human serum using CDC reference method: 6304.04 for guidance. Expanding to the plasma matrix will provide a more versatile tool for assessing PFAS exposure, enhancing our ability to monitor and mitigate the risks associated with these persistent pollutants. Plasma, unlike serum, contains fibrinogen which can potentially interfere with analytical measurements. Incorporating plasma into our testing capability would require additional verification steps to ensure the method's accuracy and precision. The verification of the plasma matrix was performed by preparing QC standards at 2 and 10 ng/mL in human plasma. These QC samples were run against our serum calibration curve and QC standards on a Sciex 6500 Qtrap LC-MS/MS with an iChrom Pico online solid phase extraction (SPE) unit with separation by an AgilentXDB-C8 column using scheduled multiple reaction monitoring mode. Accuracy and precision tests were performed, n=8, over 4 days at two target concentrations. The mean spike recovery of 2.0 ng/mL and 10 ng/mL are within ± 30%. For precision, the relative standard deviation (RSD) for each analyte does not exceed ± 20% for each concentration. These results verify that there are minimal differences in plasma matrix when running this method.

64 **Identification of the Estrogen-Inducible Proteins in Fishes from the Great Lakes Upon Exposure to Environmental Contaminants**

Krishan Weraduwage, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Taniya Jayaweera, Bernard Crimmins, Sujan Fernando, Thomas Holsen, Costel Darie

We have developed a method that allows us to identify fish exposed to endocrine disrupting environmental contaminants (EDCs) using biochemical markers. The method measures the levels of vitellogenin, a protein whose expression is induced when the fish is exposed to EDCs. A major goal of the current project is to compare the proteomes of unexposed and exposed male lake trout (Salvelinus namaycush) using quantitative proteomics. Initially, homogenates of individual fish that have elevated levels of vitellogenin and individual fish that have low levels of vitellogenin were fractionated by gel electrophoresis. The gel lanes of each fish were cut into 20-30 gel bands, and each was digested with trypsin. The resulting peptide mixtures were then subjected to mass spectrometry-based proteomics. The proteomics analysis was carried out using nanoliquid chromatography-tandem mass spectrometry using a Waters NanoAcquity UPLC coupled to a high-resolution mass spectrometer (Waters Xevo G2 QToF). A
database search was performed using the Mascot Daemon Server and vitellogenin quantitation was done by spectral counting using the in-Scaffold server. Molecular mechanisms were investigated to explain the protein dysregulated upon to exposure to EDCs.

65 Withdrawn by the author.

66 **Scalability of Solid-Core Particles for Chromatographic Analyses**

Maureen DeLoffi, Waters Corporation, 34 Maple St., Milford, MA 01757, Gabrielle Zabala, Gary Izzo, Weiqiang Gu, Thomas Walter, Cheryl Boissel, Daniel Walsh

The ability to scale chromatographic separations between UHPLC and HPLC platforms is critical for method development and can be achieved by using columns packed with stationary phases prepared on different particle sizes. Columns packed with solid-core particles have gained prominence in liquid chromatography (LC) over the last decade due to their characteristics of higher efficiency and lower operating backpressure than columns packed with fully porous particles of similar size. Waters provides a line of solid-core particle columns packed with CORTECS™ Particles in both UHPLC (1.6 µm) and HPLC (2.7 µm) formats. Utilizing CORTECS Particles provides maximize efficiency to drive separation speed, decreasing both run time and solvent waste while maintaining improved sensitivity and resolution. In this poster we explore the importance of larger solid-core particles for scalability. Prototype 5µm CORTECS Materials will be compared to CORTECS UHPLC (1.6 µm) and CORTECS HPLC (2.7 µm) C18 Materials to highlight the advantage of CORTECS Solid-core Particles and their seamless scalability. In addition, CORTECS Particles will also be compared to similar offerings on the market.

67 **Mitigation of the Non-Specific Binding during HILIC Analysis of Metal Sensitive Compounds**

Tony Reinhold, Waters, 34 Maple St., Milford, MA 01757, Paula Hong, Martin Gilar, Andrew Steere

Non-specific binding in the context of metal high performance liquid chromatography (HPLC) refers to the unintentional adsorption of metal sensitive compounds to various components of the chromatographic system, which can include the column packing material, tubing, and detector. This phenomenon can lead to decreased sensitivity, poor resolution, and inaccurate quantification, which are detrimental to the reliability of the analytical results. To mitigate non-specific binding, one can employ strategies such as using columns with inert surfaces, optimizing mobile phase composition, and utilizing complexing agents that can mask the active sites responsible for unwanted interactions. Waters Corporation has developed MaxPeak™ High Performance Surfaces Technology or HPS which reduces the chelation of oligonucleotides to the metal surfaces leading to improved sensitivity, resolution, and quantification. This poster presents the results from a hydrophilic interaction liquid chromatography (HILIC) analysis of a mixture of oligonucleotides on a system with MaxPeak HPS and a system without MaxPeak HPS to demonstrate the advantages to this technology.

68 **Evaluation of Hybrid Silica C18 End-Capped with Bidentate Silylating Reagent for HPLC**

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Silica-based reversed-phase columns have been widely used since the 1970s. Silica has the advantages of high mechanical strength and the ability to withstand high pressure, but conventional silica C18 had limited use under alkaline conditions and is only durable up to a pH of 8. In the 1990s, the end cap of silica C18 was strengthened, improving its alkaline resistance and ensuring durability up to pH 10. Since 2000, a base material called hybrid silica has been developed in which ethylene chains are introduced into the silica skeleton or bonded to the silica surface,

and hybrid silica C18 that can be used up to pH 12 has become available. Hybrid silica in which ethylene chains are introduced into the silica skeleton is said to have surprisingly high durability under alkaline conditions compared to hybrid silica bonded to the silica surface. In this study, 1,2-Bis(chlorodimethylsilyl)ethane, which are bidentate end-capping reagents that form siloxane bonds on the silica surface at two locations, was used as an end-capping agent for hybrid silica in which ethylene chains are introduced into the silica skeleton, called ethylene cross-linked silica gel. A proposed hybrid C18 was tested using standard samples to evaluate its hydrogen bonding, hydrophobicity and steric selectivity as well as peak shape of metal chelating and basic compounds. The durability under basic pH conditions was also evaluated. A commercially available hybrid type C18 column was used as a reference column.

69 Application of RP-HPLC with Fluorescence Detector for Analysis of Bisphenol Analogues in Infant and Toddler Products

Viral Shah, Intertek, 291 Route 22 East, Salem Industrial Park, Bldg. #5, Whitehouse, NJ 08888, Louis Fleck, Axel Martinez, Tyler Horvath

Bisphenol analogs (BPs) have historically been present in infant and toddler products, including pacifiers, teethers, and mouthguards. These items may expose children to bisphenol analogs, which can significantly affect their neurodevelopment, as well as impact the health of their liver and kidneys, and potentially contribute to other illnesses such as cancer. As a result, it has become crucial to analyze for the presence of BPs in these products before they are released to the market. This study aims to establish a faster and more cost-effective quantitative analysis using high-performance liquid chromatography (HPLC) with fluorescence detection (FLD) in conjunction with a reverse phase (RP) separation for fifteen BPs in infant and toddler products via solvent extraction. Sample Extraction: Homogeneous samples of individual specifiers, teethers, and mouthguards were extracted in polar organic solvent for 24 hours at 50 °C at 100 RPM. The limits of detection (LOD) and the limit of quantitation (LOQ) for the fifteen BPs were achieved at 0.5 ppb and 2 ppb, respectively. In conclusion, the RP-HPLC-FLD analytical method has been demonstrated as a faster and more cost-effective tool for analyzing bisphenols, thereby improving the quality and safety of children's products and safeguarding the health of infants and children. Fluorescence detection offers enhanced specificity and sensitivity for determination compared to UV detection.

70 Preparation of Capillary LC Columns in Tube-in-Manifold Microfluidic Devices

Christopher Piccolo, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, M. Keller, D. J. Czarnecki, T. Austin, G. Shelver, J. P. Grinias

Capillary LC provides advantages in terms of reduced sample requirements, lower mobile phase consumption, and improved heat dissipation under conditions of viscous friction. However, minimizing extra-column band broadening effects is of utmost importance in capillary LC due to the low column volumes observed with column inner diameters less than 0.3 mm. To overcome this issue, integrated systems that minimize connection tubing between instrument components and the column can be utilized. Here, progress towards developing a high-pressure microfluidic LC column platform that can be integrated with LC injection and detection modules is presented. A 0.2 x 150 mm column was packed with sub-3 µm C18 core-shell particles and tested for chromatographic efficiency in the flow range of 0.5 – 2 µL/min using a custom-designed slurry packing reservoir. Connections to the column utilizing high-pressure face-sealing fittings enabled packing pressures in excess of 1000 bar. Experimental separations of a series of alkylphenones with efficiencies up to 50,000 plates/m were then compared to CFD simulations of similar column formats to develop models that could be used in future designs of these devices.

71 Porous Graphitic Carbon Chromatography Columns: Retention Mechanisms and Applications

Clinton Corman, Sigma-Aldrich Corp., Bellefonte, PA 16823, Egidijus Machtejevas

Porous graphitic carbon (PGC) has been utilized in liquid chromatographic separations since the 1970s, particularly known for its distinctive retention of polar compounds, though its exact mechanisms remain unclear. Recent advancements in synthesis have produced a new PGC particle with unique retentive properties, differing from those previously studied. This poster will explore these polar retention effects with the new PGC particle, starting with a brief overview of past fundamental studies. We will then discuss data that highlights the differences between the two commercially available PGC particles, shedding light on how analytes interact with this new material. Application data will be presented, demonstrating how theoretical insights can guide method development, particularly for challenging compounds. Finally, we'll consider the potential of further PGC particle design improvements to enhance the resolving power for a broader range of analytes including Nitrosamines.

72 Structure Elucidation of Trace Impurities in Commercial Compounds by Nuclear Magnetic Resonance

Yao An, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901, Sloan Ayers, Ziyu Wang

There are over 300 chemical companies in the world that produce fine chemicals for the pharmaceutical industry. Many of those companies produce the same chemicals with varying degrees of purity which can be overwhelming when deciding what grade of quality is required. For example, there are currently about 26 vendors providing methanesulfonic acid and about 18 vendors offering cyclopropanol, each with an assay of greater than 95%. The synthetic routes used by the vendors may vary and each route may introduce different types of impurities. In addition, impurities at parts per million (ppm) levels with respect to analyte can significantly impact reaction yields. Additionally, due to the hygroscopic nature of the sample, the storage conditions provided by the vendor may lead to moisture absorption, which can impact the quality. In this work, we use two-dimensional and multi-nuclear NMR to investigate impurities orthogonally, with quantification NMR (qNMR) down to several ppm. The presence of cyclopropyl boronic acid in certain cyclopropanol samples is confirmed using 11B NMR. Some impurities in methanesulfonic acid are initially proposed by liquid chromatography high-resolution mass spectrometry (LC-HRMS) and further characterized by NMR. All identified impurities were verified through spiking experiments.

73 Arrayed Spin Lock Durations in One Dimensional Total Correlation NMR Spectra for Structural Characterization

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The use of arrays of 1D TOCSY and TOCSY-DEPT spectra for structural characterization is demonstrated. Spin magnetization is propagated via scalar coupling throughout a spin system, and a plot of spectral intensity v. spin lock duration for each resonance enables spectral assignment. This technique is especially applicable to the assignment of interior nuclei in long chains with minimal chemical shift resolution. A DEPT-type transfer from ^{1}H to ^{13}C permits propagation of magnetization along a ^{1}H spin system followed by detection and assignment with the improved resolution offered by ^{13}C. A novel processing routine for these spectra is presented, enabling simple visualization of the propagating magnetization. Examples include the first complete ^{13}C assignment of 1-decanol, as well as structural confirmation of more complex polycyclic hydrocarbons.

74 Sustainable Synthesis and Surface Functionalization of Cellulose Derived Carbon Quantum Dots (CQDs) for Applications in Drug Delivery

Sarah Watson, West Chester University, Department of Chemistry, 750 S. Church St., West Chester, PA 19383, Simret Asefa, Abbie Ganas

Carbon quantum dots (CQDs) account for a new, exciting class of fluorescent carbonaceous nanomaterials. Further, the CQD surface is easily functionalizable with a variety of surface moieties adding to and enhancing the myriad of advantageous physical, chemical, and optoelectronic properties of CQDs, which tune the material for many modern applications like sensing and drug delivery. Current synthesis techniques employ caustic reagents and unsustainable means to fabricate fluorescent CQDs. In this study, we aim to synthesize and functionalize CQDs through sustainable methods, thus equipping the CQDs for the desired application of drug delivery. Sustainably extracted cellulose derived from recycled paper on West Chester University's campus served as the source of carbon for the CQDs. Once extracted, we added the cellulose, urea, hydrochloric acid, and water to a microwave vessel to microwave irradiate the reaction mixture with different heating intervals. After filtration of the reaction mixture, fluorescent CQDs were made which was confirmed through Uv/Vis and Fluorimetry experiments. Utilizing SEM and DLS characterization we were able to see the variety in CQD diameter size. Further, characterization with TEM will illuminate the structure and elemental composition of the CQDs. Other characterization techniques like FTIR, Raman, and TCSPC lifetime measurements will provide further insight into the structural and optoelectronic properties of the as-made CQDs. Finally, our current research efforts into sustainable synthetic techniques for surface modification and functionalization of our CQDs are also discussed.

75 Dynamic Partitioning of Surfactants into Non-Equilibrium Emulsion Droplets Analyzed by Quantitative Mass Spectrometry

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Characterizing the distribution of molecules between fluid phases is essential for understanding chemical concentrations in heterogeneous mixtures. While partitioning is typically studied under equilibrium conditions, some mixtures may exist in multiple phases only when out-of-equilibrium. Oil-in-water emulsion droplets stabilized by surfactants are an example of such non-equilibrium systems. These droplets are thermodynamically unstable and gradually dissolve or solubilize over time. Conse-

quently, equilibrium properties may not accurately describe the behavior of these droplets. This study investigates the partitioning of nonionic surfactants between microscale oil droplets and water under non-equilibrium conditions. Using quantitative mass spectrometry, the composition of individual micro-droplets is analyzed over time with varying surfactant composition, concentration, and oil molecular structure. Nonionic surfactants rapidly partition into oil micro-droplets, reaching a non-equilibrium steady-state concentration significantly higher than in the aqueous phase. Over hours, droplets solubilize, releasing surfactants back into the water, leading to transiently high concentrations near the oil-water interface. This results in localized microemulsion phases and low interfacial tension. Since emulsions are widely used in industries such as oil recovery, pharmaceutical formulation, food, and cosmetics, understanding their behavior is crucial. These emulsions typically exist in a non-equilibrium state prior to phase separation, where traditional equilibrium partitioning coefficients are no longer representative. The partitioning dynamics revealed in this study provide insights into emulsion stability, wetting dynamics, and more. This knowledge is essential for industries to optimize the functionality of surfactant-stabilized emulsions, enhancing their application and performance in various products and processes.

76 The Challenges and Solutions to Develop the Ultra-Sensitive Hybridization LCMS Assay for Oligonucleotides

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The rapid emergence of oligonucleotides as therapeutic agents in the biopharma industry highlights their potential in treating genetic disorders, cancers, and viral infections. This surge necessitates ultra-sensitive analytical methods to detect and quantify these molecules accurately. Liquid chromatography-mass spectrometry (LCMS) assays face challenges like non-specific binding, carryover, matrix effects, selectivity, and sensitivity. Traditional sample preparation techniques such as solid-phase extraction (SPE) and liquid-liquid extraction (LLE) often struggle with low analyte concentrations and high contaminant ratios in complex biological matrices. These methods fail to adequately separate the analyte from contaminants, leading to compromised robustness and reproducibility. In contrast, the hybridization approach offers distinct advantages by employing sequence-specific probes that bind selectively to target oligonucleotides, enhancing specificity and sensitivity. This significantly reduces matrix effects and background signals, achieving superior detection limits even in complex samples. Optimizing hybridization conditions is critical for ultra-sensitive assay performance. This includes selecting high-affinity probes and formulating hybridization buffers that stabilize oligonucleotide-probe complexes while minimizing background noise. Additionally, employing internal standards (IS) that closely mimic the target oligonucleotides' behavior ensures accurate quantification and consistent results. In conclusion, hybridization-based LCMS assays represent a robust advancement over traditional SPE and LLE methods for oligonucleotide analysis. By addressing matrix effects and selectivity challenges, these assays achieve unprecedented sensitivity and reliability. However, it is crucial to address the inherent challenges of hybridization assays before these methods can be implemented in production.

77 Oligonucleotide and Metabolites Bioanalysis using LC-MS/MS Technique: Case Studies

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Oligonucleotides are nucleic acid polymers that can be used to modulate gene expression via a range of processes including RNAi, target degradation by RNase H-mediated cleavage, splicing modulation, non-coding RNA inhibition, gene activation and programmed gene editing. Many pharmaceutical companies are exploiting the usage of oligonucleotides as an emerging therapeutic modality. Numerous chromatographic methods have been developed for analysis of these oligonucleotides. The hydrophilic nature and the easily forms adducts have imposed a great challenge to the development of LC-MS/MS methods. We will see three cases where we have optimized the bioanalytical methods for analysis of oligonucleotides.

78 Probing the Structure of Guide RNA through Advanced Analytical Tools

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The guide RNA (gRNA), a pivotal component in the CRISPR/Cas9 genome editing system, confers its target specificity. gRNAs are often manufactured using the solid-phase synthesis methods, which can lead to complex impurity profile and higher order structures. The analysis of gRNA is rendered challenging due to its large size, intricate impurity profile, and complex secondary structure. In response to these challenges, we developed several liquid chromatography-based platform methods, such as ion pairing reversed phase liquid chromatography (IP-RPLC), hydrophilic interaction liquid chromatography (HILIC), ion exchange chromatography (IEC), and size exclusion chromatography (SEC) for comprehensive gRNA analysis. Furthermore, we revealed the change of higher order structure of gRNA with different chromatographic modes. Finally, we employed Microfluidic Modulation Spectroscopy (MMS), an orthogonal spectroscopic based method to demonstrate the secondary structure alterations across a temperature raise sequence of the sgRNA.

79 Recent Advances in LC-MS of Oligonucleotides

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Recent advancements in liquid chromatography-mass spectrometry (LC-MS) have greatly enhanced the analysis of oligonucleotides, especially in terms of sample preparation. This study focuses on comparing Waters OligoWorks and solid phase extraction (SPE) Kits with Phenomenex Clarity cartridges to evaluate their performance in extracting and quantifying oligonucleotides from rat plasma. The OligoWorks kits feature a detergent-free protocol that aims to simplify sample preparation by eliminating the need for evaporation and reconstitution, thereby reducing method development time. To assess the advantages, we conducted experiments that included a three-day validation study of plasma QC and standards, and an evaluation of Phenomenex Clarity lysis buffer pretreatment with Waters SPE microplates. Further experiments compared Phenomenex Clarity SPE cartridges with Waters OligoWorks protocols, focusing on aspects such as proteinase K digestion, pKa differences, and method scaling. We also investigated the performance of Waters OligoWorks total solution regarding oligonucleotide stability and digestion time courses.

80 The Evolving Role of Analytical Chemistry in PFAS Environmental Issues

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This presentation introduces the role and advancement of analytical chemistry in addressing the PFAS (per- and polyfluoroalkyl substances) challenge, an area of increasing concern due to the persistence and ubiquity of PFAS in the environment. We will trace the journey of PFAS analysis from the era preceding the commercialization of Electrospray Ionization Interface (ESI) to the present. The technological advancements that have paved the way for more refined analytical techniques will be highlighted. Regulatory requirements for maximum contamination levels (MCL's) are currently based on analytical limitations and not Health Advisory Levels (HAL's), which are much lower and practically not achievable. The current approach to PFAS analysis includes both target and non-target approaches, as well as alternative methods like total PFAS analysis using the total oxidizable precursor (TOP) method and combustion ion chromatography. Total PFAS methods will become more important as the need for closing the fluorine mass balance becomes evident. The pressing need for efficient screening methods, improved total PFAS quantification techniques, and the push towards lower detection limits and the practicality of doing this are discussed.

81 Current LC Approaches for Analysis of PFAS Referencing Short and Long Chain Mixtures

Barry Boyes, Advanced Materials Technologies, 3521 Silverside Rd., Wilmington, DE 19810, Conner McHale

Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals used to make a variety of fluoropolymer coatings and products and may be present in a variety of other consumer products, specialty chemicals, and common goods. The properties of the carbon-fluorine bond, forming the so-called "forever chemicals", have led to significant public health and environmental concerns and increased needs for diligence in surveillance, production, storage and mitigation. The current needs for analytical methods to measure the identities, quantities and transformations of PFAS cross many sample types, including diverse biological and non-biological sources. Expectations for sensitivity of detection and quantitation have increased in parallel with learnings on environmental distribution and toxicity potentials. The selectivity and sensitivity needs for PFAS analysis heavily favor LC/MS approaches. For separations, a particular challenge resides in the diversity of fluorochemicals that have been observed, and the range of polarities concerned. The unique features and great diversity of analytes has required use of several modes of LC separation, not limited to reversed-phase (RP). Polar analytes, including short chain PFAS such as trifluoracetic acid (TFA) and pentafluoropropionic acid (PFPrA) have challenged typical RP conditions, showing low retention and limited MS sensitivity. Hydrophilic interaction LC (HILIC), mixed mode-HILIC, and mixed mode variants of RP show promise for resolution and LC/MS analysis of complex PFAS mixtures. The benefits, comparative features, and potential combinations are discussed.

82 Hunting the Missing Fluorine in Aqueous Film-Forming Foams Containing Per- and Polyfluoroalkyl Substances

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Since aqueous film-forming foams (AFFFs) are major sources of per- and polyfluoroalkyl substances (PFAS), understanding the quantity and type of PFAS present in AFFFs is crucial for assessing environmental risk and remediation. We charac-

terized 25 foams from Canada and Europe, including two non-AFFFs and two fluorine-free AFFFs. We used liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) to identify novel PFAS, as well as total oxidizable precursor assays (TOP) and total organofluorine (TOF) measurements for comparison. LC-HRMS showed that the two non-AFFF foams and two PFAS-free AFFFs contained little or no PFAS, confirmed by TOF measurement using combustion ion chromatography (CIC). The PFAS-containing AFFFs, however, spanned a wide concentration range of TOF (2200–45,000 mg F/L) and contained 22 new classes of polyfluoroalkyl substances not previously reported. As a result of identifying new compounds, LC-HRMS was fully able to capture the oxidizable precursors determined by TOP assay in all tested fluorotelomer (FT) AFFFs, while unknown compounds still constituted a significant fraction (19–53 mol%) in most electrochemical fluorination (ECF) AFFFs. A fluorine mass balance was achieved by comparing the amounts of compounds identified by LC-HRMS with those detected by CIC, although LC-HRMS overestimated TOF with a recovery of 127 ± 36%.

83 A Non-Targeted Approach for PFAS Analysis Using Combustion Ion Chromatography

Jay Sheffer, Metrohm USA, 9250 Camden Field Parkway, Riverview, FL 33578

In January of 2024, the USEPA finalized Method 1621, which was optimized for the Determination of Adsorbable Organic Fluorine (AOF) in Aqueous Matrices by Combustion Ion Chromatography (CIC). This new, non-targeted method allows for the screening of a wide range of PFAS and other fluorinated compounds that may be used to understand the fuller extent and scope of fluorine-related contamination beyond what is measured via targeted PFAS analysis. EPA Method 1621 includes a two-step process. First, a 100-mL aqueous sample is concentrated onto 100 mg of granular activated carbon (GAC) using an automated preparation device. The GAC material is subsequently washed with sodium nitrate solution to remove any trace of inorganic fluorine. Following sample preparation, the GAC material is transferred directly to a sample boat for analysis of AOF using an automated CIC system. Using CIC, the GAC sample is combusted at 1050 °C in an oxygen/argon stream, and the gaseous hydrogen fluoride is absorbed into reagent water. The evolved fluoride is separated using suppressed anion exchange chromatography and quantified using conductivity detection. This talk highlights the basic principles of EPA Method 1621 and how this method differs from traditional targeted methods. Exemplary data and instrumentation from an initial MDL study will also be presented and discussed.

84 Acoustic Ejection Mass Spectrometry for Ultrahigh-Throughput Analysis of Pharmaceutical Targets

Hang Hu, Merck & Co., Inc., 126 East Lincoln Ave, Rahway, NJ 07065, Ophelia Ukaegbu, Umme Ayesa, Joseph Gouker, Jarrod Laro, Jane Wen, David McLaren, Michael Wleklinski, Chang Liu, Erik Regalado, Emmanuel Appiah-Amponsah

Fast-paced laboratories in the pharmaceutical sector, where the synthetic process route is undergoing rapid change and optimization, requires a commensurate level of analytical innovation to deliver accurate results with rapid turnaround, especially in the drug discovery and early development space. Herein, we introduce an automated ultrahigh-throughput platform using acoustic ejection mass spectrometry (AEMS) as a framework for development and optimization of modern pharmaceutical processes, such as high-throughput experimentation in medicinal chemistry, biocatalysis, protein engineering, etc. Echo triple quadrupole (QQQ) system was leveraged for multiple reaction monitoring (MRM), providing excellent sensitivity and selectivity in each of these processes. In addition, Echo quadrupole time-of-flight (QTOF) system demonstrated fast full-scan and high mass resolution to broaden the molecular coverage. The Echo-MS analytical workflow has thus been deployed to accelerate analytical turnaround while minimizing manual intervention and environmental footprint, yielding > 100-fold throughput improvement over conventional LC-MS methods.

85 Acoustic Ejection Mass Spectrometry in High-Throughput Screening

Xiujuan Wen, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, David McLaren, Chang Liu

Mass spectrometry (MS) is widely used in high-throughput screening (HTS) due to its ability to directly measure the biological reaction product without the need to alter or label the substrate or for costly detection reagents. Traditional MS platforms typically have a throughput of 8-30 seconds per sample, which can be a limitation for high-throughput screening where large numbers of samples need to be analyzed. These characteristics have typically made MS a valuable tool for orthogonal hit confirmation in HTS. Recent technological advancements have led to the development of faster sample delivery to the mass spectrometer, such as acoustic ejection mass spectrometry (AEMS). AEMS is an electrospray ionization-based mass spectrometry (ESI-MS) technique which couples acoustic ejection technology with an open-port interface (OPI) to achieve robust sample analysis speeds of one second per sample or faster in some cases. AEMS can handle a broad range of analytes with homogeneous sample preparation, making it suitable for primary screening in HTS. In this presentation we highlight some of our experiences leveraging AEMS as a primary screening technique using automated workflows in 1536w format. Excellent robustness and reproducibility have been achieved as will be illustrated through screening of a library of 300,000+ compounds against multiple enzymes. Our experience suggests that AEMS is likely to play a significant role in advancing HTS techniques and has the potential to improve the efficiency of the drug discovery process.

86 New-Generation Automated Ambient Mass Spectrometry Platform for High-Throughput Experimentation in Early Drug Discovery

Nicolas Morato, Purdue University, 207 S. Martin Jischke Dr., Room DLR 432, West Lafayette, IN 47907, Veronica Feng, Kai-Hung Huang, Kitmin Chen, Beinan Yang, Christina Ferreira, Carleen Klumpp-Thomas, Adam Gloeckner, Matt Galbraith, Csaba Hajdu, Michael Morris, Steven Pringle, Julia Balog, Andrew Mesecar, R. Graham Cooks

The early-stage drug discovery workflow, which relies heavily on high-throughput experimentation for reaction screening and bioanalysis, could benefit from the consolidation of these activities in a single closed-loop platform. Mass spectrometry (MS) is an attractive technique to achieve such consolidation due to its inherent speed, however this advantage is rarely fully utilized due to the widespread use of sample purification approaches prior to MS. Here we describe an automated system that achieves the consolidation of the early drug discovery pipeline by leveraging the advantages of desorption electrospray ionization (DESI), an ambient ionization technique that allows for the rapid and direct analysis of complex samples without any workup. This system combines custom and commercial software, robotics, and analytical instrumentation, and can achieve throughputs better than 1 Hz using high-density arrays (up to 6,144 samples per array) and 50-nL samples (<5 ng analyte). More significantly, inherent reaction acceleration in the DESI microdroplets allows reaction times to be reduced from minutes or seconds to just milliseconds. This automated platform has been utilized extensively for the screening of organic reactions for identification of optimal syntheses and late-stage functionalization of complex molecules, as well as label-free quantitative biological assays using purified targets (enzymes, receptors) and biosamples (cells, microorganisms, tissue), all with no sample cleanup. This presentation provides examples of all these capabilities within the overall context of drug discovery and will showcase the operation and capabilities of the latest generation DESI-MS system built at Purdue University within the NCATS ASPIRE initiative.

87

Withdrawn by the author

88 Quantitative Determination of Biomass Pyrolysis Products Using Micropyrolysis and GC-methanization-FID

Charles Mullen, USDA-ARS, Eastern Regional Research Center, 600 E. Mermaid Ln., Wyndmoor, PA 19038

Analytical pyrolysis using the pyrolysis-gas chromatography (Py-GC) method can be a rapid screening tool for the characterization and grading of biomass for use for production of biofuels or renewable chemical products. It can also be used to screen conditions for eventual scale up of biomass conversion processes. However, most pyrolysis experiments result in a large number of individual chemical products, and with biomass there is also significant diversity in the chemical functionality and detector response factors of the products. Therefore, retrieving the maximum amount of quantitative yield data from the experiments requires generation of numerous individual calibration curves and can be tedious. In this presentation we will discuss how Py-GC can be used in combination with mass spectroscopy (Py-GC-MS) and a commercial methanizer with flame ionization detection (FID) to eliminate the need for separate calibration curves for each compound, and the impact of that ability on the usefulness of micropyrolysis experiments. Three different cases studies will be considered. One compares and contrasts the composition and the potential yield of levoglucosan from the pyrolysis of cellulosic-pulps after lignin extraction from switchgrass and oak with organic solvents under various conditions. Both levoglucosan and side reaction product yields are determined by this method allowing for evaluation of both potential yield and purity of the products. A second evaluates the same feedstocks for catalytic pyrolysis for generation of aromatic hydocarbons. Finally, a third case study evaluates the composition of pyrolysis products of hydrochar, a by-product of oil production from hydrothermal liquefaction of food wastes.

89 Machine Learning vs. Theoretical Computational Prediction of Gas Phase Vacuum Ultraviolet/Ultraviolet (GC-VUV) Absorption Spectra

Kevin Schug, The University of Texas at Arlington, 700 Planetarium Pl., Department of Chemistry and Biochemistry, Arlington, TX 76019, Linh Ho Manh, Jay Rosenberger, Victoria Chen

A vacuum ultraviolet/ultraviolet (VUV/UV) spectroscopic absorption detector for gas chromatography (GC-VUV) has found significant use in the classification of industrial chemicals, especially fuels. Qualitative analysis relies on matching experimental spectra to library spectra. The library spectra are stable, reproducible, and high resolution (0.5 nm) gas phase measurements, free of the influence of solvent effects.

Still, chemical complexity is vast. Unknowns are encountered often. Pure chemical standards do not exist for every chemical desired to be measured. Thus, prediction of gas phase absorption spectra can be useful to aid chemical analysis. Time-dependent density functional theory (TD-DFT) has been used previously for VUV/UV spectral prediction with some success. However, the fidelity of predicted spectra do not often well capture the relative abundance of some absorption bands observed for experimentally-measured chemical compounds. Machine learning (ML) has now been investigated as an alternative to TD-DFT prediction. The ML approach requires molecular feature generation and an appropriate means to map these features onto normalized absorption spectra to create a prediction model. The .SDF files and normalized absorption spectra (125 – 240 nm) were obtained for 1397 compounds. A random forest regressor was found to outperform other ML and deep learning models. A new set of molecular features was devised to better capture features associated with aromaticity and pi-bonding, among other attributes, which are known to influence the shape of VUV/UV spectra. The ML approach to spectral prediction was found generally to outperform predictions based on TD-DFT.

90 **Simple Interfacing of Capillary Electrophoresis to Mass Spectrometry through Vibrating Sharp Edge Spray Ionization**

Lisa Holland, West Virginia University, 217 Clark Hall of Chemistry, Morgantown, WV 26506

Capillary electrophoresis (CE) - mass spectrometry (MS) is an important analytical separation technique for analyses of small and large molecules providing structural information of the analyzed compounds while maintaining a very efficient separation. CE can be coupled to MS by vibrating-sharp edge spray ionization (VSSI). VSSI is a new interface driven by acoustic energy that has been used to couple CE to MS using sheathless and nanoflow sheath designs. The nanoflow sheath CE-VSSI-MS method reduces the dependency on separation flow rates, enabling the use of narrower inner diameter separation capillaries (i.e., 25 µm) and higher separation voltage to realize faster analyses and enhance the flexibility of the separation conditions. The implementation of VSSI interfacing is simple, making CE-VSSI-MS methodology accessible. The signal attained is comparable with nanoflow sheath and sheathless CE-VSSI-MS systems. The applicability of this design is demonstrated with separations of cationic β-blockers at neutral pH over the range of 1 – 100 nM. Moreover, anionic compounds or amino acids are resolved under conditions of suppressed electroosmotic flow. Separations of peptides and proteins are also achieved.

91 No abstract submitted by the author.

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

JoAnn Buscaglia, FBI Laboratory, 2501 Investigation Parkway, Quantico, VA 22135, JenaMarie Baldaino, Kayla Moquin, Jack Hietpas

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

93 **Seldom Believe What Your Client Tells You: Make Sure to Inquire Carefully and Look More Closely, Preferably Before You Start**

Thomas Kubic, John Jay College, 8 Pine Hill Ct., Northport, NY 11768

As a forensic microscopist, I am often retained to perform a micro analysis of a sample to aid a plaintiff's or defendant's position in a legal proceeding. Many of these retentions are for civil cases involving establishing scientific proof of a defendant's fault and therefore liability. The original position of the client and the information supplied may indicate that the testing will be a simple straight forward matter. Often it turns out that when the analyses is undertaken the data generated does not conform to what the client expected and further investigation is needed to satisfactorily explain the results obtained. Many times, this investigation is not of the chemical, physical, biological or microscopical type. Rather, it is historical or geographical and not information usually supplied by the client during the initial submission. In this presentation, I will report on a number of cases where the follow up investigation revealed facts that resulted in the satisfactory explanation of the apparent incongruous analytical data. In many instances if this information were known at the onset of the analytical work, much effort and cost could have been eliminated. One of the examples covered in the presentation is a client's position that electrical fixtures were being damaged by acid rain caused by a power plant emission, when in fact it was saltwater corrosion. Other examples are discussed with the takeaway being, that the forensic consultant needs to obtain as much information as possible, hopefully, prior to the commencement of analyses.

94 **A Microscopical Approach to Problem Solving in Law and Industry**

Christopher Palenik, Microtrace LLC, 790 Fletcher Dr., Ste. 106, Elgin, IL 60123

The concept of a forensic science investigation has evolved from an application of science to purely legal matters to a wide range of other disciplines. For example, numerous food, pharmaceutical and manufacturing companies have forensic departments (in name or intent) dedicated to the investigation of problematic issues that involve tampering, counterfeiting, consumer complaints, and failure issues. The sophistication of such investigations, which are often not subject to immediate legal considerations, can vary dramatically. Many investigations begin…and end…with subjective determinations made by non-technical personnel. For example, we have experienced call center representatives in the food industry that not only arrange for the retrieval of a consumer complaint sample but who also receive, handle, and even examine returned samples to make a determination as to the validity of a claim. There are many instances where a more detailed, scientific examination can establish factual details on a timeline of hours to days. Examples of different case studies aim to illustrate the ways in which a detailed microanalytical investigation can provide manufacturers in various industries with the factual information needed to make critical decisions that may impact responses to consumers and social media posts, the release or recall of product, or production problems.

95 **Something in the Air: The Contribution of Nitrogen Oxides to the Formation of Nitrosamines from Vulnerable Active Pharmaceutical Ingredients**

Joerg Schlingemann, EMD Serono, Frankfurter Straße 250, Darmstadt, Hessen, 64293 Germany

Nitrosamines are formed from vulnerable amines and nitrosating agents under promoting conditions. Vulnerable amines can be small dialkyl amines used as reagents or present as impurities in reagents or solvents for organic synthesis. The API or its impurities may constitute vulnerable amines as well, leading to the formation of so-called nitrosamine drug substance related impurities (NDSRIs). Promoting conditions are commonly mildly acidic, allowing for the presence of unprotonated amine and the formation of nitrosating agents from their precursors. Inorganic nitrite, added as a reagent during API synthesis or as an impurity of excipients, is arguably the most relevant precursor of nitrosating agents. Nitrocellulose, a common component of printing inks and primers, has been identified as another, albeit much less relevant precursor of nitrosating agents. Until recently, only little attention has been paid to the role of nitrogen oxides, when it was demonstrated that NOx from the air can lead to the formation of NDMA from DMA during granulation of a metformin drug product blend (Fukuda et al., 2023). In the study to be presented, we look at the contribution of nitrogen oxides to the formation of NDSRIs from five model APIs in neat API, bulk tablets, and blistered tablets. 15NOx is used to distinguish from NDSRI formed via nitrite from excipients. 14N-NDSRIs and 15N-NDSRIs are measured before and after gas exposure and after one week of stress storage.

96 No abstract submitted by the author.

97 **Strategies for Overcoming Challenges in LC-MS Analysis of Nitrosamines in Pharmaceutical Products**

Jinjian Zheng, Merck & Co., Inc., 125 E. Scott Ave., Rahway, NJ 07065

Nitrosamines are potential carcinogens that have very low allowable intakes, typically ranging from 18 to 1500 ng/day based on their structures. Liquid chromatography-mass spectrometry (LC-MS) is widely utilized for nitrosamine analysis due to its high sensitivity and specificity. However, accurately quantitating nitrosamines at such low levels, particularly in complex formulated products, presents several challenges. This presentation aims to address some common challenges related to nitrosamine analysis using LC-MS, including in situ nitrosation, in-source fragmentation, false positive/negative outcomes, adduct formation, and matrix interference, etc. Several case studies will be used to demonstrate effective strategies for developing sensitive, accurate, and robust LC-MS methods for the analysis of nitrosamines in pharmaceutical products.

98 No abstract submitted by the author.

99 How Does Interfacial Water Structure Change with Increasing Surface Charge Density?

Paul Cremer, Penn State University, Department of Chemistry, University Park, PA 16803

Interfacial water structure below charged surfactant monolayers at constant temperature will be discussed in this presentation. Measurements were made in the OH stretch region of the vibrational spectrum by sum frequency generation (SFG) spectroscopy. A surfactant monolayer was formed at the air/water interface, which created a specific surface charge density. Studies were performed with arachidic acid (AA) and eicosanoyl sulfate (ESO4) monolayers, which were negatively charged, as well as with octadecyl trimethylammonium (ODTA) monolayers that possessed a positive charge. The surface charge density could be modulated at constant two-dimensional pressure by diluting the charged surfactant film with neural fatty alcohol surfactants. Moreover, salt was introduced into the subphase, which could screen the interactions between the charged headgroups. This presentation addresses how interfacial water structure evolves as the system variables are changed. Moreover, the role of ion binding versus charge screening is addressed. This problem is important for understanding whether interfacial water structure organization always follows the interfacial potential as the charge density is increased.

100 Near Infrared Vibrational Second Harmonic Generation (NIR-vSHG): A New Nonlinear Vibrational Spectroscopy of Interfaces

Eric Borguet, Temple University, Department of Chemistry, Philadelphia PA 19122

Vibrational sum frequency generation (vSFG) has emerged as a powerful probe of interfaces. However, vSFG is not simple as it requires temporal and spatial overlapping of focused laser beams, Fresnel factor correction, and time-consuming technical training. Largely eliminating these requirements, near-infrared second harmonic generation (NIR-vSHG), a single beam approach, offers distinct advantages over vSFG. By operating in the near-infrared, NIR-vSHG brings enhanced optical accessibility by avoiding substrate absorption in the mid-infrared range and simplifies the spectral analysis by excluding possible interference from Fermi resonances and Fresnel factor correction in surface-specific studies. We have used NIR-vSHG to study the overtone of several systems: the free OH at muscovite mica surfaces in air and the CH stretch in chloroform and acetonitrile at alumina interfaces. Our results provide a determination of the anharmonicity constant and dissociation energy of these CH and OH bonds at interfaces. NIR-vSHG yields important information relevant to interfacial bond activation and establishes a new tool to detect the vibrational characteristics of interfacial environments.

101 Single Molecule FRET Imaging and Deep Learning Reveal Concentration Dependence of Aggregation Pathways during Aβ42 Aggregation

Sara Sohail, Swarthmore College, 500 College Ave., Swarthmore, PA 19081, Janghyun Yoo, Hoi Sung Chung

Protein aggregation into amyloid fibrils is the hallmark of several devastating neurodegenerative diseases. Understanding disease etiology hinges on our ability to uncover the molecular mechanics of how soluble monomers assemble to form insoluble fibrils consisting of thousands of constituent monomers. Amyloid fibrils with distinct structural morphologies have been observed, and recent Fluorescence Lifetime Imaging data show that heterogeneous morphologies are formed co-presently in a single sample. Bulk biophysical methods are unable to fully characterize these fibril polymorphs. Here, we develop and implement Förster Resonance Energy Transfer (FRET) imaging to monitor the entire aggregation pathway of the Alzheimer's Disease related peptide amyloid β 42 (Aβ42) at the single fibril level in real-time. We incubated a mixture of donor-labeled, acceptor-labeled, and unlabeled Aβ42 monomers, which resulted in the formation of fibrils with distinct FRET efficiency values, indicating structural heterogeneity. FRET images reveal that increasing monomer concentration promotes the formation of a fibril assembly with FRET > 0.8, while fibrils formed at lower concentrations assemble via different pathways. At the lower concentration, two populations of fibrils emerge, with FRET values of 0.45 and 0.75. Deep learning methods (https://github.com/hoisunglab/FNet) enable segmentation of single fibrils within images of highly overlapping fibrils, allowing for quantitative analysis of the aggregation process at every frame. Photon recoloring simulations support the assignment of the observed FRET populations as fibrils with parallel and anti-parallel structures.

102 Characterizing Materials Interfaces Using Second Harmonic Generation

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Nonlinear frequency mixing (e.g. harmonic generation) and polarization rotation of electromagnetic waves are the foundation of many important and emergent applications, which include laser technologies, optical switches, photonic integrated circuits, and frequency combs, among others. The current state-of-the-art for second harmonic generation (SHG) is achieved using a sequence of multiple quantum wells that are designed to resonantly enhance transitions at both fundamental and harmonic frequencies. However, these systems are intrinsically limited to the mid infrared, precluding their operation at frequencies important for many optical imaging and telecommunications applications. Therefore, new materials that can achieve large nonlinear optical responses over a broader range of frequencies are desired. In this talk, near-infrared-to-visible (NIR-Vis) second harmonic generation mediated by two-dimensional polar metals formed from gallium, indium, and silver is described. These systems exhibit exceptionally large NIR-Vis second-order susceptibilities ($c^{(2)}$) - approaching 10 nm^2/V. This extra-ordinary response results from the unique atomic-level interfacial structure and bonding properties of one-to-three-atom-thick crystalline metal films that were formed by a process called confinement heteroepitaxy (CHet). Resonance matching in CHet-formed 2D Ag leads to competitive nonlinear excitation and scattering pathways. Coherent multi-dimensional Fourier transform SHG microscopy is used to disentangle these competing structure-dependent channels. Depending on the excitation pathway, a range of NLO responses that include saturable and enhanced harmonic generation can be induced.

103 Bacterial Model Membrane Systems Featuring Phosphatidylethanolamine and Phosphatidylglycerol as Predominant Lipids

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Supported lipid bilayers (SLBs) are a valuable tool for mimicking plasma membrane traits, aiding in detailed examination of biophysical properties and drug design research. These SLBs are also readily integrated into a wide variety of analytical sensing strategies, including optical, electrochemical, and acoustic sensing. While simple SLB formation methods prove efficient with lipids featuring the phosphatidylcholine (PC) headgroup, characterized by its cylindrical shape and neutral charge, their applicability is hindered when incorporating conical and charged lipids like phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), respectively. These prevalent bacterial membrane lipids pose challenges for SLB formation. Here, we report a novel method for forming SLBs using biologically relevant composition of PE and PG. We employ a chaotropic agent, sodium trichloroacetate (TCA), to disperse the lipids into micelles. These micelles are then introduced onto a clean glass surface. Subsequently, utilizing a solvent exchange method, we introduce a buffer, which results in the formation of the bilayer. Additionally, in our study, we introduce a novel PE-PG bilayer composition utilizing 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) and 1,2-dioleoyl-sn-glycero-3-phosphatidylglycerol (DOPG). Unlike traditional formulations such as 1-palmitoyl-2-oleoyl-glycero-3- phosphatidylethanolamine (POPE) and 1-palmitoyl-2-oleoyl-glycero-3- phosphatidylglycerol (POPG), DOPE and DOPG feature conical molecular structures, posing challenges due to their reluctance to adopt planar configurations during SLB formation by traditional methods. We evaluate the bilayer formation, diffusivity, and integrity using fluorescence recovery after photobleaching (FRAP) and quartz crystal microbalance with dissipation monitoring (QCM-D). Furthermore, we showcase the versatility of these bilayers in elucidating insights into prokaryotic membrane dynamics and their application in assaying drug-membrane interactions.

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

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

105 Extractables and Leachables Study on Self-Amplifying RNA-LNPs Manufacturing

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In the production of biological therapeutics such as Self-Amplifying RNA-LNPs, ultrafiltration and diafiltration (UF/DF) are widely regarded as effective downstream processing steps capable of removing process equipment related leachables (PERLs) introduced upstream of the UF/DF step. Herein we demonstrate UF/DF process on tangential flow filtration device (TFF) helps mitigate leachables risk not only from PERLs introduced upstream of UF/DF, but also from the TFF operation. The Extractables and Leachables study presented in this work demonstrated the major process related leachables are indeed well-cleared after TFF step, and thus present a lower overall safety risk. This is also aligned with our previous findings of TFF clearance on other biological therapeutics. The recent SARS-CoV-2 pandemic has shown the importance of developing RNA vaccines as well as the importance of cost-effective and timely vaccine production to deliver a rapid response to disease outbreaks. Having an effective RNA-based medicine requires a delivery vehicle to bring the nucleic acid into the cytoplasm and protect it from degradation, however, obtaining an efficient delivery system to achieve the full potency of RNA therapeutics remains a key challenge.

This study described and addressed the safety concerns of extractables and leachables in the single use technologies application, which is one of the important aspects in the development and design of single use technologies in biopharmaceutical and bioprocess industries. This study demonstrated that UF/DF is an effective Self-Amplifying RNA-LNPs downstream process with robust PERLs clearance capability.

106 Mass Spectrometry-Based Degradomics Analysis of Breast Milk for Early Detection of Breast Cancer

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Breast cancer (BC) is a leading cause of cancer-related deaths in women worldwide. Early detection of breast cancer is crucial. Invasive ductal carcinoma (IDC) is the most common subtype of BC, accounting for 85% of all new BC diagnoses. Human breast milk contains secreted proteins, immune cells and exfoliated epithelial cells, and can be collected non-invasively during an important time in breast development. Mass spectrometry-based (MS) proteomic experiments are ideal for the investigation of human breast milk proteins as potential BC biomarkers. Additionally, the MS-based methods allow for quantitation of the observed protein differences. Various omics can be applied to analysis of proteins to identify biomarkers for BC detection. Among them are proteomics, peptidomics, post-translational modification (PTM)-omics, degradomics, or metabolomics. One of these omics approaches, degradomics, could reveal information on how the proteins are degraded as a result of the onset of BC or the transition of BC from isolated to metastatic BC. Here, we performed degradomics analysis on 20v20 human breast milk samples using MS to identify protein biomarkers to aid in early breast cancer detection. We fractionated milk proteins using 10 kDa cut-off filters. The flow through was subjected to nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS), without trypsin digestion, to identify proteins, fragments of proteins, and peptides which are dysregulated from women with BC vs the controls. This data will be complemented with serum analysis performed the same way to draft a protein biomarker set for early detection of BC.

107 Method Development Approach to Separating Oligonucleotides Under UV and MS Detection

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Oligonucleotides continue to be of considerable interest with many applications in the bio therapeutic space, varying from neurodegenerative diseases to cancer treatments. LC and LCMS separations are routine in development and quality workflows. Often these LC method separation conditions require operation at elevated pH, high temperatures, ion pair reagents and it has been reported that oligonucleotides can be sensitive to adsorption on metal surfaces. For these reasons, traditional silica-based columns are not often utilized due to their inability to withstand elevated pH at high temperatures for extended periods of time. With a new 120Å, 2.7um, superficially porous particle (SPP), hybrid silica structure column, packed in surfaced passivated hardware, we demonstrate how this new material improves LC and LCMS separations for oligonucleotides. A range of oligonucleotide samples from 10-60mer in length were separated on multiple stationary phase columns using a Shimadzu UHPLC system. The mobile phases varied in pH along with different mobile phase modifiers. The preferred conditions were run at an elevated pH of 8-9 using TEAA as the ion pairing reagent. In order to make the conditions LCMS friendly, HFIP was substituted for Acetic acid. A range of pH's (6-9) are used to educate on the effects of pH for oligomer separations; this is demonstrated on a new high pH oligonucleotide stationary phase. Different stationary C18 phases were used to evaluate the separations as well. These columns vary in pore size and column dimension in order to highlight how SPP technologies can be advantageous in high linear velocity separations.

108 Chemical Impurity Analytical Method Development in Biopharmaceutical R&D

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As the biopharmaceutical process continues to evolve in the upstream and downstream purification space, the complexity of the process along with regulatory requirements necessitates the optimization of mammalian cell culture protein expression and analytical methodology. To facilitate this, certain compounds are added at various steps during the protein expression process. Chemical impurities are a diverse class of compounds that are intentionally introduced or produced as by-products during routine upstream biopharmaceutical process development. These impurities have temporally beneficial functions including acting as reducing agents, surfactants, and cell aggregation inhibitors. Several chemical impurities are inherently toxic, and as a result, must be removed from the final product of the drug. A regulatory need exists to demonstrate impurity clearance to ensure product and patient safety, efficacy, and compliance with regulatory standards. This presentation will highlight the pivotal role of chemical impurity analysis method development utilizing chromatography as a case study. Methods are verified and qualified, permitting method transfer to quality control laboratories to support routine testing.

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No abstract submitted by the author.

110 Successfully Implementing Key Elements of the USP <1220> and ICH Q14 Guidances in an Enhanced Analytical Procedure Development Workflow

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Current USP and ICH guidances affirm the expectation of quantitation in analytical procedure development. This course describes the key tools and methods in these guidances in detail and their use in an enhanced APLM workflow. Topics include 1) defining the ATP as a negotiated specification incorporating analytical and production variation, 2) correct robustness assessment to achieve a robust Method Operable Design Region (MODR), 3) replication strategy optimization for reportable results meeting ATP-specified performance requirements, and 4) critical knowledge transfer to APLM Stages 2 and 3. Course topics are presented in the context of LC method development, validation, and transfer.

111 Enhanced Method Development Using Empower Chromatography Data System and Quality by Design Software

Margaret Maziarz, Waters Corporation, 34 Maple St., Milford, MA 01757, Andrea Gheduzzi, Stephanie Harden, Isabelle VuTrieu

A new 'enhanced approach' to the development of analytical procedures has been recently introduced by the ICH Q14 and adopted by FDA and EMA. Since it is based on science- and risk-management principles, it represents the preferred way of communication between applicants and pharmaceutical regulatory authorities. The enhanced approach aligns with analytical quality by design (AQbD) principles and is intended to facilitate the development of fit-for-purpose and robust methods. This presentation describes the application of the enhanced approach to the development of three methods for impurities analysis. The first method was designed for the analysis of aspirin active pharmaceutical ingredient (API) and its related substances, using a statistical modeling software integrated with Empower™ CDS to run the experiments and process the data. A second method was developed to separate and analyze dexamethasone phosphate API and its related compounds using a chromatographic modeling software based on the principles of retention mechanism, also integrated with Empower. In both cases, processed data was imported to the method development software for analysis and modeling. This allowed the identification of critical method parameters and generation of a design space, also known as a method operational design region (MODR). In the third example, a method for impurity profiling, originally developed on a low-dispersion system with a sub-2 µm particle size column, was scaled to HPLC. The MODR for the HPLC method was generated to establish the acceptable performance region.

112 Challenges of Developing and Validating Analytical Procedures with the ICH Q14 Guideline

Trevor Williams, Pharmaceutics International Inc., 28 Water St., Apt D, Glen Rock, PA, 17327

Development and validation of analytical procedures under the new ICH Q14 guideline poses significant challenges yet also opportunities for the pharmaceutical sciences. This presentation explores the critical aspects of method development and validation, emphasizing the key principles and expectations outlined in ICH Q14. From analytical target profiles to method lifecycle management, the guideline encourages a structured risk-based approach that integrates quality by design principles and analytical advancements. Addressing the challenges inherent in such an undertaking effectively ensures robust, reliable, and compliant analytical methods essential for drug development and regulatory approval. This session will highlight

strategies to navigate these complexities, optimize efficiency, and enhance pharmaceutical product quality and patient safety.

113 Opportunities and Barriers in ICH Q14 Implementation: An ISPE-PQLI Survey

Qinggang Wang, Bristol Myers Squibb, Chemical Process Development, One Squibb Dr., New Brunswick, NJ 08903

ICH has recently adopted two important guidelines on analytical procedures used for the assessment of the quality of drug substances and drug products, i.e., the revised guideline Q2(R2) "Validation of Analytical Procedures" and the new guideline Q14 "Analytical Procedure Development". Q14 describes the scientific principles of development and lifecycle management of analytical procedures, and documentation of development knowledge for regulatory submissions. The revised Q2(R2) expands the scope to include validation principles for more advanced analytical procedures, as well as analytical procedures for biotechnological/biological Products. Once implemented, these two guidelines will form the basis of regulatory submission and communication of analytical procedures. Though some new elements described in Q14 and Q2(R2) have been gradually adopted by the pharmaceutical industry in the last two decades, practices vary widely among different companies. To benchmark current industry practice, ISPE-PQSI working group conducted a survey to evaluate industry readiness for Q14 and Q2(R2) implementation. The survey focused on new elements described in Q14 and Q2(R2), such as the enhanced approach to analytical procedure development and lifecycle management of analytical procedure. The survey results related to Q14 are summarized in this presentation. Opportunities and potential barriers in Q14 implementation revealed from the survey are discussed.

114 Using Alternate Light Sources to See the Unseen

Mark Witkowski, United States Food & Drug Administration, National Forensic Chemistry Center, 6751 Steger Dr., Cincinnati, OH 45237, Nicola Ranieri, Douglas Albright, John Lynch, Megan Sterling

Alternate light sources (ALSs) have been used for many years in traditional forensic science applications such as fibers, fingerprints, body fluids and document analyses. At the NFFC, its use has been expanded into pharmaceutical forensic applications (e.g., counterfeit tablet detection, diversion). The simplest ALS device, a UV lamp, emits a single wavelength of light. Although useful for some applications, it has limitations when examining more complex samples such as FDA regulated products. A multiwavelength ALS device allow for the illumination of a sample(s) over a broad wavelength range providing the analyst greater flexibility and discriminating power than a single wavelength device. The technique allows for the observation of visual differences between or within samples not easily seen under normal white light conditions. This allows the user to visually examine the sample(s) prior to the start of any traditional laboratory analysis allowing for intelligent sampling. ALS is quick, nondestructive technique that has applicability both in the laboratory and outside the laboratory. This presentation discusses different examples of ALS devices and instruments and how they have been used at the NFCC both in and outside the laboratory to examine FDA regulated products. Examples of their use and the type of visual information obtained are presented.

115 Advancing Food and Dietary Supplement Safety with 15 Years of Spectroscopic Methods at FDA/CFSAN

Betsy Jean Yakes, United States Food & Drug Administration, 5001 Campus Dr., College Park, MD 20740

During my over 15 years in the United States Food and Drug Administration (FDA) research labs, I have seen many technological innovations which have been advantageous not only to protecting the public but also to the advancing scientific knowledge. Spectroscopic instruments, especially portable devices, have been able to capitalize on wireless communication, touchscreen displays, GPS capability, rapid processors, fingerprint scanners, and advanced cameras commonly found in standard smartphones. These devices can be employed for improved screening of foods and dietary supplements; however, device efficacy must first be understood. As such, we have been exploring the feasibility of vibrational spectroscopy for evaluation of FDA regulated products using both laboratory-grade and lower-cost instrumentation. Our primary research focus has been on those products where field testing would prove most beneficial, such as those products that may be prone to mislabeling or those that are at high risk for economic adulteration. This presentation overviews near-infrared (NIR) and Raman portable spectrometers currently under evaluation. Device advantages and limitations are highlighted through brief case studies of FDA regulated products (e.g., milk powder authentication, marine oil dietary supplement label verification, and polymer tubing plasticizer evaluation) to elucidate where the technology shows promise for supporting advances in food safety.

116 Pharma in Focus: Spectroscopic Imaging for Physicochemical Insights

Daniel Willett, United States Food & Drug Administration, 645 S. Newstead Ave., St. Louis, MO 63110, Huzeyfe Yilmaz, Yeakub Zaker, Snober Ahmed, Changning Guo, Jason Rodriguez

Physicochemical properties such as particle size, constituent distribution, morphology, chemical and elemental composition are important in the assessment of the quality of complex dosage forms. Spectroscopic imaging can characterize these properties for a broad spectrum of dosage forms, often with little or no sample preparation required. For example, spectroscopic imaging can be used for in vitro bioequivalence studies as part of a sameness evaluation between generic and reference listed drug products. This presentation provides a general overview of the analytical toolbox the FDA has developed for high resolution spectroscopic imaging for physicochemical characterization at both the micro and nano size scales in combination with multivariate approaches for data analysis. These include approaches with resolutions at the micro scale such as laser directed infrared (LDIR) imaging and Raman mapping in both the fingerprint and low-frequency regions in addition to tools that can move spectroscopic imaging into the nano scale such as Nano-IR and cryogenic scanning electron microscopy (cryo-SEM) coupled with both energy dispersive spectroscopy (EDS) and Raman. Several case studies are presented encompassing a wide variety of dosage forms to demonstrate the versatility of these techniques. These applications include LDIR and Raman mapping for characterization of physicochemical distribution in solid oral dosage forms and transdermal delivery systems, in addition to cryo-SEM/Raman for characterization of ophthalmic nanoemulsions and albumin-bound nanoparticle-based formulations.

117 Analysis of Unknown (Unlabeled/Mislabeled) Drug Products for Active Pharmaceutical Ingredients at Remote Sampling Sites by FDA's Satellite Laboratory Program

Adam Lanzarotta, FDA National Forensic Chemistry Center, 6751 Steger Dr., Cincinnati, OH 45237

The FDA's National Forensic Chemistry Center (NFCC) is responsible for overseeing the use of handheld and field portable analytical devices for detecting active pharmaceutical ingredients (APIs) in unknown drug products at remote sampling sites. Phase I of this program (nationwide mail blitz) previously demonstrated that a three-device rapid screening "toolkit" consisting of a handheld Raman spectrometer, transportable mass spectrometer, and portable Fourier transform infrared (FT-IR) spectrometer was the most effective collection of instruments for identifying APIs in product types collected at international mail facilities (IMFs). Phase II of this program (pilot study) demonstrated that results generated using the toolkit at an on-site IMF satellite laboratory were as reliable as those generated by a full-service library when two or more of these instruments identify an API. Phase III of this program (production mode) demonstrated that FDA satellite laboratories yield conclusive results for approximately 85 % of the products examined, which were used to support regulatory action, and identified approximately 15 % of the products that required additional full-service laboratory analyses due to inconclusive satellite laboratory results. The satellite laboratory program is currently in Phase IV, which involves expanding to other IMF locations, supporting investigators with field operations, developing methods to identify APIs that cannot currently be identified using the toolkit, and modifying the toolkit with new instrumentation that has the potential to overcome limitations of the existing devices. This presentation describes the current progress of Phase IV and introduces future goals of the program.

118 Validation and Clinical Application of Volumetric Absorptive Microsampling (VAMS) Dried Blood Sampling Method for Phenylalanine Measurement

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Background: Dried blood sampling via volumetric absorptive microsampling (VAMS) is a useful technique for diagnosis, therapeutic drug monitoring and drug or disease response monitoring. The advantages of VAMS include the minimally invasive finger prick procedure (home sampling), the small volume collection for pediatric patients, the stability of the analyte, and the minimal impact due to individual hematocrit value. We validated an LC-MS/MS in dried human blood collected in a VAMS device and applied the method for measurement of phenylalanine to support frequent blood sampling in patients with phenylketonuria (PKU). Methods: A VAMS dried blood collection high-performance liquid chromatography-tandem mass spectrometry (HPLC–MS/MS) method was developed and fully validated for blood phenylalanine measurement in compliance with regulatory guidance. The method accuracy, precision, stability, selectivity, matrix and hematocrit effects were assessed. Results & Conclusions: A VAMS blood method for accurate and precise phenylalanine measurement was successfully validated and applied effectively to clinical studies. The validated method demonstrated excellent assay performance and method reproducibility during conduct of sample analysis and showed excellent incurred sample reproducibility.

119 Validation of Quantitation of MK-6598 Target Protein and Phenylpyruvate in Tumor Biopsies by LCMSMS to Inform Target Engagement in Phase I Oncology Clinical Trial

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Two assays were developed and validated to inform target engagement in core needle biopsies from a first-in-human clinical study. The small molecule assay measures phenylpyruvate as a proxy metabolite for H_2O_2 production in the tumor environment. The protein assay measures the target protein of MK-6598. MK-6598 is currently under evaluation in a first-in-human, Phase I trial to assess efficacy and safety and recommended Phase 2 dose (RP2D). MK-6598 is administered as monotherapy and in combination with pembrolizumab (MK-3475) in adult participants with advanced or metastatic solid tumors. MK-6598 is expected to reduce H_2O_2 levels that impact proper T cell functionality. While H_2O_2 is labile and cannot be measured accurately in tumor biopsies, phenylpyruvate was identified in preclinical metabolomics studies to serve as a viable proxy for H_2O_2 decrease by the action of MK-6598. For the clinical study MK-6598-001, the quantification of phenylpyruvate in tumor tissue was identified as a secondary objective and will be used to aid in determining the RP2D. Quantification of MK-6598 target protein is an exploratory objective and will be used in conjunction with phenylpyruvate data. Our hypothesis is that tumor samples with higher levels of target protein are more likely to be affected by MK-6598 and would be reflected by lowered phenylpyruvate concentration. Here we present the validation of two assays for the parallel quantitation of the target protein and phenylpyruvate from same tumor tissue aqueous homogenate. The assays were validated and are used to inform target engagement in the Phase I clinical study MK-6598-001.

120 Bioanalytical Assessments of a Novel Approach to Overcome Microsampling Challenges in Rodent Studies

Hsinpin Ho, Bristol Myers Squibb, Route 206 & Province Line Rd., Princeton, NJ 08543

Blood volume limitation is a widespread challenge in rodent studies. The common obstacles in regulated bioanalysis for existing microsampling technologies (i.e. VAMS, capillary tubes, or Microvette) are data bridging issues between plasma and dried blood, delicate sample handling, reduced pipetting accuracy at micro-volumes, and insufficient sample volume/aliquots for incurred sample reanalysis. Microsampling Wings (MSW2), a novel microsampling technology, may have the potential to overcome these hurdles. We have developed a liquid chromatography with tandem mass spectrometry (LC–MS/MS) method utilizing MSW2 and assessed the technology in a proof-of-concept rodent study. From the study results, the PK profiles comparing MSW2 to conventional venous blood sampling were nearly indistinguishable, demonstrating the feasibility of MSW2 approach in rodent studies. Based on the assay performance, MSW2 provided precise micro-volume for bioanalytical use without traditional micro-pipetting to measure analyte of interest in study samples. These preliminary findings pave the way for further investigations using MSW2 in regulated bioanalysis to support small rodent studies in drug development.

121 Overcoming Bioanalytical Assay Challenges to Support New Generation of Antibody-Drug Conjugates

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Antibody drug conjugates (ADCs) that combine potent payload cytotoxicity and antibody target specificity represent a significant advance in targeted cancer therapy as demonstrated by the recent regulatory approval of numerous ADCs for treatment of various oncology indications. The structural complexities of ADCs pose unique bioanalytical challenges compared to standard small molecule or therapeutic protein assay developments. To fully characterize the PK properties of each ADC and establish pharmacokinetics/pharmacodynamics, four distinct bioanalytical methods are required to measure: free payload, conjugated payload, total antibody, and DAR distribution. Each of these assays presents its unique challenges: 1) very low detection limit (pg/mL) for the free payload assay by LC-MS/MS, along with potential release of the free payload from ADC during sample collection, processing, and storage; 2) recovery of ADC during immunocapture, consistent enzymatic release of the payload and accurate quantitation by hybrid LBA, LC-MS/MS; 3) specific capture of total antibody with minimum nonspecific pulldown (for ELISA and hybrid LBA, LC-MS/MS) and consistent proteolytic release of highly sensitive surrogate peptides for quantitation (LC-MS/MS); 4) sensitive and accurate mass measurement (5-10 ppm) for in vivo DAR characterization at intact level using hybrid LBA, LC-HRMS. To support the new generation of ADCs, we have developed the required assays that cover all the mentioned challenges for timely IND tox or FIH study support. In the process, we have deepened our knowledge on the diverse linker chemistry/payloads and learned the best work practices needed for developing these high-quality ADC assays.

122 Characterization of Endogenous RNA Modifications in the Central Nervous System by LC-MS/MS-Based Epitranscriptomics

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RNA modifications are increasingly recognized for their roles in controlling the biophysical and translation properties of endogenous and therapeutic RNA biopolymers. The landscape of RNA modifications, known as the epitranscriptome, consists of more than 170 modifications of the canonical A, G, C, and U nucleotides that range in complexity from simple methylations to conjugation with cellular metabolites. Dozens of RNA modifications are encoded by any single organism, but their myriad functions remain elusive due to the lack of analytical methods capable of quantifying epitranscriptomic modifications in single cells. Here, I describe our lab's advances in mass spectrometry (MS)-based epitranscriptomics to facilitate the characterization of RNA modifications in central nervous system (CNS) tissues and single neurons. These approaches have revealed a previously uncharacterized link between RNA modifications, neuronal protein synthesis, neuron excitability, and ultimately behavioral change in the neurobiological model animal, *Aplysia californica*. Chemical and enzymatic labeling approaches for RNA are also described that enable MS-based sequencing of RNA modifications in single neurons and provide new insights toward the molecular underpinnings of CNS function.

123 Analytical Control Strategy for Therapeutic Antisense Oligonucleotides

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Antisense oligonucleotide are short single-stranded nucleic acid polymers that can bind target RNA molecules via complementary Watson-Crick base pairing to inhibit protein expression. Oligonucleotide therapeutics can address a broad spectrum of health conditions, including infectious diseases, neurodegenerative disorders, cancer, cardiovascular and kidney diseases. As the number of oligonucleotide drug applications receiving regulatory agency approval rises, and as more oligonucleotide therapeutics enter clinical trials, it becomes increasingly important to develop robust analytical control strategies to purify, analyze and characterize them. Furthermore, chemical modifications to oligonucleotide therapeutics used to evade detection by the immune system, decelerate their breakdown by naturally occurring nucleases, and enhance their binding energy can add complexity to chromatographic separation of the API and related impurities. Therefore, it is essential to establish analytical methods that can confirm the identity, and determine the purity, content and quality. Analytical control strategies for oligonucleotide therapeutics typically involve a myriad of advanced analytical techniques. As an example, the most commonly used analytical techniques for separation of impurities in antisense oligonucleotides include ion-pairing reverse phase liquid chromatography with tandem mass spectrometry. The integration of these methods into the development process is critical, not only for the optimization of the manufacturing process and the acceleration of clinical trials but also for assessing their safety and efficacy.

124 Impact of Nucleic Acids Melting Temperature on their Chromatographic Behavior

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Silencing RNA (siRNA) duplex quality control is performed in non-denaturing or denaturing conditions. Melting temperature (Tm), a mid-point transition between siRNA duplex and its single stranded constituent, is an important consideration for selection of liquid chromatography (LC) method. Tm can be estimated in-silico, but little is known how this value holds in LC conditions with mobile phases consisting of organic additives and ion-pairing buffers. We measured Tm values with differential scanning calorimetry (DSC) for siRNA sample and compared it to Tm estimated with chromatographic techniques including reversed-phase (RP) LC, hydrophilic interaction chromatography (HILIC), size-exclusion chromatography (SEC) and ion-pair RP LC. The implications of Tm on LC analysis of siRNA, sgRNA, mRNA and nucleic acids with strong secondary structure are discussed.

125 Capillary Gel Electrophoresis Separations of DNA: From Fragments to Plasmids

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Capillary gel electrophoresis is a rapid and automated approach for DNA analyses. The gel matrices used to separate DNA molecules are selected to resolve specific size ranges for different analytical methods. A wide range of gels are used for DNA sieving, including cross-linked gels, linear polymers, and nanogels. Unlike classical gels that are comprised of entangled linear polymers, nanogels self-assemble to form entangled ribbons and interconnected networks to enable sieving. Self-assembled nanogels are thermally responsive, exhibiting a low viscosity at temperatures below 24 °C and high viscosity indicative of gelling at higher temperatures. A practical benefit of self-assembled nanogels is that they are easily loaded and expelled in the capillary at low temperatures and then are reversibly gelled in-capillary for the sieving separation. Additionally, the sieving size range of a nanogel can be tuned

by controlling the temperature, concentration, and composition of the preparation. These features are demonstrated with separations of DNA for a range of applications. Practical aspects of DNA separations, including the use of modified and unmodified silica capillaries are also discussed.

126 Experiential Forensic Science Research with Portable Spectrometers

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Portable spectrometers have become important tools in a number of different industries, including forensic science, due to their ability to provide rapid, reliable, and actionable information at the sample site. These properties, in addition to having a small physical footprint, also make these chemical instruments incredibly useful tools for both undergraduate and graduate education. Each type of portable instrument (e.g., portable Raman, FTIR, NIR, GC-MS, HPMS, IMS) has their own capabilities and limitations, and thus research requires students to develop both analytical chemistry and critical thinking skills when evaluating potential applications. This presentation will focus on the ways I use portable spectrometers in university research projects for the detection and identification of illicit and counterfeit drugs, explosives, body fluids, electronic storage devices, and other forensic traces.

127 Portable Raman Spectrometers, Fluorescence, and Lego Blocks

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We have proposed the use of a set of Lego blocks as 'standard' samples to evaluate the performance of Raman spectrometers on colored and dark samples (Richard A. Crocombe, Brooke W. Kammrath, Pauline E. Leary, Thomas J, Tague and William D. P. Costa, "LEGO blocks as "Standard" Samples for Evaluation of Fluorescence Avoidance and Mitigation in Raman Spectroscopy", Appl. Spectrosc. 2024. 78(3): 340-348). These blocks have the attractive properties of being very low cost, rugged, non-toxic, easy to transport and store, and appear to be manufactured using a standard process. In addition, sample presentation to the spectrometer is straightforward and reproducible, and they appear to have uniform compositions, so that the choice of sampling location and area is not important. In that previous paper we showed unprocessed spectra obtained for these blocks using laboratory Raman spectrometers, obtained with 532, 638, 780/785 and 1064 nm excitation, and also showed their visible-near-infrared spectra. The importance of fluorescence avoidance and/or mitigation for portable Raman instruments cannot be overstated, especially as the number of portable Raman instruments sold per year (estimated as around 5000) is more than twice the unit volume of laboratory Raman instruments (estimated at around 2000). Portable Raman instruments are designed to be 'point-and-shoot' 'answer boxes', with minimal operator control over the data collection parameters. This paper describes the results of scanning these Lego blocks using ten different portable Raman spectrometers.

128 FDA's Satellite Laboratory Operations – Using a Field Deployable Toolkit for Rapid Drug Analysis at International Mail Facilities

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International mail can be a means to transport dangerous products into the United States. Because of the volume of mail processed by International Mail Facilities each year, additional scrutiny of packages will serve the interest of public health. In 2018, Congress passed the SUPPORT Act (Substance Use-Disorder Prevention that Promotes Opioid Recovery and Treatment for Patients and Communities), which led to the creation of the Satellite Laboratory Branch (SLB) within the Food and Drug Administration's National Forensic Chemistry Center. SLB scientists have developed an innovative approach to bring drug detection capabilities to points of entry throughout the country. A network of satellite laboratories located at selected international mail facilities and other remote sampling sites was established. Satellite laboratories are currently operating in Chicago, Miami, and New York City, and laboratories located in Los Angeles and Honolulu are scheduled to come online by the end of 2024. Analysts at each location employ a rapid screening "toolkit" to examine unknown (unlabeled/mislabeled) products for the presence of active pharmaceutical ingredients. The "toolkit" consists of a handheld Raman spectrometer, a portable Fourier transform infrared (FT-IR) spectrometer, a portable atmospheric pressure mass spectrometer (either a direct analysis in real-time mass spectrometer (DART-MS), or a rapid direct analysis atmospheric pressure solids analysis probe mass spectrometer (RADIAN-ASAP MS)) and a portable gas chromatograph mass spectrometer (GC-MS). Since 2021, a total of 1,699 products, 708,632 associated lot units, 1.3 kilograms of powder and 8.9 liters of liquid containing unapproved products have been prevented from entering the country.

129 Applications of Handheld Spectroscopy in Hazardous Materials Response

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In this presentation, I discuss the uses and applications of FTIR and Raman Spectroscopy handheld units for field identification of unknown solids, liquids, and gases during emergency response hazardous materials incidents. Hazardous materials incidents cover a wide variety of call types from naturals gas leaks, carbon monoxide exposures, and simple chemical spills to more complex incidents involving nefarious use of pharma-based chemicals, explosives, and chemical warfare agents. Handheld FTIR and Raman units are commonly used by technicians and specialists to identify unknown or unclassified substances for either public safety assessments, PPE selection, treatment protocols, decontamination process determination, or evidence collection. In most cases, this requires a bulk sampling technique to which FTIR and Raman handheld units are well suited. Hazardous Materials Technicians tasked with using these handheld technologies in emergency response are required to have at least a base knowledge of the strengths and limitations of each technique and understanding of how best to conduct bulk sampling with these technologies to get the most accurate results. Most field operators are not chemists and may only have a basic knowledge of chemistry and physics, therefore, many times with complicated mixtures and low absorbing or high fluorescence samples, they rely on the ReachBack services to assist in spectral analysis assistance. Often, technicians must use other presumptive classification techniques in conjunction with FTIR and Raman results to confirm the results given which takes years of training and experience to master.

130 Self-Optimized Development of Pharmaceutical Processes

Clarissa Wilding, University of Leeds, Woodhouse Ln., Leeds, LS2 9JT, United Kingdom, Richard Bourne

This talk focuses on the development of automated continuous flow systems. In particular recent research on self-optimising systems where the reactor and its process control instrumentation become an autonomous unit into which the reactants are pumped, and from which products emerge with optimized. This presentation outlines the recent grant 'Cognitive Chemical Manufacturing' and the new approaches to synthesis of fine chemicals and pharmaceutical compounds. These automated systems work without human intervention and are capable of very robust experimentation and rapid optimisation of challenging processes. This talk focuses on optimisation of multiple unit operations including optimisation of telescoped reactions and reaction followed by continuous work-up. I also explore the use of algorithms capable of optimising the trade-off between conflicting objectives such as yield and reactor productivity.

Bayesian Self-Optimization for Telescoped Continuous Flow Synthesis, Angewandte Chemie International Edition, 2022, 10.1002/anie.202214511

Automated stopped-flow library synthesis for rapid optimisation and machine learning directed experimentation, Chemical Science, 2022, 13,1208

Machine learning directed multi-objective optimization of mixed variable chemical systems, Chemical Engineering Journal, 2023, 451, 138443

131 Iterative Optimization Technology: A Calibration-Free Modeling Approach for Monitoring Active Ingredient in Pharmaceutical Blends

Md Nahid Hasan, Duquesne University, Graduate School for Pharmaceutical Sciences, Pittsburgh, PA 15217

Process analytical technology (PAT) plays a vital role in monitoring and controlling of pharmaceutical continuous manufacturing process. Spectroscopy-based PAT is commonly utilized in solid oral doses form (SODF) manufacturing. Complex multivariate data obtained from PAT sensors require chemometric model to extract process related information. The model development process sometimes may require extensive effort, time and material, which is not convenient, especially at the early product development stages. Recently, a calibration-free/minimum calibration method, called iterative optimization technique (IOT), is getting attention within academics and research and development communities due to its material-sparing, cost-effective and fast development approaches. Since the method is based on solving an optimization problem per spectrum basis, numerical solver used in finding optimal solution plays an important role. The roles of numerical solver and solver parameters used need to be explored for obtaining consistent solutions. In addition to prediction results, model diagnostics, which help distinguish unusual sample spectra from regular ones, are also important to have confidence in the predictions form the model. Choice of a suitable numerical solver, use of appropriate level of solver parameters (e.g., initial point related to feasibility region, constraint level, convergence criteria, etc.), and monitoring internal checkpoints and model diagnostics are essential for successful application of IOT algorithm in monitoring active ingredients in continuous manufacturing of SODF.

132 Deconvolution of Non-linear Surfaces Using Gaussian Mixture Models: Applications to Hyperspectral Images

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Hyperspectral imaging (HSI) is gaining traction in the chemical imaging field due to its ability to provide detailed chemical information about a material's surface. However, identifying and mapping the unique components present on a material's surface can be challenging. Techniques like Multivariate Curve Resolution (MCR) can be effective for linearly deconvoluting the contribution of each component, but they fall short when the components interact in a non-linearly. Here, we propose an alternative approach in which the pixels of the hyperspectral image are clustered based on spectral similarity using a Gaussian Mixture Model (GMM). The GMM assumes the dataset consists of mixtures of gaussian distributions, iteratively clustering data points based on parameters like cluster centroids and covariance matrices through the Expectation-Maximization (EM) algorithm. A limitation of the GMM is that it does not inherently provide an optimal number of clusters, which we address by applying the Integrated Complete Likelihood (ICL) criterion—a metric that accounts for model complexity and statistical entropy. By applying the GMM in combination with the ICL, we can obtain unique chemical maps of material surfaces. In this work, we present results from applying this approach to various HSI techniques, including Diffuse Reflectance Spectroscopy (DRS) for archaeological paintings, Raman micro-spectroscopy mapping of TiO2-II-bearing grains collected from Neoarchean spherule layers, and photoluminescence mapping of strained Ga2Se2 flakes for quantum technology applications.

133 Scalable Continuous Photochemical, Electrochemical and Thermal Reactions: Reactors and PAT Challenges: Exploring Unlocking Molecular Landscapes with Excitation Emission Matrix (EEM) Fluorescence Spectroscopy

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Photochemistry and electrochemistry are potentially very powerful tools for manufacturing not least because energy is delivered to reacting molecules far more selectively than by bulk heating. In recent years we have been addressing some of the challenges of making photochemistry and electrochemical synthesis greener, more energy efficient and more widely accessible. This presentation will cover our activity particularly aiming for generic approaches for linking and scaling up multi-step processes in the context of photo-, electro- and thermal- chemistry on the kg/day scale with emphasis on singlet O2 and the synthesis of the anti-malarial drug Artemisinin as well as recent advances in simple reactor designs for using both visible focussed on continuous photo-redox and oxidative and reductive electrochemical processes enabling 1-10 kg/day productivity in Taylor Vortex reactors with a very small footprint. Existing and new PAT approaches that exploit autonomous flow reactors and self-optimisation will be discussed with the aim of improving sensitivity, specificity, dynamic range and the speed of data acquisition, to be coupled with AI innovations. In this presentation we also focus on using Excitation Emission Matrix fluorescence spectroscopy, a rapid technique, which is not only information "rich" but also very sensitive. This work explores the potential that this approach offers to provide key insights into vital parameters, such as molecular environments, configurations and dynamics. Analysis of EEM spectra using a variety of multivariate methods, allows us gain insights into these parameters which are often obscured in traditional fluorescence spectroscopy. Here we utilize the advantages of EEM fluorescence spectroscopy alongside other spectroscopic techniques to monitor reactions in real-time and autonomously optimize, model and probe key reaction metrics for algorithmically driven process control.

134 Elastic Flow Instability Using Polymer Additives to Improve the Efficiency of Packed High-Pressure Liquid Chromatography Columns

Fabrice Gritti, Waters Corporation, 34 Maple St., Milford, MA 01757, Emily Chen, Sujit Datta

The optimal efficiency of today's packed high-pressure liquid chromatography (HPLC) columns is hindered by long-range flow heterogeneity occurring from the wall to the central region of the column. One highly attractive solution to address this structural defect of slurry-packed HPLC columns involves accelerating the rate of analyte exchange across all flow streamlines. Acoustofluidics across microchannels, turbulent flow of supercritical carbon dioxide across 250 µm open tubes, and electroosmotic flows across 5 µm open channels have been successfully applied, but they are limited to the microscale and may require external electrical power. Alternative passive solutions are needed for larger scale 4.6 mm i.d. HPLC columns, which are extensively used today in the biopharmaceutical industries.

In this study, we investigate the potential of developing elastic flow instability in the interparticle volume of a chromatographic column by adding a polymer additive to the mobile phase. The rheological measurement of 500 ppm 18 MDa partially hydrolyzed polyacrylamide (HPAM) in a 1% NaCl water solution reveals the desired stress thickening property of the mobile phase. Spatio-temporal fluctuations are generated and facilitate the transport of the analyte across the different flow streamlines. We demonstrate that elastic flow instability occurs in a 4.6 mm x 300 mm column packed with 10 µm BEHTM 125 Å particles. Permeability (friction factor versus Reynolds number) and axial dispersion (thiourea injection) measurements are presented, revealing the mitigation of the negative impact of trans-column flow heterogeneities on the efficiency of the HPLC columns through the spatio-temporal fluctuations induced by HPAM polymer.

135 Separation of Permanent Anions, Neutral Compounds, and Weak Acids Using Sequential Elution Liquid Chromatography with Tandem Columns

Lauren Lovejoy, GSK, 1250 S. Collegeville Rd., Collegeville, PA 19426, Joe Foley

Sequential elution liquid chromatography (SE-LC) separates classes of compounds by group by employing two or more selective elution modes. Advantages to using SE-LC over conventional HPLC are (i) a higher peak capacity and (ii) a reduced separation disorder. Yet, the same instrumentation employed for conventional HPLC may be used for a SE-LC. Here, the development of an analytical method by an alternative separation approach, SE-LC, to separate permanently charged anions, weak acids, and neutral compounds using anion exchange and reversed-phase columns in tandem is described. Mobile phase selection and gradient optimization are shown to be integral for the appropriate SE-LC method for separating anions, weak acids, and neutral compounds from each other, in their respective groups and will be discussed in detail in this presentation. The effects of mobile phase pH, formate concentration, sodium methanesulfonate concentration, and acetonitrile concentration on analyte retention were examined and optimized. The most successful (best resolution and repeatability) SE-LC separation was achieved by applying a low pH isocratic elution to elute the weak acids, followed by an acetonitrile gradient to elute the neutral compounds, and last a sodium methanesulfonate gradient to elute the anionic compounds using an Agilent Poroshell C18 column coupled with a Waters strong anion exchange (SAX) column. The separation of nine model analytes was achieved in less than 15 minutes, with excellent analyte peak area and retention time RSDs, for three replicate injections. This unique method of separating different classes of compounds in groups, shows promise for complex analytes for further exploration.

136 Benefits of Inert Liquid Chromatography Column Hardware for Various Applications

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Stainless steel has been the preferred material to manufacture HPLC columns due to its ease of fabrication and mechanical resistance to pressure. Metals have been known to cause metal-analyte interactions that may lead to poor peak shapes, low recoveries, and overall poor performance for metal-sensitive analytes. This type of non-specific adsorption has been documented in the literature and several solutions have been commercialized in recent years to mitigate such interactions. Inert coatings prepared via chemical vapor deposition offer one of the best solutions for improving the analysis of compounds prone to chelation. This study leverages both Biphenyl and ARC-18 stationary phases packed in inert hardware for the analysis of mycotoxins, psychedelic alkaloids, and pesticides. Results showed that the inert Biphenyl solution for mycotoxins offered significantly higher sensitivity and peak areas without the need for chemical passivation or repeat injections, while mitigating the carryover of several highly adsorptive analytes (i.e., fumonisins and tenuazonic acid). Similar results were observed for many of the phosphorylated psychedelic alkaloids found in mushrooms. Using an inert ARC-18 column, notably higher sensitivity and peak areas were observed for metal-sensitive pesticides. A survey of other LC passivation techniques will also be discussed. Overall, the implementation of inert coatings in HPLC column hardware offers robust and improved chromatographic performance without the need to alter already established methods and workflows.

137 UPLC Columns: Past, Present and Future

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

Since their introduction twenty years ago, UPLC columns have become widely used in an ever-expanding range of applications. By reducing the particle size to 1.7 µm, column efficiency per unit length is increased about three-fold relative to columns packed with 5 µm particles. Columns packed with the smaller particles also have higher optimal linear velocities, providing faster separations while maintaining high column efficiencies. The use of microbore columns (1 – 2.1 mm i.d.) enables higher detection sensitivity as well as reduced solvent consumption compared to 4.6 mm i.d. columns. In this presentation, we will review the history and evolution of UPLC columns. Examples of separations that benefit from UPLC will be highlighted, including applications using reversed-phase, hydrophilic interaction and size-exclusion chromatography for analytes ranging from small molecules to biopolymers. We

will also discuss recent advancements that further expand the capabilities of UPLC column technology.

138 **Fast RP-UHPLC Separation of Ribooligonucleotide Impurities Using Evosphere MAX C18/AR Monodisperse Fully Porous Particle Columns without Ion-Pair Reagents for Simplified Oligo Separations**

Edward Faden, MAC-MOD Analytical, 103 Commons Ct., Chadds Ford, PA 19317, Geoff Faden

Ribonucleotide synthesis, like any chemical process, can introduce impurities due to various factors inherent in the oligo-synthesis method and the nature of the reagents involved. To mitigate the presence of these impurities in synthesized ribonucleotides, rigorous purification methods, such as high-performance liquid chromatography (HPLC), gel filtration, or solid-phase extraction, to isolate and obtain high-purity products suitable for downstream applications have to be applied. Ion-pair reagents and inorganic salts are commonly used in RP-UHPLC to enhance the separation of nucleic acids. However, their presence can complicate downstream applications, increase instrument maintenance requirements and reduces the MS sensitivity due to ion suppression. This research is aimed to develop a simple and efficient ion-pair reagent-free chromatographic method for the separation of ribooligonucleotide impurities utilizing a novel bonded phase chemistry and novel monodisperse particle design packed in an inert coated hardware. We will show a robust LC-UV method for the separation of ribooligonucleotide impurities using the Evosphere MAX C18/AR stationary phase. The main advantage of the method is the use of ion-pair-free and inorganic salts-free mobile phases for this challenging separation. This new developed method in conjunction with Kopernicus University in Poland eliminates these additives, simplifying the chromatographic system and making the analysis more compatible with downstream applications such as mass spectrometry or biological assays. This approach also reduces the risk of ion suppression effects, which can occur when ion-pair reagents interfere with ionization in mass spectrometry.

139 **Accelerating High Throughput Thermodynamic Solubility Screening in Drug Discovery Using LC-MS**

Muhammad Qamar Farooq, Amgen, 1 Amgen Center Dr., Thousand Oaks, CA 91320, Adrian Carranza, Wes Barnhart, Nuria Tamayo, Imad Haidar Ahmad

Solubility is a key physico-chemical property of drug candidates because it affects absorption, distribution, metabolism, and excretion (ADME) of drugs. Solubility assays play a critical role in identifying potential drug candidates, their pharmacokinetics, and bioavailability. Solubility assays are used to identify the compounds with potential liabilities, optimize the chemical structure of the potential drug candidates and to validate the results from other assays. Thermodynamic solubility is obtained based on the concentration of the dissolved material, when reaching equilibrium with insoluble material. Thermodynamic solubility depends on several factors such as pH of solvent, incubation time, and detection method. Even though it is a time consuming process, the optimization of these parameters is critical to obtain precise, reliable, and accurate results. Herein, we used a set of meticulously chosen standards, covering a broad solubility range, to optimize the thermodynamic solubility assay currently being utilized in our discovery labs. Solubility was evaluated in different biorelevant liquids of varying pH. The pH dependent solubility of the drug can provide insights into its pharmacokinetics and bioavailability. Another important aspect of the solubility assay is the incubation time required to reach thermodynamic equilibrium, which is the bottleneck in determining thermodynamic solubility. We carefully optimized the incubation time by studying the solubility-versus-time curves for several standards to derive precise and accurate thermodynamic solubility values in minimal incubation time. Furthermore, this workflow was designed to be high throughput by using 96-well plates, a 2.2 min LC-MS analysis method, and analysis software for facile data processing and results generation.

140 **Analytical Method Development for Chromophore-Lacking Formulation Excipients in Gamma-Irradiated LAIs**

Suraj Hettiarachchi, GSK, 1250 S. Collegeville Rd., Collegeville PA 19426

Delivering efficacious medicines to patients in a highly regulated pharmaceutical industry poses technical challenges and unique opportunities. While safety and effectiveness of a drug product are paramount, manufacturing at high production volume requires adhering to stringent quality standards. For parenteral drugs, like Long-acting Injectables (LAIs), sterility is one such standard achieved through terminal sterilization using gamma-irradiation. The high penetration power of gamma-irradiation is favorable to achieve sterility in fill-finish drug product vials with minimal damage to active pharmaceutical ingredient (API). However, analyzing the stability of other formulation excipients (i.e., polyethylene glycol; PEG, and polypropylene oxide block copolymer; poloxamer) is important to preserve long term-physical stability of LAIs. The complex nature of LAI formulation matrices with multiple excipient compositions and ratios prompted development of novel analytical methods for these chromophore lacking analytes. To this end, a reverse-phase liquid chromatography (RPLC) method using a charged aerosol detector (CAD) was developed. The method was optimized for increased throughput (10 min run time), and versatility in analyzing different excipients in a single run with reduced consumable expenditure and analysis time. Key learnings from the use of this RPLC-CAD method in developing excipient degradation mitigation strategies for several LAIs in development will be discussed.

141 **Advancements Towards a Universal, Sensitive, and Selective Detection Technology for Liquid Chromatography**

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To overcome the limitations of current detection technologies, there is a pressing need for a universal, sensitive, and selective LC detector capable of accurately detecting all types of compounds, irrespective of their physiochemical properties, while providing a consistent response. Such a breakthrough enables the detection and precise quantification of a wide array of components in a sample. This technology is particularly crucial for characterizing unknown compounds, facilitating non-targeted analysis, and addressing situations where isolated standards for each component may be unavailable. This talk endeavors to shed light on a novel and promising detection technology that leverages the high energy, short-wavelength light characteristic in the vacuum ultraviolet (VUV) range of the electromagnetic spectrum to detect a wide array of molecules. The potential implications and application of this technology will be discussed using several challenging real-world examples including underivatized amino-acid analysis and cholesterol analysis both of which present unique analytical challenges using existing analytical approaches.

142 **Modernization of a Legacy Normal Phase Method on a Modern HPLC System**

Elom Pedanou, Waters Corporation, 34 Maple St, Milford, MA 01757, Lise Gauthier, Paula Hong

In regulated laboratories, particularly those within the pharmaceutical industry, high-performance liquid chromatography (HPLC) systems play a crucial role in routine analysis. However, some challenges arise due to legacy methods that were developed during the early days of HPLC technology. These methods have not kept pace with recent advancements and modernization in the field and do not fully leverage the latest technological improvements. Advancements in column chemistry and system robustness allow for better separation and detection of analytes. Modern HPLC systems are more robust, reliable, and capable of handling challenging analyses and can accommodate various chromatographic modes, including Normal Phase chromatography. While legacy methods persist, regulatory agencies such as the United States Pharmacopeia (USP) provide guidance on how to adapt and adjust these methods. This helps bridge the gap between legacy methods and modern HPLC systems. In this study, we examine the modernization of a legacy normal phase method, USP Propofol Assay, for use with a HPLC system. The original method specified an older L3 column in a non-standardized column configuration (4.6 mm x 200 mm). Given these dimensions the only available columns contained older stationary phases. Thus, the column tested did not meet system suitability criteria, including peak tailing, necessitating an upgrade. Traditionally, users might couple two columns together using a rigid column connector to meet column requirements and achieve system suitability criteria. However, our study demonstrates that coupling may not be necessary. Instead, we propose scaling the column and L/dp – within chapter 621– to achieve improved results.

143 **Improving Chromatography for Basic Analytes Using a Positive Charge Surface Material**

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Obtaining good chromatography of basic analytes in terms of peak shape can be challenging. Traditional reversed phase (RP) stationary phases can have limitations for such separations, where peaks tail due to secondary interactions between basic analytes and the underlying silica packing surface, or by other processes. Superficially porous particles (SPP) of silica modified with a positively charged ligand demonstrate improvements in RP separations of basic, small molecules as well as peptides. Silica based positively charged surface RP chromatographic packings have been previously shown to significantly improve peak asymmetry and load tolerance of basic analytes. These benefits are notable when operating under low ionic strength mobile phases common in RP LC mass spectrometry (LC-MS) applications. Examples of LC and LC/MS analysis for small molecule drugs along with synthetic peptides will be shown using the positive charge surface material demonstrating improved peak shape when compared to more traditional stationary phases.

144 **Enhancing Quantitation Accuracy and Minimizing Analyte-to-Analyte Variability for High-Throughput Liquid Chromatography - Charged Aerosol Detection (CAD) Methods**

Imad Haidar Ahmad, Amgen, 1 Amgen Center Dr., Thousand Oaks, CA 91320, Wes Barnhart, Daipayan Roy, Troy Handlovic

Charged aerosol detection (CAD) has become a valuable tool for fast and efficient quantitative chromatographic analysis of drug substances with weak UV absorption.

Previous LC-CAD studies have focused on practical applications, as well as the optimization of critical parameters such as response dependencies on temperature, nebulization process, analyte volatility, and mobile-phase composition. Optimizing the power function value (PFV) is another crucial parameter for obtaining a more linear detection signal with higher signal-to-noise ratio (S/N) and lower relative standard deviation (RSD) of area counts. Herein, we report a systematic investigation of different regression models (log-log, first- and second-degree polynomial) using both interpolation and extrapolation processes, combined with PFV optimization throughout the development of LC-CAD assays (error<5%). We also introduce the use of a system suitability probe to ensure the optimal performance of the main and inverse gradient pump in delivering an isocratic mobile phase composition to the nebulizer in the CAD detector, leading to improved reproducibility and minimized analyte-to-analyte variability. The proper selection of regression fitting and ensuring the delivery of an isocratic composition to CAD are crucial for developing quantitative LC-CAD assays for poor UV-absorbing pharmaceuticals that are sensitive, accurate, and robust across various stages of pharmaceutical development.

145 Enhancing LC and LC-MS Separations of Basic Compounds with Novel High pH Stable SPP Columns

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Many drug substances and their metabolites are basic compounds and developing separation methods to analyze them can be challenging with respect to peak tailing, inadequate retention, and unsatisfactory resolution. When these factors are combined, they have the potential to hinder the accurate quantification and purity assessment crucial for drug development and quality control. Utilizing pH increases method development flexibility for basic analytes. This presentation explores the significant advancements achieved by leveraging a high pH stable stationary phase designed on superficially porous particles. Results show that the selectivity and retention of basic compounds can be significantly improved under high pH conditions. Case studies featuring small molecule pharmaceuticals and oligonucleotides will be presented to illustrate the practical applications and benefits of high pH C18 columns in real-world scenarios. Additionally, stability data will be presented to illustrate the long column lifetimes of the high pH C18 SPP columns.

146 Authentication of Powdered Mushroom Fruiting Body by High-Performance Thin-Layer Chromatography

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Mushrooms have been part of the diet in several countries for many years and are rich in polysaccharides, dietary fibers, and other micronutrients. They have also been reported to have a wide range of human health benefits e.g. antioxidant, antidiabetic, antibacterial, antiviral among others. Triterpenoids, sterols and β-D-glucans have been described as active compounds. To ensure that the beneficial aspects of the mushrooms remain, a quality control analysis of the materials is needed. The determination of β-D-glucans and ergosterol have been targeted for this purpose. High-performance thin-layer chromatography (HPTLC) has also been used to identify mushrooms e.g. *Ganoderma lingzhi* (Reishi mushroom). HPTLC is a well-known approach to identify botanicals and has been used herein to analyze 17 mushroom fruiting bodies, including 2 varieties by using HPTLC methods targeting different groups of metabolites. The method used to ID the mushrooms herein was also transferred into a fully automated PRO system.

147 Foraging for Methods: The Right Method for the Right Spec

Khanh Tran, Alkemist Labs, 12661 Hoover St., Garden Grove, CA 92841, Sidney Sudberg, Anthony Fontana

Exploring different methodologies for testing mushrooms and mushroom containing products. Techniques such as microscopy, high performance thin layer chromatography (HPTLC), UV/Vis, and high-performance liquid chromatography (HPLC) are routinely used with mushrooms. Varied and fit-for-purpose analytical techniques to establish correct mushroom identity, to detect contamination of fungal growth, and to quantify chemical markers will be discussed.

148 Decoding Hair: Adapting Scalp Hair to Resemble Body Hair for *In-Vitro* Skin Models

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We have pioneered a novel in vitro assay that employs a silicone skin model integrated with individual scalp hair fibers for assessing the efficacy of hair removal treatments. This model, designed to mimic human body temperature, facilitates precise evaluations of depilatory products in a controlled environment. Our findings suggest that, despite having similar fiber diameter and cuticle thickness, scalp hair incorporated into our silicone model is more resistant to removal treatments than body hair. To investigate this discrepancy, we analyzed the variations in lipid and protein composition using high-performance thin-layer chromatography (HPTLC) and SDS-PAGE electrophoresis. Our research also focuses on adjusting the properties of scalp hair to mimic the behavior of body hair under depilatory treatment. This presentation will explore the differences between scalp and body hair, offering new insights into improving *in vitro* hair removal models. Our goal is to enhance the realism of depilatory testing, potentially impacting the industry by providing better testing tools for different body areas.

149 USP Standards for Passion Flower (Passiflora incarnata L.): Species and Chemotype Differentiation by HPTLC Analysis

Maria Monagas, United States Pharmacopeia, 12601 Twinbrook Pkwy., Rockville, MD 20852, Mirtha Navarro, Felipe Vargas, Eike Reich, Wilmer Perera

Passiflora L. (family *Passifloraceae*) is a very large genus comprising more than 500 species, native to North, Central, and South America, and introduced in Europe, Asia and Africa. *Passiflora incarnata, P*. edulis and *P. alata* have been traditionally used as herbal medicines. *P. incarnata* aerial parts, commonly known as Passion flower, is a popular botanical in dietary supplement applications for anxiety and sleep problems. Passionflower contains C-glycoside flavones, mainly vitexin and its isomer isovitexin, which are considered the putative bioactive or marker compounds in this plant. In addition, different chemotypes exist at the commercial level, including the swertisin chemotype or the isovitexin chemotype, which are well-known in European herbal trade. However, the existence of these chemotypes in Pan American trade needs further research. The demand for Passion flower as an adaptogen and the different challenges associated with the different species, chemotypes, and cultivation regions require creation of robust global standards for this plant and its derived ingredients. This presentation provides an overview of the development and validation of an HPTLC method for the new USP monograph family for Passion flower. A total of 76 samples of *Passiflora L.* comprising both plant materials and derived extracts from different species, chemotypes, and cultivation regions were used for this study. The method uses two derivatization reagents (NP reagent and Anisaldehyde) and detection modes (light and longwave UV light). The HPTLC method was validated according to USP General Chapter <203>. Examples of the application of this method to address the different analytical challenges are presented.

150 HPTLC Identification Tests for Botanical Dietary Supplements and Herbal Medicines in USP Monographs

Cuiying Ma, United States Pharmacopeia, 12601 Twinbrook Pkwy., Rockville, MD 20852

cGMP Practices for dietary supplements require specifications for identity, purity, strength and composition, and limits of contaminants using scientifically valid testing methods. Identification of botanical dietary supplements and herbal medicines is challenging due to their complexity which includes the number of known and unknown constituents. In USP botanical quality standards (monographs), identification includes morphological, histological, and chemical characteristics. Chemical identification tests for plant material and derived botanical extracts are mainly based on secondary metabolites, also known as marker compounds, which tend to be specific to different plants. Plant material usually contains multiple categories of marker compounds. USP botanical monographs typically include both HPLC and HPTLC tests to achieve chemical identification to test as many as possible marker components by comparison against suitable reference standards (RS). This presentation will be focused on the application of HPTLC identification test for Botanical Dietary Supplements and Herbal Medicines in USP Monographs, including test procedures with standard parameters according to <203> *HPTLC Chromatography Procedure for Identification of Articles of Botanical origin*; requirements for method development, validation and RS selection; specific monograph examples illustrating the identification of different types of botanical ingredients and the specificity of this technique to respond to different quality challenges related to identity such as the differentiation of closely related species and the detection of potential adulterants.

151 Identifying and Quantifying Pesticides in Environmental Deposition Using Gas Chromatography High-Resolution Mass Spectrometry

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Pesticides are chemical substances that are specifically designed and used for controlling, repelling, or killing pests such as insects, fungi, rodents & weeds. While pesticides are useful for things like controlling pests to allow for crop growth, pesticide deposition can ultimately cause harm for human and environmental health. This project aims to explore the presence of pesticide deposition in the environment via samples collected from wet deposition. The samples were analyzed using Gas Chromatography Quadrupole Time-of-Flight Mass Spectrometry (GC-QTOF-MS), and a targeted list of 407 compounds was created from the data set. To successfully identify pesticide/pesticide-related compounds, compounds had to have an MS/MS match, meaning the sample spectrum had to match well enough with a library spectrum, and a retention index (RI) match, meaning the library RI value had to match with the calculated RI value. A total of 14 pesticides were tentatively identified and a

total of 8 were confirmed with reference standards. We will quantify confirmed compounds and conduct statistical analysis to assess seasonal trends. These findings show that rainwater is a mechanism of transport for pesticides.

152 **Binding of Naphthenic Acids to Dissolved Organic Matter**

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The goal of this study was to investigate the binding of naphthenic acids, a major contaminant in the Canadian oil sands, to humic acid, a major component of dissolved organic matter (DOM), to achieve the long-term goal of improving remediation methods for oil sands pollution. The mechanism of naphthenic acids (NAs) binding to humic acid was investigated using Stern-Volmer analysis of fluorescence quenching data. To determine which functional groups of NAs impact binding strength to humic acid, a series of model compounds to isolate common functional groups were used, and it was found that smaller, more compact NA model compounds have higher binding constants with humic acid. To further analyze the interaction between humic acid and model NA compounds, electronic structure calculations were employed using Gauss View. Geometric optimizations were performed on the NA compounds in isolation to establish a baseline. Subsequently, these model compounds are being placed in proximity to humic acid molecules to simulate potential binding interactions and permit the identification of specific regions on the humic acid where the NA compounds are most likely to bind. Understanding the binding mechanism between NAs and humic acid in DOM may be used to inform the development of better environmental remediation methods.

153 **Using Raman MCR to Investigate Interfacial Water Signal at the Surface of Vesicles**

Phuong Ho, The Pennsylvania State University, 445 Waupelani Dr., State College, PA 16801, Alexander Lott, Olivia Fiebig, Paul Cremer

Raman spectroscopy is increasingly used in many fields because it is noninvasive and requires minimal sample preparation. It provides detailed information about the chemical composition of the sample; however, its application to lipid vesicles in solution is challenging due to the light scattering caused by the vesicles and the low interface specific water signal compared to the bulk water signal. In this work, we optimized the vesicle size to minimize scattering and obtained Raman spectra of the lipid vesicles in solution. By applying a multivariate curve resolution (MCR) algorithm to the Raman spectra, we were able to resolve the interfacial water structure around the vesicles and investigate how this changes with different vesicle and solution properties. Our results show that Raman MCR is a promising technique to study lipid vesicles in aqueous solutions.

154 **Advancing Environmental Monitoring: Rapid Quantitation of 28 PFAS in Aquatic Insect Tissue Using QuEChERS Extraction Coupled with UPLC-MS/MS**

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Per- and polyfluoroalkyl substances (PFAS) are synthetic chemicals of significant environmental concern due to their persistence, bioaccumulative properties, and toxicity. This study focuses on the detection and quantitation of PFAS in aquatic larval insects, particularly nymphal dragonflies, which are frequently exposed to contaminated aquatic environments. A novel analytical method was developed using the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) sample extraction technique, followed by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). This method enabled the detection and quantification of 28 PFAS compounds in insect tissues, with method validation demonstrating method detection limits (MDLs) ranging from 0.5 ng/g to 2.0 ng/g, recovery rates between 71.0% and 102.8%, and relative standard deviations (RSDs) spanning 2.0% to 4.6% at a 20.0 ng/mL analyte concentration. The analysis of 15 wild-caught nymphal dragonfly samples revealed the presence of several PFAS, including perfluorooctanesulfonic acid (PFOS), perfluorooctanoic acid (PFOA), and other homologs, with PFOS consistently detected across all samples. This validated method provides a robust and efficient analytical approach for the assessment of PFAS contamination in aquatic insects, offering valuable insights into the environmental distribution and potential ecological impacts of these persistent pollutants.

155 **Direct Enantiomer Differentiation of Drug and Drug-like Small Molecules Using Noncovalent Copper-Amino Acid Complexation and Ion Mobility-Mass Spectrometry**

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Drug enantiomers are "mirror image" isomers which are otherwise identical in both chemical composition and structural connectivity yet possess striking differences in pharmacological properties. There is considerable clinical interest in developing analytical methods to differentiate drug enantiomers. However, enantiomers exhibit few distinctive physicochemical properties which enable differentiation, making them one of the most challenging classes of structural isomers to resolve. Here, we report a method for differentiating chiral drug small molecules by binding enantiomers within a ternary noncovalent complex of the form $[(M)(^{L}His)(Cu^{2+}) - H]^{+}$ and measuring the resulting structural differences using ion mobility-mass spectrometry (IM-MS) incorporating a high-resolution ion mobility (HRIM) platform. Enantiomer differentiation was achieved for four drug enantiomer pairs ($^{R/S}$thalidomide, $^{R/S}$baclofen, $^{R/S}$metoprolol, and $^{D/L}$panthenol) with two-peak resolution (R_{P-P}) values ranging from 0.7 (>10% valley) to 1.5 (baseline separation). Calibration curves relating HRIM peak area to enantiomeric excess (*ee*) enable quantitation of racemic thalidomide and metoprolol *ee* with residuals of 5.7% and 2.5%, respectively. Theoretical modeling provides structural insight into specific molecular orientations enabling separations, suggesting that configuration of the Cu-His complex relative to the drug's chiral center is crucial for gas-phase stereoselectivity. Additionally, these computational results hint at specific drug structural motifs which facilitate differentiation using HRIM. Collectively, these results establish proof-of-concept for rapid differentiation of chiral drugs using noncovalent complexation combined with IM-MS. While significant hurdles remain, this work has promising implications for integrated small molecule omics strategies beyond drug quality control—enabling metabolomics, molecular diagnostics, toxicology studies, and beyond.

156 **Ion Selective Electrodes: Quantifying the Upper Limit of Detection**

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Polymeric membrane-based ion selective electrodes (ISEs) are widely used in medical, environmental, and industrial fields. Understanding the working range of these sensors is critical for any application. The lower limit of detection of an ISE in the presence of various interfering ions is well described by selectivity coefficients: Kpot(I,J). However, no such system currently exists for the upper limit of detection. The upper working range of ISEs is limited by Donnan failure, the transfer of both the target ion and an interfering ion of the opposite charge sign (counter ion) from the sample into the sensing membrane. The point at which Donnan failure occurs is dependent on both the properties of the ion-selective membrane and the identity of the counter ions. In current literature, the upper detection limits are typically reported as a single value of the target ion activity, which fails to account for the effects that interfering ions have on the sensor's working range. This project introduces a coefficient for interfering ions of the opposite charge (X) to describe the upper detection limit: KpotX(I,X). This quantitative treatment of the upper detection limit allows users to more accurately compare the working ranges of sensors measured under different conditions. The coefficient can be used as a framework to better understand the effects of the ionophore, membrane stoichiometry, and matrix on Donnan failure enabling the design of sensors with optimized working ranges.

157 **Glycosaminoglycan Imaging by IR-MALDESI**

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Glycosaminoglycans (GAGs) are linear polysaccharides composed of repeating disaccharide units that make up proteoglycans which encompass a significant portion of the extracellular matrix. Chondroitin is a type of sulfated GAG that is present in cartilage and perineural networks (PNNs). The sulfate groups are labile, and as a result GAG mass spectrometry imaging (MSI) has not been established in literature. In this work, we demonstrate the spatial distribution of GAGs within rodent brain using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) and structural similarity index measure (SSIM). Formalin-fixed paraffin embedded (FFPE) mouse brain was dewaxed and delipidated using a series of washes. Antigen retrieval was performed to undo formalin-fixation, followed by a pneumatic application of chondroitinase ABC to enzymatically cleave and release disaccharides pertaining to chondroitin sulfate. Following enzymatic digestion, negative mode IR-MALDESI was used to ionize GAG disaccharides. IR-MALDESI is a hybrid ionization technique that combines the benefits of both MALDI and ESI and has successfully detected N-linked glycans in prior studies. Here, nonsulfated Di-0S, monosulfated Di-4S and Di-6S, and disulfated Di-2,6S and Di-4,6S were detected in rodent brain. Each of these disaccharides had unique spatial distributions that colocalized to the PNNs within the brain and were most abundant in the cortex region. SSIM determined that the disaccharides underwent mixed-mode ionization and formed multiple adducts with sodium and chlorine. This is the first study to detect and spatially localize GAGs within a biological tissue using IR-MALDESI, building the path for applicational studies investigating neurodegenerative diseases and their corresponding GAG signature.

158 **Decoding CD107a and CD107b Glycosylation**

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Glycosylation is an important, diverse, and heterogeneous PTM, key to many cellular functions and dysregulated in most diseases. CD107a and b are heavily glycosylated

proteins present in the lysosome of all cells, where they shield the membrane from lysosomal contents. They also protect T-cells and NK-cells from self-degranulation upon cell killing. Importantly, cancer cells display these proteins on the cell surface, allowing for tumor metastasis and migration via selectin binding. As such, many studies have sought to characterize glycosylation changes using glycoproteomics, where distinct glycosylation patterns have been detected across multiple cancer types. That said, these studies have been conducted exclusively using tryptic digests, resulting in limited glycosite coverage. We performed the most comprehensive glycoproteomic mapping of CD107a and b to date. We digested proteins using a combination of proteases thermolysin, GluC, and trypsin, and O-glycoprotease IMPa. Peptides were desalted, separated using reverse phase HPLC, and analyzed using an Orbitrap Eclipse Tribrid. RAW data was searched with multiple search algorithms and results were manually validated. Through our analysis, we confirmed occupancy of 17 and 15 N-glycosites, and 6 and 13 O-glycosites of CD107a and b respectively. In total, we analyzed over one thousand glycopeptides, decorated by over fifty different glycan structures. Importantly, we doubled the N-glycosite coverage compared to previous studies, and revealed O-glycosites that were suspected but not confirmed. Overall, this analysis sets important groundwork for the field, which we hope will be used by other studies to enable deeper characterization of these proteins in clinical samples.

159 The Applications of Spotfire for a Pharmaceutical Forensics Database

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The forensic investigation Spotfire database is a project initiated by the Forensics and Innovative Technologies (FIT) group at BMS New Brunswick to efficiently track investigations conducted on BMS commercial products. Initially designed to house foreign matter manufacturing investigation results, the database has evolved into a comprehensive platform that provides metrics and comparative results for various investigation types addressed by FIT. FIT handles investigation requests from manufacturing sites, corporate security, and product quality complaints. Completed investigation reports contain important information such as the affected BMS product, the event origin, and the analytical results. The information is then complied into an Excel notebook and visualized using Spotfire, a web-based database platform. Spotfire visualizations include graphs and tables that illustrate key relationships between results and investigation origins. These visualizations enable users to quickly identify trends that would otherwise require manual comparison of years' worth of raw data and documents. Currently, the database encompasses five years of investigation data, totaling over 1,200 investigations. Using Spotfire has made forensic investigation information more accessible, facilitating trend analysis for investigations handled by FIT.

160 Trends in Novel Psychoactive Benzodiazepine Content of Counterfeit Alprazolam Tablets

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As the most commonly prescribed benzodiazepine in the United States, alprazolam must be properly monitored to guard against abuse and imitation of the drug. While legitimate patients may be prescribed brand-name Xanax or one of the many generic alprazolam formulations in order to treat anxiety and panic disorders, this drug is also abused illicitly for its tranquilizing and euphoric effects. As a Schedule IV compound, this is relevant to the forensic chemist who may come across it as seized drug evidence. Furthermore, the various alprazolam tablets are not immune to being reproduced by illegitimate street manufacturers. These counterfeit tablets may look like their genuine counterparts but can be composed of any number of other compounds, commonly novel psychoactive benzodiazepines (NPBs). When sold on the street, these unknown compounds can have unexpected and possibly even fatal effects. These trends are therefore not only relevant from a forensic viewpoint but also on a national health level. To that end, routine casework handled by NMS Labs dating back to 2015 was reviewed to explore the trends observed in these counterfeit tablets. Six NPBs (adinazolam, clonazolam, bromazolam, etizolam, flualprazolam, and flubromazolam) were identified as the main components of these tablets, with bromazolam comprising more than half of all counterfeit alprazolam tablets analyzed since the end of 2022. Until an evolution in the make-up of these preparations is encountered, bromazolam remains the most likely component of any counterfeit alprazolam tablet identified in casework handled at NMS Labs.

161 Gas Chromatography-Flame Ionization Detector (GC-FID) System Linearity Effects on Limit of Detection Calculations

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The construction of an analytical calibration curve within a specified range is a requirement for all analytical methods and from which values are taken to calculate Limits of Detection (LODs). The curve is a plot of the signal, r(x), vs analyte concentration, x, with the relationship between the two obtained by a linear regression analysis generating a sensitivity or slope, m, and y-intercept value, b, $r(x) = mx + b$. LODs are calculated based on these linear regressions. One of the most common, adopted in 1975, is the IUPAC definition of LOD which is the lowest concentration of an analyte that an analytical process can reliably detect, $C_L = ks_b/m$. C_L is the lowest analyte concentration that will generate a reliable signal; k is a numerical factor chosen in accordance with a desired confidence level; s_B is standard deviation of blank responses; m is the slope of the analytical calibration curve. Therefore, slope, m, is an important value that must be well defined because it is highly dependent on the working concentration range and individual chemical measurement system itself. Using GC-FID, the linearity of the coupled system was investigated using Dodecane, Pentylbenzene, Nicotine, and Caffeine at concentrations ranging the dynamic range of the detector. The values generated were then pooled into various calibration curves representing different working ranges. LOD calculations were performed using three calculations methods and compared to the experimental LOD.

162 Reliable Extraction of Fluorophore Blinking Dynamics from Wide-Field Microscopy Videos

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Blink Based Multiplexing (BBM) is an emerging technique in the field of single-molecule spectroscopy that takes advantage of varying blinking dynamics to differentiate flurophores with high accuracy. The data required for BBM is similar to the data used for stochastic optical reconstruction microscopy (STORM), in which the stochastic blinking of fluorescent dyes is used to construct nano-scale images below the diffraction limit. However, the two techniques have not been combined. This project makes a foundational step towards combining BBM and STORM by developing an end-to-end data pipeline to process videos from EMCCD or sCMOS detectors into a reliable set of blinking signals which can be differentiated using BBM. A fully automated toolchain applies existing emitter localization algorithms, tracking methods, and a novel "refitting" algorithm. This algorithm uses data generated by localization algorithms to align a secondary curve fitter exclusively for fluorescence intensity, generating an intensity signal with a higher signal-to-noise ratio than achievable using alternative methods. In addition to super-resolved imagery applications, this pipeline provides for more rapid development and usage of BBM itself. Here, we highlight an application of this new pipeline to differentiate Rhodamine-B and Rhodamine-6G fluorophores.

163 Illicit Drug Desorption and Chemical Profiling of Fingerprints using SICRIT Ion Source: A Rapid Analysis Approach

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The focus of this study centers around fingerprints and how to conduct targeted and non-targeted analysis of analytes on this complex matrix by combining novel instrumental and computational approaches. A dielectric barrier discharge ionization (DBDI) sources, such as the SICRIT Ion Source, have been demonstrated to cover a wide range of analytes. This ionization source, in combination with thermal desorption sampling allows for a rapid analysis while minimizing sample preparation. The study employed a high-resolution mass spectrometer to identify unknown compounds based on exact mass, focusing on three drugs (Fentanyl, Heroin, Cocaine) in varying absolute amounts. Direct thermal desorption of samples, completed in just 2 minutes, revealed ionization of all three compounds as protonated molecules. The limit of detection (LOD) for pure substances and spiked fingerprints, even with a complex matrix of lipids and amino acids, demonstrated sensitivity suitable for detecting trace amounts found in forensic samples. The technology's forensic potential expanded to differentiate individuals based on chemical fingerprint profiles, proving effective even for non-volatile compounds like lipids. Principal component analysis (PCA) and a machine learning pipeline demonstrated the ability to distinguish fingerprints from different individuals, even across multiple days. Overall, this study introduces a rapid SICRIT Ionization Desorption setup for forensic analysis, identifying analytes within minutes, ideal for mobile systems.

164 Enhancing Overdose Surveillance: Targeted Method Development for Analyzing Psychoactive Substances in Urine

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Drug overdoses remain a leading cause of death in the United States, imposing significant economic and psychological burdens. To combat this crisis, the CDC has launched the Overdose Data to Action (OD2A) program to gather comprehensive overdose data. At the New Jersey Public Health & Environmental Laboratories (PHEL), we have developed a qualitative method to analyze urine samples for psychoactive substances. Our approach employs Ultra High-Performance Liquid Chromatography-Tandem Mass Spectrometry (UHPLC-MS/MS) using an Agilent 6495c QQQ system, enabling the identification of 66 different compounds in human urine, including opioids, hallucinogens, cannabinoids, stimulants, benzodiazepines, and

antidepressants. The method is validated for compound detection with reporting limits ranging from 0.5 to 2.5 ng/mL, and identification is confirmed through retention times and MRM transitions. The method has undergone rigorous evaluation through proficiency tests and received 100% scores. The method parameters and results will be discussed in the poster. The developed method will be applied to the 2000 yearly remnant urine samples received from the two participating hospitals in New Jersey. Testing results will help inform the CDC and local hospitals of emerging drug trends.

165 Application of Raman Imaging in Surface Mapping of Nano Carbon Based Composite Membranes

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Raman imaging is effective in studying the spatial distributions of different chemical components based on the Raman spectra. In this study, we utilized Thermo Raman microscope DXR2xi to obtain high-quality Raman spectra, and then to generate detailed chemical images of nanocomposite membranes. Composite membranes comprising of poly (vinyl alcohol) (PVA) incorporated with carboxylated carbon nanotubes (CNT-COOH), graphene oxide (GO), and GO-CNT-COOH mixtures were developed for the dehydration of ethanol by pervaporation. A complete spectrum was acquired at each pixel of the imaged area with pixel size 0.5 micrometer. The numerous spectra were processed using analytical algorithms into informative Raman images. Characteristic Raman peak intensity distribution, Raman spectral correlation, and multivariate curve resolution MCR analysis were explored to map PVA, CNT, GO distributions on the surfaces of the composite membranes. The resulting Raman images along with optical images provided insight into the spatial distributions of GO and CNT on the surface of the PVA membranes. These Raman images clearly show that the hybrid PVA-GO-CNT-COOH membranes had uniform surface distribution of the three chemical components. The incorporation of nanocarbons was found to increase the permeation flux and separation factor. The effect of the nanocarbon distribution on the performance of the nanocomposite membrane is also discussed.

166 Determination of Toxic and Other Trace Elements in Baby Foods Using ICP-MS

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Commercial baby foods are the main source of nutrients and energy for many children around the globe. The quality and safety of baby foods are extremely important during these crucial developmental stages. Toxic elements such as: arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) have been declared harmful to human health, particularly babies and children undergoing neurological development by the Food and Drug Administration (FDA) and the World Health Organization (WHO). The Baby Food Safety Act of 2021 was introduced to regulate the presence of toxic elements in infant and toddler food products. Following this act, the US FDA published a 'Closer to Zero' plan, which outlines steps that the agency will take to reduce the toxic elements in foods eaten by babies and young children to levels as low as possible. ICP-MS offers a solution to obtaining low levels of detection for toxic elements as well as providing excellent interference removal, and being a high-speed multi-element technique with a wide linear dynamic range which may been needed for the determination of nutritional elements. This work describes a procedure for the analysis of toxic elements: As, Cd, Hg, and Pb, and other trace elements: Cr, Mn, Fe, Ni, Cu, Zn, Mo, and Tl, in baby foods (pureed and cereal) following US FDA EAM 4.7.

167 Determination of PFAS in Food Using the Automated FREESTYLE PFAS System

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Monitoring for per- and polyfluoroalkyl substances (PFAS) in food is crucial due to their persistence in the environment and potential health risks. PFAS compounds, often referred to as "forever chemicals," do not break down easily and can accumulate in the food chain, leading to long-term exposure in humans. Since these chemicals can enter food through contaminated water, soil, or food packaging, regular monitoring helps safeguard public health by ensuring that food products meet safety standards and reduces the risk of chronic exposure. Many laboratories have developed strategies for the determination of PFAS compounds from food samples. For example, scientists at the FDA recently released method C-010.02. In this study we were able to show the solid phase extraction procedure used within this method can be easily automated using the FREESTYLE-PFAS robotic system. The resulting extracts are introduced into an Agilent Ultivo LC-MS/MS instrument for detection and quantification. By the application of automated solid phase extraction, multiple samples can be processed with low demand for personnel resources. The FREESTYLE XANA-PFAS robotic system is especially suited for PFAS determination because it contains no fluorine-containing plastics such as PTFE in the flow path, thus solving the significant issue of high blank values present in other systems. In this study, no measurable blank values were seen from the system.

168 Dumas Method Application for Non-Protein Nitrogen (NPN) Analysis in Milk Products

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Non-protein nitrogen (NPN) refers to nitrogen-containing compounds present in biological samples that differ from the building blocks of proteins. NPN includes molecules like amino acids, urea, ammonia, and other nitrogenous substances crucial for various biochemical processes. It plays a pivotal role in assessing the nutritional status of food and feed products while preventing adulteration with non-protein nitrogen. Measuring NPN is not without complexity. The Kjeldahl method remains primary for determining protein and non-protein content in food. Analyzing milk via Kjeldahl yields "Kjeldahl" nitrogen, converted to "crude protein." However, 5-6% of milk's nitrogen exists as non-protein nitrogen, primarily urea, conventionally counted as protein using the standard Kjeldahl method. Determining NPN content and subtracting it from crude protein reveals the "true protein" value. Nevertheless, we have witnessed a shift towards the adoption of the Dumas method for protein analysis, gradually displacing the Kjeldahl method due to its speed, throughput, and the elimination of hazardous chemicals—albeit without established methods and norms to account for NPN. Dumas and Kjeldahl differ in principles but share nitrogen content measurement. Dumas excels in efficiency, throughput, and precision by measuring total nitrogen, encompassing both protein-bound and non-protein nitrogen. This method involves sample combustion, converting nitrogen to nitrogen gas. This abstract showcases Dumas method's application in milk samples, effectively determining "true protein" by subtracting NPN, referencing ISO 8968-4 (Kjeldahl) as the method. Importantly, Dumas method's scope extends beyond milk products.

169 Headspace Analysis of Whole Milk and Soymilk Using a SERS-Active Fiber

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Microbial and enzyme activities affect the quality and shelf-life of food. Over time, these changes result in changes in the volatile organic compounds in food, which are useful indicators of food quality and shelf-life. However, current methods for testing volatile organic compounds, like gas chromatography-mass spectrometry are expensive and laborious. Hence, a more affordable and simple method was developed in this study and tested using samples of whole milk and soymilk. A novel gold-coated surface-enhanced Raman spectroscopy (SERS)-active fiber (diameter = 0.3 mm) was inserted into the headspace of whole milk and soymilk in capped glass vials, respectively. The SERS-active fiber was incubated at room temperature for 30 minutes and then read by a Raman spectroscope for molecular spectral analysis. The samples were monitored at 1–2-day time intervals. The results showed that the SERS-active fiber was capable of capturing dimethyl sulfide, a bacterial spoilage indicator, during storage. Also, partial least squares analysis showed that the SERS spectral changes correlated well with the changes in other established indicators of quality and safety such as pH (whole milk R = 0.81; soymilk R = 0.77), microbial growth (whole milk R = 0.90; soymilk R = 0.81), optical density (whole milk R = 0.91; soymilk R = 0.90), and particle size (whole milk R = 0.79; soymilk R = 0.86). These show that the SERS-active fiber can be used to noninvasively monitor whole milk and soymilk quality and shelf-life during storage.

170 Aroma Profiling of Commercial Poi Products in Fresh and Aged States Using Comprehensive Two-Dimensional Gas Chromatography

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Taro (Colocasia esculenta L.) is a plant originating from Southeast Asia now prevalent throughout the Pacific Islands. Growing taro was and remains an important facet of Hawaiian culture. Poi is a food product prepared from steamed taro that has been macerated and allowed to ferment. While commonly prepared at home, poi can also be found in grocery stores from select Hawaiian brands. The general fermentation process of poi produces volatile organic compounds (VOCs) such as alcohols, aldehydes, ketones, organic acids, esters, pyrazines, and phenols, among others, through microbial and enzymatic reactions. The VOCs found in poi are traditionally analyzed through gas chromatography-mass spectrometry (GC-MS). This study aimed to observe changes in fresh to aged commercially purchased poi using comprehensive two-dimensional gas chromatography-quadrupole mass spectrometry with flame ionization detection (GC×GC-qMS/FID). Samples were prepared according to package instructions, extracted via headspace SPME Arrow, and analyzed in replicates of 10. All brands showed a clear distinction between fresh and aged samples. Fresh samples across all brands contained acetoin while aged samples all contained 1-pentanol, acetic acid, and 2,5-dimethylfuran. Though Hanalei and Taro brand poi had similar profiles, He Mea Ono (HMO) brand poi differed. Aged HMO brand poi contained a greater variety of compounds than aged Hanalei or Taro brand poi. Visual distinctions in chromatographic plots were supplemented by principal

component analysis (PCA) and volcano plots, identifying distinguishing compounds. Investigating the profile of poi via GC×GC-qMS/FID can enhance understanding of its unique qualities, historical context, and contemporary uses.

171 **Synthesis and Characterization of Novel Europium-Doped Ceria Nanocrystals (EuCeNCs) for Monitoring Antioxidants**

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With increased awareness of nutrition and the advocacy for healthier food choices, there exists a great demand for a simple, easy-to-use test kit that can reliably measure the antioxidant capacity of dietary products. We report the development and characterization of a portable nanoparticle-based assay, like a small sensor patch, for rapid and sensitive detection of food antioxidants. In this work, the nano catalytic therapy for healthy food choices was examined, using a molecular nanoprobe exhibiting enzymes-mimetic function and redox brings an innovation of rapid and sensitive detection of molecular targets while the antioxidants act as reducing agents. To achieve this, well-dispersed europium-dope ceria nanocrystals (EuCe-NCs) with a self-integrated catalytic and fluorescence sensing function were synthesized and characterized. The NCs have an average size of ~5 nm and exhibit bright and stable fluorescence for more than 6 months in aqueous media. This nanotechnology was developed as an assay for antioxidant activity, especially nanoceria that can switch between trivalent and tetravalent oxidation states of cerium. This assay is particularly appealing for remote sensing applications, where specialized equipment is not available, and for highly thorough analysis of a large number of samples. The sensor has been tested for the detection of common antioxidant compounds including ascorbic acid and Trolox, with future to be tested on gallic acid, vanillic acid, quercetin, caffeic acid, and epigallocatechin gallate as well as application for the assessment of antioxidant activity in real food samples.

172 **Dissolution Method Development: Lessons Learned on Tablets with Low Solubility, Sensitive to Discrimination and Metal Chelation**

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A proper designed dissolution method is indispensable at all stages of drug development, from clinical development, commercialization and successive post-market monitoring. It provides valuable information to develop a robust formulation to identify important changes in the drug product (DP) during manufacture, and to ensure DP performance. However, the method development (MD) process is usually labor-intensive and challenging especially for poorly soluble drug substances. Here, a phase-appropriate dissolution MD strategy is proposed for low solubility and metal-chelation tablet DPs together with lessons learned from multiple pre-clinical activities across different functionalities teams. To resolve the over-discrimination of the preliminary method at biorelevant conditions, micro-dissolution technique was first applied to determine the basic pH of media in contrast to traditional acidic conditions. Followed by extensive method parameters screening such as paddle speed, media volume and composition, collecting time points etc., a phase-appropriate dissolution method is proposed with justified method feasibility. More interestingly, due to the potential metal chelation nature of the compounds, salts grade, surfactant brand, and HPLC vial types all need to be carefully considered to ensure accurate data delivery. As the above method criteria would generally be ignored during MD, these lessons would therefore bring new insights for all disso method developers.

173 **Investigation of the Effects of Overexpression of Human Jumping Translocation Breakpoint (JTB) Protein Using In-Gel Digestion-Based Proteomics**

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Human JTB (hJTB) is a gene located on human chromosome 1 at q21, which is involved in the unbalanced translocation of various types of cancer. JTB protein is ubiquitously present in normal cells and is found to be overexpressed in various types of cancer including prostate and breast cancer. MCF7 breast cancer cell lines were transfected with the sense orientation of the JTB cDNA in HA, His, and FLAG tagged CMV expression vector as well as with shRNA plasmids. Proteins extracted from transient and stable transfected cells were separated using 1D-SDS-PAGE. The expression of JTB was confirmed by the western blotting technique. In-gel digested peptides were analyzed by a Nano Acquity UPLC coupled with QTOF Xevo G2 Mass Spectrometer. Data processing was done using Mascot 2.4 server and Scaffold 4.1 software. We found several proteins such as HSP's, Actin, and/or tubulin proteins are closely associated with hJTB function. Furthermore, we performed a GSEA analysis to identify the biological processes and pathways associated with the JTB protein. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the JTB protein as a potential drug target for malignancies with overexpression of the protein.

174 **East Asian Concertina Book**

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This project will catalog, analyze, and investigate an East Asian Concertina Book in the West Chester University Special Collections. The process of cataloging the East Asian Concertina Book will involve photographing the illustrations, and documenting the writings and anomalies contained within the text of the book. The project will include using micro X-ray Fluorescence (μXRF) and reflectance Fourier-transform Infrared Spectroscopy (rFTIR) to characterize the pigments, ink and the substrate of the book. As a result of this research, the WCU Special Collections will be able to expand upon the provenance of the book by addressing the potential region of origin, age, and cultural significance of this book.

175 Withdrawn by the author.

176 Withdrawn by the author.

177 Withdrawn by the author.

178 **Mitigation of Low Endotoxin Recovery (LER) Using CSE170 in Recombinant Human Acid Alpha-Glucosidase for the Treatment of Pompe Disease**

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Recombinant human acid alpha-glucosidase (rhGAA) drug product is used for the treatment of Pompe disease. For new biologics licensing application (BLA) the United States FDA requires evidence that endotoxins spiked into a drug substance (DS) or drug product (DP) can be detected using bacterial endotoxin test (BET) in *USP* <85>. If the recovery ratio of the spiked endotoxins in the finished DS or DP is below 50% in a hold-time study, it suggests the presence of low endotoxin recovery (LER). A potential LER phenomenon was observed in a hold-time study when rhGAA DS or DP samples were spiked with CSE E120 (Control Standard Endotoxin, Charles River Laboratories) and tested using the BET method. Further LER studies indicated that the LER was likely caused by components in the formulation buffer known as the masking effect, i.e., the inability to detect endotoxins, caused by chelators, detergents, and/or proteins. However, when CSE E170 (Charles River Laboratories) was spiked in DS or DP, all DS samples showed no masking on the spiked endotoxin recovery under the 2-8°C and 20-25°C hold condition for 7 days. All DP samples showed no masking on the spiked endotoxin recovery under the 2-8°C hold condition for 28 days. In conclusion, the potential LER phenomenon was successfully mitigated by spiking CSE E170 in DS/DP in hold-time studies and the validated endotoxin testing method is suitable to detect low levels of endotoxin in DS and DP samples of rhGAA from manufacturing processes.

179 **A New LC-MS/MS Method for Identification and Quantitation of an N-Nitroso Impurity (NDSRI) in a Commercial Small Molecule Drug Product Capsules**

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A novel and highly sensitive LC-MS/MS method was developed and validated to address nitrosamine durg substance related impurity (NDSRI) in related drug product capsules. For a target specification limit of 11 ppm, a quantitation limit (QL) of 1.057 ppm and a detection limit (DL) of 0.353 ppm was required. The method employed a Zorbax SB-CN 5-micron column (4.6 x 250mm). The gradient mobile phase consisted of 0.1% formic acid in water (MP A) and 0.1% formic acid in an 80:20 ratio of acetonitrile and methanol (MP B), facilitating baseline separation of the NDSRI from other compounds. Validation was performed as per ICH Q2 (R2) guideline. The specificity of the method was acceptable in the presence of API and capsule excipients. The linearity coefficient was 1.00, ensuring accurate quantification across studied NDSRI concentrations. The accuracy indicated a reliable range of 70-81%, while studies on repeatability and intermediate precision revealed low variability of 4% and 6%, respectively. The test and standard solutions were stable for up to 3 days in refrigerated conditions. Analytical Target Profile (ATP) concept was successfully used in this validated method for monitoring the analytical method performance characteristics (AMPC). The validated method was employed for confirmatory testing (CT) of NDSRI in 3 DP batches. The CT results indicated that the NDSRI was below DL, thereby allowing removal of NDSRI monitoring in routine release testing of related drug product capsules.

180 A New Analytical LC-MS/MS Method for Determination of Eight Standard Nitrosamines (NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, NDPA, NDBA) in a Commercial Small Molecule Drug Product Capsules and its API

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This analytical method describes the development and validation of an LC-MS/MS method to quantify eight standard nitrosamine (NAs) impurities (NDMA, NMBA, NDEA, NEIPA, NDIPA, NMPA, NDPA, NDBA) in small molecule API and related Drug Product capsules. Optimum resolution between the eight standard NAs and their associated internal standards were achieved on a C18 analytical HPLC column using a gradient mobile phase (0.1% Formic acid in water (v/v) MP A and 0.1% Formic acid in methanol (v/v) MP B), at 0.5 mL/min. Elution was monitored at precursor and product ions (amu) numbers. This method was successfully validated according to the ICH Q2 (R2) guideline. The method was specific for all NAs in both API and capsule matrices. The quantification of NAs ranged from 0.0215 ppm to 0.780 ppm related to the sample concentration of 20.5 mg/mL, with linearity coefficient of 0.99-1.00. The DL and QL of NAs were 0.144-0.522 and 0.438-1.590 ng/mL, respectively. The mean % recoveries (accuracies) for all standard NAs ranged between 90%-107%. The intermediate precision and repeatability for all NAs were <10%. The test and standard solutions were stable up to 5 days when refrigerated. Analytical target profile (ATP) concept was successfully used for monitoring the analytical method performance characteristics (AMPC). Confirmatory Testing indicated all NAs were below QL in all the API and DP batches, and thus enabled removal of the NA routine monitoring during batch release testing.

181 Scaling of Challenging UHPLC Compendial Methods on HPLC Systems

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In day-to-day operations, pharmaceutical laboratories may encounter the need to adjust liquid chromatography (LC) methods. This can involve method adjusting between LC platforms, such as between ultra-high-performance liquid chromatography (UHPLC) and high-performance liquid chromatography (HPLC) systems, or across columns of different dimensions and particle sizes. For example, method modernizations, such as conversions from HPLC to UHPLC separations, typically scale method conditions to use columns with smaller particle sizes for higher throughput and reduced mobile phase consumption. In contrast, in a regulated environment, the conversion from UHPLC to HPLC conditions may be more prevalent as HPLC separations may be perceived as more robust for routine analyses. However, with the growing acceptance of UHPLC separations, sub-2-µm particle size columns are becoming more common in regulated methods. In this study, two compendial LC assay methods, specifying sub-2-µm columns, from the United States Pharmacopeia (USP) are examined on an HPLC. Both USP assay methods utilize challenging conditions more similar to UHPLC separations than HPLC, with low flow rates, small injection volumes, and columns with small inner diameter and particle size. Additionally, the small injection volume coupled with the organic content of the sample diluent could pose a challenge for meeting system suitability. To simulate the method scaling that a QC laboratory may perform, the two assay methods are first evaluated on HPLC without modification and then scaled up to use columns with larger particle size. Results between the original and scaled methods are compared.

182 Combining Droplet Microreactor and Mass Spectrometry for Efficient Detection of Antihistamine Drugs in Oral Fluid

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Surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS) has received considerable attention as a complementary approach to matrix-assisted laser desorption/ionization mass spectrometry, offering substantial potential for analyzing molecules in the low-mass region. Herein, we propose a facile method a microreacror for the synthesis of two types of barium ferrite ($BaFe_2O_4$ and $BaFe_{12}O_{19}$) nanoparticles (NPs) within droplets for detecting antihistamine (AH) drugs in oral fluid (OF). The synthesized $BaFe_2O_4$ and $BaFe_{12}O_{19}$ NPs exhibited small particle size, good ultraviolet absorption, and excellent performance in SALDI-MS, as determined by survival yield measurements. The limit-of-detections for AH drugs were in the range of 1 pg mL^{-1} to 100 ng mL^{-1}, and spot-spot reproducibility of the SALDI substrates was satisfactory. Moreover, when analyzing cetirizine in the OF, the obtained recoveries of cetirizine were 101% and 99% using $BaFe_2O_4$ and $BaFe_{12}O_{19}$ NP, respectively. Furthermore, the proposed method was validated by analyzing OF samples from a healthy volunteer who consumed a 5-mg levocetirizine tablet for seven days. SALDI-MS analysis confirmed the successful detection of endogenous components, the parent ion of cetirizine, and other exogenous substances. This study reports an advanced application of droplet microreactor technology for designing and synthesizing a wide range of novel and efficient SALDI-MS substrates for various applications.

183 Chemometrics Assisted GC-FID Analysis of Fatty Acids in Blood Plasma Under Varying Dietary Conditions Towards Clinical Applications

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This study evaluates the concentration of fatty acids (FAs) in plasma across different dietary conditions using gas chromatography-flame ionization detector (GC-FID). The fatty acids in plasma of mice intaking a high-fat (HFD), low-fat (LFD) and normal diet (ND) under varying sucrose conditions were extracted, derivatized to fatty acid methyl esters (FAME) and analyzed using GC-FID. The concentrations and abundancies of FAs in each sample were computed. Between the ND and HFD groups, 11 prominent FAs were detected, including palmitic, stearic, and behenic acid. The concentrations of these fatty acids were notably 60-90% higher in the plasma of the HFD group, indicating a strong correlation between high-fat diet intake and elevated fatty acid levels in the blood. Upon introducing sucrose levels as a variable, multivariate statistical analysis revealed three distinct clusters, specifically with all high-fat diet (HFD) groups in close proximity within the plot. Particularly, the low-fat diet (LFD) groups under normal sucrose conditions exhibited a more pronounced clustering pattern, wherein the LFD groups without sucrose and with high sucrose clustered together. This suggests that sucrose alone may not exert a significant effect on fatty acid (FA) levels in the blood, whereas the dietary fat content likely dominates its influence. These findings offer potential insights into the intricate interplay between FAs, carbohydrates, and their relation to insulin sensitivity and other chronic metabolic diseases.

184 Applications of Synthetic Carbon for Pharmaceutical Industry

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The use of synthetic carbon adsorbents in pharmaceutical applications is of significant interest due to its potential impact on biotherapeutic drug production. This study explores two applications of Carboxen® synthetic carbon adsorbents in pharmaceutical manufacturing: host cell protein (HCP) removal during monoclonal antibody (mAb) purification and metal scavenging for active pharmaceutical ingredient (API) synthesis. Firstly, the study evaluates Carboxen® synthetic carbon adsorbents for depleting HCPs during mAb purification, addressing the challenges associated with HCP depletion. The research aims to enhance HCP removal during mAb purification, contributing to the highest possible purification levels. Secondly, the study investigates the efficacy of Carboxen® synthetic carbon adsorbents for scavenging rhodium and palladium metals, commonly used as process catalysts for API synthesis. The research compares the scavenging efficiency and API yield of Carboxen® synthetic carbon adsorbents with a leading metal scavenger, Silica-Thiol, in methanol and N,N-dimethylformamide. Preliminary results show the potential of synthetic carbon adsorbents to efficiently scavenge metals while maximizing API yield, offering promising alternatives for pharmaceutical manufacturing. Overall, this research provides valuable insights into the diverse applications of Carboxen® synthetic carbon adsorbents in pharmaceutical manufacturing, highlighting their potential to enhance critical processes and contribute to the production of high-quality biotherapeutic drugs and APIs.

185 Deviations of Expected Split Ratio of Active Pharmaceutical Ingredients

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The inlet is where the injection of the sample takes place. A syringe enters the inlet and injects said sample. The inlet is then heated with the goal of evaporating both the solvent and the solute. When a split injection occurs, the sample is split into two parts. The smaller part of the sample is transported onto the column by the carrier gas. The larger portion of the sample is vented through the split exits. Ideally, the amount of sample that goes onto the column is proportional to the split flow. If the split ratio is 100:1, 1% of the sample reaches the column. When these inlets were developed, the main analytes used to test these splitting were alkanes. Alkanes are considered an ideal analyte due to their chemical properties. Gas chromatography has since branched out to various analytes, thanks to capillary columns, which use the capillary effect. This is required for active pharmaceutical ingredients to elute below its boiling point. Now that other compounds can be analyzed, would they be similar to the ideal alkanes, or would they have deviations? This research seeks to provide an understanding of the various effects that the inlet creates when trying to apply gas chromatography methods to active pharmaceutical ingredients in the hopes that the pharmaceutical industry can become greener.

186 Extended Retention and Separation capacity of Nitrosamines Utilizing a Polar Endcapped Monodisperse Fully Porous Particle (MFPP) HPLC Column

Edward Faden, MAC-MOD Analytical, 103 Commons Court, Chadds Ford, PA 19317, Mark Woodruff

Widely suspected of being a human carcinogen, Nitrosamines must now be moni-

tored for their presence and potential introduction in manufacture since being found in Ranitidine and Metaformin, amongst other drugs products, by the US FDA. Although hundreds of nitrosamines exist, they can vary widely in their chemical nature being hydrophilic or hydrophobic in nature. Therefore, presenting a challenge to developing a method that can function for many of these groups. In this poster we show the ability of the new Evosphere® AQUA column in conjunction with a simple mobile phase to produce full resolution and offer good sensitivity. Evosphere is built around a new Monodisperse Fully Porous Particle (MFPP) which is designed to provide more you get a much-elevated backpressure and the potential for blockage and robustness issues, which can lead to a lack of confidence in the method. The MFPP will provide better packed columns, less band broadening and 40-50% greater efficiency than other equivalent silica particles in HPLC, therefore giving higher resolution and sensitivity. Bonded to this MFPP is a selection of new stationary phase efficiency than traditional polydisperse particles. So in this poster a 3µm Evosphere particle is providing the efficiency and sensitivity that would be expected if using a sub 2µm UHPLC particle. If you run with a UHPLC particle then choices, providing the ability to enhance resolution for critical pairs of closely related compounds, ideal for the Nitrosamines class of compounds which can have very similar structures.

187 Investigation on *In-Vivo* Degradation Rate of Compound A in Normal Human Stomach pH Range via Design and Execution of *In-Vitro* Biorelevant Dissolution to De-Risk a Potential PK Variability due to Varied Degradation Levels

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Compound A is a BCS ClassII weak base and is prone to acid/base catalyzed hydrolysis. It has a steep solubility dependent curve, with solubility dropping sharply at neutral physiological pH. An amorphous solid dispersion formulation was developed which exhibits higher apparent solubility but also lower chemical stability when compared to the formulation containing crystalline drug. Using a stability indicating UPLC method and high-resolution mass detector, it was observed that the major degradation pathway of Compound A is acid/base catalyzed hydrolysis to form major degradant compound D. Given that API is sensitive to pH dependent acid/base hydrolysis which is the major degradation pathway, the understanding of the degradation rates of API in conditions encompassing possible stomach pH ranges is considered critical to capture any potential risk of varied bioavailability of API in different pH conditions which might have an impact on PK profiles in vivo. The phase I dissolution method did not provide this information, hence there was a need to understand in-vivo API degradation profile. A method was developed to immediately quench the samples to neutral pH. The quenching solution was a combination of aqueous buffer with organic solvent to prevent in-vial precipitation of API. This experiment encompassed various dosage strengths and physiological stomach pH (ranging from pH 1.2-3). This study helped us understand potential limitations to drug availability post acid-catalyzed degradation in the stomach of clinical patients.

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

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Breast cancer (BC), one of the most frequent malignancies, is the top cause of mortality among women in the United States. In the United States, around one in every eight women may acquire breast cancer over her lifetime. Triple negative breast cancer (TNBC) is characterized as minimal levels of specific receptors, including those for estrogen and progesterone, along with HER2. , It the most aggressive type of breast cancer. As a result, early diagnosis and therapy are critical, and protein biomarkers may be a solution if significant dysregulations are detected in the sera. Serum gives information about what is happening in the body at the moment of collection, making it a suitable 'tissue' for cancer screening investigations. Mass spectrometry (MS)-based proteomic technologies are useful for investigating protein biomarkers, and they were employed in this work to look for protein variations in serum between women with breast cancer, matched controls, and biological replicates. 16 blood samples from women with TNBC and matched controls (8 vs 8) were analyzed utilizing an in-gel and in-solution digestion, followed by nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS) with a NanoAcquity UPLC and a QTOF Xevo G2 XS MS. The raw data was examined with ProteoWizard MSConvert (v. 3.0), Mascot Daemon server (v. 2.5), and Scaffold 4.3 software. This study's dysregulated proteins include apolipoproteins, alpha-1-antitrypsin family proteins, and serpin peptidase inhibitors, all of which have previously been reported in breast milk research, in addition to various glycoproteins and immunoproteins.

189 The Use of Human Breastmilk as a Bio-Fluid for Protein Biomarker Identification for Breast Cancer

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Breast cancer (BC) is a common malignancy in women, with approximately one in every eight getting an aggressive type of BC during their lifetime. Mammography is currently the primary way of screening for breast cancer, however it is not practical for younger women or those with denser breasts. Human breastmilk is an easily accessible biofluid that provides information about the function of the breast. The majority of past research on breastmilk has concentrated on its content and effects on child growth and development, with little information about the proteins, immune cells, and epithelial cells present that could indicate BC formation. Human breastmilk from breast cancer patients and controls was studied using liquid chromatography tandem mass spectrometry (LC-MS/MS). Multiple proteins were found dysregulated in the BC-pateients' milk compared to control samples. Both 1D-PAGE and 2D-PAGE analyses were done, and disturbed proteins were detected in BC-related samples from both investigations, including zinc-alpha-2-glycoprotein, caseins, fatty acid-binding proteins, apolipoproteins, and anti-trypsin family proteins. Each milk sample set includes both within- and across-women comparison groups of BC and controls to discover dysregulated proteins in breastmilk from mothers with and without BC. Within-woman comparisons reveal changes in protein levels between one breast with BC and the healthy breast of the same woman, resulting in a stronger comparison group with the least amount of genetic variation. The proteins found to be continuously dysregulated in breastmilk could be employed as biomarkers for BC diagnosis.

190 Achieving High Recovery & Reproducibility in High Throughput Sample Preparation Using Silica/Polymer Composite 96-Well SPE Plates

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Achieving reproducible, high-recovery sample preparation is essential in high sample throughput laboratories, such as clinical, drug development and food analysis labs. In these areas, 96-well Solid Phase Extraction (SPE) has become one of the main high-throughput sample preparation techniques, owing to its capability to selectively extract analytes from complex sample matrices, ensuring greater assay sensitivity and robustness, as well as reducing downtime with valuable instrumentation. Typically, SPE sorbents use functionalized loose-packed silica particles secured by frits in a plastic housing, whereas utilization of a solid silica/polymer-based composite media, removing the need for frits, enables higher analyte recovery at lower elution volumes, and greater repeatability. In addition to the cost-saving and environmental benefits of lower elution solvent usage, fritless composite SPE media enables analysts to greatly reduce or remove lengthy dry-down steps from their SPE workflows, saving precious time. This poster showcases the benefits of fritless composite SPE media via a variety of application-based data that demonstrate the advantages that can be realized for analysts who operate with limited sample quantities, are looking to reduce workflow time, or choose a greener sample preparation technique than loose-packed sorbent SPE.

191 Scalable and Automatable Benchtop Scale Protein A Based mAb Purification Using AffinEx SPE Columns with Resolvex A200 Sample Processor

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Diverse technology platforms are used for bench scale affinity mAb purification in protein science labs. It is often due to scaling issues, that different technologies are used during different phase of the project, such as gravity feed, centrifuge, vacuum manifold, magnetic beads, Te-Chrom™, and Akta FPLC. It is often involving agarose based Protein A sorbent filled Self-packed column, spin columns, Predictor™ plates, magnetic beads, Robo-columns®, HiTrap™, and HiScreen™ columns. Method transfer during scaling is challenging across different technology platforms. Also, refrigeration is usually required for agarose-based devices during storage and transportation. Positive pressure processed solid phase extraction (PPSPE) technology has been well accepted in clinical, forensic, and toxicological laboratories for decades. We would like to introduce a bench top mAb purification platform, AffinEx Protein A columns and 96-wellplates using PPSPE on a Tecan Resolvex A200. This process is easily scalable, and automation friendly. With sorbent fill volume ranging from 50uL to 1000uL, these Protein A columns provide a protein binding capacity from 1mg to 20mg of IgG equivalent, in a single tube volume of 1-6mL. In addition, these products are not required for refrigeration during storage and transportation. IgG extraction recovery of AffinEx columns will be reported, along with stability test results, and reusability of the products after over 20 times of alkaline cleanings and renewals. Protocols for mAb extraction and alkaline cleaning and renewal are provided.

192 The Importance of Temperature on Complete Digestion of High-Fat Foods for Metals Analysis

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Toxic heavy metals such as arsenic, cadmium, lead, and mercury are known to cause adverse effects on human health; therefore, it is critical to regulate certain elements within food products. High-fat foods such as peperoni, mayonnaise, and more must be tested for the presence of these elements to ensure consumer safety and product quality. Metals analysis in these types of samples can be challenging due to their high-fat content. Trace metals analysis relies heavily on a robust, reliable, and reproducible sample preparation technique. Given the diversity of sample matrices that can have a high-fat content and the need for one digestion method that is suitable for all, the digestion parameters are crucial. In this study, the importance of temperature to achieve a complete, microwave-assisted acid digestion of a variety of different high-fat foods is explored. This approach provides a rapid, efficient and simple process for trace metals analysis of high-fat foods.

193 Bio-Inspired Technologies for Monitoring Human and Environmental Health

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Significant effort has been devoted to developing technologies that effectively mimic biological processes, but these methods often fail to replicate the efficiency and selectivity of native systems. We have found that, by combining chemistry with the inherent activity of biomolecules and microbes, we can improve upon conventional technologies for human and environmental health monitoring. Specifically, by combining biomolecular activity with inexpensive sensing, we can detect viral infections. Similarly, using synthetic biology, we can engineer microbes to both degrade and detect harmful environmental pollutants. Through these technologies, we have consistently found that the combination of chemistry and biomolecular engineering affords advantages beyond the capabilities of either technology alone.

194 A Career in Analytical Chemistry

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Chemistry has always been a fascinating field for me. During my life I can say that I have pursued many fields of chemistry, starting with Organic, Bio, Physical, and finishing with Analytical Chemistry, a field which I pursued for more than 40 years. I got my Ph.D. in Romania at the University Al. I. Cuza, Iasi, Romania in 1983. I pursued a Postdoctoral Fellowship at the Weizmann Institute of Science in Rehovot, Israel, with the late Professor Emanuel Gil-Av. Professor Gil-Av invented the separation of enantiomers. This experience was followed by a second Postdoctoral Fellowship with Professor Barry Karger at Northeastern University, where I was able to explore the synthesis of stationary phases for protein separation. After completing my post-docs, I was hired by Merck Research Laboratories. I worked there for 16 years, leaving as a Senior Research Fellow. I subsequently worked for 13 years at Boehringer Ingelheim Pharmaceuticals, achieving the position of Distinguished Research Fellow. I am a recipient of the Kolthoff Fellowship and am an Honorary Member the University Senate of the University Alexandru Ioan Cuza, Iasi Romania. During my career, my main interest has been in the separation of enantiomers, with a particular focus on the interaction of enantiomers with chiral stationary phases as well as analytical process research. I have been active in, and held leadership roles in, several organizations supporting chromatographic research. In addition to an extensive list of publications, I am Editor-in-Chief for a journal and several book series and do consulting for a number of pharmaceutical CROs. I will describe some of the approaches I took to understanding the interactions underlying the separation of enantiomers with stationary phases, with a focus on using thermodynamic and spectroscopic methods.

195 Variety of Vibrational Spectroscopy: From Fundamental Research to Practical Applications for Forensic Purposes and Medical Diagnostics

Igor Lednev, University at Albany-SUNY, 1400 Washington Ave, Albany, NY 12222

Vibrational spectroscopy has been undergoing a significant development during the last two decades. A great variety of spectroscopic methods have been developed with a wide range of spatial and temporal resolution. Further advancement of the field resulted from utilizing Machine Learning for the analysis of complex spectral data. The development of robust and relatively inexpensive instruments including portable devices opened exciting new opportunities for various practical applications of vibrational spectroscopy. This presentation will begin with a brief overview of the variety of vibrational spectroscopic techniques. Then, the benefits of using several complimentary methods for tackling a complex scientific problem are discussed using the investigation of the structure, formation mechanism and stability of amyloid fibrils as an example. The discussion of practical applications of vibrational spectroscopy is focused on the commercialization of the first universal test for the identification of all main body fluids for forensic purposes. Additional potential capabilities of the method include determining the time since deposition and the phenotypic profile, sex, race, and age of the donor specifically. A novel two-step method for a fast detection of organic and inorganic gunshot residue using fluorescence spectroscopy followed by a confirmatory identification by Raman microspectroscopy will be also discussed. The development of a universal approach for diseases diagnostics based on Raman hyperspectroscopy and Machine Learning is also discussed briefly including the commercialization of saliva and blood screening tests for Alzheimer's disease, as well as tests for Sjögren's disease, Duchenne muscular dystrophy, and Celiac disease.

196 Development of Quantitative Mass Spectrometry Approaches for Analyses of Protein and RNA Modifications

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Histones are small proteins that package DNA into chromosomes, and a large number of studies have showed that several post-translational modification (PTM) sites on the histones are associated with both gene activation and silencing. Along with DNA and small non-coding RNA, histone PTMs make up epigenetic mechanisms that control gene expression patterns outside of DNA sequence mutations. Dysregulation of these chromatin networks underlie several human diseases such as cancer. Here I will give an update on technology advancements that have allowed for high-throughput quantitative mass spectrometry analyses of histone PTMs using rapid scanning mass spectrometry instrumentation. We are also continuing to develop approaches for intact protein analyses as well. To expedite discovery of new modifications, we have developed data independent acquisition mass spectrometry approaches for glycan and RNA quantification as well. We will demonstrate the feasibility of these approaches with applications to various biological and biomedical fields.

197 Probes with a Purpose: NMR Instrumentation for Biological Semisolids and Partially Ordered Samples

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NMR spectroscopy provides a useful set of techniques for studying complex systems, including biological macromolecules and oriented samples. NMR provides exquisite chemical specificity and unparalleled capabilities for measuring dynamics and ligand binding. NMR data can also be integrated with other methods for multi-modal studies of proteins under conditions that are close to their biological contexts. The push toward higher magnetic fields, coupled with recent focus on large biomolecules and heterogeneous materials have increased the stringency of experimental requirements for NMR instrument designs and sample preparation methods. A major research direction for my group focuses on the development of instrumentation, particularly probes for specialized experiments. Our philosophy is to make the experimental methodology (including the instrumentation) fit the sample rather than altering the sample to fit the method. I will describe the design, construction, and testing of specialized NMR probes for studying semisolid biological materials, an important class of samples that includes membranes, hydrogels, and condensates. For example, my group has developed switched-angle spinning probes for dipolar recoupling in partially ordered systems, as well as multi-channel magic angle spinning probes for multinuclear NMR in extensively deuterated samples. Probe design parameters, benchmarks, and illustrative experimental results will be presented for representative applications. I will also discuss future directions, including our efforts to add additional nuclei to the protein NMR toolbox, chemical biology techniques for selectively incorporating isotope labels, and using 3D printing to make instrumentation development more accessible, enabling a variety of specialized experiments for different biological and materials problems.

198 Spectroscopic Approaches for Tracking Degradation of Organic Semiconductors Important for Organic Optoelectronic Devices

Jeanne E. Pemberton, University of Arizona, Department of Chemistry & Biochemistry, 1306 East University Blvd., Tucson, AZ 85721

Next-generation organic semiconductor (OSC)-based optoelectronic devices have experienced continual improvement in performance metrics such as power conversion efficiency. However, these devices still suffer inadequate lifetimes in the field due to often poorly-understood and complex degradative processes. Different approaches to characterization of such degradative chemistry using vibrational spectroscopy have been explored in this laboratory. This presentation will provide an overview of efforts in areas. The use of FTIR spectroscopy for elucidating the role of different stimuli, including voltage and radiant exposure, in initiating such chemistry in operando device-like architectures, will be explored. Possibilities for diagnostic use of FTIR spectroscopy for identifying degradation in operating devices will be highlighted. In addition, efforts to use Raman microscopy to define the heterogeneity of degradative chemistry across a device and as a probe of OSC polymer morphology, a critical characteristic for device efficiency, at different points along device fabrication will be described. Collectively, these studies exemplify the power of vi-

brational spectroscopy coupled with advanced data analysis methods for delineating this complex chemistry in operating OSC devices.

199 **Nonlinear Optical Tools for Analysis of Macromolecular Assemblies**
Garth Simpson, Purdue University, Department of Chemistry, West Lafayette, IN 47907
The puzzling sensitivity of nonlinear optics to molecular chirality opens the door for new spectroscopic and microscopic methods for probing macromolecular assemblies. However, doing so hinges on the development of meaningful frameworks for bridging molecular and macromolecular structure back to experimental observables. As one prominent example, the ordering of biomolecules at surfaces and in 3D fundamentally changes the "rules of the game", enabling new classes of spectroscopy and microscopy measurements selective for ordered chiral architectures. The fundamentals of the underlying light-matter interactions and instrumental methods to leverage them will be the central subject of the presentation.

200 Withdrawn by the author.

201 **CryoTEM - A Powerful Multi-Attribute Characterization Tool for Nanoparticle Formulations**
Mandy Janssen, NanoImaging Services, 4940 Carroll Canyon Rd., Ste. #115, San Diego, CA 92121, Sheng Wang, Brent Wood, Giovanna Scapin, Eric Bushong, Brianna Fisher, Kalyn Kallio
Successful nanoparticle-based vaccines and therapeutics require optimal stability, encapsulation efficiency, and therapeutic efficacy. This is achieved via careful consideration of the formulation components, but also the choice and optimization of the manufacturing processes as they can directly influence the nanoparticles' physicochemical characteristics. To highlight this, we present the use of cryo transmission electron microscopy (cryoTEM) to characterize LNPs that were generated using four different mixing systems and using various downstream manufacturing processes. Cryo-TEM images in combination with advanced image analysis tools revealed subtle differences in size and payload distribution as well as significant variations in morphological features. Although size distribution, lamellarity, and presence of payload can potentially be assessed via other individual orthogonal techniques, cryoTEM has the unique advantage of requiring only parsimonious amounts of sample, directly observing individual particles at high resolution and in their near-native state and providing information on multiple LNP quality attributes simultaneously. Besides LNPs, we will give an example of protein-based as well as viral vector-based nanoparticle formulations where cryoTEM can be a remarkably useful characterization tool for ensuring optimization of formulation parameters, product consistency, and quality control.

202 **Weighing in the Biophysical Characterization Power: From Discovery to In-Depth Understanding to Biologics Product**
Jing Song, Merck, 126 E. Lincoln Ave., RY-870, Rahway, NJ 07065
Biophysical characterization plays critical roles in various stages of biologics product development. This abstract presents two studies that covers an in-depth understanding of these protein products via biophysical characterization which contributed valuable insights into the assessment, screening, and characterization of protein solution properties. In the first study, water proton nuclear magnetic resonance (wNMR) was employed as a noninvasive, in situ method to assess the aggregation propensity of a high-concentration drug product, Dupixent® (dupilumab). The wNMR results demonstrated concentration-dependent reversible association and irreversible aggregation under different stress conditions. Importantly, these findings were correlated with established biophysical analytical tools commonly used in the pharmaceutical industry. This application of wNMR offers a promising approach for analyzing high-concentration protein formulations directly in their primary containers. The second study introduced a Reciprocal Injection Device (RID) for accelerated formulation screening under intensified stress conditions within prefilled syringes. This versatile device allows for the assessment of concentration-dependent protein stability and interfacial interactions that are commonly encountered in combination drug products. The RID demonstrated efficacy in evaluating protein stability under diverse stress conditions and has the potential to expedite the formulation screening process. These studies contribute valuable insights into the assessment, screening, and characterization of high-concentration protein formulations. The novel applications and biophysical methodologies presented here have the potential to enhance drug development and improve quality assessment in critical areas of biologics formulation.

203 **Use of Digital Representations of Skeletal Remains for Forensic Analysis**
Katie Steigerwalt, Cedar Crest College, 770 East James St., Lehighton, PA 18235, Matthew Kieber-Emmons, David Webb, Carol Ritter
The accurate and efficient development of biological profiles is crucial for identification of remains that are too degraded for DNA analysis. Biological profiles are built based on osteometrics, or the statistical analysis of skeletal landmarks. At present, nearly all measurements are taken by hand; however, implementing 3D modeling would allow for a more thorough evaluation as it would assist in preserving fragile evidence and provide new avenues for collaboration amongst anthropologists. Modeling from computed tomography (CT) has seen limited applications in anthropology but lacks the resolution necessary for use in linear discriminant analysis due to variables like slice thickness and signal to noise ratio created by the X-ray. Surface scanning techniques that have been incorporated in archeological work would allow for the elimination of these variables and create a more detailed model. This study assessed the use of digital modeling techniques using measurements made by expert and amateur analysts. Digital representations were constructed using photogrammetry and LiDAR scanning on complete human crania and common anthropometric markers were measured by participants. These measurements were then compared to those obtained from the authentic skulls to assess the efficacy of surface scanning techniques. In applying these methods to forensic anthropology, faster and more accurate analysis will be possible, which will allow for faster identification of unknown remains by analysts

204 **Assessment of Blood and Semen Detection and DNA Collection from Swabs up to Three Months after Deposition on Five Different Cloth Materials**
Francisco Medina-Paz, New Jersey Institute of Technology, Department of Chemistry and Environmental Sciences, 323 Dr. Martin Luther King Jr Blvd., Newark, NJ 07102, Brandon Kuba, Emily Kryvorutsky, Gabriela Roca, Sara Zapico
Blood and semen are the most relevant body fluids in forensic sciences due to their wide presence in crime scenes. Based on antigen–antibody reactions binding unique proteins for each body fluid, serological assays represent one of the most rapid and highly specific tests for blood and semen. Few studies have assessed the factors affecting body fluid identification by applying these assays. This work aimed to study the effect of different fabrics from clothes and time since deposition on identification through immunochromatographic tests for blood and semen and STR profiling from these samples. Body fluids were deposited on black- and white-dyed denim and cotton fabrics, and on leather. Blood and semen were sampled at 1 day, 30 days, and 90 days after deposition and identified by using the SERATEC® Hem-Direct Hemoglobin Test and the PSA Semiquant and SERATEC® BLOOD CS and SEMEN CS tests, respectively. Laboratory and crime scene tests presented similar performances for the detection of blood and semen stains on every tested fabric. No differences were found on band intensities between timepoints for all fabrics. It was possible to recover and identify blood and semen samples up to three months after deposition and to obtain full STR profiles from all the tested fabrics. Both body fluid STR profiles showed differences in their quality between 1 and 90 days after deposition for all fabrics except for black cotton for semen samples. Future research will expand the results, assessing body fluid identification on other substrates and under different environmental conditions.

205 **Differentiating Black Powder Manufacturers through Post Blast Residue Utilizing Semd-Eds and Drifts**
Damian Niescior, Rutgers University-Camden, 602 Steamboat Sta, Southampton, PA 18966, Ken Hand, Georgia Arbuckle, Kimberlee Moran
Black powder explosive (BPE), an inorganic low explosive, is a popular material used by criminals and terrorists to build bombs and improvised explosive devices (IEDs). Although it is at least eight hundred years old, the simple explosive mixture is still produced today. The explosive is utilized for the manufacture of fireworks, by hunters, and by hobbyists. This easily acquired explosive currently has no added identifying markers, or taggants, to trace the post-blast black powder residue (BPR) to the original manufacturer. This differentiation can be crucial for forensic and criminal investigators to identify the manufacturer as this is valuable information that can aid in the investigation of cases involving these explosives. Utilizing diffuse reflectance Fourier transform infrared (DRIFTS) and scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS), a method to analyze the solid products of manufactured black powder was designed to determine a distinction between five different brands and varieties of purchased BPE. These solid products allowed for the identification of characterizable distinctions in the primary component analysis (PCA) of the post-blast residue and allowed for the preliminary differentiation of five different black powder products from three different manufacturers. The five different manufacturer's products were analyzed in both the pre-burn and post-burn states using PCA of the DRIFTS data. This analysis allowed for some preliminary distinction of black powder residues by manufacturer through the post-blast residue FTIR spectra.

206 **Analysis of Urine for Cannabinoid Presence**
Kourtney Albert, Arcadia University, 611 Stewart Rd., Hatboro, PA 19040, Heather Harris, Samuel Krug, Karen Scott
This research aims to successfully determine the absence or presence of select cannabinoids in donated urine samples from 24 different cannabidiol (CBD) product users. This will be achieved by using a validated method for quantifying Δ9-Tetrahy-

drocannabinol (Δ9-THC) and its metabolites, CBD and its metabolites, and cannabinol (CBN) on an Agilent 6495 liquid chromatography- tandem mass spectrometry (LC-MS/MS). After testing a variety of different sample preparation techniques, liquid-liquid extraction (LLE) was determined to be the most effective in analyte recovery. Once method validation is complete, the donated samples will analyzed and their results will be used to determine what truly lies within these products as well as reveal potential conversion within products used over an extended period of time.

207 Machine Learning Prediction of Best Chiral Stationary Phase for Chromatographic Enantioseparation Based Upon Chemical Structure

Chris Welch, Indiana Consortium for Analytical Science & Engineering, 410 W. 10th St., #1020H, Indianapolis, IN 46202

Current practices in chromatographic method development often employ rote screening of columns, mobile phases and other conditions to identify effective combinations. While useful, this strategy has long been recognized as slow and wasteful of solvents and other resources, with the development of dependable predictive models for chromatographic performance being a long-sought goal. Over the past 8 years we have collaborated to develop machine-learning based predictive models for the chemical structure-based selection of chiral stationary phases (CSPs) for the chromatographic separation of enantiomers based on the more than 200,000 literature-reported chiral separations documented in the ChirbaseTM database. While early success in this effort was encouraging, the performance of the models was less than desired. We now report significant progress in this longstanding problem arising from an NSF Center for Bioanalytic Metrology-sponsored collaboration with the Haixu Tang group at Indiana University in applying their 3DMol approach to the problem, as recently reported in *Analytical Chemistry*.

208 Application of Retention Prediction to LC Separation Problems

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Retention prediction algorithms are used for method development and optimization. We show that retention prediction is accurate and can be also used for method transfer between the different LC systems, where the retention of early eluting peaks is affected by parameters such as gradient delay and gradient distortion by a mixer. The complexity of some samples (protein digest, RNA digests) is so high that a complete resolution of all components cannot be achieved even with retention prediction software. In such applications the prediction goals are different. In proteomic research the peptides diverging from their predicted retention are excluded from the set as false positive results, which improves the protein identification reliability. In RNA mapping application the retention prediction helps to identify the short isobaric oligonucleotides with the same nucleotide composition using their unique retention behavior.

209 Fuel for the Engine - High Throughput Retention Measurement to Enable Predictive Models for Liquid Chromatography

Dwight Stoll, Gustavus Adolphus College, 800 West College Ave., Saint Peter, MN 56082, Sarah Rutan, Trevor Kempen, Bob Pirok

Efforts to model and simulate various aspects of liquid chromatography (LC) separations (e.g., retention, selectivity, injection breakthrough) depend on experimental retention measurements to use as the basis for the models and simulations. Often these modeling and simulation efforts are limited by datasets that are too small because of the cost (time and money) associated with making the measurements. Other groups have demonstrated improvements in throughput of LC separations by focusing on “overhead” associated with the instrument itself – for example, between-analysis software processing time, and autosampler motions. In our wikiChrom Project we are focused on improving the throughput of retention measurements such that datasets of hundreds of thousands of measurements can be made on a practical timescale (e.g., a few years). In this presentation I will describe our approach, which is focused on the use of low volume columns operated at high flow rates, as well as instrumentation and informatics infrastructure that enable the approach. At the core of our current platform is an algorithm that enables unsupervised operation – a “self-driving” LC that makes its own decisions about the conditions needed to make retention measurements with pre-defined characteristics. I will then discuss some observations we’ve made during the course of about 150,000 retention measurements, and the characteristics of a Hydrophobic Subtraction-type selectivity model based on our recently released, public dataset of about 43,000 measurements. We believe this research trajectory has high potential to profoundly impact the quality of models and simulations that can be developed for many aspects of LC separations.

210 Structure and Graph Based Machine Learning Prediction of Retention Times for LC Method Development of Pharmaceuticals

Jonathan Fine, Merck & Co., Inc., 126 E. Lincoln Ave, Rahway, NJ 07065, Pankaj Aggarwal, Amanda Mann, Armen Beck

A significant amount of resources are spent on developing Liquid Chromatography (LC) methods, a process that requires a large amount of data to be generated. While computer assisted modelling has been successfully implemented to decrease resource spend, current modelling approaches are data driven and require systematic, project-specific screening data to predict component retention time as function of chromatographic parameters. To address this gap, cheminformatics techniques, such as Quantitative-Structure Retention Relationships (QSRR), have been successfully used to predict the retention time of component mixtures. We have shown that these structure-based techniques can be combined with data-driven techniques to add structure into data driven models. In this presentation, we will show how QSRR, traditional machine learning techniques, and graph-based machine learning can be used to create predictive models for structurally diverse training sets. When using QSRR descriptors to vectorize chemical structures, Random Forest (RF) outperformed other traditional machine learning techniques in both cross-validation studies and a blind test set. Once hyperparameter optimized, RF predicted the retention time of a randomized test set with a median percentage error less than 4% for four gradient conditions used for method development. Graph-based methods further improve upon the RF performance by allowing for structure-based featurization to become a learned component of the model. These models can be used to predict the retention time of unknown peaks in the method development runs, allowing one to generate new optimum LC conditions via data-driven modelling without the need for additional experimental method development work.

211 Building Bridges: Turning Differences into Strengths

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

An important challenge for lab managers is to get people with different mindsets to work together. Just like the old proverb that it takes a village to raise a child, it takes a team to innovate in science. Getting that team to align around the problem, share their expertise, work together, and tackle the challenges of the science can be difficult. Enabling teams to perform requires patience, resilience, and persistence. The lab manager will need to provide a solid foundation for the team to be successful. That foundation is built from respect, dignity, safety, and purpose. For people to effectively work together they must have mutual respect, treat everyone with dignity, especially during difficult times, have sufficient psychological safety to share ideas and observations, and be very clear about the purpose of their work. Upon that foundation, effective teams can be built. This presentation highlights the expectations that the lab manager can instill in the team to enable them to bring their diverse experiences together to solve the technical challenges, including: 1. The importance of active listening; 2. Developing and using strengths; 3. Seeking win/win outcomes; 4. Engaging in creative conflict; 5. Enhancing belonging; 6. Building trust.

212 Supporting Diversity and Inclusion in the Workplace

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

Many times, we as lab managers can get so caught up in our everyday employment duties, we forget that we have the opportunity to enhance our personal and professional environments by creating a workforce that is both diverse and inclusive. Lab managers who are aware of diverse experiences (both positive and negative), as well as what can be done to minimize/eliminate obstacles, will be able to create an environment that has greater innovation, an improved brand image, and better employee engagement. They will also be able to facilitate and sustain higher employee morale, improve employee productivity, and improve creativity and innovation throughout the organization. Knowing the difference between diversity and inclusion (as well as why one cannot be effective without the other), as well as implementing creative strategies to provide opportunities for all, can also foster a more diverse employment setting. It is not only our responsibility as managers to create a workforce environment that is welcoming and enticing to all, it is also of great benefit to us as individuals and organizations.

213 How to Manage Conflict

May Adaeze Chinda, University of Ghana Medical Centre Ltd, Legon, Accra, Ghana, PO Box CO 2300 Community One, Tema/Ghana West Africa, 233

The purpose of the presentation is to understand what Conflict is all about, appreciate the types of conflict, identify what causes conflict, consequences of conflict which could be negative or positive and various management styles that managers can exploit to resolving the conflict that may negatively affect productivity. In today’s fast-paced and interconnected world, conflicts are an inevitable part of any workplace, which arises from disagreements, as a result of difference of opinions, competing interests, or tensions that can manifest as various levels from interpersonal relationships to global affairs (Elgoibar *et al.*, 2017). Conflicts can arise and impact productivity, employee morale and overall organizational success. For any organization to boost productivity, create a positive environment and improve communication, management of conflict is key. Effective conflict management requires the employment of a variety of styles. The manner in which dispute is managed determines the volume and intensity of the conflict Meyer S. (2004). Conflict Management is the use of processes, tools, and skills to find creative and respectful ways to manage

disagreements and disputes. It includes the ability to resolve conflict collaboratively through effective communication skills like active listening, assertive speaking processes such as mediation, negotiation etc. In conclusion, Conflict management is a critical skill for managers due to diverse teams and competing priorities in modern workplaces. Poor conflict management leads to stress, absenteeism, and low morale. Effective conflict resolution promotes collaboration and productivity. Managers must understand workplace conflicts and resolution approaches to navigate challenges successfully.

214 Finding the Holy Grail: Engagement

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

Managers face challenges such as engagement which is increasingly recognized as a critical factor and cornerstone in achieving success in effective communication and interaction. Some key factors to foster engagement are tapping into the digital technological platforms, real-time interaction, feedback, personalization, and analyzing data from outcomes to determine its effectiveness or ineffectiveness. It is imperative to build transparency and trust to ensure that the data and information is authentic while simultaneously sustaining and continuously improving engagement. This presentation aims to provide comprehensive understanding of effective engagement strategies, offering actionable insights and practical tools for applying these approaches in various settings. Come and join in to gain understanding of how to navigate and leverage these engagement strategies for lasting impact.

215 Exploring the Analytical Capabilities of LIBS-ICPMS for Geological, Energy, Pharmaceutical, and Medical Applications

C. Derrick Quarles Jr., Elemental Scientific Inc., 7277 World Communications Dr., Omaha, NE 68122, Benjamin T. Manard

The use of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) has become an intriguing option for elemental imaging. This technique offers high-speed (≥1000 Hz), highly-spatial (nm to µm) information with excellent detection limits (sub-ppb to ppb). The only negative for ICP-MS is that it utilizes an atmospheric plasma generated by argon gas, therefore, atmospheric elements (H, N, O) and F (ionization potential is higher than that of Ar) are not accessible. Laser-induced breakdown spectroscopy (LIBS), when operated in a helium purged atmosphere can excite and detect H, N, O, and F, in addition to all other elements on the periodic table. Thus, combining LIBS and LA-ICP-MS provides elemental coverage for the entire periodic table. In this work, a 193 nm nanosecond excimer laser (imageGEO) is combined with a high-speed laser ablation cell and two LIBS detectors (multi-channel CMOS detector and an ICCD based detector). This presentation goes over how this combined LIBS-ICPMS technology works, in addition to going into applications that cover the geochemistry, battery, nuclear materials, and life science areas.

216 Standard-Free Absolute Quantitation of Drug Metabolites via Elemental F and Cl Detection by Plasma-Based Mass Spectrometry

Kaveh Jorabchi, Georgetown University, 37th and O St. NW, Washington, DC 20057, Samuel White, Grace Hahm, Matthewy Cerny, Zahra Afsharsaveh

Absolute quantitation of metabolites is essential in drug development for characterizing drug safety and pharmacokinetic properties with applications spanning from early in-vitro studies to animal and clinical investigations. However, lack of authentic standards fro metabolites presents significant challenges for these measurements, requiring radioisotope labeling of the parent drug to circumvent the need for analytical standards. Radioisotope labeling in turn leads to additional difficulties, namely long (sometimes months) and expensive synthesis as well as radiation safety concerns, heightening the need for alternative analytical technologies. Here, we present plasma assisted reaction chemical ionization mass spectrometry (PARCI-MS) as a potential solution for rapid absolute quantitation of metabolites that contain F and Cl atoms. Compounds separated by LC are introduced into an inductively coupled plasma where F and Cl atoms are quantitatively converted to HF and HCl. These species are then ionized by reagent ions produced from a nano-ESI and are detected by advanced MS techniques such as high-resolution MS. We show compound-independent response factors with simultaneous F and Cl measurement capabilities, and present a strategy to calibrate response factor variation across gradient chromatography, leading to a rapid quantitation method. Examples of metabolite quantitation for *in-vitro* experiments are also presented. Given the prevalence of F and Cl in drug structures, PARCI-MS offers an attractive alternative to radioisotope labeling for applications in drug development.

217 Advancing Nanomaterial Measurement Science with Single Particle Inductively Coupled Plasma Mass Spectrometry (spICP-MS)

Antonio R. Montoro Bustos, National Institute of Standards and Technology, 100 Bureau Dr., Gaithersburg, MD 20899, Monique E. Johnson, George C. Caceres, Karen E. Murphy, Michael R. Winchester

Accurate measurements at the nanoscale are critically needed to refine manufacturing processes, assess efficacy, and ensure the responsible use of engineered nanomaterials. The ability to control and harness phenomena that occur on a dimensional scale below 100 nm has led to exponential growth in environmental and nanomedicine applications. However, the expected low levels of engineered nanoparticles in environmental systems preclude reliable detection by conventional analytical strategies. Thus, novel approaches for reliable in situ nanomaterials characterization are needed. In this context, single particle inductively coupled plasma-mass spectrometry (spICP-MS) provides rapid, simultaneous measurement of size distribution and number concentration of nanoparticles suspended in liquids, with detection capability down to 10 nm, at environmentally relevant number concentration levels. While the use of spICP-MS for the characterization of nanomaterials in different fields has been recently reported, important challenges and limitations in obtaining accurate and consistent size and particle number concentration measurements still remain, particularly with respect to the lack of reference materials and standardization of spICP-MS methodologies. In the past years, the Inorganic Chemical Metrology Group has engaged in several activities to build a robust nanomaterial spICP-MS measurement infrastructure. Activities that will be the focus of this talk include validation of spICP-MS for routine characterization of gold nanoparticles of different sizes, coatings, and surface charge at environmentally relevant concentrations in water; analysis of silver and titanium dioxide nanoparticles in water; characterization of silicon dioxide food additive materials in aqueous media; and multimethod characterization of gold nanoparticles uptake in the model organism, *Caenorhabditis elegans*.

218 Ditch the Argon! Plasma-Based Atomic Spectrometry Using Ambient Air

Steven Ray, State University of New York at Buffalo, Department of Chemistry, Buffalo, NY 14260

The instrumental techniques that have been developed for trace and ultra-trace elemental analysis are powerful and well-suited to common applications of the methodology. Consequently, several researchers have turned towards the development of instrumental technologies that can meet the needs of these common analytical methodologies using experiments that offer reduced complexity, reduced cost, and that are more applicable to remote deployment. Several of the emerging techniques for analytical atomic spectrometry will be discussed in this presentation. A common means of reducing the ongoing cost of analytical measurements using plasma-based spectrometry is by replacing the commonly-used argon gas with cheaper and more plentiful air or nitrogen. These nitrogen plasma sources for atomic emission and atomic mass spectrometry are much simpler than the conventional radio-frequency counterparts, supporting lower capital cost, more efficient in operation, and also can operate continuously using ambient air for the discharge. The microwave inductively-coupled atmospheric plasma (MICAP) and other microwave-based plasmas will be discussed as one such alternative source. Liquid-based glow discharge plasmas offer operation without the need for argon support gas, but also can be supported directly atop the sample surface to permit direct sampling of the liquid sample. Moreover, these liquid-based glow discharged require only modest power (e.g. 100W) for operation, and boast limits of detection and linearity that is competitive with extant techniques for routine water analysis. The operation and analytical performance of one such source, the solution-cathode glow discharge, will be detailed.

219 Transmission Infrared Microscopy and Machine Learning for the Forensic Analysis of Automotive Paint

Barry Lavine, Oklahoma State University, Department of Chemistry, 107 Physical Science, Stillwater, OK 74078, Haoran, Elizabeth Donkor, Collin White, Thomas Hancewicz

In the forensic examination of an automotive paint chip, each layer of paint after hand sectioning via a scalpel and dissecting microscope is analyzed individually by FTIR spectroscopy. In this presentation, new developments from our laboratory for the infrared imaging of automotive paint are highlighted in five specific areas. First, cross sectioning of the paint chip is performed without epoxy or other embedding media. Second, using an ultramicrotome instead of a conventional microtome allows for so-called “small” paint chips that are often found on the clothing of a pedestrian in a hit-and-run to be cross sectioned and analyzed than what is practical by conventional FTIR. Third, the use of a new multivariate curve resolution technique (modified alternating least squares or MALS) allows for more accurate spectral reconstructions of the IR spectra of the individual paint layers from an image line map of these thin peels. Fourth, an adaptive approach to baseline correction that integrates three well known methods (rubber band, Whittaker filter, and asymmetrically reweighted penalized least squares smoother) is integrated into MALS to generate a better snapshot of the background of each IR spectrum in the image line map. Fifth, an automotive paint library containing all four paint layers is integrated into MALS thereby allowing spectral library matching to be coupled with the recovery of the IR spectra. Using this multifaceted approach, the recovered background corrected IR spectra of each layer are obtained as well as the background corrected IR spectral library for each sample line map.

220 No abstract submitted by the author.

221 **The Use of Near Infrared Spectroscopy and Machine Learning to Assess the Quality of 3D Printing Tablets**

Keith Freel, University of Maryland-Baltimore, School of Pharmacy, 20 N. Pine St., Baltimore, MD 21201, Yihan Wang, Ahmed Ibrahim, Sharon Flank, Jon Schupp, Stephen Hoag

Quality models using traditional and machine learning algorithms were established using near-infrared (NIR) to support 3D drug printing for point-of-care personalized medicine. The goal was to create a model to assess the quality of 3D printed medicines, i.e., differentiate acceptable from unacceptable. In addition, the spectra were collected using a laboratory and at line micro spectrometer, and the results of these two spectrometers were compared. Tablets were printed using the FABRX M3DIMAKER2 with the semisolid extrusion (SSE) print head, loaded with an extrudable paste composed of 0%, 20% 30% or 40% w/w hydrochlorothiazide (HCT) with Lactose monohydrate, polyvinylpyrrolidone k90 and sodium carboxymethyl cellulose at 3:1:3 mass ratio. Tablets were printed for each HCT level and were dried. NIR measurements were collected in duplicate using the Metrohm RCA and the Viavi® Micro. In addition, the physical property of tablet dissolution was measured using standard USP dissolution methods. Initial Principal Component Analysis (PCA) showed PC1 and PC2 were linked to variation in API content and moisture. Respectively, and the placebo and four levels of API could be classified by PCA1 and PCA2 scores for both the laboratory and micro spectrometers. Dissolution times at 20 minutes could also be distinguished via PCA. To compare algorithms, PCA, K-Nearest Neighbor (KNN), Support Vector Machines (SMV) and Artificial Neural Networks (ANN) were used. Each algorithm produced models which will be compared and contrasted. NIR shows potential as a quality tool for 3D drug printing including predictive modeling to support pass/fail real time release testing.

222 **Next Generation Machine Learning Technologies to Accelerate Pharmaceutical Process Research and Development**

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

Developing pharmaceutical processes to supply medicines and vaccines around the world is a critical task to enable the continued improvement of human health. A deep scientific understanding of our pharmaceutical processes aids in their rapid and improved development. Maximizing the knowledge gained during each conducted experiment and extracting all useful information embedded within the generated experimental data are both vital aspects of pharmaceutical research and development. In order to achieve this desired scientific understanding, novel data-rich technologies at the intersection of data science and digital tools with experimental and spectroscopic methods is needed. In this work, we offer a next generation of machine learning technologies to accelerate and improve process research and development with demonstrated applications across small molecule, biologic, and vaccines modalities. Specifically, we herein provide novel machine learning efforts in conjunction with Raman hyperspectral imaging to molecularly identify and spatially resolve immobilized enzymes for biocatalysis and protein engineering. Further, we offer process analytical technology (PAT) coupled with machine learning to elucidate real time information in an *in situ* manner with applications across large molecule research and development. These new enabling technologies ultimately help uncover important scientific findings that facilitate development of optimal pharmaceutical processes. Overall, our interdisciplinary research presented herein highlights advancements in both digital sciences and data-rich experimentation via showcasing the potential next frontier of process enabling technologies that help meet the needs of the current and future pharmaceutical landscape across modalities.

223 **Automated Solvent Extraction Method of PFAS From Difficult Food and Food Packaging Matrices**

Alicia Stell, CEM Corporation, 3100 Smith Farm Rd., Matthews, NC 28106, Benedict Liu

There is increasing concern of per- and polyfluoroalkyl substances (PFAS) in our environment as a whole, due to their persistent nature. More and more regulations regarding PFAS are being implemented. Having a harmonized method to accurately determine the PFAS content in food as well as other matrices is important to this industry. The extraction of PFAS can be challenging given the susceptibility to contamination and the low levels in which these compounds are present. Existing techniques are predominately manual methods that are not rapid, simple, and efficient. In this study the EDGE PFAS, an automated solvent extraction system, is explored. This method offers efficient extraction of PFAS from challenging food and food packaging matrices in one simple process, where the final extract is filtered and ready for further cleanup and analysis. Excellent extraction recovery and reproducibility of PFAS from a variety of matrices, including food and its packaging, is presented. The EDGE PFAS method offers a rapid, simple, and efficient solvent extraction solution for PFAS testing.

224 **A High-Efficiency Approach to Quantitating Pesticides in Challenging High-Pigmented Food Matrices with GC/MS/MS Using a New Electron Ionization (EI) Source for Maximized Uptime**

Anastasia Andrianova, Agilent Technologies, 2850 Centerville Rd., Wilmington, DE 19808, Samuel Haddad, Limian Zhao

A newly developed and validated high-efficiency workflow solution offers sensitive and robust quantitation of over 200 volatile and semi-volatile pesticides in high-pigmented, challenging matrices with GC/MS/MS. This approach allows for meeting the established maximum residue levels (MRLs) with LOQs as low as 0.01 ppb. Matrix-matched calibration ensures excellent accuracy over a wide dynamic range, spanning up to five orders of magnitude from 0.01-1,000 ppb. Method ruggedness is validated by maintaining measurement accuracy and precision for matrix samples spiked at 2 ppb across 800 runs over 17 days of continuous analysis. Additionally, the presentation discusses key strategies for using hydrogen as an alternative carrier gas, optimizing injection conditions and EI source technology to prevent undesirable in-source reactions and achieve high-quality results. Method translation and retention time locking techniques allow the use of MRM transitions and retention times from a helium carrier gas database. A panel of 200 pesticides was tested in challenging high-pigmented food matrices to evaluate LODs under optimized GC/MS/MS conditions, demonstrating accurate quantitation at or below 10 ppb in QuEChERS extracts. Key components of the robust workflow include efficient sample preparation and cleanup, innovative GC hardware and functionality, GC supplies, the novel EI source technology, and built-in GC/TQ intelligence and software functionality. This comprehensive approach addresses the needs of analytical laboratories in navigating complex matrices and achieving uncompromised results with emerging alternative carrier gases.

225 **Non-Invasive Raman Spectroscopy for the Authentication of Food Products**

Alexander Rzhevskii, Thermo Fisher Scientific, 2 Radcliff Rd, Tewksbury, MA 01876

Raman spectroscopy is now well established as one of the most powerful analytical techniques for characterizing samples of a very complex molecular composition from natural sources. This technique offers a unique capability for a non-invasive and non-destructive spectral analysis of various ingredients, additives, stabilizers, and chemical contaminants within the food chain. Ensuring the authenticity of food products has become an increasingly challenging task for the industry due to the globalization of food markets, widespread economically motivated adulteration, and shortages of valuable food sources. Thus, assessing the authenticity of food products has become paramount, requiring fast and reliable methods. In the presentation, the recent advances in Raman spectroscopy are shown to allow authentication and distinguishing adulteration and contamination of beverages, edible oils, and honey directly in primary packaged (such as in plastic and glass bottles and containers) food goods. Examples of the applications of benchtop Raman microscopes and field-deployable portable dispersive Raman spectrometers for food authentication/adulteration are shown. It is demonstrated that among many analytical methods, Raman spectroscopy offers great potential in the characterization of the nutritional components, quality, and safety of foodstuff as a non-invasive, non-destructive, easy-to-install, ease-to-operate, sensitive, and rapid analytical method at any level from production to the market.

226 **Replicating Water and Fat-Soluble Vitamins Analyses on a Modern HPLC System**

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With the increasing popularity of vitamin supplements, there is a need to ensure the products meet the content descriptions. High performance liquid chromatography (HPLC) is an essential analytical tool to measure the supplements, ensuring products meet label claims. However, the wide range of chemical characteristics make analysis of vitamins with a single mode of chromatography challenging. For highly non-polar, fat-soluble vitamins (e.g., vitamin E) analysis by normal phase is a preferred mode of chromatography, while water soluble vitamins are often analyzed by hydrophilic interaction chromatography (HILIC) to address retention of highly polar vitamins1. In addition, vitamins can pose many analytical challenges as the samples can come in complex matrices and sample preparation can often be laborious. In regulated labs, the ability to migrate these methods to different systems can be essential as systems are updated and replaced with newer systems. In addition, having a single system that can perform both types of analyses, with minimal changes, is crucial. In this work, we will document the ability move methods for both water-soluble vitamins under gradient HILIC conditions and fat-soluble vitamins under normal phase conditions across systems. This work will demonstrate the ability to achieve the same quantitative results on legacy HPLC systems and newer LC systems.

226 A Illicit Drug Desorption and Chemical Profiling of Fingerprints using SICRIT Ion Source: A Rapid Analysis Approach

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The focus of this study centers around fingerprints and how to conduct targeted and non-targeted analysis of analytes on this complex matrix by combining novel instrumental and computational approaches. A dielectric barrier discharge ionization (DBDI) sources, such as the SICRIT Ion Source, have been demonstrated to cover a wide range of analytes. This ionization source, in combination with thermal desorption sampling allows for a rapid analysis while minimizing sample preparation. The study employed a high-resolution mass spectrometer to identify unknown compounds based on exact mass, focusing on three drugs (Fentanyl, Heroin, Cocaine) in varying absolute amounts. Direct thermal desorption of samples, completed in just 2 minutes, revealed ionization of all three compounds as protonated molecules. The limit of detection (LOD) for pure substances and spiked fingerprints, even with a complex matrix of lipids and amino acids, demonstrated sensitivity suitable for detecting trace amounts found in forensic samples. The technology's forensic potential expanded to differentiate individuals based on chemical fingerprint profiles, proving effective even for non-volatile compounds like lipids. Principal component analysis (PCA) and a machine learning pipeline demonstrated the ability to distinguish fingerprints from different individuals, even across multiple days. Overall, this study introduces a rapid SICRIT Ionization Desorption setup for forensic analysis, identifying analytes within minutes, ideal for mobile systems.

227 Understanding the Role of CD68 in the Tumor Microenvironment

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Glycosylation is a post-translational modification characterized by extensive heterogeneity and its critical for a myriad of cellular functions. Mucin-domain glycoproteins have the highest density of glycosylation, and their glycans have been shown to be important in human health. Amongst these is CD68, a histochemical marker for inflammation and tumor associated macrophages (TAMs). Here, we sought to characterize the glycosylation of CD68, how it changes in different macrophage states, and which glycan-binding proteins can interact with CD68. To do so, we expressed and purified CD68 and subjected it to digestion by a series of mucinases, proteases, and glycosidases. Digests were desalted and analyzed via LC-MS/MS using HCD-triggered-EThcD on a Thermo Orbitrap Eclipse. RAW data was searched using and the resulting spectral matches were manually validated. Through our workflow, we were able to localize 51 O-glycosites, with structures ranging from single monosaccharides to doubly-sialylated core 2 O-glycans. Further, we were able to confirm occupancy of 8 out of the 9 plausible N-glycosites, carrying all types of N-glycans. Using inactive point-mutants of our mucinases, we were also able to isolate CD68 and other mucins from monocytes and macrophage lysates. Following digestion and MS analysis, the glycosylation of these mucins was mapped, and their profiles compared. Finally, we conjugated CD68 onto beads, isolated binding partners from peripheral blood mononuclear cells and HeLa cells, digested using Trypsin and analyzed with MS. This work enables further studies to target CD68 and its glycosylation for cancer, immune exhaustion, and inflammation treatments.

228 Peptidomic Analysis of Breast Milk and Sera from Women with Breast Cancer and Equivalent Controls to Identify Potential Biomarkers for Early Diagnosis

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Breast cancer (BC) is one of the top causes of death for women. The early stages of breast cancer may include dysregulation of proteins and peptides, which can be exploited as biomarkers for early identification, particularly in reproductive-age women. Serum and milk analysis have the potential to aid in the detection and analysis of tumor-secreted proteins and peptides, as well as overall disease responses. Mass spectrometry (MS) is useful in peptidomics research because to its high sensitivity and capacity to detect low-abundant and smaller peptides. Serum and milk samples (50 BC vs 50 control serum samples and 20 vs 20 breast milk samples) were separated using 30kDa, 10kDa, and 3kDa MWCO filters, resulting in three fractions for analysis from each sample. The 10k and 30k samples were digested in-solution with trypsin and analyzed using nanoliquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) to detect peptides and proteins that differed between the BC and control samples. The 3 kDa fraction was not digested, but instead Zip-tipped and analyzed directly using nanoLC-MS/MS. The raw data were then analyzed using Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. Fibrinopeptide A (gil229185), a thrombin-cleavage fragment of the Fibrinogen A (FGA) protein (gil18088463), was frequently overexpressed in BC samples compared to controls. Fibrinogen is known to be involved in cancer cell proliferation; however, FGA may also play a role in cell killing. This suggests that FGA has potential as a biomarker of, or progression marker for BC.

229 Proteomics Investigation of Human Breast Milk with Invasive Ductal Carcinoma Breast Cancer and Match Control for Early Detection of Breast Cancer: A Mass Spectrometry Approach

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Breast cancer (BC) is a leading cause of cancer-related deaths in women worldwide. Early detection of breast cancer is crucial. Invasive ductal carcinoma (IDC) is the most common subtype of BC, accounting for 85% of all new diagnoses of BC. It starts in the milk ducts of the breast and invades the surrounding breast tissue. Human breast milk contains secreted proteins, immune cells and exfoliated epithelial cells, and can be collected non-invasively during an important time in breast development. Mass spectrometry-based (MS) proteomic experiments are ideal for the investigation of human breast milk proteins as potential BC biomarkers. Additionally, the MS-based methods allow for quantitation of the observed protein differences. In this study, we performed proteomics analysis on 20v20 lactoferrin depleted human breast milk samples using mass spectrometry to identify protein biomarkers to aid in early breast cancer detection. Samples were collected from women who were lactating for both the BC and their matched controls. In-solution digestions were preformed after depleting the lactoferrin from milk samples followed by nano-liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) to identify proteins which were dysregulated from women with BC and the matched control. Proteins were found to be differentially expressed between breast milk samples from women with BC and without BC. This study will be confirmed with a larger cohort of breast milk samples for validation. If these proteins are found to be consistently dysregulated between BC and the controls, a draft protein biomarker could be determined to aid in early detection of BC.

230 Integration of Innovative Biochemical and Instrumental Methods to Enable Structural Elucidation and Spatially Resolve Labile Carbohydrates

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Glycosylation is one of the most important co- and post-translational modifications since despite less than 2% of genes coding for glycosylation, it impacts more than 50% of the eukaryotic proteome. Glycans are key biological components that make up a significant portion of cells and the extracellular matrix. Consequently, glycans have a wide variety of both physiological and pathological functions. Mass spectrometry imaging (MSI) is a powerful analytical technique in which the spatial distribution of ions can be visualized within a biological tissue section. The most common ionization technique used in MSI is matrix-assisted laser desorption ionization (MALDI). MALDI can successfully image *N*-linked glycans. However, there are certain charged carbohydrate moieties that are labile in nature and require chemical derivatization to prevent loss. Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) is an ambient ionization technique that combines the benefits of MALDI and electrospray ionization (ESI). Due to the ESI-like ionization mechanism, IR-MALDESI deposits lower amounts of internal energy onto the sample compared to MALDI and can ionize and detect intact glycans without the use of chemical derivatization. This grants IR-MALDESI the unique ability to achieve deep glycomic coverage while also preserving crucial spatial information. In this presentation, we report enhanced coverage of the brain glycome, a new rule to predict sialic acid (NeuAc) content, novel insights into the role of sialylated glycosylation in fetal and adult blood clotting, and the adaptation of the IR-MALDESI platform to image glycosaminoglycans (GAGs).

231 Optimizing Surface Plasmon Resonance (SPR) Characterization of the Binding Between Individual Tandem Repeats of MUC16 (CA125) and Clinically Relevant Antibodies

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Survival rates for ovarian cancer are improved if diagnosed at an early stage, but most cases are not diagnosed until late stage. CA125, a peptide epitope on the large mucin protein MUC16, is a US FDA-approved biomarker to monitor the recurrence of ovarian cancer. The epitope is located within a region of many tandem repeats with similar amino acid sequences, but the exact epitope location has long been an important gap in our knowledge. Previously our group and others have demonstrated that the antibodies used in the clinical test (OC125 and M11) display variable binding to a subset of individually expressed recombinant tandem repeat proteins. To determine the location of the CA125 epitope, we performed the most comprehensive study to date characterizing the binding of individual recombinant tandem repeat proteins with clinically relevant antibodies. The repeats were expressed in E. coli and binding kinetics were measured with surface plasmon resonance. We optimized immobilization and pre-concentration parameters, assessed non-specific binding, and scouted regeneration conditions. As we anticipated from our previous work, we see variable binding responses across the repeats and a range of binding affinities,

giving us a more detailed understanding of where CA125-specific antibodies are and are not binding to MUC16. A more comprehensive characterization of the tandem repeat domain will yield important insight into why CA125 may be under detected in some patients. Additionally, this knowledge will offer the chance to develop new affinity reagents that target the currently undetected regions to increase the utility of this biomarker.

232 Developing a Method to Monitor the Estrogen-Inducible Proteins in Fishes from the Great Lakes upon Exposure to Environmental Contaminants

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A major goal of the current project is to monitor expression of specific liver proteins in lake trout (Salvelinus namaycush) and walleye (Sander vitreus), upon exposure to environmental contaminants (within the Great Lakes). Some of these contaminants, like polychlorinated biphenyls (PCBs), have estrogenic activity (Endocrine Disrupting Compounds or EDCs). Two important fish proteins whose levels change upon exposure to EDCs are vitellogenin and zona radiate proteins. These are prime candidates for our study for several reasons: a) the levels of these proteins increase in female fishes upon exposure to EDCs, b) these proteins' expression is induced in males only after exposure to EDCs. c) both proteins are produced in only male fish upon exposure to EDCs, d) both proteins are produced only in the liver. We used mass spectrometry-based proteomics to analyze fish homogenates from three of the Great Lakes (Erie, Huron and Michigan) and to identify the vitellogenin and zona radiate proteins. We were successful in identifying vitellogenin in all samples, but have not been able to identify the zona radiate protein yet. The study is ongoing and will attempt to identify the relationship between expressions of vitellogenin due to the exposure to EDCs and allow us to monitor the influence of EDCs in the lake trout from Great Lakes.

233 Development and Qualification of a Greener High pH Generic HPLC Method

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Open-access HPLC systems within a small molecule process chemistry laboratory provide fit for purpose analytical data to guide chemists through process development. Two categories of methods run on these systems: generic methods and compound specific methods. Generic methods are designed as first intent separation methods, to determine the yield and purity of most reaction mixtures and materials. Desirable metrics for generic methods are a minimum peak capacity of 100 and an analysis time within 5 minutes. A recent push for more environmentally sustainable methods has warranted re-development of these methods with an aim to reduce solvent usage. Herein is presented an updated high pH generic HPLC method and its qualification. Compared to its predecessor, the Analytical Method Greenness Score (AMGS) improved, and method performance was the same or better. Relevant validation parameters were examined through three case studies of representative compounds from a small molecule pharmaceutical process development laboratory.

234 Electron Transfer Reactions of Transition-Metal Complexes for Solar Energy Conversion and Storage

Annie Shen, Bryn Mawr College, 101 N. Merion Ave, Bryn Mawr, PA 19010, Michael Eberhart

The possibility for hydrogen as a clean source of energy is limited by its production from fossil fuels. An avenue to generate hydrogen fuel sustainably is through electron transfer chemistry involving a photosensitizer to split water into hydrogen and oxygen favorably. Our research focuses on the synthesis and electrochemical experimentation of transition-metal complexes with multiple charge accumulating sites. Splitting water into hydrogen and oxygen requires four individual electron transfers, so the accumulation of electrons in our transition-metal complexes will overcome the tendency for the reverse reaction. Co^{2+}, Fe^{2+}, Ni^{2+}, and Pd^{2+} di-nuclear metal complexes were synthesized according to literature reactions and confirmed with NMR spectroscopy before ligand variations were made to observe changes in reduction potentials. Cyclic voltammetry (CV), UV-visible spectroscopy, and spectro-electrochemistry experiments were conducted to compare respective electrochemical properties. Data gathered from these experiments were used to predict electron transfer behavior of the synthesized compounds with a Ruthenium photosensitizer during a photo-induced reaction.

235 Sensing Copper (II) Ions with Coumarin Dyes

Revathi Variar, West Chester University, 218 Iron Lake Dr., Chester, PA 19341, Jingqiu Hu

Monitoring copper levels in food, water, and environmental samples is crucial, as copper is an essential micronutrient for both plants and animals. In this project, the photophysical properties of two coumarin compounds have been studied to explore their potential as fluorescent sensors for copper (II) ions. Coumarin 1 and Coumarin 35 displayed weak fluorescent emission in pure water with emission maxima of 470 nm for Coumarin 1 and 540 nm for Coumarin 35, respectively. Upon addition of PMAA in acidic conditions, the emission maximum of Coumarin 1 shifted to 430 nm with over 41-fold enhancement while the emission maximum of Coumarin 35 blue shifted to 460 nm with over 1300-fold enhancement when the R/D ratio is 1000. The sensitivity of coumarin dyes to microenvironments makes them promising chemical sensors. The presence of copper (II) ions effectively turned off the emission of Coumarin 35/PMAA mixture. The linear response range is determined to be 5.0 - 35 micromolar of copper (II) ions when R/D ratio is 50 in acidic condition. This method has a good selectivity toward copper ions over other metal ions.

236 Assessing Algal Toxin Contamination in Connecticut Freshwater Systems Utilizing Liquid Chromatography Tandem Mass Spectrometry

Slawomir Piela, Drexel University, College of Medicine, Wyomissing, PA 19610, Anthony Provatas, James Stuart

Algal toxins are a diverse group with a variety of toxicological profiles. They can have a variety of medically significant effects and pose a significant public health hazard. Many common medically significant toxins are found in freshwater systems including recreational areas and drinking reservoirs. The intersection of algal growing seasons and freshwater resource usage, presents a dangerous exposure risk. The presence of many algal toxins cannot be correlated with an active bloom. Methods to quantify toxin presence in water bodies are complicated by regional variation of algal species and toxins. The variability of algal species and the toxic products they express are related to overall species factors as well as individual environmental influences. In essence, broad national studies are unable to succeed due to the inherent highly region-specific nature of this issue. Public health authorities and medical literature are additionally not in agreement with what constitutes dangerous levels of algal toxins creating an unprecedented lack of clarity for policy makers and clinical decision makers. This study serves to quantify and speciate a specific class of algal toxin in a temperate well-developed freshwater environment. Additionally, this study serves to apply broad WHO guidelines on exposure and tolerance limits to generate basic exposure guidelines for the impacted region.

237 Qualitative Analysis of Polycyclic Aromatic Hydrocarbons in Vehicle exhaust and Roadway Surfaces Utilizing GC-MS/MS

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Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants and known carcinogens, primarily originating from the incomplete combustion of organic matter. This study focuses on the petrogenic production of PAHs that pollute our roadways and will be done qualitatively for 27 predetermined PAHs. Samples of both varying car exhausts and different locations on the roadways were collected. Analysis was conducted using gas chromatography-tandem mass spectrometry (GC-MS/MS).

238 A Green Chemistry Methodology for the Degradation of Malathion

Michael Eckel Santos, Seton Hall University, 14 Hillary Terrace, Succasunna, NJ 07876, Marius Pelmus, Sergiu Gorun

Malathion is a widely used organophosphate pesticide employed for killing mosquitoes and flies. Its metabolite, Malaoxon, which is about 60x more toxic and is also produced during wastewater chlorination treatment, thus raising the issue of finding more environmentally friendly ways to degrade Malathion. We report that Malathion is photodegraded by reactive oxygen species (ROS) generated photochemically by the robust hexadecafluoro zinc phthalocyanine $F_{16}PcZn$. The anchoring of this photocatalyst on alumina (Al_2O_3) resulting in a hybrid material which degrades Malathion by ~99.4% in 2 hours using visible light. A new GC-FID analytical method was designed and used to reveal that the undesirable Malaoxon was not present amongst the photodegradation products. Taken together the results define a green chemistry methodology for the removal of Malathion from wastewater without creating more toxic products.

239 Precise Water Analysis in Cannabis: Karl Fischer (KF) Titration Method

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The interest in cannabis and its derivatives for recreational and medical applications has surged, prompting the need for stringent quality control measures to prevent recalls due to contamination or incorrect cannabinoid profiles. The 2018 Agriculture Improvement Act introduced a new framework for defining cannabis and its products, notably distinguishing hemp from marijuana based on delta-9 tetrahydrocannabinol (THC) concentration. Hemp, with a THC concentration of not more than 0.3% on a dry weight basis, is now excluded from the Controlled Substances Act, emphasizing the importance of precise water concentration measurements due to potential microbial growth risks. While traditional methods like loss of drying (LOD) at 40°C over 24 hours under vacuum over molecular sieves are used to determine water content in

regulations such as the European Pharmacopeia or Deutsches Arzneibuch (DAB), they also capture volatile substances, including essential oils, leading to inaccurate readings. The Karl Fischer (KF) titration method emerges as a superior alternative, offering rapid and selective water content determination without interference from volatile compounds. Utilizing a double platinum electrode and iodine solution for titration, the KF method provides automated endpoint detection through a reversible redox system of iodine and iodide, ensuring precise measurements. This study aims to validate the efficacy of the KF titration method by comparing its results with those obtained through conventional oven techniques, demonstrating its accuracy and efficiency in cannabis water content analysis.

240 An Automated Dynamic Headspace Approach for the Determination of Ignitable Liquid Residues from Mock Arson Evidence

Megan Harper-Kerr, GERSTEL, Inc., 701 Digital Dr., Ste. J, Linthicum, MD 21090

In forensic investigations, analyzing ignitable liquid residues (ILRs) obtained from crime scenes is critical for establishing whether a fire was deliberately set and potentially identifying a perpetrator. Traditional methods for extracting ILRs from fire debris, such as solvent and headspace extractions, often have significant drawbacks. These methods can destroy the sample, involve lengthy manual procedures, and require harmful solvents. This study explores the efficacy of an automated dynamic headspace (DHS) extraction technique using sorbent-filled tubes to isolate ignitable liquids from mock arson evidence. The DHS method offers several advantages over traditional extraction methods, including non-destructive sample handling, improved sensitivity, and the elimination of hazardous solvents. This study demonstrates an automated DHS approach to extract three commonly used ignitable liquids from mock arson evidence.

241 Investigating Odor Signatures of Electronic Storage Devices

Samuel Friday, University of New Haven, 300 Boston Post Rd., West Haven, CT 06516, Jon Naples, Pauline Leary, Marisia Fikiet, Alyssa Marsico, Brooke Kammrath

Electronics are integral tools for many aspects of our everyday lives, and thus may contain valuable evidence for criminal investigations. Recent developments in canine detection have shown that dogs are capable of distinguishing characteristic odor signatures/profiles of electronic mass storage devices (MSDs) such as micro cell phones, USB drives, and SD cards with levels of accuracy identical to when they detect drugs, explosives, and people. However, the specific chemistry of said detection is unknown, since the sensitivity of a dog's nose far exceeds that of modern instrumentation. Current research on electronic device odors is minimal and contradictory. At issue is the role of triphenylphosphine oxide (TPPO) in canine detection of MSDs. TPPO is a flame retardant which coats all electronic printed circuit boards to prevent their overheating, and was identified as a target odor to train canines by the Connecticut State Police K9 unit in collaboration with a forensic chemist. Other research using room temperature headspace analysis via solid phase microextraction (SPME) concluded that MSDs do have characteristic odor profiles, making detection with minimal false alerts feasible for trained canines. However, no TPPO was detected or identified in this study, contradicting prior research and the demonstrated of TPPO in canine detection training. This research aims to resolve this question through an investigation of different GC-MS sampling methods of MSDs.

242 Achiral Separation of Fluorofentanyl Derivatives on Chiral Stationary Phases in Varying Mobile Phase Modes

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Drug overdoses have consistently risen in the United States over the last two decades. The CDC estimates nearly 108,000 deaths in 2022 alone, with fentanyl and other synthetic opioids making up nearly 70% of those deaths. Derivatives of fentanyl, like fluorofentanyl, are appearing more frequently in seized samples when tested for composition. Other than filler substances like lactose and mannitol, fluorofentanyl now constitutes the most commonly found adulterant in seized fentanyl samples. Para-fluorofentanyl is the most common regioisomer of fluorofentanyl, however it's possible for two other regioisomers, meta- and ortho-fluorofentanyl, to be present. While there are currently several methods published for the separation of these isomers, none are able to separate all 3 isomers in the same method. It's imperative that a reliable testing method for the identification of each isomer is established.

This study is focused on establishing such a method by using Daicel polysaccharide-based chiral stationary phases (CSPs). Although most often used for the separation of optical isomers, CSPs have also demonstrated their ability to separate a wide range of isomers, including diastereomers, regioisomers, structural isomers, and atropisomers. To provide utility and flexibility in the application, the method development was conducted with both high-performance liquid chromatography (HPLC) mode with normal phase and reversed phase solvents, as well as with super-critical fluid chromatography (SFC) mode. Optimization of each mode as well as single isomer peak identification for each method are shared.

243 Analysis of Volatile Organic Compounds from Submerged Animal Tissue Decomposing at Varied Temperatures

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The search for human remains is important in forensic science and often involves searching bodies of water. There is currently no forensic procedure to use the chemical analysis of water to identify potential areas of interest to find decomposing remains, and searches often must solely rely on scent detection canines or visual observation. This study involved the analysis of volatile organic compounds (VOCs) in the headspace of water associated with the decomposition process of animal tissue using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS) in water at different temperatures. Pieces of pork belly (n=3 replicates) were decomposed in jars filled with tap water at three different temperatures (32˚C, 22˚C, and ~5˚C) for 12 days and compared to jars containing no pork belly. Water samples were taken from each jar and analyzed using GC×GC-TOFMS. ChromaTOF Tile software was used to see the similarities and differences in the sample environments. The most prominently differing chemicals were octathiocane and 1-dodecanol. The longer the pork decomposed the fewer similarities there were between the pork samples and the water samples. Samples at warmer temperatures started becoming more dissimilar from the room and cold temperatures later in the trial. The room temperature samples did not differ prominently from the coldest temperature. GC×GC-TOFMS was shown to be able to differentiate differences in decomposition status between the environments. These findings provide direction for additional research into the use of headspace water analysis by GC×GC-TOFMS for future use in forensic investigations.

244 Analysis of Green Gunshot Residues Using Scanning Electron Microscopy with Energy-Dispersive X-Ray Spectroscopy and Comprehensive Two-Dimensional Gas Chromatography

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

245 Chemical Fingerprint of Ignitable Liquid Residues by DART-MS: More Than Volatile Organic Compounds

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The detection of IL is critical to the arson investigation process, which may potentially identify the cause of the fire and the ILs used to initiate the fire. The most commonly used technique to analyze IL is GC-MS; however, it has a major limitation of analyzing only the volatile organic compounds (VOC) in IL. The non-volatile or less volatile components in IL are likely to be contained in the fire debris and hence could yield corroborating evidence on the use of specific ILs in the investigation. Direct analysis in real time mass spectrometry (DART-MS) is an emerging analytical technique in the forensic community that has shown promising capability in drug analysis. However, its potential in other forensic fields, such as IL analysis, has not been fully explored. In this study, the IL, such as gasoline, paint thinner, and biodiesel were analyzed by DART-MS, and the results were compared with the traditional GC-MS method. In comparison to the DART-MS data, the GC-MS profiles changed significantly among weathered IL samples because the weathering process alters the relative quantities of the IL. The DART-MS data of ILs provided less variable profiles and, therefore, may not be used to predict the weathering percentage of IL. However, the capability of DART-MS in detecting the less volatile IL constituents, such as fuel

additives in gasoline and glycol ethers in paint thinner, imparts its unique ability to discriminate among different types of ILs.

246 The Role of Diphenylamine in Enhancing Fluorescence Analysis of Smokeless Powder and Gunshot Residue for Forensic Purposes

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Smokeless powder (SP) is a common type of propellant used in firearms. Gunshot residue (GSR) is a type of trace evidence that refers to the residual particles left on the hands, clothing, or surfaces post firearm discharge. The most common tool for the analysis of IGSR is SEM/EDS, but this technique is expensive, destructive, and time-consuming. The analysis of SP can be challenging because the formulation is protected by intellectual property laws. To optimize the novel, two-step method for GSR detection and identification developed in our lab, the luminophore(s) present in GSR must be known. The objective of this study is to combine the use of preparative HPLC, steady-state fluorescence, and QTOF LC/MS to determine the characteristic luminophore(s) responsible for the fluorescence of SP and GSR. The fluorescent spectra of HPLC purified fractions have an excitation lambda max at 385 nm and an emission lambda max at 420 nm. The QTOF LC/MS results have consistent retention values between runs, and this peak corresponds to the same molecular ion $[M+H]^+$ at m/z = 170. This three-pronged approach suggests that diphenylamine (DPA) was found to be responsible for the fluorescence of SP and GSR. DPA is a common stabilizer, and our results suggest it survives combustion since it was found in both SP and in GSR. SP and GSR analysis are important for criminal justice because they provide critical evidence that can help to establish the guilt of a suspect in a shooting-related crime.

247 Free Tools to Support Liquid Chromatography Teaching, Learning, and Method Development

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Liquid chromatography is a powerful analytical tool used in diverse application areas ranging from separation of enantiomers to characterization of complex natural products. Fully leveraging the power of the technique requires in-depth understanding of how LC works, as well as its limitations, and is facilitated by simulation tools that extend the imagination of the analyst. In this poster we will introduce and demonstrate three free, web-based tools that are useful for teaching and learning about LC, and in the process of method development. They are useful to users at all experience levels, ranging from absolute beginners to experts. These tools are: 1) an easy-to-use LC simulator for reversed-phase separations that enables exploration of important parameters including mobile phase composition, temperature, flow rate, column length, and particle size (www.multidlc.org/hplcsim). Teaching resources (e.g., homework assignments) have been developed around this tool, and are freely available to educators; 2) an advances LC simulator for reversed-phase separations – this tool extends the capabilities of the first simulator by allowing the user to change column chemistry, and simulation two-dimensional separations (www.multidlc.org/multiSimLC); 3) an LC columns database that enables exploration of similarities and differences between over 750 different commercially available reversed-phase columns (www.hplccolumns.org). Importantly, these tools are being coupled with experimental retention data that is flowing from our wikiChrom Project that aims to bring the power of big(ger) data to chromatography simulation tools (wikichrom.multidlc.org). We are eager to share these free tools with the chromatography community, and open to collaboration to improve their utility.

248 2.7-um Superficially Porous Particles for Chiral Chromatography in HPLC and SFC

Melissa Wilcox, Regis Technologies, 8210 Austin Ave., Morton Grove, IL 60053

The use of superficially porous particles is an effective means to improve analysis speed and column efficiency for both high pressure liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). The technology is also applicable to the separation of enantiomers using chiral stationary phases (CSPs). In this poster, we discuss practical approaches to chiral separations using superficially porous particles in order to improve analytical throughputs and production rates. Comparisons between separations using the same Pirkle-type CSP Whelk-O1 bonded to both fully porous and superficially porous particles are shown. The data highlights many differences in speed, retention, and resolution between the particle types. Sample loadability on superficially porous and fully porous particles is also compared, and the implications for the use of superficially porous particles for chiral preparative applications is discussed.

249 Enhancing Basic Thin Layer Chromatographic Analysis: Application-Driven Capabilities of the TLC Explorer

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The TLC Explorer is a comprehensive documentation system designed for manual thin-layer chromatography (TLC). It facilitates data capture, analysis, and archiving of chromatograms, thereby enhancing usability and reliability of chromatographic data processing. Through various application, the TLC Explorer's capacity to streamline the identification of substances following separation via TLC is demonstrated. Four examples from different application fields are presented: in-process control for catalytic synthesis, quantification of a cosmetic formulation, fingerprint analysis of a natural extract, and method development workflow for the replacement of non-sustainable solvents with sustainable alternatives. Additionally, we illustrate its utility in ensuring compliance with the stringent requirements outlined in the European Pharmacopoeia (EP) and the United States Pharmacopeia (USP). By presenting the seamless integration of the TLC Explorer into chromatographic workflows, this poster underscores its pivotal role in advancing fast and convenient classical thin layer chromatographic analysis in modern laboratory practices.

250 Evaluation of Bidentate End-Capping Silylation Reagents for HPLC

Scott Sliver, Pyvot, 1040 1st Ave., Ste. 330, New York, NY 10022, Norikazu Nagae, Ryuji Koyama, Tomoyasu Tsukamoto

Silica-based reversed-phase columns have been widely used since the 1970s, and end-capping by trimethylsilylation has been performed to reduce the influence of residual silanol groups after alkyl group bonding. End-capping reagents have also been improved, and efficient end-capping methods have been proposed. In this study, 1,5-dichlorohexamethyltrisiloxan and 1,2-Bis(chlorodimethylsilyl)ethane, which are bidentate end-capping reagents that form siloxane bonds on the silica surface at two locations, were evaluated as C18 stationary phases using three types of silica-based materials, including a hybrid type. The influence of residual silanol groups was compared using amitriptyline as well as hydrogen bonding, hydrophobicity and steric selectivity. A commercially available hybrid type C18 column was used as a reference column. The durability under acidic pH and basic pH conditions was also evaluated.

251 In-Process LCMS Analysis of Polysorbate 80 Biodegradation

Jixin Liu, Croda, Inc., 315 Cherry Ln., New Castle, DE 19720, Kate McEvoy, Wenyi Yee

Sustainability is a critical focus across various industries, including consumer care, agriculture, and pharmaceuticals. Ongoing research into biodegradable surfactants aims to reduce environmental impact by ensuring these compounds break down naturally. Understanding their degradation pathways and by-products is vital for assessing their environmental fate. High-resolution mass spectrometry (HRMS), combined with chromatography, plays a crucial role in identifying compounds and analyzing complex mixtures. This powerful technique enables the characterization of biodegradation products, providing essential insights into the biodegradation process. Polysorbates (PS), commonly known by their trade name Tweens, are non-ionic surfactants with a long history of safe use in industries such as pharmaceuticals, agriculture, and beauty, due to their low toxicity and high biocompatibility. This study presents an analytical investigation into the biodegradation process of Polysorbate 80 (PS 80), utilizing OECD 301F methodology combined with in-process LC-MS analysis. Our findings identify the degradation products and elucidate the degradation mechanism of PS 80, including ester hydrolysis and the formation of carboxylated polyethylene glycol (PEG). This research enhances our understanding of the environmental fate of PS and informs the design of innovative biodegradable surfactants.

252 A "Trimmed-Down" Look into Using Analytical Techniques for Lipid and Protein Composition in Aging Indian Hair

Nusrat Islam, TRI Princeton, 601 Prospect Ave., Princeton, NJ 08540, Ernesta Malinauskyte, Vanessa Castro, Daniel Strzeszewski, Lijing Xu

This study explores the use of HPTLC, SDS-PAGE, and HPLC to understand the roles of proteins and lipids in the aging of Indian hair fibers. The research aimed to fill gaps in the existing literature by analyzing lipid composition variations among different age ranges of hair fibers and examining changes in proteins and lipids along the hair shaft. This study revealed that compared to other ethnicities, Indian hair has higher concentration of specific lipids such as triglycerides and free fatty acids. Protein analysis suggested increased crosslinking within aging hair shafts, likely due to UV or heat treatments. These results may help to tailor hair care strategies for specific ethnicities and emphasize the need for early protection against environmental damage to preserve hair health and enhance the efficacy of hair care products.

253 Analysis of Chloro-thioethers Photodegradation by Fluoro-phthalocyanines in Homogenous and Heterogenous Systems

Sean Scally, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079, Sergiu Gorun, Marius Pelmuş

Chloro-thioethers, environmental polluters, exhibit acute toxicity to humans. Their selective, partial oxygenation is of interest. We report that symmetric F_{64}PcZn, and functionalized, asymmetric $F_{48}H_7$(COOH)PcZn fluorinated phthalocyanines oxygenate Chloro-thioethers via a $^1O_2$ pathway to produce mostly less toxic sulfoxides, as determined by GC-MS.

254 Automated Biorelevant Solubility Workstation for Long-Acting Injectable Drug Development

Michael Rerick, GSK, 1250 S. Collegeville Rd., Collegeville, PA 19426, Luis Herran

High-throughput automation is essential for the advancement of analytical screening in pharmaceutical drug development by improving experimental quality, turnaround time, and resource management. Assay requirements for active pharmaceutical ingredients (API) and solvents can be reduced through the miniaturization of sample preparation steps, resulting in either a greener process or an improvement to the dataset quality by introducing additional replicates. The incorporation of automation is particularly beneficial when applied to long-acting injectable (LAI) drug development. These compounds exhibit slow dissolution rates and low solubility (< 10.0 µg/mL) under biological conditions to control extended release once administered, requiring highly sensitive chromatographic methods to study solubility in biorelevant media. Automation technologies can be implemented to enable full automation of the sample handling steps for these assays, resulting in highly accurate and efficient workflows. In this work, we developed a custom workstation using the Hamilton Vantage, tailored for LAI solubility screening. Training of new analysts has been streamlined by using bespoke interfaces specific for each sample handling step, while still maintaining the flexibility needed to meet a diverse range of experimental requirements. UHPLC method optimization was performed on an Agilent 1290 to reduce limits of quantification for LAI drug substances down to 10 ng/mL through solvent focusing chromatographic techniques. Overall, this method has been utilized to screen a diverse range of LAI candidates to-date and highlights how automation can enhance analytical workflows in pharmaceutical sciences.

255 Enhancing the Automated Screening of Physicochemical Properties of the Discovery Portfolio

Jordan De Jesus Silva, Analytical Research and Development, MRL, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Susana Morais, Alexander S. Chin, Dorothy Levorse, Devan McCoy

Merck's drug discovery pipeline relies on the ability to perform ADMET assays for early-stage drug development. Triaging according to desired physicochemical properties enables decision making on where to allocate resources and time to advance drug candidates to (pre)clinical testing. In that realm, logD and solubility are crucial properties that not only impact the bioavailability, adsorption, and toxicity of drugs, but also give insights into their dosing and formulation strategies. Over the last 15 years, Discovery Analytical Research has spearheaded the safe, efficient, and reliable execution of solubility and logD studies leveraging high-throughput automation. Addressing the needs of their stakeholders in Discovery Chemistry and Pharmaceutical Sciences, high-throughput Soly/logD (HTS) experiment was designed in collaboration with Discovery Sample Management (DSM) to facilitate the request and analysis of over 400 samples resulting in the solubility of each in 3 buffer solutions and their logD, weekly. The advent of macrocyclic peptides has caused a paradigm shift within our company. Apart from the synthetic challenges that limit the amount of material available to perform ADMET assays, this class of "*small molecules*" has been comparatively underexplored and offers opportunities to implement novel analytical techniques and redesign of established procedures. Herein, we discuss the development of an enhanced HTS (eHTS), restructured to deliver 11 solubilities at lower sample cost and improved carbon footprint. We highlight the administrative challenges overcome in collaboration with DSM, boosted efficiency in sample preparation and the design of automated analysis methods to accommodate for a ~4-fold increase in weekly data output.

256 Development of Novel Technologies as Enabled by Pre-Competitive Collaborations: The Enabling Technologies Consortium

Rahul Sangodkar, Amgen Inc., 360 Binney St., Cambridge, MA 02142

Solubility measurement and analyses are integral to pharmaceutical process development at various stages, including chemical route selection and optimization, crystallization design, and formulation development. Advances in automation and innovative technologies offer avenues to improve and address accuracy, reproducibility, and robustness for measurement of solubility in bio-relevant and process-relevant using manual and automated platforms. The Solubility Working Group in the Enabling Technologies Consortium (ETC) is pre-competitive forum for participating companies to identify technology gaps across the industry and evaluate opportunities, partner with experts, and develop new high-throughput automated technologies, which can be deployed broadly for solubility measurement.

257 Leveraging an Automated Standardized Approach to LC Method Development.

Scott Hartzell, Eli Lilly and Company, 1400 W. Raymond St., Indianapolis, IN 46221

Leveraging an automated standardized approach to LC method development accelerates the delivery of safe and efficacious therapies to patients by shortening the path to commercialization. This approach tackles challenging analytical separations within a limited timeframe, ensuring patient safety through a Quality by Design (QbD) framework. By integrating Liquid Chromatography-Mass Spectrometry (LCMS) alongside robust method development strategies, the process emphasizes modeling, expert knowledge, and advanced software tools. Using these advanced tools and a standardized systematic approach can enhance analytical controls, improve efficiencies, and aid in the separation of complex analytes. The methodology analyzes chromatographic data to recommend optimal LC conditions, taking into account the intended use and constraints like resolution, run time, peak shape, and robustness. These comprehensive analytical separation databases or well-defined design spaces, facilitates quick and efficient development of dependable analytical methods. Continuous updates to the database are crucial when unexpected impurities are identified, thereby enhancing Chemical Manufacturing and Control (CMC) strategies and ability to iterate on best separations for the appropriate developed method. Ultimately, an automated standardized approach propels the innovation of robust and reliable analytical methods, crucial for advancing CMC speed and ensuring high-quality pharmaceutical products.

258 Materials for Inhaled Aerosol Treatment of Disease: Optical Photothermal Infrared Microscopy (O-PTIR) as an Advanced Characterization Method for Assessing the Emitted Dose of Complex Dry Powder Inhaler Formulations

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Dry Powder Inhalers (DPIs) are used every day by millions of people to treat symptoms of asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and other lung diseases. The aerosols emitted by the DPIs into the patient's lungs are highly engineered nano- to microparticles containing a mixture of excipient(s) and drug(s). Improved characterization of these materials offers an opportunity to better understand current formulations and the factors contributing to aerosolization performance. Optical photothermal infrared spectroscopy (O-PTIR) offers rapid IR, Raman, and fluorescence imaging capability with a resolution of ~300-500 nm. This allows assessment of relative ratios of drug/drug/excipient at the particle level and, thus an understanding of the distributions of particle composition as a function of particle aerodynamic characteristics within the ~ 1-5 micron size range of interest for delivery to the lung. We will present data illustrating how particle composition can vary with as function of formulation, inhaler actuation conditions, and aerodynamic particle size distribution (APSD). O-PTIR is a powerful tool for characterization of dry powder inhalers and offers substantial promise for name brand/generic comparisons and optimization of formulations during product development.

259 Applications of Combined O-PTIR and Raman in the Analysis of Microplastics from Environmental Samples

Bangshuai Han, Ball State University, 2000 W. University Ave., Muncie, IN 47306, Moayad Yacoub, Samuel Tenney

Microplastic pollution is an emerging concern to the environment and public health. However, the identification of microplastics has been challenging, time consuming, with limited confidence. Until recently, microplastics characterization had been largely limited to either optical characterization or analysis by a single spectroscopic technique such as Raman scattering, which can make particle identification challenging. The recent development of multimodal optical-photothermal infrared (O-PTIR) and Raman spectromicroscopy provides a unique solution for the identification of microplastics. Infrared spectroscopy (IR) can be useful in the identification of unknown compounds and plastics, however the wavelength-dependent spatial resolution required by most IR microscopy methods can prove challenging, if not impossible, for sparsely dispersed and small microplastic samples. The multimodal O-PTIR and Raman tool provides wavelength-independent sub-500 nanometer spatially resolved infrared and Raman spectra from the exact same location and at the exact same time, while concurrent optical imaging allows for fast screening and identification of particles of interest. We will introduce the O-PTIR and Raman technique and discuss how it has been utilized to identify the microplastics present in environmental samples.

260 O-PTIR Submicron Spectroscopy and Imaging of Bone Tissue Mineralization

William Querido, Temple University, Department of Bioengineering, 1947 N. 12th St., Philadelphia, PA 19122

Tissue biomineralization is remarkably fascinating, resulting in composite matrices in which properties of organic molecules and inorganic crystals drive tissue function in health and disease. In bone, the basic building blocks of its mineralized tissue are type I collagen and apatite (calcium phosphate), whose molecular and crystal composition and structure are essential determinants of bone strength and quality. In this talk, be ready to delve into bone mineralization through the novel application of optical photothermal infrared (O-PTIR) spectroscopy and imaging—which offer excellent tools to investigate tissues and biomaterials at the chemical and molecular level with an unprecedented 500 nm spatial resolution. I will share some key findings and exciting directions of my quest using O-PTIR spectroscopy, combined with machine learning analysis of spectral data, to elucidate how bone submicron-level composition may contribute to the reduced bone quality and increased risk of fractures affecting individuals with bone diseases, such as osteoporosis and osteogenesis imperfecta. I hope this talk will highlight how a deep dive into the building blocks of tissue quality through innovative techniques may offer a new perspective to improve the clinical diagnosis of diseases and the design of optimized strategies for tissue regeneration.

261 Spatially Resolved Spectroscopy for MOF-Based Resist Development

Andrea Kraetz, Johns Hopkins University, Department of Chemical and Biomolecular Engineering & Institute for NanoBioTechnology, Baltimore, MD 21210, Prerna Prerna, Mueed Ahmad, Ilja Siepman, J. Anibal Boscoboinik, Michael Tsapatsis, Samuel Tenney

The solvent- and electron-beam- (e-beam-) sensitivity of Zeolitic Imidazolate Frameworks (ZIFs) can be detriments for their potential uses as adsorbents and catalysts and for the determination of their structure using electron microscopy and diffraction. However water-soluble ZIFs upon e-beam exposure are rendered insusceptible to facile dissolution by water, enabling their use as resists for electron-based lithography. Here, we use spatially resolved spectroscopy with polarized infrared to determine spectroscopic signatures of e-beam induced changes in a prototypical ZIF, called ZIF-L. We confirm an earlier suggested two-stage dose-dependent evolution consisting of an amorphization stage at low doses, followed by a chemical altering stage at higher doses, and suggest that the former is characterized by interlayer hydrogen bond breaking, while in the later, high dose stage, e-beam induced 2-methyl-Imidazole (2mIm) ring opening and dehydrogenation lead to the formation of a zinc cyanide amorphous framework. The structural changes caused by e-beam treatment inform the choice of solvent for pattern development. Notably, while the dissolution of ZIF-L in water slows down at high pH, ZIF-L treated at the low-dose regime (between 2-3 mC/cm2) undergoes accelerated dissolution in these basic solutions allowing the realization of positive tone resist behavior (i.e., dissolution of the e-beam treated areas and retention of the crystalline untreated ZIF-L).

262 Forensic Soil Analysis by Particle Correlated Raman Spectroscopy (PCRS): Comparison to Traditional Methods

Jasmine Kaur, University of New Haven, 59 Front Ave., West Haven, CT 06516, Joshua Christensen, Drew Kuroda, Ella Galvan, Ethan Groves, Christopher Palenik, Peter De Forest, Marisia Fikiet, Virginia Maxwell, Brooke Kammrath

Particle correlated Raman spectroscopy (PCRS) is a powerful tool for the analysis of mixtures of discrete particles, such as the population of mineral grains in a soil sample. This is because PCRS combines image analysis and Raman spectroscopy to provide information regarding both morphological characteristics and chemical composition, thus furnishing comprehensive and distinguishing material insights. This presentation will focus on a comparison of the PCRS results of the mineral fractions with those obtained through traditional forensic soil analysis methods for 10 soil samples collected in the Northeast United States. Findings unveil both similarities and disparities between the PCRS results and those produced by traditional soil analytical techniques, which includes polarized light microscopy, X-ray diffraction, and infrared spectroscopy. This research delves into the evaluation of an emerging technique for forensic soil analysis, hinting at the potential of PCRS not only in forensic contexts but also in diverse material examinations, including other geological, clinical, or industrial analyses.

263 Towards Determining the Limit of Detection for a Universal Body Fluid Identification Method for Forensic Purposes

Riley Alpuché, University at Albany-SUNY, 1400 Washington Ave., Albany, NY 12222, Ben Taubner, Nathaniel Cady, Igor Lednev

A genetic profile that could uncover crucial evidence for solving a crime can be created with as little as 50 picograms of DNA. We have recently developed a universal method for the identification of all main body fluids using Raman spectroscopy. It is of great practical importance to determine the detection limit of the method and compare it with the sample volume required for DNA analysis. We have already reported that our method can detect a blood volume much smaller than required for DNA analysis; if the crime scene has a bloodstain sufficient for DNA analysis, our method should detect and confirm the identity. The DNA concentration in body fluids most often relevant to crime scenes – semen, saliva, sweat, and vaginal fluid – was quantified, and the volume of sample necessary for an adequate DNA profile was estimated. Semen has shown the greatest concentration of genetic material, while sweat shows the least. The limit of detection of this model is currently under investigation with samples of the investigated body fluids being deposited in sets of either 1, 5, or 10 droplets of volumes between 300-360 picoliters each, by the sciFLEXARRAYER S3. This instrumentation utilizes piezo drivers to create an electric force which deposits droplets in such small quantities. These samples were then tested using Horiba XploRA Raman confocal microscope and after preprocessing, spectra were applied to the identification model. Results currently show that samples of saliva, semen, and vaginal fluid are accurately predicted as such.

264 An Evaluation of the Forensic Readiness of Comprehensive Two-Dimensional Gas Chromatography Towards Organic Trace Analysis

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

Many forensic analysis techniques rely on targeted detection of analytes in complex matrices. Nontargeted analysis approaches are less common in routine forensic analysis but have vast implications in finding unknown compounds which may be pertinent to understanding the case at hand. Often, complex samples must undergo procedures involving extraction or derivatization to reveal their underlying complex chemical composition. More recently, comprehensive two-dimensional gas chromatography (GC×GC) has presented promise in forensic research for simplified nontargeted analysis, especially from matrices involving volatile analytes. This research has aimed at developing methods to analyze decomposition odor, latent fingermarks, and organic gunshot residue using GC×GC hyphenated with mass spectrometry. This talk covers extraction, analysis and data processing methods developed in our research for GC×GC analysis of organic traces. An evaluation of each application was conducted, using technology readiness levels to characterize research from fundamental to applied. While the majority of GC×GC applications fall within a technology readiness level indicating development, the major gaps in the progression of this technology for routine use provide an avenue for future work. Approaches to characterizing necessary figures of merit, uncertainty, and error, along with inter-laboratory validation will be required to continue advancing the use of nontargeted analysis by GC×GC in the forensic sciences. This talk presents current methods under development for organic trace analysis and place each application in the context of technology readiness for broad forensic usage.

265 What is in a Fingermark? A Nontargeted Analysis Using Comprehensive Two-Dimensional Gas Chromatography

Emma Macturk, William & Mary, Chemistry Department, 540 Landrum Dr., Integrated Science Center 1053, Williamsburg, VA 23185, Katelynn A. Perrault Uptmor

Ridge characteristics from a deposited fingerprint are routinely used by forensic specialists to individualize a fingerprint to a suspect. Partial fingerprints consisting of sweat and oil that lack a complete ridge pattern are often not fit to undergo the identification process, although their residue may still contain valuable chemical information about a suspect or their actions. Initial studies analyzing fingermark residue with gas chromatography- mass spectrometry (GC-MS) demonstrated that fingermark residue contains biomarkers like alcohols and lipids that can distinguish between the sex and age of donors. However, there has been little research in full, nontargeted characterization of fingermark residue using advanced chromatographic methods. This study aimed to develop a method for the collection and nontargeted analysis of fingermark residue using comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS). Fingermarks were collected on a microscope slide after hand washing and regeneration of residue. The residue was dissolved in solvent and analyzed using GC×GC-TOFMS. Peaks for common fingermark compounds such as cholesterol and supraene were identified and exhibited peak areas with inter-individual relative standard deviation (% RSD) of approximately 56.53 % and 33.21% across eighteen fingermarks. Additional method development for residue extraction and full recovery will be presented to improve reproducibility. Other compounds included octadecanol, various C_{15}-C_{34} esters, and the common sunscreen ingredients avobenzone and octocrylene. The characterization of analytes beyond expected fingermark compounds provides a baseline for future probing of residue to identify additional external contaminants such as explosives or gunshot residue that may be relevant in forensic casework.

266 Discrimination of a Self-Reference Algorithm Threshold with ROC Curve to Distinguish Between Human and Animal Blood Using Raman Spectroscopy for Forensic Purposes

Alexis Weber, SupreMEtric, 420 Sand Creek Rd., Apt. 420, Albany, NY 12205, Harrison Dickler, Igor K. Lednev

Determining whether a bloodstain originates from a human or non-human source is crucial in forensic investigations. Bian et al. introduced a pioneering self-reference peak algorithm to analyze Raman spectra of bloodstains, demonstrating its potential in distinguishing between human and non-human blood. However, their initial model only incorporated three non-human species. This study extends the algorithm's capabilities to differentiate between human and eighteen non-human species based on blood sample Raman spectra. By comparing intensity ratios between bands at 1003 and 1341 cm-1 across species, the study sought to identify a threshold for distinguishing human from non-human samples. The self-referencing algorithm accurately categorized spectra from donors of all eighteen non-human species. Its simplicity and minimal training requirements make it accessible for forensic use, in contrast to more complex computational methods. Leveraging Raman spectroscopy, this technique offers rapid, non-destructive, and highly accurate analysis, holding promise for forensic applications.

267 Forensic Fingerprinting of the Unseen: Revealing the Dark Secrets of PFAS with High-Resolution Ion Mobility

Frederick Strathmann, MOBILion Systems, 4 Hillman Dr., Ste. 130, Chadds Ford, PA 19317, Thomas Lubinsky

Per- and polyfluoroalkyl substances (PFAS) have become a widespread global contaminant. Owing to their extensive usage over decades, these 'forever chemicals' permeate various environmental settings, raising public health alarms. The current analytical tools often miss a segment of PFAS compounds, referred to as PFAS 'dark matter.' The objective of this study was to explore the use of high-resolution ion mobility (HRIM) coupled to high resolution mass spectrometry (HRMS) for PFAS forensic fingerprint analysis to delve deeper into this 'dark matter' and illustrate an unparalleled combination of analytical techniques for understanding PFAS distribution, impact, and possible mitigation. We incorporated the MOBIE HRIM System (MOBILion Systems, Chadds Ford, PA) with existing liquid chromatography (LC) and HRMS methods, aiming to reveal the hidden aspects of PFAS. By analyzing a technical perfluorooctanoic acid standard, we employed HRIM to identify and fully resolve eleven isomers, interrogate an authentic aqueous film forming foam (AFFF) sample, and reliably distinguish electrochemical fluorination from fluorotelomerization generated PFAS. The ability of HRIM to convert arrival times to unique molecular identifiers, collision cross section (CCS), was also harnessed. This gave rise to a 2-dimensional plot, the "confirmational space map", depicting the relative size and mass of each compound, as well as 3-dimensional representations of chromatographic retention time, CCS, and *m/z* for a comprehensive fingerprint of each sample. Overall, the combined use of LC, HRIM, and HRMS emerges as a potent toolset, capable of unveiling the broader spectrum of PFAS in the environment aimed at identifying and attributing sources of PFAS.

268 Digitally Enabled Generic Analytical Framework Accelerating the Pace of Liquid Chromatography Method Development for Vaccine Adjuvant Formulations

Mohamed Hemida, Analytical Research and Development, MRL, Merck & Co., Inc, 126 E. Lincoln Ave., Rahway, NJ 07065, Rodell C. Barrientos, Caleb Kinsey, Nathan Kuster, Mayank Bhavsar, Armen Beck, Heather Wang, Andrew Singh, Pankaj Aggarwal, Arthur Arcinas, Malini Mukherjee, Emmanuel Appiah-Amponsah, Erik L. Regalado

The growing use of adjuvants in vaccine formulation has created need for analytical assays that deliver reliable analysis for complex vaccine adjuvants. Due to their complex chemical and physical properties, the separation of vaccine adjuvants is considered a highly challenging and laborious task. Reversed-phase liquid chromatography (RPLC) is among the most important techniques for the characterization of adjuvant content, identity, and purity profile. However, the time constraints of developing "on-demand" robust methods to changes in formulation led to analysis becoming a bottleneck in vaccine development. Herein a simple and efficient generic analytical framework capable of chromatographically resolving the most commonly used non-aluminum-based adjuvants is introduced. This was designed to seek a more proactive approach for fast-paced assay development endeavors that evolved from extensive stationary phase screening in conjunction with multifactorial in silico simulations of adjuvant retention time (RT) as a function of gradient time, temperature, organic modifier blending, and buffer concentration. The multifactorial retention models provided excellent baseline separation of all adjuvants in a single run, which was found to be very accurate, with differences between experimental and simulated retention times of less than 1%. The analytical framework also introduced a new approach to method development via building a dynamic RT database for adjuvants with broad vaccine formulations. The framework was demonstrated with numerous analytical assays that can be generated rapidly from simulations in vaccine development. The developed analytical assay covers content, purity profile by RPLC-UV-CAD, and component identification (RPLC-MS) across complex vaccine formulations.

269 Improved Assay Development of Pharmaceutical Modalities Using Feedback-Controlled Liquid Chromatography Optimization

Andrew Singh, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Fatima Naser Aldine, Heather Wang, Devin Makey, Rodell Barrientos, Michelle Wong, Pankaj Aggarwal, Erik Regalado, Imad Haidar Ahmad

Development of meaningful and reliable analytical assays in the (bio)pharmaceutical industry can often be challenging, involving tedious trial and error experimentation. In this work, an automated analytical workflow using an AI-based algorithm for streamlined method development and optimization is presented. Chromatographic methods are developed and optimized from start to finish by a feedback-controlled modeling approach using readily available LC instrumentation and software technologies, bypassing manual user intervention. With the use of such tools, the time requirement of the analyst is drastically minimized in the development of a method. Herein key insights on chromatography system control, automatic optimization of mobile phase conditions, and final separation landscape for challenging multicomponent mixtures are presented (*e.g.*, small molecules drug, peptides, proteins, and vaccine products) showcased by a detailed comparison of a chiral method development process. The work presented here illustrates the power of modern chromatography instrumentation and AI-based software to accelerate the development and deployment of new separation assays across (bio)pharmaceutical modalities while yielding substantial cost-savings, method robustness, and fast analytical turnaround.

270 Does pH Matter? Comparing multidimensional Design Spaces of Volatile and Non-Volatile Buffer Systems

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

The choice among various pH-modifiers for separating ionizable compounds often raises intriguing questions that remain unaccounted by most HPLC practitioners. Indeed, in reversed-phase (RP) applications, defining pH proves challenging due to the continuous presence of organic solvent, which impacts the dissociations of buffering agents, ionizable analytes, and residual silanol groups of the silica base material. Temperature variations and varying buffering capabilities further complicate the interpretation of pH relationships, potentially leading to changes in relative peak positions and peak-overlaps. This prompts a logical question: can a volatile acetate buffer replace an equimolar non-volatile phosphate buffer within the equivalent pH range? To address this question, we employed an AQbD-based modeling methodology (DryLab) to construct and compare 3-dimensional (tG-T-pH) separation models of terazosin and selected impurities. Spanning the pH range of 6.0-8.0, our Design Space (DS) models comprehensively captured all the dynamic developments occurring within each separation system. Surprisingly, we not only identified expected equivalences, as indicated by shared Method Operable Design Regions (MODRs), but also clear differences between the two buffer systems.

271 The Relevance of Modeling in Pharmaceutical Submissions

Imre Molnár, Molnár-Institute, Schneegloeckchenstrasse 47, Berlin, 10407 Germany, Arnold Zoeldhegyi

HPLC-methods for new pharmaceutical agents are carefully investigated by regulatory agencies worldwide. These investigations should be based on looking at a large number of experimental factors such as pH, temperature, gradient time and steps, column dimensions, flowrate, etc., how they influence the separation of the active pharmaceutical ingredient (API) from its side products and impurities. This highly complex matter can be reviewed by modeling much faster and judged chromatographically reliable on a high level. The talk presents case studies how this process can save a great amount of time and speed up the process of creating new medicines for the patient.

272 Could Light Ruin your RPLC Robustness – Lessons from API Method Development

Anna Calkins, Bristol Myers Squibb, 15 Treeman Dr., Hillsborough, NJ 08844, Jonathan Shackman, Elizabeth Yuill

Testing analytical methods on various robustness parameters is crucial during method development to ensure long-term suitability. As part of this process, when developing an API purity/impurity method, we utilized DryLab modeling to identify ideal method parameters and determine the method operable design region. We successfully optimized conditions and developed a method meeting all our robustness criteria, including temperature, gradient, flow rate, and column batch-to-batch variability; however, we observed large peak drifting between runs before and after a long weekend, leading to coelution of two key impurities. Preparation of fresh mobile phase (MP), which was simply aqueous ammonium acetate and acetonitrile (pH adjusted to pH 5.5), restored the separation, but was not a preferred long-term solution. Therefore, we systematically investigated this phenomenon to determine the root cause and make any necessary method adjustments. We monitored the

retention and separation of the drifting impurities using MP exposed to various degradation conditions including heat, UV-light, acid, base, and oxidation. The MP at low volumes (ca. 200 mL) exposed to UV light for 24 hours best matched the peak drift pattern we observed and was subsequently analyzed by atomic spectroscopy, NMR, and GC-MS for differences between exposed and unexposed MP. After implementing foil covering for in-use MP bottles and dark storage for other MP bottles, we saw little to no peak drifting over a week-long period and were able to implement this method condition when transferring the method to the external manufacturing site while we continue to explore possible degradation products.

273 Reduction of Wet Experiments by Use of Drylab for Simulation and Prediction of Chromatographic Separations

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Developing a robust, reproducible, and reliable HPLC or UHPLC method is time-consuming and cumbersome, even for an experienced liquid chromatographer. The traditional method development process typically involves a significant amount of work, consumes large quantities of organic solvents, and generates considerable waste. Therefore, optimizing the entire method development process to enhance efficiency and environmental friendliness would be highly beneficial. DryLab is analytical design modeling software rooted in solvophobic theory. By using minimal experimental data inputs, Drylab builds multi-dimensional resolution models based on retention time and peak area. These models allow for the computation of an optimal theoretical method and the prediction of chromatograms across a wider range of experimental conditions that cannot be practically achieved in the laboratory. Drylab enables efficient assessment of separation changes while simultaneously varying multiple method parameters, such as pH, temperature, buffer concentration, and more without the traditional tedious trial and error process. Drylab offers significant advantages to those developing HPLC methods, especially for optimizing complex samples. Drylab provides substantial benefits by optimizing resource usage and promoting environmental sustainability.

274 Deep Learning to Enhance Investigative Lead Information from Automotive Clear Coats

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Automotive paint consists of a thin undercoat and color coat layer protected by a thicker clearcoat layer. All too often, the clearcoat is the only layer of automotive paint recovered at the crime scene of a vehicle related fatality. Searches of OEM paint libraries for clear coats using commercial search algorithms typically return a large and unusable number of hits. To address this problem, we are applying convolutional neural networks to create a non-linear embedding of the data in a shared semantic feature space for vehicle manufacturer and assembly plant classification. An in-house infrared spectral database of 3320 OEM clear coats of automotive vehicles manufactured at Chrysler, Ford, GM, Honda, Nissan, and Toyota assembly plants was employed to develop a library search prefilter. The training set was divided into ten sets of approximately equal size. The training and evaluation approach followed a 10-fold cross validation using two validation sets. A fold consists of combining eight partitions into a training set. A model is trained on the eight-partition training set with the first validation set being used to select the best model and the second validation used as a test set to evaluate the model that was selected by the first validation set. A neural network consisting of three convolutional layers and four dense layers yielded a mean accuracy of 95.3%. Restricting the network to the fingerprint region increased mean accuracy to 96.6%. Currently, we are integrating residual network blocks and a semantic feature space to further improve search prefilter performance.

275 Shift Invariant Tri-Linearity Algorithms for Fast, Flexible Blind Source Separation in Hyphenated Chromatography

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Ideally, hyphenated chromatography measurements follow the parallel factor analysis (PAFAFAC) model. However, measurements exhibiting shifting in the chromatogram elution profiles do not follow the PARAFAC mode. PARAFAC2 is an alternative model designed to account for shifting profiles. However, even PARAFAC2 has trouble fitting data with shifting profiles – most notably when factors are not present in every sample. Shift invariant tri-linearity (SIT)a is an alternative algorithm that has significant advantages over PARAFAC2. In comparison to PARAFAC2, SIT has been found to be ~20 times faster and requires fewer factors to extract the same chemical information. SIT also allows for non-negativity constraints in the shifting mode and allows for relaxation of the tri-linearity constraint in factors that do not exhibit tri-linearity. These advantages result in a flexible blind source separation algorithm for hyphenated chromatography measurements. A disadvantage of SIT is that it does not account for changes in elution profile shapes. A modification to the SIT algorithm, shift invariant soft tri-linearity (SIST), has been demonstrated to account for shape changes.b This talk will introduce the SIT and SIST algorithms. Comparisons to common factor-based methods such as multivariate curve resolution will also be provided using simulated data and laboratory measurements. The talk will show that SIT and SIST are flexible alternatives that allow constraints in all modes and allows tri-linearity to be relaxed on factors such as baseline shifts that do not follow tri-linearity.

[a]Schneide, et al., J. Chemom. 37(8) (2023); e3501. doi: 10.1002/cem.3501

[b]Schneide, et al., Chemometr. Intell. Lab., (under revision 2024).

276 Expanding the Utility of a Virtual Method Development Tool

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The development and optimization of liquid chromatography (LC) separations can be time consuming and costly, often requiring many steps including literature research, method scouting, method development, and method optimization. To reduce cost and save time, an instrument-free software modeling tool was developed allowing users to select compounds from a database and instantly model a separation by adjusting parameters such as instrument/system effects (dwell and extra column volume), temperature, and mobile phase additives. Previous utility has been demonstrated for the virtual development of a method for the analysis of drugs of abuse (DoA). Since the initial successful demonstrations of the DoA library, the tool has since been expanded to include additional libraries, per- and polyfluorinated substances (PFAS) and cannabinoids, and expansion to the existing DoA library. Using these libraries, method parameters were developed within the software and virtual chromatograms generated. Generated method conditions were transferred and set up on the instrument for analysis. To assess the accuracy of the modeler, experiments comparing compound retention time values between experimental and modeled data were conducted. To determine the software's ability to model separations, acceptance criteria was chosen based on a retention time window of ±15 seconds, selected to represent half of a typical MRM window. Results show that this virtual tool can be used across a variety of analyte classes to develop methods quickly and accurately. This novel, virtual method development tool can improve turnaround time, increase throughput to existing methods, offer an on-demand consultative user experience, and a greener solution for development.

277 Feature Extraction Algorithm for Conventional and Comprehensive Two-Dimensional Gas Chromatography Coupled with Mass Spectrometry

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In chromatographic analysis, dealing with regions of significant peak overlap poses a challenge, hindering reliable identification and quantification. Various chemometric deconvolution techniques aim to address this issue by modeling instrumental responses originating from different analytes based on the original data. In the domain of GC-MS data analysis, the raw instrumental response can be represented as a matrix, originated from the outer product of its chromatographic elution profile by mass spectra. This second-order structure enables the application of bilinear chemometric decomposition methods, allowing deeper insights and characterization of complex profiles. In this context, we introduce a methodology tailored for extracting features from GC-MS and GC×GC-MS data. These chromatograms are segmented into discrete time windows, within which the mass spectra are observed. The methodology then estimates the underlying factors within each window to discern relevant spectral features (i.e., the purest m/z). Subsequently, it employs pure-component analysis (i.e., SIMPLISMA) to isolate these features. Following this, it applies constraints for peak characterization and quality assessment, ensuring that only peaks common to all samples are considered. The final output is a deconvoluted peak table containing the pure and more reproduced contributions of each feature. One of the primary advantages of this method lies in its elimination of data alignment requirements. By autonomously locating maxima of the most pertinent deconvoluted peaks, our algorithm streamlines the analysis process. Additionally, leveraging the information related to pure m/z values greatly accelerates the analytical process and enhances scalability avoiding reiterating all steps.

278 Streamlining Chromatographic Method Evaluation and Ensuring Data Quality through Advanced Tools

Farrel Borden, Mestrelab Research, 5154 Woodward Dr., Feliciano Barrera, Santiago de Compostela, Spain, Gary Sharman, Mitcheell Maestre, Agustín Barba, Nicola Tonge

At a large pharmaceutical company, Design Make Test cycles produce thousands of molecules annually, necessitating purification and testing. The substantial volume and repetitive analyses pose challenges, making the process time-consuming and error-prone. These challenges prompted the search for an innovative solution to automate data processing steps while integrating the resulting data with existing IT infrastructure which allows the purification workflow to become automated and autonomous. Here, we highlight the solution developed and implemented at a sepa-

ration science lab, to expedite chromatographic method selection and enhance the reliability of liquid chromatography-mass spectrometry systems. The solution comprises two modules. The first rigorously examines test spectra, evaluating critical parameters such as retention time, limit of detection, peak resolution, and instrument pressure against predefined acceptable ranges. This ensures optimal instrument functionality. The second module assesses peak resolution, position, size, purity, and other criteria, employing a comprehensive scoring system that considers nonlinear relationships. This aids in classifying methods and streamlining the selection of optimal conditions, reducing time and resource investments. The successful implementation, involving collaboration between chromatography experts, data scientists, and IT specialists, yielded undeniable benefits. During an assessment of more than 400 compounds, the new solution correctly identified the best separation method for more than 95% of the samples. Furthermore, the instrument performance testing instills an extra layer of confidence in the validated instruments. The integration of these tools significantly improved the approach to method development and instrument validation, marking a substantial advancement in chromatographic processes.

279 Time-Resolved Spectroscopy for Quantitative Characterization of Surface-Modified TiO2

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Surface modification of metal oxide nanoparticles is essential for improving the quality and performance of pigments, cosmetics, and composite materials. TiO_2, widely used as a white pigment in paints, plastics, papers, and cosmetics, faces challenges due to its photocatalytic activity, causing matrix degradation and pigment photo-graying upon light exposure. To address this, alumina, zirconia, and silica shells are commonly used for TiO_2 surface modification to suppress photocatalytic activity and enhance photo-stability. Characterization of modified nanoparticles typically involves techniques such as AFM, SEM, TEM, XRD, and FTIR, but these methods are hindered by complex sample preparation and specialized instrumentation, limiting their suitability for rapid large-scale characterization. Therefore, developing efficient techniques for surface-modified powder characterization is crucial for process control. Here, we propose time-resolved photoluminescence (TR-PL) spectroscopy as a versatile method for determining metal oxide surface coating coverages on colloidal nanoparticles. This approach relies on coating-dependent fluorescence quenching of fluorophores via interfacial electron transfer (IET), enabling rapid characterization of coating properties on large ensembles without the need for selective fluorophore binding. Moreover, the method can be applied to various coatings on diverse nanoparticle substrates. We demonstrate this approach with Al_2O_3 and SiO_2 coatings on TiO_2, utilizing reference samples produced through an improved wet chemical deposition process for controlled deposition of Al_2O_3 patches on TiO_2 particle surfaces.

280 Understanding the Degradation and Strain Effects of Thin GaSe

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Harnessing quantum mechanics and the unique optical properties of materials like transition metal dichalcogenides (TMDCs) and post transition metal chalcogenides (PTMCs), has led to significant advancements in quantum photonic technologies, such as quantum key distribution for secure communication. Here the specific PTMC of interest is gallium selenide (Ga_2Se_2) because its 2D nature should allow it to be incorporated into a wide range of solid-state photonic devices, its transition to a direct bandgap as a function of increasing film thickness makes it less sensitive to the number of monolayers transferred onto a device, and its decrease in bandgap as a result of strain provides a mechanism to create localized emission of single photons. However, one concern with the use of Ga_2Se_2 is its rather quickly degraded optical signal. To better understand this process, photoluminescence (PL) and Raman spectroscopy were performed on thin Ga_2Se_2 samples stored under various conditions to measure how the overall optical and structural properties were affected over time. Atomic force microscopy (AFM) was used to determine the height of each flake to directly investigate the relationship of flake thickness, overall PL intensity, and signal degradation. While all samples reached a baseline degradation within approximately 8 hours, a key finding suggests that the relationship between PL degradation and thickness changes as a function of time. Understanding the strain and degradation of thin Ga_2Se_2 may allow for it to be utilized in continued advancements for quantum technologies.

281 Fluorescent Dyed Gold Core-Silicon Shell Nanoparticles for Biological Applications

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Fluorescent microscopy has become an invaluable tool in the study and research of biological specimens on a nanoscale, which provides both sensitivity and specificity to cellular imaging techniques.[1] By using these techniques into living cells has opened up the possibility to study dynamic processes with unprecedented spatial and temporal resolution.[2] The metal core – shell nanoparticles, are very desirable class of matter because of their unique nanostructure.[3] Enhancement and customizability of the size and shape of the core as well as the shell thickness and shape, allows to create different surface interactions.[4] The purpose of this research is to synthesize and analyze fluorescently dyed metal core-shell nanoparticles for applications in cancer cell research. In this study we present the structural study and analysis of these nanomaterials using instruments such as fluorescent confocal microscopy, spectra fluorometer, ultraviolet-visual spectroscopy, transmission electron spectroscopy, and scanning electron microscopy.

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282 Silver Nano Raspberries Stabilized by Cyclic Silanes

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In this research, we present the investigations of cyclic hydro silanes as reducing and stabilizing agents in the synthesis of silver nano raspberries. Through the manipulation of silane concentration and functionality we are able to tailor this passivation to enable a diffusion of small molecules for a controlled catalytic activity. Previously our group investigated the preparation of nano-sized metal systems involving long alky chains silanes with successful results.[1,2] Using similar one pot synthesis, two cyclic silanes,1,3,5,7-tetramethyl cyclotetrasiloxane $D4H$ a cyclic substituted siloxane and 1,2,3,4,5,6,7,8-octamethyl cyclotetrasilazane $D4H$ cyclic substituted silazane were studied. The variation of the silane compound to the metal salt ratios resulted in nanoparticles of different sizes and morphology. The characteristic features of products were obtained using IR, UV-Vis, and TEM.

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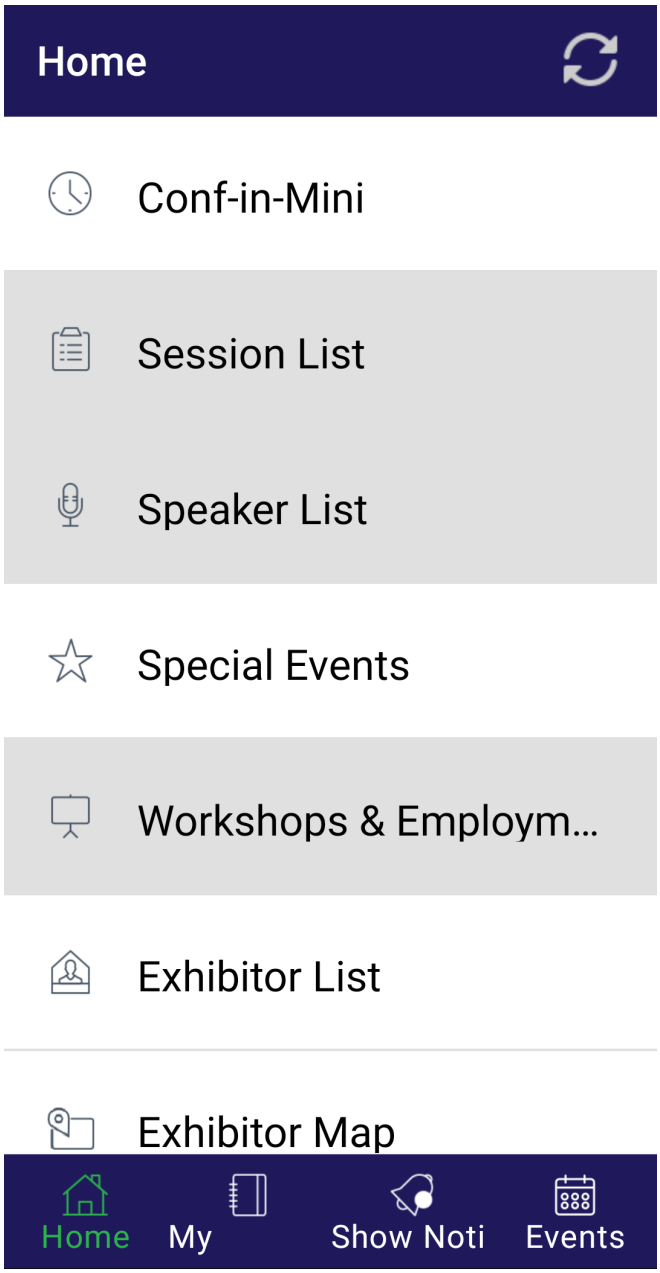

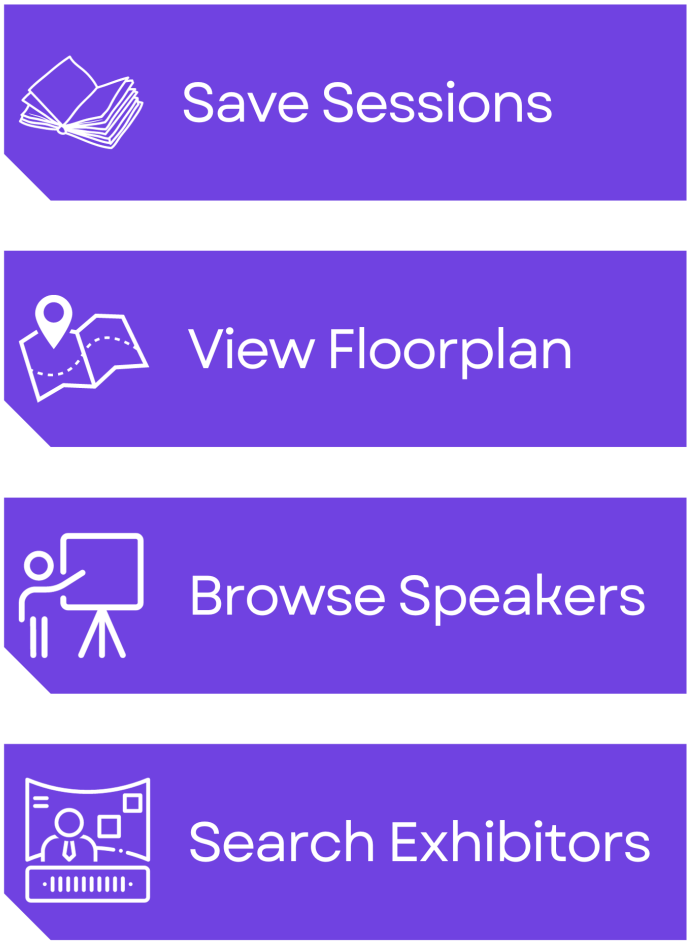

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