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November 14–16, 2022

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EASTERN ANALYTICAL SYMPOSIUM & EXPOSITION

2022 EAS Abstracts

This volume contains the final abstracts for the oral and poster presentations which take place Monday, November 14, through Wednesday, November 16, 2022. If an abstract is not provided in this volume or the Addendum, then the presenting author did not supply an abstract. For each abstract provided, a complete mailing address for the presenting author is shown. Additional authors are indicated, however, their mailing addresses are not provided.

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1 **Oxidation and Deposition Processes with Metal Nanoparticles**

Francis Zamborini, University of Louisville, 2320 South Brook St., Department of Chemistry, Louisville, KY 40292

The controlled synthesis of metal nanoparticles (NPs) has led to many fascinating fundamental studies of their unique properties and applications based on those properties, which are tunable by their size, shape, composition, and environment (stabilizers). The electrochemical properties of metal NPs are no exception, exhibiting unique electrocatalytic, oxidation, deposition, charge transport, and restructuring properties based on the specific type of metal or alloy NPs. The electrochemical properties can be measured on NPs in solution or when assembled as arrays on electrode surfaces, either as the average of many NPs or with an individual NP, one at a time. The unique oxidation behavior of arrays of Au NPs attached to glass/ITO allows the use of anodic stripping voltammetry (ASV) as a size analysis method to monitor ripening, thermal properties, deposition, and separations. This provides a faster and cheaper method for analyzing the size, purity, and aggregation of Au NPs as compared to electron microscopy, and also allows the study of unique electrochemical behavior directly on electrode surfaces. As the size of the Au NPs decreases below 2 nm, their electrochemical and thermal stability decreases dramatically as determined by ASV. ASV also enabled successful separation of 1.6 nm Au NPs from a disperse population containing NPs up to 4 nm by solvent fractionation and fast analysis. Voltammetry shows that Au NPs exhibit size-dependent electrocatalytic properties towards electrochemical deposition of Au, or seed-mediated growth. Voltammetric methods provide valuable characterization of Au and alloy NPs for size, composition, purity, aggregation, and catalysis.

2 **Integration of Dielectrophoretic Selective Single-Cell Capture at a Wireless Electrode Array with On-Chip Analysis of Single Circulating Tumor Cells**

Robbyn Anand, Iowa State University, 1605 Gilman Hall, 2415 Osborn Dr., Ames, IA 50011

Circulating tumor cells (CTCs) are cells that have escaped from a localized tumor mass and entered the bloodstream. A fraction of these cells survive to seed metastatic growths, and therefore, the detection of CTCs and analysis of their characteristics is valuable for diagnosis, tracking disease progression, and selection of therapeutic agents. Due to the extreme rarity and heterogeneity of CTCs, the development of selective isolation techniques and subsequent single-cell analysis continues to be an important challenge. Dielectrophoresis (DEP) accomplishes antibody-independent separation of CTCs from blood cells. However, many of the current approaches suffer from low throughput and are not amenable to on-chip single-cell analysis. We previously reported a DEP strategy that addresses these concerns. First, the use of arrays of 'wireless' bipolar electrodes (BPEs) allows for otherwise unattainable device formats. Second, pockets extruding from either side of the microchannels render the design applicable for high-fidelity single-cell capture. Third, extension of each pocket into reaction chambers allows interaction of single cells and reagents required for subsequent assays. In this presentation, we describe several advancements to this platform including 1) AI-driven label-free identification of CTCs, 2) an electropolymerizable, hydrophobic ionic liquid to create a robust seal at each chamber, 3) methods for co-encapsulation of reagent beads with individual cells, and 4) quantification of clinical performance. Finally, we demonstrate assay of cytosolic and secreted enzymes from captured cells. In combination, these advancements create a robust and versatile platform for individual analysis of CTCs as a liquid biopsy.

3 **Serial and Parallel Approaches to High-Throughput Electrochemistry**

Lane Baker, Texas A&M University, Department of Chemistry, 580 Ross St., College Station, TX 77843

Rapid screening of chemical libraries has been an important driving force for the development of novel instruments and methods. Instrumentation aimed at achieving this goal via electrochemical methods has consistently lagged behind spectroscopic or mass spectrometric platforms. Coupled with the recent renaissance in electroorganic synthesis, high-throughput methods for screening electrochemical properties are of significant interest. Systematically testing different substrates, electrodes, cell geometries, electrochemical methods (i.e., constant potential vs. constant current vs. alternating current), and conditions one at a time quickly increases the length of time required to sufficiently characterize a reaction. The gap in electrochemical approaches toward high-throughput measurements exists for good reason. Specifically, electrochemical instrumentation proves to be a significant bottleneck. Here, we will demonstrate two methods to carry out high-throughput electrochemical measurements, one in parallel and one in serial. The two methods rely on advanced electrochemical instrumentation which are described. Selected applications in synthesis of materials and electrochemical transformations are discussed

4 **Magnetic and Electrochemical Preconcentration: A Route to Home-Based, Picomolar Detection of a Heart Failure Biomarker**

Richard Crooks, The University of Texas at Austin, Department of Chemistry, Austin, TX 78712

The objective of the project described in this presentation is the creation of a new, low-cost, appropriately sensitive paper diagnostic device for quantitative electrochemical detection of a heart failure marker known as NT-proBNP. The device is based on a metalloimmunoassay in which NT-proBNP (a short peptide) is sandwiched between two antibodies. One antibody is conjugated to a magnetic microbead and the other is linked to a silver nanoparticle. Upon formation of the sandwich, the complex is concentrated onto an electrode surface via a magnetic force induced by a magnet on the backside of the electrode. A series of electrochemical processes are then initiated that result in amplified detection of the silver nanoparticle label. This talk will describe the key iterations and improvements the sensor has undergone over the past several years, leading now to a device that detects NT-proBNP in serum throughout the risk stratification range for heart failure of 53 pM to 590 pM. The coefficient of variation of the assay is around 10% and the assay time is less than 10 minutes

5 **Novel Strategies for Targeted Protein Quantification in Biomatrices**

Bo An, GlaxoSmithKline, 1250 S. Collegeville Rd, Collegeville, PA 19426

Despite recent advances in liquid chromatography mass spectrometry (LC-MS) based targeted protein quantification, it remains a daunting challenge to achieve high sensitivity with excellent robustness and throughput. High sensitivity protein measurements usually require immuno-affinity enrichment, which relies on critical reagents and is often difficult to develop and to multiplex. Several novel antibody-free techniques will be discussed here, permitting high-throughput and ultra-sensitive analysis of protein target in plasma/tissues with a short, straightforward development cycle. Firstly, an orthogonal-dimension solid-phase extraction (SPE) based sample cleanup method has been developed and practiced to improve robustness and sensitivity by strategically manipulating pH and ionic/solvent strengths to selectively enrich the target while eliminating most of matrix components. Secondly, the novel trapping micro-LC-MS strategy that selectively enrich targets and eliminates matrix components, which achieves ultra-sensitive analysis with high speed, has been developed. The method provides high sensitivity comparable or better than nano-LC-MS, while achieves high throughput and excellent robustness even superior to a conventional-high-flow LC-MS, for quantification of protein targets in biomatrices. Finally, an antibody-free platform, which was developed based on multi-dimensional sample cleanup/enrichment, will be discussed. This method enables quantification of multiple protein targets with high sensitivity that is comparable to hybrid IC-LC-MS methods. More importantly, the increased throughput of this platform compared to previous multi-dimensional strategy makes it suitable for industrial pharmaceutical investigation, without the need for antibodies or other affinity reagents. All the methods are universally applicable in any biomatrix and permits large-scale quantification with high sensitivity, accuracy, and robustness.

6 **In Silico Multifactorial Modeling for Streamlined Development and Optimization of Chromatography Methods**

Imad Haidar Ahmad, Merck & Co., Inc., 126 East Lincoln Ave., Rahway, NJ 07065

Modern pharmaceutical processes can often lead to multicomponent mixtures of closely related species that are difficult to resolve chromatographically, even worst in preparative scale settings. Despite recent improvements in column technology and instrumentation, there remains an urgent need for creating innovative approaches that address challenging coelutions of a critical pair and lack of chromatographic productivity of purification methods. Computer-assisted modeling using ACD Labs/LC Simulator software has been introduced in our labs as an initial analytical framework to isolation and purification workflows enabling rapid increase of scale-up productivity of target pharmaceuticals in multicomponent mixtures. This allows to achieve dramatic increase in productivity while minimizing solvent consumption and hazardous waste. The use of multifactorial and polynomial models to predicting the retention behavior of small and large molecules using different chromatographic techniques at analytical and preparative scale will be discussed in this presentation.

7 **Improving Oligonucleotide Separations and Impurity Analysis Using LC Systems and Columns with Hybrid Surface Technology**

Martin Gilar, Waters Corporation, 34 Maple St., Milford, MA 01757

Antisense oligonucleotides (ASO), silencing RNA (siRNA), single guide RNA (sgRNA), and mRNA are classes of nucleic acid-based therapeutic compounds. Their characterization, and quantitation is typically performed by ion-pair reversed-phase liquid chromatography (IP-RP-LC), anion-exchange chromatography (AEX-LC) or recently by hydrophilic interaction chromatography (HILIC) and size exclusion chromatography (SEC). It has been shown that acidic biopolymers such as nucleic acids, peptides or acidic small molecules can be adsorbed on metallic chromatographic hardware. This leads to analyte loss and has a detrimental impact on sample quantitation and carryover. This is especially noticeable for oligonucleotides and nucleic

acids. In this work we demonstrate oligonucleotide loss due to column hardware and LC system tubing. We show that a single stainless steel column frit is responsible for an oligonucleotide loss of between tens to hundreds of picomoles, depending on the frit size (column i.d.) mobile phase pH and buffer concentration. We demonstrate that chemical modification of metal surfaces (high performance surfaces, HPS) improves analysis of oligonucleotide drugs. HPS modified columns and LC hardware were applied for analysis of ASO, mRNA and other analytes in IP-RP-LC, HILIC and SEC modes.

8 **Empower mRNA-Based Medicines by HPLC**

Penggao Duan, Moderna, 2 Brookside Ave, Lexington, MA 02421

The development of one of the first mRNA vaccines (Moderna COVID-19 Vaccine) was made successful globally by understanding the critical quality attributes (CQAs) of the mRNA product. The presentation will describe the potential of mRNA medicines and will focus on the role of HPLC analysis in evaluating CQAs of mRNA-based medicines. In addition, some considerations and findings of HPLC analysis of mRNA will be discussed.

9 **BCEENET: Creating a Collaborative Network to Support Course-Based Undergraduate Research Experiences (CUREs) Using Digitized Natural History Collections**

Janice Krumm, Widener University, 1 University Place, Chester, PA 19013

Biological Collections in Ecology and Evolution Network (BCEENET) supports the development and implementation of Course-based Undergraduate Research Experiences (CUREs) using digitized natural history collections. Undergraduate research has been shown to increase student research and professional skills, development of a science identity, and sense of belonging in the scientific community. Embedding research experiences in science coursework is especially important in increasing access for low income, first generation, and historically marginalized student populations unable to dedicate time to research outside their normal course load due to personal and financial barriers. In collaborative teams, BCEENET members including undergraduate educators, education experts, natural history collections professionals, and data experts developed four CUREs that only require student access to computers, the internet, and open-access resources. This was possible through the use of digitized natural history collections data, a rapidly growing, open-access resource freely available to all students, increasing the accessibility of CURE experiences at both 2- and 4-year institutions. This presentation will discuss the development of the collaborative BCEENET network and how it supports faculty at diverse institution types in developing and implementing CUREs using freely available open-access resources.

10 **Student Outcomes and Perceptions of Specifications Grading in a First Semester General Chemistry Course**

Stephen Habay, Salisbury University, Department of Chemistry, 1101 Camden Ave., Salisbury, MD 21801

Assigning grades based on accumulation of points and partial credit can be problematic. Points-based grading does not directly measure if a student has achieved the learning outcomes of the course and shifts student focus toward getting the desired grade and away from achieving learning goals. The specifications grading method has emerged as an alternative that better reflects achievement of learning outcomes, increases student engagement, reduces student stress, and promotes a growth mindset through cycles of revision and reassessment. Through specifications grading, final letter grades can be assigned based on groups of assessments successfully completed, rather than points accumulated, that are directly linked to learning outcomes achieved. This talk compares the results of specifications grading to traditional points-based grading in a first semester general chemistry course over the course of four semesters. Differences in student achievement and success rates, perceptions of the specifications grading method, and student behavior in the course will be highlighted.

11 No abstract submitted by the author.

12 **Transforming the Chemistry Lab Experience**

Shirley Fischer-Drowos, Huy Dao, Widener University, Kirkbride Hall, One University Place, Chester, PA 19013

The value of an experiential laboratory experience was tested during the recent COVID-19 pandemic. Educators had to pivot quickly to accommodate the lack of in-person teaching. For lectures, Zoom and Teams allowed for relatively seamless transitions of content delivery. Though, changing to this format required significant adjustment for both students and faculty. However, laboratory courses were much more challenging to transform to the online domain. An evaluation of available platforms demonstrated a number of irreconcilable issues ranging from correlating available experiments to the lecture, to the lack of materials in a number of topics. Often more time was needed to learn and apply the software than to learn the lesson being conveyed. In order to make the transition as smooth as possible, our first-year laboratories were filmed and edited to provide “real-lab” content. Break-outs were embedded to allow students to perform the same manipulations they would have done in the laboratory. Instructor guides were prepared to make multi-section delivery uniform. At the upper-level, creative social distancing coupled with peer learning assistants allowed for an experiential experience in an environment following recommended safety guidelines. An overview and examples will be provided.

13 **Getting New Correlations from Old Spectra-Covariance NMR to Rescue Challenging Biomolecular Projects.**

Dominique Frueh, Johns Hopkins University School of Medicine, 725 North Wolfe St., Baltimore, MD 21205, Kenneth Marincin, Aswani Kancherla, Subrata Mishra

Nuclear magnetic resonance (NMR) lays a central role in elucidating biomolecular mechanisms because the technique permits monitoring systems in a non-invasive manner and with atomic resolution. This information is provided by a variety of correlation maps reporting structural, thermodynamic, kinetic, and dynamic parameters impacting nuclei in molecules. However, spectral complexity challenges data analysis for macromolecules. Here, I will describe how we use NMR spectra as matrices or multi-dimensional arrays and employ mathematical operations to produce novel correlation maps conveying the information otherwise provided separately by these spectra. I will showcase applications for a variety of proteins, with an emphasis on NMR resonance assignments, while describing the procedures necessary to minimize artifacts in correlation maps. The method provides alternative NMR readouts from existing spectra to facilitate biomolecular NMR investigations.

14 **Characterization of Biotherapeutics by Chemometrics and Machine Learning Analysis of NMR Spectra**

Frank Delaglio, National Institute of Standards and Technology, Institute for Bioscience and Biotechnology Research, 9600 Gudelsky Dr., Rockville, MD 20850

Protein therapeutics are a highly successful class of drugs that are used to treat a number of serious and life-threatening conditions such as cancer, autoimmune disorders, and infectious diseases including COVID-19. These therapeutics have numerous critical quality attributes (CQA) that must be evaluated to ensure safety and efficacy, including that they must adopt and retain the correct structural fold without forming unintended aggregates. Furthermore, since these therapeutics are manufactured in living cells, where small variations in growth conditions can have a large therapeutic impact, analytics for cell growth metabolomics are also needed. This need is even more critical for therapies such as CAR-T, where the cells themselves are administered directly to the patient. Nuclear magnetic resonance spectroscopy (NMR) is powerful and diverse tool to help meet these measurement needs, because NMR spectra are sensitive to molecular shape and interactions as well as chemical structure, and NMR can provide this information at atomic resolution for proteins as well as for the small molecule mixtures comprising the metabolome. Exploiting NMR for these biomanufacturing needs leads to a series of computational challenges which we review, including spectral reconstruction of incomplete data, metrics of spectral similarity, detection and quantification of spectral features, and spectral analysis of mixtures.

15 **Shifting-Corrected Regularized Regression Model for NMR Metabolomic Identification**

Thao Vu, Colorado School of Public Health, 285 N Sable Blvd, 6205, Aurora, CO 80011, Yuhang Xu, Yumou Qiu, Robert Powers

The process of identifying metabolites in complex mixtures plays a critical role in metabolomic studies to obtain an informative interpretation of underlying biological processes. Manual approaches are time-consuming and heavily reliant on knowledge and assessment of nuclear magnetic resonance (NMR) experts. We propose a shifting-corrected regularized regression method, which identifies metabolites in a mixture automatically. Using a novel weight function, the proposed method is able to detect and correct peak shifting errors caused by fluctuations in experimental procedures. Simulation studies show that the proposed method performs better with regard to the identification of metabolites in a complex mixture. We also demonstrate real data applications of our method using experimental and biological NMR mixtures.

16 **Using Deep Learning to Unleash the Potential of NMR Spectroscopy**

D. Flemming Hansen, University College London Gower Street, London, United Kingdom, WC1E 6BT

Artificial intelligence (AI) and deep learning are now established as some of the most important technologies of our time. In magnetic resonance, AI methods are deployed extensively for tasks such as image enhancement and classification, whereas the uptake of AI in nuclear magnetic resonance (NMR) spectroscopy has been slower - but this picture is now swiftly changing. It will initially be shown how deep neural networks (DNNs) can be trained for homonuclear decoupling in protein NMR spectroscopy involving the detection of 13C nuclei. 13C-detected methods can be advantageous because they offer superior resolution, however, the spectra are

often complicated by homonuclear scalar couplings, which reduce the sensitivity and resolution. Our recent work shows that decoupling of 13C-detected spectra can be achieved by passing a single spectrum through a DNN to yield a singlet spectrum of high quality. Another area, where we have focused our developments is for autonomous analysis of complex NMR data. Many NMR tools have been developed to characterize dynamic and exchanging systems; however, analyses of the resulting NMR data often hinge on complex least-squares fitting procedures and human intuition. DNNs will be presented for the analysis of complex 1H chemical exchange saturation transfer (CEST) data, where the DNN not only accurately predicts the chemical shifts of nuclei in the exchanging species, but it also determines the uncertainties associated with these predictions. All the DNNs developed do not contain any parameters for the end-user to adjust and the methods therefore allows for autonomous analysis of complex NMR data.

17 **Forensics and Innovative Technologies (FIT): How FIT Fits in Bristol-Myers Squibb**

Ravi Kalyanaraman, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903

Forensics describes the scientific methods used in an investigation. Pharmaceutical forensics is looking for evidence and using your scientific knowledge and know-how to find proof that will help solve issues in manufacturing, patient safety, and crimes. Forensics and Innovative Technologies (FIT) group was created in 2021 within Global Quality Analytical Science and Technologies (GQAS&T) to support commercial operations for investigation support in manufacturing, patient complaints, and to screen suspect and counterfeit products. Several state-of-the-art analytical tools and techniques are used to support pharmaceutical forensics. These include but not limited to Energy Dispersive x-ray Spectroscopy (EDS), Scanning Electron Microscopy (SEM), confocal Raman, portable and benchtop Raman, Infrared (IR), Near Infrared (NIR) micro-spectroscopy, and Quantum Cascade Laser (QCL) IR spectroscopy. The talk will feature case studies along with the support provided by FIT for commercial products which includes pharma, biologics and cell therapy products.

18 **HPTLC Separation of Novel Psychoactive Substances**

Thomas Brettell, Cedar Crest College, Department of Chemical, Physical, & Forensic Sciences, 100 College Dr., Allentown, PA 18104, Marianne Staretz

In attempts to circumvent laws, clandestine laboratories modify the chemical structures of common drugs of abuse. This results in the synthesis of several different classes of novel psychoactive substances (NPSs) which include synthetic cannabinoids, cathinones, benzodiazepines, cathinones, phencyclidine analogues, tryptamines, and several others. Historically, traditional thin-layer chromatography (TLC) has been used for the analysis of seized drugs. Using traditional TLC does not always provide a satisfactory level of sensitivity, resolution, or documentation to analyze these new compounds many of which may be closely related structural isomers/analogues. This presentation will discuss the use of high-performance thin-layer chromatography (HPTLC) for the analysis of several classes of NPSs. A CAMAG automatic TLC system, HPTLC Silica gel 60 F254 20 x 10-cm plates and HPTLC-Platten 10 x 10-cm RP-18 WF254s plates were used in all analyses. A CAMAG TLC visualizer was used to visualize developed plates and take pictures with white light, 254 nm and 366 nm wavelengths. RF values demonstrated good intra- and inter-day reproducible (CV% <10%) with detection limits in the range of 25-100 ng/band. The results suggest that HPTLC is a useful and suitable method for separating many different classes of NPSs submitted to crime laboratories. HPTLC has demonstrated to be a green separation technique with adequate sensitivity, good resolution, along with providing proper documentation for peer review.

19 **Identification of Fibers Using Raman Microspectroscopy: A Case Study**

Sergey Mamedov, HORIBA Scientific, 20 Knightsbridge Rd, Piscataway, NJ 08854

Raman microspectroscopy is very applicable in the field of forensics. Only a small amount of sample is required, and little or no sample preparation is necessary. It allows for trace analysis, whereas sampling can be done directly through transparent evidence bags and packaging, such as glass and plastics. It covers a wide spectral range from 10 cm-1 to 4000 cm-1, making the technique ideal for identifying organic and inorganic substances, including fibers, drugs, pharmaceuticals, explosives, inks, paint, etc. To aid law enforcement personnel, investigations have been geared toward the ability of Raman microspectroscopy to identify a variety of polymers used in fibers. This is very important, as the presence of fibers at a crime scene has often been instrumental in the process of solving crime. “Fingerprints” of nylon 6, Kevlar, polystyrene, PET, polypropylene, and some other fibers along with different types of nylon (nylon 6, nylon 6/6, nylon 12, and others) will be highlighted in the presentation, as well as the ability to identify fiber mounted on a substrate. The capability of Raman spectroscopy to differentiate between fibers of similar chemical structures will be demonstrated. Spectral data of the fibers were collected using 532 nm, 633 nm, and 785 nm laser excitations. A comparison of the Raman spectra of the fibers taken with different excitation wavelengths will be discussed. It will be shown that search can quickly identify materials whose spectra have been collected in a library or matched to suspect material samples.

20 **Examination of Pigmented Fibers for Trace Evidence Applications**

Christopher Palenik, Microtrace LLC, 790 Fletcher Dr., Suite 106, Elgin, IL 60123, Kelly Beckert, Ethan Groves, Otyllia Abraham

Pigmented (i.e., solution dyed) fibers are colored through the addition of fine, solid pigment particles prior to extrusion and are encountered in an increasing range of applications. As this presentation will demonstrate, a variety of different pigments may be present within a single fiber, and thus the identity, relative concentration, morphology, and particle size of each represent additional points of comparison and potential significance during a forensic fiber comparison. Despite this wealth of information, pigmented fibers have never been systematically researched and as a result are rarely, if ever, exploited in a forensic analysis. This research aims to expand this knowledge base by presenting a systematic, microscopical study of pigmented fibers using forensic methods accessible to a trace laboratory. The fibers selected for study have been drawn from an internally developed, curated collection obtained from fiber manufacturers that span a variety of commercial applications, polymer types, and colors. This presentation will summarize results arising from a critical study of this sample set by polarized light, oil immersion, and fluorescence microscopy. This direct study of pigments within fibers is supported by research into sample preparation techniques, including longitudinal mounts and cross sections, optimized to maximize the resolution of individual pigment particles that often approach, or at times exceed (are less than) the resolution limits afforded by light microscopy. The result is the development of a systematic technical method to recognize and study pigmented fibers that also provides insights into questions of interpretation and significance.

21 **Differentiation of Structurally Similar Fentanyl Analogues with Theoretical and Experimental Analysis by Surface-Enhanced Raman Spectroscopy (SERS)**

Sevde Dogruer, Florida International University, 11200 SW 8th St., CP 175 Miami, FL 33199, Emily Hernandez, Bruce McCord

New synthetic opioids, especially fentanyl and its analogues, have produced an acceleration in opioid abuse. The presence of fentanyl analogues as mixtures in illicit drugs makes it hard to estimate their potencies. This makes the detection and differentiation of fentanyl analogues critically significant. Most screening methods in current use have difficulty detecting the full range of opioid analogues due to a wide variety of structural variations. However, Raman spectroscopy, specifically surface-enhanced Raman spectroscopy (SERS) is capable of detecting and identifying previously known and unknown fentanyl analogues. It can also differentiate structurally similar fentanyl analogues due to its ability to yield spectroscopic fingerprints for the detected molecules. Certain fentanyl analogues such as carfentanil, furanyl fentanyl, acetyl fentanyl, 4-fluoroisobutyryl fentanyl, have gained popularity and constitute 76.4 percent of the fentanyl analogues identified in drug seizures. Several have been already described using Raman spectroscopy. However, there are many fentanyl analogues that are structurally similar to these compounds. Thus, it is important to differentiate drug analogues from similar molecules to track and identify trends in illicit distribution. In this presentation we develop methods for the differentiation of structurally similar fentanyl analogues using theoretical and experimental methods. To do this, a set of fentanyl analogues were examined using density functional theory. These results were then compared with normal Raman and SERS techniques and analyzed using statistical methods. The ultimate goal of the project will be to assist law enforcement in differentiating fentanyl analogues individually and in drug mixtures.

22 **Highly Selective Differentiation of Organic Gunshot Residues Combining their Elemental and Molecular Signatures**

Shelby Khandasammy, University at Albany-SUNY, 1400 Washington Ave., Albany, NY 12222, Lenka Halámková, Matthieu Baudelet, Igor Lednev

Firearm related evidence is greatly significant to forensic researchers. Recently, many researchers have explored organic gunshot residue (OGSR) evidence. The forensic value of OGSR is bolstered by many factors—notably, OGSR analysis has shown the potential to allow differentiation based on ammunition brands and/or calibers. Raman spectroscopy is a vibrational spectroscopic technique which has been investigated for OGSR analysis specifically. Raman spectroscopy is a specific, simple, and rapid analysis technique. Laser-induced breakdown spectroscopy (LIBS) is a simple, robust, and rapid analytical method which requires minimal to no sample preparation. In this study Raman spectroscopy and LIBS were used together to achieve the specific identification and characterization of OGSR particles from closely related ammunition types. The main goal was to determine if this method had the potential to differentiate between various types of ammunition stemming from the same caliber, and produced by the same manufacturer, based on the analysis of OGSRs generated under identical firing conditions. Raman spectroscopy was first

used to identify particles as OGSR. Subsequently, high-resolution microscopy documented the OGSR particles' morphologies. Finally, LIBS analysis of the particles was carried out. Advanced chemometric techniques allowed for successful differentiation between the OGSR samples analyzed. This project was supported by Awards No. 15PNIJ-21-GG-04153-RESS (I.K.L.) and 2019-R2-CX-0035 (S.R.K.) awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect those of the U.S. Department of Justice.

23 **Raman Spectroscopy of TiO_2, WO_3, and Y_2O_3 Nanoparticles**

Sergey Mamedov, HORIBA Scientific, 20 Knightsbridge Rd, Piscataway, NJ 08854

Metal oxide nanoparticles are attractive for many applications but have recently become an essential part of dye-sensitized solar cells and highly efficient catalysis materials. The material's efficiency depends on size, shape, and surface chemistry, which critically determine their properties and interaction. Raman spectroscopy is a powerful method to investigate nanoparticles' vibrational properties, as the Raman band's peak and width are very sensitive to the local structure. Besides, phonons' behavior at the nanoparticle boundary strongly depends on the particle size and is a critical factor in creating a highly efficient material. It was shown that the Raman peak of TiO_2 at 142.9 cm^{-1} shifted to a high-frequency region with a decrease in the nanoparticles' size. However, nanopowders of WO_3 and Y_2O_3 were not investigated yet. The samples of TiO_2, WO_3, and Y_2O_3 with a mean size ranging from 5 to 40 nm were investigated by Raman spectroscopy in the broad spectral range. The model of phonon confinement was used to describe the experimental data. The correlation length of the phonons calculated from the spectra of nanoparticles shows a good correlation between grain sizes obtained from Raman spectra and XRD. Raman spectra are more sensitive to nanoparticles' structural motive compared to XRD. The Raman spectra may differ even if X-ray diffraction shows the same particle size. It reflects the differences in the surface structure of nanoparticles.

24 **Root Cause Spectroscopic Failure Investigation Aided by High Resolution SEM/EDS, FT-IR, XPS Instruments**

Jeanette vajki Vass, Auto & Materials, 120 Watson Mill Rd., Landenberg, PA 19350

My talk demonstrates the consequences that arise when Quality Control and Testing Protocols are absent or not followed. This investigative study describes a devastating failure costing over $2 billion and affecting more than 100,000 newly built Southeastern US coastal homes where living conditions became dangerously toxic due to a highly corrosive but mysterious substance that poisoned the indoor atmosphere of the homes. Our laboratory team was forewarned about this potentially severe, widespread environmentally induced and amplified disaster which quickly led to a very thorough investigation to identify the corrosive and potentially deadly substance and propose further remedial actions. The scope of my presentation is to show the vital role of the Systematic Analytical Characterization Approach through applied and Integrated use of Standard and Micro-Scale Spectroscopic Tools in Failure Analysis (e.g., FT-IR, SEM/EDS, XPS, and more). With the aid of the above specialized spectroscopic and microscopic approaches, including special surface techniques, the source(s) of the emitted corrosive substance was properly identified, and the root cause of the problem was determined. By identifying the emitted corrosive substance, our investigation enabled area residents to file and win Class-Action-Lawsuits, thereby securing due compensation and repairs for their losses. In closing, the enormous health and reconstruction cost of this devastating failure could have been easily prevented. This presentation shows you how a systematic approach with practical and preventative measures could have avoided this enormous problem.

25 **Smart Biosensors with Machine Learning for Objective Pain Assessment**

Omowunmi Sadik, New Jersey Institute of Technology, BioSensor Materials for Advanced Research & Technology (BioSMART Center),161 Warren St., University Heights, Newark, NJ 07102

Conventional methods of assessing pain are mainly subjective, and the challenge of objectively quantifying pain is a daunting task. As pain levels increase, so do the amount and variety of pain killers required to manage pain successfully. Pain killers vary widely in their action and potency mechanisms, ranging from non-steroidal, anti-inflammatory drugs to opioids. The ability to objectively measure pain serves as a guide to more effective analgesic therapies, especially in the acute clinical setting. This work aims to push the frontiers of pain diagnosis by developing a multidimensional, multimodal pain expression database (M2PED) for use in clinical settings. This presentation will discuss the modalities, challenges, and prospects of M2PED for pain diagnosis using molecular (biosensors), imaging (facial expressions), and visual analogs.

26 **Light-Addressable Electroanalysis with Semiconductor/Metal Nanoparticle Junctions**

Glen O'Neil, Montclair State University, 1 Normal Ave, Montclair, NJ 07043

Light-addressable electrochemical sensing (LAES) is a photoelectrochemical sensing technique that uses light to activate a faradaic electrochemical reaction at the surface of a semiconducting photoelectrode. Using LAES we can confine an electrochemical reaction to a microscopic portion of a macroelectrode using focused illumination, enabling photoelectrochemical imaging of biological processes. LAE sensors use semiconductors as the light-absorbing electrode material, and as a result understanding and controlling the electrochemical response of these sensors is considerably more challenging than with metallic electrodes. These challenges are due to the nature of the band structure of the semiconductor, the interfaces between the semiconductor/electrolyte, and the nature of charge transfer across these interfaces. Moreover, when silicon is used as the electrode material, steps must be taken to protect the sensor from corrosion in aqueous electrolytes.Here we present recent results from our group showcasing semiconductor/metal junctions (Schottky junctions) as LAE sensors. We show that Schottky junctions formed between nSi and Au, Pt, or Ni nanoparticles are excellent candidates for LAE sensors. These junctions can be prepared with a simple benchtop electrodeposition procedure and demonstrate excellent electrochemical properties with near-reversible cyclic voltammetry observed with a number of outer-sphere redox couples. We apply these sensors to the detection of sub-μM concentrations of neurotransmitters and other biologically relevant species (e.g., H2O2). We will also show how alternative voltammetric waveforms can be used to provide richer electrochemical information about the interface and increase sensitivity of the sensors.

27 **AI for Model Exploration of Molecular Equilibria in VR**

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The practice of chemistry involves discovering chemical reactions to understand the chemical and biological processes of the world around us and using these to formulate the synthesis of new compounds. However, discovering chemical reactions remains a manual, subjective, and destructive process using complex instruments or requires large amounts of training data for a target task. And developing their mathematical models is also challenging because of the often-different knowledge domains required in math, computer science, engineering, chemistry, and biology—many study molecular systems using apparent information obtained from less reliable mathematical models. Here, we demonstrate a method by which our newly developed machine learning algorithm, AI for molecular equilibria (AI4ME), can aid chemists in discovering new chemical mechanisms. The model architecture design was motivated by reinforcement learning taking advantage of combinatorics, grid search, Newton-Raphson, and Newton-Gauss-Levenberg-Marquart algorithms for action optimization toward the predefined signal goals. We propose using our algorithm to discover the reactions between molecules with little input, understand them with attribution interrogates, and use these observations to guide intuition and propose chemical mechanisms. We validate our approach on several simulated and measured pH data. Quantitative results accurately demonstrate the correct chemical mechanism as the first chosen model among the five top optimized chemical models with optimized thermodynamic parameters, initial molecules, and concentrations. We show how it potentially led to designing new biomolecular sensing systems using detected chemical mechanisms. We also built an interactive VR environment that deploys AI4ME for visual learning.

28 **Novel LC-MS-based Platform for Extensive Investigation on Antibody-Drug Conjugates Induced Ocular Toxicity by Integrating Global Proteomics and Targeted Drug Disposition Analysis**

Xiaoyu Zhu, SUNY at Buffalo, Department of Pharmaceutical Sciences, 701 Ellicott St., Buffalo, NY 14203, Min Ma, Sangita Patel, Ming Zhang, Jun Qu

Antibody-drug conjugate (ADC)-induced ocular toxicities have been widely reported clinically while the underlying mechanisms, presumably complicated, are largely unknown. To enable a comprehensive understanding of ADC-induced ocular toxicities, quantitatively studying tissue disposition of ADC/toxin and the toxic mechanism are desired. However, technical difficulties need to be addressed including blood contamination, small sample amount, sensitivity and specificity of ADC ocular tissue disposition bioanalysis. Moreover, due to the complicated pharmaceutical effects of ADCs, an in-depth global protein quantification is preferred for toxic mechanism exploration. Here we developed a novel LC/MS-based platform addressed these challenges and enabling investigation of ADC/toxin disposition and the detailed molecular-level mechanisms of ocular toxicity. The platform consists of a streamlined antibody-free sample preparation workflow combining an ultra-sensitive trapping-micro-LC-SRM/MS method for quantification of total mAb, conjugated-toxin and

free-toxin with high sensitivity, accuracy and robustness. Meanwhile, with the same set of samples, the UHR-IonStar strategy enabling in-depth, accurate and reproducible global protein quantification was employed for an unbiased discovery and measurement of the key proteins dysregulated by ADC treatments. Following intravenous administration of clinically-equivalent doses of ADCs with DM4 or MMAE payload into rats, ocular syndromes were assessed with ophthalmologic examination. Ocular tissues were collected and subjected to tissue disposition analysis and global protein quantification using the LC/MS-based platform. Our findings provide useful perspectives for ADC-induced ocular toxicities.

29 **Green Chemistry Initiatives at MilliporeSigma for a Sustainable Future**

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MilliporeSimga (The life science business of Merck, KGaA, Darmstadt, Germany) developed and launched DOZN™2.0, a unique web-based greener alternative scoring matrix, also known as a quantitative green chemistry evaluator based on the 12 principles of green chemistry for customers to evaluate their relative greenness of their processes. The 12 principles of green chemistry provide a framework for learning about green chemistry and designing or improving materials, products, processes and systems. DOZN™2.0 scores products based on metrics for each principle and aggregates principle scores to derive a final aggregate score. The system calculates scores based on manufacturing inputs, GHS and SDS data which provide a green score for each substance. DOZN™2.0 is flexible enough to encompass the diverse portfolio of products ranging from chemistry to biology to material science-based products. The DOZN™2.0 system has also been verified and validated by a third party to ensure best practices are applied and also published. This new Greener Chemistry Initiative offer customers' an increased breadth of Greener Alternative products with confirmatory documentation to validate greener characteristics. Through DOZN™2.0 customers now have access to calculate the green scores of their own processes and products. This free, web-based tool provides users with even more data so that they are properly equipped to increase their sustainability. DOZN™2.0 keeps data privacy top of mind—allowing customers to score their processes/products in a safe and secure manner.

30 **Growth Rate Dependence of Secondary Organic Aerosol on Seed Particle Size, Composition, and Phase**

Devon Higgins, University of Delaware, 063 Brown Lab Newark, DE, 19716, Michael Taylor, Justin Krasnomowitz, Murray Johnston

Atmospheric nanoparticle growth, which is a key process associated with formation of cloud condensation nuclei (CCN), is thought to be dominated by the condensation of condensable organic vapor (COV), as produced in autooxidation reactions when a biogenic precursor is oxidized. However, it has been recently established by our group that seed particle size, phase, and composition affect particle growth in the 40-100 nm size range. When seed particles composed of ammonium sulfate or freshly generated α-pinene SOA were exposed to a-pinene and ozone, they were found to grow by different amounts, which varied with aerosol liquid water (ALW) content. Seed particle growth is represented by the growth yield (GY), which is defined as the fraction of a-pinene molecules that react with ozone to give a product that grows the particle. Overall, SOA seed particles gave the lowest GY. Effloresced ammonium sulfate particles gave somewhat higher GYs and increased with increasing relative humidity while deliquesced ammonium sulfate particles gave the highest GYs. The GY for effloresced ammonium sulfate particles was independent of particle size, while liquid-like seed particles (SOA and deliquesced ammonium sulfate) gave somewhat higher growth yields for largest particles studied. The observed growth dependences on particle size, phase, and composition suggest that particles grow by both surface- and volume-limited kinetics. The particle growth results will be presented alongside molecular composition measurements via mass spectrometry to discuss the reaction mechanisms responsible for growth under the various conditions studied.

31 **Unraveling the Complex Composition of Produced Water by Specialized Extraction Methodologies**

Emanuela Gionfriddo, The University of Toledo, 2801 West Bancroft St., Toledo, OH 43606, Ronald V. Emmons, Govind S. Shyam Sunder, Tiffany Liden, Kevin A. Schug, Jon R. Kirchhoff

Produced water (PW), a waste byproduct of oil and gas extraction, is a complex mixture containing numerous organic solubles and elemental species; these constituents range from polycyclic aromatic hydrocarbons to naturally occurring radioactive materials. Identification of these compounds is critical in developing reuse and disposal protocols to minimize environmental contamination and health risks. In this study, versatile extraction methodologies were investigated for the untargeted analysis of PW. Thin-film solid-phase microextraction with hydrophilic–lipophilic balance particles was utilized for the extraction of organic solubles from eight PW samples from the Permian Basin and Eagle Ford formation in Texas. Gas chromatography–mass spectrometry analysis found a total of 266 different organic constituents including 1,4-dioxane, atrazine, pyridine, and PAHs. The elemental composition of PW was evaluated using dispersive solid-phase extraction followed by inductively coupled plasma–mass spectrometry, utilizing a new coordinating sorbent, poly(pyrrole-1-carboxylic acid). This confirmed the presence of 29 elements including rare earth elements, as well as hazardous metals such as Cr, Cd, Pb, and U. Utilizing chemometric analysis, both approaches facilitated the discrimination of each PW sample based on their geochemical origin with a prediction accuracy above 90% using partial least-squares-discriminant analysis, paving the way for PW origin tracing in the environment.

32 **A Screening Test for Pollution of Lakes with Perfluoroalkyl Substances (PFAS): Raman Spectroscopy of Fish Blood**

Luis Pérez-Almodóvar, University of Albany, 1400 Washington Ave., LS 2033, Albany, NY 12222, Igor Lednev

There is a critical need for a fast and onsite method for evaluating per- and polyfluoroalkyl substances (PFAS) pollution. The detection of PFAS in fish blood allows for the evaluation of time-integrated pollution as well as fish health. However, current PFAS detection methods are complicated, involve several steps, and are costly. As such, Raman spectroscopy with Chemometrics is investigated as a novel alternative method for detecting different concentrations of PFAS. Here, blood plasma obtained from smallmouth and largemouth bass located in several lakes with various concentrations of PFAS were analyzed. Partial least squares discriminant analysis combined with receiver operating characteristic curve analysis was able to separate the two groups (Low vs. High PFAS concentration) with 100% accuracy at the donor level during external validation. In addition, the Genetic Algorithm (GA) analysis identified the spectroscopic components responsible for the differentiation of fish classes. While further work is ongoing, these results imply there is a potential for Raman spectroscopy to be used in the future as a successful method for PFAS detection, which can be essential as a diagnostic tool to predict fish health and monitor environmental contamination.

33 **The Importance of High-Resolution Ion Mobility Mass Spectrometry to Accurately Read Back the Complex Language of Biology**

David Muddiman, North Carolina State University, 2620 Yarbrough Dr., Department of Chemistry, Dabney Hall, Raleigh, NC 27695, Jeffrey Enders, Taufika Williams, Kenneth Garrard

Protein glycosylation plays important roles including protein folding, cell signaling, enzymatic activity and adhesion and thus, is a key co- and post-translational modification. In humans, ~2% of the human genome is dedicated to the molecular machinery to modify proteins with a diverse array of glycan structures with > 50% of human proteins being glycoslylated. Given our experience over the past decade in measuring *N*-linked glycans cleaved from mAB's, pure glycoproteins as well as from complex mixtures (e.g., plasma proteins, brain tissue) by LC-ESI-HRAM-MS, we know the limitations of measuring glycans using currently available mass spectrometry platforms – the isomer barrier. Accurate definition of the *N*-linked glycans from a mAB requires a multi-faceted approach including: retention time (LC), accurate mass (defines composition), tandem-MS and ion mobility (resolves isomers). Collectively, this high dimensionality data can accurately reflect and differentiate the glycans on different mAB's being produced. Our approach is based on LC coupled to a SLIM-QTOF-MS; importantly, this is a high-resolution ion mobility platform (HRIM) compared to other approaches such as drift-tube ion mobility (DTIMS) mass spectrometry, which has an ion mobility resolution in the range of 50-85; going beyond that requires multiplexing techniques making data reduction more complicated. In contrast, the SLIM device from MOBILion has an ion mobility resolution of 250 (without multiplexing) allowing baseline resolution of glycan isomers. Results will be shown for each aspect of the overall strategy and how this platform can be an integral part for the characterization of protein therapeutics.

34 **Statistical Approach for System Suitability Testing for Mass Spectrometry Imaging by Infrared Matrix-Assisted Laser Desorption Electrospray Ionization (IR-MALDESI)**

Olivia Dioli, North Carolina State University, FTMS Laboratory for Human Health Research, Department of Chemistry, 2620 Yarbrough Drive, Raleigh, NC 27695, Hellena Bai, Emily Hector, Kenneth Garrard, David Muddiman

Mass spectrometry imaging (MSI) is a powerful analytical tool that can be leveraged to generate 2D and 3D molecular images of biological specimens. However, a statistical analysis to determine the quality of acquired data has been rarely evaluated. Here, we aim to present a system suitability testing method to provide objective data about the quality and reliability of MSI experiments. A workflow was developed with an MSI platform using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) as the ionization source coupled to an Exploris Orbitrap 240. The goal was to quantitatively determine how instrument usage affects data quality over time. Glass slides were homogeneously sprayed with fluconazole and analyzed by IR-MALDESI. An initial criteria matrix was determined, and the threshold of these criteria was selected based on their distribution, which was simulated by 4000 data-

sets collected with instrument in working order. Decision of instrument performance was made at false discovery rate of 5%. Then, data was collected after the instrument had been used for a series of tissue experiments, after routinely cleaning the instrument's ion cage, and finally once again after a cleaning of the instrument's ion cage and quadrupole. Based on the criteria mentioned, our results indicated that sprayed glass slides can be used as an effective SST strategy for IR-MALDESI-MSI and shows promise for implementation with other MSI platforms. Current efforts are focused on quantifying this data into a single statistic that can be used to quantify instrument performance.

35 **Quantitation of Antibody Deamidation Degradation and Host Cell Proteins by Coulometric Mass Spectrometry**

Yongling Ai, New Jersey Institute of Technology, Department of Chemistry & Environmental Science, Newark, NJ 07102, Hao Chen

Targeted proteomic absolute quantitation strategies rely on the use of synthetic stable isotope-labeled peptides or proteins as internal standards for quantitation, which are costly and time-consuming to synthesize. To circumvent these limitations, we recently developed a coulometric mass spectrometric (CMS) approach for absolute quantitation of proteins without the use of standards, based on the electrochemical oxidation of oxidizable surrogate peptides followed by mass spectrometric measurement of the oxidation yield. However, the performance of CMS for a protein mixture has not been evaluated. This study presents the unprecedented quantitative analysis of CMS for therapeutic protein mixtures, including absolute quantitation of low abundant host cell protein (HCP) in the presence of highly dominant monoclonal antibody (mAb) as well as simultaneous quantitation of deamidated peptide products and intermediates generated from mAb deamidation. First, we demonstrated the feasibility of quantifying multiple proteins (β-Lactoglobulin B, α-lactalbumin and carbonic anhydrase) in a mixture with CMS, for the first time, as validated by isotope dilution method. Second, down to 500ppm of PLBL2 (mAb: PLBL2 ratio = 2000:1), a problematic HCP, was successfully quantified by CMS in the presence of NIST 8671 mAb. Third, CMS was also applied for the evaluation of deamidation on mAb, allowing simultaneous quantitation of native, deamidated, and succinimide intermediate peptides from NIST 8671 heavy chain. Overall, our data suggest that CMS for protein absolute quantitation without using any standards has the potential to support the rapid method development and control strategy during biotherapeutic drug discovery and bioprocess development.

36 **A Novel Chromatographic Approach to Microplastics Analysis Using Pyrolysis-GC-MS: How Your GC/MS Can Be Adapted for Microplastics Research**

Khadiza Mom, Quantum Analytics, 27635 Commerce Oaks Dr, Oak Ridge North, TX 77385

Microplastics are at the forefront of environmental research. There is a growing demand by researchers for tools and techniques that can characterize and quantify the polymer analytes that dominate the microplastics problem – analytes like polyethylene, polyvinyl, nylon, and rubber. Although traditional gas chromatography-mass spectrometry offers incredible analytical power, GC/MS alone cannot adequately analyze the range of compounds critical to robust microplastics research. Additional challenges include achieving peak resolution in a sample with multiple polymers or matching unknown peaks to a known polymer using limited, traditional libraries. During this session, we will present how a vertical microfurnace pyrolyzer, specialized column, and microplastic-specific search engine are used to enhance the sample introduction and interpretation capabilities of GC/MS systems.

37 **Tall Versus Wide Data and the Promise of Machine Learning**

Peter Harrington, Ohio University, Department of Chemistry & Biochemistry, Harrington Center for Intelligent Chemical Instrumentation, 133 University Terrace, Athens, OH 45701

Many advances in modern society are due to embedded machine intelligence. These innovations include voice recognition in phones and personal assistants, image recognition for identifying faces in photos, language translation, and digital handwriting transcription. These advances all rely on deep neural networks (DNNs). A history from a single neuron through shallow to deep learning networks. Three important configurations of DNNs will be presented: restricted Boltzmann Machines (RBMs), convolutional neural networks (CNNs), and generative-adversarial networks (GANs). Discussion on database quality will be briefly presented, emphasizing the "garbage in leads garbage out" principle. Most of the machine intelligence successes are based on Big Data, also called tall. Most analytical data sets are wide because they have many more variables than objects. Tall data sets prevent overfitting caused by the many adjustable parameters of DNNs. However, regularization methods can mitigate overfitting. Techniques for understanding the decision-making processes of the trained networks are essential because they put the chemistry back into the chemometrics and allow for discoveries. Still, more importantly, it ensures that the network is not making decisions on artifacts in the analytical measurement or flaws in the experimental design.

38 **Tools for Final Model Selection**

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A plethora of options exist for developing multivariate calibration models. There are many choices for modeling method, both linear and non-linear, and most methods involve one or more tunable meta-parameters. Multiply this by the many options for preprocessing data and selecting variables and the number of possible models becomes huge. The rejection of many of these combinations is obvious, but generally that leaves many options that have trade-offs between fit error, prediction error, degree of overfitting, model robustness and model complexity. So how to choose a final model for deployment? One possibility is to plot $R^2 - Q^2$ versus Q^2 (where Q^2 is the cross-validation equivalent of the calibration R^2). Unlike the root-mean square error of calibration and cross-validation (RMSEC and RMSECV), R^2 and Q^2 are not in the units of the variable to be predicted and have a non-linear relationship with them. An alternative is to plot plot the ratio of RMSECV/RMSEC versus RMSECV. This plot makes it easy to find models that are not overfit and still have a small error of cross-validation. Contours can be added to find the model which is "closest" to the "perfect model." Model robustness plots are also considered. Models can be tested to shifts in the wavelength axis and to synthetic interferents. This shows how sensitive the model is to an unstable instrument or to new minor components in the test samples. A final possibility is to not select a single model but rather average the output of a collection of models.

39 **How Machine-Learning Tools Complement Applications of Absorbance-Transmittance Excitation-Emission Matrix (A-TEEM) Spectroscopy for Food, Pharma and Water Quality**

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This study reviews the key concepts of the patented A-TEEM spectroscopy method focusing on the orthogonality of the simultaneously acquired absorbance and fluorescence data. The resultant synergistic effects of resolving the extinction coefficients and fluorescence quantum yields, in addition to the respective absorbance spectra and the fluorescence excitation-emission spectra, are discussed. Additionally, the importance of applying NIST traceable instrument bias and fluorescence inner-filter effects corrections is explained. The ability to minimize and stabilize solvent matrix effects by working in a Lambert-Beer (L-B) linear concentration range is discussed along with the inherent A-TEEM capacity to identify and quantify low concentration analytes among higher concentrations of background components under L-B linear conditions. Key applications in food, pharma and water quality are used to illustrate how Machine Learning (ML) tools complement the multidimensional and orthogonal A-TEEM data analysis for effective quantification and discrimination. Central to the effectiveness of ML for A-TEEM is Multi-Block (MB) data fusion which leverages all the unique absorbance and fluorescence properties of individual chemicals in solution. Case studies for food, pharma and water quality all concur with the improved performance of MB data fusion for Partial Least Squares (PLS) discrimination and linear regression as well as Extreme Gradient Boost discrimination and regression, which in cases of sufficient sample population may yield better performance than PLS. The fit-for-purpose aspects of the A-TEEM and ML analysis are presented as working industrial standard operating protocol documents and an international standard method for water contamination monitoring.

40 No abstract submitted by the author.

41 Withdrawn by the author.

42 **Cannabis Potency Testing - Which Column Dimension is Right for You?**

Jamie York, Restek, 110 Benner Circle, Bellefonte, PA 16823, Melinda Urich, Justin Steimling, Cathy Hetrick,

When starting method development for potency testing, it's important to choose the right column dimension for the target analysis. In this work, different column dimensions of the Raptor ARC-18 phase were utilized to develop methods to meet various labs' needs using HPLC-UV. To demonstrate the powerful resolving capabilities of Raptor ARC-18, a 50 x 3 mm, 2.7 µm column was used to analyze 7 cannabinoids including CBD, CBDA, delta-9-THC, delta-8-THC, (6aR, 9S)-delta-10-THC, (6aR, 9R)-delta-10-THC, and THCA. This method utilizes gradient conditions, methanol as the organic modifier, and an overall cycle time of 8 minutes. This methodology is ideal for labs that are only interested in the required testing needed to be compliant with specific state testing regulations. Next, additional cannabinoids including CBDV, THCV, CBG, CBN, CBGA, and CBC were added to the previous analytes for a total of 13 cannabinoids. Using the same column dimension and mobile phases, a method was developed to resolve all analytes in 10 minutes. Finally, to include exo-THC and CBNA, a 150 x 3 mm, 2.7 µm column dimension was used to demonstrate the utility of a longer column dimension. The organic modifier used was 0.1% formic acid in acetonitrile, where a total of 15 cannabinoids were able to be resolved in 10 minutes. Each of these methods was applied to hemp matrix to demonstrate the applicability of these methods in real world samples.

43 Shape and Frequency-Based Peak Identification Techniques for Chromatography

Cable Warren, The University of Texas at Arlington, Department of Chemistry and Biochemistry, 700 Planetarium Place Arlington, TX 76019, Akinde Kadjo, Purnendu Dasgupta

Mass spectrometry provides unambiguous identification of analyte peaks. However, mass spectrometers are high-maintenance, expensive instruments whose use are not warranted unless essential. In addition, for low m/z ions, MS detection limits are often unattractive. Other chromatographic detectors used for quantitation generally do not provide enough information to identify analytes. Thus, retention time matching is used for identification, comparing retention times of unknown compounds to those of standards. However, retention times shift from column degradation and co-eluting analytes that may be unanticipated or unknown. To account for this uncertainty, methods of identity confirmation are increasingly useful. The most cost effective and convenient methods of identity confirmation are those that avoid large changes to chromatographic procedure. Previously, we presented a method of shape-based identity confirmation examining peak widths at various normalized peak heights and established that chromatographic peaks have measurable differences in their shape. Through construction of "shape calibration matrices", the *sameness* of chromatographic peaks to those in a library of standards could be confirmed. This proved quite reliable for identity confirmation and utilizes the same standards already present for retention time matching. As shape can be interpreted and derived from frequency characteristics, we investigated the utility of Fast Fourier Transform (FFT) results for identity confirmation. FFT allows for deconvolution of characteristic frequencies in chromatographic peaks for comparison. Based on some predetermined criteria, the proposed identity of the unknown peak can then be confirmed or denied. The relative effectiveness of the FFT and shape-based methods are compared.

44 Electrochemical Method for the Detection of Gunshot and Metal Residues

Molly Trautman, Lebanon Valley College, 960 Mine Rd., Lebanon, PA 17042, Donald Dahlberg, Michelle Rasmussen

As violent crime rates have increased in the last few years, the need for quick, precise, and accurate lab results has also increased. As a result, this has brought attention to the scientific methods behind evidence analysis in forensic science. Gunshot residue and residue from metal weapons are crucial pieces of evidence that get left behind at most crime scenes, whether that's on a suspect or object. The purpose of this study is to develop an electroanalytical method to characterize metal residues using a portable instrument, making it possible to evaluate samples in the field. Most firearm ammunition companies make bullets that contain a specific trace amount of both organic and inorganic chemicals such as antimony, copper, manganese, lead, tin, zinc, iron, and diphenylamine. Handling metal objects to use as a weapon can also leave behind residues. Ideally, the proposed sensor will allow a specific brand of bullet or alloy of metal to be quickly and accurately recognized on scene from a swab of gunshot or metal residue. Electrochemical sensors function by recording the amount of electrons accepted or donated in a sample through redox reactions that occur directly on, or near, the sensor. This study utilizes square wave anodic stripping voltammetry to generate a "fingerprint" voltammogram for a given sample. The change in current is related to the relative concentrations of the constituents in the residue sample, allowing the collected evidence to be compared to the known constituents of potential weapons or ammunition used in a violent crime.

45 The Separation of Dextro- and Levomethorphan on CHIRALPAK® Immobilized Chiral Columns

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

Dextromethorphan (DXM) is a cough suppressant found in several common over-the-counter medications. DXM is in the class of compounds referred to as morphinans. This class of compounds also contains well-known opioids like morphine, naloxone, and levorphanol. As such, it does exhibit some mild opioid-like characteristics if taken in excess, but not to the same degree as its enantiomer, levomethorphan (LVM). LVM is a Schedule II narcotic in the US, characterized as being 5x stronger than morphine itself; DXM, as previously mentioned, is OTC. Because of the highly potent nature of the LVM, it is important to have an analytical HPLC method for accurate quantification of it in DXM raw material to ensure OTC cough suppressants meet the regulatory requirements for acceptable levels of this narcotic. To date, only one separation has been reported in the literature, a normal phase separation with Hexane-Isopropanol-Diethylamine using CHIRALCEL® OJ-H. While the separation is baseline, it requires a low flow rate (0.5 ml/min) and therefore elongated analysis time (11 mins) to obtain good resolution. This poster presents several new separations on CHIRALPAK® immobilized columns, as well as an updated normal phase separation on CHIRALCEL® OJ-3, which offer significantly reduced analysis times, while maintaining good baseline resolution.

46 Non–Invasive Discrimination Between Pregnancy and Pseudopregnancy in Giant Panda Using Near–Infrared Spectroscopy (NIRS)

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The endangered giant panda *(Ailuropoda melanolueca)* is a flagship species for wildlife conservation. Due to this species' pseudopregnancy, where a non–pregnant female's reproductive hormones show a similar pattern as a pregnant one, pregnancy detection can be challenging. Traditional fetal detection methods include ultrasound and hormonal biomarkers in urine and blood, which are limited to late–stage pregnancy and are time–consuming. Our objective was to test whether NIRS with the mode–cloning technique can differentiate between pregnancy and pseudopregnancy using field–ready fecal samples. Fecal samples (n=74) from 2 captive female pandas were collected during pregnancy and pseudopregnancy. During fecal processing from wet to dry to ground, NIR spectra were collected for model calibration and validation with an ASD FieldSpec®3 Vis–NIRS portable spectrometer. Another female experiencing pseudopregnancy (n=7) provided samples for external validation. Principal component analysis (PCA) and partial least squares–discriminant analysis (PLS–DA) were applied, and mode–cloning was performed with piecewise direct standardization (PDS) on spectral transformations between processing states. Models using highly–processed ground samples had a pregnancy prediction accuracy of 93.8% and 100%, for validation and external validation, respectively. Similarly, the accuracy of modeling less–processed dry samples reached 93.7% and 85.71%, and unprocessed wet samples reached 87.5% and 100% when mode–cloning was applied. NIRS with mode–cloning of field–ready wet fecal samples could be used for *in–situ* giant panda demographics and reproduction assessment.

47 Understanding Photo-Chemical Properties and Degradation Pathways of Cadmium-based Pigments Using Pump-Probe Microscopy

Yue Zhou, Duke University, Department of Chemistry, 124 Science Dr., Durham, NC 27708, Marta Ghirardello, Daniela Comelli, Warren Warren, Martin Fischer

Cadmium-based pigments, including cadmium yellows and cadmium reds, are a group of significant pigments broadly applied in historical paintings. They can offer a nearly continuous spectrum of colors from light yellow to deep red by adjusting solid solutions of CdS with ZnS and CdSe. The tendency of cadmium pigments, especially cadmium yellows, to degrade over decades has attracted interest in the investigation of possible degradation mechanisms. Conventional research is mainly based on chemical composition analysis and photoluminescence studies of cross-sectional samples taken from degraded paints. However, different synthetic methods of pigments and heterogeneous components of the paints could lead to different degradation behaviors, which researchers have yet to fully understand, making it difficult for art conservators to preserve historical artworks. Here, we demonstrate the use of pump-probe microscopy, a recently developed optical analytical technique, to provide high-resolution, chemical-specific images of cadmium-based paint samples. Commercial cadmium pigments with various hues were studied under pump-probe microscopy to reveal the decay dynamics of pure pigments. In addition, reproduced cadmium yellow pigments following different historical manufacturing recipes are investigated and exhibit slightly different transient absorption signals, indicating that synthetic methods could influence the physical-chemical properties of cadmium pigments. In the future, artificial degradation will be induced on mock-up samples for both self-made and commercial cadmium yellows and compared with historically degraded paints. We expect the pump-probe technique can shed light on the degradation mechanisms of cadmium yellows, and noninvasively diagnose early-stage discoloration.

48 Kinetic and Equilibrium Studies on the Adsorption Cadmium and Lead Adsorption with Biowaste Adsorbent from Aqueous Solutions for Environmental Pollution Control

Taha Allah, St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439, Enju Wang, Kaltrina Jusuf, Ali Shohatee, Louis Trombetta

Over the last few years, pollution of water is a high concern from natural and anthropogenic sources. Another apprehensive fact of pollution is the high presence of contaminants in the environment, especially those with toxic and hazardous properties. Since we are dealing with a high amount of contaminants, remediation of the environment has an enormous cost, and reducing pollution is critical. The big challenge currently is to find the most eco-friendly path that leads to the decontamination of the environment. This study presents the study using environmentally friendly, low-cost waste adsorbents, such as potato and pumpkin peels, shells of peanut and sunflower seeds, chamomile tea residues, and coffee bean waste, as a cadmium and lead ions removal from an aqueous solution. The biowaste sorbents were also treated with acid (HCl) or base (NaOH). A known concentration metal ion solution was introduced into the biowaste sorbents at various pH ranging from 5-9, the pH and metal ion were monitored with pH and cadmium or lead ion-selective electrode continuously for two hours, the final concentration for the metal ions concentration after 24

hours was measured with the ISE and then confirmed with inductively coupled plasma atomic emission spectroscopy (ICP-AES). The adsorption characteristics of the sorbents were also studied with scanning electron microscope, infrared spectra and differential scanning colorimetry. Preliminary results of our research showed promising outcomes using base treated potato and pumpkin peel derived sorbents for the removal of cadmium and lead from an aqueous solution with five minutes of contact.

49 **The Commercialization Effort for a Universal Method for Body Fluid Identification for Forensic Purpose**

Alexis Weber, University at Albany, SUNY, 420 Sand Creek Rd., Apt 420, Albany, NY 12205, Igor Lednev

The ability to identify body fluid traces at crime scenes, while preserving any DNA present, is critically important in forensic science. Currently in forensic science laboratories, the identification can be difficult and many of the current techniques are specific to one body fluid. Additionally, typical biochemical methods are destructive – preventing any further analysis. When there is a problem within the scientific field, research laboratories are the main group to solve this problem. After conducting research in the laboratory, the next step in the process is to commercialize the research. Commercialization is bringing a product to market and selling it for financial gain. Within the Lendev Laboratory, in order to develop a universal, confirmatory, nondestructive, approach that can be used to differentiate and identify body fluids, the specificity of Raman spectroscopy was combined with the analytical power of statistical modeling. All six forensically relevant body fluids (blood, semen, saliva, sweat, urine, and vaginal fluid) were successfully discriminated by coupling Raman spectroscopy and chemometrics. This technique is both reliable and nondestructive, offering substantial advantages over the current techniques used to identify body fluids. This development of this product has occurred over several years to prepare it for sale, with the culmination of this being the creation of the start-up company SupreMEtric LLC. SupreMEtric's mission is to streamline the forensic analysis of biological stains by creating a universal nondestructive method for the identification of all main body fluids. This presentation covers the process from research to commercialization process of this technology.

50 **Considerations for HILIC Method Migration**

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

Hydrophilic Interaction Chromatography (HILIC) methods can be found in regulated laboratories that have different chromatographic systems present for analysis. HILIC methods can be migrated from system to system but a lack of knowledge about the chemistry of HILIC separations and the considerations may lead to challenges when migrating the methods across systems. Given their unique characteristics, properly setting up a system including washes, for HILIC separations can be complex. One issue is the use of an incorrect needle wash solvent composition on the system which may stem from monographs not specifying wash solvent compositions. In this presentation, the USP Cetirizine Hydrochloride Assay and Organic Impurities method was used to demonstrate the impact of various needle wash solvents on a HILIC chromatographic separation. In this evaluation, different needle wash solvent compositions were evaluated and the resulting effects on the chromatography were analyzed on a UHPLC system. The method was migrated across multiple LC systems to determine the impact of LC design and needle wash combination on chromatographic performance. Although needle wash considerations are made to reduce carryover, this study provides an example where carryover is not the only critical parameter impacted by the needle wash. The needle wash can also impact the peak shape or chromatography. Due to differences in LC system mechanical designs, specifically the autosampler, an inappropriate wash solvent may impact HILIC method migration.

51 **Investigation of RPLC Method Migration Risks using Chromatographic Simulator**

Zhimin Li, Waters Corporation, 34 Maple St., Milford, MA 01757, Lise Gauthier, Norris Wong, Kaveh Amini, Corey Reed, Fabrice Gritti, Martin Gilar

Investigating liquid chromatography (LC) method migration problems can be a challenging process for analytical laboratories. Method migration within the organization or to external contract organizations may encounter difficulties, not limited to poor method robustness, operator error, or complex samples. Complex samples that possess a broad range of physico-chemical properties may be especially difficult to retain and resolve in reversed-phase (RP) LC. Successful method migration of gradient methods depends on LC system characteristics, such as gradient delay, system dispersion, gradient fidelity, and others. Common LC method migration tools provide simple guidance, but often disregard the parameters listed above. Therefore, in practice, successful method migration is not always achieved. In this study, we developed an LC method migration simulator based on the Linear Solvent Strength (LSS) model that can visualize chromatographic separations and illustrate the impact of LC system characteristics on chromatographic separation. The simulator requires user inputs of LC system parameters (system dispersion, gradient delay, and gradient mixer dispersion) and sample parameters (S – slope of retention change with change of organic mobile phase composition and $\log k_{00}$ — analyte retention at aqueous mobile phase). Using these inputs, the simulator can predict the chromatographic separation of the sample of interest and visualize the impact of LC system choice and other critical LC parameters in chromatograms. This simulator enables the analysis of critical LC parameters to facilitate successful method migration. The difficulties and challenges during the method migration can be foreseen, and appropriate mitigation and adjustment can be implemented to improve method migration success.

52 **Automation of Analytical Methods for Oral Compressed Tablets**

Calvin Huang, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065

Analytical testing is an essential part of all research and development, clinical, and commercial drug products. Current drug product methods: assay/degradation, content uniformity, and dissolution, are used to support drug product clinical release as well as drug product clinical and registration stability studies. The most difficult analytical challenge in the execution of these methods involves the time consuming manual sample preparation steps which often requires an analyst's full attention, thus reducing overall efficiency of resources. In order to decrease the overall manual effort needed for sample preparation, the Analytical Team utilized available equipment to automate the sample preparation portion of the methods in order to free up analyst's time, allowing efforts to be focused on other essential project deliverables. The Tablet Processing Workstation (TPW) was used to automate Assay/Degradation and Content Uniformity experiments, while the Sotax AT-70 was explored to automate dissolution experiments. The Analytical Team evaluated and finalized the equipment parameters and was able to successfully qualify the automated assay/deg and content uniformity methods to be used in the development environment. The next step will be to further develop automated methods that are qualified for a GMP-environment. Incorporating a fully automated sample preparation will reduce analyst variability, ergonomic stress, increase efficiency and increase safety compliance by decreasing the risk of analyst exposure to high-potent classified product.

53 **Digitalization of Laboratory Processes with Cloud Based Solution, Tablets, and QR Codes**

Henry Tat, Merck & Co., Inc., 126 E. Lincoln Ave, Rahway, NJ 07065

The pharmaceutical industry is a highly regulated field which requires extensive change control and validation of processes. Because of this, modernization of controlled processes often falls behind current technology. Implementation of a flexible and central cloud based system not only allows for increased efficiency and compliance, but it creates one system that reduces training burden, localized processes, and the need to switch between multiple systems. This work will describe the implementation, strategy, and benefits of using a cloud based system with tablets and QR codes for modernizing day to day laboratory activities in an analytical lab.

54 **Development of a Dual Electrospray Ionization Source with In-Line Absorbance-Based Voltage Control**

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

To increase throughput and/or method complementarity, multiple HPLC columns can be used in parallel arrays. A challenge with this approach is the need for multiple detectors, which can be especially challenging for LC-MS methods due to the high cost and large size of MS instrumentation. Dual electrospray ionization (ESI) sources that enable spray from multiple fluidic channels can be used to direct two eluent flows to the MS. However, this can lead to higher background noise and lower analyte signal during method segments when compounds are only eluting from one channel. To mitigate this issue, control of the high voltage power supply (HVPS) can be coupled to an in-line absorbance detector, so that spray is only initiated when compounds are known to be present. In this experiment, a low-volume capillary-scale absorbance detector is coupled to a miniaturized HVPS through a Raspberry Pi control system to enable this strategy. Details on the electronic, fluidic, and 3D printed support components of the system are presented, along with a demonstration of flow injection experiments to test various operating conditions.

55 **Application of Trapped-Ion-Mobility Spectrometry (TIMS) Time-of-Flight (TOF) Mass-Spectrometry in Expediting Conventional Food Analysis of Simple and Complex Carbohydrates**

Artem Filipenko, Bruker, 40 Manning Rd, Billerica, MA 01821

Trapped Ion mobility offers high resolution of complex mixtures with effective fragmentation rates beyond 100Hz. It provides accurate collisional cross section (CCS) values as well adds to the sensitivity of QTOF (Quadrupole Time-Of-Flight) mass spectrometry. Established MS methodologies underutilize these advances and take a longtime in required detailed analysis of biological and food samples. We describe here a very rapid method of direct infusion of samples for comprehensive analysis of mono-, di-, and trisaccharides and malto-oligosachhrides (4-10 monomer length). The method provided a baseline separation of mono-, di- and tri-saccharides in ion mobilograms with high resolving power. The CCS value measurements of the di- and

tri-saccharide are in excellent agreement with reference literature values. Malto-oligosaccharides separation was demonstrated over a wide mobility range at ~1 Hz and resolution allows to see their different branching points. TIMS/MS heat maps allow to screen unknown samples in real time with direct injections and identify ion groups of interest in the real time. The method shortens the analysis time to minutes from hours in availability of results translating into savings not only in time but also in production cost.

56 **The Detection of Flavonoids in Hemp Flower by LC-MS/MS**

Jamie York, Restek, 110 Benner Circle, Bellefonte, PA 16823, Justin Steimling, Cathy Hetrick

Flavonoids are a class of compounds that are endogenous in hemp and cannabis that are antioxidant rich and can affect the flavor profile. Routine testing in cannabis labs oftentimes does not include flavonoid testing, but as the market for cannabis grows, industry leaders may choose to implement new tests to differentiate their services from their competitors. In this work, a method to detect 19 flavonoids in CBD and CBG dominant hemp flower was developed using LC-MS/MS. Extraction of flavonoids was performed by weighing 0.5 g of ground hemp flower and adding 80/20 methanol/water (10 mL) to the hemp flower and vortexing. The samples were sonicated for 15 min, centrifuged at 4200 rpm for 5 min, and diluted 50-fold. The analytical method was developed using LC-MS/MS with electrospray ionization in positive and negative ion modes. The method uses gradient conditions on a Raptor Biphenyl 100 x 2.1, 2.7 µm column equipped with a guard cartridge. 10 flavonoids were detected in the CBG hemp flower sample and 12 flavonoids were detected in the CBD hemp flower samples. Single point recovery experiments were also performed at 100 ppb using two deuterated internal standards. The results of the single point recovery ranged from 80 – 104%. The developed method was able to resolve isobars within a cycle time of 7 minutes and acceptable recoveries were achieved for both hemp flower samples, indicating this is an effective procedure for the extraction of flavonoids from hemp flower.

57 **Cannabinoid Extraction Efficiency for Potency Analysis: An in Depth Look of Multiple Techniques**

Melinda Urich, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16841, Justin Steimling, Cathy Hetrick

There are many different extraction techniques on the market, and oftentimes new labs are confused as to where to begin and which technique would best suit their needs. In this study, six different extraction methods were investigated to determine overall efficiency, as well as sample preparation cost and sample preparation time. Two types of chemovars were used for the study, CBD and CBG, to ensure extraction efficiency is comparable across different varieties. Due to its robust nature, a UV-Vis was used and monitored at 228 nm. All data was obtained by using a Raptor ARC-18 2.7 µm, 150 x 4.6 mm column with accompanying 5 x 4.6 mm EXP guard. A total of sixteen cannabinoids were monitored under 25: 75 isocratic conditions consisting of mobile phase A 5 mM ammonium formate in water with 0.1% formic acid and mobile phase B of 0.1 % formic acid in acetonitrile, for total cycle time of 9 minutes.

58 **Understanding Dispersion in HPLC Absorbance Detectors**

Cable Warren, University of Texas at Arlington, Department of Chemistry and Biochemistry, 700 Planetarium Place, Arlington, TX 76019, Charles Shelor, Purnendu Dasgupta

Band dispersion, before, in, and after the column, are undesirable; unfortunately, they prove unavoidable in real chromatographic systems. Dispersion in a HPLC absorbance detector represents an interesting dilemma, while increasing the path-length improves detection sensitivity, it necessarily increases the cell volume and increases dispersion. “High Dynamic Range” systems with two completely independent detectors, in which a short path cell is followed by a long path cell, are commercially available. Efforts have been made to understand dispersion in long path cells investigating if mathematical processing may yield the higher resolution of short path data while conserving sensitivity. Such efforts largely relied on observational mathematical models without fundamental phenomenological understanding of the dispersion. We report results from an improved experimental system which in addition to a D2-lamp photodiode array-based absorbance detector, several UV-LED based detectors are placed perpendicular to the direction of flow. This design allows the progress of dispersion along the cell to be quantitatively measured through a range of bandwidths as observed in real chromatographic situations. In practice, this unique configuration combines long path length, high sensitivity detection with short path length, high efficiency detection. This flow cell provides an exciting opportunity for understanding and correcting for peak broadening within an absorbance detector and may permit long path length absorbance detection without the drawback of excessive dispersion.

59 **Non-Destructive Discrimination of Starch Adulteration in Ginger Powder Using Digital Images and Tree-Based Algorithms**

David Stefany, Rutgers University, 5 Picardy Rd., New Brunswick, NJ 07876, Thomas Hartman

Ginger (Zingiber officinale) is not only used as a flavor in many cuisines but for its medicinal properties.[1] Methods to authenticate ginger powder have been developed using a range of analytical techniques. [2,3] A report by Jahanbakhshi demonstrated the use of deep learning to detect fraud in ginger powder using digital images.[4] This research proposes new rapid, low-cost, non-destructive methods that can discriminate between levels of starch adulteration in ginger using summary statistics from digital images. A compact digital microscope was used to capture mages of the powder samples. Summary statistics of RGB and HSV color channels from the images are used as model inputs. This research focused on the use of tree-based algorithms (Random Forest, Gradient Boosting Machines) to discriminate between levels of starch in ginger powder (range 0% – 30%).

60 **Investigation into Noise-Suppressed First Derivatives for Rapid Symmetrization and Deconvolution of Peaks in Chiral Chromatography**

Troy Handlovic, The University of Texas at Arlington, Department of Chemistry and Biochemistry, 700 Planetarium Place, Arlington, TX 76019, M. Farooq Wahab, Daniel Armstrong

All peaks from enantioseparations show some degree of asymmetry, which is detrimental for quantitative and semi-quantitative chiral analysis. Methods have been proposed to correct this asymmetry but often are computationally exacting and require knowledge of convolution mathematics to obtain correct results. In this work, we propose a straightforward and fast peak symmetrization algorithm for correcting or reducing peak tailing or fronting. This method corrects the peak shape by adding a fraction of the peak’s first derivative to the chromatogram. The area remains invariant through this process since the area under the first derivative is numerically close to zero for highly asymmetric peaks. Analytical chemists have been historically skeptical about using derivatives since differentiation is a noise amplification process. Here, insight into how pre-smoothing the data with a modified Whittaker smoother (“perfect smoother”) is provided to yield a fast noise-suppressed first derivative. The central difference method is also used to compute the first derivative numerically, reducing the root mean square of the noise by 40% compared to the standard forward difference method. A survey of forty chiral separations is presented, demonstrating the range of asymmetry observed on a supercritical fluid chromatograph, UHPLC, and HPLC for multiple chiral stationary phases. Examples of symmetrization of the peaks from enantiomers in comparable and disproportionate concentrations are provided. Artifacts of deconvolution are discussed, along with approaches to avoid such artifacts.

61 **Adsorption of Amine Compounds on Glass Surface and Their Impact on the Development of Analytical Method and Pharmaceutical Process**

Xuejun Xu, Bristol Myers Squibb Company, One Squibb Dr., New Brunswick, NJ 08903, Jennifer Lott, Kathleen Kelly, Zhongping Shi

A diamine compound (3-{2-[2-(3-aminopropoxy)ethoxy]ethoxy}propan-1-amine) was demonstrated to impact downstream steps in a pharmaceutical synthetic process, and control of this compound to less than 0.015 wt% (or 150 ppm) was necessary. A novel, simple, and sensitive LC-MS method without the derivatization of the diamine was developed for in-process control (IPC) and demonstrated to be suitable for use when experiments were run at small lab scale. During the development of the LC-MS method, the diamine was found to significantly adsorb to the surface of glassware, and the adsorption constant was estimated to be (5.4 - 10.4) × 10^8 mL/mol SiOH. This phenomenon impacted the analytical method recovery and the control of the diamine in the process. The diamine adsorption was investigated and procedures were developed to mitigate adsorption. The addition of 0.1% triethylamine in sample diluent was found to significantly reduce adsorption of the diamine and improve LC-MS method recovery. However, at kilo plant scale, the impurities that were initially inhibited in development work appeared. It was determined that the diamine adsorbed on glassware more strongly than previously expected, including the reactor. This presentation will discuss steps taken to reduce adsorption on glass both for the analytical scale as well as the plant glass reactor.

62 **Performance Improvement of Ultra-High Pressure Liquid Chromatography Mass Spectrometry Using Vacuum Jacketed Column Technology**

Fabrice Gritti, Waters Corporation, 34 Maple St., Milford, MA 01757, Sornanathan Meyyeppan, Jason Hill, Thomas McDonald, Rob Plumb

Significant losses in peak resolution are encountered in ultra-high performance liquid chromatography (UHPLC) coupled to mass spectrometry (MS) detection. This problem is caused by the excessive post-column dispersion from the column outlet to the direct/infusion valve, and to the ionization probe. This issue is of great concern for short (2-5 cm long) narrow-bore (2.1 mm i.d.) columns packed with sub-2 µm

particles. A solution that consists in deploying the column very close to the point of sample ionization while running the column in absence of a LC oven is presented. First, a vacuum jacket is placed around the column in order to maintain the column temperature nearly uniform along its length. The vacuum jacket column (VJC) prevents most of the heat exchange between the lab air environment and the column wall. Secondly, a Joule heater is placed locally at the column outlet to 1) compensate for possible heat leaks at both column extremities and 2) reduce the nefarious impact of radial temperature gradients expected in UHPLC columns at high speeds/pressure. The advantage of the VJC directly connected to the ionization probe of the MS detector over classical columns in the oven of standard UHPLC-MS systems is demonstrated for the separation of drugs (RPLC, 2.1 x 100 mm column) and the ultra-fast detection of four metabolites (RPLC, 2.1 x 50 and 30 mm column) by gradient elution. The gain in peak capacity (+130%) is explained from the reduction of post-column dispersion and the increase of column efficiency.

63 **Characterization of the Composition of 3-D Printed Devices by Using Pulsed Gas Direct Analysis in Real Time Mass Spectrometry**

Brian Musselman, Bruker, 999 Broadway, Ste. 404, Saugus, MA 01906, William Fatigante, Artem Filipenko, Jenna Covey

The rapid adoption of 3-D plastic printer technology has led to a proliferation in production of unique devices. The devices fabricated range from simple toys to dangerous firearms. Characterization of the material utilized to produce these devices can permit identification of the plastic, however, the fact that these materials are often common polymeric materials limits the utility of many analytical methods to simple identification of the core plastic. In this work, analysis of trace samples of the starting materials and printed products in seconds per sample by using differential temperature desorption ionization by direct analysis in real time (DART) mass spectrometry. The experiment permits a determination of the polymer type and minor components related to color and plasticity. Historically, detection of trace levels of leachable components and colorants in food packaging has been completed by using DART-MS. Here we demonstrate and improved method which utilizes DART with pulsed gas ionization to generate background free molecular fingerprints of these materials.

64 **Targeted Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Seawater, Plankton, and Shellfish Tissue Using UPLC-MS/MS**

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

Per- and polyfluoroalkyl substances (PFAS) are an extensive group of anthropogenic chemicals characterized by strong fluorine-carbon bonds that make them resistant to biotic and abiotic degradation. PFAS are also unique in that they are hydrophobic, oleophobic, and lipophilic, which promotes their bioaccumulation in water, sediment, and biota. Airports and military bases are often direct and indirect sources of PFAS contamination into the aquatic environment due to their high-volume use of PFAS-containing aqueous film-forming foam (AFFF). Once in the aquatic environment, PFAS can be bioaccumulated by organisms at the base of the food web and transferred between trophic levels, potentially resulting in high concentrations in tertiary consumers. To evaluate and quantify the extent of PFAS trophic transfer in marine food webs near airport and military bases, we used Solid-Phase Extraction (SPE) and a Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method for the determination of 28 PFAS in seawater, plankton, and shellfish tissue from Long Island Sound, Connecticut followed by Ultra-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UPLC-MS/MS). Analysis of plankton samples is ongoing, however reporting limits for seawater ranged from 0.001 ng mL-1 to 0.009 ng mL-1 and 0.2 ng g-1 to 5.81 ng g-1 for shellfish tissue. Moreover, seven out of eight seawater samples and 10 out of 12 composite shellfish samples had detectable PFAS levels, with shellfish tissue having the highest concentrations.

65 **Determination of Total Chlorine in Palm Trees for Early Detection of 3-MCPD in Refined Oil Using ICP-OES**

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

In recent years the use of refined palm oil in food and cosmetic products has grown rapidly. However, due to the possible presence of 3-monochloropropane-1,2-diol (3-MCPD) in the refined palm oil it can pose potential health risks to the consumer. Europe, the US and Malaysia (one of the largest palm oil producers) have set the limit for the maximum daily intake to 2 µg/kg 3-MCPD of body weight. Therefore, the palm oil industry has instituted a maximum allowable concentration of 2 ppm chlorine in the refined oil, as the precursor for 3-MCPD formation. This poster describes the sample preparation and analysis of different parts of the palm tree for chlorine using the PerkinElmer Avio 220 Max ICP-OES.

66 **Determination of Total PFOS/PFOA: Evaluation of Calibration Standard and Integration Technique**

Cynthia Srigley, United States Food & Drug Administration, 5001 Campus Dr., College Park, MD 20740, Susan Genualdi, Wendy Young, Lowri DeJager

Per- and polyfluoroalkyl substances (PFAS), specifically the branched and linear isomers of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), are persistent compounds of human health concern which are known to bioaccumulate in wildlife and agricultural products in areas of environmental contamination. The accurate and precise determination of these compounds is an important methodological consideration when evaluating concentrations relative to toxicological reference values. This work investigated different quantification techniques for the measurement of total PFOS and PFOA in incurred samples of cow's milk, deer meat, and clam, previously collected as part of targeted surveys. Data generated using an established liquid chromatography tandem mass spectrometry (LC-MS/MS) method were analyzed using three different integration approaches: a total PFOS/PFOA technique involving a single calibration curve; a linear/total branched technique involving two calibration curves, and an individual isomer technique which quantified linear and branched isomers relative to the corresponding calibration curves for each isomer (eight calibration curves). Analytical standards varying in linear and total branched isomer compositions were also tested with the goal of identifying the optimal combination of calibration standard and integration approach with least amount of integration bias. Relative response factors provided insight into differences observed among quantification techniques.

67 **High-Throughput Analysis of Small Molecules Using Custom Capillary LC Instrumentation**

Deklin Parker, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Samuel Foster, James Grinias

Capillary LC is gaining popularity as a greener liquid-phase separation technique due to a significant reduction in operating flow rate and mobile phase waste generation compared to traditional LC methods. However, it has not been widely adopted for high-throughput screening, which is typically performed using spectroscopic, mass spectrometric, or analytical-scale LC techniques. Here, a new capillary LC-based method was developed to enable sub-10 s separations for eventual use in a high-throughput screening platform. The system consists of compact LC pump capable of achieving low µL/min flow rates, a low-volume internal loop injector, and a novel LED-UV absorbance detector. Data acquisition and system control are achieved through a home-built Java program operated on a low-cost single-board computer. With a 0.3 x 50 mm column operated at 50 µL/min, an 8 second separation window was achieved. As a preliminary investigation of potential use in high-throughput screening, a series of 96 replicate injections (representing a 96-well plate) were completed in 12.8 minutes. The total mobile phase required for this injection sequence was less than 1 mL, representing a significant reduction in solvent waste compared to similar high-throughput strategies using larger diameter LC columns.

68 **Impact of Instrument Design on Absorptive Carryover**

Kaveh Amini, Waters Corporation, 34 Maple St., Milford, MA 01757, Lise Gauthier, Corey Reed, Paula Hong

Carryover is a common problem encountered when running methods on high pressure liquid chromatographic systems, particularly for quantitative analysis and/or sensitivity analyses. For many methods, absorptive carryover is particularly problematic as it is caused by the analyte sticking to the surface of the system. To reduce or eliminate absorptive carryover, most modern HPLC systems incorporate some type of a needle wash during the injection cycle. The choice of needle wash solvent is critical since it should be of a suitable composition to dissolve any compound of interest which can then be flushed to waste. Depending on the autosampler design, needle washing can occur prior to and/or after injection, or prior to and/or after sample aspiration. The mechanism under which the needle is washed and the timing and duration that this step occurs can have a significant impact on carryover. In this presentation, we will investigate absorptive carryover on HPLC systems, and evaluate the impact of injector design. Specifically to assess the impact of carryover in a typical assay, the USP monograph for chlorhexidine hydrochloride will be performed on several HPLC systems with UV detection. Since USP monographs do not specify a needle wash solvent, the systems will be evaluated using different needle washes compositions. Furthermore, the needle wash mechanisms and timing among the different HPLC systems will be assessed. The results will demonstrate strategies for successful method migration across systems and control strategies that can be implemented to ensure carryover is addressed for a range of systems.

69 **Method Optimization and Validation of PFAS in Human Serum Using On-line SPE UHPLC-MS/MS**

Elizabeth Pelczar, New Jersey Department of Health, 3 Schwarzkopf Dr., Ewing, NJ 08628, Carrie Xu, Linbin Zhong, Shawn O'Leary, Chang Ho Yu, Tina Fan

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are emerging environmental contaminants that have received much attention recently due to their persistence, abundance, and toxicity. To date, human exposure studies have primarily focused on measuring a limited number of conventional PFAS in serum. However, growing research data indicates that PFOA and PFOS are present as branched and linear isomers whose accurate measurement is important to supporting health studies and source identification. With the phasing out of conventional PFAS and the rise of newer replacements (such as GenX, ADONA, and 9Cl-PF3ONS), it is imperative to develop methods that accurately measure the emerging PFAS in the human body. We recently optimized an on-line SPE method for accurately identifying and quantifying 18 PFAS, including linear and branched PFOS and PFOA as well as three replacement PFAS, in human serum. This newly optimized method uses a Chronos on-line SPE unit, coupled with a UHPLC (CHRONECT SPH1299) by Spark Holland and a Sciex 7500 mass spectrometer. The method is based on CDC Method #6304.09; but has been significantly modified to operate under UHPLC conditions and greatly improved to give much lower LODs (0.05 vs 0.1 for most compounds). All target compounds are baseline-separated with an excellent linearity (R2>0.99) in the range of 0.05–40 ng/mL. Superior accuracy (91-114%) and precision (RSD=2-19%) were also obtained across the compounds. A high background encountered during the development was successfully controlled by strategic selection of SPE cartridge, column, and solvent adjustment as well as having a thorough cleaning protocol in place

70 **Applications for Microscale Separations of Small Molecules at Genentech**

Crystal Ye, Mengling Wong, Genentech, 1 DNA Way, South San Francisco, CA 94080

With the increasing complexity of small molecule modalities and their syntheses, a need for separations of sub 2 milligram samples become more imminent. The Purification group at Genentech is exploring the use of the 1260 Infinity II Analytical Scale Fraction Collector coupled to a UHPLC-MS system to enable microscale separations for small molecules, peptide-like molecules, and impurity isolation. This workflow allows us to save on solvents (at least 50%) and improve recovery in comparison to a preparative HPLC method.

71 **Cloud-Based Enterprise Solution for Visualization and Reporting of Liquid Chromatography Data**

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

HPLC is a vital technique used from early discovery through commercialization. HPLC data are recorded and stored by the Empower software package, which provides the ability to both record chromatograms and process them. These results are used to verify a chemical synthesis, determine product stability, write regulatory filings, monitor the quality of products, and much more. These results are siloed and need to be manually exported and reformatted by scientists. Furthermore, Empower data lack critical metadata attributes related to the samples they represent, leading to issues in their organization and downstream data analytics and erects barriers to collaboration. Due to these issues, scientists must conduct manual data transcription and metadata tagging. This process is both time-consuming and error prone, yielding mistakes which can lead to potential product recalls and delays. To address these issues, we have developed a cloud-based, enterprise-wide data pipeline for automated transcription and storage of Empower data into a standardized data format that is technique and vendor agnostic. Thereby allowing downstream users and applications to analyze the results both immediately and accurately. These downstream applications include several data visualization, analysis, and reporting dashboards. Our work not only enables more sophisticated data analytic workflows, but it also decreases the amount of time required for routine scientific activities. Example data workflows include analytical method and product life cycle management, stability trend analysis, and cross analysis of experimental results using different techniques.

72 **An Investigation of Robust Sample Preparation on an Automated Tablet Processing Workstation, and Lesson Learned**

Ujala Patel, Merck & Co., Inc., 90 E Scott Ave., Rahway, NJ 07065

Robust sample preparation is an essential consideration for analytical methods, as this enables reliable and reproducible results throughout testing. During the development of highly potent sterile solution for injection formulations, the Tablet Processing Workstation (TPW) automation platform was used for sample preparation due to low volumes and the highly potent API. In several sequences, wide replicate spreads were observed, especially for samples run later in the preparation sequence. Formulation samples (1ml) were transferred into test tubes closed with metal caps to run on a TPW. HPLC analysis of the analytical solutions occasionally found higher assay values for samples prepared at the middle and end of the sequence. Because high assay values can be an indication of solvent evaporation and concentration of the analyte, the TPW process was monitored carefully. Indeed, heating of the samples by the TPW electronics underneath the sample tray caused slow evaporation of the sample solutions, with subsequent condensation of water on the metal caps. This effectively increased the API concentration in the test solutions. An insulating layer of Styrofoam was added below the sample trays to minimize heating, and the metal caps were replaced with plastic, to minimize condensation. A vortexing step was added at the beginning of each preparation so any water droplets would dislodged into the solution prior to dilution. Implementation of these steps resolved the high replicate spread and high assay. These simple, yet highly effective strategies are generally applicable to other programs with low sample volumes that would be sensitive to evaporation.

73 **Higher Resolution and Higher Sensitivity for Solid-State NMR Spectroscopy**

Lyndon Emsley, École Polytechnique Fédérale de Lausanne (EPFL), Institut des Sciences et Ingénierie Chimiques, Lausanne, CH-1015, Switzerland

Limited sensitivity and resolution are the two main bottlenecks to wider application of NMR to solids. To improve resolution, coherent averaging methods are at the heart of modern NMR spectroscopy, and they are used in many applications to selectively modulate particular interaction terms. Examples range from simple spin echoes to remove heteronuclear J-couplings in solution, to rotor-synchronized symmetry-based pulse sequences to remove dipolar couplings under MAS in solids. Coherent averaging schemes are however never perfect. Residuals are always present, and much effort has been put into schemes to make the residuals as small as possible. Here, we suggest that instead of optimizing and perfecting a coherent averaging scheme, we could approach the problem by parametrically mapping the error terms due to imperfect averaging in a k-space representation, in such a way that they can be removed in a multi-dimensional correlation leaving only the desired pure isotropic signal.1We illustrate the approach here by determining pure isotropic 1H spectra from a series of MAS spectra acquired at different spinning rates.

74 **Scaling Analyses of Hyperpolarization Transfer from Paramagnetic Centers in Solid Media**

Nathan Prisco, University of California, Dept. of Chemical Engineering, Santa Barbara, CA 93106, Arthur Pinon, Lyndon Emsley, Brad Chmelka

An energy-conserving constitutive model, with analogies to heat conduction and mass transfer, quantitatively describes the propagation and dissipation of non-Boltzmann spin polarization in diverse heterogeneous solids. The approach provides insights on hyperpolarization processes, including those that occur in dynamic-nuclear-polarization NMR measurements, which lead to enhanced signal sensitivity, and opens opportunities to characterize the compositions and structures of diverse materials, especially near surfaces. In such systems, spin polarization is generated by microwave excitation of unpaired paramagnetic electron spins and transferred via hyperfine interactions to nearby nuclear spins. This produces a locally high level of non-Boltzmann polarization that can be relayed via dipole-dipole interactions over distances ranging from <1 nm to tens of μm, depending on the relative rates of polarization generation, spin diffusion, and spin relaxation in a given medium. Scaling analyses lead to dimensionless parameters that are based solely on measurable or known physical properties and enable the evaluation of the rates at which polarization propagates and dissipates within large coupled spin ensembles. The resulting analytical expressions agree closely with experiment and account quantitatively for transient and steady-state polarization transfer within solids and across interfaces. This enables the determination of near-surface compositions and structures of heterogeneous materials that were previously challenging or infeasible to investigate. The results and approach yield general design criteria for predicting, analyzing, and optimizing polarization transfer in diverse heterogeneous solids for applications in materials science.

75 **Custom-Made Magnetic Resonance: An Application-Driven Instrumentation Approach**

Dimitrios Sakellariou, KU Leuven - cMACS, Celestijnenlaan 200f - Box 2454, Leuven, Vlaams Brabant, 3001 Belgium

Low-field NMR is as old as Magnetic Resonance. Recent advances in magnet design have allowed for bringing this technology to the laboratory bench for routine high-resolution NMR spectroscopy. In parallel to this extrapolation from high-field NMR, many new options became available pushing magnetic resonance closer to the chemical and medical analysis. Miniaturization, low-cost and hyphenation are some of the most straightforward attributes of such transportable technology. Our inspiration for NMR and MRI developments can originate thus directly from relevant problems and associated questions rather than the needs for a versatile and universal technique. Similarly the methodology can be crafted to accompany the tailor-made instrumentation. In this talk I will be presenting recent developments from

our laboratory in custom-made magnetic resonance as an application-driven tool to answer specific questions, focusing on the structure and function of porous materials in petrophysics, in civil and in materials engineering, catalysis and biomedicine.

76 **Statistical Learning in NMR Non-Crystallography**

Philip Grandinetti, Ohio State University, Department of Chemistry, Columbus, OH 43210

Glass is an extraordinarily versatile material and ubiquitous in our society. While most worldwide production focuses on windows and containers, there is a growing demand for improved specialty glasses, which have become vital to an extensive range of technological applications. Despite this importance, a complete theory for the properties of glasses and glass transition remains one of the top unsolved problems in chemical physics. Contributing to this problem is the scarcity of quantitative details about structural order and disorder in glass---yet all of the macroscopic properties of a glass are a direct result of its underlying structure. Often, the structure of a non-crystalline material is likened to that of a liquid that has "locked" into a particular configuration of phase space—one consistent with all observable macroscopic properties. While we have no hope of knowing this configuration, we can aspire to determine various multi-variate probability distribution functions, p(Z), where Z are specific structural features and establish their connections to macroscopic properties. This can be accomplished in two steps: inverting an NMR spectrum of a glass into its underlying NMR tensor parameters distribution, $(\nu) \rightarrow f$ (R), and inverting the NMR tensor parameters distribution into the local structure distribution, f (R) → (Z). The challenge with this approach is that both steps are ill-posed problems. We have been developing statistical learning methods for the model-free linear inversion of NMR spectra of glasses into NMR parameter distributions. In this talk, I present the details of our linear-inversion methods and illustrative applications on experimental spectra of silicate glasses.

77 No abstract submitted by the author.

78 **From Ideas to Globally Accessible Instruments**

Benedict Diederich, Leibniz-IPHT, Albert-Einstein-Str. 9, Jena 07743, Germany

Pandemics, extinction of species and antibiotic crises: These are not dystopian scenarios of the distant future, but current, observable problem situations that people in global ecosystems are now trying to deal with and have to find a way through scientific analysis. Science is responsible here for researching connections on a global level and, by disseminating its findings. However, a high level of technical expertise, as well as high financial investments for laboratory equipment and experiments is often required. What follows from this is a resource-related limited accessibility to the scientific tools that are necessary for researching socially relevant topics. "Frugal Science" thinks of scientific instruments from a whole new perspective. Limited by the available budget and parts, one has to get creative to solve a scientific question posed by society. Following this idea, we have applied this method to the - in many situations - very expensive field of microscopy to provide a deeper insight into the microcosm for everyone, everywhere. With our modular, open-source optics toolbox UC2 (You.See.Too.), we provide not only teaching materials in STEM subjects, but also powerful tools that put optical tools in the hands of those who need them most: Biologists, Chemists, Physicists, Computational scientists and students. We proof its capabilities as a rapid prototyping tool for optics, by rapidly developing a new method to capture large amount of plankton in 3D using a light sheet and a holographic setup. The "HoLiSheet" costs less than the laptop that processes the data and is fully open-source.

79 **PlanktoScope: Affordable Modular Quantitative Imaging Platform for Citizen Oceanography**

Thibaut Pollina, Stanford University, Shriram Center for Bioengineering and Chemical Engineering, Stanford, CA 94305, Adam Larson, Fabien Lombard, Hongquan Li, David Le Guen, Sebatien Colin, Colomban De Vargas, Manu Prakash

The oceans represent 97% of all water on Earth and contain microscopic, drifting life, plankton, which drives global biogeochemical cycles. A major hurdle in assessing marine plankton is the planetary scale of the oceans and the logistical and economic constraints associated with their sampling. This difficulty is reflected in the limited amount of scientifically equipped fleets and affordable equipment. Here we present a modular hardware/software open-source strategy for building a versatile, re-configurable imaging platform - the PlanktoScope - that can be adapted to a number of applications in aquatic biology and ecology. We demonstrate high-throughput quantitative imaging of laboratory and field plankton samples while enabling rapid device reconfiguration to match the evolving needs of the sampler. The presented versions of PlanktoScope are capable of autonomously imaging 1.7 ml per minute with a 2.8 µm/px resolution and can be controlled from any WiFi-enabled device. The PlanktoScope's small size, ease of use, and low cost - under $1000 in parts - enable its deployment for customizable monitoring of laboratory cultures or natural micro-plankton communities. This also paves the way toward consistent and long-term measurement of plankton diversity by an international fleet of citizen vessels at the planetary scale.

80 **Analytical and Operational Challenges in Counterfeit Case Studies**

Ravi Kalyanaraman, Bristol-Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903

Counterfeit drugs are potentially dangerous and serious threats to patients globally. It is estimated that approximately 10 to 20% of the world's pharmaceutical drugs are counterfeited. Even in countries that are generally considered to have a more regulated environment, such as USA, UK and Canada, counterfeit medicines have entered the legitimate supply chain due to increasing international trade and sales via the internet. Due to this infiltration, pharmaceutical manufacturers and health authorities have found ways to authenticate and identify their product by various technologies. These technologies include covert and overt features along with serialization added to the packaging material and authenticating the suspect drug product for these features. One of the effective ways to authenticate and verify the chemical contents in a pharmaceutical product is by using Raman and Near Infrared (NIR) spectroscopy and by obtaining a unique chemical fingerprint of the drug product using these techniques. Using these product fingerprints, for the past 15 years we have shown that it is possible to test a suspect sample and identify if it is a counterfeit and also to authenticate if it is a legitimate product. Solid (tablets, and capsules) and liquid dosage forms (biologics) were analyzed in a non-destructive way with little or no sample preparation. Even though these technologies have evolved and has improved in the past 15 years, there are still some challenges both from analytical and operational perspective. This talk highlights these challenges by providing specific case studies.

81 **Microscopical Analysis Applied to the Detection and Sourcing of Counterfeit Products**

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

Certain key questions are common to counterfeiting investigations. Is a product authentic? Can contravened goods from multiple shipments be associated? Can the origin of goods be attributed to a specific geographic source (geolocation/source attribution/route attribution)? The microscopy and microanalysis of pharmaceutical products, specifically the characterization of the individual components that comprise the drug product and its packaging can be used to answer, or at least place constraints on, such questions. In the drug product, the size, distribution and composition of the API and excipients and the structure of the product (e.g., tablets, films, liquids, and lyophilized product) can provide insights. Trace reaction products may also indicate that a different synthetic reaction pathway was utilized. Drug packaging can be composed of different materials, layer structures, coatings, adhesives, and marking inks. Finally, contaminants in both the product and packaging, which may arise from different ingredients sources or less pristine manufacturing environments can also be used to identify and discriminate counterfeit from authentic products. Ultimately, the specific information and relevant analytical approach applied to a given sample depends on the product, its components, and the specific questions being asked; however, information of this type can be used to confirm authenticity, recognize counterfeit products and intellectual property transgressions, group counterfeit products from different sources, and constrain their geographic origin. This presentation illustrates these applications of microanalysis through examples that span counterfeiting investigations in the pharmaceutical, food, and consumer goods industries, forensic criminal investigations, fine art and high-value collectibles, and intellectual property litigation.

82 **Unsafe Pharmaceuticals: Fake or Counterfeit**

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Unsafe medicines are a fundamental patient safety, public health, and a global security problem. Formidable challenges impede protecting the medication supply chain in the United States and around the world. In this presentation, we will discuss recent advances in materials-based microscale and nanoscale anti-counterfeiting technologies for both drug package labeling and in-drug product labeling. Analytical authentication technologies with emphasis on microscopy, chemical microspectroscopy and nanospectroscopy are considered the most effective methods to identify both active pharmaceutical ingredients and impurities in genuine and counterfeit drugs.

83 **The Role of Instrument Detection Level in the Development of Sustainable Trace Level Methods**

James Stry, FMC Corporation, Stine Research Center, 1090 Elkton Rd., Newark, DE 19711

Regulatory agencies require analytical methods to achieve predetermined limits of quantitation and detection. Method development scientists must work within these constraints to optimize throughput, reproducibility, and accuracy, while simultaneously trying to minimize the environmental impact of their procedure. The ability of

the analytical instrumentation to detect an analyte will dictate the complexity of the method developed. For analytes that are sensitive in the analytical instrument used: rapid, efficient, and sustainable methods can be developed. Conversely, for analytes that are not sensitive in the analytical instrument used: time consuming, complicated, and solvent consuming methods are developed. In this presentation the role of instrument detection level on the method development process will be discussed.

84 **Greening Separation Science**

Christopher J. Welch, ICASE, 410 W. 10th St., Room 1020H, Indianapolis, IN 46202

Chromatography is a powerful enabling technology for chemical research, providing important platforms for both chemical analysis and purification. However, chromatography has long been recognized as a significant contributor to laboratory waste. In this presentation we survey improvements and techniques for 'greening' chromatography, from the use of alternative solvents to new sample preparation techniques and the use of miniaturized columns. Progress and trends in the field are noted and prospects for future developments are discussed

85 **A Rapid Automated Extraction Platform to Assess Drug Product Potency by Online LC**

Stephen Groskreutz, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, Grodon Lambertus

In this work, we present an automated active pharmaceutical ingredient (API) extraction platform with integrated analysis by online liquid chromatography (LC) to bridge the analytical resourcing gap for continuous drug product manufacturing. Development of an API extraction method with online LC represents a significant labor savings in development and an improvement in data quality and confidence, as an extraction constitutes a model-free "absolute" path to measurement of API concentration. The platform consists of an extraction module for repeated "dynamic" pressurized solvent extractions of tablets placed into 2.5-mL extraction vessels. Extractions rely on temperature and pressurized solvents (50:50 0.1% TFA/acetonitrile, 100 bar, 3.0 mL/min) to dissolve the tablet coating (if present), disperse and solubilize material. Upon exiting the vessel, solvent carrying extracted material is sampled every 15 s using a dual loop LC injection valve. The valve allows direct injection of drug product extract onto a short LC column for online monitoring of the extraction process. High frequency sampling results in an "extract-o-gram". Auto-integration of API peaks allows direct calculation of tablet potency. To assess the performance of the assay we conducted a series of experiments with four drug products spanning a wide dosage range: 200 mg ibuprofen, 81 mg aspirin, 12 mg and 1 mg tablets of development materials (DM). Results were encouraging; using a generic set of extraction conditions quantitative recoveries were obtained for ibuprofen (99.3% label claim, 0.5% RSD), aspirin (103.2%, 1.1% RSD), DM1 (98.5%, 1.1% RSD) and coated DM2 (100.8%, 1.3% RSD) and core DM 2 (100.9%, 2.4% RSD) tablets.

86 **Transferring Analytical-Scale LC Separations to Compact Capillary LC Instrumentation**

James Grinias, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028

The use of portable and compact instrumentation has expanded the possibilities of integrating capillary-scale liquid chromatography (LC) techniques into realms typically dominated by analytical-scale methodology. Low-volume detector flow cells and UV-LED light sources allow for improvements in absorbance detection for columns with internal diameters in the 0.1 – 0.3 mm range. Compact single-quad mass spectrometers with integrated vacuum systems also allow for LC-mass spectrometry (MS) measurements when combined with compact capillary LC platforms. Considerations for column selection (in terms of length, internal diameter, particle size, and particle morphology) include pressure limits (both column and instrument), required efficiency for a given separation, and the balance between operating flow rate and the maximum volume that can be delivered from the pumping system in a single method. We have employed compact capillary LC instrumentation in a wide variety of application areas that are discussed. The analysis of pharmaceutical compounds has focused on QA/QC methodology (including impurity monitoring) and strategies for on-line reaction monitoring. Compact LC-MS has also been used for targeted screening of illicit drug compounds towards implementation in point-of-care settings. Common educational demonstrations of food and beverage analysis using LC have also been translated to the compact capillary-scale instrument, with a new capability for outreach activities achieved through the use of remote instrument control over the internet. The common LC methods used in these and other application areas have the potential to be transformed through this technology that is greener and simpler to operate while still providing efficient chromatographic separations.

87 **Functional Biosensors for Infectious Disease**

Ariel Furst, Massachusetts Institute of Technology, 77 Massachusetts Ave. 66-462, Cambridge, MA 02139

Electrochemistry is an exceptionally powerful tool to monitor chemical and biological interactions. By harnessing the inherent activity and function of biomolecules, we have significantly improved the selectivity and sensitivity of electrochemical biosensors. Specifically, by combining electrochemistry with synthetic biology, we have developed a platform to detect biologically-active small-molecule environmental pollutants at sub-micromolar levels. Similarly, by harnessing the inherent metabolic activity of pathogens, we can detect bacteria that cause food- and water-borne illnesses. By combining improved chemistries for biomolecule modification with unique signal amplification strategies, we have successfully detected targets from extremely complex solutions and directly incorporated remediation with detection.

88 **Using Bioelectrocatalysis for Analysis**

Shelley Minteer, University of Utah, 315 S. 1400 E, Salt Lake City, UT 84112

Biocatalysts have been used as electrocatalysts (termed bioelectrocatalysis) for over a century, but mostly with a focus on energy applications. This talk discusses the use of bioelectrocatalysis for analytical applications ranging from biosensing to electroanalytical evaluation of enzyme kinetics and mechanism. It discusses the advantages and disadvantages of bioelectrocatalysis for analytical applications.

89 **Revealing the Heterogeneity in Metal Dissolution Reaction via Colocalized Electrochemical and Structural Imaging**

Hang Ren, University of Texas at Austin, 102 East 24th St., FNT 2.102, Austin, TX 78712

Electrochemical metal dissolution reactions are fundamentally important in battery and corrosion processes. Their kinetics is highly dependent on surface structures and the presence of passive films. In this talk, I present the study on the initiation of metal dissolution reactions on Ag and Ni, representing model systems for oxide-free and oxide-covered metals, respectively. The local dissolution kinetics is voltammetrically mapped via scanning electrochemical cell microscopy (SECCM). Co-localized characterization of crystal orientation reveals slower dissolution on {111} close-packed planes. The local dissolution kinetics on grain boundaries can also be directly measured, which shows a faster dissolution rate on some but not all grain boundaries. The dependence of passive film breakdown on the thickness of the passive film is also revealed, which is obtained from colocalized TOF-SIMS mapping. We demonstrate that correlative electrochemical and structural imaging are powerful tools for studying heterogeneity at complex electrochemical interfaces.

90 No abstract submitted by the author.

91 **Determining the Time Since Deposition of Menstrual Blood Stains Utilizing Raman Spectroscopy**

Alexis Weber, University at Albany, SUNY, 420 Sand Creek Rd., Apt. 420, Albany, NY 12205, Igor Lednev

Blood traces are commonly found at crime scenes and can provide substantial information about the event that occurred, and individuals involved. There are two main types of bloodstains, peripheral blood and menstrual blood. There are several techniques that can be used to discriminate between peripheral and menstrual, but once identified it would be ideal to obtain more information from the sample. Determining the time of crime is an important goal for crime scene investigations, which can be achieved by estimating the time since deposition (TSD) of bloodstains. If crime scenes contain multiple sets of bloodstains, the calculated TSD should allow for the selection of bloodstains relevant to the crime; and therefore, reduce the number of samples which should be collected, documented, and processed. Vibrational spectroscopy paired with chemometrics has shown provide reliable, rapid, and non-destructive methodologies to determine the TSD of bloodstains. However, most of the work conducted thus far have been on peripheral bloodstains. Menstrual blood is also commonly found at bloodstains and can be present as a result of tissue damage from an assault or a natural cause. Determining the TSD of the menstrual bloodstain can help to reconstruct the event and differentiate between stains that are relevant vs those that are extraneous. At a crime scene both peripheral and menstrual bloodstains can be present. To fill this gap in the research, a novel method for the prediction of TSD of menstrual bloodstains, based on Raman spectroscopy was created.

92 **The Role of Micro Spectroscopic Analysis Tools in Industrial Problem Solving**

Jeanette vajki Vass, Auto & Materials, 120 Watson Mill Rd, Landenberg, PA 19350

Detecting, testing, preventing and controlling failure mechanisms are the keys to ensuring reliability of commercial and consumer products. The characterization and identification of micron and submicron size particles is essential for understanding and managing certain manufacturing processes. Supplying quality products with consistent, reliable performance within a competitive price range requires careful planning and the selection of appropriate methods. The objective of this presentation is to demonstrate the immense value of SEM/EDS, FT-IR, Raman and other non-destructive micro-spectroscopic tools. The analysis results will reveal both chemical and structural composition information while providing a valid platform for solving manufacturing problems along with promising quality assurance. My presentation

will classify, compare and contrast both the Advantages and Limitations of the most common micro-scale analytical instruments. The audience will be provided with a convenient instrument selection guide and necessary reasons for having several different instruments on hand in order to generate an accurate and complete analysis report. Through specific case studies, I will demonstrate the challenges that spectroscopists face with the practical application of Micro-Scale Analysis Tools for solving problems involving the characterization of a chemical and structural composition. Furthermore, my talk will reveal how the knowledge of product-specific critical performance parameters is essential for selecting the most appropriate instruments. In conclusion, we will have learned that each instrument has a specific role in the particle identify characterization and that the final analysis result represents a cumulative problem-solving process.

93 **Phenotype Profiling based on Raman Spectroscopy of a Blood Deposit: The Effect of Hormone Replacement Therapy on Sex Determination**

Emily Miller, University of New Haven ,300 Boston Post Rd., West Haven, CT 06516, Alexis Weber, Igor Lednev, Brooke Kammrath

Hormone replacement therapy (HRT) is a common treatment for women taking estrogens or progestogens to alleviate the symptoms of menopause, men taking testosterone to combat the natural decrease in production with aging, and transgender or nonconforming individuals taking hormones to more closely align their secondary sexual characteristics with their gender identity. In the case of transgender hormone therapy, there are two types: masculinizing hormone therapy and feminizing hormone therapy. Meaningful research has been published on the use of Raman spectroscopy for the determination of sex from blood deposits. Differences in the makeup of blood between females and males have been found in plasma, specifically in the coagulation factor FV, α1-antitrypsin, and ß2-macroglobulin. Thus, the ability of Raman spectroscopy combined with chemometrics to differentiate blood samples by biological sex of the donor is seen. Given that HRT introduces exogenous hormones affecting a person's biochemistry, it is important to investigate the effects of HRT on the classification of sex by Raman spectroscopy. This research investigated how chemometric models that have been used for sex classification work when tested with samples from transgender individuals undergoing HRT. Transgender individuals are disproportionately victims of violent crimes; however, they are not often included in scientific research. It is essential to include this underrepresented group to ensure they are represented in all forensic science, especially in research such as this where analytical chemistry is being used to determine the sex of the donor of a blood deposit.

94 **Biophysical Characterization of Advanced Therapeutic Modalities: Antibodies, Nucleic Acids and AAVs**

Yelena Pyatski, BioTools, 17546 BeeLine Hwy, Jupiter, FL 33478, Kimberly Quinn, Maksim Mezhericher, Rina Dukor

Biotherapeutics are the fastest growing and the most challenging area of pharmaceutical industry. New modalities are always emerging, such as bispecifics, ADCs and nucleic based. Adeno-associated viral (AAV) capsids are rapidly emerging vector technology for several novel gene therapy modalities. The slightest change in antibody's Higher Order Structure (HOS), can significantly impact the efficacy and immunogenicity. Hence HOS characterization is one of many Critical Quality Attributes (CQAs) used to identify the structure, purity, functionality, and stability of a potential biotherapeutic candidate. Unpackaged AAV genomes, partial genomes, and genomes packaged into AAV aggregates cannot be assumed to have the full efficacy of intact genomes packaged in a single intact, unaggregated capsid. Hence one of the most pressing CQAs during AAV manufacturing is proper assessment of genome packing efficiency in the capsid. Many techniques on the market are used for monitoring HOS and genome packing efficiency. However, these techniques are expensive, high maintenance and hard to interpret. Vibrational spectroscopy - FTIR, Raman and Raman Optical Activity (ROA) have recently been shown as sensitive and specific. The specificity stems from availability of a large spectral range (compared to such techniques as UV, MMS (IR with QC laser) and enabling detection of all critical functional groups: amides, sidechains, disulfide bonds for peptides/proteins and base stacking, phosphate backbone and other features for nucleic based molecules. In this presentation, we showcase examples of different modalities and the incredible power of FTIR, Raman/ROA for structural characterization of biologics in R&D and QC.

95 No abstract submitted by the author.

96 **Exploring the Contours of A-TEEM Spectroscopy for Food Analysis**

Linda Kidder, HORIBA Scientific Instruments, 3793 Plum Spring Lane, Ellicott City, MD 21042, Adam Gilmore, Cary Davies

A-TEEM is a 3D fluorescence method that incorporates UV/Vis for a 2-in-1 measurement spectroscopies for rapid and cost-effective analysis of food items. Typically, food analysis is dominated by separations approaches, liquid (HPLC) or gas (GC) chromatography alone or couple coupled with mass spectrometry (MS) for enhanced sensitivity. These methods are expensive on a per-measurement basis by the time that solvents, columns, standards, and waste disposal fees are considered. Molecular spectroscopic methods, most commonly near infrared (NIR), but including Fourier transform infrared spectroscopy (FT-IR) and Raman are useful for certain measurements, with low per-measurement costs, easy sample preparation, and rapid measurement time. However, the sensitivity of these techniques is low compared to LC-MS or GC-MS, limiting the types of problems that can be tackled. We will present applications showing robust classification and quantification of low concentration samples in complex matrices, including: quantification of polyphenols in wine; detection of degradation in olive oil; rapid determination of THC well below legal limits in hemp samples; and detection of adulterants in natural products used in nutraceuticals.

97 **Mid-Infrared Solutions for Rapid Sensing of Food Contaminants**

Luis Rodriguez-Saona, The Ohio State University, 110 Parker Food Science Building, 2015 Fyffe Rd, Columbus, OH 43210

Optical technology is rapidly developing, and instruments are available commercially as portable and handheld devices that can be used when it is not practical or economical to use the more sophisticated and costly instruments used in research laboratories. We will present information on the feasibility of portable infrared systems in applications relevant to the food industry. We have evaluated the performance against benchtop systems directed at developing fingerprinting strategies towards providing reliable tools for assessment of safety. Food applications have been targeted on detection of chemical food contaminants through development of spectral signature profiles permitting the chemically authentication of raw materials. This technology can enable the food manufacturer for real-time and field-based measurements to control the raw material stream, addressing safety and brand equity. Portable infrared technology could save time and money to the food industry helping to implement risk management systems.

98 **Raman Spectroscopy for Food Applications**

Zili Gao, University of Massachusetts Amherst, 102 Holdsworth Way, Amherst, MA, 01003, Lili He

Raman spectroscopy, owing to its advantages of providing chemical signature of a sample analyte in a quick and non-destructively way, as well as the capability of integrating with nanotechnology for enhanced performance (i.e. surface-enhanced Raman spectroscopy, SERS), has been applied for a variety of applications for food analysis, including the analysis of food components, additives, and contaminants. In this presentation, the author will talk about using Raman spectroscopy for differentiating antioxidant isomers, quantifying mixture components, characterizing chemical responses to thermal processing, and measuring the chemical distribution in encapsulation. In addition, the applications of two innovative SERS substrates, nanoparticle mirror and SERS needle will be presented. The nanoparticle mirror substrate can be used as a coating for surface pesticide analysis. The SERS needle can be used for volatile spoilage marker detection in package headspace, multiphase detection, and minimum invasive analysis.

99 **Application of a Software-Assisted Mass Spectrometry (MS) Data Interpretation Workflow for Cyclic Peptides**

Jiaxuan Yan, Merck & Co., Inc., 126 E. Lincoln Ave, Rahway, NJ 07065, Xing Yin, Wendy Zhong, Douglas Richardson, Hillary Schuessler

Cyclic peptides represent a major class of chemically and biologically diverse compounds. In recent years, synthetic and semi-synthetic cyclic peptides have drawn attention in the pharmaceutical industry because of their drug-like properties and huge potential as drug candidates1. Mass spectrometry (MS) and tandem MS fragmentation (MS/MS) based analytical techniques have been widely used in linear peptide analysis; however, the structural complexity for cyclic peptides brings extreme challenges for MS/MS analysis since the fragmentation of cyclic structures can be extremely complicated. Extensive efforts will be needed to analyze the fragments and understand the fragment structures thoroughly. Therefore, a workflow was developed that uses software for spectra deconvolution and fragments assignment to overcome the aforementioned challenges. Daptomycin was studied as a model compound and analyzed under different MS/MS fragmentation modes (higher energy collision dissociation (HCD) MS/MS, electron induced dissociation (EID) MS/MS etc.), resulting in different bond cleavages and different fragmentation patterns. This new workflow can enable us to identify diagnostic ions and fingerprint fragmentation patterns under different MS/MS fragmentation modes rapidly; allows us to save time on extremely time-consuming manual MS/MS data interpretation; and owns great potential to be utilized in concurrent fast-paced drug development and analysis.

100 **2-Pyridine Carboxaldehyde for Semi-Automated Soft Spot Identification in Cyclic Peptides**

Joe Cannon, Bristol-Myers Squibb, Department of Metabolism and Pharmacokinetics, Princeton, NJ 08648, Haiying Zhang, Zhigang Lyu, Silvi Chacko

Cyclic peptides are an attractive option as therapeutics due to their ability to disrupt crucial protein-protein interactions and their flexibility in display type screening strategies, but they come with their own bioanalytical challenges in metabolite identification. Initial amide hydrolysis of a cyclic peptide results in a ring opening event in which the sequence is linearized. Unfortunately, the mass of the singly hydrolyzed sequence is the same (M+18.0106 Da) irrespective of the initial site of hydrolysis, or soft spot. Soft spot identification at this point typically requires time consuming manual interpretation of the tandem mass spectra resulting in a substantial bottleneck in the hit to lead process. To overcome this, derivatization using 2-pyridine carboxaldehyde, which shows high selectivity for the alpha amine on the N-terminus, was employed. This strategy results in moderate to high efficiency derivatization with a unique mass tag and diagnostic ions that serve to highlight the first amino acid in the newly linearized peptide. The derivatization method and analytical strategy are demonstrated on a whole cell lysate digest, and the soft spot identification strategy is shown with two commercially available cyclic peptides, JB1 and Somatostatin. Effective utilization of the automated sample preparation and interpretation of the resulting spectra shown here will serve to reduce the hit-to-lead time for generating promising proteolytically stable peptide candidates.

101 **Host Cell Protein Characterization Methodology and Use within Downstream Process Development**

Stephanie Lehman, GlaxoSmithKline, 1250 South Collegeville Rd., Collegeville, PA 19426, Josue Baeza

The purpose of downstream process development is to purify monoclonal antibodies from a complex mixture, resulting in a highly purified therapeutic antibody. However, there are host cell derived proteins that can remain in the final drug substance which are referred to as host cell proteins (HCPs). HCPs in the drug product can have deleterious side effects on the patient, such as unintended immunogenic consequences, or on the drug product, such as protein clipping and disulfide bond scrambling. Identification of individual HCPs is valuable tool towards understanding problems as they arise and is only performed with mass spectrometry. However, mass spectrometry is limited by the ability to measure samples with 4-5 orders of magnitude. HCPs are often over 6 orders of magnitude less abundant than the monoclonal antibody (mAb). To improve detection of low-level HCPs, an optimized “native” digestion method is employed along with orthogonal high pH separation. The EvoSep HPLC is used for nanoflow separation and a Thermo Eclipse for detection. Additionally, a data independent acquisition (DIA) workflow is utilized as needed. This talk will present a review of our current method for HCP identification and quantitation, characterization of the sensitivity of the method, and provide strategies for collaborations with downstream process development teams. Additionally, a case study of HCP characterization during downstream process development is presented.

102 **Two-Dimensional Liquid Chromatography-Mass Spectrometry (2DLC-MS) for Simultaneous Multi-Attribute Characterization of Adeno-Associated Viruses**

Zhijie Wu, Regeneron Pharmaceuticals, Inc., 777 Old Saw Mill River Rd., Tarrytown, NY 10591, Hongxia Wang, Andrew Tustian, Haibo Qiu, Ning Li

Adeno-associated virus (AAV) is a non-enveloped, single-stranded DNA virus, and has emerged as an attractive class of therapeutic agents to deliver genetic materials to host gene therapy applications such as gene editing, replacement, addition, and silencing. The use of AAVs has several advantages including its ability to transduce a wide range of species and tissue in vivo, low risk of immunotoxicity, and mild innate and adaptive immune responses. As AAV is a new and complex therapeutic modality, novel analytical methods for product testing and characterization are needed. Here, we developed a two-dimensional liquid chromatography-mass spectrometry (2DLC-MS) platform for AAV characterization, which can simultaneously monitor multiple critical quality attributes. In the first dimension, high-resolution anion-exchange chromatography (AEX) was utilized to separate empty and full capsids and measure the relative percentage of each component. In the second dimension, reversed-phase liquid chromatography coupled with mass spectrometry (RPLC-MS) was employed to separate and characterize viral proteins. Using this technique, the peaks of interest shown in the first-dimensional AEX chromatogram were selected. The viral capsids in the peak of interested were subjected to online AAV capsid denaturation and removal of MS-incompatible salt, followed by protein separation on second-dimensional RPLC and characterization by MS. Characterization of an AAV8 sample using the 2DLC-MS method revealed different viral protein 2 (VP2) phosphorylation levels among different peaks shown in the AEX chromatogram. In summary, this 2DLC-MS method allows for high-throughput and multi-attribute AAV characterization in a single run, with minimal sample handling required for different AAV serotypes.

103 **Forensic Capabilities for US Trade Enforcement at the USDHS Customs and Border Protection’s New York Laboratory**

Adam Hutter, DHS/CBP, 1100 Raymond Blvd., Ste. 550, Newark, NJ 07102

The presentation will provide an overview of the forensic chemistry capabilities of the Department of Homeland Security, Customs and Border Protection (CBP), Laboratories and Scientific Services, New York Laboratory, located in Newark, NJ. The New York Laboratory provides scientific and forensic testing that helps CBP enforce trade and narcotics laws, detect and intercept Weapons of Mass Destruction and other hazardous materials, and protect Intellectual Property Rights. Scientific services include the mechanical and/or chemical analyses of full range of commodities from inorganics, organic chemicals and products, food, petroleum, textiles, footwear, raw sugar, polymers, plastics, and paper. Laboratory chemists perform analyses for Proper identification and tariff classification, Anti-Dumping/Countervailing Duty enforcement, Country of origin/trans-shipment circumvention, Admissibility/Import safety, Intellectual Property Rights, Petroleum Program, Quota scope determinations. The New York Laboratory specializes in National Import Specialist rulings, food, import safety, and non-human DNA (e.g., seafood, enumerated woods, and natural textile fibers).

104 **Pain Biosensors in Forensic Identification of Physical Trauma**

Omowunmi Sadik, BioSensor Materials for Advanced Research & Technology (BioSMART Center), Department of Chemistry & Environmental Science, New Jersey Institute of Technology, 161 Warren St., University Heights, Newark, NJ 07102, Gaddi Eshun, Christopher Henni, J Schaffer, Walker Land

This research seeks to develop and validate arrays of biosensors for biomarkers of pain, pleasure, and stress (P2S) that could aid forensic or medicolegal investigators to uncover physical trauma. We have previously developed and validated biosensors with machine learning for Cyclooxygenase-2 (COX)-2) and inducible nitrous oxide synthase (iNOS) as biomarkers for an objective measure of pain level in ~ 800 patients. Preliminary results from our laboratory indicated that Contactin-1 (CNTN-1) could also be a promising pain biomarker. Previous studies pointed to CNTN-1 as a pain suppressor and found antibodies against CNTN-1 in chronic inflammatory demyelinating polyradiculoneuropathy patients. Conventional methods of wound documentation are superficial and qualitative, employing cameras and diagrams, thus highlighting the need for a more in-depth analysis that uses empirical and quantitative evidence. Analogous to STRs kits, where the combination of twenty or more markers objectively identifies a person, utilizing an array of biosensors with machine learning could provide a complementary tool for forensic examiners/forensic nurses to confirm or refute physical or sexual abuse allegations. When used in conjunction with standard protocols, self-report, photographic documentation of victim’s physical trauma, and other forensic evidence, the biomarker P2S levels could be used to corroborate victim testimony objectively.

105 **Illicit Drugs: A Guide for Analysis**

Kristi Bartok, Union County Prosecutor’s Office Forensic Laboratory, 400 North Ave., East Westfield, NJ 07090

The analysis of illicit drugs presents many challenges in today’s forensic laboratories. Not only does the modern laboratory need to be able to identify the dozens of “classical” illicit drugs, but also the myriad of novel psychoactive substances (NPS) that are constantly emerging as a way to produce a “legal” high. A minimum amount of testing is required to be performed in order to definitively identify a controlled substance. These requirements have been put in place by the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) as a way to provide consistency among forensic laboratories that analyze seized drugs. SWGDRUG has placed analytical techniques into three separate categories based on their ability to provide selective information about a molecule based on its class information, chemical or physical characteristics, or structural information. By performing these analytical techniques in order from least to most selective, an analyst can gain important preliminary information on the identity of the molecule before confirming with a more selective technique.

106 **Quantitation of Protein Deamidation Degradation by Coulometric Mass Spectrometry (CMS) and Its Potential Application for Determining Post-Mortem Interval (PMI)**

Hao Chen, New Jersey Institute of Technology, Department of Chemistry and Environmental Science, 161 Warren St., Tiernan Hall, 1st Floor, Rm. 151, Newark, NJ 07102

Proteomic absolute quantitation strategies mainly rely on the use of synthetic stable isotope-labeled peptides or proteins as internal standards, which are highly costly and time-consuming to synthesize. To circumvent this limitation, our laboratory recently developed a coulometric mass spectrometric (CMS) approach for absolute quantitation of proteins without the use of standards, based on the electrochemical oxidation of oxidizable surrogate peptides followed by mass spectrometric measurement of the peptide oxidation yield. Previously, CMS was only applied for single

protein quantitation. In this study, taking one step further, this study conducted the unprecedented quantitative analysis of deamidated peptide products arising from mAb heavy chain deamidation reaction. In particular, the deamidation succinimide intermediate which had not been measured before due to lack of standard was quantified by CMS, for the first time. This preliminary result suggests that CMS could be a method for determining protein deamidation with application for determining post-mortem interval (PMI) in forensics.

107 **Making Progress with Social Justice**

Raychelle Burks, American University, 4400 Massachusetts Ave. NW, Washington, DC 20016

While working for social justice and designing sensing systems may seem unrelated endeavors, they share a common core. Doing better. In this talk, Dr. Burks how equity guides her analytical work in forensic science.

108 **The Dark Side of Science: Misconduct in Research**

Elizabeth Biks, Harbers Bik LLC, 648 Sheraton Dr., Sunnyvale, CA 94087

Science builds upon science. Even after peer-review and publication, science papers could still contain images or other data of concern. If not addressed post-publication, papers containing incorrect or even falsified data could lead to wasted time and money spent by other researchers trying to reproduce those results. Several high-profile science misconduct cases have been described, but many more cases remain undetected. Elisabeth Bik is an image forensics detective who left her paid job in industry to search for and report biomedical articles that contain errors or data of concern. She has done a systematic scan of 20,000 papers in 40 journals and found that about 4% of these contained inappropriately duplicated images. In her talk she will present her work and show several types of inappropriately duplicated images and other examples of research misconduct. In addition, she will show how to report scientific papers of concern, and how journals and institutions handle such allegations.

109 **Spectrometers in Wonderland: Shrinking, Shrinking, Shrinking**

Richard Crocombe, Crocombe Spectroscopic Consulting, 30 Thornberry Rd., Winchester, MA 01890

This paper will give a brief overview of the major portable spectroscopic techniques: those based on optical spectroscopy techniques, including near-infrared (NIR), mid-infrared (MIR), and Raman; mass spectrometry (MS) systems, including high-pressure MS (HPMS), gas chromatography-MS (GC-MS), ion mobility spectrometry (IMS); and elemental techniques, including X-ray fluorescence (XRF) and laser-induced breakdown spectroscopy (LIBS); and emerging miniaturized techniques like nuclear magnetic resonance (NMR). The above are all "conventional" spectroscopic techniques, reduced to a rugged portable format, and with self-contained data systems. They provide specific, actionable, information to their non-scientist operators working with them outside the laboratory-in the field-and these instruments have well-defined value propositions. A recent development is the availability of very low cost (< $100) multispectral sensors operating in the visible and NIR regions. This low cost enables them to be embedded into consumer products, for instance, smart "white goods" appliances, personal care, fitness products, and even "wearables." An amazing amount of optical technology is now packed into smart watches, and even ring-sized wearables, and we are only just starting to see the possibilities of exploiting all the data these device produce. In the future, miniature and portable spectrometers will be ubiquitous-outside the laboratory, and in your home and pocket, and even in your toilet (!)

110 **Safety and Security Dependence on Vibrational Spectroscopy**

Pauline Leary, NOBLE, 1934 Bulls Head Rd., Stanfordville, NY 12581

Portable spectrometers revolutionized the safety and security industries. These systems enable identifications of threats in real time to protect the public and remediate scenes where exposure to them has occurred. The breadth of portable spectrometers available range in complexity from simplified devices like pH paper to sophisticated systems like gas chromatograph-mass spectrometers. Simplified devices are valuable because they are inexpensive and easy to deploy: Their challenge is that results may only classify a sample and/or provide a presumptive result. The benefit of the more sophisticated systems is that they enable selective identification of target threats and provide a high level of confidence in the result: Their challenge is that they tend to be more expensive and difficult to operate and maintain in the field. Infrared and Raman systems lie in the middle of this range and are especially valuable because they reconcile the typical benefits of systems on either end in that they are easy to operate and maintain, but also provide selective results that the operator can be confident in reporting. They were among the first portable spectrometers deployed after 9/11 to address the challenges faced by safety and security professionals. Initially used to respond to white-powder calls, they have continually adapted to meet the ever-evolving detection challenges faced by the community and have become an integral part of most response protocols. The history of these deployed systems as well as the current state of their designs and capabilities will be presented.

111 **Advancing the On-Scene Detection and Identification of Illicit Drugs with Portable Technologies**

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

Portable spectrometers present a powerful tool for combatting the illegal drug trade. Both in the field and in the laboratory, portable spectrometers provide rapid and reliable analysis of suspected illicit drugs while creating a reviewable record which are necessary for adjudications. The complexity of the modern illicit drug trade, and specifically the range of components and concentrations, means that a tool box approach is optimal. It is critical to use the right tool, for the right job, in the right way. Given the diversity of portable spectrometers available for the on-scene detection of illicit drugs, it is important to understand the limitations and advantages of each instrument in order to maximize their use for successful forensic investigations involving illicit drugs.

112 No abstract submitted by the author.

113 **Global Protein-Turnover Quantification in Escherichia coli Reveals Cytoplasmic Recycling under Nitrogen-Limitation**

Martin Wuhr, Princeton University, Department of Molecular Biology, Princeton, NJ 08540

Protein turnover is a critical regulatory mechanism for proteostasis. However, proteome-wide turnover quantification is technically challenging and, even in the well-studied E. coli model, reliable measurements remain scarce. Here, we quantify the degradation of ~3.2k E. coli proteins under 12 conditions by combining heavy isotope labeling with complement reporter ion quantification and find that cytoplasmic proteins are recycled when nitrogen is limited. Furthermore, we show that protein degradation rates are generally independent of cell division rates, and we used knockout experiments to assign substrates to the known ATP-dependent proteases. Surprisingly, we find that none are responsible for the observed cytoplasmic protein degradation in nitrogen limitation, suggesting that a major proteolysis pathway in E. coli remains to be discovered. Thus, we introduce broadly applicable technology for protein turnover measurements. We provide a rich resource for protein half-lives and protease substrates in E. coli, complementary to genomics data, that will allow researchers to decipher the control of proteostasis.

114 **Proteomics Analysis Combined with Pulsed-Metabolic Labeling Reveals New Targets and Mechanisms of Host Protein Degradation Mediated by Herpes Simplex Virus Type 1**

Katarzyna Kulej, The Children's Hospital of Philadelphia Research Institute, 4200 Colket Translational Research Building, 3401 Civic Center Blvd., Philadelphia, PA 19104, Matthew Charman, Joseph M. Dybas, Namrata Kumar, Edwin Halko, Simone Sidoli, Benjamin A. Garcia, Matthew D. Weitzman

Herpes simplex virus-1 (HSV-1) causes contagious and persistent infections that affect about 60% of the human population. Our previous work demonstrated that HSV-1 infection leads to changes in histone marks of host chromatin and alters the host cell proteome. We aimed to define the viral activities that mediate selective host proteins and histone modification alterations. Here we have developed a pulsed-metabolic labeling approach based on mass spectrometry and proteomics to determine how HSV-1 lytic infection induces degradation of host targets across the total cellular proteome. Analysis of histone post-translational modifications in infected cells revealed that host chromatin is enriched in histone marks of decondensed heterochromatin after infection. To discriminate between protein degradation versus arrested gene expression, we compared protein abundance with labeling rate. Proteins reduced in abundance, but which maintained incorporation of heavy amino acids during infection, were classified as degraded rather than no longer translated. Importantly, this protein group included all known degraded targets of the viral ubiquitin ligase ICP0. Interestingly, we did not observe reduced abundance of histone deacetylases (HDACs) despite a significant increase in histone acetylation. Therefore, we performed phosphoproteomics analysis to identify regulation of chromatin via phosphorylation and inactivation of chromatin modifying enzymes. Our analysis detected a significant enrichment of phosphorylation on HDAC1 and HDAC2, which are known to be associated with their inhibition or subcellular localization. Our data demonstrate that combining pulsed-metabolic labeling of cellular proteome together with phospho-proteomics and analysis of histone marks reveals multiple levels of host protein regulation during HSV-1 infection.

115 **Quantitative Mass Spectrometry for Understanding Chromatin Mutations in Human Disease**

Benjamin Garcia, Washington University, 660 S. Euclid Ave., St. Louis, MO 63110

Histone are small basic proteins that regulate gene expression through the use of post-translational modifications (PTMs) or variant proteins. Histone H3.3 is one such specialized histone variant that has been associated with active gene expression and can be incorporated into chromatin at any given moment. Recently, mutations to histone H3.3 have been found in some human diseases, especially pediatric gli-

omas. Therefore, understand the role of histone H3.3 in human health and disease is of great importance. Here I will describe our latest efforts to develop mass spectrometry based proteomics for characterization of histone PTMs and variants. These methods are crucial for detection of modifications on histone H3.3. The application of these methods will be described to study histone H3.3 during embryonic stem cell differentiation, and also in a new neurodevelopmental disorder found in children which cause speech delays, seizures and a host of other neurological disorders.

116 **A New Perspective for Aging Research: The Proteome that Decorates Reactivated Heterochromatin**

Simone Sidoli, Albert Einstein College of Medicine, 1300 Morris Park Ave., Ullmann 405, Bronx, NY 10461

Chromatin state drives DNA readout, including gene expression and DNA repair. Chromatin state and dynamics is modulated by chromatin interacting proteins, in particular histones and their post-translational modifications (PTMs). Histones are rarely modified by a single PTM, but they are rather decorated by at least 3-5 co-occurring ones. For this reason, the equation one PTM = one function is insufficient to describe the fine tuning of chromatin regulation. Our lab investigates how anomalous combinations of histone PTMs decorate the chromatin of stressed cells. We focus on aging models to understand the fundamentals of phenotype frailty that potentially makes us more susceptible to disease development. Aging is characterized by an overall increase in genomic instability and chromatin decondensation, so we focus on loci that we labeled "reactivated heterochromatin." These are chromatin domains undergoing anomalous decondensation, which are decorated by histones co-modified by PTMs benchmarking both condensed heterochromatin and active transcription. We optimized our mass spectrometry methods on 3D cell models, and then applied them on B-cells retrieved from Ashkenazi Jews donors. This cohort includes offsprings of exceptional longevity (OPEL), i.e., individuals in their 70s with projected longer lifespan due to their centenarian ancestors. Preliminary data on 60 individuals revealed the enrichment of unusual histone modifications in the OPEL group, the group we utilize as indicator of projected longer lifespan. We have then applied biochemistry techniques to identify the role of these unusual modifications on chromatin readout. Together, our work aims to understand the role in longevity of unique chromatin markers.

117 **D8-THC Distillates Analysis Using High Resolution and Ion Mobility Mass Spectrometry**

Douglas Stevens, Waters Corporation, 34 Maple St., Milford, MA 01757, Marian Twohig, Andrew Baker, Andrew Aubin, Christopher Hudalla

Though delta-9-THC is the main pharmacologically active component in cannabis, its psychoactive isomer, delta-8 THC, naturally occurs in the cannabis plant at low levels. Bulk delta 8-THC is often produced from hemp-derived CBD. The conversion of CBD to delta-8 THC requires harsh conditions leading to multiple reaction by-products which need to be characterized to enhance understanding of the chemical components produced. Distillates were diluted in acetonitrile and analyzed using ESI+ LC/MS with a high-resolution quadrupole time-of-flight mass spectrometer (QToF MS). Additional work was performed on a QTof-ion mobility hybrid MS. A C18 column, 2.1 x 100 mm, 1.6 µm at 250 C was used to separate cannabinoids using isocratic elution and a mobile phase of 0.1% formic acid in water and acetonitrile. Purity of samples measured by UV ranged from 79.0%-93.6%. Several known cannabinoids were identified based on RT and UV spectra, including delta-8 THC, delta-9 THC and exo-THC. However, unidentified peaks were also detected in the UV data. In the subsequent HRMS analysis, the software highlighted m/z 315.23186 as the base peak for several unknowns with proposed elemental compositions of C21H30O2. Fragmentation data suggests the components share structural characteristics with the C21 neutral cannabinoids including delta-9 THC. Ion mobility spectrometry, which separates species on the basis of size, shape, and charge suggests that these species are additional isomeric forms of the C21 neutral cannabinoids. A chlorinated compound, with a proposed elemental composition of C21H31ClO2 and common fragments with delta-9 THC and its isomers, was also observed.

118 **Case Studies where Regulations Drive Cannabis- Laboratory Failure**

Susan Audino, S.Audino & Assoc. LLC, 408 Canal Way W., Bethany Beach, DE 19930

Consumers rely on regulations to ensure all products on store shelves meet certain safety and information specifications. Imagine if prescription medicines were improperly labeled, or if consumers were not informed of pet food or human food contamination. Regulations exist for the benefit of all consumers. Cannabis regulations are promulgated as a result of legislation that require states to provide oversight of products designated for medicinal and/or adult consumers as a way to ensure consumer safety. Unfortunately, the implementation of these regulations prompt businesses to operate in a gray-zone where requirements are easily over-looked, exploited, or even ignored. Laboratory testing is often to blame for products that fail consumer expectations. This session will provide insight and case-studies where regulations provide the foundation for laboratories to fail in business, fail in their provision of effective testing services, and fail in meeting the very basis of consumer safety from which the regulations were established.

119 **Compliance Testing of *Cannabis sativa L.* for Delta-9 THC and CBD Using Gas Chromatography with Flame Ionization Detection and Liquid Chromatography with UV detection**

Anuja Bharadwaj, The Connecticut Agricultural Experiment Station, 123 Huntington St., New Haven, CT 06511, Terri Arsenault

The Agricultural Improvement Act passed in December of 2019 legalized the growing of Cannabis sativa with <0.3% total delta-9 THC at the federal level. In addition, many states have legalized high THC Cannabis sativa varieties for medical and/or adult-use. The changing regulatory landscape has necessitated testing of Cannabis sativa for cannabinoids. In addition, states may require testing for pesticides, heavy metals, terpenoids, mycotoxins, etc. Laboratories are working to meet these regulatory requirements, but there is a lack of reference materials, proficiency testing, and standard procedures. Our laboratory began testing for total delta-9 THC in 2020 to meet the regulatory requirements for hemp production. In 2021 our laboratory began developing methods for testing marijuana products to meet the requirements of the medical and adult-use marijuana programs. This presentation will compare gas chromatography with flame ionization detection to liquid chromatography with ultraviolet detection for the analysis of cannabinoids. In terms of hemp, testing assures that plant material is below 0.3% total delta-9 THC to meet the legal definition. In terms of medical marijuana, the testing of numerous matrices such as oils, slips, pills, etc., assures consistent dosing of cannabinoids. For adult-use marijuana, testing ensures products are free from contamination and cannabinoids match the label claims. The data presented will compare the two methods of analysis.

120 **Expanding the PROVEDIt Set with Next Generation Sequencing Data: Supporting Foundational Forensic Research Initiatives**

Ami Reader, Rutgers University-Camden, 116 Stanley Ave., Bellmawr, NJ 08031, Jessica Dominguez Lopez, Catherine Grgicak

Forensically relevant Next Generation Sequencing (NGS) pipelines have the potential to supersede traditional capillary electrophoresis (CE) methods. Though NGS has clear genealogical and phenotyping applications, forensic identification with NGS requires additional research to demonstrate that any genetic resolution gained by sequencing short tandem repeats (STRs) is not diminished by potential decreases in analytical sensitivities. In addition, with the forensic DNA domain transitioning from manual to probabilistic genotyping methods, adaptable models and algorithms need development. To assist with these endeavors, we are preparing for the expansion of PROVEDIt database with an additional 223 NGS profiles – ranging in template mass from 1 to 0.0063 ng – prepared over the course of 2 years and run on an Ion GeneStudio S5 after library preparation with the Precision ID GlobalFiler NGS STR Panel v2. We first focus on publishing data that are of single source and from 40 distinct genotypes. By varying the load concentrations [pM], amplification cycle numbers and the number of samples loaded onto a chip, a total of 11 NGS laboratory treatments are represented. We report on the relationships between allele coverage, coverage ratios, allele drop-out, and treatment therein highlighting the value of an open database to the forensic community. This work is supported by NIJ2018-DN-BX-K0185 and NIST-60NANB19D140 awarded by the National Institute of Justice, Office of Justice Programs. The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not reflect those of the Departments of Justice or Commerce.

121 **Optimization of Fellatio Sample Analysis**

Brianna Gregory, Cedar Crest College, 68 Water Lane, N Levittown, NY 11756, Janine Kishbaugh

The presented research establishes a way to maximize results of orals swabs from sexual assault casework by determining best practices for sample collection and analysis. Fellatio, commonly performed during sexual assault, was investigated to determine if male DNA can persist in a female's mouth to obtain DNA results up to 24 hours later. Three oral collection areas were sampled using both cotton and nylon flocked swabs to determine which yields better results. Additionally, different time intervals up to 24 hours were tested. Participants were asked to record activities including eating, drinking, and acts of oral hygiene to help interpret the results. DNA extraction containing DTT was performed, followed by PCR amplification with Y-STR primers. The amplicons were analyzed on a 3130 genetic analyzer. Full male DNA profiles were found at each time interval. An increase in observed alleles was obtained by increasing the electrophoresis injection time to 15 seconds. When looking at the 144 swabs after Post PCR purification was performed, 57.6% of total swabs had a full profile. Determination of successful sample collection methods, including swab type and sampling areas, could improve sexual assault investigation.

122 **Development and Validation of a GC-QQQ Method for Smokeless Powder Additives**

Blake Kerstetter, West Chester University of Pennsylvania, 156 Middle Creek Road, Selinsgrove, PA 17870, Monica Joshi

Smokeless powders are low explosives that are used as propellants in firearm ammunition. They are of forensic interest within the analysis of gunshot residue (GSR) as a form of trace evidence. Presence of GSR indicates that a person handled a firearm, was in the vicinity of a firearm discharge event, or was in contact with an object or person containing gunshot residue. The organic gunshot residue (OGSR) component of GSR requires sensitive methods for its detection due to the volatility of smokeless powder additives. Analysis of OGSR is not routine practice in forensic laboratories and the forensic community is making efforts to encourage it as a supplemental technique to the analysis of inorganic GSR. The trace levels of OGSR found on shooters hands and the lack of sensitivity of traditional GC-MS techniques has deterred the use of this technique. In this presentation, we will discuss the use of a triple quadrupole (QQQ) mass spectrometer to increase sensitivity, thereby allowing for trace analysis of smokeless powders. The complete method development and validation process for a GC triple quadrupole mass spectrometer (GC-QQQ) will be discussed. The presentation will demonstrate the ability of the method to detect trace levels of 15 smokeless powder additives such as nitroglycerin and nitrodiphenylamine. The method developed with chemical reference standards was verified with smokeless powder extracts, post combustion extracts, and hand swabs of shooters. Attendees of this presentation will learn about the method development and validation process for GC-QQQ instruments with smokeless powders as the target analyte.

123 **Method Development and Validation for the Determination of Fentanyl and Fentanyl-Related Compounds on United States Paper Currency by LC-QQQ-MS**

Matthew Hewes, Thomas Jefferson University & NMS Labs, 2300 Stratford Ave., Willow Grove, PA 19090, Alex Krotulski, Donna Papsun, Barry Logan

Previous research evaluated the extent to which cocaine has been detected on currency in the United States. The literature was in agreement that the majority of money analyzed was contaminated with cocaine. This study set out to replicate that research with fentanyl given its increasing prevalence in forensic casework over the past decade. A quantitative liquid chromatography triple quadrupole mass spectrometry (LC-QQQ-MS) method was developed and validated for this purpose with the ability to detect six analytes: fentanyl, 4-ANPP, acetylfentanyl, benzylfentanyl, cocaine, and methamphetamine. Sample preparation involved soaking the bills in a solvent followed by performing a liquid-liquid extraction. Optimal chromatography was achieved using a C18 stationary phase and 5mM pH 3 ammonium formate in water and 0.1% formic acid in acetonitrile mobile phases. The working range for this method was 1 ng/mL to 100 ng/mL (or 0.1 µg to 1.0 µg per bill). Samples used for this research were one-dollar bills collected from thirteen cities throughout the United States. Fentanyl was found in a majority (63%) of samples, with 61% of samples having at least 0.1 µg of fentanyl and 4% having at least 1.0 µg of fentanyl. Cocaine and methamphetamine were found in virtually all samples, typically in amounts greater than 1.0 µg per bill. The remaining three analytes were detected only a handful of times and in trace amounts, exclusively in the presence of fentanyl. Areas of the country with a higher incidence of fentanyl use yielded a higher frequency and magnitude of contaminated bills.

124 **Sustainable Analytical Methodology for Residual Dextran Sulfate in Biopharmaceutical In-Process Samples by UV-Vis Spectrophotometry**

Lee Oliver, GlaxoSmithKline, 709 Swedeland Rd., King of Prussia, PA 19406

The biopharmaceutical manufacturing process is comprised of a series of upstream protein expression and fermentation and downstream protein purification and polishing steps. Chemical residuals, a diverse class of compounds that are intentionally introduced or produced as byproducts during upstream process development, benefit the process to increase protein production efficiency. Due to the inherent toxicity of many chemical residuals, clearance via downstream purification is essential to ensure patient safety. One such residual, dextran sulfate, functions to inhibit mammalian cell aggregation during large molecule protein expression. This paper will highlight two analytical methods developed utilizing sample derivatization with UV-Vis detection. The SoloVPE platform, often used in the biopharmaceutical space to quantitate protein concentration, utilizes the Beer-Lambert Law to calculate absorbance values. The Unchained Labs Lunatic platform performs UV/Vis spectral analysis utilizing microfluidic circuits molded onto 96-well plates. To reduce waste and promote sustainability, the initially developed SoloVPE method was transferred to the Lunatic platform. This transition significantly improved throughput while reducing reagents consumed and analysis time from ~4 minutes/single reading to ~5 minutes/96-well plate. Methods were verified and qualified, permitting method transfer to quality control laboratories for routine testing.

125 **Modernized Impurity Analysis of the Kinase Inhibitor Imatinib by High-Resolution LC with MS-Compatible Mobile Phases**

Peng Chen, Waters Corporation, 34 Maple St., Milford, MA 01757, Bonnie Alden, Matthew Lauber

A new pipeline of cancer-fighting drug candidates has emerged, many of which take aim at kinase inhibitors as their druggable target. These APIs and their impurities are constructed with heteroatoms and conjugated substituents that mimic amino acid residues. Their chemical diversity requires high-resolution impurity analyses. A UPLC method has been developed with a charged surface phenyl stationary phase for the separation of Imatinib and nine of its related impurities, seven of which are documented in the EP monograph. Imatinib was the first kinase inhibitor approved by the FDA in 2001. The European Pharmacopeia (EP) has issued a monograph HPLC method for its analysis as well as a set of its related impurities. This EP method uses three different HPLC conditions and two different HPLC columns to separate Imatinib from seven of its impurities. Additionally, the EP method calls for a non-volatile ion-pairing agent (sodium octane sulfonate) to facilitate the separation, which precludes the use of mass spectrometry. We have taken advantage of a charged surface hybrid phenyl column and MS-compatible mobile phases to achieve significantly higher separation efficiency and selectivity for Imatinib and nine of its related impurities. Moreover, the Waters structural elucidation tools helped derive molecular formulas of known or unknown MS signals and subsequently analyzed the MS/MS fragmentation spectra for structural assignments. The approaches outlined here give an example of modern chromatographic separation hyphenated with MS structural elucidation in the analysis of Imatinib and related impurities, which would be adaptable to other kinase inhibitors.

126 **Root Cause Identification of Unexpected Toluene Ingress Enables Commercial Process Validation for the Synthesis of a GMP Pharmaceutical Intermediate**

Jackson Hall, Merck & Co., Inc., 126 E. Lincoln Ave., Rahway, NJ 07065, Robert Franklin, Pratiq Patel, Holst Halsey, Zhu Liu, Linda Zheng, James Corry, Lisa Jellett, Hanlin Luo, Morgan Crawford, Cheol Chung, Nadine Kuhl, Rebecca Arvary, Feng Tan, Sachin Lohani

During process validation preparation for the commercial-scale production of a GMP intermediate at an international contract manufacturing organization (CMO), an unknown peak, equivalent in area to limit of quantification peaks, was observed in dry cake gas chromatograms. The process intermediate is synthesized using a multi-reaction flow step that employs two separate organolithium reagents (methyllithium and n-butyllithium (nBuLi)). In order to de-risk the production, a comprehensive investigation was conducted to identify the unknown peak, determine root cause, and implement a control strategy for future batches. Historical data did not contain this unknown peak, suggesting ingress was specific to CMO generated material. GC-MS analysis identified the peak as toluene, which is not used in the step and is an ICH Class 2 solvent. Thus, ascertaining the ingress point and process purge were vital before progressing to process validation. Purge capability testing was conducted in-house, while root cause analysis was performed at the CMO, where the raw materials were located. The highly reactive nature of organometallic reagents presented numerous engineering and analytical challenges, including limiting in-process sampling and analysis of bulk raw materials due to safety hazards. Coordination with the CMO permitted analysis of organometallic factory lots and other commodity raw materials. Testing revealed the root cause was residual toluene in the nBuLi in hexanes, stemming from hexanes recycling at the nBuLi manufacturer. The toluene levels in the nBuLi were low enough for sufficient purging during the isolation. Process validation was successfully concluded on five commercial-scale batches with <50 ppm toluene.

127 Withdrawn by the author.

128 **Predicting Pharmaceutical Product Performance through Modeling, Machine Learning and Statistics**

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

The delivery of novel pharmaceuticals to address unmet medical needs to patients is a long and arduous path, historically on the order of 10 years from target identification to commercial launch. There is a compelling argument for accelerating this timeline. In the case of some therapeutic areas, such as cholesterol lowering therapeutics, the impact of these time scales on human health is not always readily obvious. In oncology, long development times can have a stark impact literally measured in lives lost. We have also seen firsthand in the last few years how advances in the development of vaccines and the associated diagnostics can dramatically alter global morbidity numbers. This talk will focus on advances in modeling, machine learning and the application of statistics to challenges in developing pharmaceutical products. Our group is focused on advancing novel computational approaches, in concert with experimentation that can help us forecast product performance over long time-scales, typically physical and chemical stability, or drug release rates, or extrapolating across compositional space or across scales. These concepts are

illustrated through a few examples ranging from machine deep learning applications applied to 3D XRCT imaging of small molecule formulations and coarse grain modeling applied to protein therapeutics in understanding protein aggregation risk. These examples illustrate how experimental measurements on fresh samples can be used in conjunction with modeling to predict macroscopic behaviors at longer time scale or, in contrast, modeling can be applied to predict experimental observables at later times.

129 **Automated High-Throughput Biophysical Methods for Higher Order Structure (HOS) Analysis of Protein Biopharmaceuticals**

Anne H. Kim, Pfizer, 1 Burtt Rd., Andover, MA 01810

Protein higher order structure (HOS) is an important product quality attribute that governs the structure-function characteristics, safety, and efficacy of therapeutic proteins. Circular Dichroism (CD), Differential Scanning Calorimetry (DSC), Infrared (IR), and Fluorescence have long been recognized as powerful biophysical tools in determining protein secondary and tertiary structure and monitoring the dynamic structural changes. Such biophysics analyses help establish process and product knowledge, understand the impact of upstream (cell culture) and downstream (purification) process conditions, monitor product stability, and assess product comparability when process improvements are implemented. In this presentation, we are going to describe an overview of biophysical analysis methods for HOS and highlight an automated high-throughput biophysical workflow with automated CD and microfluidic modulation mid-IR for protein characterization and comparability/biosimilarity studies for biopharmaceutical process and product development.

130 **Computational Tools for Modeling Critical Quality Attributes in Biologics**

Naresh Chennamsetty, Bristol Myers Squibb Co, 1 Squibb Dr., 48-WSA013E, New Brunswick, NJ 08901

Critical quality attributes (CQAs) such as aggregation, oxidation and deamidation pose significant challenges in the development of biologics. These CQAs can adversely affect biologic activity, immunogenicity, protein kinases (PK) or manufacturability. Furthermore, biologics have been increasing in complexity with each passing year from antibodies to fusion proteins, bi/tri-specifics and ADCs. With increasing complexity, there is a lack of historical data and a growing need for understanding their CQAs at earlier stages of development. However, there is limited or no material to assess these experimentally at these early stages. We demonstrate that in-silico computational tools can help in very early assessment of CQAs. These can be applied for even highly complex assets. We apply these models on several model proteins and therapeutic candidates and demonstrate their accuracy based on comparison with experimental data. Furthermore, we demonstrate that these models help in understanding the underlying mechanistic cause of degradation at a molecular level. Thus, we show that these models can aid in early risk assessment and to perform targeted experiments.

131 **NMR as Integral Part of Innovative, Smart Solutions to Increase Automation from R&D to Manufacturing - New Compact, Mobile, Affordable Approach to API Manufacturing**

Anna Codina, Bruker UK Ltd, Banner Lane, Coventry, West Midlands, CV4 9GH, United Kingdom, Luis Carrillo, Julien Marin, Philippe Robin

The current global supply chain issues are clearly an unresolved challenge that the pharmaceutical industry is also facing. The pandemic has put under stress supply chain and manufacturing forcing the increase of agility, mobility and adoption of new technologies regardless of the regulatory burden that this implies. The concept of portable, continuous, miniature and modular manufacturing units is a reality,[1,2] but perhaps not yet accessible to the pharmaceutical and biopharmaceutical ecosystem. Bruker has therefore joined forces with 3 other companies (NovAliX, Alysophil, De Dietrich Process Systems) to bring to market a new approach to active pharmaceutical ingredient (API) production. The partnership will leverage its complementary skills to provide a complete, standalone, and location-independent API manufacturing solution to a pharmaceutical company or contract manufacturing organization (CMO). The partnership will leverage the combination breakthrough synthesis, continuous flow chemistry and in-flow analysis with artificial intelligence to create this next generation, autonomous and optimal production unit.[3] During this talk, we give an update on progress made towards the project objectives. We also discuss the automation of other applications of magnetic resonance which makes the technique more accessible to a wider variety of users.

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[3] https://www.dedietrich.com/en/news/corporate/pipac-collaboration-enables-new-compact-mobile-and-frugal-approach-active, accessed 03Jul22

132 **Laboratory Automatization is No Silver Bullet**

Pascal Wambua, Pwani Oil Products, Wahunzi St., PO Box 81927-80100, Mombasa, Kenya

With the increasing need to revamp efficiency back in the laboratory. Many laboratories and research organizations have opted to integrate automation into their process. This, however, has been faced with imminent challenges such as lack of funds, required skills, and global uncertainty on new technology. Laboratory Automation is an automation system for the performance of highly repetitive tasks in the Laboratory. It replaces human operators in the preparation and transport of specimens, with robotic devices. Laboratory automation consolidates the control of multiple different analytical instruments to a smaller number of operators. This way the automation reduces the costs in laboratory testing while improving efficiency. This presentation aims at discussing the benefits of laboratory automation, sustaining the automation process, and why laboratory automation might not be the silver bullet.

133 **Empowering Staff through a Constructive Performance Review**

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

Most lab staff find the annual performance review frustrating, demoralizing, and not helpful to actually improving their performance. This activity is often treated as a required HR function, and managers are just completing the forms, and trying to get it over with as quickly as possible. They often focus on the shortcomings, and produce no forward-looking value. Despite its poor reputation, the performance review can be a useful tool in improving staff performance and morale. By providing equal focus to accomplishments and individual development opportunities, the annual performance review can be both a time to celebrate what when right and a time to discuss the most important areas for further development and improvement. Providing constructive criticism promptly during the year will both provide more opportunity and time for improvement, and enable the performance review to be more strategic and meaningful for both the employee and the organization. This talk will highlight ways to conduct positive performance reviews that celebrate accomplishments, builds development plans on staff strengths and enables the discussion to be more forward looking and supportive.

134 **A Diverse and Collaborative Workforce: Starting it and Keeping it**

Maria Dennis, Weill Cornell Medicine, Duke Human Vaccine Institute, 1320 York Ave., Apt. 18K, New York, NY 10021

What is science without collaboration? What is progress if inclusivity is ignored? As scientists, as researchers, as people striving for change, we should have inclusivity and collaboration at the forefront of our minds. When I started my research career in vaccine development 6 years ago, I was not aware of how much collaboration plays a part. I was a part of a large, diverse lab overseen by two intelligent women scientists, and seeing how they worked together seamlessly inspired me to take a more active interest in improving research environments for the better. But how can one do that? By listening to the struggles and accomplishments of underrepresented minorities in science. By creating a safe and open atmosphere for difficult yet important conversations to be had. By educating the masses on cultural and community practices that are used to make those feel more at home in their workplace. But none of us are born an expert; to create this environment, and to keep it, we must listen to each other. Speak to each other with the confidence that we are trying to reach the same goal. And most importantly, be honest with each other. The impact of our research can be made stronger if we choose to work together.

135 **Motivating and Retaining Staff**

May Adaeze Chinda, University of Ghana Medical Centre, AHPC/GAMLS, PO Box CO 2300, Community One Tema, FL 134 Golf City Main St., Greater Accra, Ghana GK-0237-4804

The purpose of the presentation is to establish the relationship between motivation and staff retention as well as establish the key factors that influence employee retention and what Managers should look out for when conducting recruitment. Retaining skilled staff is one of the major challenges employers have to deal with to achieve their goals, so employers must know what their staffs look at for and encourage the achievement of both the organizational as well as staff personal goals. According to survey conducted in Northern Region of Ghana by Eunice Ayeremusah (2018). she established that the most important motivational packages are salary, promotional opportunities, Job security and Work environment. Salary proved to have the greatest effect on retention. Fairweather (2005) says employees will feel better and happier and work better, stay long if they perceive their employer are reasonable and fair. Employee likes to work and stay in an organization where their opinion counts. The higher the employee involvement in decision making the higher their organization retention level. In conclusion, Motivation plays an important role in employee satisfaction and eventually retention. Motivation is the catalyst for the success of any individual. Therefore, it is the responsibility of managers and Team leads to ensure that employees are motivated regularly to bring out the best in them.

139 **Recent Developments in Tandem-Column Liquid Chromatography and Chiral Capillary Electrophoresis**

Zhiyang Liu, Drexel University, Department of Chemistry, Philadelphia, PA 19104, Eric Buchhalter, Joe Foley

In conventional one-dimensional column chromatography, a single column is employed in the overwhelming majority of instances. However, two columns have occasionally been utilized in series over the years in liquid chromatography to improve resolution, either by increasing the plate number/peak capacity or by increasing the selectivity. Focusing on the latter which employs two different columns and is termed tandem-column liquid chromatography (TC-LC), we describe results obtained to answer the question of whether the use of two (or more) different columns in series is fundamentally better from the perspectives of selectivity and resolution than conventional one-dimensional liquid chromatography that utilizes a single column, i.e., single-column liquid chromatography (SC-LC). Our results indicate that, depending on the number of columns (SC-LC) or column combinations (TC-LC) investigated, the increase in resolution provided by TC-LC for the separation of a given sample ranges from 0.1 to 0.75 resolution units [1]. In chiral capillary electrophoresis (CE), the separation of a pair of enantiomers is typically achieved via the addition of a chiral selector such as a neutral or charged cyclodextrin to a conventional background electrolyte (BGE) or, in electrokinetic chromatography, by the incorporation of a chiral surfactant, co-surfactant, and/or chiral oil into a surfactant-based pseudostationary phase (PSP). Assuming ongoing preliminary experiments are successful, we describe an alternative approach to chiral CE that requires neither a chiral BGE nor a chiral PSP and then share representative separations of common enantiomeric drugs such as NSAIDs.

Reference:

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

137 **Capillary Electrophoresis Coupled to Mass Spectrometry through Vibrational Sharp-Edge Spray Ionization**

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

Coupling capillary electrophoresis (CE) to mass spectrometry (MS) is a powerful strategy to leverage a high separation efficiency with structural identification. Traditional CE-MS interfacing relies upon voltage to drive this process. Additionally, sheathless interfacing requires that the electrophoresis generates a sufficient volumetric flow to sustain the ionization process. Vibrating sharp-edge spray ionization (VSSI) is a new method for interfacing capillary electrophoresis to mass analyzers. In contrast to traditional interfacing VSSI is voltage-free, making it straightforward to CE and MS. A new nanoflow sheath CE-VSSI-MS is introduced in this work to reduce the reliance on the separation flow rate to facilitate the transfer of analyte to the MS. The nanoflow sheath VSSI spray ionization functions from (400 to 900 nL/min). The use of a nanoflow sheath enables greater flexibility in the separation conditions. The nanoflow sheath is operated using aqueous solutions in the background electrolyte and in the sheath demonstrating the separation can be performed under normal and reversed polarity in the presence or absence of electroosmotic flow. This includes the use of a wider pH range as well. The versatility of nanoflow sheath CE-VSSI-MS is demonstrated by separating cationic, anionic, and zwitterionic molecules under a variety of separation conditions. The detection sensitivity observed with nanoflow sheath CE-VSSI-MS is comparable to that obtained with sheathless CE-VSSI-MS as well as CE-MS separations with electrospray ionization interfacing.

138 **Liquid Chromatography Column Considerations in Pharmaceutical & Biopharmaceutical Analysis**

James Grinias, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028

Liquid chromatography plays an important role in the characterization of both small molecule pharmaceuticals and larger biopharmaceutical products. However, different considerations in column selection and method development must be made when shifting between these molecular size domains. Particle pore size can impact the amount of stationary phase surface availability that a given molecule has, adding to the complexity of retention mechanisms in reversed phase separations. The internal diameter of a column affects method development and method transfer. Flow rate scaling to maintain linear velocity between columns with different diameters can lead to increases in peak signal and sensitivity, although extra-column effects that can hinder separation performance must be considered. The primary focus of these comparisons is with analytical-scale columns, but implications for extending these observations to capillary-scale columns are also discussed.

139 No abstract submitted by the author.

140 **Investigation of the Presence and Migration of Perfluoroalkyl Substances (PFAS) from Nonstick Cookware**

Kaylie Kirkwood, North Carolina State University, 2620 Yarbrough Dr., Raleigh, NC 27607, James Dodds, Erin Baker

Perfluoroalkyl substances (PFAS) are a class of thousands of organofluorine chemicals with unique properties including thermal stability, resistance to degradation, and surfactant capabilities. PFAS have thus been used in a variety of industrial and household applications, including coatings for nonstick cookware. While traditional nonstick coatings such as polytetrafluoroethylene (PTFE), known as Teflon, are well-characterized, little is known about the composition of alternative nonstick coatings often advertised as PFAS-free. Thus, given the known toxicity and environmental persistence of PFAS, it is important to investigate both their presence within the new coating materials and potential migration to food. Here, one PTFE and eight PTFE-alternative nonstick frying pans were analyzed for PFAS presence in 4 different sample types including: 1) their nonstick coating material; water heated in 2) pre- and 3) post-scratch pans; and methanol warmed in post-scratched pans. PFAS in each sample were then quantified using liquid chromatography, ion mobility spectrometry and mass spectrometry (LC-IMS-MS) and external calibration. All nine pans illustrated detectable levels of PFAS in at least 2 of the 4 samples collected. Specifically, all pans that produced enough solid coating material for analysis had between 2 to 10 ppb summed PFAS concentrations including 6-, 7-, 11-, and 12-carbon perfluorocarboxylic acids. Additionally, the pre- and post-scratched water samples had sub-ppt sum PFAS concentrations, indicating that PFAS migration into boiling water was minimal but still present. These concentrations consistently increased following scraping, showing the importance of disposing scratched nonstick cookware, regardless of whether it is advertised as PFAS-free.

141 **Next Generation Infrared Matrix-Assisted Laser Desorption Electrospray Ionization Source for Mass Spectrometry Imaging and High-Throughput Screening**

Kevan Knizner, North Carolina State University, FTMS Laboratory for Human Health Research, Department of Chemistry, Raleigh, NC 27695, Jacob Guymon, Kenneth Garrard, Guy Bouvrée, Jeffrey Manni, Jan-Peter Hauschild, Kerstin Strupat, Kyle Fort, Lee Earley, Eloy Wouters, Fan Pu, Andrew Radosevich, Nathaniel Elsen, Jon Williams, David Muddiman

Infrared matrix-assisted laser desorption electrospray ionization mass spectrometry (IR-MALDESI-MS) is an effective analytical platform for mass spectrometry imaging (MSI) of biological tissues and high throughput direct analysis of biochemical samples without tedious sample preparation. An entirely new architecture was designed and constructed to improve IR-MALDESI-MS studies, improve source versatility, and overcome the limitations of the previous source. This NextGen IR-MALDESI source includes a vertically mounted IR-laser to increase laser energy applied to the sample, a computerized 3D translation stage for more repeatable MSI analyses, an aluminum enclosure to reduce plastic incorporated in the source, and a versatile mass spectrometer interface plate so the NextGen source can be implemented into a wider range of research labs. In this work, we present the characterization of the NextGen IR-MALDESI source as an improved source for MSI and direct analysis. Design of Experiments (DOE) is an efficient method to optimize an analytical platform in a minimized number of experiments. DOE was used to optimize the internal component geometries of the NextGen source to maximize measured analyte abundances and minimize ESI variability. The updated geometries resulted in increased measured analyte abundances by at least 2x for larger *m/z* analytes and ~24x for smaller *m/z* analytes. Finally, a complete parts list and their respective CAD 3D models will be released in the future publication so the NextGen IR-MALDESI source can be implemented into any research lab interested in HTS or MSI by IR-MALDESI-MS.

142 **Cannabis Potency Testing - Which Column Dimension is Right for You?**

Jamie York, Restek, 110 Benner Circle, Bellefonte, PA 16823, Melinda Urich, Justin Steimling, Cathy Hetrick

When starting method development for potency testing, it's important to choose the right column dimension for the target analysis. In this work, different column dimensions of the Raptor ARC-18 phase were utilized to develop methods to meet various labs' needs using HPLC-UV. To demonstrate the powerful resolving capabilities of Raptor ARC-18, a 50 x 3 mm, 2.7 µm column was used to analyze 7 cannabinoids including CBD, CBDA, delta-9-THC, delta-8-THC, (6aR, 9S)-delta-10-THC, (6aR, 9R)-delta-10-THC, and THCA. This method utilizes gradient conditions, methanol as the organic modifier, and an overall cycle time of 8 minutes. This methodology is ideal for labs that are only interested in the required testing needed to be compliant with specific state testing regulations. Next, additional cannabinoids including CBDV, THCV, CBG, CBN, CBGA, and CBC were added to the previous analytes for a total of 13 cannabinoids. Using the same column dimension and mobile phases, a method was developed to resolve all analytes in 10 minutes. Finally, to include exo-THC and CBNA, a 150 x 3 mm, 2.7 µm column dimension was used to demonstrate the utility of a longer column dimension. The organic modifier used was 0.1% formic

acid in acetonitrile, where a total of 15 cannabinoids were able to be resolved in 10 minutes. Each of these methods was applied to hemp matrix to demonstrate the applicability of these methods in real world samples.

143 **Modeling and Optimization of Multiple-Quantum Magic-Angle Spinning NMR Spectra**

Lexi McCarthy, Ohio State University, Department of Chemistry, 100 W 18th Ave., Columbus, OH 43210, Brendan Wilson, Deepansh Srivastava, Jay Baltisberger, Philip Grandinetti

Multiple-Quantum Magic-Angle Spinning (MQ-MAS) is a popular method for obtaining isotropic solid-state NMR spectra of quadrupolar nuclei, but optimum experimental conditions for excitation of forbidden multiple-quantum transitions are not intuitive. Nutation behavior for excitation and mixing of triple-quantum coherences is a complicated function of experimental and sample parameters, including quadrupolar coupling constant and rf field strength. Additionally, the relative integrated intensities are often not quantitative, particularly for sites with significantly different quadrupolar coupling constants. Furthermore, non-uniform excitation of crystallite orientations can lead to severely distorted anisotropic line shapes, further complicating spectral analyses. To address these deficiencies, we have developed a simplified theoretical description of multiple quantum excitation and mixing for half-integer quadrupolar nuclei in the static limit approximation, where pulse durations are less than 10% of a rotor period. This theoretical approach recasts the complexity of multiple quantum nutation behavior in terms of universal excitation and mixing curves with an appropriate scaling by the quadrupolar coupling constant. With this scaling, there is only a slight dependence on the quadrupolar asymmetry parameter remaining. From these universal curves and an approximate quadrupolar coupling constant, one can determine the optimum rf field strength and pulse durations that maximize sensitivity. Additionally, this approach leads to an efficient algorithm for rapidly simulating the triple-quantum-filtered central transition spectrum for arbitrary excitation and mixing rf field strengths, pulse durations, and MAS rates within the static limit approximation. This algorithm enables the accurate determination of relative site populations and quadrupolar coupling parameters in least-squares analyses of MQ-MAS spectra.

144 **Mrsimulator: An Object-Oriented and Open-Source Software Package for Fast Solid-State NMR Spectral Simulation and Analysis**

Matthew D. Giammar, Ohio State University, Department of Chemistry and Biochemistry, 100 W 18th Ave., Columbus, OH 43210, Philip J. Grandinetti, Deepansh Srivastava, Alexis McCarthy, Maxwell C. Venetos

The free and open-source Python package, mrsimulator is presented as a simple-to-use, easy-to-install, versatile library with a permissive (BSD) license capable of simulating one- and higher-dimensional NMR spectra under static, magic-angle, and variable-angle conditions. Computational efficiency in spectral simulations is achieved by limiting simulations to situations where analytical solutions are available for transition frequencies and coherence transfers between transitions. This approach is generalized to multi-dimensional NMR spectra simulations using symmetry pathway concepts for describing multi-pulse NMR experiments[1]. The mrsimulator package supports the simulation of uncoupled and coupled spin systems with nuclei of arbitrary spin. Coupled spin systems, however, are limited to those well described by inhomogeneous Hamiltonians, that is, we avoid simulations involving homogeneous interactions as defined by Maricq and Waugh[2]. Fortunately, this constraint only prevents mrsimulator from modeling spectra from a small fraction of popular solid-state NMR methods. The efficiency gains with this approach are essential for modeling spectral of non-crystalline materials where subspectra from thousands of spin systems are needed for accurate modeling. The mrsimulator package is fully documented with numerous examples (https://mrsimulator.readthedocs.io). It easily integrates with other scientific and machine learning libraries to create new opportunities for data science with solid-state NMR spectroscopy.

References:

[1] Grandinetti et al. Prog NMR Spect. 59 (2011) 121-96.

[2] Maricq, M. Matti and J. B. S. Waugh. J. Chem. Physics 70 (1979): 3300-3316.

145 **Functionalized Gold Nanoparticles with Halogen Bonding Capability – an Avenue for Molecular Detection Schemes**

Quang Minh (Harry) Dang, University of Richmond, Chemistry Department, 410 Westhampton Way, Richmond, VA 23173, Samuel T. Gilmore, Karthik Lalwani, Richard Conk, Jeffrey Simpson, Michael C. Leopold

Fundamental study of molecular detection schemes enables the practical application of science with important implications for measurement of species relevant to the environment, national security, and clinical diagnostics. Essentially, these schemes rely on (1) specific interactions between synthetic host molecules and their targeted analytes and (2) an indicator that the molecules are interacting. Halogen bonding (XB)—an orthogonal, electrostatic interaction between a region of positive electrostatic potential (δ+) on a halogen atom (XB donor) and a Lewis base (δ–) (XB acceptor)—can serve as the specific interaction. Herein, a thiolate molecule/ligand with strong XB-donor moiety (–C6F4I) is successfully synthesized and characterized with NMR, IR, and HRMS. The XB-donor ligand is then used to functionalize hexanethiolate-protected gold nanoparticles (AuNPs) through place-exchange reactions. The XB-functionalized AuNPs (XB-fAuNP) can engage in strong XB interactions with XB-acceptor analytes, which results in AuNP aggregation events (measured via UV-Vis, TEM imaging, and DOSY NMR measurements) with a limit of detection in the nanomolar range. XB interactions between the fAuNPs and analytes are also shown electrochemically. The developed XB-fAuNP system can serve as the basis for future molecular detection schemes that utilize visual, electrochemical, and spectroscopic methods and target analyte molecules of interest in the forensic, environmental, and medicinal fields.

146 **Impact of Electrolyte Formulations on Potassium Deposition Morphology in Potassium Ion Batteries**

Naiara A. Munich, Barnard College, Department of Chemistry, 4259 Altschul, 3009 Broadway, New York, NY 10027, Lauren E. Marbella

Lithium-ion batteries (LIBs) are currently playing a key role in the electrification of ground transportation in the effort to reduce carbon emissions in the wake of climate change. However, lithium's low natural abundance in the earth's crust has raised questions about LIBs' role as a long-term energy storage solution since an increased demand for lithium will lead to a large strain on lithium supply. Potassium-ion batteries (KIBs) have been proposed as a naturally abundant fast-charging alternative to LIBs as potassium metal is two orders of magnitude more abundant than lithium. We know that metal anodes offer high energy density for any battery chemistry, yet, despite decades of research on Li stripping and plating we do not have evidence that these lessons will translate to K deposition behavior. Here we are using scanning electron microscopy (SEM) imaging to compare the electrodeposition and electrochemistry of potassium in carbonate-based and ether-based electrolyte formulations using KPF6 and KFSI, respectively. Preliminary data has demonstrated that carbonate-based electrolytes lead to better plating structures than ether electrolytes. We hope to further elucidate our findings by characterizing the film of decomposition products on the surface of the potassium anode known as the solid electrolyte interphase (SEI) using solution nuclear magnetic resonance (NMR) spectroscopy and x-ray photoelectron spectroscopy (XPS). Together, these findings will help to provide a fundamental understanding of KIB performance and degradation mechanisms.

147 **Elucidating *Pseudomonas Aeruginosa* Infection Biomarkers Using Proteomics, Metabolomics, MALDI, and Cyclic-IM-MS**

Samuel Krug, University of Maryland, 20 N Pine St., Pharmacy Hall, Baltimore, MD 21201, Saba Shahzad, William Temple Andrews, Ludovic Muller, Weiliang Huang, Angela Wilks, Maureen Kane

Pseudomonas aeruginosa (PA) is a gram-negative opportunistic pathogen and has multi-drug resistant activity. In order to better understand virulence during infection, our lab has constructed PA mutants to dyregulate the heme oxygenase system (HemO), which is essential for iron processing. Untargeted proteomic data for these mutants was acquired using a Waters Nano-LC system coupled to a Thermo Orbitrap Fushion Lumos Tribrid and revealed several key regulator pathways were altered in the mutant, slowing PA growth and proliferation. We have quantified some of these metabolites using a Waters Acquity LC couple with a Waters TQ-XS system and shown that alkylquinolone n-oxides (AQNO) are significantly reduced and may be a promising biomarker. We have also been able to image bacterial culture of these mutants by MALDI using a Bruker solariX and spatially visualized metabolite excretion. At the conclusion of this experiment, we were not able to distinguish between classes of quorum sensing molecules that are isobaric, so we are currently developing methodology on the Waters SYNAPT G2-Si Cyclic IM-MS in order to better resolve these species. In future studies, we will be looking to move from a cell culture model to an acute infection mouse model and analyze lung tissue.

148 **Investigation and Identification of an Atypical Ghost Peak in a Gas Chromatography Analysis Involving Dimethylsulfoxide (DMSO) as Diluent**

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Testing for residual solvents in a Drug Substance by Gas Chromatography (GC) was performed at an external manufacturing site as part of process familiarization gave an Out Of Trend (OOT) result, in which an atypical impurity peak was observed at 18 minute from the sample injection. This peak was above the limit of quantitation (LOQ) in the sample, but it was less than the limit of detection (LOD) in blank and standard injections. The root cause of the OOT needed to be immediately addressed so that the actual production batch could proceed without any delay. The OOT investigation excluded the possibilities of glassware contamination, carry over or a system peak. The sample preparation procedure at the manufacturing site was reviewed and it was noticed that the sample was extensively sonicated during the sample preparation. Investigation experiments were carried out and it was confirmed that when DMSO is sonicated for 20 minutes, a large peak with retention time of 18 minutes showed up in the blank injection. Review of development method and development data revealed that this peak was present in blank injections at random levels between various DMSO batches, depending on the grade/quality of the solvent. Identification of this artifact and the lesson learned from the event will be discussed.

149 **Multiple Analyte Quantitation Using a Polyarc® for Universal Carbon Detection**

Dana Zeigler, Arkema Inc., 900 First Ave., King of Prussia, PA 19406

Quantitation of analytes using a gas chromatograph with flame ionization detection (GC-FID) is a routine analysis in many labs. However, when the samples are complex and contain many analytes of interest, quantitation can be more challenging. Using external standardization to establish an FID response for each analyte yields robust but labor-intensive results. Additionally, this approach requires standards of each analyte, which can prove to be expensive, and relies on reliable sourcing of chemicals- a distinct challenge with recent supply chain issues. In order to reduce overall analysis time and increase lab throughput, a Polyarc® system was installed. The Polyarc® system is a commercially available (Activated Research Company LLC) methanizer that converts all carbon atoms to methane for a uniform response in the flame ionization detector. These responses are then compared against a known amount of internal standard in each sample to quantify the amounts of each analyte of interest. When applied to the quantitation of residual monomers in polymer latexes, the GC-Polyarc®-FID was found to have comparable limits of detection and linearity. This system was found to eliminate the need for costly and time consuming external standardization and led to improved efficiency in the lab.

150 **A Comparison of Normal versus Reversed-Phase Chiral Methodology for an Agrochemical Compound**

Austin Whittington, FMC Corporation, Analytical Sciences, Stine Research Center, 1090 Elkton Rd., Newark, DE 19711, Gloria Chung, Mary Ellen McNally

Chiral impurities in a technical grade agrochemical compound have been determined by both normal phase and reversed-phase chiral separation to establish and validate the analytical methods. Methods are similar in the sample concentration and wavelength used to examine the enantiomers, and their subsequent epimers. The differences between methods can be seen in chromatographic mode, solvent system, column, gradient, and linearity range. GLP method validation for these studies included data for linearity, precision, accuracy, specificity, limit of quantitation, and confirmation of identity. This comparison demonstrates that either reversed or normal phase methodologies can be employed to support registration of the manufactured technical grade material.

151 **Simple Green Synthesis and Characterization for Nano-Sized ZnO**

Nazharie Brandon, The University of the District of Columbia, 4200 Connecticut Ave, Washington, DC 20008, Uche Udeochu

Green chemistry has an important role in creating environmentally conscious pathways to engage in the applied sciences. Here we report the green synthesis of Zinc Oxide using plant extracts and Zinc salts, without environmentally toxic reagents or waste. In this method, aloe and other fruit and vegetable plants are prepared into an aqueous solution using heat and deionized water. Solid Zinc Oxide was collected via precipitation and characterized through IR and UV spectroscopy as well as SEM imaging. Our collected samples demonstrated mixed sizes and morphologies ranging from granular to hexagonal. The sizes and shapes varied in this method according to altered temperatures, solution concentrations and precipitation methodology. This work is aimed at synthesis and characterization of materials with possible applications in solar energy designs. By understanding the electrochemical properties that define our materials, synthesized with green methods, effective applications of these materials will be clearer.

152 **Diffusion-Ordered NMR Spectroscopy of Sweet Sorghum Bagasse Lignin Isolated After Low Moisture Anhydrous Ammonia (LMAA) Pretreatment**

Gary Strahan, United States Department of Agriculture/ARS/Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, Charles Mullen, Ryan Stoklosa

Lignin is an aromatic biopolymer found in plant cell walls and is largely responsible for the strength and rigidity of plants. It typically makes up 15-40 wt% of lignocellulosic plant tissues. Lignin has been difficult to convert to higher value products, and this has been a roadblock to the economic viability of cellulosic biorefineries. Lignin's properties vary depending upon both the plant species and the method of fractionation from the carbohydrate (cellulose and hemicellulose) portion of biomass. Developing new methods to characterize and understand the various structures and molecular sizes present in lignin is critical to improving the conversion processes. In this work, lignin that was isolated from sweet sorghum bagasse after pretreatment with low moisture anhydrous ammonia (LMAA), was characterized by diffusion-ordered nuclear magnetic resonance (NMR) spectroscopy, as well as 1D-1H and multinuclear 2D NMR, and elemental analysis. We discuss how these structural characterizations are related to molecular weight, and by comparison to a commercial soda lignin product. Discuss the effects of LMAA pretreatment on the lignin molecular weight.

153 **Effect of Organic Solvent in Mobile Phase on Dipole-Dipole Interaction Using Biphenyl Phase**

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It is well known that phenyl and biphenyl phases have dipole-dipole interactions that are absent in alkyl phases such as C18 and C8. Biphenyl phase exhibits stronger dipole-dipole interaction than phenyl phase, so that biphenyl phase was used for effect of organic solvent on dipole-dipole interaction in this study. Methanol, 2-propanol, acetonitrile and tetrahydrofuran as an organic solvent in the mobile phase were compared to separate isomers of methylhippuric acid. It has been confirmed that acetonitrile and tetrahydrofuran counteract dipole -dipole interaction and 2-propanol enhance dipole -dipole interaction more than methanol. Additionally, retention time could be controlled by adding acetonitrile in the mobile phase. Although C18 phase could not separate iso-butyraldehyde-DNPH and n-butyraldehyde-DNPH, biphenyl phase could control the degree of separation of iso-butyraldehyde-DNPH and n-butyraldehyde-DNPH by changing the composition ratio of methanol and IPA as organic solvents in the mobile phase.

154 **Monoclonal Antibody Analysis with Compact Capillary LC Instrumentation**

Benjamin Libert, Advanced Materials Technology, 3521 Silverside Rd., Wilmington, DE 19810, Samuel Foster, Taylor Harmon, Barry Boyes, James Grinias

The analysis of monoclonal antibodies (mAb) and their various critical quality attributes (CQAs) by LC-MS is a critical aspect of biopharmaceutical characterization. Optimally, these techniques could be coupled directly to bioreactors for real-time process monitoring of biopharmaceutical manufacturing by LC-MS. Recently, a method for on-line monitoring of small molecule reactions was demonstrated using compact capillary LC-MS instrumentation. Here, the same compact capillary LC instrument is utilized for a variety of analytical methods typically applied for the characterization of mAbs. A variety of columns with inner diameters in the 0.2 – 0.3 mm range and packed with 2.7 µm superficially porous particles were used for separations. Particles with 1000 Å pores were used for the analysis of intact mAbs and mAb fragments and 160 Å pore size particles were used for the separation of peptides from a mAb tryptic digest. Details regarding both instrument and method development are described.

155 **Tandem Column-High Performance Liquid Chromatography Achiral Separation of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)**

Megan Malvoisin, Drexel University, 305 Disque Hall, 32 South 32nd St., Philadelphia, PA 19104, Joe Foley

A liquid chromatographic method was developed to separate nine non-steroidal anti-inflammatory drugs (Aceclofenac, Aspirin, Celecoxib, Diclofenac, Flurbiprofen, Ibuprofen, Ketoprofen, Naproxen, Salicylic Acid) using an achiral tandem column configuration under reversed-phase conditions. An NSAID test mix was prepared with 0.5 mg/mL thiourea as a t0 marker and 0.5 mg/mL Aceclofenac, Aspirin, Celecoxib, Diclofenac, Flurbiprofen, Ibuprofen, Ketoprofen, Naproxen, and Salicylic Acid in 1:1 acetonitrile: 0.2% formic acid. Three different columns in two different tandem configurations are being investigated. The three columns are a 5-cm C8 column, a 5-cm perfluorophenyl (PFP) column, and a 5-cm biphenyl column. The two tandem configurations are a 5-cm perfluorophenyl column followed by either a 5-cm biphenyl column or a 5-cm C8 column.

156 **Elucidation and Rejection of a New Process Impurity Formed in the Commercial Route to a GMP Pharmaceutical Intermediate**

Alison McQuilken, Merck & Co., Inc., 126 E. Lincoln Ave, Rahway, NJ 07065, Erin McCarthy, Nelo Rivera, Ben Turnbull, Justin Newman, Taylor Behre, Ryan Cohen, Samantha Burgess, Jiaxuan Yan, Zhixun Wang, Nadine Kuhl, Jimmy DaSilva, Erik Regalado, Derek Henderson, Fuh-Rong Tsay

The 8-step supply route to generate a process intermediate was optimized to 2 steps. This optimized process introduced a new impurity at high levels (3 A%) that carried into the final API step, resulting in elevated levels of a new API-related impurity. Identification and isolation of this new impurity was further complicated by the polarity of the process intermediate and its lack of chromophore. An ion-pair HPLC-CAD method was developed to separate the desired intermediate from impurities on a reverse-phase C18 column to better understand the fate and purge of the new impurity. Through close collaboration between cross functional teams, the new API-related impurity was confirmed to be a dimer and traced back to a new intermediate-related impurity. This understanding enabled timely process improvements to control the level of this dimer impurity at the penultimate step.

157 **Separation of Bispecific Antibody Variants Using Wide Pore, Small Particle Reversed Phase Chromatography**

Erin Wilson, GlaxoSmithKline, 709 Swedeland Rd., King of Prussia, PA 18079, Jeff Roberts, Byron DiPaolo

Bispecific antibodies are becoming more prevalent in biopharmaceutical research and development due to their potential to provide superior clinical therapeutic effects and broad applications in tumor immunotherapies and other diseases as compared to traditional monoclonal antibody therapies. Bispecifics are comprised of two binding sites on the Fab region of the antibody. These binding sites can be directed at two different antigens or two different epitopes of the same antigen. This construct requires intentional heterodimerzation of the light chain and heavy chains of each antibody, creating a unique analytical challenge for analysis of product-related variants such as homodimers and halfbody fragmentation. Using reversed-phase chromatography, bispecific product-related variants, including but not limited to homodimer and halfbody species, were shown to be optimally separated based on hydrophobicity using a wide pore (1000A), small particle size (2.7um) diphenyl column.

158 **Development of Tandem-Column Liquid Chromatographic Methods for Pharmaceutical Compounds Based on Hydrophobic Subtraction Model Simulations**

Zhiyang Liu, Drexel University, 32 South 32nd St., Philadelphia, PA 19104, Yiyang Zhou, Qinggang Wang, Joe Foley, Dwight Stoll, Jonathan Shackman

Chromatographic separations are most affected by selectivity as compared to efficiency or retention. Generally stationary phase and mobile phase pH (for small molecule ionizable analytes) will have the greatest effect on reversed phase liquid chromatography (RPLC) selectivity, with “fine tuning” afforded by gradient profile, temperature, organic eluent type, ionic modifier type, ionic strength, etc. Hence, method development begins with column choice. We have previously shown that in-line, tandem-column formats can offer some advantages over equivalent length single column serial separations. With potentially hundreds of phases available in a variety of geometries and particle sizes, in silico prediction of tandem column pairs to evaluate experimentally can be more effective than extensive column screening approaches. As a first demonstration, a diverse set of 14 common over-the-counter drugs, such as acetaminophen, aspirin, ibuprofen, and omeprazole were predictively modeled in tandem-column LC by leveraging the hydrophobic subtraction model (HSM). These mixtures’ simulated separations were then verified experimentally. As a more practical example, an active pharmaceutical ingredient (API) compound in development at BMS and its related compounds (including process impurities and decomposition product(s) of the API compound) were modeled and a successful and rapid tandem-column stability indicating method was achieved. Simulation errors and their effects on method development results will also be discussed.

159 **Spectroscopic and HPLC-UV Studies: Porphyrin Aluminum Metal-Organic Framework Reacting with Organosulfur Compound Diethyl Sulfoxide**

Amarachukwu Agbim, Morgan State University, Chemistry Department, Baltimore, MD 21251, Alexander Samokhvalov

Metal-Organic-Framework (MOFs) have been extensively investigated for the sorption of hazardous substances from air, water, and soils. MOFs are highly porous advanced coordination polymers with characteristic metal cations and organic “linkers.” Aluminum-MOFs, Al-MOFs with porphyrin linkers show high sorption capacity and stability, and they are promising as chemo-sensors for hazardous compounds. Diethyl sulfoxide (DESO) is environmental contaminant and a major by-product of oxidation of organo-sulfur compounds from petroleum and natural gas, such as dimethyl sulfide (DMS). DESO is used in biomedical research, drug formulations, and as a solvent for reactions. Also, DESO structurally resembles diethyl sulfide (DES), the oxidation product of the surrogate chemical warfare agent (CWA) sulfur mustard. In this research, we synthesized by facile hydrothermal route an Al-MOF with tetrakis (4-carboxyphenyl) porphyrin (TCPP) as linker; it is denoted compound 1 aka asisAl-MOF-TCPPH2. Next, we removed “guest” molecules from compound 1 by thermal activation in a vacuum to obtain its activated form actAl-MOF-TCPPH2 (compound 2). Further, we characterized the composition and structure of the compounds by ATR-FTIR spectroscopy and powder XRD. Then, we investigated the interaction of compound 2 with liquid DESO giving adsorption complex aka compound 3. In compound 3, isolated “guest” DESO molecules are bonded to functional groups in actAl-MOF-TCPPH2 as “host” material. Finally, sorption of DESO from diluted aqueous solution by compound 2 was studied by HPLC-UV method.

160 **UHPLC-QToF Detection, Identification and Quantification of PFAS in Face Masks**

Hannah Levasseur, University of Connecticut, 3107 Horsebarn Hill Rd, Storrs, CT 06269, James Stuart, Noah Liguori-Bills, Anthony Provatas

We report on the development of a convenient, simple and reliable sample preparation method to perform untargeted UHPLC-QToF testing to detect whether any per- and/or poly- fluorinatedalkylsubstances (PFASs) may be present in representative facemasks, commonly used in the Covid-19 pandemic. Of the six types of common face-masks tested, after mild sample vortexing with methanol, only the common blue, single-use, disposable face masks were found to have very low amounts, 0.26 ng/g of PFOA and 0.32 ng/g of PFBS. However, all of the masks, except for the N95masks, were found to have higher levels of such substituted Bisphenols, as Bisphenol B (BPB) and Bisphenol C (BPPC). Hence certain facemasks, whether treated or somehow contaminated with PFAS, worn for extended periods of time may be a notable source of exposure and may contribute to health complications in the long term.

161 **Selectivity Examination of Stationary Phases for Hydrophilic Interaction Chromatography (HILIC) and Use of Multivariate Analysis to Classify Materials Based on Their Chemical Modification**

Clinton Corman, MilliporeSigma, 595 N. Harrison Rd., Bellefonte, PA 16823, Cory Muraco, Michael Ye, Martin Ross, Alok Kuma

Hydrophilic Interaction Chromatography (HILIC) is a powerful separation mode allowing for the analysis of a diverse range of polar molecules. In this separation technique, analytes retain and elute based on multiple factors – partitioning into and from labile water layer(s) enriched off the hydrophilic stationary phase surface, ionic interactions with charged analytes, hydrogen bonding, dipole-dipole interactions, and adsorption in some cases. The complexity of HILIC retention mechanisms, combined with a vast array of stationary phases to choose from, makes column selection potentially time consuming. In this work, we examine over fifteen columns under HILIC conditions using a standardized approach that gives information about the hydrophobic selectivity, hydrophilic selectivity, shape selectivity, cation exchange and anion exchange selectivity, and surface acidity/basicity. The use of principal component analysis allowed for clustering of the columns based on their chemical modification. The combination of selectivity data and multivariate analysis tools has helped elucidate retention properties of different stationary phases in HILIC. In addition, the methodology has been especially useful as an initial column screening approach for HILIC method development as well as an effective tool for the development of new HILIC stationary phase materials.

162 **Study on Matrix Preparation for MALDI-Imaging of Synthetic Polymer Samples**

Artem Filipenko, Bruker, 40 Manning Rd., Billerica, MA 01821

MALDI-Imaging is an analytical method used to visualize the spatial distribution of various molecular species using MS as detector. Few studies use MALDI imaging in the polymer industry. The lack of basic know-how for MALDI matrix preparation remains a significant barrier to such applications. Here we test the use of a manual airbrush and an automated device to generate matrix deposition via spraying a matrix solution. From the comparison between automatic spraying using a commercial sprayer and manual spraying using an airbrush, it has been found that automatic spraying is more uniform, showing a low point-to-point variation in peak intensity, especially after the data sets have been normalized to base peak intensities. Automatic spraying produces a more uniform signal with a higher average intensity than manual spraying. The improved data quality with the automatic spray device was used to optimize the experimental conditions. We have found that the highest signal intensity was achieved with a high THF ratio, which allows the polymer of the sample to be easily dissolved. In addition to the varying mixing ratio of the solvent, the additional spraying of tetrahydrofuran without matrix after spraying with the matrix solution was tested and found to be effective, giving almost best results in terms of both average signal intensity and signal intensity variation. We have varied the number of spray cycles between 2 and 20. Depending on the size of the polymer, we have found that 4 or 6 spray cycles give the highest intensity.

163 **Analysis of Perfluoroalkyl and Polyfluoroalkyl Substances in Drinking Water: Validation Studies of EPA Method 537.1 Using the QSight 220 UHPLC/MS/MS**

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

Growing environmental and health concerns about Per- and Polyfluoroalkyl Substances (PFAS) have led to stricter and more extensive regulations of these substances in drinking water, ground water, soil and food over the past decade. PFAS are man-made chemicals used in a wide variety of commercial products like nonstick cookware, food packaging, paints, clothing, fire retardants and surfactants since the 1940’s. Due to their inert nature, PFAS are persistent and have been found to accumulate throughout the environment. Originally considered biologically inactive, recent research has revealed their toxicity to humans and wildlife leading to stricter global regulations restricting their levels in food, water, air and soil. EPA Method 537.1 is a widely used method for the determination of selected PFASs in drinking water by solid phase extraction and liquid chromatography/tandem mass spectrometry. This poster will discuss the validation of EPA 537.1 on the QSight 220 LC/MS/MS and the many pitfalls of implementing this method. The QSight 220 demonstrates excellent sensitivity, precision and accuracy running EPA Method 537.1 that provides accurate results for PFAS lower than any current regulatory limits.

164 **Simultaneous Quantification of Methotrexate and its Metabolites via Coated Blade Spray-Tandem MS**

Diego Lopez, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823, RyaMicklitsch, Shane Stevens, German Gomez-Rios, Tom Kane

Coated blade spray (CBS) is a sample preparation technology that can be directly interfaced with MS instrumentation for rapid screening and quantitation. As a proof-of-concept, we have developed a CBS method for the quantitation of MTX and its metabolites 7-Hydroxy Methotrexate (7OH-MTX) and DAMPA in human serum via tandem mass spectrometry. Our preliminary results revealed that CBS could deliver performance comparable, or better, than those typically achieved with technologies generally used in the clinical laboratory such as immunoassay and liquid chromatography-MS. Glucarpidase inactivates MTX by cleaving it into two non-toxic metabolites: DAMPA and 7OH-MTX. It is well known that these metabolites interfere with commonly used MTX immunoassays, which means the assays can offer inaccurate and potentially misleading results. Therefore, more specific methods, such as those based on mass spectrometry, are preferred for the determination of MTX in patients under Glucarpidase therapy. Whilst several LC-MS/MS methods for MTX have been previously developed, these tend to require long chromatography times or complex sample prep workflows. Thus, having faster and specific assays for MTX and metabolites is ideal. In this study, we demonstrate the quantitation capabilities of CBS-MS/MS towards MTX and metabolites in serum. The CBS method have proven to be very robust with at least 250 consecutive injections (CV < 3.7% with IS correction), which can be completed by an analyst standing in front of the MS in less than 2 hours and without having to replace/clean the mass spectrometer inlet.

165 **Multiplicity-Edited 19F-13C Heteronuclear Single Quantum Coherence Experiment**

Sara Maute, Chemours, 201 Discovery Boulevard, Newark, DE 19713, Alexander Marchione, Elizabeth Diaz

The popular multiplicity-edited heteronuclear single quantum coherence experiment has been modified for ^{19}F-^{13}C experiments by insertion of broadband inversion and refocusing pulses in ^{19}F. This experiment has particular utility in distinguishing CF_3 from CF_2X, and CF_2 from CFX (X = O or halide). Examples are shown of a mixture of poly(hexafluoropropylene oxide), trichlorotrifluoroethane, and perfluoro-1-heptene. Phase distortions are avoided by careful selection of the value of J_{CF} for which delays are optimized, where a slight overstatement of J_{CF} yielded no distortions, while an understatement did. A one-dimensional version of the experiment has also been developed.

166 **Spatially Resolved and Operando Detection of Cathode Degradation in Li-Ion Batteries**

Julia Hestenes, Columbia University, Department of Applied Physics and Applied Mathematics, 500 W 120th St. SW, Mudd Building, New York, NY 10027, Richard May, Jurek Sadowski, Naiara Munich, Lauren Marbella

The high energy densities of Ni-rich and Mn-rich lithium transition metal oxide cathodes have garnered immense interest for their potential to improve the performance of Li-ion batteries. Unfortunately, degradation at the cathode/electrolyte interface leads to poor cycling performance of these materials. The chemical mechanisms underpinning this degradation are difficult to study due to the delicate and highly heterogeneous nature of the cathode electrolyte interphase (CEI) formed on composite cathode films (containing the electrochemically active material plus performance-enhancing carbon additives like conductive carbon and polymer binder). Here, we use a combination of ex and in situ nuclear magnetic resonance (NMR) spectroscopy, X-ray photoemission electron microscopy (XPEEM) and electron paramagnetic resonance (EPR) spectroscopy to characterize chemical and spatial heterogeneities in the CEI deposited on LiNi0.8Mn0.1Co0.1O2 (NMC811) and LiNi0.5Mn1.5O4 (LNMO) cathodes. We find that on NMC811, LiF and carbonate-containing Li salts selectively deposit on NMC particles, while alkyl carbonates are randomly distributed on the surface, suggesting that these species participate in anode/cathode crosstalk during electrochemical cycling. Meanwhile, in LNMO, MnF2 is distributed indiscriminately on the surface of the cathode film after electrochemical cycling due to the high voltage degradation processes of HF generation and transition metal dissolution. With four-line probe electronic conductivity measurements, we find that MnF2 greatly decreases the electronic conductivity of the cathode film. Together, these spatially resolved and time resolved characterization techniques reveal the reaction mechanisms at play in CEI formation which are closely related to the performance decline that is characteristic of these materials.

167 **A Comparison of Techniques for Sampling of Plant Volatiles in Four Plant Varieties**

Megan Harper, GERSTEL, Inc., 701 Digital Dr., Ste. J, Linthicum Heights, MD 21090, Jack Stuff

In this study, passive air sampling with Thin Film Solid Phase Microextraction (TF-SPME) devices coated with divinylbenzene/polydimethylsiloxane (DVB/PDMS) and the GERSTEL Twister® coated in PDMS were used. Active air sampling with PDMS foam and Tenax® TA thermal desorption tubes was also employed. Violet star petunias, oakleaf hydrangeas, citronella, and lemon thyme plants were used for this study. Overall, passive sampling with the TF-SPME device covered a broad range of plant volatiles and with lower detection limits compared to the other techniques but required a longer sampling time.

168 **Structure Elucidation of Three Non-Ionizable Impurities Formed in an Alternate Processing Route**

Xiaoyan Wang, FMC Corporation, 1090 Elkton Rd., Newark, DE 19711, Dawn Pierce, Carlos Amezcua

Three new impurities were formed during the phosgene chlorination step in an alternate processing route of an intermediate of an agrochemical compound. A high-resolution mass spectrometer (LC-QTOF) was initially utilized to acquire the accurate masses of the new impurities to propose possible chemical formulas and structures thereafter. However, the impurities would not ionize even under multi-mode (mixed ESI and APCI) ionization. Preparative HPLC was then used to isolate these non-ionizable impurities. Each peak of interest was collected into 3 fractions: before, at, and after the apex. The collected fractions were analyzed by the original analytical method to confirm they were the peaks of interest. The samples were also analyzed by GC/MS and the acquired mass spectra were searched against the NIST library. The apexes of the peaks (more concentrated sample) were first analyzed by 1H NMR directly using a solvent pre-saturation technique to suppress the strong signal from the eluent. The structures of the impurities were assigned based on the 1H NMR spectra and the GC/MS results. Further sample treatment was performed by liquid-liquid extraction (LLE) of the combined fractions (before, at, and after the apex) with dichloromethane, followed by nitrogen evaporation and solvent exchange into DMSO-d6. The enriched samples were analyzed by 2D 1H/13C NMR to confirm the 1H NMR-based structure assignment results. In conclusion, the structure elucidation strategy using preparative HPLC fractionation, LLE, and solvent reduction/exchange followed by NMR analysis successfully revealed the definitive structures of three small non-ionizable impurities.

169 **Development of an Open-Source Automated Derivatization Process for Fatty Acid Analysis by GC-MS**

Joeachin Obasi, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Mita Ray, Leah Notarfrancesco, James Grinias

Free fatty acids (FFA) are an important part of the lipid metabolism and many of these compounds are widely monitored biomarkers. Specific FFAs have been linked to various physiological disorders, including diabetes and coronary heart disease, and thus are routinely measured in clinical diagnostics. FFAs are typically converted to fatty acid methyl esters (FAMEs) prior to analysis by gas chromatography-mass spectrometry (GC-MS), as these analytes are more easily quantified and separated. However, this process can be time- and labor-intensive. Automated approaches have been developed, but frequently require expensive robotic sampling systems. Therefore, to increase sample preparation throughput in a lower cost platform, a simple open-source syringe pump was used to perform automated derivatization of FFAs to fatty acid methyl esters. A Python program and microcontroller were used to control the pump: FFAs were delivered to one input of the device and methanolic-HCl was delivered through a second input. FAME products were formed as the reagents were pumped through a reaction channel held at 80 °C. The development of this platform, the characterization of the automated derivatization process by GC-MS, and its comparison to traditional manual techniques are described in this presentation.

170 **Signal Enhancement of Organic Acids in Supercritical Fluid Chromatography-Mass Spectrometry Using a Piperidine-Aniline Derivatization Tag**

John Boughton, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Faith Wroniuk, Yih Ling Saw, Lark Perez, James Grinias

The analysis of polar organic acid metabolites by traditional LC-MS techniques often proves challenging. The need for long equilibration times in the HILIC separations required for these polar compounds and the poor ionization efficiency frequently observed in negative mode electrospray ionization used for their detection by MS are key disadvantages. Here, a high proton affinity derivatization tag is used to label carboxylic acid groups and increase the detection sensitivity of these organic acid metabolites. The piperidine-aniline tag contributes a tertiary amine that significantly improves electrospray ionization signal in the positive mode. However, the tag was observed to make separation under traditional liquid-phase separation techniques more difficult. Instead, an SFC-MS method was developed that can be used to separate and detect the derivatized compounds. Data related to signal enhancement and method development for a mixture of commonly measured organic acids with 1-3 carboxylic acid groups is described.

171 **Experimental Design and Chemometrics in Undergraduate Quantitative Analysis**

Emily Manna, Lebanon Valley College, 1421 Mumma Rd., Annville, PA 17112, Michelle Rasmussen

The principles of chemometrics are not typically covered in undergraduate analytical chemistry curriculum. The goal of this work is to develop a laboratory activity for an undergraduate quantitative analysis lab that will introduce the students to experimental design and chemometrics. We developed a method for fabricating paper-based sensors using inexpensive lab materials. Our sensors have two reaction sites that can be used to detect two analytes simultaneously. We chose two simple colorimetric reactions for detecting two analytes. Students in the course were assigned varying conditions that were chosen using a two-factor central composite design by Design Expert. The goal of the activity is to maximize the sensitivity of each reaction. The students learned how to make the sensors and run them under their given conditions. Their results were then used to determine the optimal conditions for each reaction.

172 **Sensing Biothiols Using Luminescent Water-Soluble Au(I) Complexes Through Photoluminescence and Electrochemical Studies**

SunJin Kim, Lebanon Valley College, 5555 Merivale Court, Harrisburg, PA 17112, Michelle Rasmussen, Mukunda Ghimire

Biothiols play crucial part of cellular functions such as oxidative stress, metal binding, biocatalysis, detoxification etc. Such cellular functions are associated with a wide variety of chronic and degenerative diseases due to the changes in the level of biothiol. Studies have also demonstrated the importance of biothiols such as cysteine (Cys), homocysteine (Hcy) and glutathione (GSH) in metabolism and thereby affecting the function of immune system. Therefore, this project focuses in developing tools to monitor biothiol levels in immune cells in clinical samples using luminescent water-soluble Au(I) complexes with long lifetime and higher photoluminescence quantum yield (PLQY) when compared to organic fluorophores-based probes of bithiol sensors that are widely explored. These photophysical properties of Au(I) complexes should allow an increase in the selectivity and sensitivity towards biothiols including glutathione (GSH). Moreover, we will also explore an electrochemical method to simultaneously measure the biothiols levels to provide a suitable sensor for rapid, specific, and inexpensive sensors.

173 **Large-Scale Supercritical Fluid Chromatography Purification of Unstable STING Agonist Intermediates**

Dawn Sun, Bristol Myers Squibb, Department of Discovery Synthesis, Research and Early Development, PO Box 4000, Princeton, NJ 08540, Dauh-Rurng Wu, Peng Li, Henry Yip, Bei Wang, Xiaoping Hou, Rulin Zhao, Huiping Zhang, James Kempson, Arvind Mathur

A regioisomeric mixture of the nucleoside derivative, Intermediate 1, required resolution by preparative supercritical fluid chromatography (SFC) in order to obtain the desired regioisomer as a key intermediate in a STING agonist program. Various chiral columns and organic modifiers in carbon dioxide at different temperatures were screened to obtain the best regioisomeric resolution. A key issue associated with interconversion between the regioisomers via silyl migration during purification was investigated in methanol, acetonitrile, and the mixture of acetonitrile and isopropanol, and the optimal organic modifier in CO2 was established to mitigate the interconversion to an acceptable level (<5%). Taking into account peak resolution, throughput, interconversion and operation robustness, an efficient SFC method for large-scale purification was successfully developed and scaled up onto a 5 cm I. D. Chiralcel OJ-H column using 25% acetonitrile: isopropanol [1:1 (v/v)] with 0.1% ammonium hydroxide as the modifier in CO2 at a total flow rate of 270 mL/min and a temperature of 30 °C. In addition, continual evaporation (i.e. every hour) of the desired isomer fraction stream post-separation ensured minimal further interconversion. A total of 258 grams were separated at a high throughput of 8.6 g/h. Regioisomeric purity of the desired isomer of Intermediate 1 was ≥98.2% and the recovery was ≥90.2%. A similar purification strategy was applied to the regioisomeric resolution of Intermediate 2, an analog of Intermediate 1.

174 **Development of a Spectroscopic Screening Tool to Determine Optimal Sampling Sites for DNA Recovery from Human Skeletal Remains**

Cody Silverman, University of Albany-SUNY, 107 Beverwyck Dr., Apt. 8, Guilderland, NY 12084, Kathleen Smith, Angie Ambers, Brooke W. Kammrath

Numerous challenges exist with forensic genetic testing of human skeletal remains due to diagenesis patterns in bone microstructure, DNA degradation, and the presence of PCR inhibitors. Diagenesis is the microscopic breakdown of the bone matrix, which consists primarily of mineralized calcium hydroxyapatite and collagen. The process of diagenesis occurs in a heterogeneous, non-uniform manner along the diaphysis of a long bone, and determining the region with the most intact bone microstructure is not possible with the naked eye. Therefore, taking cuttings from the diaphysis for DNA testing is a blind process, and decades of research and casework have demonstrated that differences in DNA recovery do exist between cuttings along the shaft of the same long bone. An additional consideration is that forensic genetic testing of bones is a time-consuming and labor-intensive process. Development of an effective screening method to determine the optimal sampling site(s) on the diaphysis could reduce time, labor, costs, and the degree of destructive sampling necessary to obtain a DNA profile. Non-destructive Raman spectroscopy could serve as a reliable screening tool to obtain information about bone microstructure and stage of diagenesis which, according to previous research, often correlates to the quantity and quality of endogenous DNA within that region of bone. This approach could help maximize DNA recovery and improve success rates in unidentified human remains (UHR) investigations.

175 **The Importance of a Comprehensive Raman Spectral Library for the Identification of Minerals in Soil**

Chase Notari, University of New Haven, 33 Northwoods Ln., Middletown, CT 06457, Brooke Kammrath

Raman spectroscopy is a valuable tool for elucidating the chemical structure and more of an unknown sample. A comprehensive collection, or library, is required for the proper identification of any material. Searchable spectral libraries have demonstrated value for the identification of a plethora of forensic samples, such as drugs, organic pigments and polymers. Mineral analysis is another opportunity where a comprehensive searchable Raman spectral library could aid in the identification of samples for both geological and forensic purposes. However, while there have been several collections of mineral spectra created, there remains to be one comprehensive searchable Raman spectral library. Programs like KnowItAll currently only have a few hundred profiles, and other databases like RRUFF do not have a capability to compare an unknown sample to the library, thus a comprehensive database is required. Another important consideration is the challenge associated with the natural variations within a mineral variety which can cause spectral differences. This research aims to create a comprehensive spectral library of minerals that addresses these issues and also is evaluated for its ability to accurately identify samples from a known set of 60 common soil minerals.

176 **Investigating Pharmaceutical Frozen Solution Using 31P and 1H Solid-State NMR**

Yong Du, Merck & Co., Inc., Rm 2810, Building 80B, 126 E. Lincoln Ave, Rahway, NJ 07065, Jinghan Li, Raj Suryanarayanan, Yongchao Su

A substantial number of protein therapeutics and vaccines are stored in a frozen state (e.g., at temperatures below -20 °C) in order to enhance product stability. However, the freezing process can induce stresses including cold temperature, solute concentration due to the ice formation, eutectic crystallization of buffer solutes, and resultant pH shifts. These microenvironmental conditional change may result in instability of proteins. For example, a pH decrease of 3-4 units has been reported in phosphate buffered protein solutions during freezing. Therefore, a mechanistic understanding of freeze-thaw cycling is of vital importance for the rational design of biological drug products. In the present study, we, for the first time, have developed a solid-state nuclear magnetic resonance (ssNMR) protocol to characterize the phase behaviors of mobile and frozen phosphorous species in sodium phosphate buffered frozen solutions. Using 31P ssNMR, the selective crystallization of disodium hydrogen phosphate as a dodecahydrate ($Na_2HPO_4{\cdot}12H_2O$) can be readily identified and quantified. The pH shift as a consequence of $Na_2HPO_4{\cdot}12H_2O$ crystallization was correlated to the freezing temperatures. Moreover, 1H NMR was shown capable of quantifying the unfrozen water content. The effect of freezing temperature, buffer and trehalose concentration, and freezing speed on the unfrozen content was systematically investigated. In summary, this study demonstrates that ssNMR is a promising biophysical technique in the characterization of frozen solutions, which can be widely applied to obtain critical knowledge of the physiochemical stability of therapeutical proteins in their frozen state.

177 **Innovative Chromatographic Approaches to Improve the Characterization of Complex Biopharmaceutical Products**

Davy Guillarme, University of Geneva, School of Pharmaceutical Sciences, CMU – Rue Michel-Servet 1, 1211 Geneva 4, Switzerland, Amarande Murisier, Szabolcs Fekete

The therapeutic success of monoclonal antibodies (mAbs) in the treatment of cancer has contributed to their rise, ranking six mAbs and derived products among the 10 best-selling drugs in 2020. From production to patient administration, a complete characterization of therapeutic proteins and their variants must then be performed by analytical methods, to ensure product safety and inter-batch reproducibility. The aim of this presentation will be to introduce some innovative chromatographic approaches recently developed in our laboratory, to improve the possibilities offered by chromatographic techniques for the analysis of therapeutic proteins. A first innovative approach is based on the use of ultra-short columns (only a few millimeters) in RPLC and IEX, to obtain separations as efficient as with standard size columns, but with significantly reduced analysis times. This behavior is due to the fact that large

proteins have very high S values (slope of the log k relation as a function of %ACN), and an on-off (also known as bind-elute) retention mechanism. Analyses of mAbs and immunoconjugates (ADCs) have been successfully performed in only a few tens of seconds. The second approach is based on the use of special gradient conditions, allowing to significantly improve the selectivity between different protein isoforms. One or more isocratic steps were added during the RPLC analysis to increase the selectivity between the chromatographic peaks. Thanks to this principle, we were able to demonstrate infinite selectivity between protein chromatographic peaks, simply by adding suitable isocratic step.

178 **Three More Chromatographic Questions Needing to be Answered**

Mark Schure, Kroungold Analytical, Inc., 1299 Butler Pike, Blue Bell, PA 19422

In this award section honoring Fabrice Gritti, I will cover three questions with short presentations on each. These questions are: Q1: How to relate noise filter properties used in chromatography to the loss of resolution and plate height?. Q2: What type of pore dynamics and/or retention energetics causes tailing? Q3: Is it possible to make a statistical overlap theory with multiple channel detectors like mass spectrometry? What does peak capacity mean in this context? These questions will be answered with simple formulas, examples and consequences!

179 **Fabrice Gritti: Chromatographic MythBuster**

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

Fabrice Gritti published over 300 peer reviewed papers since receiving his Ph.D. (2001). In his publications (often with his mentor professor Guiochon) he investigated phenomena affecting the liquid chromatography (LC) performance such as adsorption isotherms, mass transfer kinetics, chromatographic peak shapes, ionic interactions, and packing efficiency. In the process he dispelled many myths and incorrect interpretations of LC principles. For example, Fabrice proved that on column frictional heating has strong impact on separation performance of columns packed with sub two-micron particles. His work altered the design of UPLC column and instruments. Fabrice also studied the core-shell particle technology and shown that performance gains of these columns is driven by B-term of the vanDeemter curve rather than by a narrow particle size distribution (A-term). In the series of publications, he built on work of others and clarified the mechanism of reversed-phase columns de-wetting, a phenomenon that puzzled separation scientist for decades. He also illustrated the impact of column packed bed heterogeneity on the analyte zone shape and the apparent column efficiency. The list of Fabrice's accomplishments can (and will) continue, since he is young and productive. Fabrice, my congratulations to the EAS Award for Outstanding Achievements in Separation Sciences!

180 **Retention Mechanism in Reversed Phase Liquid Chromatography. Past, Recent, and Future Research Investigations**

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

Most liquid chromatography methods developed in the industry (pharmaceutical, biological, and food) are based on reversed-phase liquid chromatography separation methods. After more than fifty years of research aiming at speeding up RPLC method development in these industries, the practitioners are still missing a sound and robust chromatography software based on the true retention mechanism yet to be revealed in RPLC for small molecules. This ongoing situation has led method development to be mostly supported by empirical, statistical, and artificial intelligence-based models essentially void of physical sense. Therefore, it requires training and calibration steps which remain expensive, time consuming, and risky. The determination of the true retention mechanism in RPLC has been the source of many theories and controversies in the last five decades. In this presentation, we will then first review past and recent research achievements that aimed at delivering a sound description of the retention mechanism in RPLC. Their debunk (from experimental data) or their intrinsic flaws (from the laws of chemistry) will be discussed. To date, there is still no clear and obvious retention models capable of accurately predicting retention in RPLC. In the second part of this presentation, we will then propose and discuss some future alternative tools (pore reconstruction, molecular dynamics simulation) that could possibly unravel the long lasting hidden secrets of retention in RPLC since the very first silica-C18 material has been synthesized in the late 1960s (by Halasz) and early 1970s (by Kirkland).

181 **How to Crack the Glass Ceiling: Diversity and Inclusion in Chemistry**

Kevin Middleton, Cannabiz Labs, 446 Midway Point Ellenwood, Atlanta, GA 30294

A diverse workforce and inclusive workspaces are critical for the advancement of Science and for the future of Chemistry as an Industry. Diversity provides the potential for greater innovation and creativity in the workforce. Inclusion is what enables organizations to realize the social, business and economic benefits of its diversity potential. Achieving diversity and inclusion in Chemistry has never appeared more pressing or more challenging. Companies need the innovation and superior performance that a diverse workforce yields. Throughout history, the chemical industry has come together to enhance understanding of and provide solutions for the world's largest challenges. The good news is that a majority of companies are now seeking more innovative ways to pursue diversity but for some under-represented minorities, the universal challenges of diversity, inclusion, equity and respect can be compounded by a lack of representation, discrimination, a general sense of feeling alone, and many other unconscious and insidious biases. The purpose and goal of this lecture is to discuss my personal experiences and challenges with Diversity and Inclusion in Chemistry, the barriers and best practices for creating a more diverse environment and looking at diversity and inclusion from an employer and employee standpoint.

182 **My Journey to Discovery Chemistry and Drug Regulatory Affairs**

Sherrie Pietranico-Cole, Novartis Pharmaceuticals Corporation, One Health Plaza, East Hanover, NJ 07936

Sherrie Pietranico-Cole is an alumnus of Rutgers University, Douglass College, where she received a BA in Chemistry in 1986. After graduating from Rutgers, Sherrie became the first African American woman to graduate from the University of Pennsylvania with a PhD in Chemistry in 1992. Sherrie joined Discovery Chemistry at Hoffmann-La Roche in Nutley, NJ where she rose to the position of Sr. Research Leader and spent 20 years discovering drugs for the treatment of inflammatory diseases, metabolic diseases, and viruses. Sherrie led the chemistry team that discovered Resmetirom (MGL-3196) which is currently in Phase 3 clinical trials for the treatment of NASH. In 2012, when the Roche site in Nutley closed, Sherrie joined Novartis Pharmaceuticals Corporation in East Hanover, NJ and transitioned to a career in Drug Regulatory Affairs. Sherrie will take you through her journey to Discovery Chemistry and Drug Regulatory Affairs and provide insights about careers in Medicinal Chemistry and Drug Regulatory Affairs. *The views and opinions expressed in this presentation are those of the author and do not necessarily reflect the official policy or position of Novartis Pharmaceuticals Corporation, Madrigal Pharmaceuticals, and Hoffmann-La Roche or any of its officers.

183 **Portable Raman Spectroscopy for Screening of Phthalate Plasticizers in Food Contact Materials via Chemometrics and Library Spectral Matching**

Joshua Moskowitz, University of Maryland, Joint Institute for Food Safety and Applied Nutrition, 2134 Patapsco Building, College Park, MD 20742, Katherine Carlos, Luke Lindahl-Ackerman, Kristen Reese, Betsy Yakes

Plasticizers may be present in food contact materials such as packaging, containers, and production line tubing to lend softness and flexibility to the materials. Specifically, ortho-phthalate plasticizers (hereafter referred to as phthalates) have received attention for potential adverse health effects. Portable and rapid analyses of plasticizers in food contact materials are advantageous, allowing for onsite detection of plasticizers at various stages of the food production process. Here, we investigate the application of portable Raman spectroscopy to the identification of various plasticizers in PVC food contact materials. This is accomplished with the use of either "on-instrument" chemometric modelling with a 785 nm Raman instrument or "on-instrument" library spectral matching with a 1064 nm Raman instrument. The library and chemometric methods function via the comparison of Raman spectra between unknown food contact tubing samples and a panel of previously scanned, known tubing samples. These two methodologies will be compared, as each offers distinct advantages in portable analysis. In addition, gas chromatography-mass spectrometry (GC-MS) and direct analysis in real time-MS (DART-MS) methodologies are used to confirm plasticizer identities and evaluate the portable Raman spectroscopic methods. This work may provide information regarding the prevalence of various plasticizers in PVC food contact materials.

184 **Rapid Screening of New Psychoactive Substances in Suspect Counterfeit Tablets using SERS, FT-IR and DART-TD-MS**

Martin Kimani, United States Food & Drug Administration, 6751 Steger Dr., Cincinnati, OH 45237

The Centers for Disease Control and Prevention estimated 100,306 drug overdose deaths in the U.S. during the 12-month period ending in April 2021, mostly attributed to synthetic opioids. The smuggling of illicit synthetic drugs such as fentanyl and 2-benzylbenzimidazole analogs (nitazenes) through the international mail is a growing concern and has negatively impacted the fight against the opioid epidemic in the United States. In 2018, the Substance Use-Disorder Prevention that Promotes Opioid Recovery and Treatment [SUPPORT] Act was enacted to combat the ongoing opioid crisis and importation of 'articles of concern'. In response, the FDA's Forensic Chemistry Center launched a satellite laboratory at the Chicago International Mail Facility (IMF) that uses field portable analytical instruments to screen suspect pharmaceuticals. The satellite laboratory houses a three-device "portable toolkit" consisting of a handheld Raman, direct analysis in real-time mass (DART-TD-MS) and Fourier transform infrared (FT-IR) spectrometers and has examined over 1,500 samples and helped prevent over 350,000 dangerous lot units from reaching the US supply chain. FDA's Office of Enforcement and Import Operations seized several suspect oxycodone HCl tablets at the Chicago IMF between December 2021 and May 2022,

which were transferred to FDA's on-site satellite laboratory for analysis. The laboratory confirmed the presence of 2-benzylbenzimidazole analogs in these tablets. This presentation discusses ongoing work at the Chicago satellite laboratory and detail the analysis of 2-benzylbenzimidazole analogs in suspect counterfeit tablets using a combination of complementary and orthogonal techniques, including surface enhanced Raman scattering spectroscopy (SERS), FT-IR, and DART-TD-MS.

185 **Portable Instrumentation for the Screening of Explosives**

Gina Guerrera, United States Federal Bureau of Investigation, Redstone Arsenal, 4940 Fowler Rd., Huntsville, AL 35898

This presentation discusses the importance of portable instrumentation in the field of explosives and the various kinds of instruments that are currently being used. Where explosives are suspected, portable instrumentation allows for rough, quick information that can help investigators/bomb techs on scene. They can assess how serious a given situation, along with what evidence should be collected and sent to the laboratory. These portable instruments also serve as a form of protection for those handling unknown material. It provides screening data to help ascertain how sensitive a material may be. This information aids in determining the most safe and efficient way of removing or rendering safe explosives on-scene. In addition, field screening for explosives provides an avenue for leads in investigations. The FBI Laboratory has worked with other agencies and manufactures to add Raman and infrared (IR) spectra of explosives to these portable instrument libraries from material identified in casework. Portable instrumentation for the screening of explosives serves a vital purpose and its technology continues to improve and develop for safer and more efficient ways of detecting and screening for explosive material.

186 **Street Chemistry: How are Portable Handheld Raman and Infrared Spectroscopy are being used by law Enforcements to Solve Crimes**

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

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

187 **Application of Electron Transfer Dissociation in Phosphoproteomics to Identify Rewiring of Kinase Substrate Specificity**

Danielle Caefer, University of Connecticut, 75 N Eagleville Rd., Storrs, CT 06269

When pathogenic mutations to protein kinases are investigated, two main effects are generally considered, loss of regulation or loss of catalytic activity. Here, we present multiple examples of pathogenic mutations that result in a third distinct outcome, a rewiring of substrate specificity. Okur-Chung Neurodevelopmental Syndrome (OCNDS) is broadly characterized by delayed psychomotor development and intellectual disability. It has been linked to multiple *de novo* heterozygous mutations to the CSNK2A1, the gene which encodes protein kinase CK2, a well characterized acidophilic, serine/threonine kinase. We applied the Proteomic Peptide Library (ProPeL) approach to generate high resolution specificity motifs to investigate the effect of OCNDS-associated mutations on CK2. Constructs containing CK2WT and OCNDS-associated mutants were expressed in *Escherichia coli* and were subsequently allowed to phosphorylate the endogenous proteome according to their native specificity. Phosphorylated proteins were identified using a ThemoFisher Orbitrap Eclipse Tribrid Mass Spectrometer. The specificity motifs derived in these experiments revealed that multiple OCNDS-associated mutations cause a rewiring of substrate specificity, shifting the preferences of CK2 away from the canonical wild-type preference for acidic residues at either the +1 or +3 positions relative to the central phosphoacceptor residue.

188 **Mucinomics as the Next Frontier of Mass Spectrometry**

Stacy Malaker, Yale University, 350 Edwards St., Rm 224, New Haven, CT 06511

Mucin domains are densely O-glycosylated modular protein domains found in a wide variety of cell surface and secreted proteins. Mucin-domain glycoproteins are key players in a host of human diseases, especially cancer, but the mucinome remains poorly defined. This is largely due to the challenges associated with studying mucin-domain glycoproteins, especially by mass spectrometry (MS). Recently, we characterized a suite of bacterial mucinases and demonstrated that their use in MS workflows enhanced sequence coverage, glycosite localization, and glycoform identification. We also employed inactive point mutants of these enzymes to strategically enrich mucin-domain glycoproteins from complex samples like cell lysates and crude ovarian cancer patient ascites fluid. Yet, many issues associated with studying mucin-domain glycoproteins remain. My laboratory focuses on developing methodology to analyze mucin-domain glycoproteins by MS. We also study mucin domains in a biological context since they play integral (yet poorly understood) roles in many clinically relevant glycoproteins. This presentation will highlight our current advances toward these goals, including optimized mucinase and enrichment methodologies, glycoproteomic software comparisons, and biological investigations into immune modulating mucin-domain glycoproteins.

189 **Analysis of Intact Proteins with Electron Transfer Dissociation, Proton Transfer Charge Reduction, and Parallel Ion Parking**

Seamus Kelley, University of Virginia, Department of Chemistry, McCormick Rd., Charlottesville, VA 22904, Jeffrey Shabanowitz, Donald Hunt

Electron transfer dissociation (ETD) is a useful analytical tool for protein primary structure elucidation. In ETD, a radical anion is reacted with a multiply charged macromolecule to transfer an electron and generate complementary fragments along the peptide backbone. ETD performs well on highly charged species and retains labile post-translational modifications (PTMs), like phosphorylation and O-linked glycosylation. When using ETD on intact proteins, higher-order fragmentation can occur. These higher-order ETD fragments are typically non-sequence informative and increase the amount of noise in a spectrum. To inhibit higher-order fragmentation, a supplemental waveform can be applied to the ion trap to kinetically excite the first-generation fragment ions. The kinetic excitation effectively "parks" these ions to prevent further reactions, hence the name parallel ion parking (PIP). Even with improvements to ETD with PIP, analysis of large species can still be limited by spectral congestion. Proton transfer charge reduction (PTCR) is a non-fragment generating reaction that removes a proton from a polypeptide, reducing its charge state. By performing PTCR on first-generation ETD fragment ions, we are able to prevent the overlapping of ions in the m/z plane, and spread the ion current across the entire scan window. In this presentation, I will detail the kinetic concerns with intact protein ETD reactions, demonstrate how PIP can alleviate these concerns, outline the utility of coupling ETD with PTCR, and discuss the results of pipETD/PTCR experiments on an intact protein.

190 **Addressing Biological Questions with Electron-Transfer Dissociation and High Field Fourier Transform Ion Cyclotron Resonance Mass Spectrometry**

Lissa Anderson, National High Magnetic Field Laboratory, 1800 E Paul Dirac Dr., Tallahassee, FL 32310, Chad Weisbrod

Intact protein analysis provides proteoform-specific understanding of biological phenomena that cannot be achieved by analysis of digested peptides. However, top-down analysis is complicated by incomplete protein separation, low fragmentation efficiency, slow spectral acquisition rate, low S/N, and high spectral complexity. These effects limit proteoform detection and characterization and grow exponentially worse as mass increases. The 21 tesla (T) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer at the National High Magnetic Field Laboratory (NHMFL) offers high mass resolving power, mass accuracy, dynamic range, and scan rate, and achieves unprecedented performance with respect to intact protein sequence analysis. The 21 T is part of the NHMFL FT-ICR User Facility and is available to all qualified users. Electron-transfer dissociation (ETD) offers several benefits for proteoform analysis, often outperforming collision-based methods with regard to confidence (q-value) in protein identification and degree (percent sequence coverage) of proteoform characterization. However, as protein size increases, total ion current is distributed among more fragment ion channels and greater ion populations or additional signal averaging are needed to achieve desired signal-to-noise ratios in ETD product ion spectra, lengthening experiment time. We will provide performance benchmarks of the 21 T FT-ICR mass spectrometer for characterization of intact proteins, describe fundamental theory, instrumentation and methods for performing time-efficient ETD experiments, and highlight results from top-down proteomics projects employing ETD.

191 **Creating more Efficient, Less Hazardous Syntheses of Pharmaceuticals Using the 12 Principles of Green Chemistry**

Loyd Bastin, Widener University, 1 University Place, Chester, PA 19013

We have used the 12 principles of green chemistry to redesign the syntheses of two classes of pharmaceutical compounds: 3,5-diarylisoxazoles and phenylmaleimides. Isoxazoles are a group of pharmaceuticals that are used as anti-convulsant, anti-epileptic and anti-inflammatory drugs. The conventional process used to make these molecules uses a number of hazardous chemicals and produces large amounts of waste. We have developed a more environmentally-friendly 4-step synthesis that yields multiple isoxazoles, uses less hazardous reagents, reduces waste, is more energy efficient, and is cost effective. Maleimides are essential building blocks for a number of pharmaceuticals and herbicides. We have developed a three-step synthesis for a variety of phenylmaleimide derivatives that has high atom economy, is energy efficiency, reduces waste and use of hazardous reagents and solvents, and is extremely cost effective.

192 **Green Chemistry: From Fundamentals to Applications**

John Wasylyk, Bristol Myers Squibb, One Squibb Dr., New Brunswick, NJ 08620, Robert Wethman, Ming Huang

Over twenty years ago, the twelve green chemistry principles were introduced to influence the chemistry field with the goal of lessening the negative impact on the environment. As a result, a wide range of researchers have embraced as many of the principles as possible, accepting green chemistry as an approach to the design, development, and manufacture of chemical products by intentionally reducing or eliminating toxic and hazardous chemicals and minimizing the waste generated during all stages of development and ultimately, manufacturing. This is accomplished by finding creative, alternative routes in all aspects of chemistry. There are numerous guides available that provide valuable insight into determining the hazardous level of solvents, reagents, catalysts, and starting materials. Likewise, tools are available to track greenness of chemical reactions and processes that allow the user to track the impact of the synthetic scheme will have on the environment. This presentation will cover the green principles, applications of various guide and tools used during the entire development cycle leading to manufacturing and will cover organic, inorganic, and analytical chemistries.

193 **Raising Awareness: The Successful Implementation of Natural Plant Based Medicines Used as Adjunct Therapies with Standard Treatments for Metastatic Breast Cancer**

Jaime Brambilla, Grace Health and Wellness, Weston, CT 06883

My wife Nicole, at 49, was diagnosed with ER+, PR-, HER2+, and BRCA- invasive ductal carcinoma, which progressed metastatically to include bone, liver, and lymph node involvement. At the end of a five-month cannabinoid treatment period, PET/CT investigations revealed no evidence of metastatic disease, and chemotherapy was withdrawn. Over the last 15 years, a considerable body of in-vitro and in-vivo evidence supports the anti-neoplastic properties of cannabinoids and, more recently, psychedelics. Real-world evidence is reported of the therapeutic effect of cannabinoids and psychedelics in reducing tumor proliferation and aiding as a palliative medicine to treat pain and psychological distress associated with cancer and chemotherapy. The data presented here, on data published on Nicole's treatment, indicates the potential therapeutic utility of such adjunctive pharmacological interventions in individuals with metastatic breast cancer. Careful cannabis genetic selection, controlled environment cultivation, and extraction techniques were required and chromatographic analysis across each batch to consistently reproduce these specialized combinations. Cannabinoid drug discovery and standardized reproducibility are necessary for FDA/EMA/MHRA oversight and approval of these formulations to make these treatments legally available without restriction and prescribed by physicians. Only the most sophisticated and sensitive methods for chemical and molecular analysis can be used to identify all the compounds present in botanical drug preparations. Further, this is needed to screen for the therapeutic indication and discover the specific sets of molecules activating the different mechanisms of action producing the anticancer effect that we have observed in Real-World evidence.

194 **Is it Marijuana? Is it Hemp? Perhaps? A Brand's perspective on cannabinoid analysis**

Robert Rankin, Nice Cannabis, Farm Foundry, 1053 E Whitaker Mill Rd., Raleigh, NC 27064

A critical component to bring product to market in the cannabis industry is a successful certificate of analysis, CoA. Here, an overview on the importance of lab testing from a perspective of a hemp/cannabidiol (CBD) brand will be presented. Specifically, what are the consequences of inaccurate or indadequate products testing and how does this set the cannabis industry back in making progression.

195 **Leveraging Advanced Mass Spectrometry Tools to Explore Complex Cannabinoid Distributions.**

Alexander Aksenov, University of Connecticut, 55 North Eagleville Rd., Unit 3060, Storrs, CT 06269, Alexey Melnik

Mass spectrometry (MS)-based metabolomics have been increasingly driving discoveries in many areas, particularly biological research. Over past several years, multiple amazing breakthroughs in ways to extract new knowledge from existing MS data have been made. However, adoption and thus taking advantage of these new methods has been slow in disciplines that also utilize MS, such as cannabis research and analysis. Gas chromatography-mass spectrometry (GC-MS) is the most common MS technique, and the key analytical technology used in cannabis testing. Surprisingly, researchers still use decades-old data analysis strategies. We have developed an approach to explore GC-MS data via molecular networking, which allows considering molecular families as opposed to individual annotations [1]. The molecular networking has existed for number of years for liquid chromatography-tandem mass spectrometry (LC-MS/MS) and has led to many groundbreaking discoveries. Molecular networks can then be visualized for in-depth exploration of the data. We show how complex chemistry of cannabis can be explored with molecular networks. The scope of applications of networks is likely to be expanded as community finds new and innovative ways to utilize molecular networking to further enhance GC-MS data analysis.

Reference:

[1] Aksenov. A. et al. Algorithmic Learning for Auto-deconvolution of GC-MS Data to Enable Molecular Networking within GNPS. Nature Biotechnology. 39 (2), 169-173 (2021).

196 **Cannabinoid Composition Analysis by Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry**

Gregory Sotzing, University of Connecticut, Institute of Materials Science, 97 N. Eagleville Rd., Storrs, CT 06269

The certificate of analysis utilizing techniques such as high-performance liquid chromatography and gas chromatography using cannabinoid standards for both identification and content analysis is required for the sale of cannabinoid products. Nuclear magnetic resonance (NMR) spectroscopy is a gold standard for the analysis of organic compounds using both 1H and 13C nuclei chemical shifts and spin-spin coupling to identify structure. Phytocannabinoids, naturally occurring terpenophenolic compounds, are particularly unstable to heat, oxygen, light and acid. For example, heating of cannabidiol (CBD) under anaerobic conditions will result in the formation of both delta-8 and delta-9 tetrahydrocannabinol (THC). Further, heating of CBD in the presence of acid will catalyze this transformation. Shelf-life, heat and solution stability need to be considered when handling most cannabinoids. The analysis of cannabinol (CBN) from CBD will be reported comparing 1H NMR with results obtained from certificate of analysis (HPLC). Focus will be on comparing both techniques, considering both their pros and cons.

197 **Systematic RPLC Method Development for an Important Class of Pharmaceutical Compounds Possessing Ketoamide Group**

Nilusha Padivitage, Merck & Co., Inc., Analytical Research & Development, MRL, Rahway, NJ 07065, Charlie Wolstenholme, Steve Castro, Brittany Kassim, Yong Liu, Jinjian Zheng, Paul Bulger

The organic compounds with ketoamide functionality have been widely exploited to a huge range of biological targets as effective therapeutic agents such as anti-viral, anti-bacterial, anti-HIV, anti-tumor and anti-inflammatory drugs etc. These compounds can undergo hydrolysis, keto-enol tautomerization, cis-trans interconversion, dimerization through Michael addition mechanism in aqueous conditions. Thus, it is very challenging to analyze these compounds and their related impurities/isomers by Reversed Phase HPLC (RPLC), the most versatile analytical method for pharmaceutical analysis. In this work, we present a systematic approach for RPLC method development to address these challenges. Hybrid columns (Waters ACQUITY BEH C18, BEH Phenyl) were used due to their applicability to mobile phase with wide range of pH and temperature. Since ketoamide is prone to dimerize/oligomerize and epimerize at basic pH, mobile phase pH was optimized. At acidic pH, the ketone group can undergo hydrolysis to diol or hemiacetal/acetal if alcohol based solvents are used as mobile phase. This results in broad peak shape, which is detrimental for accurate analysis of impurities. To overcome the effect, high column temperature was employed to accelerate the kinetic of interconversion from ketone to diol or hemiacetal/acetal, consequently, sharper peak shape was obtained. Finally, the gradient was further optimized to achieve well-resolved peaks of interest.

198 **Exploring the Improvements Enabled by 1.5 mm ID UHPLC SPP Columns**

Stephanie Schuster, Advanced Materials Technology, Inc., 3521 Silverside Road Quillen Building, Ste. 1-K Wilmington, DE 19810, Peter Pellegrinelli, Conner McHale, Benjamin Libert

The LC separations community is trending to the use of UHPLC instruments with reduced-bore analytical columns. Even for analysts using 3.0 mm or 2.1 mm (ID) analytical columns, an additional boost in sensitivity and further solvent savings are highly desirable. These two advantages – increased sensitivity and reduced solvent consumption – are typically associated with using smaller ID columns in LC and LCMS analyses. However, moving to smaller ID columns requires making delicate connections to instruments with miniscule volumes that are highly susceptible to extra column volume effects of the LC system. Additionally, specialized pumps are required to accurately deliver very low mobile phase flow rates. Without moving to a specialized HPLC system, these impediments present a significant chromatographic challenge. To meet this challenge, novel 1.5 mm ID columns have been developed for use with low dispersion UHPLC instruments. The impact of reduced dispersion through LC and LCMS system optimization including proper selection of connecting tubing will be highlighted. These novel columns exhibit exceptional performance, offer ease of implementation due to their hardware design, and are designed for all commercial UHPLC instrumentation in both small molecule and bio workflows. Benefits of moving from 2.1 mm to new, robust 1.5 mm ID columns will be demonstrated for low molecular weight pharmaceuticals and other established methods. A case study with 1.5 mm columns will be presented using LCMS analysis of drugs of abuse and metabolites where sample size can be limited.

199 **Trace Corrosion of Stainless Steel HPLC Components from Common Mobile Phase Additive and the Deleterious Impact on Separations**

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

At the 2021 Eastern Analytical Symposium, we presented on the impact that mobile phase solvents such as water, methanol, and acetonitrile can have on a variety of commonly used metal alloys like Stainless Steel, MP35N, and titanium for HPLC equipment. A question on mobile phase additives was brought up, and this talk will be a follow-up from the 2021 talk. Here, we investigate the impact that mobile phase additives such as TFA, TEA, HFIP, etc. have in the form of corrosion or metal ion leaching from a stainless steel HPLC component. We look at both aqueous and organic solvents as well as the impact that coatings deposited via chemical vapor deposition can have to reduce the impact to the steel substrate. The issues these metal ions and corrosion from various components can have on separations as well as solutions from leading equipment manufacturers will be discussed.

200 **Characterization of Zwitterionic HILIC Columns Based on Ethylene-Bridged Hybrid Particles**

Thomas Walter, Waters Corporation, Chemistry R&D, 34 Maple St., Milford, MA 01757, Bonnie Alden, Kenneth Berthelette

Hydrophilic Interaction Chromatography (HILIC) is one of the most effective approaches for separating mixtures of polar analytes. Of the stationary phases used for HILIC, zwitterionic chemistries have been among the most popular due to their good retention for a wide range of polar compounds, including neutrals, anions and cations. We recently developed a zwitterionic stationary phase based on ethylene-bridged hybrid (BEH) particles that exhibits strong retention and stability from pH 2 - 10. We have characterized the water layer thickness for this stationary phase as a function of the water content and buffer concentration of the mobile phase, as well as temperature. These results will be compared to those for other HILIC stationary phases, and the correlation with retention will be discussed. We also investigated the equilibration volumes required to achieve stable retention for mobile phases having different water concentrations. The relationship between the equilibration volume and the water layer thickness will be discussed. Applications of the hybrid zwitterionic HILIC columns for separating several important classes of polar analytes will also be shown.

201 **High-Performance Thin-Layer Chromatography and Morpho-Anatomy and of *Monteverdia Ilicifolia* "Espinheira-Santa" and its Adulterants**

Kevin Antunes, State University of Ponta Grossa, Ponta Grossa, Paraná, Brazil, Valter Paes de Almeida, Luciane Mendes Monteiro, Wilmer Perera, Christopher Howard, Eike Reich, Gustavo Heiden, Ernestino de Souza Gomes Guarino, Vera Lúcia Pereira dos Santos, Vijayasankar Raman, Jane Manfron

Monteverdia ilicifolia (Mart. ex Reissek) Biral (syn. Maytenus ilicifolia), commonly known as "espinheira-santa", are widely used in South American folk medicines to treat gastritis and ulcers. Several herbal products containing the leaves of M. ilicifolia are sold on the market. Many other species with similar leaf morphology are also called espinheira-santa and used for the same purpose. The most common adulterants that show morphological similarities to M. ilicifolia are Monteverdia aquifolia (Mart.) Biral [Celastraceae], Sorocea bonplandii (Baill.) W.C.Burger, Lanj. & Wess. Boer, [Moraceae], Zollernia ilicifolia Vogel [Fabaceae], Jodina rhombifolia (Hook & Arn.) Reissek (recognized as espinheira-de-três-pontas) [Santalaceae], and Citronella gongonha (Mart) R.A.Howard [Cardiopteridaceae]. This study aimed to differentiate M. ilicifolia from its adulterants by morphological, microscopic and HPTLC techniques. The morpho-anatomical studies of the leaves and stems of M. ilicifolia and its adulterant species have revealed noteworthy features that can help species identification. In addition, the comprehensive HPTLC analysis enables unambiguous identification of M. ilicifolia and quality control of commercial espinheira-santa.

202 **Hair, Hair Follicle, and Sebum Lipids Evaluation Using HPTLC**

Ernesta Malinauskyte, TRI Princeton, 601 Prospect Ave., Princeton, NJ, 08540, Katerin Mateo

Lipids are responsible for the maintenance of the structural integrity of skin and hair. We utilized silica gel high-performance thin-layer chromatography (HPTLC) to separate the lipids and densitometry to quantify the levels of free sterols, free fatty acids, ceramides, triacylglycerol, and squalene in hair, hair follicle, and skin. The talk will reveal differences amongst them, challenges, and solutions for such analyses.

203 **HPTLC 4.0 - The Future of Planar Chromatography?**

Eike Reich, HPTLC Association, Dianastrasse 10, Rheinfelden, AG, CH-4310 Switzerland

With the commercial introduction of plates coated with fine particles by MERCK in the late 1970s, the foundation for the evolution of HPTLC was laid. Over the last four decades, significant progress has been seen in the development of instruments. Most recently, even fully automatic systems became available. The available tools - instrument and software - have supported scientific research of all aspects of planar chromatography, stimulated curiosity, and expanded the range of what is possible. Yet, it seems that it was the discussion of a rigorous concept of standardization, its subsequent implementation into pharmacopoeias, and the resulting enforcement by authorities, which made HPTLC a distinct and accepted technique for routine analysis, particularly in quality control of herbal materials. What will be next? This presentation connects fundamental concepts, such as standardization and comprehensive HPTLC fingerprinting, with current ideas about the evaluation of system suitability, comparison of data across multiple plates, and the use of complementary developing solvents, leading to HPTLC 4.0. This vision may be developed into a global platform for collaboration of researchers and routine users of HPTLC. It has the potential of taking the inherent advantages of planar chromatography into a bright future.

204 **Psilocybe: Potency of Active Compounds, Psilocybin and Psilocin. A Single Lab Validation Using HPTLC, LC/MS/MS.**

Sidney Sudberg, Alkemist Labs, 12661 Hoover St., Garden Grove, CA 92841

Psilocybe mushrooms have been in the news lately reflecting a growing interest from the psychology sector for its potential therapeutic value with Post Traumatic Growth (PTG) and other psychological issues. In this presentation a HPTLC phytochemical constituent profile will be demonstrated, as well as the quantitation and validation of the analysis of the major active components, psilocybin and psilocin. The results of HPTLC quantitation will be compared with HPLC & LC/MS/MS data.

205 **MicrobeMASST - Detection of MS/MS Spectra in a Bacterial and Fungal Reference Database**

Simone Zuffa, Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, 9500 Gilman Dr., La Jolla, CA 92093, Robin Schmid, Anelize Bauermeister, Andres Mauricio Caraballo Rodriguez, Emily Gentry, Paulo Wender Portal Gomes, Michael Meehan, Mingxun Wang, Pieter Dorrestein

Bacteria, fungi and other microorganisms play a fundamental role in the regulation of complex ecosystems, from the oceans to the human gut. The rapidly developing metabolomics field aims to characterize the biochemical profiles of diverse samples using high-throughput technologies, such as mass spectrometry (MS). This highly-sensitive technique can generate thousands of tandem MS (MS/MS) spectra per sample but spectral annotation remains one of the main bottlenecks, leading to hundreds of unannotated features per experiment. We developed microbeMASST to facilitate understanding if detected features of interest from complex biological samples have microbial or fungal origins. This new search tool allows users to look for MS/MS spectra against a curated reference database of more than 60,000 monocultures of bacteria and fungi, returning results into a taxonomically informed tree. We showcase an example of a newly identified conjugated bile acid, phenylalanine-cholic acid (Phe-CA), which has been associated with inflammatory bowel disease (IBD). Phe-CA was found in cultures of bacteria that have been previously associated with IBD, such as *Enterococcus faecium*, *Fusobacterium nucleatum*, and *Burkholderia*. MicrobeMASST is a powerful tool that complements the already established Global Natural Product Social Molecular Networking (GNPS) ecosystem, allowing users to postulate mechanisms and develop hypothesis-testing follow-up studies.

206 **Toward High-Throughput Metabolic Phenotyping in Synthetic Biology with Desorption Electrospray Ionization-Mass Spectrometry Imaging**

Hawkins Shepard, Vanderbilt University, 2201 West End Ave., Nashville, TN 37235, Jody May, John McLean

Current advances in synthetic biology have allowed for genetic engineering strategies to outpace the screening capabilities of traditional analytical workflows. To improve the throughput of screening chemical signatures obtained from genetically-edited microorganisms, an untargeted analytical workflow using desorption electrospray ionization-mass spectrometry imaging (DESI-MSI) was developed for the direct analysis of microorganisms in situ. This DESI-MSI method can differentiate individual phenotypes from directly imaged bacterial co-cultures using an unbiased segmentation algorithm which identifies statistically unique chemical regions within the MS images using spatial shrunken centroids. While direct DESI-MSI analysis alleviates sample preparation requirements compared to traditional LC-MS, the resulting MS images needed for the segmentation approach still require several hours to generate, which is not amendable to high-throughput screening. To further accelerate metabolic phenotyping, a single raster "fast pass" strategy is described which implements line scans to reduce data acquisition to the minute timescale. In order assess the effectiveness and spatial resolution of this fast pass method, several different analyte classes were evaluated including free fatty acids, carbohydrates, peptides, and exogenous drug compounds. Preliminary results demonstrating an increase in the sample throughput by an order of magnitude while obtaining similar chemical information from individual sample spots in comparison with a traditional imaging approach. Current work involves the application and adaptation of the work-

flow to bioengineered bacteria in order to aid in the characterization of genetic edits associated with the production of economically-important chemicals.

207 **D-Alanine in Mammals: Where Does it Come from? Exploring the Microbiome-Gut-Brain Axis with LC-MS**

Chen Huang, University of Illinois, Neuroscience Program, Roger Adams Laboratory, 600 South Mathews Ave., Urbana, IL 61801, Tian Qiu, Cindy Lee, Stanislav Rubakhin, Dongkyu Lee, Jonathan Sweedler

While proteogenic L-amino acids are well studied in animals, the presence and function of several d-amino acids in mammals is much less understood. The sources of D-amino acids include intake of foodstuff, endogenous synthesis (racemization), and absorption from the gut-microbiome. Recently, D-serine, D-aspartate and D-cysteine have been reported in the mammalian brain and endocrine systems and endogenous synthesis were documented. D-alanine, like the more extensively studied D-serine, binds to NMDA receptors and regulates NMDAR activity. It has been implicated in several mental disorders and can be found in many parts of the body. However, for D-alanine, the source is not well known. Here we explore the sources of D-alanine including from diet, endogenous synthesis and the gut-microbiome. To determine its source, we use germ-free mice that lack a healthy gut-microbiome, and introduce stable isotopically labeled D-Ala. We then assay various tissues using quantitative mass spectrometry. We demonstrated that D-alanine can be produced by the gut-microbiome, absorbed by the gut and small intestines, and distributed throughout the body including the Islets of Langerhans and the brain.

208 **Metabolomics - A Discovery-based Approach in the Infection Relevant Environment**

Neha Garg, Georgia Institute of Technology, 950 Atlantic Dr., Atlanta, GA 30332, Andrew Mcavoy

The *Burkholderia cepacia* complex (Bcc) consists of over 20 phenotypically diverse Burkholderia species that can cause opportunistic infections in immunocompromised patients and patients with cystic fibrosis, resulting in disparate clinical outcomes. Using untargeted metabolomics, we discovered a strain-specific new pathway for thiomethylation of trimethoprim and several endogenous metabolites. Furthermore, comparative metabolomics of several strains revealed strain-specific and antibiotic-dependent personalized responses to sub-lethal exposure to several antibiotics in chemical and physical environments representative of infection sites. Several of the strain-specific metabolites and their biosynthetic origin was identified using untargeted metabolomics of cultures grown in the presence of isotope-labeled amino acids. This work lays emphasis on the functions of antibiotics at sub lethal concentrations as signaling molecules leading to the induction of biochemical pathways involved in cell-to-cell communication positioning pathogens for colonization. We highlight the use of state-of-the-art untargeted metabolomics-based approaches as a discovery tool for identification of phenotype-specific metabolites and their biosynthetic pathways.

209 **Infrared Chemical Imaging: Uniting Theory, Modeling and Instrumentation for New Capabilities**

Rohit Bhargava, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, 405 N. Mathews Ave., Urbana, IL 61801

Infrared (IR) spectroscopic imaging allows the recording of molecular and structural properties of complex samples without the need for prior information on composition, dyes or stains. The spatial and spectral domains are coupled, presenting both opportunities and challenges for recording high-content, high-fidelity data that determine image quality. We first provide an optical theoretic and information theoretic approach to present a roadmap for the development of optical microscopy-based imaging. We show how the spatial quality is related to spectral data and provide explicit relationships to devise imaging configurations at performance limits. We show how molecular content over large areas can be recorded by new high speed microscopy configurations and present rigorous approaches to extending molecular understanding with linear and circularly polarized light. Finally, we present a detailed theoretical understanding of image formation in nanoscale spectroscopic imaging using an atomic force microscope in contact mode. A new method, based on null deflection of the cantilever, is introduced to provide high quality data.

210 **Nanoscale IR Spectroscopy: From Recent Technical Advances to Nanoscale Mapping and Identification of Metal Soaps in Oil Paints**

Andrea Centrone, National Institute of Standards and Technology, 100 Bureau Dr., Bld. 216, Rm. A219, Gaithersburg, MD 20899

Oil paints are mixtures of pigments, drying oils and additives. Painted artworks don't last indefinitely because humidity and temperature driven chemical reactions between oils and pigments form "metal-soaps" that, whether over a few years or centuries, can cause paint degradation. Though these compounds have long been found in oil paintings, for example by submicron-Fourier transform infrared spectroscopy μ-FTIR, researchers do not fully understand how they form and then proceed to damage artworks. In this talk, I will discuss the working principles and applications of two novel photothermal IR spectroscopy methods (AFM-IR[1-3] and O-PTIR[3]) that use, either an atomic force microscope (AFM) tip, or a visible laser beam, respectively, to bypass the diffraction-limit and to push the benefits of IR spectral analysis to the nanoscale (≈10 nm for AFM-IR, ≈500 nm for O-PTIR). As examples, I will determine the nanoscale distribution of metal-soaps in 1) a Zinc-containing oil paint of known average composition naturally aged for 23 years and 2) in the top layer of a French nineteenth-century painting (Gypsy Woman with Mandolin by Corot, c.1870) that contain lead-white and cobalt-green pigments along with metal-soap. Our measurements offer an unprecedented nanoscale composition-sensitive observation window on oil paints which will be critical to better understand chemical reactions in paints and to identify species with low average concentrations that are undetectable by FTIR. Finally, I will discuss recent AFM-IR advances from my lab.

References:

[1] Chem. Soc. Rev. 2022, 51, 5248.

[2] Chem. Soc. Rev. 2020, 49, 3315.

[3] Anal. Chem. 2022, 94, 3103.

211 **Stimulated Raman Scattering Microscopy: From Label Free to Metabolic and to Super-Multiplex Imaging**

Wei Min, Columbia University, Department of Chemistry, 3000 Broadway, New York, NY 10027

All molecules consist of chemical bonds, and much can be learned from mapping the spatiotemporal dynamics of these bonds inside cells, tissue and animals. Since its invention in 2008, stimulated Raman scattering (SRS) microscopy has become a powerful modality for imaging chemical bonds with high sensitivity, resolution, speed and specificity. The past dozen years have witnessed the blossoming of SRS microscopy, where advances in both optical instruments and imaging probes have found broad applications in life sciences. Here I present the exciting development in our group, from label-free imaging to metabolic imaging in animals to super-multiplexed imaging and to single-molecule vibrational imaging.

212 **Advancing Development of Biotherapeutics: New Tools for Emerging Modalities**

Rina Dukor, BioTools, Inc., 17546 Bee Line Hwy, Jupiter, FL 33478

Biotherapeutics is an ever-changing landscape of novel molecules. Over the last 20+ years, the field has seen commercialization of over 100 antibodies, ADC's and Fc-fusion proteins. But the current 'it' molecule is a nucleic acid – RNA, DNA or 'derivative'. More than two dozen have been approved in the form of siRNA, ASO's, vaccines and over one hundred are currently in clinical trials. And this list doesn't include delivery technologies such as carbohydrates, LNPs and AAVs. With new modalities come new critical quality attributes (CQAs) and structural characterization challenges. With antibodies, and peptides / proteins in general, the two most widely used structural techniques have been CD and FT-IR. But as biologics evolved to ADC's, chimeric and other forms, the Raman / ROA has gained significant momentum because it provides enhanced sensitivity to structural changes not seen with other techniques. ROA is a powerful combination of Raman + CD. The merger of two techniques brings together molecular specificity with 3-dimensional structure as each measurement simultaneously provides two spectra: Raman & ROA. The result is complete structural characterization. Because each molecule has its own, unique Raman / ROA spectra, each therapeutic product has its own signature including stereoisomerism. In this presentation, we will briefly discuss the science behind the technologies and demonstrate how advances in vibrational spectroscopy expedite and help advance development of biotherapeutics no matter the modality.

213 **Fast Food to a Slow Cooked Home Meal: Non-Targeted Analyses as Seen Through the Eyes of a High-Resolution Mass Spectrometer**

Gene Hall, Rutgers, The State University of New Jersey, Department of Chemistry and Chemical Biology, 610 Taylor Rd., Piscataway, NJ 08854, Hyunji Yu, Alexi Ermakov

Gas phase ion-molecule reactions have been taking place since the beginning of time. Today, mass spectrometer engineers have harnessed these reactions and captured and confined them to a small enclosure called an "ion source." This ion source is operated at atmospheric pressure under what is known as atmospheric pressure chemical ionization (APCI). The APCI device used to analyze various foods is the atmospheric solids analysis probe (ASAP). We operate our ASAP source under temperature program with N2 gas as the heat source in the presence of a constant flow of an aqueous solution of acetonitrileformic acid with a lock mass standard. This unusual combination of reagents results in a complex chemical ionization source of both NO^+ and H_3O^+ to produce adducts. The NO+ adducts produce fragments that reflect the chemical structure of the ionized molecules in more detail. The study presented will focus on a technique we describe as un-targeted dip and stick temperature program atmospheric solids analysis probe (ASAP). We will discuss the use of ASAP to analyze non-destructively and no sample preparation of various food samples from apples to zucchini. Analysis of desserts will also be presented.

In addition, the discussion will also focus on using ASAP to compare cooking plant-based foods with meat foods using an air fryer. Optimizing the ASAP method under atmospheric pressure chemical ionization using a corona pin and different matrices acting as Bronstead acids to induce proton transfer that competes with NO^+ adduct production will also be discussed to demonstrate structure determination.

214 **Non-Targeted Analysis of Foods Using Liquid Chromatography High-Resolution Mass Spectrometry**

Christine Fisher O'Donnell, United States Food & Drug Administration, 5001 Campus Dr., College Park, MD 20740, Ann Knolhoff

Most chemical analyses of food focus on specific analytes that are of interest. While invaluable, these targeted analyses are inherently limited because new, unknown, and/or unexpected analytes of interest are not likely to be detected/observed. To address this, non-targeted analysis (NTA) using liquid chromatography high-resolution mass spectrometry (LC/HR-MS) can be implemented. LC/HR-MS enables the detection of a wide variety of analytes across a broad range of concentrations, which is extremely beneficial in complex samples such as foods. Specifically, HR-MS enables measurement of exact mass and isotopic ratio distribution of individual analytes, which is critical information for aiding unknown identification. Although these NTA methods are extremely powerful, it can be challenging to process and interpret the information-rich data sets they generate (e.g., thousands of compounds may be detected in a single food sample). This presentation will provide an overview of NTA using LC/HR-MS to analyze foods, beginning with a general NTA workflow including sample preparation, data acquisition, and data processing. The presentation highlights different tools that can be used to help categorize samples, prioritize compounds of interest, and identify unknowns. In addition, the presentation addresses some of the challenges with assessing data quality and method performance throughout these complex NTA workflows. This discussion also highlights the collaborative efforts of the Benchmarking and Publications for Non-Targeted Analysis (BP4NTA) working group to address these challenges.

215 **Authentication and Standardization of Botanicals by MALDI-TOF Mass Spectrometry**

Christian Krueger, Complete Phytochemical Solutions, LLC, 317 South St., Cambridge, WI 53523

Authentication of foods, beverages and dietary supplements of botanical origin is a critical regulatory component of current good manufacturing practice (cGMP). Selection of quality raw botanical materials and consistency of manufacturing efficacious ingredients/formulated products requires standardization of the phytochemical profile. Matrix-Assisted Laser Desorption/Ionization Time-of-Flight mass spectrometry (MALDI-TOF MS) is ideally suited for characterizing complex heteropolymeric phytochemical structures such as proanthocyanidins and hydrolysable tannins that are unique hallmarks of the original botanical material. MALDI-TOF MS data processing using advanced chemometric software and multivariate statistical procedures have been successfully applied to categorize foods, beverages and dietary ingredients of botanical origin.

216 **Ensuring Food Ingredient Quality with Mass Spectrometry**

Uwe Nienaber, Kalsec Inc., 3713 W. Main St., Kalamazoo, MI 49006

To ensure the highest quality, consistency and authenticity of its products, a food ingredient company employs mass spectroscopy in many ways. Numerous examples are given starting with the use of ultra-performance liquid chromatography mass spectrometry (UPLC-MS) for the quality control of chili pepper extracts through the quantification of capsaicinoids over a wide concentration range. The same instrumentation is employed to analyze the complexity of advanced hop extracts. Using gas chromatography (GC)-MS, a predictive model was developed to screen for the addition of fossil fuel derived material to garlic essential oils. A continuing issue is the illegal use of synthetic food colors and dyes to enhance the appearance of numerous spices, which can be screened using UPLC-MS/MS. Applications using DHS-GC-quadrupole time-of-flight (QTOF) with untargeted data acquisition, and semi-targeted multivariate data analysis to discover compounds contributing to off-flavors in plant-based milks, and components contributing to sensory differences between non-alcoholic and full strength beers will be demonstrated.

217 **Microelectrophoretic Separations for Studies of Microbial Stress Response**

Michelle Kovarik, Trinity College, 300 Summit St., Hartford, CT 06106

While gel electrophoresis is a common tool in molecular biology, free zone capillary electrophoresis has had fewer applications in this area. However, capillary and microchip electrophoresis have a number of advantages for biological measurements, including precise quantitation and single-cell capability. While many bioassays measure fluorescent signals using microscopy or flow cytometry, adding a separation step permits simultaneous measurements of many fluorescent indicators in the same spectral channel, allowing for internal standards and multiplexed measurements. In our lab, we apply this principle to two main areas: kinase activity in response to nutrient deprivation and oxidative stress response. I present capillary electrophoresis separations of peptide substrate reporters for protein kinase B and their application to cell signaling during social development in the amoeba *Dictyostelium discoideum* and during mating in the ciliate *Tetrahymena thermophila*. I also present microchip electrophoresis separations of reactive oxygen species indicators from single *D. discoideum* cells, illustrating the capability of this technology to reveal non-genetic heterogeneity through single-cell assays. Results from both projects demonstrate the power of combining microelectrophoretic separations and existing molecular tools to extend biological research.

218 **Development of Gas and Liquid Chromatographic Methods for the Determination of Cannabinoids in Cannabis Samples**

Walter Wilson, National Institute of Standards and Technology, 100 Bureau Dr., MS: 8392, Gaithersburg, MD 20899, Jerome Mulloor, Andrea Yarberry

The National Institute of Standards and Technology (NIST) has established a Cannabis Laboratory Quality Assurance Program (CannaQAP) to improve the comparability of the analytical measurements in forensic and cannabis testing laboratories. CannaQAP is an interlaboratory study mechanism that is similar to a proficiency testing scheme; however, the focus is towards education without assigning pass/fail grades to anonymized participants. All CannaQAP studies are evaluated by NIST and summarized in publicly available NIST Internal Reports. These materials are characterized at NIST for up to 17 cannabinoids (e.g. Δ9-THC, THCA, CBD) by liquid chromatography coupled to a photodiode array detector (LC-PDA) or tandem mass spectrometry and gas chromatography mass spectrometry. In this presentation, the method development and initial validation studies will be summarized. The LC-PDA method implemented at NIST was the Cannabis Analyzer high sensitivity method from Shimadzu Scientific Instruments, which separates 11 cannabinoids in 10 min with a reversed-phase C18 stationary phase and an acetonitrile-water gradient mobile phase program. The LC-MS/MS method development utilized MS/MS selectivity to reduce necessity of LC separation of cannabinoids with distinct ion transitions, allowing for focused chromatographic separation of the isomeric cannabinoids. The GC-MS method focuses on the baseline separation of nine neutral cannabinoids while limiting the total run time to less than 10 minutes, permitting a higher sample throughput in laboratories. As part of the method development, a detailed comparison of GC column stationary phase and oven temperatures were evaluated with an emphasis placed on optimizing separation and sensitivity for Δ9-THC.

219 **Rapid Screening and Confirmation of Target Analytes in Biological Fluids with CBS-MS Using a Modified Automated Liquid Handling Robot**

Thomas Kane, Restek Corporation, 110 Benner Circle Bellefonte, PA 16823, Ryan Micklitsch, Shane Stevens, Tracey Peters, Matt Lininger

Coated blade spray (CBS) is a solid phase microextraction (SPME)-based analytical technology that performs collection of analytes of interest from a sample and subsequent direct ionization to mass spectrometry systems. The device comprises a small, thin conductive emitter having a general blade shape with a flat surface terminating into a point. As a SPME device, the blade is coated at the terminal end with an extraction sorbent bed. Performing as a direct-to-MS device following sample extraction, the blade is positioned horizontally in front of the MS inlet and a small amount of mobile phase solvent is applied to the flat portion of the blade. A DC potential is then applied to the blade relative to the MS inlet. Analytes are eluted from the sorbent bed and ionized via an electrospray Taylor cone. There are two basic stages to substrate electrospray emitters-based chemical analysis: (1) analyte collection followed by (2) instrumental analysis. In an effort to maximize blade-to-blade precision performance required for laboratory adoption of the CBS technique, we have adapted commercial liquid handling automation to perform the entire analysis workflow. In this work we present CBS applications employing the robot automation approach towards the quantitative determination of several controlled-substances/pain-management drugs. We will also present a modified pipettor device capable of moving the blade device from a vertical position (required for analyte collection steps) to a horizontal position (required for electrospray analysis).

220 **Building Robustness into a Drug Substance Stability-Indicating Method with QbD – A Case Study**

Elizabeth Yuill, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903, Yande Huang, Jonathan Shackman, Hua-Chia Tai, Peter Tattersall, Jia Zang

Methods to monitor active pharmaceutical ingredient (API) quality are of crucial importance and thus, development can be particularly resource intensive. For example, drug substance stability-indicating methods require thorough development to successfully track impurities formed during the manufacturing process as well as degradants that may be formed upon storage or handling of the API. Further, these conditions must withstand the stresses of validation, transfer to external laboratories, and routine execution throughout the method lifecycle. An analytical quality-by-design strategy incorporates robustness into method development through tools such as screening workflows, modeling software, and risk assessments to stress meth-

od conditions during parameter selection, rather than at validation stage. This case study highlights how an up-front investment to capture key knowledge of impurity separation through chromatographic modeling can save time that may otherwise be spent on troubleshooting during method transfer. Center point chromatographic conditions were selected based on simulated robustness regions, which could be later referenced to understand potential method failure points. Methods were successfully validated for support of long-term stability studies of a drug substance and commercial manufacturing.

221 **Leveraging Advances in Mass Spectrometry Instrumentation and Techniques to Address PFAS Contamination**

Craig Butt, SCIEX, 500 Old Connecticut Path, Framingham, MA 01701, Karl Oetjen, Simon Roberts, Megumi Shimizu, Amy Rand

During the past 20 years, PFAS have emerged as a global environmental contaminants, garnering increasing attention from scientists and regulators. Despite significant progress, many challenges remain which demand novel analytical strategies and instrumentation. The early days of PFAS research primarily focused on the development of robust, targeted quantitation methods with the aim of improving overall data quality as well as achieving low detection limits. Many targeted PFAS methods have become routine and standardized, and the evolving instrument technology has continued to push detection limits lower. Further, the widespread adoption of accurate mass spectrometry has brought new opportunities to address PFAS contamination. For example, the ability of QTOF instruments to collect MS/MS fragmentation patterns has improved confidence in suspect screening workflows as well as novel PFAS identification during nontarget analysis. This presentation will cover a broad range of applications to highlight advantages of modern nominal mass LC-MS/MS and accurate mass QTOF instruments. For example, applications involving the new electron activated dissociation (EAD) fragmentation mechanism of the ZenoTOF 7600 will be discussed. Also, the nontargeted analysis of PFAS in cosmetics will be described, including the detection of novel PFAS compounds.

222 **Remediation of PFAS from a Variety of Environmental Matrices**

Jay Meegoda, CEE, New Jersey Institute of Technology, University Heights, Newark, NJ 07102

The Polyfluoroalkyl substances (PFAS) are a family of highly toxic emerging contaminants that have brought attention and awareness to the public and private organizations due to its adverse health impacts on society. The scientific community has been laboriously working on two fronts; (1) adapting already existing and effective technologies in destroying organic contaminants to the PFAS remediation; and (2) developing new technologies to remediate the PFAS. A common characteristic in both fronts would be the separation/removal followed by destruction. The widely adopted separation technologies can momentarily remove the PFAS from being in contact with humans, it remains in the environment and continues to impose risks to society. On the other hand, destructive technologies discussed here can effectively destroy PFAS compounds and fully address society's urgent need to remediate this harmful family of chemical compounds. This presentation reports the manure PFAS destruction technologies. Some of the technologies presented in this review are still under development in lab-scale, while others have already been tested in the field.

223 **Collaborative PFAS Research Using High Resolution Mass Spectrometry: Challenges and Progress**

Sara Nason, Connecticut Agricultural Experiment Station, 123 Huntington St. New Haven, CT 06511

Per- and polyfluoroalkyl substances (PFAS) are an emerging class of environmental contaminants that have toxic effects at extremely low concentrations. They have been used in consumer products and industrial applications since the 1940s, and are frequently detected in drinking water, soil, and human serum and blood. Complicating their analysis, thousands of PFAS exist, though most methods target less than 50 compounds. This talk discusses tips for implementing new PFAS analyses, methods for detecting as many PFAS as possible using liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS), and results from projects on complex matrices with community impacts.

224 **Challenges in Method Development of PFAS in Food**

Susan Genualdi, United States Food & Drug Administration, CFSAN, 5001 Campus Dr., College Park, MD 20740, Cynthia Srigley, Wendy Young, Christine M. Fisher, Lowri deJager

In order to better understand the dietary exposure of per-and polyfluoroalkyl substances (PFAS), more data is needed on highly consumed foods in the US diet. The FDA's Total Diet Study (TDS) routinely monitors composite samples of table-ready highly consumed foods each year. Samples collected through this program in 2018 were used to develop a Quick, Easy, Cheap, Effective, Rugged, Safe (QuEChERS) extraction method for 16 PFAS with analysis by liquid chromatography mass spectrometry. This method was expanded to processed foods in 2020 and modifications were made due to challenges that arose with false positives and interferences. High-resolution mass spectrometry was used to further investigate these interferences as well as to confirm their detection. To date, the FDA has analyzed 532 foods from both regional and national collections, which revealed detections of PFAS primarily in seafood products. In 2021, the method was extended to 20 analytes for a survey of highly consumed seafood products in the US. Additionally, the quantification of the sum of perfluorooctanesulfonic acid (PFOS) and perflurooctanoic acid (PFOA) isomers can vary dependent on the choice of analytical standard and ratios of the branched versus linear isomers. Investigations into these differences were examined between analytical standards and in incurred residue samples.

225 **Screening for Generality in Asymmetric Catalysis**

Spencer McMinn, Merck & Co., Inc., 33 Ave Louis Pasteur, Boston, MA 02115

Research in the field of asymmetric catalysis over the past half century has resulted in landmark advances, enabling the efficient synthesis of chiral building blocks, pharmaceuticals, and natural products. A small number of asymmetric catalytic reactions have been identified that display high selectivity across a broad scope of substrates; not coincidentally, these are the reactions that have the greatest impact on how enantioenriched compounds are synthesized. We postulate that substrate generality in asymmetric catalysis is rare not simply because it is intrinsically difficult to achieve, but also because of the way chiral catalysts are identified and optimized. Typical discovery campaigns rely on a single model substrate, and thus select for high performance in a narrow region of chemical space. Here, we put forth a practical approach for using multiple model substrates to select simultaneously for both enantioselectivity and generality in asymmetric catalysis from the outset. Multi-substrate screening is achieved by conducting high-throughput chiral analyses via supercritical fluid chromatography-mass spectrometry (SFC-MS) with pooled samples. When applied to Pictet–Spengler reactions, the multi-substrate screening approach revealed a promising and unexpected lead for the general enantioselective catalysis of this important transformation

226 **Chiral Method Development and Optimization on Daicel Polysaccharide Chiral Stationary Phases**

Weston Umstead, Chiral Technologies, 1475 Dunwoody Dr., Suite 310, West Chester, PA 19380

One of the most frequently asked questions about chiral separations is where to start for method development. With so many different chiral stationary phases and mobile phase combinations, this can be quite challenging for new or inexperienced users. After 30+ years of work in this field, Daicel continues to streamline and optimize the most efficient processes for determining ideal separation conditions. The most efficient way to answer the question is to perform a method development screening, which allows you to quickly assess a wide range of separation conditions. From this, optimization is typically much faster and more effective. This presentation will cover a number of topics aimed to help a user better understand the nature of chiral separations and the method development process. This includes a discussion on the selection mechanism that governs polysaccharide-based chiral separations, a discussion of chiral method development strategies for HPLC and SFC, and an in-depth look at a few relevant HPLC and SFC examples of interest.

227 **Accelerating Chiral Supercritical Fluid Chromatography with 3- and sub-2-um Fully Porous Particles and 2.7-um Superficially Porous Particles**

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

Supercritical fluid chromatography (SFC) is an effective and often preferred means to separate enantiomers in pharmaceutical and other settings. Its advantages over liquid chromatographic approaches in terms of productivity, solvent consumption, operating costs, and environmental impact are well-documented. These advantages result primarily from the use of inexpensive, low-viscosity supercritical carbon dioxide as the major mobile phase component. Because it can be difficult to predict which chiral stationary phases (CSPs) will resolve chiral compounds of interest, it is often necessary to screen many different columns with different mobile phase conditions before proceeding to method optimization. The speed advantages of SFC are particularly helpful at this time-consuming screening stage, and the process can be further accelerated by using state-of-the-art instrumentation and modern column technologies that employ small, fully porous particles (FPP) or superficially porous particles (SPP). In this work, practical approaches for fast chiral methods using SFC and modern particle technologies are considered. The effects of instrument volume and pumping capabilities are used to rationalize column dimension selection. Experimental results obtained using columns packed with a variety of 3- and sub-2-µm FPP and 2.7-µm SPP chiral stationary phases (polysaccharide-based, Pirkle-type, macrocyclic antibiotic, etc.) are examined.

228 Application of Functionalized Cyclofructan for Enantioselective Sub/Supercritical Fluid Chromatography of Ru(II) and Os(II) Coordination Complexes

Troy Handlovic, The University of Texas at Arlington, Department of Chemistry and Biochemistry, 700 Planetarium Place, Arlington, TX 76019, M. Farooq Wahab, Houston Cole, Nagham Alatrash, Elamparuthi Ramasamy, Frederick MacDonnell, Sherri McFarland, Daniel Armstrong

Cyclofructan-6 (CF6) functionalized with (R)-naphthyl ethyl (RN) groups has been proven to have unique selectivity for the enantiomers of bidentate polypyridyl octahedral coordination complexes in HPLC. These compounds contain helical chirality and exist as delta (Δ) and lambda (Λ) enantiomers. Recently, a large amount of research has been centered on polypyridyl octahedral complexes with Ru(II) and Os(II) metal centers due to their ability to serve in cancer treatment, photodynamic therapy, and other biological activities. Sub/supercritical fluid chromatography (SFC) is a green separation technique used to separate a wide variety of compounds and is proven immensely useful for chiral separations. Currently, SFC is not considered applicable for ionic compounds, even with additives and organic modifiers. We sought to expand the CF6-RN column chemistry to SFC to provide the first chiral SFC separation of a wide variety (23 complexes in total) of ionic octahedral polypyridyl complexes. Unexpected behavior for mixing methanol and acetonitrile as the organic modifier is shown, along with the effects of additives. Enantioselectivity on CF6-RN chemistry is shown to depend on the metal complexes' conjugation level and rigidity. Mass transfer kinetic behavior is investigated, and high-efficiency baseline resolved rapid separations for fast screening or quantitation of representative coordination complexes are presented.

229 Challenges in Applying Chemometrics to Data from Handheld Instrumentation

Barry Lavine, Oklahoma State University, Department of Chemistry 107 Physical Science, Stillwater, OK 74078, Collin White, William Gilbert, Wesley Carson, Karl Booksh, James Jordon

The illegal timber trade poses a threat to the survival of endangered tropical hardwoods such as rosewood. In the present work, the potential of hand-held X-ray fluorescence (XRF) instruments to identify rosewood was investigated using a genetic algorithm (GA) for pattern recognition to identify features characteristic of rosewood in a plot of the two largest principal components of the XRF spectral data. For this study, we incorporated model inference directly into the variable selection process to identify wavelengths that minimize error across the entire model. This was accomplished by assessing the uncertainty associated with the sample scores in each PC plot using the jack-knife to generate estimates of dispersion. During each generation, the fitness function evaluates thousands of PC plots, one for each feature subset (i.e., chromosome) in the solution population. For each score plot, the training set samples are removed one at a time, and the score and loading matrices for the resampled training set are recomputed. Because of the rotational ambiguities of principal component analysis (PCA), the loading matrix for each resampled training set is first rotated using a Procrustean rotation to match the loading matrix associated with the score plot containing all samples. For each sample, scores across all leave-one-out score plots are projected onto the original PC plot, which is then scored by the fitness function of the pattern recognition GA. The jack-knifed scores of each sample effectively comprise an error cloud to depict the uncertainty associated with each point in the PC plot.

230 Handheld Laser-Induced Breakdown Spectroscopy, Chemometrics, and the Supply Chain

Nancy McMillan, New Mexico State University, Department of Geological Sciences, Box 30001, MSC 3AB, Las Cruces, NM 88011

Laser-Induced Breakdown Spectroscopy (LIBS) is a laser ablation optical emission analytical technique; both scientific and industrial application are growing. Handheld LIBS instruments now produce high-quality spectra with point-and-analyze convenience. Rapid in situ analysis opens opportunities to explore scientific questions more fully. However, most instruments have fixed laser wavelength, laser power, and Q-switch delay time, making it impossible to optimize performance. The most common laser is Nd:YAG operated at its fundamental wavelength of 1064 nm; thus, these instruments are most useful for materials with high concentrations of transition metals. LIBS spectra contain an enormous amount of information on the concentrations and isotopes of naturally occurring elements and material structure, making them ideal for chemometric analysis. In all NMSU LIBS lab projects, spectra from unknown samples are chemometrically compared to a database of spectra acquired from known samples. This methodology has been applied to a variety of problems, including identification of bacterially-influenced speleothems in modern cave systems and the identification of heavy minerals in river sediments to determine paleogeographic river patterns. Chemometric analysis of complex handheld LIBS spectra (immune to fraudulent simulation) has the potential to provide an enhanced level of verification to supply chain security measures such as blockchain. Cotton, lumber, gems, and other critical minerals are examples of materials that would benefit from the ability to identify and remove illegal and fraudulent sources. This is not without challenge: development and maintenance of the database is a large undertaking that requires cooperation between governments, their agencies, and scientific organizations/businesses.

231 Self-Optimizing Support Vector Classifiers Applied to the Analysis of Maca Metabolomic Mass Spectral Profiles

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Support vector classifiers are often used in conjunction with kernels to accommodate classifications when there is no linear separation. The most popular kernel used in combination with the support vector classifier (SVC) is the radial basis function (RBF). The RBF function is optimized with a width parameter σ and the SVC with a cost parameter C. These parameters are usually optimized by a grid search of the training data that may lead to overfitting. Bootstrapped Latin partitions (BLPs) provide a comprehensive and general approach for calculating average prediction errors. The optimal parameters can then be obtained from response surface modeling (RSM) of a grid formed from the design points of (σ, C). This self-optimizing RBF-SVC (SO-RBF-SVC) is easy to employ and compared favorably to other classifiers on a dataset comprising direct infusion negative ion high-resolution mass spectra of Maca extracts from Peru and China. Table of external validation using 100 bootstraps and 4 Latin partitions for classifying Maca extracts by their country of origin using normalized negative ion mass spectra.

SVC-Tree	SO-PLS-DA	SO-RBF-SVC	SO-SVC
93.8±0.5%	86.5±0.9%	95.1±0.8%	93.8±0.5%

232 Chemometrics & Portable Instrumentation: From Environmental Forensics to Art Conservation

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Though field portable instrumentation yields a wealth of previously unattainable data, this comes with diminished spectral resolution. When paired with chemometric analysis, portable instruments are still viable for solving complex analytical problems. Here, portable instrumentation and chemometrics will be discussed in terms of two applications: classification of tropical hardwoods analyzed with laser induced breakdown spectroscopy (LIBS); and cluster analysis of historical textiles using fiber optic reflectance spectroscopy (FORS) and x-ray fluorescence spectroscopy (XRF). Two LIBS datasets of Dalbergia spp. and its lookalikes were analyzed using two signal-to-noise ratios (S/N) and classified with 5 algorithms – Partial Least Squares Discriminant Analysis (PLS2-DA), k-Nearest Neighbors (k-NN), Classification & Regression Trees (CART), Random Forests (RF), and Support Vector Machines (SVM). It was found that SVM & RF have similar performance, S/N ratio does have a distinct effect, and imbalanced classes do not impede SVM/RF algorithm performance. Implementation of classification algorithms for field detection of Dalbergia spp. is thus viable. A dataset of 198 historical textiles from Norwich Textile Books were measured with portable FORS and benchtop XRF for chromophore content. Application of Principal Component Analysis (PCA) and a flat classifier decision rule based on the 380-469nm peak maximum showed 3 clusters, while PCA of XRF data of the same samples showed no clustering. These results indicate that samples can be classified by color, however, dyes are present in quantities too small to be detected with FORS in textiles.Ca

233 HPLC- and UHPLC-MS Analysis of Pharmaceutically Relevant Bio-Macromolecules on the Analytical and Capillary Scale

Hayley Herderschee, University of Michigan, Chemistry Department, 930 N University Ave., Ann Arbor, MI 48,109, Tian Lu, James Deng, Ping Zhuang, Robert Kennedy

Biomacromolecules are important in the current landscape of the pharmaceutical industry and academic research. Analyzing these macromolecules is challenging due to their structural complexity and size. Additionally, their biological origin means they are usually present in complex biological matrices, requiring high-resolution separations to characterize them. We describe liquid chromatography-mass spectrometry (LC-MS) methods directed at polysaccharides and proteins. We used a 1.9 µm nonporous reversed phase analytical column for polysaccharide analysis to resolve six serotypes chromatographically. In-source decay enabled the detection of characteristic fragments of each serotype by TOF-MS.[1,2] A quantitative method was developed for these compounds based on this approach.[3] High-resolution chromatography is desirable for more complex mixtures, such as those encountered in top-down proteomics. One route to improved resolution is long columns (100-150 cm) with small particle sizes (d_p < 2.0 µm).4 The high back pressure of such columns can be overcome using ultrahigh-pressure liquid chromatographic (UHPLC) systems. With our home-built UHPLC system, we can perform routine separations at 20 – 50 kpsi, allowing us to maximize the increase in efficiency provided by longer columns with smaller particles. We explore packing 100 cm long capillary columns

with 1.1 and 1.7 µm particles, via kinetic plot modeling, to achieve separations of complex intact protein mixtures as an initial step towards improved resolution for top-down protein analysis.[5]

234 **LPH-C18: A C18 Column Alternative**

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High pressure liquid chromatography (HPLC) columns can have a wide range of pore sizes, particle sizes, column dimensions, and stationary phases. The C18 stationary phase is one of the most popular phases due to its excellent performance for a broad range of analyte polarities. It is important to note that all C18 columns do not behave the same. For example, some C18 columns are designed for differences in selectivity, aqueous compatibility, peak shape improvements based on the end capping, and low/ high pH compatibility. Different chemical characteristics can create difficult separations; therefore, it is important to choose the right C18 column in order to achieve the best separation. A low pH compatible, 90 Å, superficially porous particle C18 phase is introduced as an addition to the chromatographer's toolbox. This sterically protected ligand enables low pH mobile phases to be used without sacrificing column retention loss over time. This reversed phase column is available in both 2 and 2.7 µm particle sizes and is designed for a wide range of small molecule applications including polyphenols, pesticides, and cannabinoids. The 90 Å LPH-C18 is an excellent option for LC-MS/MS applications due to its low column bleed and should be included as part of column screening sets during method development when using low pH mobile phases.

235 **Clear as a Diamond: Fundamentals and Strategies for Using Porous Graphitic Carbon Columns in Liquid Chromatography**

Cory Muraco, MilliporeSigma, 595 North Harrison Rd., Bellefonte, PA 16823, Michael Ye, Clinton Corman

High performance liquid chromatography (HPLC) separations of both small and large molecules have mostly been performed with silica-based particles since the 1960's. Reasons for this preference include the mechanical stability of silica, the ease of silica particles to be bonded to by alkylsilanes, and the ability to manufacture these particles with high lot-to-lot reproducibility. Even though other types of particles have been packed into columns for use in analytical separations, the difficulty in completely understanding the inherent mechanisms and controlling them have led chromatographers to gravitate, repeatedly, back to silica-based columns. This seminar will focus on recent advances, in the past two years, in utilizing columns packed with porous graphitic carbon (PGC) particles in the analysis of compounds classically associated with being difficult to separate by conventional silica-based columns. An explanation of the fundamental mechanisms on how the stationary phase interacts with analytes, and how these differ from silica-based columns, will be offered. The fundamentals will be further explained using application examples demonstrating how PGC particle packed columns can expand the method development space for the analyst and be orthogonal to conventional chromatography columns. Finally, aspects of method development will be shared further illustrating the utility of the column in solving challenging analytical separations.

236 **Development of Robust 2D RPLC-NPLC Methods to Support Simultaneous Achiral-Chiral Analysis in High-Throughput Experimentation**

Steven Chin, Genentech, 1 DNA Way, South San Francisco, CA 94080, Karissa Cruz, Kenji Kurita

Typical chromatographic analysis of chiral compounds requires the use of achiral methods to evaluate impurities/related substances along with separate methods to evaluate chiral purity. As such, the use of two-dimensional chromatography to support simultaneous achiral-chiral analysis has become increasingly popular over the past few years. Especially in the field of high-throughput experimentation, where low reaction yields and/or side reactions can lead to challenging direct chiral analysis, two-dimensional chromatography can eliminate the need to purify mixtures to determine chiral selectivity. Advancements in multi-dimensional chromatography have led to the development of robust two-dimensional reversed phase liquid chromatography (2D RPLC) instrumentation to achieve this simultaneous analysis, but in the event that a chiral RPLC method cannot be readily developed, options become more limited (ie. 2D RPLC-SFC). One particular technology, 2D RPLC coupled with normal phase liquid chromatography (NPLC) continues to remain elusive due to solvent immiscibility between water in RPLC and nonpolar solvents (ie. hexanes) in NPLC, leading to band broadening, poor resolution, poor peak shapes, and baseline issues in the second dimension. Experimentation on NPLC was conducted to understand the effect of various water-containing diluent injections and applied to the development of robust 2D RPLC-NPLC methods. Following thoughtful consideration and modifications to the design of a 2D system in regards to mobile phase selection, sample loop sizing, targeted mixing, and valve/pump compatibility, proof of concept has been demonstrated with the development of robust, working methods to perform simultaneous achiral-chiral analysis by 2D RPLC-NPLC of these complex chiral compounds.

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

Mary Donnelly, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Hannah Yorkey, Danielle Whitham, Costel Darie

The field of proteomics is becoming more common in modern applications. Current experimental techniques for proteomics experiments must be efficient and accurate. The current protein digestion and peptide extraction method from an SDS-PAGE are time consuming and labor-intensive. Optimization of this method could save time and effort while yielding similar or acceptable results in terms of protein identification. To optimize the digestion protocol, we used two model proteins: bovine serum albumin (BSA) and lysozyme. Both proteins vary in their molecular weights and number of disulfide bonds, and are easily identifiable, which make them ideal proteins for this investigation. The trypsin digestion variations include various digestion times and temperatures. Peptide extraction variations include shaking and sonicating with varying times and steps. Several concentrations of both proteins were also tested to assess the sensitivity. The peptide mixtures were then analyzed by nanoLC-MS/MS using a NanoAcquity UPLC coupled with a QToF Xevo G2 MS, and the raw data was analyzed using Mascot Daemon (v. 2.5) server. Both the protein score and type of protein found was taken into consideration with data analysis. Variations in the parameters (digestion time, extraction time and number of steps, extraction method, etc.) for protein digestion and peptide extraction allowed us to determine which method is the fastest and the least labor-intensive while resulting in acceptable protein identification compared to the current protocol. This study is still underway.

238 **Investigation of the Effects of Human Jumping Translocation Breakpoint (hJTB) Protein for Potential use as a Cancer Biomarker**

Madhuri Jayathirtha, Clarkson University, Box 5810, 8 Clarkson Ave, Potsdam, NY 13699, Danielle Whitham, Shelby Alwine, Hannah Yorkey, Costel Darie

Human JTB (hJTB) is a gene located on the human chromosome 1 at q21, which is involved in the unbalanced translocation in various types of cancer. JTB protein is ubiquitously present in normal cells and is found to be overexpressed in various types of cancer including prostate and breast cancer. Hence, this protein could be a biomarker for tumor malignancies and a potential target for their treatment. However, the biological function and the pathway through which this protein causes increased cell proliferation is not entirely clear. Investigation and comparison of the proteomes of cells with upregulated and downregulated JTB can be a good approach to understand the function of the protein and its contribution to tumorigenesis. In this study, MCF7 and HEK293 cell lines were transfected with the sense orientation of the JTB cDNA in a CMV expression vector as well as with shRNA plasmids. Proteins extracted from the transfected cells were separated using SDS-PAGE. The expression of JTB was confirmed by western blotting technique. In gel and in-solution 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 that were dysregulated. Furthermore, we performed Gene Set Enrichment Analysis (GSEA) to identify the pathways that are associated with the JTB protein. These studies could help us elucidate the mechanism through which JTB induces cell proliferation and test the protein as a potential drug target for malignancies.

239 **Proteomic Analysis of Human Breast Milk Using Mass Spectrometry to Reveal Protein Biomarkers for Early Breast Cancer Detection**

Danielle Whitham, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Roskanak Aslebagh, Devika Channaveerappa,Brian Pentecost, Kathleen F. Arcaro, Costel C. Darie

Breast cancer (BC), one of the most common cancers, is a leading cause of death for women in the United States. An estimated 1 in 8 women in the United States will develop BC in their lifetime. Early diagnosis and treatment of BC is crucial, and protein biomarkers for this disease could make this possible. Mass spectrometry (MS)-based proteomic methods are ideal for the investigation of dysregulated proteins from women with BC and matched controls. If significant protein dysregulations are revealed, they could be considered potential future protein biomarkers of BC for diagnosis and treatment. In this study, human breast milk samples were investigated by a combination of a 1D-SDS-PAGE and 2D-SDS-PAGE followed by in-gel trypsin digestion, and then analyzed by nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS), data processing, database search, statistical analysis and bioinformatic interrogation. For 1D-SDS-PAGE, all milk proteins were analyzed and compared between BC-related and control sample, while for 2D-SDS-PAGE, only the dysregulated proteins were subjected to analysis. The dysregulated proteins found in this study are being further investigated as BC biomarkers for future clinical methods for early diagnosis and treatment of BC.

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

Isabelle Sullivan, Clarkson University, Biochemistry & Proteomics Group, Department of Chemistry & Biomolecular Science, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Panashe Mutsengi, Danielle Whitham, Costel Darie, Brian Pentecost, Kathleen F. Arcaro

Breast cancer (BC), found most commonly in women, is a leading cause of death in women in the United States. Nearly 13% of women will develop BC in their lifetimes. Triple-Negative Breast Cancer (TNBC) in particular, is an incredibly aggressive form of cancer due to its lack of specific receptors commonly observed in breast epithelium and BC. This form of BC lacks estrogen receptors (ER), progesterone receptors (PR), and HER2 (human epidermal growth factor receptor 2) receptors, making it the hardest BC to treat. It is imperative to diagnose BC efficiently and quickly. A human serum test, utilizing protein biomarkers would allow for earlier diagnosis and treatment. We are using mass spectrometry (MS) based proteomics methods, to identify serum biomarkers that differ between controls and breast cancer cases. In this study, serum samples from one Asian American woman with TNBC (classified as PT2 pN0), and a race- & age-matched control (1 vs 1) were analyzed using in-gel and in-solution trypsin digestion, followed by nano-liquid chromatography-tandem mass spectrometry (nanoLC- MS/MS) analysis, using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. The raw data was then analyzed using ProteinLynx Global Server (v 2.4) Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. The dysregulated proteins found in this study are being compared with dysregulated proteins found in previous breast milk and serum proteomics studies. This study is ongoing.

241 No abstract submitted by the author.

242 **Assessing the Limit of Linearity of Cannabinoid Analogs (Δ8-THC, Δ10-THC, and CBD) and their Major Metabolites in Six Commercial Homogeneous Cannabinoid Urine Screening Kits**

Ashley Pokhai, Virginia Commonwealth University, 323 W. Broad St, Richmond, VA 23220, Justin Poklis, Grace Williams, Carl Wolf

Background: Following legalization of hemp, there has been increased use of cannabinoids. This growth is not only attributed to Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD) but also other THC analogs such as Δ8-tetrahydrocannabinol (Δ8-THC) and Δ10-tetrahydrocannabinol (Δ10-THC). These analogs are positional isomers of Δ9-THC. Therefore, their detection may be amenable to current immunoassays used for the detection of Δ9-COOH-THC in urine. Objectives: To assess the limit of linearity (LOL) of Δ8-THC, Δ10-THC, and CBD and their major metabolites in six commercial cannabinoid urine screening kits. Methods: Six urine homogeneous immunoassay kits (Abbott Diagnositcs, Lin-Zhi International, Thermo Fisher Scientific, Roche Diagnostics, and Siemens Healthineers) were evaluated to detect cannabinoids at two cutoff concentrations: 20 or 25ng/mL (lower cutoff) and 50ng/mL (federal cutoff). Δ8-THC, Δ10-THC, and CBD along with their major metabolites were evaluated. Previously, the precision at a decision point (DP) was assessed for detectable analytes. LOL was assessed for detectable analytes by preparing an eleven-point curve. Results: LOL for the lower and federal cutoffs are as follows: 175ng/mL and 250ng/mL, respectively, for Δ8-THC; 150ng/mL for both cutoffs for Δ8-COOH-THC and Δ8-OH-THC; 150ng/mL and 300ng/mL, respectively, for each stereoisomer of Δ10-THC; and not observed for 6-OH-CBD and 7-OH-CBD in the range tested for either cutoff. Conclusion: For Δ8-THC, Δ8-COOH-THC, Δ8-OH-THC, and each stereoisomer of Δ10-THC, the LOL was a higher concentration than the decision point for each assay. For 6-OH-CBD and 7-OH-CBD, the LOL is outside of the range tested.

243 **Analysis of Cannabis Plant Materials by Near Infrared (NIR) Spectroscopy and Multivariate Data Analysis for Differentiating Low-THC and High-THC Cannabis**

Aaron Urbas, National Institute of Standards & Technology, Chemical Science and Technology Laboratory, 100 Bureau Drive, Gaithersburg, MD 20899, Ewelina Mistek-Morabito, Walter Wilson, Igor Lednev

The passage of the Farm Bill in December of 2018 removed hemp from the schedule I of the Controlled Substances Act at the federal level and established a threshold for total delta-9 tetrahydrocannabinol (Δ9-THC) content of 0.3% on a dry weight basis to be classified as hemp. In this work, near infrared (NIR) spectroscopy combined with statistical data analysis was explored as a rapid method for differentiating cannabis plant materials with two specific aims, (i) differentiation between low-THC and high-THC cannabis samples and (ii) quantitative estimation of total-Δ9-THC. Partial least squares discriminant analysis (PLSDA) was used to build classification models for low-THC and high-THC cannabis plant materials. These models exhibited excellent separation between the two classes. No misclassifications were observed from evaluation with a test set (based on 60/40 calibration/test split). Partial least squares (PLS) regression was used to develop quantitative models for total Δ9-THC, the performance of which was evaluated in a 60/40 calibration/test set split. The predictions of the test set resulted in a coefficient of determination (r2) of 0.962 and the root mean squared error of prediction (RMSEP) of 0.859% total Δ9-THC. While the overall performance of the model across the full total Δ9-THC calibration range was good, the accuracy and precision were not sufficient for reliable determinations of low total Δ9-THC content based on a 0.3% (mass%) criteria. However, the methods may be suitable for other purposes, such as rapid screening or field analysis, where less stringent method requirements may be suitable.

244 **Peak Tailing Investigation of Organic Acids in Reverse Phase Liquid Chromatography**

Yiyang Zhou, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901, Qinggang Wang

Peak tailing of pharmaceutical compounds in reverse-phase liquid chromatography (RPLC) can greatly impact peak resolution and integration, which significantly lowers quantitation accuracy. Understanding of solute-stationary phase interactions which lead to peak tailing is important for column selection in RPLC method development. While peak tailing caused by ionic interaction has been well studied, peak tailing caused by hydrogen bonding, especially of acidic compounds, is less understood. In this work we demonstrated hydrogen bonding between an H-donating solute and a stationary-phase acceptor is a probable cause for the tailing of weak organic acids (e.g. sulfinic acids and carboxylic acids). Six different commercial C18 RPLC columns (including end capped and non-end capped) were tested with a sulfinic acid and a sulfonic acid compound under pH ranging from 1.7 to 3.7. Tailing factor and retention factor measurements indicated that the protonated form of sulfinic acid compound has a strong hydrogen bonding interaction with stationary phase. Hydrophobic-subtraction models with several carboxylic acid probes were further employed systematically to examine acid-surface hydrogen bonding interaction of the commercial RPLC columns. Through a bi-Langmuir adsorption simulation model (CADET), we were able to determine the population of the strong interaction site and estimation of the interaction strength. A possible mechanism for weak organic acid peak tailing involving surface vicinal silanol pairs and chains is proposed and validated preliminarily by acidic hydrothermal treatment of column bonded phases.

245 **New Porous Monodisperse Particles for Increasing Resolution in Liquid Chromatography**

Edward Faden, MAC-MOD Analytical, 103 Commons Ct., Chadds Ford, PA 19317, Yvonne Walsh, Ken Butchart, Mark Woodruff

In this poster we discuss the use of a new range of stationary phase chemistries allied with a fully porous monodisperse silica particles. One of the major challenges in LC and LC-MS is achieving full resolution of compounds especially when metabolites and or isomeric species are involved. By combining the high efficiency of a monodisperse silica with new diverse stationary phase ligands, we have the potential to gain more selectivity and resolution between analytes. Whilst C18 and C8 alkyl chain stationary phases are the most common choice for starting method development, they cannot achieve all separations with the required resolution sufficient to provide accurate qualitative results. The use of orthogonal stationary phases containing halogenated, aromatic or polar character allows differing mechanisms other than just hydrophobicity to be employed. We discuss the use of several mixed stationary phases which allows more mechanisms of interaction to be used in the separation process to gain more resolution. We highlight applications where these mixed stationary phases can be utilized to alter selectivity of complex samples, gaining resolution over a traditional C18 bonded phase.

246 **New Porous Monodisperse HPLC Particles**

Edward Faden, MAC-MOD Analytical, 103 Commons Ct., Chadds Ford, PA 19317, Yvonne Walsh, Ken Butchart, Mark Woodruff

In this poster we discuss the use of a new fully porous monodisperse silica particle and its application in HPLC and UHPLC. Silica particles have been the mainstay in HPLC for 50 years and various innovations have taken place in terms of particle size, particle composition and particle morphology with the goal of improving chromatographic stability and efficiency. We look at the use of a fully porous monodisperse particle in terms of the increased efficiency that it can provide. We look at the advantages over UHPLC particles and core-shell particles, in particular sample loading, method scaling and column backpressure. We discuss the van-deemter curve and band dispersion in relation to the tighter d90/10 size distribution provided by a monodisperse particle. Combining this monodisperse particle with a range of selectivity's can then provide the ultimate in terms of sample resolution and sensitivity when trying to develop a new and improved HPLC methods.

247 **Optimizing Your Ion Exchange Chromatography Instrument and Process**

Jodie Wall, Inorganic Ventures, 300 Technology Dr, Christiansburg, VA 24073, James King

Ion chromatography (IC) is a time-tested method of analyzing cations and anions in solution with many applications for both clinical and industrial settings. More applications are being developed continuously with a particular focus in the pharmaceutical

world. These systems are popular due to their reliability, accuracy, selectivity, and low cost of consumables. Optimizing both the hardware of the IC system and the methods that are utilized are key to getting the most accurate results. Perhaps your original instrument setup worked well, but now technicians and/or samples have evolved, and your data is not looking conclusive. Maybe your instrument has aged and an update to your methods seems appropriate. This presentation will look at optimization in three parts by examining the current market hardware options and how to choose the right instrument for your application, examining the settings of your method and how it may affect your data, and looking at some common mistakes we have made in our laboratory and how you can avoid making the same errors. Putting together the best instrument and method while avoiding common pitfalls will help to ensure that you get the best results for your laboratory.

248 **Proteomic Analysis of Human Breast Milk using Mass Spectrometry to Reveal Protein Biomarkers for Early Breast Cancer Detection**

James Lowe, Clarkson University, Department of Chemistry & Biomolecular Science, 10 Clarkson Ave,, Potsdam, NY 13699, Danielle Whitham, Roshanak Aslebagh, Devika Channaveerappa, Brian Pentecost, Kathleen F. Arcaro, Costel C. Darie

Breast cancer (BC), one of the most common cancers, is a leading cause of death for women in the United States. An estimated 1 in 8 women in the United States will develop BC in their lifetime. Early diagnosis and treatment of BC are crucial, and protein biomarkers for this disease could make this possible. Mass spectrometry (MS)-based proteomic methods are ideal for the investigation of dysregulated proteins from women with BC and matched controls. If significant protein dysregulations are revealed, they could be considered potential future protein biomarkers of BC for diagnosis and treatment. In this study, human breast milk samples were investigated by a combination of a 1D-SDS-PAGE and 2D-SDS-PAGE followed by in-gel trypsin digestion and then analyzed by nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS), data processing, database search, statistical analysis, and bioinformatic interrogation. For 1D-SDS-PAGE, all milk proteins were analyzed and compared between BC-related and control samples, while for 2D-SDS-PAGE, only the dysregulated proteins were subjected to analysis. The dysregulated proteins found in this study are being further investigated as BC biomarkers for future clinical methods for early diagnosis and treatment of BC.

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

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

Cancer is among the top 5 causes of death in the U.S and Breast cancer (BC) is the second most frequently diagnosed form of cancer with 271,270 new cases in 2019. BCs can be grouped into two main categories; in situ BC and invasive BCs. There are two main types of invasive BCs: Invasive Ductal Carcinoma (IDC) and Invasive Lobular Carcinoma (ILC). In IDC, the cancer cells begin in the ducts and then invade other parts of the breast tissue. Comparison of IDC serum samples with matched controls could identify dysregulated proteins that can potentially be used as protein biomarkers. In this investigation, we used a proteomics approach to compare sera from 3 African American donors with IDC and 3 matched healthy controls. The IDC samples were ER+PR+ and the tumors were classified as PT1c pNx, PT1a pNx, and PT2 pN1a. The samples were prepared according to an in-gel digestion protocol and analyzed by nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. In a second experiment, the samples were prepared according to an in-solution digestion protocol and analyzed by nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. The resulting raw data were analyzed using ProteinLynx Global Server (v 2.4), Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. The dysregulated proteins that were identified are currently being interrogated. This is an ongoing investigation.

250 **Structural Characterization of Snakes Skins: A Proteomics Investigation**

Celeste Darie, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Danielle Whitham, James Wait, Alisa G. Woods, Arzu Colak, Costel C. Darie

Skins protect most living organisms from the surrounding environment. Snakes shed their skin, only to develop stronger, more protective skin. However, the proteins responsible for building snakes skin are not known. Furthermore, the proteins responsible for the unique morphology of the skin of each snake are also not known. Here we used a roteomics-based approach to investigate the proteomes of four types of snake skins: brasilian rainbow (BR), Dumerils Boa (DB), Hog Island Boa (HIB) and Ball Python (BP). Initial analysis by electrophoresis revealed that all four snake skins have 3-4 major protein bands and several minor ones. Mass spectrometry analysis of these major protein bands identified one protein, common in all snakes' skins. Additional analysis suggests that each skin analyzed also has a unique set of major proteins. Further analysis of the snakes' skins using transmission electron microscopy and atomic force microscopy revealed unique patterns specific to each snake skin analyzed. Data obtained so far is still being analyzed.

251 **Investigation of the Effects of Human Jumping Translocation Breakpoint (hJTB) Protein for Potential Use as a Cancer Biomarker**

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

Human JTB (hJTB) is a gene located on the human chromosome 1 at q21, involved in the unbalanced translocation in various cancers. JTB protein is present in normal cells and found to be overexpressed in various cancers including prostate and breast cancer. This protein could be a biomarker for tumor malignancies and a potential target for their treatment. The biological function and the pathway through which this protein causes increased cell growth and proliferation is not entirely clear. Investigation and comparison of the proteomes of cells with upregulated and downregulated JTB can be a good approach to understand the function of the protein and its contribution to tumorigenesis. In here, MCF7 breast cancer cell lines and HEK293 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 SDS-PAGE. The expression was confirmed by western blotting. In gel and in-solution digested peptides analyzed by 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 that were dysregulated. Furthermore, we performed GSEA analysis to identify the biological processes and pathways that are 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.

252 **Low-Cost Microfluidic Platform to Assay Bacterial Biofilm Formation in Flow**

Christopher Piccolo, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Dylan Winkens, Tajrian Khan, Aarsh Patel, James Grinias, Lark Perez

Bacterial biofilm formation can be a catastrophic consequence for numerous bacterial infections. The increasing prevalence of antibiotic-resistant bacterial strains makes understanding the fundamental processes behind this formation a critical aspect in designing new strategies for limiting biofilm growth. Existing methods to measure biofilm formation suffer from lengthy assay times and difficulties in performing observations under flow conditions. By employing low-cost additive manufacturing techniques and open-source microcontrollers, a new microfluidic platform for real-time simulation and observation of biofilm formation in vivo has been developed. 3D-printed molds are used to cast PDMS microfluidic devices that contain a parallel array of replicate serpentine channels with dimensions on the order of 150 µm. The inlet of the device is connected to a reservoir of cell culture media containing Pseudomonas aeruginosa, a protobacteria, which is delivered to the device using gravimetric flow. The outlets of the device are connected to a droplet-counting-array (controlled by an Arduino Mega) as a simple method of tracking decreases in flow rate that occur due to biofilm formation within the microfluidic channels. An adjustable magnification stage was also developed for visualization of the device in real-time. All data is logged to a Raspberry Pi single-board computer, which is used to compile visual data into time lapse videos and export numerical droplet data in CSV format. Approaches to using this device to identify a "time-to-clog" marker that can serve as a key attribute of biofilm formation studies are described here.

253 **The Impact of Pomalyst® Capsule Size Change on API Release – A Comparative Dissolution Study**

Lyudmila Khalatyan, Bristol Myers Squibb, 556 Morris Ave., Summit, NJ 07901, Naseer Alam, Minshan Shou, Emma Ianutolo, Evan Bekos

For all strengths, the manufacturing process of Pomalyst® fills a blended powder into capsule shells having similar volumes. During closing, the body-cap junction space can trap some powder, which can then leak outside the capsule shell during the packaging and result in product complaints. In 2021, larger size capsule shells for all strengths were introduced as a replacement to mitigate this problem. Per health authority guidelines, process-related changes such as changes to capsule size can trigger concerns on comparability and in vivo performance. Therefore, a comparative multimedia dissolution study was performed to evaluate the release of Pomalidomide from both current and the newly introduced larger capsule shells. The results demonstrated that the dissolution profiles from both capsule shells can be considered equivalent. The work was summarized as a part of the supplementary documents to support the filing of Pomalyst® in Japan.

254 **Determination of Impurity Profile for Vidaza (Azacitidine for Injection) by Forced Degradation**

Matthew Feliciano, Bristol Myers Squibb, 556 Morris Ave., Summit, NJ 07901, Sangeeta Dey, Minshan Shou, Evan Bekos

A forced degradation study was conducted for Vidaza (Azacitidine for Injection) to demonstrate the stability indicating function of the related impurity method. Vidaza was found to be stable under photolytic conditions, while degradation was observed under acidic, basic, oxidative, thermal/humidity and metal ion conditions. Oxazolidinone impurity was formed mainly under thermal/humidity and thermal dry conditions and detected with a charged aerosol detector (CAD) since it does not contain UV chromophores. Acceptable mass balance and the spectral homogeneity of Azacitidine peak were achieved for all stressed samples.

255 **Risk and Control Strategy Development for Small Molecule Drug (API-1) Potential Aldehyde Adducts Through the Disruptive qNMR Method in Combination of Small Scale Formulation Processing**

Zhengyang (Allen) Xin, Merck & Co., Inc., MMD - ACT, 126 E. Lincoln Ave. Rahway, NJ 07065, Ryan Cohen, Zhixun Wang, Kweku Amponsah-Efah, Cyndi Qixin He

Acetaldehyde and propionaldehyde, known as trace level impurities in acetone, could form aldehyde adducts during spray drying (SD) process of small molecule drug substance where acetone is used as solvent. Risk assessment and control strategy are needed for these potential aldehyde adducts in small molecule drug product (DP). This work describes the combination of a disruptive qNMR analytical method with ultra-sensitivity and small scale SD process experiment to fulfill the needs using a small molecule model compound (API-1). A qNMR homodecoupling (HD) method was developed to collapse aminal proton multiplets into singlets leading to a 5X S/N improvement as well as resolving interference from low level polymer peaks in the SDI (LOD at 0.03% is achieved). Results demonstrated that API-1 aldehyde adducts can't persist in the SD process owing to their intrinsically poor stability. Thus, the risk of API-1 aldehyde adducts formation in API-1 DP is low. Furthermore, control strategy not to test API-1 aldehyde adducts in either SDI or tablets, but rather limit aldehyde levels in acetone solvent is established.

256 **Assessing Syringe Filter Performance for Liquid Chromatography Samples**

Geoff Faden, MAC-MOD Analytical, 103 Commons Ct, Chadds Ford, PA 19317, Mark Fever, Matt James, Tony Edge

Many samples analysed by liquid chromatography (LC) contain particulates which may damage the analytical column and LC system. Syringe filters provide a quick and convenient solution for particulate removal and are routinely applied in many industry sectors. The filter must provide effective particulate removal, whilst preserving sample integrity by not contaminating the sample during use. The quality of syringe filters is easily overlooked but can be highly impactful on the analytical results obtained. Previous work in our laboratory found syringe filter quality to be potentially highly variable, for example, the extraction efficiency of various 0.45 µm filters was found to range from 18.6 to 102.4%. This work defines a series of simple analytical tests to assess filter efficiency and cleanliness. Extraction efficiency can be determined by filtering solutions of latex beads of well-defined size (0.3, 0.46 and 1.1 µm), followed by UV analysis. Assessment of leachables and extractables was achieved by filtering an aliquot of solvent, followed by gradient analysis by LC-UV. These tests were used to assess multiple batches of a premium range of syringe filters containing various specifications of membrane materials. Extraction efficiency ranged from 97.0 to 99.3 % (RSD = 0.16-1.66%). Low levels of extractable components were determined. The potential impact of extractable syringe filter components was demonstrated by comparing high- and low-quality filters for a trace-level nitrosamine pharmaceutical assay. Whilst the cleaner filter allowed accurate determination of the spiked nitrosamine impurities, the lower-quality filter leached components that co-eluted with three of the spiked nitrosamine impurities.

257 **Optimizing Sample Throughput in Bioanalytical Workflows**

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

Bioanalysis involves the analysis of complex biological samples for exogenous or endogenous components. It commonly employs a degree of sample preparation, followed by LC-MS/MS analysis. The analytical challenges associated with the analysis of a biological fluid will be examined, with a focus on the impact that not removing matrix components can have on the detection system, specifically the phenomena of ion suppression. Three different approaches will be investigated, namely protein precipitation, supported liquid extraction and solid phase extraction. The impact of using these different approaches on the removal of phospholipids, an important matrix component when considering ion suppression, will be discussed, as well as other considerations ranging from financial to method development times. For SPE a decision tree is presented which guides stationary phase selection for particular analytes. Finally, a fully optimised method is shown, together with the resulting performance characteristics, for the analysis of fluticasone in rat plasma. The data provided shows excellent linearity, reproducibility and accuracy across a range from 1 to 200 ng/mL.

258 **The Impact of Plasmonically Driven Hot Carrier Generation on Surface Enhanced Raman Spectroscopy (SERS) Signal**

Chelsea Goetzman, The Ohio State University, 296 Park Rd., Columbus, OH 43081, Zachary Schultz

Plasmonic nanostructures have paved the way for the development of surface enhanced Raman spectroscopy (SERS); a technique that enhances the Raman signal specific to the vibrational modes of a molecule. SERS enhances the Raman signal up to 109-fold allowing for lower limits of detection. Through the illumination of the nanostructure with a laser, a localized surface plasmon resonance (LSPR) is excited and enhances the electric field at the surface of the nanostructure. While the excitation of the LSPR enhances the Raman signal, it can also generate hot carries that cause the formation of transient species that can change the Raman signal. Photoproducts and transient species have been reported for various nanostructures in different SERS experiments and can include cross-linking/dimerization, fragmentation, and radical formation. Understanding the parameters and dynamics of the formation of transient species will allow for generation of desired intermediates for further applications. Previously, our group has reported on transient species formation with the common Raman reporter molecule, 4-mercaptobenzoic acid, as well as the amino acid tryptophan. This work will use changes in the SERS signal to elaborate on the conditions and dynamics of hot carrier reactions associated with the plasmonic activity of nanostructures.

259 **Evaluation of Pump Performance for Long Shallow Gradient Peptide Mapping Analysis**

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

Peptide mapping is a critical tool in biopharmaceutical analysis for characterization and impurity testing. Biotherapeutics are typically complex and contain peptides with a wide range of chemical properties. The samples found in these analyses require demanding method conditions to achieve chromatographic separation, including low flow rates and long, shallow gradients. These are the challenging conditions to produce repeatably for high and ultra-high performance liquid chromatography system (HPLC/UHPLC) pumps. As a result, accurate and precise pump performance is critical to success with these types of reversed-phase protein digest separation methods. Manufacturers have released systems to address these types of samples, with more robust flow paths better equipped to handle demanding conditions. In this study, an enolase digestion standard is used as a representative complex sample across multiple HPLC and UHPLC systems designed for bio related applications. Injections were made using a long shallow gradient method where organic content was increased at a rate of 0.5 %/min over 96 minutes. Peaks were selected based on sufficient sensitivity and resolution for quantitative purposes and were limited by resolution differences across systems. The Arc™ Premier System was studied in both binary and quaternary configurations and was compared to other bio LC systems for peak retention time standard deviation and signal-to-noise.

260 No abstract submitted by the author.

261 **Advanced Mass Spectrometric Approaches to Pharmaceutical Product Development**

Elizabeth Pierson, Merck & Co., Inc., 126 E Lincoln Ave., Rahway, NJ 07065, Josey Topolski, Alyssa Stiving, Dave Foreman, Huaming Sheng

Chemical stability is a key measure of pharmaceutical drug product robustness. Chemical degradation in drug products is most commonly a result of environmental conditions (temperature, humidity, light) or sample matrix (drug-drug interactions, drug-excipient interactions). Environmental conditions can be easily correlated with chemical degradation trends through forced stress studies, however correlations between the chemical degradation product and the sample matrix components are less obvious. Historically, chemical stability is established through laborious and time consuming HPLC experiments. For oral compressed tablets in particular, the methods involve disintegrating the tablet and dissolving the active pharmaceutical ingredient in a solvent mixture. Although these tests provide reliable quantitative results, much useful information is lost during the destructive sample preparation. Mass spectrometry imaging of tablets, which provides spatial maps of chemical species, offers an additional perspective to glean these correlations. Here, we show desorption electrospray ionization mass spectrometry imaging (DESI-MSI) and secondary ionization mass spectrometry (SIMS) imaging have been used to probe drug-drug and drug-excipient interactions within oral compressed tablets and other pharmaceutical dosage forms. Identification of colocalized degradation products and matrix components, through spatial mapping of chemical species, has yielded greater insight into matrix driven chemical degradation mechanisms, drug uniformity, and other critical quality attributes. Additionally, studies employing commercial charge detection mass spectrometry (CDMS) measurements on pharmaceutically relevant samples will be discussed.

262 **Native Ion Mobility-Mass Spectrometry for Studies of Membrane Protein Complexes**

David Russell, Texas A&M University, Department of Chemistry, College Station, TX 77842

Native ion mobility-mass spectrometry (IM-MS) is rapidly evolving as important characterization technique for structural biology owing to its high sensitivity, dynamic range and overall performance metrics. Most commercially-available IM-MS instruments were developed for omics applications, consequently the performance for studies of intact macromolecule (100 kDa – MDa) complexes do not meet the needs of structural biology research. Our research aims focus on development and optimization of advanced MS instrumentation for structural characterization of protein:protein, membrane protein:lipid, and protein:RNA complexes in terms of their stabilities and conformational/compositional heterogeneity. Our experimental approaches focus on development of variable-temperature ESI for determination of accurate thermodynamics, viz. enthalpy-entropy compensation of protein-ligand and protein-protein interactions and assembly, and structural characterization of the resulting complexes. To achieve the IM-MS resolution required for these studies we are developing ultra-high resolution digital quadrupole (DigiQ)-Orbitrap instruments. The prototype (vT-ESI-DigiQ-FT-IMS-Orbitrap) instrument has sufficient resolution to monitor individual ligand binding events, e.g. binding ATP to 802 kDa. GroEL chaperonin. We project a minimum of 10X gain in resolution is possible for the next-generation DigiQ-Orbitrap. This presentation will present results using vT-ESI for determination of the thermodynamic of (i) ATP-GroEL binding, an entropically driven reactions, (ii) binding of GroES to the GroEL (single-ring and tetradecamer) and the effects of conformational heterogeneity on these reactions. The general utility of these approaches will be demonstrated for several other model systems.

263 No abstract submitted by the author.

264 **A-TEEM - A Spectroscopic Tool for the Rapid Characterization of Low Concentration Therapeutics**

Linda Kidder, HORIBA Scientific, 3793 Plum Spring Lane, Ellicott City, MD 21042, Adam Gilmore

The commercial deployment of biotherapeutics depends on advanced analytical tools to ensure safety and efficacy. Many of these products (vaccines in particular) are formulated at quite low concentrations, in aqueous solutions, and with confounding excipients, all characteristics that make these very challenging samples for the "standard" spectroscopic toolbox (Raman, FT-IR, NIR). A-TEEM is a multimodal spectroscopic approach that incorporates both 3D fluorescence and UV/Vis spectroscopic measurements for a 2-in-1 measurement approach that has shown significant capability in rapid and cost-effective analysis of biotherapeutics. We will present several examples illustrating how A-TEEM can be used for the following: vaccine formulation development, vaccine quality screening, viral vector characterization (empty/full ratio and serotype differentiation), and exosome screening.

265 **Point-of-Care Diagnostics Devices for Targeting Emerging Biomarkers**

Samuel Mabbott, Texas A&M University, 600 Discovery Dr., 3006 TAMU, Nanotheranostics Lab Department of Biomedical Engineering, College Station, TX 77843

The continuous development of biosensors, assay formats, analytical platforms, and small-form-factor readers has contributed to significant advances in point-of-care diagnostics that are essential for the timely detection of disease-associated biomarkers within the immediate vicinity of a patient. Furthermore, as POC devices are generally more low-cost and portable than traditional platforms, they enable diagnoses to be made in remote or under-resourced environments thus increasing healthcare accessibility. My laboratories research focuses on the development of diagnostic platforms for targeting emerging biomarkers such as miRNA using surface-enhanced Raman scattering (SERS). In my presentation I will give insight into the platforms we are developing to aid the early diagnosis of preeclampsia and other cardiovascular-related diseases.

266 **Chemically Defined Media Analysis by Absorbance-Transmission & Fluorescence Excitation Emission Matrix (A-TEEM)**

Andrew Lewis, Janssen R&D, 200 Great Valley Parkway, Malvern, PA 19355

The analysis of chemically defined media (CDM) powders presents numerous challenges due to the complex array of chemical components. Unique identification of a specific formulation often requires multiple analytical techniques for high confidence. Rapid determination of CDM quality, lot-to-lot consistency, and the suitability of prepared medium solutions is similarly difficult. Here we present the application of a spectroscopic method – Absorbance-Transmission & fluorescence Excitation Emission Matrix – for unique ID and characterization of CDM powders used in cell culture processes. Proof-of-concept of a chemometric modeling approach is described, in which an established spectral library is used to ensure specific identification and demonstrate suitable material quality on a qualitative basis. Challenges with modeling subtle differences in similar formulations and statistical analysis approaches are highlighted.

267 **Exendin-4 Analog for Optoacoustic Imaging of the GLP-1 Receptor**

Sheryl Roberts, Wayne State University, Molecular Imaging, Oncology, School of Medicine, Detroit, MI, 48201, Eshita Khera, Crystal Choi, Tejas Navaratna, Jan Grimm, Greg Thurber, Thomas Reiner

Limitations in current tools have long challenged the imaging of small pancreatic islets and ß-cell mass (BCM) quantification in animal models[1]. Alternatively, in vivo multi-spectral optoacoustic tomography (MSOT), represents a viable option to image at greater depths than other optical techniques[2]. Here, we report the first development of a broad spectrum and high absorbance near infrared (NIR) optoacoustic agent, $E4_{x12}$-Cy7. The tracer is based on the sequence of exendin-4 that targets the glucagon-like peptide-1 (GLP-1) receptor[3]. The introduction of an unnatural amino acid at the K(12) position of exendin-4, a clinical drug which is an analog of GLP-1 agonist, allowed us to successfully conjugate NIR Cy7 *via* biorthogonal copper(I)-catalyzed azide-alkyne cycloaddition reaction (CuAAC). Probe characteristics and accumulation were assessed *in vivo* with healthy Foxn1 nude mice *allografted* MIN6, a pancreatic beta cell which have naturally occurring glucose-stimulated insulin secretion. MSOT and fluorescent validation showed targeted uptake to the allografted MIN6 cells. The combination of the high spatial resolution achieved with the MSOT and targeted NIR probe (and other similar analogs) could prove a crucial technique for monitoring diabetes and progression in both preclinical and clinical contexts.

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268 **LIMS, Automation Software and Data Integrity: Why it Matters**

Christine Paszko, Accelerated Technology Laboratories, Inc., 496 Holly Grove School Rd., West End, NC 27376

The amount of data that is produced each day is growing at an incredible rate. Over 2.5 quintillion bytes of data are created every single day, and this number continues to grow every single day. In (2020) people generated 1.7MB of data every second for every person on Earth according to the Digital Information World Blog. In the analytical testing laboratory, we have entered a new age in the scale and complexity of data as well as the pace and volume at which the data is generated. Today laboratories require secure, compliant Laboratory Information Management Systems and powerful tools for analysis and to extract both laboratory and business intelligence from their data. More and more organizations will begin feeding their LIMS data as well as their unstructured data into a Data Lake. The key to making good decisions is having high quality data. This presentation will examine the importance of data integrity, along with the role of LIMS and other tools that can be used to help analytical laboratory professionals to produce, manage, analyze and maintain high quality data. We will also examine the impacts of poor data integrity and poor laboratory data management solutions and systems with a few examples and understand what it means to the laboratory.

269 **Data Integrity and Compliance – A Lab Scientist's Perspective**

Sharla Wood, Bristol Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08901

The generation of data continues to evolve as new technologies and ways of working are introduced. However, the expectations of health authorities and our patients remain unchanged – confidence in the quality and integrity of data generated to support GMP decisions. Data Integrity and compliance are ultimately about our patients and enable us to ensure the delivery of high-quality products and patient outcomes. However, remaining compliant is not without its challenges. Guidelines may be interpreted differently, responses are often reactive rather than proactive, and constant continuous improvements can be difficult to stay on top of. A lab scientist's perspective will be shared on how to stay ready and make compliance less of a burden and more just a part of everyday life in the lab.

270 **Delivering Secure and Reliable Data with LIMS**

David Minicuci, Thermo Fisher Scientific, 1601 Cherry St., Suite 1200, Philadelphia, PA 19019, David Manning

Laboratory software has a responsibility to deliver secure and reliable data, empowering scientists to focus on advancing research and delivering high-quality treatments. As pharma organizations digitally transform their operations, data integrity and security are crucial considerations. To help manage this transformation, organizations must have a good understanding of potential challenges, including cybersecurity, data security, and data reliability. With ever-increasing concerns around cyber security, it is essential that critical software solutions are developed according to a robust product security program, to minimize the risk of malicious attacks. Using granular configuration tools, data can be made available only to people that have authority to see and use it – by team, group, function, or individual. Organizations looking to adopt a cloud-based system can be assured of security and reliability, including specific considerations to optimize scalability and compliance for scientific data management and analysis. Through instrument and system integration, manual data transfer is eliminated, driving data integrity from acquisition through to reporting, archival, and disposal. The security and integrity of our customer's data is a top priority for Thermo Fisher Scientific. Join our talk to hear how we develop, implement and manage our solutions to help keep your lab's most valuable asset safe.

271 No abstract submitted by the author.

272 No abstract submitted by the author.

273 **Phase-Appropriate Implementation of AQbD Method Development**

Jinjian Zheng, Merck & Co., Inc., 125 E. Scott Ave, Rahway, NJ 07065, Xiaohua Zhang, Pankaj Aggarwal

In this presentation, we will discuss the application of AQbD concept to the development of HPLC methods for pharmaceutical analysis at different stages. Several real life examples will be used to demonstrate the design and development of efficient analytical methods to take advantage of the regulatory flexibilities provided by the new regulatory guidelines including USP <1220>, ICH Q2, Q12 and Q14.

274 **Expanding the Use of AQbD Tools to Address Small Molecule Pharmaceutical Development Challenges**

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

The Analytical Quality by Design (AQbD) is a comprehensive and systematic approach for method development that provides a broad knowledge about the impact of the critical method attributes on the quality of the data in terms of accuracy and precision. An important aspect of the AQbD approach is risk assessment. In the risk assessment step, critical method parameters are identified and assessed for the highest impact on the quality of the data generated by the method. The high-risk parameters that may affect the method's ability to meet the goals are assessed based on the sound chromatographic science and published research and of course, previous knowledge and experience. These parameters may include, column chemistry, mobile phase pH, solvent type, detection method, and aspects of sample preparation. The material of the LC system hardware may also have a great impact on the quality of the data especially if the analytes are metal sensitive compounds. In this presentation, the impact of the material of the LC hardware on the peak shape, analyte recovery, and the precision of the analysis of metal chelating compounds will be demonstrated. The advantages for using surface treated LC hardware for the analysis of metal sensitive compounds will also be presented and discussed.

275 **Effective Use of Strategic Analytical Quality-by-Design Tools in Stage 1 of the Analytical Procedure Lifecycle Management Workflow**

George Cooney, S-Matrix, 1594 Myrtle Ave., Eureka, CA 95501

This presentation will describe the effective use of strategic Analytical Quality-by-Design (QbD) tools and approaches in Stage 1 of the Analytical Procedure Lifecycle Management (APLM) workflow. The information will be presented within the context of LC method development, validation, and transfer. The tools will include a) Design of Experiments (DOE) approaches for Chemistry System Screening and Optimization of both the LC method and the associated Sample Preparation method, b) integrated Robustness Simulation for establishing the robust Method Operable Design Regions (MODR) for both methods, and c) Replication Strategy experiments which incorporate <USP 1210> Interval Metrics to identify the optimal strategy for the LC method in terms of most efficiently generating Reportable Values which meet the method precision requirements stated in the ATP. The presentation will also describe how the strategic knowledge gained in Stage 1 directly supports the activities carried out in APLM Stages 2 and 3.

276 **Visualizing Reactions and Particle Transformations Using Online and Offline Raman, FTIR and Optical Microscopy**

Charles Goss, GlaxoSmithKline, 1250, S Collegeville Road, Collegeville, PA 19426, Daniel Green, Anthony Nocket, Andrew DiPietro, Kevin Chu, Swetha Ainampudi, Alexis Venere, Alicia Potuck, Kaitlyn Lehman, Nick Radziul, Connor Faith, Luke Huelsenbeck, Anjan Pandey

This presentation will illustrate how analysis techniques like Raman spectroscopy, IR spectroscopy and optical microscopy can be used both in-situ and offline to visualize reactions, crystallization profiles, and particle transformations (e.g., size reduction, crystal form) to determine process profiles, endpoints, stability and sometimes reveal unexpected behavior. Representative Drug Substance and Drug Product example data will be presented and discussed to illustrate how these tools have helped project teams improve their process understanding and accelerate development.

277 **The Driving Sustainable Research: Maximizing Spectroscopy and Spectrometry Tools**

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

Over the last several decades, tremendous advances have been made in sustainable chemistry, with a heavy focus on the synthetic arena. Complementary, are the more recent advances in enhancing green approaches in analytical chemistry for supporting synthesis at all levels from discovery to scale-up to manufacturing. The analytical focus has been on the prevention of waste generation; safer solvents and auxiliaries; design for energy efficiency; and the development of instrumental methods. The application of vibrational-spectroscopy-based techniques, coupled with multi-component analysis greatly enhances process knowledge and control without adding additional assays. This includes the careful selection of the most appropriate technique based on experience, pre-screening, and greenness. The limitations of vibrational-spectroscopy can be address in certain cases by in-line mass spectrometry without the need for any pre-treatment including gas column separation. Sampling can occur through the headspace of reactors and can be used to ensure scrubber efficiency as well as for drying end-point determinations. We will show examples of how we select the appropriate analytical technique, simplified sampling systems, and the resulting data that provides enhanced process knowledge, as we prepare to drive the sustainable analytical approach into manufacturing.

278 **Highly Selective Small Molecule Impurity Monitoring Using Molecular Rotational Resonance: From Residual Solvents to Challenging Isomers**

Alexander Mikhonin, BrightSpec, Inc., 770 Harris St., Ste. #104B, Charlottesville, VA 22903, Reilly Sonstrom, Justin Neill

Spectroscopic techniques are of high demand for process analytical technology (PAT) applications because of their fast feedback. However, most of these techniques including FTIR, Raman, NIR, UV-VIS and fluorescence suffer from insufficient chemical selectivity, background variations, or matrix effects. Selectivity can be addressed by applying multivariate chemometric models. However, these models are unfriendly to even minor variations of process parameters or sample matrix and, thus, require continuous maintenance. As a result, offline laboratory-based analyses with high resolution but relatively slow feedback, such as chromatography (GC, LC, SFC) and NMR, may still be required to fully characterize a system or process. For this reason, search for highly-selective and online-capable spectroscopic methods is still ongoing. This talk will present the application of molecular rotational resonance (MRR) spectroscopy to directly resolve multi-component mixtures without advance sample preparation. MRR has extraordinary chemical selectivity, high spectral resolution, and minimal matrix effects. As a result, MRR can cover analytical gaps of conventional spectroscopic methods and, also, simplify method development workflows and improve routine analysis reliability by eliminating the need for chemometrics. MRR is capable of addressing a number of analyses that are challenging for conventional analytical methods, including analyzing troublesome residual impurities or structurally similar isomers (regioisomers, diastereomers, enantiomers, and isotopic variants). Other ongoing MRR developments include crude-mixture quantitative analysis of essential oils and distillation process monitoring and optimization. As a result of its analytical capabilities and performance metrics, MRR can likely become a valuable tool for developing next-generation PAT-based quality control strategies.

279 **Do you really Understand Your Crystallization – The Value of PAT**

Norman Wright, Mettler Toledo, 6708 Alexander Bell Drive Columbia, MD 21046, Brian Wittkamp, Charlie Rabinowitz

Process Analytical Technology (PAT) has benefitted from real-time analysis as seen by the increasing number of applications successfully developed over a broad range of academic and industrial syntheses in pharmaceutical, biopharma, chemical and polymer sciences. With success, comes the push to investigate increasingly complex chemistries that then require innovative reaction analysis technologies to help describe both the reaction mechanism and intermediates that occur during the process. While significant advances in particle size characterization have recently occurred and will be discussed, the availability of infrared and Raman spec-

troscopies provides tools that when used in concert can provide a higher level of reaction and process understanding often necessary for challenging chemical reactions. The many advantages that on-line spectroscopic analysis provide include: readily integrated analytics yielding data rich, structurally specific information from reaction intermediates and final products. This level of understanding is increasingly important in the drive to reduce overall experiment time and lower cost while gaining more information from fewer experiments. This paper will present examples of PAT technologies in real-time reaction analysis. Highlighting the ability to easily extract and combine information from multiple sources, enabling the identification of control parameters that are required for optimizing a reaction without compromising product quality and critical when optimizing and scaling up reaction processes.

280 **Follow that Particle: Applying Morphological and Spectral Analysis to Developing Pharmaceutical Product and Process Understanding**

Anne Virden, Malvern Panalytical, Grovewood Rd., Malvern Worcestershire, WR14 1XZ, United Kingdom, Deborah Huck-Jones

Particle size and shape matter, but for complex formulations they're not easy to measure. You can measure the properties of the raw ingredients, but what happens to those particles before they get to the patient? We're going to follow particles through production processes and through devices to enable accurate assessments of bioavailability and bioequivalence. In nasal sprays, the drug particle size affects the dissolution rate and availability to sites of action within the nose. The FDA recommends measuring drug particle size distribution but also the impact of actuation on the degree of agglomeration of the drug. That creates two challenges: identifying agglomerates and differentiating drug particles from excipients. Morphologically-Directed Raman spectroscopy (MDRS) can help. Particle morphology is measured by image analysis. The particle shape can tell us if the particles are agglomerates or primary particles. Then using MDRS we can return to particles and chemically identify them using Raman spectroscopy. But characterizing individual particles takes time. This is where the morphologically-directed bit helps. Using the differences in particle shape we can classify particles that are definitely excipient, and then target the Raman analysis on the rest – saving time. So MDRS provides particle size, particle shape and chemical identification, enabling the degree of agglomeration of the active ingredient to be assessed before and after actuation. MDRS can also be used to follow the active ingredient through processing steps. We'll present another case study showing how the combination of process and excipient affects the morphology of the active ingredient.

281 **Automated Particle Correlated Raman Spectroscopy: Case Studies from Microplastics and Pharma to Illustrate Correct Methodology for Diverse Samples**

Bridget O'Donnell, HORIBA Scientific, 20 Knightsbridge Road, Piscataway, NJ 08854

Complete particle characterization is critical for a variety of applications and materials, including pharmaceuticals in various dosage forms (powders, aerosols, tablets, and ointments/creams) and microplastic contamination in a variety of complex matrices (drinking water, marine water, sediment, and tissue). For pharmaceuticals, particle size, distribution, and shape are critical factors for product performance and can have an effect on solubility, dissolution rate, and stability. In the case of microplastics, smaller sized microplastics have strong implications for health effects in organisms, including human beings. While there are a variety of available techniques for particle characterization including dynamic light scattering (DLS) and laser diffraction, Particle Correlated Raman Spectroscopy (PCRS) enables chemical identification in addition to particle morphology and size characterization. In this presentation, PCRS will be described with a focus on sample preparation and presentation for a range of diverse sample types. Depending upon the sample and particle size being studied, preparation techniques can include size fractionation using sieves, vacuum induced dispersion, and vacuum filtration. Complete particle characterization is realized with PCRS through the combination of multimodal optical microscopy techniques with Raman spectroscopic chemical identification. The combination of particle morphology and size with chemical identification enables a more complete understanding of particulate as it relates to drug performance in pharmaceuticals and potential health implications in microplastics.

282 **Raman Spectroscopy of Sedimentary Grains Shows Potential for Use in Provenance Analysis**

Tim Prusnick, Renishaw Inc., 1001 Wesemann Dr., West Dundee, IL 60118, Sarah Shidler, Lucy Grainger, Achim Hermann

Raman spectroscopy for the study of Rutile geochemistry shows potential for use in provenance analysis, characterizing sedimentary samples and allowing for the determination of potential source areas that are not possible with traditional methods. This method relies on the identification of TiO_2 mineral polymorphs and other minerals that are present. Raman spectroscopy is ideal for mineral samples, providing clear chemical identification of common minerals and their polymorphs. The non-contact, non-destructive technique preserves the sample for additional analysis methods. In this study, heavy mineral separates were embedded in 1" epoxy mounts with the mineral surfaces exposed and polished. The 80-300 micron sediment grains were analyzed using confocal Raman spectroscopy. Targeted particle analysis allowed for the analysis of grains of interest without analysis of the surrounding epoxy. The Raman results were combined with other analytical techniques to provide a more complete understanding of the sedimentary grains for provenance analysis.

283 **Cannabinoid Separation: A New HPLC System Suitable for Cannabis Research**

Alicia Stell, CEM Corporation, 3100 Smith Farm Rd., Mathews, NC 28106, Benedict Liu, Candice Cashman

The efficient, accurate, and reliable quantification of cannabinoids has increasingly become a burgeoning field of research. Many are interested in these compounds due to their well-known therapeutic properties. In addition to the stringent production and labeling regulations that accompany the legalization of cannabis and cannabis products throughout the United States. Undergraduate programs have started to incorporate courses and hands-on research specific to cannabis chemistry. One limiting factor includes the expense and expertise characteristic of the separation and quantification of cannabinoids. Typically, HPLC equipped with a UV detector is utilized to analyze both acidic and neutral forms of cannabinoids. Often, these systems require significant upfront expenditures and a designated analyst. Herein, we highlight the use of Lucidity's new HPLC-UV system, the miniLC, to separate and quantify a mixture of 16 cannabinoids. Quantification is performed using an internal standard and pure reference compounds for the construction of calibration curves. All calibration curves displayed R2 values >0.999. Advantageously, the miniLC boasts a small footprint, allowing for easy movement and point of need analysis. The system is also affordable and equipped with software designed to be accessible to all users. The miniLC has proved to be a valuable analytical tool, opening this form of analysis to educational laboratories and to scientists in the cannabis industry. The miniLC is an excellent option for laboratories seeking a compact, simple, and affordable HPLC-UV system.

284 **Addressing Secondary Interactions in Size Exclusion Chromatography of Protein Therapeutics**

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Size exclusion chromatography (SEC) is often plagued with non-specific electrostatic and hydrophobic secondary interactions. This affects the quality of the separation and ability to characterize and monitor important product-related impurities in protein therapeutics. To overcome this challenge, researchers have often needed to perform extensive method development experiments to find the appropriate additives, salt concentrations, and/or organic solvent strengths for accurate quantitation of aggregates and fragments. In this presentation, we will demonstrate how a novel packing material based on a high coverage hydroxy-terminated polyethylene oxide (PEO) bonding and column hardware manufactured to have a hydrophilically modified organosilica surface improved column surface inertness. This technology when paired with an easily prepared physiological buffered mobile phase, is shown to significantly minimized extensive method development. Material science and biochemistry-based considerations will be explained to provide context to the past and alternative use of metal hardware as well as diol and methoxy polyethylene oxide bonded particles. Separations of protein therapeutics, such as monoclonal antibodies, antibody drug conjugates, and fusion proteins have been explored using physiological mobile phase buffers. In an example of application to the analysis of ado-trastuzumab emtansine, the PEO bonded packing material and hybrid surface hardware has quickly afforded sharper, more symmetrical monomer peaks along with higher, more accurate, relative abundance measurements on aggregation compared to diol bonded packing material in conventional hardware. It is shown that improved SEC column inertness is enabling for accurate characterization of CRISPR Cas proteins along with their aggregates and ribonucleoprotein complexes.

285 **Applying Method Operable Design Region (MODR) and Replication Strategy Optimization Results to Support Analytical Procedure Lifecycle Management (APLM) Stage 2 Method Validation and Transfer and APLM Stage 3 Procedure Monitoring**

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Establishing the robust Method Operable Design Region (MODR) and the optimum replication strategy for the analytical procedure are two critical elements of Analytical Procedure Lifecycle Management (APLM) Stage1. The knowledge obtained from robust MODR characterization directly supports the Analytical Control Strategy, also developed in Stage 1, and the statistical control charting done in APLM Stage 3. Replication strategy optimization defines the most efficient strategy for generating reportable values which meet the analytical method precision requirements defined in the Analytical Target Profile (ATP). The optimized analytical method and optimized replication strategy can then be used in combination with USP <1210> interval metrics to assess the success of method transfer activities done in APLM Stage 2. This

presentation will describe the use of these analytics and workflow within the context of the development, validation, and transfer of a liquid chromatography method.

286 Withdrawn by the author.

287 **Microscopy & Microanalysis of Temporary Tattoos**

Michelle D. Miranda, Farmingdale State College-SUNY, The Center for Criminal Justice, 2350 Broadhollow Rd., Farmingdale, NY 11735

The examination and analysis of permanent tattoos and tattoo inks has been well studied across both medical and forensic fields. Conversely, studies concerning temporary tattoos and their chemical compositions have been very limited in number and scope. This presentation will address recent studies concerning the microscopy and microanalysis of both children's and adult temporary tattoos in a forensic context. Samples were examined macro- and microscopically and analyzed using micro-FTIR [ATR and R-A] in an effort to identify the chemical composition of temporary tattoos with the goal of assessing inter- and intra- brand variation for forensic identifications and comparisons. During the course of the studies presented, it became clear that observation was a critical component of the analytical scheme, with microscopy playing an important role in the broader process of scientific research.

288 **Hammer Bounce**

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When the trigger is pulled on a loaded revolver, the firing pin strikes the primer of the cartridge in a chamber to initiate a sequence of events that culminate with the discharge of a bullet. A phenomenon known as hammer bounce has been noted in the literature as a partial rearward movement of the hammer just after firing, followed by its forward return. Recently, photographs have appeared that show a revolver at the instant of discharge with the hammer at its fully rearward position. This seemingly improbable coincidence prompted this research to be undertaken. In addition to determining the amount of rearward movement of the hammer using high-speed photography, the indentation of the primer was examined with both stereomicroscopy and incident-light comparison microscopy. This was done to determine the ramifications of potential double strikes of the firing pin on the primer and its effect on the evaluation of the resulting tool mark.

289 **The Application of Electron Backscatter Diffraction to the Forensic Analysis of Minerals**

Tiffany Millett, John Jay College, CUNY, The Graduate Center, 118 Gauldy Ave., Staten Island, NY 10314

To date, Electron Backscatter Diffraction has found few uses in the forensic sciences. One of the few utilizations of this technique in forensic science is in firearm serial number restoration as a complementary technique to the time-consuming and tedious chemical etching methods. This research aims to expand the uses of electron backscatter diffraction in the forensic sciences as it applies to other types of samples, specifically as it pertains to forensic mineral analysis. Electron Backscatter Diffraction offers the analyst information regarding crystal structure of a material, so long as the sample is crystalline or polycrystalline in nature. Minerals, many of which are crystalline in nature, can be analyzed using Electron Backscatter Diffraction. The Electron Backscatter Diffraction instrumentation used to achieve these patterns is coupled with a Scanning Electron Microscope and Energy Dispersive X-ray Spectrometer. These three instruments provide the analyst information of morphology, elemental composition, and of course, crystal structure. This combination of information in one single system is the most attractive feature of this method.

290 **Look Before You Leap**

Peter De Forest, Forensic Consultants, PO Box 141, Ardsley, NY 10502

Analytical chemistry problems can be divided into two broad categories. These are high-volume routine testing and open-ended non-routine investigative analyses such as those for general unknowns. The former can be automated and make use of auto-samplers. This presentation will focus on the latter where the analytical problem often benefits from an approach designed and developed de novo. Simply looking at the sample under low power magnification can be a valuable first step. Much can be learned in this way. Recall Louis Pasteur's classic experiment with ammonium sodium tartrate crystals. Under magnification he observed two distinct crystal symmetries which allowed him to manually pick through the sample and isolate the two distinct symmetries. When placed in solution each of these caused the beam of polarized light in a Biot polarimeter to rotate in opposite directions. This led to the discovery of chirality and enantiomers. Beginning ~75 years ago a revolutionary approach to analytical chemistry began with instrumental methods based on physical properties slowly replacing those based on chemical reactions. Some physical methods such as emission spectroscopy dated from the mid-nineteenth century but were used for research and only gradually became tools of the analytical chemist. Their adoption and development in the analytical laboratory was a boon to high-volume routine analyses, but led to the unfortunate neglect of chemical microscopy. This paper argues for re-assessing the inherent value of techniques in chemical microscopy and the recognition of their value for non-routine chemical analyses. Case examples from forensic science will be used.

291 **Automated Platform Analytical Method to Determine Polysorbate 80 Content in Biopharmaceutical Drug Product Using the Andrew Robot: A Practical Approach to Automation**

Sharon Matamoros, *GlaxoSmithKline,* 1250 S Collegeville Rd, Collegeville, PA 19426, Katie Carnes, Dao Nguyen, Kaitie Grinias

The implementation of laboratory automation can accelerate the chemistry, manufacturing, and control (CMC) activities, as well as build quality into the process. Automated liquid handling, for example, eliminates human error associated with different pipette techniques between analysts, and dramatically reduces variability in analytical test results. Experiment time can be reduced, and safety-related harm due to repetitive movements can be eliminated for the scientists. The Andrew robot enables automated pipetting, flexible programmable settings, and simple drag-and-drop protocol designs. This liquid handler robot is user friendly and ideal for analysts and scientists with limited robotics knowledge. This talk describes a practical approach and steps to automate a platform analytical method to determine polysorbate 80 (PS80) content in biopharmaceutical drug products. Automation development challenges and solutions are discussed, to include: standard solution preparations for a calibration curve, approaches to address samples with varying viscosities, and the preparation of assay controls. Analyst versus robot performance is discussed which includes the analysis of linearity, precision, and accuracy. Scientists and analysts interested in automating an analytical method will benefit from this talk.

292 **Enhanced Sensitivity for Peptide and Protein Applications Using the 1.5mm ID Column**

Peter Pellegrinelli, AMT, 3521 Silverside Rd., Suite 1-K, Wilmington, DE 19810, Stephanie Schuster, Conner McHale

Chromatographers continue to explore alternate avenues to improve their chromatography. These avenues can encompass different parameters which can be manipulated. Notably method parameters, connection tubing, instrumentation configuration, column choice, amongst others. If the method requirements permit freedom for change, adjusting method parameters or exchanging the column are simple changes. It is well known that by switching from larger ID columns to a smaller ID can improve sensitivity and reduce overall solvent consumption. Previously, in order to improve the method sensitivity using a 2.1 mm ID, chromatographers made the switch to a specialized micro or nano flow LC system using capillary columns. From a routine operational and maintenance standpoint this can be an expensive venture to undertake to improve one's chromatography. Incorporating the new 1.5mm ID column a chromatographer can skip the expense of a specialized system and gain improvements in both sensitivity and reduced solvent consumption with a commercial UHPLC system. This new column dimension shows an increase in sensitivity through both UV and LC-MS applications. With greater response and higher ionization efficiencies problematic separations can be overcome. Advantages to the bio workflow with proteomic and peptide mapping applications, specifically intact and reduced mAbs will be shown.

293 **A Proteomic Investigation of Human Serum from Donors with Triple Negative Breast Cancer and Matched Controls to Identify Protein Biomarkers for Breast Cancer Detection**

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

Breast cancer (BC), is a leading cause of death for women in the United States. An estimated 1 in 8 women in the United States will develop BC in their lifetime. Triple negative BC (TNBC) is BC in which the cancer cells are devoid of estrogen receptor, progesterone receptor and Her2 receptor. Therefore TNBC is the most aggressive type of BC. Early diagnosis and treatment of BC is crucial. One way to detect BC in its early phase is through identification of proteins that are dysregulated due to the onset of BC (i.e. protein biomarkers). . Mass spectrometry (MS)-based proteomic methods are ideal for the investigation of protein biomarkers. This study utilizes MS-based proteomics to study the protein differences in the human serum from women with TNBC and matched controls. If significant protein dysregulations are found, this could lead to a protein biomarker signature for breast cancer to aid in diagnosis and treatment. In this study, 16 human serum samples from women with TNBC and matched controls (8 vs 8) were investigated by in-gel and in-solution digestion, followed by nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. The raw data was then analyzed using ProteinLynx Global Server (v 2.4) Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. The dysregulated proteins in this study are currently being compared with the dysregulated proteins found in various breast milk proteomics and serum proteomics studies. The study is underway.

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

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

Cancer is among the top 5 causes of death in the U.S and Breast cancer (BC) is the second most frequently diagnosed form of cancer with 271,270 new cases in 2019. BCs can be grouped into two main categories: *in situ* BC and invasive BCs. There are two main types of invasive BCs: Invasive Ductal Carcinoma (IDC) and Invasive Lobular Carcinoma (ILC). In IDC, the cancer cells begin in the ducts and then invade other parts of the breast tissue. Comparison of IDC serum samples with matched controls could identify dysregulated proteins that can potentially be used as protein biomarkers. In this investigation, we used a proteomics approach to compare sera from 3 African American donors with IDC and 3 matched healthy controls. The IDC samples were ER+PR+ and the tumors were classified as PT1c pNx, PT1a pNx, and PT2 pN1a. The samples were prepared according to an in-gel digestion protocol and analyzed by nanoliquid chromatography tandem mass spectrometry (nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. In a second experiment, the samples were prepared according to an in-solution digestion protocol and analyzed by nanoLC-MS/MS), using a NanoAcquity UPLC coupled with a QTOF Xevo G2 XS MS. The resulting raw data were analyzed using ProteinLynx Global Server (v 2.4), Mascot Daemon server (v. 2.5), Mascot Distiller Workstation, and Scaffold 4.3 software. The dysregulated proteins that were identified are currently being interrogated. This is an ongoing investigation.

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