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ABSTRACTS OF TECHNICAL PAPERS

1 Multidimensional Gas Chromatography: Do More Acronyms Really Give Better Analysis?
Nicholas H. Snow, Seton Hall University, 400 South Orange Ave., South Orange, NJ 07079

Today's gas chromatographic methods often include multiple dimensions of separation that can both help and hinder the separation and analysis. New detection technologies, such as tandem mass spectrometry (MS-MS) and VUV, which combined with new column technologies such as gas chromatography (GC)xGC and IL columns and selective sample preparation can generate up to six dimensions. While this adds considerably to the achievable separation power, it adds similarly to the chemistry that must be understood and applied by the analyst. For example, an solid-phase microextraction (SPME)-GCxGC- time-of-flight (ToF)MS analysis includes the full six dimensions; each has the potential to either aid or disrupt the separation: sampling, extraction, injection, two dimensions of GC and MS detection. Examples from pharmaceutical and organic contaminant analysis are used to examine multi-dimensional separations in terms of the selectivity that can both be obtained and lost as dimensions are added or if any, such as the injection technique are not fully understood or optimized. In gas chromatography, additional dimensions of separation provide both more capability and more challenge: more dimensions may not always be better.

2 Advances in the Application of Vacuum Ultraviolet Spectroscopic Detection for Gas Chromatography
Kevin A. Schug, The University of Texas-Arlington, Box 19085, 700 Planetarium Pl., Arlington, TX 76019, ChangleiQiu, Ling Bai, Jamie Schenk, Jonathan Smuts, Phillip Walsh, Harold M. McNair, Jack Cochran Vacuum ultraviolet (VUV) absorption spectroscopy is a new tool for gas chromatography (GC) detection. It provides means for qualitative speciation, as well as quantitation, of volatile and semi-volatile organic compounds. The VUV detector measures gas phase absorption in the 120 – 240 nm wavelength region, where all molecules absorb and have unique spectral signatures. GC-VUV complementary to mass spectrometry and provides improved means for detecting low molecular weight, isomeric, and labile species. Because of the well characteristic VUV absorption signature of molecules, some interesting qualitative and quantitative treatments are possible. Co-eluting compounds for which VUV reference library spectra are available can be easily deconvoluted into individual response signals. In fact, a more comprehensive time interval deconvolution (TID) procedure for automated speciation and classification of compounds eluted during a chromatographic run is now possible. TID has been applied to both gasoline PIONA and Aroclor polychlorinated biphenyl classification analyses. Further, the potential for pseudo-absolute quantitation will be discussed. Because the absorption cross-section is a physical property for a given molecule, it is possible to delineate exactly how many molecules give rise to an absorption event for that molecule in the flow cell, if all other variables are known. This provides some interesting possibilities for system diagnosticians and calibrationless quantitation, which is also demonstrated.

3 Designing Flow-Based Unit Operations for Sample Prep
Graham D. Marshall, Global FIA, Inc., 684 Sixth Ave., Fox Island, WA 98333, David J. Hoidich, Don C. Olson

A review of flow-based analysis literature will quickly reveal that most papers are focused on the actual measurement: chemistry, hardware, optimization studies, etc. Few papers have been devoted to the use of flow-based sample manipulation for sample preparation. In many instances these steps are manual. A notable exception is the work of Prof. Purnendu Dasgupta who has pioneered several novel pieces of equipment for sample preparation. Some of these are briefly mentioned in honor of Prof. Dasgupta’s award for Outstanding Achievements in the Fields of Analytical Chemistry. This is followed by a discussion of some of the unit operations that we have developed for transforming real world samples so that they can be handed off to an appropriate flow-based analytical application. Included in these examples is a self-cleaning filter, a solvent extraction unit operation that actually works, and a dilution and quenching module that can handle both homogenous and heterogeneous samples and also allow monitoring of super-saturated crystalization studies.

4 Cheating Mr. Beer: Augustus Has Had It Too Good!
Purnendu K. Dasgupta, University of Texas-Arlington, Department of Chemistry and Biochemistry, Arlington, TX 76019, Ruchika P. Bhatwal, Yin-Huan Li

By far the most common quantitation technique in analytical chemistry’s optical absorbance measurement. With single path multireflection cells such as a “White Cell”, the limit of detection (LOD) improves but not the dynamic span. Partially reflective mirrors on each side of a measurement cell, was proposed in 1987. This leads to greatly increased effective pathlengths at low absorbance levels. Interferometric principles were originally applied incorrectly to derive the transmission behavior. In 1988 O’Keefe introduced Cavity Ring Down and in 1998 extended it to continuous wave (CW) measurements using the same arrangement proposed in 1987. O’Keefe’s derivations of transmission behavior are also approximate. We show that the limiting gain is 1/(1-R), R being mirror reflectance. We further show how to attain substantial gain in detection limits by this technique in a very simple and inexpensive. Broadband cavity enhanced absorption spectroscopy is only now coming into being although the feasibility was shown 25 years ago.

5 Utilizing NIRS When the Analyte is Obscure
Samuel Coleman, ColeSpec Solutions, 138 Deer Chase Ct., Azle, TX 76020

Since near-infrared spectroscopy (NIRS) is the measurement of absorbed energy from specific vibrating bonds, the more specific and precise the chemical analyte determined in the reference laboratory, the greater the relationship between laboratory values and spectral measurements. In the agricultural field we notice this by comparison of the relationship of nitrogen (or crude protein) content as compare to fiber analytes such as acid detergent fiber or neutral-detergent fiber, both of which are much less chemically defined than nitrogen. Yet successful NIRS equations have been developed for the fibrous analytes as well as other ill-defined, chemically speaking, constituents. When it comes to bioassays, which comprise the more interesting and useful assays for feeds and forages, the chemical representation is even more obscure. The determinant is often an interaction of feed chemistry and the animal that eats and digests it. For ruminant animals, such as a cow or sheep, the situation is more complex because microflora contained in the gastrointestinal tract, particularly the rumen, are the primary processors, and their interaction with the host animal influences digestion and absorption of nutrients. For voluntary intake, one of the more important entities for determining growth and efficiency, the animal exerts a significant role the amount eaten, and the feedstuff and its chemistry largely function as constraints. We examine some of these obscure analytes and present a case for a functional NIRS relationship, even though the goodness of fit statistics may suggest otherwise.

6 Routine and Non-Routine Use of NIR Spectroscopy in Forage Crop Analysis
Craig Roberts, University of Missouri, 108 Waters Hall, Columbia, MO 65211

Forage crops, sometimes called “forages,” are plants that support livestock production. Analysis of forages by near-infrared (NIR) spectroscopy can be daunting. As this presentation explains, forage samples present an extremely diverse matrix and are subjected to a wide array of direct and indirect measurements. The diverse matrix is the result of multiple species that include annuals and perennials, warm-season and cool-season plants, as well as grasses and legumes. The diverse matrix is also affected by these species being planted in mixtures, harvested several times during the year, and grown in different environments. The wide array of measurements includes routine analysis for nutritive value, known as “quality components,” such as fiber, protein, and digestibility. Measurements also include “antiquality components,” such as tannins, toxins, and molds. In addition, forage measurements include physiological, morphological, taxonomic, and genetic traits. To address the difficulties of using NIR technology in forage crop analysis, American scientists formed various collaborative organizations. As discussed in another presentation, United States Department of Agriculture researchers organized the Forage Testing Network to explore the feasibility of NIR technology for routine applications. Forage crop educators followed by forming the NIRS Forage and Feed Testing Consortium, a nonprofit user group to develop equations of common interest and monitor accuracy. As these collaborative efforts enjoyed success in routine measurements, forage scientists began attempting NIR applications for non-routine analytes, such as compounds that occur in minute concentrations. These non-routine analytes, as well as the principles necessary for their successful calibration, are discussed.

7 Taking NIRS Outdoors
Patrick Starks, United States Drug Administration-Agricultural Research Service, 7207 W. Cheyenne St., El Reno, OK 73036

Forage nutritive value (i.e., forage quality) impacts livestock health and performance, but determining the quality of forages for grazing animals is difficult. In the 1970s, development and application of bench-top near-infrared spectroscopy (NIRS) techniques to assess forage quality proved to be a great leap forward, as it replaced the more costly, labor-intensive, and less timely wet chemistry procedures.
used in proximate analysis. However, even with this advancement, NIRS evaluation of forages still required manual sample collection and preparation, the analytical results lagged sampling dates, and results were (and remain) point-based. Therefore, we evaluated the possibility of "taking NIRS to the field" to rapidly assess the nutritional landscape of grazing livestock via hand-held spectroradiometers (which we refer to herein as remote sensing). In this presentation we describe: 1) experiments designed to test the feasibility of quantifying selected forage quality indicators, 2) application of remotely sensed crude protein to determine timing of supplementation, and 3) application of remote sensing to assess relative feed value of alfalfa (Medicago sativa L.). The final part of the presentation addresses plans to use unmanned aerial vehicles (UAVs) coupled with inexpensive spectroradiometers to provide field to landscape level assessments of forage quality.

8 Early Days of Forage NIR
Franklin E. Barton II, LLS Instruments, 165 Sunnybrook Dr., Athens, GA 30605
Near infrared spectroscopy got its big start in agriculture. Specifically, it was used to measure protein content in wheat. That, however, was not the application that launched all the successive ones. NIR had many detractors in the early 1970's and the United States Drug Administration, Agricultural Research Service (ARS) began an in house research program to vet the technology. This research program was called the National Near Infrared Forage Research Network and was comprised of research in six locations, State College, PA, Beltsville, MD, Athens, GA, Minneapolis, MN, El Reno, OK and Logan, UT. These locations were ARS locations with ongoing research program in agronomy/plant breeding, animal science, forage livestock, dairy, rumen microbiology, spectroscopy/chemometrics and instrument engineering. Protein in wheat was an easier task in that Kjeldahl analysis had been around over a century and provided very precise results and in the case of wheat constituted 12-14% of the sample. In the case of forages protein was 4-6% and the other assays for fiber, lignin and digestibility were far less precise and the composition variable because of differences in the plant chemistry between warm and cool season grasses and legumes. We look at the problems, the solutions and the early efforts to build the applications. Finally, we discuss the documentation of these efforts in Handbook 648.

9 Past, Present and Future Analytical Techniques to Detect Counterfeit Drugs
Ravi Kalyanaraman, Bristol-Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903
Counterfeit and adulterated pharmaceutical drugs have caused a serious threat to patients in the United States after the recent counterfeit Avastin® injection incident. Counterfeit drugs have entered the pharmaceutical supply chain due to increasing international trade and sales via the internet. Pharmaceutical industry and federal agencies have found ways to counteract the increasing threats caused by pharmaceutical counterfeiting. Recent estimates are that perhaps 15% of pharmaceutical drugs world-wide are counterfeited. Several technologies have been put to use to detect counterfeit drugs rapidly and efficiently. These include covert and overt features added to the packaging material and to the drug product itself, and authenticating the suspect drug for these features. In the past ten years or so, near-infrared (NIR) and Raman spectral techniques, which are complimentary in nature, have been used widely to detect counterfeit drugs. Both techniques are rapid, noninvasive and nondestructive that can be used for the analysis of many classes of pharmaceutical dosage forms. In the past five years, portable versions of infrared (mid and NIR) and Raman spectrometers have paved the way to take the ‘lab’ closer to where the counterfeit activities are taking place, such as deceitful manufacturing facilities and pharmacies. Unique spectral fingerprints are developed for authentic product using these portable spectrometers to test the suspect product against these fingerprints in order to spot counterfeiters and also to authenticate legitimate products. More recently, Raman spectral fingerprints have been developed for protein based biologics drugs to screen for counterfeits. This talk highlights the past, present and future analytical techniques to detect counterfeit drugs with specific examples.

10 Protecting Patients: A Case Study in Counterfeit Medicines
Anthony Zook, Merck, MS: WP53F-104, 770 Sunnynowt Pike, West Point, PA 19486
The threat to global public health associated with counterfeit, diverted, and stolen medicines continues to escalate. Collected intelligence on the subject for calendar year 2015 reported 3,002 unique pharmaceutical counterfeiting, diversion, and theft events, involving 1,095 different medicines, and spanning 128 countries. In response to this threat, Merck executes an intelligence-led anti-counterfeiting program with the primary goal of protecting patient safety. The strategy focuses on securing the supply chain, investigations and enforcement actions, and creating advocacy and awareness to this critical issue. A recent case study involving the significant penetration of counterfeit medicines into a developed market is discussed. The discussion includes how forensic applications of analytical chemistry have aided the investigations, and further supported the protection of patients from this threat to public health.

11 No LC? No problem! Quantitative Assay of Beta Lactam Antibiotics with a Paper Test Card
Marya Lieberman, University of Notre Dame, 271 Stepan Hall of Chemistry, Notre Dame, IN 46556, Nicholas M. Myers, Jalien Carpenter, Doaa Auldulaimi, Margaret Berta, Mercy Maina, Phelix M. Were, Jamie M. Luther
Many developing countries have registered thousands of brands of pharmaceuticals for sale in their markets, but lack the resources needed to ensure the quality of these products. Poor quality beta lactam antibiotics are a particular public health risk in these settings. These inexpensive medications make up a significant fraction of the anti-infective market, and when underdosed they cause poor clinical outcomes and contribute to development of resistant pathogens. Underdosing can result from degradation in poor storage conditions, repackaging of expired products, sloppy or negligent manufacturing, or outright falsification. In response to the unacceptable levels of substandard beta lactam antibiotics found in our 2013-2015 sample pool collected by covert shoppers in Kenya, we have developed a paper test card to detect beta lactam antibiotic pills that contain less than 90% of the stated active pharmaceutical ingredient (API). A sample of the antibiotic undergoes base hydrolisis to form free thiol groups, which are then oxidized with excess iodine. The remaining iodine is back-titrated on the card by thiosulfate. The test takes about 10 minutes and can be done in a laboratory equipped with a centigram balance. The accuracy and precision of the paper test card were measured for ampicillin and amoxicillin tablets collected in Kenya in 2014-2015, and the results were compared with a high-performance liquid chromatography (HPLC) assay. While the HPLC is clearly a better analytical tool, the paper cards were able to detect most of the substandard products, and could be used as a quick screening method for monitoring post-market antibiotic quality.

12 Authentication of Pharmaceutical Products with Spectroscopic Solutions
Jeffry Denault, Eli Lilly, Lilly Corporate Center, Indianapolis, IN 46221, Robert Beal
The challenges associated with chemical authentication of suspect counterfeit pharmaceutical products are becoming more difficult as (1) counterfeit samples have become closer replicates of authentic products and (2) reporting requirements to regulatory agencies are increasing. A range of rapid spectroscopic technologies have become more prevalent with the commercialization of portable/handheld analyzers and these technologies provide an opportunity to generate high quality data to address both issues quickly. This talk addresses general challenges and the implementation of spectroscopic technologies for authentication of pharmaceutical products.

13 NMR Based Screening Methods for Hit Finding in Medicinal Chemistry
Hugh Eaton, Merck Research Labs, MS: K15-LL0950, 2015 Galloping Hill Rd., Kenilworth, NJ 07033, Yan Hou, Mark McCloose, Daniel Wyss, Payal Sheth, Todd Mayhood, Christopher Tan, Marc Labrorg, Jing Su, Terry Roemer, Andrew Cooke, Craig Stump, Darrell Henze, John Sanders, Berengere Sauvagnat, Elliot Nickbang, Yang Xianshu, Hua-Poo Su
Fragment based drug discovery (FBDD) has become an increasingly important method for finding starting points for new leads in the pharmaceutical industry. Among nuclear magnetic resonance (NMR) methods used for FBDD, ligand-detected saturation transfer difference (STD) spectroscopy is one of the most common, and has been used extensively to screen libraries of low molecular weight (MW) fragments against a variety of targets. This talk describes a STD fragment screening targeting an allosteric binding site of the receptor tyrosine kinase TrkA. Another approach used to find new leads in the pharmaceutical industry is phenotypic cell-based screening. This talk describes an NMR-based triage procedure used to evaluate putative hits found in an anti-bacterial phenotypic screen. The triage procedure includes a 1D 1H-NMR functional assay to measure the activity of screening hits against the bacterial epimerase MnaA, STD experiments, and protein-detected 1D 1H-NMR ligand binding experiments.

14 Quantitative NMR in Solids: Towards Uniform Enhancement in CP-MAS NMR Spectroscopy
Guangjin Hou, University of Delaware, Department of Chemistry and Biochemistry, Newark, DE 19716
One of the outstanding advantages of nuclear magnetic resonance (NMR) spectroscopy is its capacity of providing quantitative measurement for systems from molecular to macroscopic scales, compared to the other spectroscopic methods. The
most straightforward technique for quantitative NMR spectroscopy is to perform the single pulse excitation experiment, but generally it is quite time-consuming, especially for those rare nuclei such as 13C, 29Si, and 15N, due to the low sensitivity and long spin-lattice relaxation times (T1). The cross polarization magic angle spinning (CP-MAS) method plays a crucial role in extending the analytical capabilities of solid-state NMR to the studies of such ‘rare’ nuclei, where the sensitivity can be enhanced dramatically and the experimental time is reduced greatly as well. However, one of the major drawbacks of CP is that the present techniques and approaches are not applicable for quantitative measurement or analysis. In this presentation, the recent advances on quantitative determination of CP-based MAS NMR spectroscopy have been introduced. Among these techniques, the heteronuclear/homonuclear recoupling technique or multieCP technique has mostly been used, the non-uniformity CP-enhanced magnetizations of dilute spins are redistributed, and finally the uniform enhancement can be obtained. It is applicable for quantitative measurement or analysis. Specifically, the experimental schemes have been demonstrated to be applicable for obtaining quantitative CP-MAS spectra no matter what the recycle delay is. More noticeably, high efficiency gains can be obtained with relatively short recycle delays. These schemes are suitable for abundant or isotope-labeled systems as well as the systems with rare nuclei in natural abundance.

15 Absolute Concentration of Choline Derivatives in Biologically Relevant Media Using 14N-NMR
István Pelczer, Princeton University, Frick Chemistry Building, Washington Rd., Princeton, NJ 08544, Julianne Goff
Choline is an essential metabolite, which is part of many important pathways and is a biomarker for cancer, its development and response to treatment. Therefore, it is an important issue to measure its absolute concentration in various biological media, such as biofluids, cell- and tissue/biopsy samples. The sample applies to a variety of food and food ingredients, where choline content could be characteristic to authenticity or condition of the substance. There are many analytical ways to approach this issue, yet nuclear magnetic resonance (NMR) is the only one, which would handle natural samples directly and is fully quantitative by nature. This presentation is focusing on using 14N-NMR, primarily 14N,1H-HSQC-based correlation methods to measure absolute concentration of choline in various biologically relevant conditions. In choline the quadrupolar moment of 14N collapses due to high symmetry of the nitrogen substitution, which makes the correlation experiments feasible, while this special filter removes all background, resulting in a clean spectrum. We have been testing DR BENCE, an in-house developed indirect reference method and PULCON, as well as incremental addition of known components for liquid state samples, and the latter two for HR-MAS. We shall compare the results so far and discuss the technical benefits and potential disadvantages of the various methods.

16 Quantitative NMR Analysis Made Faster and more Accurate
Michael A. Bernstein, Mestrelab Research, Feliciano Barrera 9B - Bajo, Santiago de Compostela – 15706, Spain
The use of nuclear magnetic resonance for quantitation (qNMR) is well accepted, and has myriad applications. High-resolution NMR data is one such case that exploits the accepted notion that all signals in an NMR spectrum have the same response factor. This extends qNMR to being a powerful technology for the (often) quite complex case of mixtures analysis. For qNMR to be accurate and applicable, care must be taken at every stage: sample preparation, data collection, processing, and analysis. The effect of these factors and required uncertainties, precision, accuracy, etc., are well known, and the requirement will therefore be predicated on need. Here, we discuss qNMR signal processing and analysis in the context of qNMR, from high-precision applications where uncertainties >1% are required, to more complex analyses where an uncertainty of perhaps 2-5% is adequate. Requirements will determine the allowed workflow. In the context of signal processing and analysis, this is often linked with the degree of automation that becomes available. We discuss the options and approaches that are available for NMR-based quantitation of simple compounds to more complex mixtures.

17 Environmental Chemistry Compound Identification Using High Resolution Mass Spectrometry Data Integrated to the EPA Chemistry Dashboard
Antony Williams, United States Environmental Protection Agency (EPA), 109 T.W. Alexander Dr., Research Triangle Park, Durham, NC 27711, Jon Sobus, Mark Strynar, Andrew Mace, John Barr, Christopher Smith, Jordan Foster, Michelle Krzyzanowski, Jeff Edwards, Kamel Mansouri
There is a growing need for rapid chemical screening and prioritization to inform regulatory decision-making on thousands of chemicals in the environment. We have previously used high-resolution mass spectrometry to examine household vacuum dust samples using liquid chromatography time-of-flight mass spectrometry (LC-TOF-MS). Using a combination of exact mass, isotope distribution, and isotope spacing, molecular features were matched with a list of chemical formulas from the EPA’s Distributed Structure-Searchable Toxicity (DSSTox) database. This has further developed our understanding of how openly available chemical data-bases, together with the appropriate searches, could be used for the purpose of compound identification. We report here on the utility of the EPA’s ICSS Chemistry Dashboard for the purpose of compound identification using searches against a database of over 720,000 chemicals. We also examine the benefits of quantitative structure–activity relationship models (QSAR) prediction for the purpose of retention time prediction to allow for alignment of both chromatographic and mass spectral properties. This abstract does not reflect United States Environmental Protection Agency (EPA) policy.

18 Mechanistic Study of Gas Phase In-Source Hofmann Elimination of Doubly Quaternized Cinchona-Alkaloid Based Phase-Transfer Catalysts by (+)-ESI/Tandem Mass Spectrometry
Huan Li, Linda C. H. Li, Wai Y. M. Ip, Mark Strynar, Jennifer Smith, 126 E. Lincoln Ave. , Rahway, NJ 07065, Rong-Sheng Yang, Katrina W. Leka, Edward S. Sherer, Li-Kang Zhang, Bangping Xiang, Roy Helmy
A new class of cinchona alkaloid phase-transfer catalysts (PTC), which are quaternized at both quinuclidine and quinoline nitrogen, were reported by Merck. In this presentation the study on the formation of Hofmann elimination product ions [M2+ + H+] for bisquaternary ammonium salts. Hence, efforts were made towards the understanding of the mechanism of formation of Hofmann elimination product ions [M2+ + H+]. Using liquid chromatography high resolution mass spectrometry–mass spectrometry (LC-HRMS-MS), structure elucidation and density-functional-theory (DFT) calculations. Based on the experimental and calculation results, the in-source fragmentation pathways of PTCs were summarized as below. Under positive electrospray ionization (ESI), The PTC would lose BrnBr or HBr to form mono-charged product ion due to strong cumbic repulsion. The in-source Hofmann elimination was the major fragmentation pathway leading to the formation of [M2+ + H+] and HBr neutral loss. Although there were four possible beta-hydrogen for elimination, calculation and MSMS results indicated that only one major Hofmann elimination product was formed in the gas phase. This results help us better understand the MS in-source fragmentation mechanism of doubly charged ammonium salts.

19 Mass Spectrometry Based Proteomics to Investigate and Characterize Human Jumping Translocation Breakpoint (hJTB) Protein
Devika Channaveerappap, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Kangning Li, Costel C. Darie
Human JTB (hJTB) is a gene located on the human chromosome 1 at q21 which is involved in the unbalanced translocation in various types of cancer. JTB protein is ubiquitously present in normal cells and is found to be overexpressed in different types of cancer including prostate and breast cancer. Hence this protein could be a tumor biomarker for different types of malignancies and a potential target for their treatment. However, 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 function of the protein and also its contribution to tumorigenesis. In this study, MCF7 breast cancer cell lines were transfected with the sense and antisense orientation of the JTB cDNA in HA, His and FLAG tagged CMV expression vector. Proteins extracted from transiently transfected cells were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The expression of JTB was confirmed by Western blotting technique. In gel digested peptides were analyzed by a Nano Acquity ultra-performance liquid chromatography coupled with Xevo G2 Mass Spectrometer. Data analysis was done using Mascot server and Scaffold 4.1 software. Furthermore, stable transfection will also be done to evaluate the JTB gene function and regulation mechanism. Two other isoforms of JTB will be analyzed and characterized to study their function and will be compared with the wild type JTB. 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.

20 Analytical Challenges during the Formulation Development of an Unstable Active Pharmaceutical Ingredient
Matthew Janson, Genentech, 465 East Grand Ave., South San Francisco, CA 94080, Mohammad Al-Sayah, Connie Chan, Janan Jona, Nathaniel Segrees, Karthik Nagappan
The degradation mechanisms of active pharmaceutical ingredients (APIs) are typically assessed by conducting forced degradation studies. The outcome of these studies would guide formulation development. In this study, we present the analytical challenges encountered during the formulation development of an unstable API. The API was relatively unstable in solution but to achieve the required exposure conditions, it was relatively unstable in solution but to achieve the required exposures, the API was relatively unstable in solution but to achieve the required exposures...
a solution/suspension formulation had to be developed for the preclinical toxicology studies. A thorough stability investigation was conducted and a mitigation plan was implemented to achieve an acceptable formulation shelf life. For clinical studies, tablet development employed a dry granulation process. During the initial excipient compatibility study, significant amounts of a new unknown degradation product were observed. Liquid chromatography-mass spectrometry (LC-MS) was conducted to identify this new degradation product. N-1 formulation blends were then assessed to identify the root cause for the formation of this degradation product. Once the degradation mechanism was fully understood, the degradation product was formed in significant quantities and the absolute structure was confirmed by nuclear magnetic resonance spectroscopy (NMR). The degradation product was identified as a dimer formed by the reaction of the API with residual formaldehyde present in some excipients. Kinetics of degradation product formation, excipient selection, and storage conditions will be discussed to illustrate the successful development of tablets to support Phase 1 clinical studies.

21 Complying with USP<232> and ICH Q3D: The Application of Automated Sample Preparation for ICP-MS Analysis in Pharmaceutical R&D
Jonathan L. Sims, Perkin Elmer, 710 Bridgeport Ave., Shelton, CT 06484, Carol Moynihan
The adoption of new requirements for controlling elemental impurities in Pharmaceutical products both in the USA (United States Pharmacopeia (USP)<232>) and globally (International Conference on Harmonization (ICH) Q3D) has created the need for laboratories to produce a significant number of methods and process potentially 1000’s of samples ahead of January 2016. The possibility of automating these processes will increase the ability of scientists to meet the looming deadline. A Sotax Automated Sample Processing Station (APWS) has been investigated for use in preparing samples for analysis by inductively coupled plasma mass spectrometry (ICP-MS). As the components of the automated system are mainly made from metal and ICP-MS is a very sensitive technique designed to analyse trace (ppb) levels of elemental contamination within samples, it is necessary to identify potential contaminant species and their origins prior to optimizing methods for specific sample types.

22 Determination of Antibacterial Activity in Medicinal Plants Using UHPLC-HRMS
Gaganpreet K. Monga, Kean University, 1000 Morris Ave., Union, NJ 07083, Mima E. Giron, Anima Goshal, Dil Ramanathan
In recent years, there has been a problem with pathogens developing antibiotic resistance. Failure to obtain molecules with resistant properties has lead scientists to examine phytochemicals and other natural antibiotics as a source for novel drugs. With advances in high resolution mass spectrometry (HRMS), it has become possible to detect desired masses. Prior work used gas chromatography (GC)-MS to profile Persia americana leaf and Visnina macrophylla bark samples to confirm the presence of antibacterial compounds (i.e., acetic acid and eucalyptol). A 3.5 minute gradient method was developed to detect Acetic acid using a linear trap quadropole (LTQ)-Orbitrap Mass spectrometer coupled with an ultra-high-performance liquid chromatography (UHPLC) system and a positive electrospray ionization (ESI) source. Other than that, antibacterial activity of acetic acid (concentrations of 5%, 2%, 1%, 0.5%, & 0.25%) and methanolic extractions constituted in different concentrations (methanol) were tested against the bacteria E. coli, using the Kirby-Bauer agar disk diffusion method. Further testing of methanolic extractions (1 gram of dried powder in 10 ml MeOH, rotavopped and reconstituted in 20% DMSO) was done in a 96 well plate assay against E. coli (4 X 106 CFU/ml). This portion used 0.1% lodonitrotetrazolium (INT) as an indicator of bacterial death.

23 Watching Dissolution on the Sub-Micron Scale: Utilization of AFM Imaging in Liquids Using Biorelevant Media
Amanda Mann, Merck & Co., 126 E. Lincoln Ave., Rahway, NJ 07065, Matthew S. Lam, Andre Hermans, Justin Pennington
The biorelevant dissolution of active pharmaceutical ingredient (API) particles and their intermediate states (e.g., spray dried particles or extrudates) is integral to predicting the in-vivo performance of pharmaceuticals. However, in-vitro studies often do not accurately predict actual performance and require a more in-depth understanding of the dissolution mechanism. Dissolution mechanisms can be deconvoluted via testing of a large set of conditions, such as evaluating the effect of compaction forces, granulation forces, API particle sizes, disintegration tests, etc. However, it would be useful to complement these experiments with direct in-situ observation of dissolution on the sub-micron scale. Atomic force microscopy (AFM) has been utilized to map topographical features, measure sample hardness, and evaluate phase distribution of a wide variety of samples, including nanoparticles, films, and biological materials with sub-nanometer resolution. Previously, AFM has been utilized to monitor the dissolution of API particles at different exposure times. Due to the challenge of collecting AFM images in liquids, these samples are typically exposed to the dissolution medium, dried, and then imaged. Here, we describe the in-situ monitoring of the dissolution of Compound A in biologically relevant media (FaSSIF) via liquid AFM imaging. The challenges and approaches to successfully preparing high quality samples and imaging the dissolution of crystalline API surfaces are discussed; as well as the utility of these sub-micron observations to better understand the mechanisms observed in the bulk-scale dissolution experiments.

24 Predictive Capability of DDD Plus for In-Vitro Dissolution of Immediate Release Formulations
Zongyuan Huang, Bristol Myer Squibb, 1 Squibb Dr., New Brunswick, NJ 08901, Xin Lu, Limin Zhang, Lili Lo
The aim of this study is to apply the Dose Disintegration and Dissolution Plus (DDD Plus) simulator program to dissolution profiles of immediate release tablet formulation in order to evaluate the prediction scope and accuracy of the program. Film coated tablets produced from two active pharmaceutical ingredients (API) were used as model drugs for the evaluation. The simulated dissolution profiles were compared to the experimental data using formulations with the needed API particle size, levels of disintegrants and lubricants, and tablet hardness. The effect of dissolution parameters such as media pH, surfactant level, and hydrodynamics were also evaluated by comparing simulation to experimental data. The results from these comparative tests provided rationalization to adjust the constants in the mathematical models for better accuracy. The usefulness, advantages and disadvantages of DDD Plus to assist dissolution method development are discussed.

25 Application of Two-phase Dissolution-Permeation Test Method for Predication of In-Vivo Performance of Poorly Water Soluble Drugs
Yi Shi, Abbvie, 1 N. Waukegan Rd., North Chicago, IL 60064
No abstract submitted by the author.

26 Mechanistic Approach for Assessing the Impact of Buffer Properties and Volume on Dosage Form Performance in the Presence of an Absorption Compartment
Deanna Mudie, Bend Research, 4550 Research Rd, Bend, OR 97701
No abstract submitted by the author.

27 An Integrated Analytical Approach to Solving Complex Polymer Problems
Scott Hanton, Intertek, 7201 Hamilton Blvd., MS: RD1, Dock 5, Allentown, PA 18195, Menas Vratsanos, Dale Willcox
Polymers and plastics play an increasing role in providing the needed material properties to address a wide range of products today. Polymers are found in just about everything we use in modern life. The incredible diversity of polymer products is driven by sustained innovation in polymer chemistry. The drive for new polymer chemistry and formulation presents analytical science with new and more complex measurement problems. In this presentation we show how complex polymer problems can be investigated using an integrated analytical approach. By using multiple analytical techniques, such as gel permeation chromatography, mass spectrometry, Rheology, nuclear magnetic resonance, Fourier transform infrared, and thermal analysis, we can enable improved characterization of polymer materials.

28 Characterization of Surfactants Used for Emulsion Polymerization Using Liquid Chromatography Mass Spectrometry (LC-MS)
Christie Bowden, Arkema, 900 First Ave., King of Prussia, PA 19406
Surfactants are amphiphilic compounds that contain a hydrophobic “tail” and a hydrophilic “head” and can be non-ionic, anionic, cationic, or amphoteric. They are used in emulsion polymerization and effect the overall polymerization process as well as the final latex properties. Common surfactants used for emulsion polymerization are non-ionic fatty alcohol ethoxylates and anionic fatty alkyl ether sulfates and fatty alkyl ether sulfosuccinate esters. These molecules are complex due to the presence of various alkyl chain lengths, varying degrees of ethoxylation, and impurities that can be also present. Being able to fully characterize these surfactants is key to optimizing emulsion polymerizations as well as final latex performance. This presentation discusses how ultra-high-performance liquid chromatography coupled with electrospray ionization mass spectrometry can be utilized to characterize these complex mixtures of non-ionic and anionic surfactants. Using a totally porous C18 column along with an ammonium acetate and methanol gradient, surfactants can be separated based upon their alkyl distribution and their degree of ethoxylation. By coupling the separation to an electrospray ionization source and performing accurate mass analysis, the alkyl distribution and degree of ethoxylation can be determined. In addition, this technique can be utilized to identify minor impurities present in the surfactants, which might not be possible using other analytical techniques.
29 Analysis for Low Level Polymeric Components Using Pyrolysis-GC-MS
Jocelyn White, The Dow Chemical Company, 400 Arcola Rd., Collegeville, PA 19426

The presence of low level functional monomers is often critical to the performance of polymer products. Detecting these monomers by spectroscopic methods such as FTIR and NMR can be difficult due to peak overlap with higher level monomers. Pyrolysis-GC-MS offers unique advantages in identifying and quantifying low level components of polymeric products. The thermal degradation into monomers and other small molecules allows for separation of the components in a polymer and lower detection limits for many functional monomers. With appropriate reference samples, these low level components can also be quantified. Examples are given demonstrating the detection of low level monomers using pyrolysis-GC-MS.

30 Applications of Coupled Rheology – FT-IR to Polymer Analyses
Dana Garcia, Arkema Inc., 900 First Ave., King of Prussia, PA 19406, Sara Reynaud, Zeena Cherian, Mark Lavach, Chuck Crabb, Robert Barsotti, Florence Mehlmann, Francesca Devito, Fabian Meyer

Coupling rheology with Fourier transform infrared (FT-IR) spectroscopy affords the opportunity to directly correlate rheological properties with structural changes on the molecular level in a dynamic approach. This presentation explores applications of the technology to examine the viscosity increase observed for styrene-butadiene copolymers under isothermal conditions at 250°C and rheological changes of poly[(lactic-co/glycolic acid)/poly(methyl/hexylacrylate) (PLA-PMMMA) blends in a temperature ramp above the upper critical solution temperature. The coupled experiments were carried out using the Rheonaut® module for the Thermo Scientific HAAKE™ MARS™ rheometer interfaced with a Thermo Scientific™ Nicolet™ IS10 FT-IR Spectrometer. The styrene-butadiene example showed spectral evidence for cross-linking while in the case of the PLA-PMMMA evidence was gathered for changes in interactions between components. Thermo Scientific™ Nicolet™ IS10, HAAKE™ MARS™ are trademarks of Thermo Scientific. Rheonaut is a trademark of Resultec Analytic Equipment.

31 Chemical Analysis in Packaging Development and Redesign
Alan Sentman, Polymer Solutions, 135 Technology Dr., Christiansburg, VA 24073

No abstract submitted by the author.

32 Determination of the Cause of Discoloration in Hard Gelatin Capsules Containing FD&C Blue #2 (Indigotine Colorant) Through Forced Degradation Studies Using BiliCareOptima Mathematical Model
Ajith S. Nair, BiliCare Research, 1389 School House Rd., Delaware City, DE 19706, Matthew Fiore

FD&C Blue #2 is one of the major colorants used in the preparation of colored hard gelatin capsules used extensively for pharmaceutical products including over-the-counter (OTC) medication. Publications have indicated that FD&C Blue #2 could potentially fade when the dye is exposed to light, acid, base, oxidizing agents, extreme temperature and moisture. In studies, filled hard gelatin capsule shells containing FD&C Blue #2 demonstrated fading while stored in accelerated International Conference on Harmonization (ICH) conditions (40°C/75% RH) after 6 months, although no signs of fading were observed when stored in intermediate ICH conditions (30°C/65% RH). This study is set up to determine the cause of discoloration for hard gelatin capsules containing FD&C Blue #2 colorant observed when stored in accelerated ICH conditions through forced degradation studies. It is identified that the capsule undergoes color change when its moisture content reaches certain levels. The moisture threshold values are determined at different temperatures and the data are used to develop a packaging system that contain enough moisture barrier to prevent this discoloration at the exposure conditions and protect the drug product from failing in stability. Moisture barrier required in the package to prevent the product from discoloring were determined using BiliCareOptima mathematical model and determined the optimum packaging material for commercialization.

33 A Robust Approach for Dealing with Problematic Sample Types and Matrices Encountered in the Modern Pharmaceutical Laboratory whilst Using Ion Chromatography as an Analytical Tool
Stuart J. Proctor, Metromin USA, 6555 Pelican Creek Circle, Riverview, FL 33578

Ion chromatography (IC) in the field of pharmaceutical sciences is still dominated by aqueous based suppressed conductivity methodologies. This is largely due to limitations of older generation IC equipment which forces method development chemists to develop sample preparative or column regeneration procedures to deal with hydrophobic samples or sample matrix problems. Furthermore, suppression reactions required by older generation conductivity detectors limit chemistry choices and hinder the detection of weak acids and weak bases. Improvements in conductivity detection, column chemistry and suppression technology now delivers supreme flexibility to the method development chemist enabling the choice of conditions best suited to their sample type and matrix. Choosing between silica or polymer based columns, the solvent makeup of the mobile phase or running either suppressed or non-suppressed conductivity detection can have a drastic impact on your analysis. The correct combination can simplify sample preparation or eliminate a tough sample matrix improving the precision and accuracy of the measurement whilst reducing method development and validation times. This presentation focuses on how modern IC technology can be utilized to develop a more robust approach to dealing with typical sample types and matrices encountered in the modern pharmaceutical laboratory.

34 HPLC Methods Transfer across Multiple Chromatographic Systems: The Impact of Instrument Design
Paula Hong, Waters, 34 Maple St., Milford, MA 01757, Patricia R. McConville

Transfer of high-performance liquid chromatography (HPLC) reversed-phase methods across both HPLC and ultra-HPLC chromatographic instrumentation requires careful consideration of the operating parameters and design of each instrument. For example, gradient formation can be influenced by dwell volume, while linearity and quantitation can be affected by the mechanism for sample injection, as well as the detector. Lastly, thermostating can alter retention—whether due to frictional heating affects, mobile phase pre-heating or thermal gradients. To understand the effect these factors may have on methods transfer, both instrument characteristics and specific method conditions must be factored and evaluated when transferring HPLC methods. In this presentation a number of HPLC methods, including United States Pharmacopeia (USP) monograph methods, are analyzed across HPLC and UHPLC systems. Testing is conducted to measure specific characteristics of the system, and demonstrate how these characteristics can impact methods transfer. Comparison of different instrument configurations is also performed, for example quaternary and binary pumps are evaluated as well as flow through and off-line injection modes. System suitability criteria are used to evaluate the separations. Based on the effects of individual system characteristics, strategies for successful methods transfer are described. In these examples, consideration is made to conduct method transfer in accordance with regulatory guidelines for allowable adjustments to methodological parameters.

35 USP Method Modernization Using “Equivalent L/dp” and “Equivalent N” Allowed Changes with CORTECS C8 and CORTECS UPLC C8 Columns
Thomas Swann, Waters Corporation, 34 Maple St., Bldg. B, Milford, MA 01757, Jennifer M. Nguyen

Many United States Pharmacopeia (USP) monograph liquid chromatography (LC) methods were created years ago when longer columns, packed with larger fully porous particle sorbents, were the norm. Methods using these columns can now be considered “outdated” in resolving power and speed. Switching the stationary phase particles from larger to smaller and from fully porous to solid-core can greatly improve method resolution and speed. Better resolution arises from the narrower peaks (higher efficiency) that these particles provide. Quicker methods originate in the ability to use shorter columns with such particles without sacrificing efficiency. This presentation illustrates how an analyst can use solid-core CORTECS columns to modernize a USP method. We seek to address the key USP assay method for improvement and demonstrate the benefit of adopting two different USP General Chapter <621> allowed changes to isocratic LC methods. In this work, the “equivalent L/dp” guideline leverages the efficiency improvement from using the smaller CORTECS particles and the “equivalent N” guideline exploits the efficiency advantages of the solid-core CORTECS particles. The result is a much shorter analysis time (92% decrease) and lower solvent consumption (90% reduction) compared to the original method.

36 Advances in the Analysis of Oral Care Consumer Products Using Benchtop Nuclear Magnetic Resonance
Robin Gordon, Colgate-Palmolive, 909 River Rd., Piscataway, NJ 08854, Michael Knapp, Susan Friedman

At Colgate Palmolive, the majority of the products produced for our consumers are for Oral care and contain fluoride. Fluoride is a key release specification and is regulated by the United States Food and Drug Administration (FDA). We are constantly seeking more rapid and efficient methods for analyzing fluoride. Fluoride-containing mouthrinses were previously assayed by ion chromatography (IC), a relatively time consuming and costly analysis. This procedure requires the analyst to prepare and analyze multiple standard solutions and system suitability solutions before a sample analysis is performed. In most cases, results will not be available for hours, or even the next business day. Additionally, the IC method uses expensive consumables and requires frequent system maintenance. This presentation shows how the latest benchtop nuclear magnetic resonance (NMR) technology has provided Colgate with a more efficient and cost effective way of analyzing fluoride in oral care products, especially in mouthrinses. The assay does not require a sample preparation and...
results can be obtained in 12 minutes or less. This methodology requires less analyte time, making it an extremely efficient tool for our research and QC laboratories.

37 Approaches to Develop USP Quality Monographs for Over-the-Counter Drug Products
Richard B. Nguyen, United States Pharmacopeial Convention, 12601 Twinbrook Pkwy, Rockville, MD 20852

Over-the-counter (OTC) medicines include hundreds of active ingredients and thousands of different formulations; and many OTC drug products do not require pre-market approval by the United States Food and Drug Administration (FDA). Recognizing the need for standards that ensure good quality for OTC drug products, the United States Pharmacopeial Convention (USP) has made compendial standards for OTC medicines a major focus. Develop monograph for each OTC drug product is impossible due to thousands of products and rapid changes in reformulation. An optimal way to provide better coverage and control of drug product quality is through a streamlined monograph development process of using the General Chapters. USP General Chapter is desired to organize related tests and procedures in one place for simplified reference in related individual monographs. The presentation provides an overview of regulation pathways for OTC drug products, outline the challenges in establishing USP quality standard for OTC medicines, and to provide USP current approaches to develop monographs for broader coverage of OTC drug products through the use of General Chapters.

38 USP Addresses Safety Concerns Associated with Acetaminophen Formulations
Olydeyn W. Anthony, United States Pharmacopeia, 12601 Twinbrook Pkwy., Rockville, MD 20852, Richard B. Nguyen

Acetaminophen is an active ingredient found in hundreds of over-the-counter (OTC) and prescription medicines. It is the most common drug used to relieve pain and fever. However, it is also combined with other active ingredients in medicines for the treatment of allergy, cough, colds, flu, and sleeplessness. In prescription medicines, acetaminophen is formulated with other classes of active ingredients to treat moderate to severe pain. Acetaminophen can cause serious liver damage if more than directed is used, and even rare skin reactions have been reported in some instances. The United States Pharmacopeial Convention (USP) has updated its documented standards by developing new experimental procedures to monitor harmful and potentially life threatening impurities in these formulations. The USP laboratories have also introduced new reference standards to assist in the identification and quantification of other impurities that were not previously monitored. A comprehensive overview of the initiatives currently in progress at USP to address these issues will be provided; including the use of Expert Panels; ongoing partnerships with United States Food and Drug Administration (FDA) and Consumer Health Products Association (CHPA); and the introduction of innovative approaches to bring articles in its compendium up-to-date.

39 Towards practical IR imaging for rapid digital molecular pathology
Rohit Bhargava, University of Illinois-Urbana-Champaign, 405 N. Mathews Ave., Urbana, IL 61801

Here we focus on the needs and progress in using infrared (IR) spectroscopic imaging for biophysical, especially translational, applications. We first discuss various theoretical approaches and comparison metrics to understand the ultimate capabilities of IR imaging instrumentation. These allow us to make important trade-offs in various configurations and design instrumentation that is fit for purpose. We report on several new configurations in IR imaging that provide information at different length scales and different chemical functionalities. Next, we describe challenges in pathology and where IR imaging may be appropriate for applications. We report on the application of new capabilities for histopathology, demonstrating the increased capabilities of instrumentation that can lead to practical solutions. Showing the impact of fundamental advances and their translation as building blocks in developing robust protocols for cancer pathology, we conclude with a discussion on emerging directions in instrumentation and applications.

40 Synchrontron Infrared Nano Spectroscopy (SINS) and 3D FTIR Tomography
Michael C. Martin, Advanced Light Source, Lawrence Berkeley National Laboratory, 1 Cyclotron Rd., Bldg. 62100, Berkeley, CA 94720

I describe two new techniques we have recently developed and demonstrated. First, by combining scattering-scanning near-field optical microscopy (s-SNOM) with mid-infrared synchrontron radiation, Synchrontron infrared nano-spectroscopy (SINS) enables molecular and phonon vibrational spectroscopic imaging, with rapid spectral acquisition, spanning the full mid-infrared (300-5000 cm⁻¹) region with nanoscale spatial resolution. This highly powerful combination provides access to a qualitatively new form of nano-chemometric analysis with the investigation of nano scale, meso scale, and surface phenomena that were previously impossible to study with infrared (IR) techniques. We have installed a SINS end-station at Beamline 5.4 at the Advanced Light Source (ALS) at Lawrence Berkeley National Laboratory, making the s-SNOM technique widely available to non-experts, such that it can be broadly applied to biological, surface chemistry, materials, or environmental science problems. We demonstrate the performance of synchrontron infrared nano-spectroscopy (SINS) on semiconductor, biomimural and protein nanostructures, providing vibrational chemical imaging with sub-zeptomole sensitivity. Second, 3D FTIR tomography provides spectrally rich, label-free, non-destructive visualizations of distinctive chemical compositions throughout intact biological or materials samples. The technique has combined Fourier transform infrared (FTIR) spectroscopy with computed tomography (CT) to create a non-destructive 3D imaging technique that provides molecular-level chemical information of unprecedented detail on biological and other specimens with no need to stain or alter the specimen. We have installed a new IR imaging and tomography endstation at beamline 2.4 of the ALS. I describe how the technique works and present application examples spanning a variety of scientific disciplines.

41 Nanoscale Infrared Spectroscopy and Chemical Imaging with AFM-IR
Craig B. Prater, Anasys Instruments, 325 Chapala, Santa Barbara, CA 93101

Atomic force microscope based infrared spectroscopy (AFM-IR) is a rapidly emerging technique for performing chemical analysis and compositional mapping with nanoscale spatial resolution. The AFM-IR technique uses the tip of an AFM probe to locally detect infrared absorption be measuring thermal expansion of absorbing regions of the sample while being irradiated with a tunable mid-infrared laser source. IR absorption spectra with nanoscale spatial resolution are achieved by measuring the AFM probe response as a function of the wavelength (wavenumber) of the incident radiation. Compositional maps can be created by tuning the mid-IR laser to a specific absorption band, thus mapping the distribution of specific chemical species. This presentation overviews the underlying technology of AFM-IR, discusses recent advances in the field and then survey diverse applications in materials and life sciences. We shall specifically discuss AFM-IR measurements of polymeric materials (blends, multi-layer films, membranes, coatings), and biological applications including sub-cellular spectroscopy and amyloid protein aggregation/secondary structure. Complementary AFM-based measurement techniques including scanning near field optical microscopy, nanoscale thermal analysis, and nanoscale mechanical spectroscopy are also briefly discussed.

42 Building an Open Source Toolkit for Parallel and Out-of-Core Analysis of Large Hyperspectral Images
David Mayerich, University of Houston, 4726 Calhoun Rd., Houston, TX 77204, Rupali Mankar, Sebastian Berisha

Infrared spectroscopy is commonly used in the areas of materials and forensics. This is due primarily to the ability of vibrational spectroscopy to provide quantitative measurements utilizing intrinsic contrast. These features make spectroscopy a potentially useful tool for historical research and disease diagnosis. However, acquiring and analyzing sufficient data to make these tools practical for clinical applications continues to be a bottleneck. Continued advances in detector technology combined with the increasing availability of tunable quantum cascade laser (QCL) sources are significantly increasing the throughput of infrared spectroscopic imaging systems. However, as throughput increases we quickly encounter difficulties imposed by computationally intensive and complex data management schemes. Standard solutions to these problems include utilizing cluster and supercomputing. However, biomedical professionals often lack the resources and expertise to take full advantage of these systems. In this talk, I will discuss an open-source framework that we are building to make the analysis of large spectroscopic data sets practical on benchtop systems. We focus our efforts on two fronts. First, our software uses of out-of-core processing techniques that minimize data transfers from secondary storage and hide calculations behind data fetches. This allows our data processing times to approach the time required to transfer data from a secondary storage device, such as a hard drive or NAS. Secondly, we optimize complex calculations through the use of graphics processing unit (GPU)-based parallel processing. This provides significantly increased throughput for linear calculations, which are common in spectroscopic image processing, by using inexpensive hardware available for desktop workstations.

43 Fundamental Investigations into the Retention and Selectivity Observed on Biphenyl Stationary Phases for Reversed-Phase HPLC
Daniel Shollenberger, MilliporeSigma, 505 H. Harrison Rd., Beltsfie, PA 16823, Dave Bell, Stacy Shollenberger, Gary Oden, Hugh Cramer

It is well known that aromatic stationary phases provide alternative reverse phase selectivity, but recently there has been growing interest in new phenyl type chemistries driven by the changing landscape of small molecule compounds utilized in drug development and the subsequent assays of their metabolites. While phenyl and phenyl oxyl stationary phases are well studied in the literature, there is not as much detailed characterization for biphenyl phases. This study seeks to elucidate
the interactions specific to biphenyl stationary phases that provide unique selectivity and retention in reversed phase chromatography. Pi-pi interactions are often cited as the major phase attribute for aromatic stationary phases, and so this study also seeks to better understand the nature of pi-pi interactions that have been the justification for observed differences in selectivity between aromatic and alkyl phases, and the differences in selectivity associated with changing the organic components of the mobile phase. With the complexities of isolating lone or specific interactions, this study also seeks to evaluate the thermodynamic relationships useful in building a model that correlates solute properties with proposed retention mechanisms.

44 New Method to Automate Shake Flask Determination of LogD with Biphasic Partitioning in an HPLC Vial
Mark Mitchell, Reaction Analytics Inc., 2711 Centerville Rd., Wilmington, DE 19808, Mike Lopez.
LogD is a critical measure in the evaluation of early stage pharmaceutical drug candidates to move ahead for further development. Shake Flask and other methods such as comparable run-time depend on operator skill and interpretation when speed and accuracy are critical. This new method uses only a few milligrams of active pharmaceutical ingredient (API) in a biphasic mixture in the sample vial. The high-performance liquid chromatography (HPLC) has been prepared with a high dynamic range detector and autosampler with advanced controls to manage the extremes in concentration with high LogD compounds now more common in the drug development pipeline. This ability to measure LogD was demonstrated with the iChemExplorer hardware and software on the Agilent HPLC. The method could be reproduced on any HPLC platform now available in the pharmaceutical development laboratory.

45 Multiple Injection Techniques in Pharmaceutical Analysis and in Support of High-Throughput Experimentation
Kerstin Zawatzky, Merck & Co., 126 E. Lincoln Ave., MS: RY800-C367, Rahway, NJ 07065, Christopher J. Weich.
High-throughput analysis using MISER (multiple injections in a single experimental run) and similar techniques allows rapid evaluation of large sample sets coming from high-throughput experimentation (HTE) in pharmaceutical process research. While most investigations into HTA MISER have focused on achiral analysis, the technique can also be suitable for studying enantiopurity. Since supercritical fluid chromatography (SFC) is currently the preferred method for fast enantiopurity analysis, with analysis times of only a few seconds being possible in some cases, MISER SFC experiments are presented, showcasing the power and versatility of the technique, with 96 well-plate analysis times of about 33 minutes being possible in the best cases. Various further multiple injection strategies for high-throughput analysis and their advantages and disadvantages are discussed to meet the challenges of HTA in pharmaceutical analysis and process research like improving speed or sensitivity.

46 Molecular Isotopic Engineering (MIE): Industrial Manufacture of Small Molecules and Biologics of Predetermined Stable-Isotopic Compositions for Novel Intellectual Property Coverage as Well as for Authenticity and Security Protection
Joseph P. Jasper, Nature’s Fingerprint / MIT LLC, 8 Old Oak Ln., Natick, CT 06357, Peter S. Mezes, Peter Farina, Anna Pearson, Anthony Sabatelli Molecular Isotope Technologies LLC has developed four patented or patent-pending generations of stable-isotopic methods and technologies: 1) product characterization (for both small molecules and biologics), 2) process characterization (naturally, process patent protection), 3) in-process (continuous) analysis, and now 4) molecular isotopic engineering. Early work in cooperation with the United States Food and Drug Administration on the product characterization of naproxen revealed manufacturer-level isotopic provenance of this small analogsae molecule (Wokovich et al., 2005) which was referred to as “The Manufacturer’s Fingerprint.” This isotopic provenance represented the convergence of the effects of the stable-isotopic compositions of starting materials and isotopic effects of the synthetic process. Rather than merely accepting the random effects of variable sourcing and synthetic process on the stable-isotopic compositions of products, we take a proactive approach to purposefully determine the stable-isotopic composition of bio/pharmaceutical products. The main rationale for molecular isotopic engineering (MIE) is to predetermine the isotopic ranges of products for reasons of product identification and of product security, and also for intellectual property considerations. As an example of MIE, we analyzed the products of the isotopic-synthetic reactions for the last two steps of naproxen synthesis: 2-Bromo-6-methoxynapthalene + Bromopropionate → ν α-α-Sodium naproxen pre-selection of the stable-isotopic compositions for the starting material. 2-Bromo-6-methoxynapthalene yielded the product of discrete stable-isotopic ranges. In principle, the MIE approach should be readily adapted to existing bio/pharmaceutical manufacturing units. The main difference in the manufacturing process would be the use of starting materials or synthetic intermediates of pre-measured stable-isotopic compositions. The manufacturing apparatus would remain unchanged. This approach could have broad application in securing drug identity/provenance from manufacturing plant to consumer.

47 Extremely High-Throughput Headspace and Gas Analysis by Integrating Autosamplers with SIFT-MS
Barry J. Prince, SytTech Systems, 3 Craft Pl., Chathamchurch 8024, New Zealand, Daniel B. Milligan, Vaughan S. Langford, Murray J. McEwan, Chuck Renner
Autosampler integration is the simplest and most cost-effective way to leverage high sample throughput from the rapid, direct gas analysis provided by selected ion flow tube mass spectrometry (SIFT-MS). Furthermore, an autosampler improves repeatability and reproducibility compared to manual analysis and it eliminates operator error. In this paper, the SIFT-MS technique is introduced briefly and its unique requirements for automation are described. In particular, because SIFT-MS is a direct mass spectrometry technique (that is, one that has no pre-separation using chromatography), its requirements for an autosampler differ from those used very commonly with mass spectrometry (GC). GC-based techniques require rapid injection to achieve good chromatographic separation; chromatography necessarily leads to prolonged analysis as individual compounds elute from the column over a period of time. However, SIFT-MS requires steady sample injection for the duration of the analysis, because analysis is carried out simultaneously with injection. Selectivity in SIFT-MS is achieved very elegantly by coupling mass spectrometry with ingenious application of ultra-soft chemical ionization; eight reagent ions are available and automatically switched by software control of a quadrupole mass spectrometer. The very diverse reaction mechanisms provided by the reagent ions provides detection of a very wide range of compounds, including chromotically challenging ones such as ammonia, formaldehyde, and hydrogen sulfide. Autosampler integration opens diverse new applications of SIFT-MS for both contract and research and development laboratories. Various applications are used to illustrate the power of automating the technique, including routine Headspace analysis, multiple Headspace extraction and sample bag analysis.
graphical separation and quantitation of 25(OH)D2, 25(OH)D3, and their C3-epimers. Beagle serum was fortified with 25(OH)D2, 25(OH)D3, 3-epi-25(OH)D2, and 3-epi-25(OH)D3, and extracted with hexane by liquid-liquid extraction (LLE). Extracted samples were evaporated to dryness, reconstituted with 50:50 water:methanol, and injected on a Shimadzu Nexera XR UHPLC coupled to a Sciex API 4000™ mass spectrometer. Calibration standards and QC samples were prepared in synthetic human serum and subjected to the LLE procedure. Good linearity was obtained for fortified samples with a concentration range of 1 to 100ng/mL, with 1/x weighting. Three quality control (QC) levels of fortified synthetic human serum yielded acceptable accuracy, recovery, and relative standard deviation (RSD). Fortified beagle serum was analyzed using the validated method with acceptable accuracy and precision. The Raptor™ FluoroPhenyl column provided unique selectivity for accurate and differential quantitation of 25-hydroxyvitamin D and C3-epimers in serum with a 7-minute analysis time.

50 GC-MS and UHPLC-HRMS Analysis of Bromelia Pinguin Rhizome Against E. coli and S. aureus
Marc E. Giron, Kean University, 1000 Morris Ave., Union, NJ 07083, Anima Ghosal, Dil Ramanathan
Since scientists are constantly developing methods to combat effects of pathogens and their strains, it is no wonder that pathogens are adapting and gaining resistance to existing drugs. Plants have provided medicinal treatment on a worldwide scale for generations. Each plant part contains different percentages of phytochemicals that provide unique selectivity for accurate and differential quantitation of 25-hydroxyvitamin D and C3-epimers in serum with a 7-minute analysis time.

54 Practical Method Development and Optimization for UHPLC and HPLC Separations of Peptides for Peptide Mapping Analyses Using Different Mobile Phase Additives
Thomas J. Waeghe, MAC-MOD Analytical, 103 Commons Ct., Chadds Ford, PA 19317, Stephanie A. Schuster, Barry E. Boyes, Benjamin Libert
Peptide mapping is a critical competency and technique for both research and development and for quality assurance and product release for many new biopharmaceuticals including peptide drugs, monoclonal antibody drugs and antibody-drug conjugates (ADCs). New 2-μm superficially porous columns, capable of pressures up to 1000 bar, can deliver high resolution / peak capacity separations, and can be used at temperatures up to 60 °C. Moreover, a variety of mobile phase additives include trifluoroacetic acid (TFA), ammonium formate/formic acid buffers and a new additive, difluoroacetic acid (DFA), can be applied for effective development and optimization of rugged and robust separations of complex enzyme digestes. We demonstrate the usefulness of these columns and mobile phase additives in the development of peptide mapping separations, and offer a stepwise method development and optimization strategy for such separations.

56 Thromboelastography (TEG) for Analysis of Blood Clots and Platelet Aggregation Analysis
Diego M. Ferreira, University of São Paulo - FMUSP, Av. Dr. Arnaldo, 455 - Cerqueira César - CEP: 01246-903 - São Paulo - SP 18550-000, Brazil, Vinicius A. Machado, Joel A. R. Filho, Estela R. R. Figueira, Cristiany B. Ludwig, Evelin T.B. Serpa, Jessica M. S. Rodrigues
In the early studies, our group evaluated three different methods of analysis Thromboelastometry, Thromboelastography and platelet aggregetometer, Rotational Thromboelastometry (ROTEM) is an instrument that provides an overview of the coagulation status and additional information about detecting function and platelet aggregation using aggregometry impedance. Rotational Thromboelastometry provides great stability with a drive easy and fast tool can use even in a surgical center dispensing aid experts in the field, being able to do the same function as other traditional instruments but with more ease. Your driving is easy and safe containing for four channels for testing. For the use, are needed single-use reagents for rapid and reliable results, its operation becomes easy because through touch screen all results are presented step by step in graphical. Your differential information it can provide to the patient as Hyperfibrinolysis, dilutional coagulopathy, fibrinogen replacement, factors or platelets, Heparin control or protamine. Several reagents are available for ROTEM, among them are, factor activation tissue, elagic acid phospholipid activation, heparinase, cytochalasin and aprotinin fibrinolysis inhibitor. After one hour, Rotational Thromboelastometry gives us all the parameters we need to complete verification about possible hemostatic disorders.

60 MS Clarus SQ showed that it contained antibacterial compounds, e.g., acet- tic acid. Experiments were performed to determine minimal inhibitory concentration (MIC) of the plant extract using a 96-well plate against Staphylococcus aureus and Escherichia coli (E. coli). Two samples of B. pinguin rhizome were extracted with methanol (1 g of dried powder in 10 mL MeOH), one for 72-hours and the other for 48-hours. Both samples were centrifuged and evaporated to dryness using a rotovap and reconstituted in 20 μM dimethylsulfoxide (DMSO). Both bacteria were treated separately with plant extract and the bacterial growth was examined using two color changing indicators (resazurin and liodonitrotetrazolium chloride (INT)) for comparison. Indicators were used at a concentration of 0.1% to determine the MIC values against E. coli and S. aureus. The MIC of E. coli was determined to be 12.35 mg in 95 μL, however, the MIC value determination of S. aureus is currently in progress. Studies are ongoing to evaluate the use of phospholipids of the bacterial membrane as a substitute for the indicators using an ultra-high-performance liquid chromatography high resolution mass spectrometry (UHPLC-HRMS).

680 and MS Clarus SQ showed that it contained antibacterial compounds, e.g., Ace- tic acid. Experiments were performed to determine minimal inhibitory concentration (MIC) of the plant extract using a 96-well plate against Staphylococcus aureus and Escherichia coli (E. coli). Two samples of B. pinguin rhizome were extracted with methanol (1 g of dried powder in 10 mL MeOH), one for 72-hours and the other for 48-hours. Both samples were centrifuged and evaporated to dryness using a rotovap and reconstituted in 20 μM dimethylsulfoxide (DMSO). Both bacteria were treated separately with plant extract and the bacterial growth was examined using two color changing indicators (resazurin and liodonitrotetrazolium chloride (INT)) for comparison. Indicators were used at a concentration of 0.1% to determine the MIC values against E. coli and S. aureus. The MIC of E. coli was determined to be 12.35 mg in 95 μL, however, the MIC value determination of S. aureus is currently in progress. Studies are ongoing to evaluate the use of phospholipids of the bacterial membrane as a substitute for the indicators using an ultra-high-performance liquid chromatography high resolution mass spectrometry (UHPLC-HRMS).
57 Anti-Arthritic Activity of N-(2-hydroxy phenyl) Acetamide in Collagen-Induced Arthritis Rats
Bimal Kunwar, H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan, Shabana Usman Simjee

Rheumatoid arthritis is a chronic inflammatory joint disease that may affect the other parts of the body as well. Collagen-induced arthritis (CIA) rat model was employed for studying the effects of the test compound. The body weights and paw volumes were monitored to evaluate the progression of the disease. All animals were humanely sacrificed as an arthritic score of 4 was observed in the arthritic control group. Blood, knee joints and brain samples were collected and processed per requirement. It was observed that NA-2 significantly reduced paw edema volume, and prevented the body weight loss in the treatment group receiving (5 mg/kg) dose compared to the arthritic control group. The H&E stained sections demonstrate an increase in inflammatory score and bone erosion in arthritic control compared to normal group. A prominent inhibition of both infiltration of inflammatory cells and bone erosion was observed in NA-2 treatment group. The serum samples analyses clearly demonstrated that the treatment of NA-2 markedly reduced the signs of arthritis. N-(2-hydroxy phenyl) acetamide treated group significantly reduced NO (p < 0.05), PO (p < 0.05) and elevated GSH (p < 0.05) level in serum samples of treatment group. Further, treated group significantly reduced proinflammatory cytokines (p < 0.05 for IL-1b), (p< 0.05 for TNF-α) as compared to the arthritic control group. These findings suggest that NA-2 might be a good candidate for further development as anti-arthritis drug. Our next goal is to analyze membrane metalloproteinas- es (MMPs) and correlate its expression level with the levels of ROS and cytokines needed as anti-arthritic drug. These findings suggest that NA-2 might be a good candidate for further development as anti-arthritis drug.

58 Unsaponifiable Matter in Carnuba (Cera Carnuba) Wax, a Modification of the USP/NF and FCC Methods
Yusuf Yildiz, Complete Analysis Laboratory, 1259 Rte. 46, Parsippany, NJ 07054

Carnauba wax consists chiefly of myricyl cerotate (MW 817.4) and small quantities of free ceric acid (C26H52O2, Mw 396.7) and myricyl alcohol (C30H62O, mp 90 oC). Of the two common extraction solvents, ethyl ether or petroleum ether, Lewkowtisch prefers the former. Concerning separation of phases, he advocates addition of small amounts of alcohol or caustic, and he also states that formation of a flocculant layer between the aqueous layer and the solvent does not interfere with the correct estimation of the unsaponifiable matter. These statements were not corroborated in the hands of this chemist. Carnuba wax, a rather expensive wax, may be adulterated with less expensive paraffin by dishonest merchants. American Society for Testing and Materials (ASTM) has a method for determining paraffinic material in carnauba wax. It uses heptanes at its boiling point to dissolve the wax, apply it to a silica gel column, and elute only the nonpolar (i.e., alkane) material. The method has the disadvantage of using a large volume of hamptane, nor it is called for by either United States Pharmacopoeia or the Food and Drug Administration.

59 Method Development for Identification of Adulterated Spirits Using Field Portable GC-MS
Bill Hahn, Perkin Elmer, 710 Bridgeport Ave., Shelton, CT 06484, Thomas Mancuso

European spirits are premium global products, and are therefore a major target for illegal counterfeiters. Counterfeiting has an impact on direct sales: it damages the reputation of brands and spirit categories. Safety issues are also a concern, and these impacts are therefore relevant to all spirit producers. Counterfeiters use denatured industrial alcohol to produce fake and adulterated spirits, which degrades the product and can pose a significant health risk to those consuming it. In this paper we focus on the complete method development to rapidly detect and identify denatured alcohol in Scotch whisky and other spirits using the PerkinElmer Torion T-9. The T-9 is a fully self-contained, ruggedized, field-portable GC-MS. Battery-operated, with an internal carrier gas supply, and easy to use software, the system can be easily operated in the field by a non-chemist. Successful deployment of the T-9 to the field is also dependent on development of a sampling method that is compatible for the task, and easily present the data analysis of the results to the analyst in a clear actionable form.

60 Withdrawn by the author.

61 Steep Time and Temperature Effects on Flavor and Flavonoid Extraction of Black Tea
Anne Jurek, EST Analytical, 503 Commercial Dr., Fairfield, OH 45014, Wade Stephenson, Kelly Cravenor

The steeping time of tea can have a profound effect on flavor. Most tea products have a recommended steeping time and temperature. The steeping temperature of the water can vary from “bring water to a rolling boil” to a “very light steam”, while the steeping time can fluctuate from two to five minutes. If a tea is steeped for too long or at too high of a temperature; it can become bitter. However, longer steeping times enhance the health benefits of tea by increasing the amount of flavonoids extracted from the tea. This application looks at the effects of time and temperature on the extraction of tea flavors and flavonoids.

62 HILIC-MS/MS Method Development for the Analysis of Paralytic Shellfish Poisoning Toxins in Shellfish Harvested from New Hampshire Coastal Waters
Jessica Henry, University of New Hampshire, 23 Academic Way, Durham, NH 03824, Madhumita Chatterjee, Juliane Nassif, Sterling Tomellini

Paralytic shellfish poisoning (PSP) results from ingesting shellfish contaminated with potent neurotoxins that are produced by toxic algae found in marine environments. The PSP toxins are made up of saxitoxin (STX) and its analogs. Due to the severity of PSP, regulatory bodies worldwide monitor the presence of the toxins in harvested shellfish to insure harvested shellfish meat is within the international regulatory limit of 80 µg STX equivalents per 100 g shellfish meat. In the state of New Hampshire, the New Hampshire Public Health Laboratories (NHPHL) monitor PSP toxins using a bioassay; however, it is desired to move to an instrumental method of analysis. The use of hydrophilic interaction liquid chromatography (HILIC) coupled to mass spectrometry (MS) offers a sensitive and specific alternative method and has been demonstrated as a viable alternative for the analysis of the polar PSP toxins. Previous research at the NHPHL used HILIC-MS to measure PSP toxins in harvested shellfish, yet the method was limited by its ability to adequately detect all of the toxins within the regulatory limit. The current study focuses on developing a HILIC-MS-MS method to improve the separation and the detection limits for three PSP toxins currently being monitored in New Hampshire coastal waters: STX, neo saxito- xin (NEO) and decarbamoylsaxitoxin (dcSTX). The separation of the analytes was achieved on a core-shell, bare silica column (Supelco, Ascentis Express HILIC). The developed HILIC-MS-MS method will be applied to harvested shellfish samples archived from past shellfish harvesting seasons.

63 Near-Infrared Spectroscopy for the Rapid Screening of Economic Adulterants in Food
Hui Li, Galaxy Scientific, 14 Celina Ave, #17, Nashua, NH 03063, Qian Wang, Richard Jackson

Food safety is of increasing concern in the developed world. This includes accidental contamination by pathogens and other toxins, as well as deliberate fraud. Detection of accidental contamination often requires very low detection limits, but the economics of food fraud mean that the food is usually either mislabeled, or mixed with a relatively high concentration of adulterant. This makes the detection of economic adulteration amenable to spectroscopic techniques. Near infrared (NIR) spectroscopy is particularly well suited for this because it samples a relatively large volume, meaning the spectra of inhomogeneous samples are more reproducible. The principle difficulty in using NIR spectroscopy for the detection and identification of adulterants is that the spectrum is dominated by the spectral contribution from the matrix (i.e., the food), and that the spectrum of the matrix exhibits variability between samples. We have developed an algorithm that can extract the adulterant spectrum from the spectrum of the mixture, thereby facilitating the identification of the adulterant. Details of the algorithm, and several examples of its application to the detection of adulterants in food are presented.

64 Nutrients, Flavors, Non-Volatile Chemical Profiles and Chemistry in Daily Consumed Onion Products
Yang Yang, International Flavors and Fragrances, 1515 Highway 36, Union Beach, NJ 07735, Richard Hiserodt

Comprehensive non-volatile chemical profiling for different types of onion extracts become available with the use of high resolution liquid chromatography tandem mass spectrometry (LC-MS/MS), in conjunction with large-size of natural product database. By using metabolomics approach, the major chemicals in fresh onion juice, cooked onion juice and processed onion material could be found and identified. Naturally-occurring amino acids were found to be present in the onion products in a relatively rich level. A series of cysteine derivatives were dominated in the sulfur-containing chemicals. The MS intensity change of cysteine derivatives from fresh onion juice to cooked onion and processed onion suggested oxidation reaction occurred. The cysteine derivatives as flavor enhancers and therapeutic agents in different types of onion products were discussed.
Gas chromatography-flame ionization detection (GC-FID) analysis for quantitation of residual solvents in drugs and intermediates is one of the most important and frequently used tests in the pharmaceutical industry. However, as currently practiced, the technique requires significant sample preparation time, in addition to having a very poor 'green factor' (analytical method volume intensity, or AMVI). In this study, a simple and fast protocol using multi-stable solvent standards mixed with a seven minute universal GC-FID method (using both He and H2 as carrier gas) and empower data analysis is presented. We demonstrate that standard mixtures of solvents commonly used in process chemistry workflows can be stored in crimped high-performance liquid chromatography (HPLC) vials at -10 °C for at least 31 months. The 31 months stability data showed over 97% recovery for all 25 solvents, with overall relative standard deviation below 5%. Our approach simplifies tremendously the tedious task of residual solvent quantitation, resulting in significantly less labor, greater reliability, faster time to result and at least a 300 fold reduction in solvent consumption and hazardous waste disposal.

**65 GC-FID Method for High-Throughput Analysis of Residual Solvents in Pharmaceutical Drugs and Intermediates**

Erik L. Regalado, Merck & Co., MS: RY818-B218, 126 E. Lincoln Ave., PO Box 2000, Rahway, NJ 07065

Gas chromatography-flame ionization detection (GC-FID) analysis for quantitation of residual solvents in drugs and intermediates is one of the most important and frequently used tests in the pharmaceutical industry. However, as currently practiced, the technique requires significant sample preparation time, in addition to having a very poor ‘green factor’ (analytical method volume intensity, or AMVI). In this study, a simple and fast protocol using multi-stable solvent standards mixed with a seven minute universal GC-FID method (using both He and H2 as carrier gas) and empower data analysis is presented. We demonstrate that standard mixtures of solvents commonly used in process chemistry workflows can be stored in crimped high-performance liquid chromatography (HPLC) vials at -10 °C for at least 31 months. The 31 months stability data showed over 97% recovery for all 25 solvents, with overall relative standard deviation below 5%. Our approach simplifies tremendously the tedious task of residual solvent quantitation, resulting in significantly less labor, greater reliability, faster time to result and at least a 300 fold reduction in solvent consumption and hazardous waste disposal.

**66 Stability-Indicating HPLC Method Development and Validation for Cetylpyridinium Chloride**

Ashraf Khan, United States Pharmacopeia, 12601 Twinbrook Pkwy., Rockville, MD 20852, Shane Tan, Fatkhulla K. Tadjimukhamedov, Alan R. Potts

Cetylpyridinium chloride (CPC), a cationic quaternary ammonium compound, is used in dental hygiene. United States Pharmacopeia (USP) 38 monograph uses a non-specific titration procedure for assay and has no organic impurities (OI) procedure. To modernize the assay and introduce an OI procedure, a new high-performance liquid chromatography (HPLC) method has been developed and published in USP 39 First Supplement. Chromatographic separation was achieved on a C18 column (L1 packing, 2.1 x 100 mm, 5 μm) operating at 40 °C with an isocratic elution of mobile phase of 0.1% TFA in water and acetonitrile. The flow rate was 0.6 mL/min. The UV detection was at 258 nm. Four impurity peaks detected at the analytical wavelength in four active pharmaceutical ingredients (API) samples were completely separated. The API samples and forced degraded samples were analyzed for peak purity by HPLC- photodiode array and liquid chromatography-mass spectrometry. Only the oxidatively-stressed sample showed degradation (~6%) with the formation of few well-separated peaks. The CPC peaks in all stressed samples, as well as in API samples, were free from co-elution. For the OI procedure, the validation covered a range of 0.05% to 1% of the sample solution concentration. The lower level is consistent with United States Food and Drug Administration regulatory requirements for impurities. The linear regression correlation coefficient was 0.999. The method met acceptance criteria for specificity, linearity, standard solution stability and quantitation limit. For the assay, results of linearity, precision, and accuracy met acceptance criteria. A new reversed phase and stability-indicating HPLC method has been developed for CPC. The single method is suitable for quantitative analysis of Assay and OI.

**67 Ultra-Fast GC for Residual Solvents in Drug Substance Scale-Up Using a Low Thermal Mass GC**

Yuwen Wang, Boehringer Ingelheim Pharmaceuticals, 900 Ridgeway Rd., Ridgefield, CT 06877

Temperature programming in gas chromatography (GC) is considered the second most important parameter to control the selectivity of a column. A new GC technology to achieve ultrafast temperature programming with an unprecedented cool down time has recently become available. This technology is referred as low thermal mass GC which represents a significant break-through reducing sample analysis time. Conventional GC has been used for decades to determine residual solvents in drug substance and intermediates. It usually takes thirty to sixty minutes long for a single GC run. This is not sufficient for in-process testing, which requires a quick delivery of results to ensure the quality of the products. Our lab has developed fast GC methods for in-process testing using a narrow bore column which reduces the GC run time to eight minutes. Due to the slow cool down time, the GC cycle time is still around fifteen minutes. A low thermal mass GC was evaluated in our lab for the determination of residual solvents for drug substance scale-up and manufacture. The GC run time was reduced to two minutes for eighteen commonly used process solvents and the total GC cycle time was reduced to three minutes. The method was validated in terms of specificity, repeatability, sensitivity, recovery and linearity. Examples of process samples in drug substance scale up are also presented.

**68 Withdrawn by the author.**
mo-5-chlorobenzaldehyde by comparison of the unknown peaks to the HPLC and GC retention times and GC- mass spectrometry (MS) fragmentation pattern of an authentic standard. Further studies determined that the amount of 2-bromo-5-chlorobenzaldehyde was both solvent and imine intermediate concentration depend-ent. The 2-bromo-5-chlorobenzaldehyde amount was also greatly reduced in the presence of butylated hydroxy toluene (BHT) and absent when in the presence of 2,2,6,6-tetramethylpiperidinoxyl (TEMPO), therefore indicating a radical mech-anism. Additionally, UV solution treatment studies demonstrated the production of 2-bromo-5-chlorobenzaldehyde in acetonitrile, the HPLC method diluent, thereby also explaining its earlier observation in the large and small scale batches of the imine. This example highlights one of the many situations confronted by the analyti-cal scientist during the analysis of unstable intermediates at external manufacturing sites and the importance of effective collaboration between innovative pharmaceuti-cal companies and manufacturing contract research organizations.

72 An Evaluation of X-Ray Fluorescence as an Alternative Technique to Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) for Elemental Impurity Analysis of Pharmaceutical Samples

Tiffany M. Brucker, Vertex Pharmaceuticals, 50 Northern Ave., Boston, MA 02210

Heavy metal impurities are critically assessed in drug substance, drug products, and inclusive excipients due to possible toxicological effects. The International Confer-ence on Harmonization (ICH) lists permitted daily exposures (PDEs) for each ele-ment, and drug products must be within these specifications. Currently, inductively coupled plasma (ICP) spectrometry is the most commonly used method to measure metals within the pharmaceutical industry. However, ICP has several drawbacks in-cluding, but not limited to, difficult sample preparation (using strong acids, for diges-tion of samples), sample destruction, daily calibration, potential carry-over between samples, and the need for skilled operators. Wavelength-dispersive X-ray fluores-cence (WD-XRF, XRF) is an alternative technique for elemental impurity analysis with common applications in areas such as the arts, petroleum, and environmental industries. However, there is limited information evaluating the capabilities of the technique for pharmaceutical applications. Advantages of WD-XRF include minimal sample preparation, non-destructive analysis, and absence of daily calibration. In addition, simple to use software and generic methods simplify analyses. This post-er evaluates the benefits and capabilities of WD-XRF as an alternative technique, comparing quantitative performance with ICP-OES methods. Throughput and flexi-bility considerations are also discussed.

73 The Ion Exchange Selectivity of Amines whilst Using Non-Suppressed Conductivity Detection and an IC Controlled with the Empower Chromatography Data System

Stuart J. Procter, Metrohm USA, 6555 Pelican Creek Circle, Riverview, FL 33578, Ashley Witig

Ion chromatography (IC) in the field of pharmaceutical sciences is still dominat-ed by aqueous based suppressed conductivity methodologies. This is largely due to limitations of older generation IC equipment which forces method development chemists to develop sample preparative or column regeneration procedures to deal with hydrophobic samples or sample matrix problems. Furthermore, suppression reactions required by older generation conductivity detectors limit chemistry choices and hinder the detection of weak acids and weak bases. This poster describes how a modern IC technology operating in Empower, a commonly used chromatography data system within the pharmaceutical industry, can be used for the analysis of low molecular weight amines that are widely used in raw materials or intermediates in the synthesis of numerous pharmaceuticals. Their monitoring is crucial as most of them are toxic or chemicals that cause irritation to skin, mucous membranes and the respiratory tract. Furthermore, secondary amines can react with nitrite forming carcinogenic nitrosamines.

74 Optimization of Solubility Evaluation Procedure for Drug Development

Lily Du, Boehringer Ingelheim Pharmaceuticals, MS: PSB 1, 39 Briar Ridge Rd., Danbury, CT 08811, Lalane Bodhipaksha, Cindy Qin

During drug development, solubility data of drug substances is used for determining crystallization/recrystallization parameters, investigating solid form landscape, and evaluating dissolution/bioavailability. Solubility of the drug substance is evaluated in various solvents and/or solvent mixtures at different temperatures. Conventional method used in the solubility evaluation involved sample preparation for steps such as dilutions, centrifugations, and/or filtrations, which are time consuming and potentially introduce more errors in the results. To overcome these challeng-es, we investigated the feasibility of using high-performance liquid chromatography coupled with an automated sample preparation unit named iChemExplorer to de-termine solubility. This automated samples preparation unit is capable of controlling temperature and providing proper mixing and filtration. In addition, a high dynamic range diode array detector (HDR detector) was used to increase linear dynamic range of the detection. The wide linear UV range of the HDR detector provides the quantification of much broader concentration range in a single run compared to a typical UV detector. This optimized approach significantly reduced the overall solubility measurement time and potentially reduced the variation of the results. The solubility data obtained from this automated procedure was comparable with that obtained with the conventional method.

75 Maximizing a Labs Budget for Instrumentation

Jon Welsh, Agilent Technologies, 2850 Centerville Rd., Wilmington, DE 19808

Agilent Technologies has a recent program that can help a lab get the most out of its budget for analytical instrumentation and keep us with fast changing technolo-gy. Agilent’s refurbished instrument business enables customers to trade-in older instruments, for crediting lowering the barrier to upgrade to new instrumentation. This Technology Refresh can allow a lab to increase throughput and sensitivity by using the latest technology instrumentation. Agilent can also buy under-utilized instru-ments for cash helping the lab operating budget. When the need is for instruments on a budget that are equal to the labs current instrumentation, the lab can purchase refurbished versions of its current and last generation instruments fully refurbished to factory standards with full Agilent support and one year warranty. This program can allow a lab to expand its capacity with a limited budget as well as reduce train-ing costs by purchasing instruments that the lab is already familiar with even if they have been obsolete.

76 Innovative Strategies and Solutions to Monitor and Control Cross Contamination of Solvent during Vendor Drumming Process

Jia Zang, Bristol-Myers Squibb, MS: B50-202K, 1 Squibb Dr., New Brunswick, NJ 08903, Yun Ye, Peter Tattersall, Jason Hamm, Michael Randazzo, Michael Cassidy, John Sarik, George Gossett, Dana Cazzullo, Thomas Raglione

Organic solvent quality can have a significant impact on the intermediate and/or active pharmaceutical ingredient (API) quality during manufacturing process. Re-cent uses of solvents that had been cross contaminated, during vendor repack-ag-ing, have had significant impact on the quality and timelines for the production of clinical API materials. A joint effort cross different functional groups developed a strategic workflow to monitor for potential solvent cross contamination and prevent the acceptance of solvent drums that had been inadvertently contaminated by the vendor. The presentation will demonstrate the challenges encountered during the development of an appropriate analytical control method to detect all of the differ-ent potential solvent contamination combinations that were possible at the vendor’s site. To overcome the method development challenges, an innovative and strategic two-step process was developed for monitoring all incoming solvent drums. The presentation also includes a discussion on the challenges encountered in the suc-cesful implementation of the new vendor drumming and monitoring strategies, as well as, the challenges of implementing a sensitive analytical method in an industrial packaging site.

77 Microwave Digestion of Fish Oil Samples - Reducing the Risk of a Runaway Exothermic Reaction

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The digestion of fish oil, in a gel cap format, was done using a NovaWAVE micro-wave digestion system. During the digestion, the fatty acids of the fish oil may result in an exothermic reaction within the vessel leading to a loss of the sample. Several temperature profiles are presented highlighting the effectiveness of the NovaWAVE system to reduce the risk an exothermic reaction (runaway sample). For each sam-ple temperature is monitored and optimized in real-time by applying the correct mi-crowave energy when needed. The flexibility of the NovaWAVE system, of digesting different samples in one rack, is also discussed.

78 Solid-Phase Extraction (SPE) is Liquid Chromatography (LC): Open It As Such, Get Better Results, and Learn

Mark Hayward, ITSP Solutions, 212 Northlake Dr., Hartwell, GA 30643, Jonathan Ho, Tom Moran, Kim Gamble

The fundamentals of achieving high performance operation in LC have always been well packed sorbents, precise flow control, and minimizing dispersion. ITSP SPE cartridges with the usual LC or SPE packed sorbents can be operated with a syringe pump to achieve behavior and function that mirrors that of LC in all respects with regard to the van Deemter model of column chromatography. Flow experiments can be performed, dispersion measured (recovery), the distinct optimum velocity determined for each separation type to be performed. With method optimization experiments performed in the same ways as done for LC (including smaller particles for higher reverse phase velocities), SPE recoveries can be systematically made both high and precise. Performing SPE in this way also may provide opportunity for increasing knowledge of LC and SPE. When solvent chemistry is varied under ion exchange conditions, optimum velocity remains constant, but acceptable velocity
ranges near the optimum observed in the van Deemter curves narrow under solvent conditions less favorable to the adsorption/desorption reaction and broaden under conditions more favorable to the adsorption/desorption reaction. Furthermore, these effects can vary between and be observed separately for the sample load (adsorption) and elute (desorption) steps. In a simple collision theory view of the process, this suggests that the distance at which adsorption/desorption can be initiated with adsorption sites (collision radius) varies and can be measured as a function of solvent chemistry.

69 Unified Drug Testing by Online SPE-LC-MS-MS: One Totally Automated Method Measures ALL the Drugs in Urine and/or Oral Fluids
Mark Hayward, ITSP Solutions, 212 Northlake Dr., Hartwell, GA 30643, Jonathan Ho, Tom Moran, Kim Gamble
Measurement of drugs of abuse in urine and/or oral fluids (OF) is common for pre-employment screening, DOT / federal mandated testing, law enforcement, and compliance/diagnostic determinations by physicians (with the latter two growing rapidly). In an effort to meet these needs, we have developed an automated on-line solid-phase extraction liquid chromatography tandem mass spectrometry (SPE-LC-MS-MS) method. It uses SPE to clean and pre-concentrate samples so that low dose drugs at or near 1 ng/g concentration are easily measured at S/N ≥ 20. The method’s design is balanced to address (identify/measure) all of the drugs (acidic and basic drugs as well as polar and non-polar drugs), as well as either urine or OF samples, all in one method, all in one workflow. It is completely automated from sample plates/vials to results (with no change in workflow) while still using only the native MS software and can process up to 96-well plates of samples overnight per LC-MS-MS. The results will be waiting for you in the morning. Total automation is achieved using the PAL system LC autosampler which performs all sample preparation, including the SPE in parallel to LC-MS-MS analysis, and injects the sample into the LC-MS-MS. The cycle time achieved for online SPE-LC-MS-MS is 4.5 minutes for 71 drugs (opiates, metabolites, illicits, opioids, barbs, benzos, and THC) in urine. The SPE method precentrates the drugs so they are easily measured by the LC/MS/MS (high intensity, low background LC peaks) and high success rates are achieved for automatic integration of LC peaks. The entire process described has validated in both clinical/forensic labs and is used for daily production work.

80 Quick Extraction and Quantification of Polychlorinated Biphenyls and Organochlorine Pesticides in Dry Bloodspots on Protein Saver Cards Using GC-MS-MS
Stephen Jiang, University of Connecticut - Center for Environmental Science and Engineering, 3107 Horsebarn Hill Rd., Storrs, CT 06269, Anthony Provatas, James Stuart, Christopher Perkins
Many organic pollutants are persistent in the environment and prone to bioaccumulation. Certain of these pollutants are carcinogenic and inhibit an organism’s metabolic processes thus are linked to an array of degenerative and reproductive diseases. Their lipophilic nature cause bioaccumulation that widely affect the health of the environment including humans. Polychlorinated Biphenyls (PCB’s) and Organochlorine pesticides (OCP’s) are examples of such pollutants. Traditionally, testing for the presence of organic pollutants within wildlife animals is done using whole blood or plasma. Whole blood however, can be viscous and contiguate making the quantification and transfer difficult cause difficulties in shipping. The utilization of dry bloodspots using protein saver cards alleviates these. Bloodspots allows for ease of handling in a small lightweight media that is just as effective as liquid whole blood when analyzing for pollutants after extraction. Compared to tradition or conventional testing methods, such as liquid-liquid extraction (LLE) followed by solid-phase extraction (SPE), using bloodspots minimizes both the amount of blood, critical when taking samples from small or endangered organisms, as well as solvent needed for analyte extraction. Our method also allows for faster sample preparation requiring only 2-3 hours to complete sample preparation compared to LLE and SPE. Highly sensitive and selective gas chromatography tandem mass spectrometry (GC-MS-MS) is then used to identify and quantify the targeted organic pollutants.

81 Automating the Accurate Transfer of Highly Volatile to Highly Viscous Liquids Using a Bench-Top Workstation.
Fredrick Foster, Gerstel, 701 Digital Dr., Ste. J., Linthicum, MD 21090, John Stuff, Edward Pfannkoch
The manual transfer of liquid standards and solutions is usually part of the daily activities throughout the analytical laboratory. Oftentimes, the samples need to be transferred when creating calibration standard samples, pipetting solvents, and combining liquids. The accurate and precise transfer of liquids can be critical to the analytical results. Liquids with low boiling points or high viscosities pose several challenges to achieving accurate and precise delivery of desired volumes. Verification of the volumes of liquids transferred would lend support to the quality of the analytical procedure and ensure the high quality of the resulting data. A single robotic X-Y-Z coordinate autosampler commonly used for sample introduction in gas chromatography (GC) or high-performance liquid chromatography (HPLC) can be used to perform a wide variety of sample preparation techniques using a single instrument and controlling software. The sampler can be configured as part of a GC or LC system or can be configured as a bench-top workstation and can also include an analytical balance to provide weight verification of liquid transfers. In this report, the automated transfer by the robotic autosampler of a variety of liquid standards having a wide range of volatility and viscosity properties is discussed. Examination of a new vent tool that allows liquid samples to be transferred while venting a sealed vial is described. Resulting weight verification, precision, and accuracy data from the assessment of example compounds transferred by the autosampler are provided that demonstrate the dramatic improvement in accuracy and precision for transferring these type liquids.

82 Super-Fast Extractions: Unifying Sample Preparation and Chromatography
Bill Hedgepeth, Shimadzu Scientific Instruments, 7100 Riverwood Dr., Columbia, MD 21046, Kenichiro Tanaka
Supercritical fluid extraction is a quick and convenient technique for the sample preparation of a number of compounds from a variety of sample matrices. The automation of this technique can make it much faster and more reproducible that manually intensive sample preparation techniques like QuEChERS (quick, easy, cheap, effective, rugged, and safe). Recently, a new system was introduced that automatically sends the extracted sample components directly to an analytical column for testing. A number of applications that include the analysis of explosives in soil samples and pesticides in food that use this technique are shown.

83 Challenges in Sample Solution Preparation of New Topical Formulations: What are Representative Samples for Development?
Avi Rosenberger, Teva Pharmaceutical Industries, Hatarufa 12, Netanya 4250483, Israel, Maher Kaadan, Mazzi Dagan-Lion
Sample preparation is a crucial step in analytical method development. Analysis of ointment and cream products can be challenging. The aim is to develop a simple and practical method, such as mixing or shaking using a polar organic solvent compatible with the chromatographic conditions and the desired detection technique. The main challenge is to ensure that the active pharmaceutical ingredient (API) is fully extracted from the matrix, which is often practically insoluble in polar organic solvents or organic/aqueous mixtures. During formulation development several formulations are explored and the question is which sample(s) are considered to be representative for development of sample solution preparation. Ultimately, sample preparation and formulation development are initiated together and analysis is performed on newly produced formulations. However, minor changes in the formulation, strength(s), manufacturing process and storage conditions may have significant effect on extraction efficiency of the API, therefore requiring re-assessment of the procedure. During preliminary development of new ointment/cream formulations, problems with extraction efficiency arose for the cream formulation lation, accompanied by texture change, upon storage under accelerated conditions. Problems were also encountered with the lowest strength ointment at release and with the highest strength ointment during intermediate stability. Additionally, other challenges were encountered during scale-up of the production to manufacturing facility. In response, optimization of sample solution preparation was performed on release and stability samples, as well as on lab- and manufacturing-scale samples of various strengths.

84 Carbohydrate Biopolymer Profiling by Nanopore Sensors
Buddini I. Karawidiyia, University of Rhode Island, 140 Flag Rd., Kingston, RI 02881, Y.M. Nuwan D.Y. Bandara, Jason R. Dwyer
Structural characteristics of biomolecules play a crucial role in determining their unique function in biological systems and therefore molecular structure determination has gained extensive attention from the analytical science world. Rapid and reliable sensing platforms are required to meet the current analysis demands of these molecules for many fields, like the pharmaceutical industry and biomedicine. Our main focus is on carbohydrate biopolymers (polysaccharides) —an important class of biopolymers associated with mediating many biological functions including cell-cell interactions, cell proliferation, apoptosis and microbial interaction with the body. While the field of biomolecular sensing has devoted tremendous effort to the study of DNA and proteins, structure determination of polysaccharides has lacked the attention it deserves mainly due to the complications associated with tackling the complexities in monomer composition, crosslinking, polymerization, isomeric forms and branched structures. Along with this, the need for effective and robust non-optical sensing device—operated on an ostensibly simple principle, sensitive to molecular structures by reading unique current fluctuations when molecules translocate through the nanopore. With the intention of extending the sensing capabilities of these novel sensors for sugar determination, we used a silicon nitride nanopore sensor, enabling routine profiling of as little as one molecule of sugar at a time. Given the importance of intermolecular interactions in biology, we used carbohydrate biopolymers where we could chemically control biopolymer aggregation
to differentiate, as a proof-of-principle experiment, between single and complexed biopolymer strands. We intend to establish the fundamentals of using nanopore sensors for single molecular sugar characterization.

85 A Simple Determination of Absolute Zero through Pressure-Temperature Extrapolation
Jacob M. Newman, Touro College Lander College for Men, 7531 150th St., Flushing, NY 11367
A fast and straightforward experiment for the determination of absolute zero via extrapolation of the pressure/temperature curve for air was developed, targeting high school or introductory college chemistry students. Using off the shelf data logging equipment and sensors, students measure the pressure and temperature of a sample of air of known volume, contained in a commercially available stainless steel sphere equipped with pressure and temperature sensors, at four different temperatures above, at, and below room temperature. They then repeat the experiment with a variable quantity of air obtained by opening the pressure relief valve of the sphere at elevated or reduced temperature. Students then plot a pressure temperature curve and extrapolate to determine values of absolute zero. Typical results are 15K +/- 5K. Students are asked to analyze why their experiment does not yield the expected value of 0 K. The experiment helps introductory students to understand the postulates and limitations of the kinetic molecular theory of gases. Students spend an average of 1.5 hours working on the experiment, and the experiment could be shortened further to fit into an hour-long class period.

86 Performance of Cairpol CairClip NO2 and Combined O3-NO2 sensors
Ashley A. Cole, Rutgers University, Environmental and Occupational Health Sciences Institute, 170 Frelinghuysen Rd., Piscataway, NJ 08854, Soowen Jeong, Clifford Weisel
Cairpol CairClip NO2 and O3-NO2 portable air quality sensors detect the NO2 and combined O3-NO2 air concentrations, respectively, in an amperometric manner by a Faraday electrochemical cell (FET), and 6) simple, automated formaldehyde analysis.

87 Naked-Eye Coulometric Sensor Using a Longitudinally Oriented Ag Band Electrode
Kwok-Fan Chow, University of Massachusetts-Lowell, 1 University Ave., Lowell, MA 01854, Jung-Min Oh
In this poster, we demonstrate a coulometric sensing platform that is capable of sensing analytes on a working electrode and providing a visual readout of the analyte concentration on a silver (Ag) band counter electrode in a microchannel. The display mechanism relies on the electro-oxidation of metallic Ag as a complementary reaction to the sensing reaction. The Ag band counter electrode is arranged longitudinally in a microchannel while the frontal tip of the band electrode directly faces a gold (Au) working electrode, which lies across the microchannel. The Ag oxidation always occurs at the band electrode’s tip region that faces the working electrode due to the Ohmic potential drop across the solution in the microchannel. The decrement of the Ag electrode, which is clearly measurable with the naked-eye, correlates linearly with an analytic concentration and with an analytic feeding rate.

88 Automation of Rapid SIFT-MS VOC and Inorganic Headspace Analysis
Chuck Renner, Quantum Analytics, 3400 E. Third Ave., Foster City, CA 94404
Integration of autosampler technology from Gerstel with selected ion flow tube mass spectrometry (SIFT-MS) instrumentation from Syft Technologies provides opportunities for unprecedented throughput of VOC and inorganic headspace and gas analysis. In this paper, the integrated autosampler-SIFT-MS solution is introduced and various high-throughput applications presented that are of relevance to both contract research and research and development laboratories in a range of markets. Applications include: 1) residual solvent analysis in packaging and pharmaceuticals, 2) residual monomer analysis in polymers, 3) multiple headspace extraction (MHE), 4) rapid, objective sensory screening for the food industry, 5) full evaporative technique (FET), and 6) simple, automated formaldehyde analysis.

89 SpeedMixer Capabilities
Drew D. Tyger, FlackTek, 1708 Hwy 11, Landrum, SC 29356
The SpeedMixer is the most advanced mixing technology available today, fully capable of mixing a variety of materials—from thick to thin in any combination—quickly and repeatably. The SpeedMixer eliminates air bubbles and mixes in disposable containers. It is used to mix colloids, fluids, powders, pastes, creams, grease, resins, inks, paints and silicones mixtures. Our lab size, small batch, manufacturing mixers have the capacity to mix in samples in seconds. Sample sizes range from, <1g - 10Kg, and can be mixed in cups and cartridges, including Semco. Absolutely no cleanup required.

90 High-Speed, Multi-Element Imaging using Laser Ablation ICP/TOF Mass Spectrometry
Olga Borovinskaya, TOFWERK, Uttigenstrasse 22, Thun 3600, Switzerland, Martin Tanner, Ernie Pettit
The scan speeds (pixels/s) of commercially available laser ablation (LA) inductively coupled plasma mass spectrometry (ICP-MS) imaging hardware can significantly limit experimental throughput—particularly if multiple elements are to be recorded. Among other factors, long ablation cell wash-out times and dispersion of laser-generated aerosol force extended delays between the measurements of successive pixels in order to avoid spectral blurring. With this in mind, multiple groups are making efforts to improve hardware for transporting aerosol from the point of ablation to the ICP-MS torch. The recently developed dual concentric injector (DCI) from Electro Scientific Industries (ESI) and the Aerosol Rapid Introduction System (ARIS) from Teledyne enable rapid aerosol washout of <50 ms—increasing achievable imaging speed and sensitivity. The advantages of these high-speed LA sampling systems are fully exploited when combined with the unique capabilities of the TOFWERK icpTOF mass spectrometer, which simultaneously measures all isotopes and records a complete mass spectrum every 30 µs. This work demonstrates multi-element LA-ICP-MS imaging using the TOFWERK icpTOF coupled with the latest laser ablation imaging hardware from ESI (NRW213 laser / TwoVoz2 cell / DCI) and Teledyne (Analyte G2 laser / HelEx II cell / ARIS). Complete mass spectra were acquired for individual laser pulses at times between 10 and 20 Hz, enabling multi-element images to be recorded at more than 10x the rate of other commercially available systems. Synchronization of the icpTOF and laser on a single-pixel basis simplifies image reconstruction and provides sharp images with no blurring of pixels.

91 Analyzing Multi Component Dissolution Samples using in-situ Fiber Optic UV Spectrophotometry
Andrew Kielt, Distek, 121 North Center Dr., North Brunswick, NJ 08902
Traditionally, analyzing more than one active pharmaceutical ingredient (API) with ultra violet (UV) spectrophotometry poses a challenge as both species often absorb over the same spectral region, causing deviations from Beer’s Law. This linear relation between absorbance and the absorbing species is used to calculate concentrations based on the measured absorbance at a specific wavelength. Separation techniques such as high-performance liquid chromatography (HPLC) are often reverted to when analyzing mixture samples with more than one API due to the concentration calculation errors caused by the spectral overlap. However, Multicomponent Analysis (MCA) software and complete spectral profiles collected using a fiber optic UV dissolution analyzer overcome these obstacles. This is accomplished using the classic m x n least squares form of multiple linear regression to analyze the two spectrally overlapping components. The software uses a calibration matrix of extinction coefficients derived from the spectra of up to seven standard solutions to calculate component concentrations in an unknown mixture. The MCA module can also be used to measure one API in the presence of strong interference from excipients, coatings, etc. This study demonstrates the MCA software’s capability, used in tandem with in-situ fiber optics, to accurately monitor and quantify the dissolution profile of a commercial product containing two APIs, eliminating the need to draw samples for HPLC analysis.

92 Application of USP Apparatus 7 to In-Vitro Drug Release in Silver Foam Dressing
Jensen Lee, Logan Instruments, 19 Schoolhouse Rd., Somerset, NJ 08873, Hock S. Tan
We studied the in-vitro drug release characteristics of dressing pads containing silver complex, over a seven-day period, using both a United States Pharmacopeia (USP) Dissolution Apparatus 7 (12-sample carousels) and a Franz diffusion cell (6-cell) system, both manufactured by Logan Instruments, Somerset, NJ. The results demonstrate that the drug release profiles obtained using the two systems are equivalent, though the USP Apparatus 7 produced data with less variability. These results indicate that Logan Instruments’ Apparatus 7 assembly, which is cost-effective, and easier to operate, can replace the conventional Franz cell set-up to generate drug release data for wound dressing pad and possibly transdermal patch products.
Column Performance: Comparison of the Superficially Porous Particle (SPP) to the Fully Porous Particle (FPP)
Sharon Lupo, Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

Superficially porous particles (commonly referred to as SPP or "core-shell" particles) have been proven to provide fast and efficient separations. These particles feature a solid, impermeable core enveloped by a thin, porous layer of silica that decreases the diffusion path and reduces peak dispersion. As a result, significant improvements in efficiency and sensitivity can be achieved over fully porous particles of similar dimension. In this presentation the performance of 5-µm SPP particle columns will be compared to columns packed with traditional 5-µm and 3-µm fully porous particles (FPP). The relationship between pressure and efficiency will be explored. In addition, run time, signal to noise ratio, peak width, and resolution will be evaluated in several chromatographic experiments. Each experiment will be performed on the same instrument using identical method conditions for each particle. Through these experiments we hope to demonstrate the following advantages of 5-µm SPP particle columns over columns packed with traditional 5-µm and 3-µm FPP particles. When used in the development of new assays, they allow the chromatographer to obtain fast run times and excellent method performance without changes in instrumentation. When substituted into existing methodologies which utilize 5-µm and 3-µm FPP columns, 5-µm SPP columns have the potential to dramatically decrease analysis times while improving efficiency and sensitivity.

Barcode Tracking System for Automated ICP-ICPMS Analysis According to USP <232> and <233> Protocols
Kevin J. Hahn, Eimco Scientific, 7277 World Communications, Omaha, NE 68122, Paul Field

A fully-automated two-dimensional (2D) barcode sample tracking system is evaluated for United States Pharmacopeia (USP) <232> and <233> sample preparation and analysis using tandem inductively coupled plasma mass spectrometry (ICP-ICPMS). The system, commercially known as PlasmaTraxTM, uses 2D barcoded sample and digestion tubes to store, reference, and transmit data to LIMS (Laboratory Information Management Systems) for report generation. Four distinct stations are integrated with one complete software package. 1) TraxGPTM is a handheld scanner used to initially define USP samples. 2) TraxMassTM is a balance with integrated barcode scanner for recording sample weights. 3) TraxPrepTM is an offline sample preparation station which automatically prepares and spikes samples according to USP protocols. 4) TraxSCTM is an autosampling platform which utilizes barcode decoded information to build appropriate J calibration curves prior to intelligently and uniquely diluting and analyzing samples by ICP-ICPMS. The ability of PlasmaTraxTM to automatically perform a complete USP <232> and <233> protocol is investigated for a variety of oral drugs. The daily dose and sample mass of drugs ranges from 0.01-20.0 g/day and 0.180-0.220 g, respectively. The system automatically calculates and prepares unspiked and J-spiked solutions according to USP oral PDE (permissible daily exposure) limits. The autodilution system selects the correct J standard (High-J or Low-J) and calibrates at the 0.5 and 1.5J concentrations defined by each drug’s daily dose. The final results indicate samples are run according to USP protocol and pass validation criteria of accuracy, repeatability, suitability, and specificity. Barcode information is permanently associated with the results, ensuring sample integrity throughout the USP preparation and analysis process.

Rapid Automated Protein Crashing Method Using Novel Tip-on-Tip Filtration
William E. Brewer, DPX Technologies, 541 Main St., Ste. 116, Columbia, SC 29201, Kaylee R. mastrianni

We propose a novel automated alternative to protein precipitation/centrifugation which uses wide bore tip mixing to ensure thorough protein precipitation coupled to "tip-on-tip filtration." The wide bore tip mixes the sample with acetonitrile and then transfers the protein crashed sample into a secondary filter tip. The sample is then dispensed through both tips into a clean well plate for further sample preparation and/or analysis. For increased sample clean-up during the filtration process, different solid phase extraction sorbents can be added. We present three filtration options: filtering only, after the addition of phospholipid removal sorbent. The first is ideal for drug discovery or samples that need alternative sample preparation post-filtration. The addition of C18 sorbent acts as a guard cartridge for liquid chromatography tandem mass spectrometry analysis of small molecules, like drugs of abuse and pharmaceuticals. Anything that would stick irreversibly on the analytical column will stick on the C18 sorbent instead, essentially making this filter tip a "disposable guard cartridge." Lastly, the addition of a phospholipid removal sorbent is ideal for larger molecule analysis where phospholipid matrix effects are prominent. This automated process can perform blood protein precipitation, filtration and potentially clean-up 96 samples at a time, drastically increasing the throughput and ease of performing blood analysis.

93 Synthetic Drugs of Abuse Compound Fingerprinting by VUV Spectroscopy
Paul Johnson, VUV Analytics, 715 Discovery Blvd., Ste. 502, Cedar Park, TX 78613

The abuse of synthetic designer drugs by teenagers, young adults, and the home-less population is a growing problem across the United States (US). Inert herbal blends and pills sprayed with synthetic compounds have led to thousands of hospitalizations and overdose deaths over the past several years. Synthetic chemicals sold by international manufacturers are imported into the US legally due to their chemical identity differing from compounds restricted by the United States Drug Enforcement Administration. VUV Analytics presents the GC-VUV analytical solution for the rapid identification and quantitation of illicit compounds at the 2016 Eastern Analytical Symposium. The VGA universal gas chromatography (GC) detector provides excellent measurement sensitivity and unmatched selectivity of co-eluting isomers without the need for chromatographic baseline resolution or calibration. All compounds absorb strongly in the VUV spectrum, and their inherent absorbance cross sections lead to unique spectral fingerprints. GC-VUV data is presented demonstrating the ability to verify the chemical identity and compound class of components found in designer drugs. VUV Analytics is the world leader in vacuum ultraviolet (VUV) absorption spectroscopy. VUV Analytics manufactures the V100, VG101, & SVGA-100 universal gas chromatography and streaming gas detectors.

94 Comparing BromoPhenyl to FluoroPhenyl Stationary Phase in Core-Shell HPLC Columns
Ken Tseng, Nacali USA, 10225 Barnes Canyon Rd., Ste. A103, San Diego, CA 92121, Toshi Ono, Tsunehisa Hirose

FluroPhenyl phase high-performance liquid chromatography (HPLC) columns have gained popularity in recent years for difficult-to-separate compounds. The separation mechanisms are composed of reversed-phase, hydrophobic interaction liquid chromatography, and ion-exchange. This unique selectivity has proven to be useful as an orthogonal method to C18 in method development. BromoPhenyl phase HPLC column is a newer column with 5 bromides on a phenyl group as the stationary phase. It has similar selectivity as FluroPhenyl phase with stronger interaction. This difference in selectivity has proven useful in the purification of very polar molecules under reversed mode condition. Conditions affecting selectivity are tested and compare between the two phases for neutral, acidic, and basic compounds.

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spectral features from low-filed data.[1] We present work towards the use of online, low-field NMR for reaction monitoring with spectral reduction based on CRAFT analysis of the data.

Reference:

100 NMR and Configuration
R. Thomas Williamson, Merck & Co., 126 E. Scott Ave., Rahway, NJ 07065
No abstract submitted by the author.

101 Evolution of NMR Capabilities Across the Breadth of a Career: Revision of the Structure of Cryptospiroline and Elucidation of the Structure of Homodimericin A
Gary E. Martin, Merck & Co., 126 E. Scott Ave., Rahway, NJ 07065
Nuclear magnetic resonance (NMR) capabilities have evolved from simple proton spectra acquired in a single continuous wave (CW) sweep to sophisticated two-di-mensional (2D) NMR experiments involving multiple nuclei that can facilitate the elucidation of complex molecular structures. Following a brief survey of key developments in the evolution of NMR technologies and experimental methods, the revision of the structure of the complex spiro nonacyclic alkald cryptospiroline, which required nearly 25 years, are discussed. Nearly a decade after the initial 1993 report, chromatographic interrogation of the original sample showed that it had totally degraded into 26 components, two of which were fully characterized in 2002. One degradant could be rationalized from the reported structure, the other could not, strongly inferring that the original structure was incorrect. Advances in probe technology and experimental methods that would enable the remaining sample of the alkaloid to be characterized took until late 2014. Using 1.7 mm MicroCryoProbe™ capabilities coupled with newly developed LR-HSQMBC, 1,1- and 1,2-HD-ADEQUATE experiments allowed the structure to be finally and unequivocally established. The second example from the realm of chemical ecology, homodimericin A, is a severely proton-deficient natural product that defined characterization by conventional 2D NMR techniques including computer-assisted structure elucidation. The hexacyclic central core of the molecule incorporates 20 carbons, 14 of which are non-protonated. Characterization of the structure required the utilization of 1-HD-ADEQUATE, LR-HSQMBC, J-HCA, DFT calculations and residual chemical shift anisotropies (RCSAs) in addition to the array of conventional NMR experiments and provides an example of what is now possible with modern state-of-the-art NMR methods.

102 Biomedical Applications of Optical Coherence Tomography
James G. Fujimoto, Elihu Thomson Professor, Department of Electrical Engineering and Computer Science and Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, MA 02139
Optical coherence tomography (OCT) is an emerging imaging modality which uses echoes of light to generate micron resolution, cross-sectional and three-dimensional images of tissue architectural morphology. OCT imaging depth is limited to ~2 to 3 mm in most tissues, however imaging can be performed using catheters and endoscopes. OCT enables “optical biopsy”, visualizing tissue microstructure in situ and in real time without the need for tissue excision or histological processing. Three-dimensional functional imaging of Doppler blood flow and vascular contrast is also possible. OCT has applications in multiple clinical situations, where biopsy is hazardous or impossible, guiding conventional excisional biopsy to reduce sampling errors, and guiding interventional treatment or monitoring treatment response. OCT is now a standard diagnostic in ophthalmology, with over 30 million procedures yearly. It is also being developed in applications ranging from intravascular to endoscopic imaging. This presentation reviews the development of OCT technology and gives an overview of applications across different medical and research specialties. In addition to research advances, we also discuss the translational process for medical instrumentation technology.

103 Fundamental Underlying Light Propagation for Optical Imaging in Model and Tissue Scattering Media
Robert Alfano, Institute for Ultrafast Spectroscopy and Lasers (IUSL), The City College of New York, 160 Convent Ave, MR-41, New York, NY 10031
This talk focuses on fundamental understanding of light propagating in tissue and tissue like scattering media. I discuss light’s salient properties of: wavelength, polarization, coherence, ultimate speed, and short laser pulses; and how light pulse propagates in a scattering medium by breaking up into three components called the Ballistic, Snake and Diffusive components. The ballistic retains the coherence and polarization properties of the light; the snake follows with less; and then the diffusive which retains none of coherence or polarization. Light plays an important role in photonic applications in biomedical optical spectroscopy and optical imaging using linear and nonlinear optical interactions, such as second harmonic and multiphoton fluorescence (2PEF) imaging. The second part of talk reviews the most important applications using light salient properties. Some of the key important outcomes of using light in optical biopsy for medical applications are: fluorescence spectroscopy, Raman spectroscopy and coherence optical tomography (OCT). The OCT discovery and advances to clinical use was pioneered by Gold Medal winner Dr. Jim Fujimoto using the ballistic component of the light in backscatter geometry for the eye, arteries and colon.

104 Intra-Operative OCT Imaging and Sensing Devices for Clinical Translation
Yu Chen, University of Maryland, 3142 Kim Engineering Bldg., College Park, MD 20742
Stereotactic procedures that require insertion of needle-based instruments into the brain serve important roles in a variety of neurosurgical interventions, such as biopsy, catheterization, and electrode placement. A fundamental limitation of these stereotactic procedures is that they are blind procedures in that the operator does not have real-time feedback as to what lies immediately ahead of the advancing needle. Therefore, there is a great clinical need to navigate the instrument safely and accurately to the targets. Towards that end, we developed a forward-imaging needle-type optical coherence tomography (OCT) probe for avoiding the hemorrhage and guiding neurosurgical interventions. The needle probe has a thin diameter of 0.7 mm. The feasibility of vessel detection and neurosururgical guidance were demonstrated on sheep brain in-vivo and human brain ex-vivo. In addition, we further reduced the probe size to 0.3 mm using an optical Doppler sensing (ODS) fiber probe to detect the blood vessels lying ahead to improve the safety of this procedure. Furthermore, to overcome the field-of-view limitation of OCT probe, we developed an MRI-compatible OCT imaging probe for neurosurgery. MRI-OCT multi-scale imaging integrates micro-resolution optical imaging with wide-field MRI imaging, and has potential to further improve the targeting accuracy.

105 Noninvasive Optical Imaging and Stimulation of Drosophila Heart Function
Chao Zhou, Lehigh University, Sinclair Lab Room 225, 7 Asa Dr., Bethlehem, PA 18015
Electrical stimulation is the clinical standard for cardiac pacing. Although highly effective in controlling cardiac rhythm, the invasive nature, non-specificity to cardiac tissues and possible tissue damage limits its applications. Optogenetic pacing of the heart is a promising alternative, which is non-invasive and more specific, has high spatial and temporal precision, and avoids the shortcomings in electrical stimulation. Drosophila melanogaster, which is a powerful model organism with orthologs of nearly 75% of human disease genes, has not been studied for optogenetic pacing in the heart. Here, we developed a non-invasive integrated optical pacing and optical coherence microscopy (OCM) imaging system to control the heart rhythm of Drosophila at different developmental stages using light. The OCM system is capable of providing high imaging speed (130 frames/s) and ultrahigh imaging resolutions (1.5 μm and 3.9 μm for axial and transverse resolutions, respectively). A light-sensitive pacemaker was developed in Drosophila by specifically expressing the light-gated cation channel, channelrhodopsin-2 (ChR2) in transgenic Drosophila heart. We achieved non-invasive and specific optical control of the Drosophila heart rhythm throughout the fly’s life cycle (larva, pupa, and adult) by stimulating the heart with 475 nm pulsed laser light. Heart response to stimulation pulses was monitored non-invasively with OCM. This integrated non-invasive optogenetic control and in-vivo imaging technique provides a novel platform for performing research studies in developmental cardiology.

106 Synthesis and Applications of pH Reversible Ion Exchange Materials
Christopher Pohl, Thermo Fisher Scientific, 1228 Titan Way, Sunnyvale, CA 94085
Ion exchange materials are widely used in chromatography for the separation of a range of analytes, ranging from low atomic weight species such as lithium to high molecular weight proteins such as monoclonal antibodies. Generally, ion exchange materials used in chromatography are designed to work in a single operating mode, separating either anionic species or cationic species. Here we investigate the utility of ion exchange materials designed so that the net charge of the stationary phase can be reversed through proper choice of monomer composition such that manipulation of the mobile phase pH results in a reversal of the stationary phase net charge. The manipulation of stationary phase composition to achieve control of the net stationary phase charge state is accomplished through the use of a mixture of monomers, each with a distinct pKa and/or intrinsic charge state. Use of monomers with opposite intrinsic net charge state is useful for the control of net charge distribution. With the addition of a third zwitterionic monomer, it is possible to use pH to manipulate the net charge of the zwitterionic monomer incorporated into the polymer network and with judicious choice of polymer composition, the charge state of the stationary phase. One advantage of pH reversible ion exchange materials is
its ability to eject highly retained species difficult to elute by other means through the use of electrostatic repulsion. The utility of such materials for chromatographic separations are demonstrated.

107 Recent Advances in Suppressed Ion Chromatography with Carbonate Eluents
Kannan Srinivasan, Thermo Fisher Scientific, 1228 Titan Way, Sunnyvale, CA 94087, Brittany Omphroy, Mirnal Sengupta
It is well accepted to use a suppressor when pursuing ion analysis with an ion chromatograph. The function of the suppressor in ion chromatography is to remove the counter ion to the eluent and convert the eluent to a weakly dissociated form. When pursuing anion analysis with hydroxide chemistry the product of suppression is water and has a low conductivity background and low noise. When pursuing anion analysis using carbonate and/or bicarbonate eluents, the suppressor product is carbonic acid which results in a greater than 10 fold higher background than hydroxide eluents and relatively high noise. In the chemical mode of operation, the noise is not impacted by the suppressed conductivity background; however leakage of the chemical reagent can compromise the operational dynamic capacity of the suppressor and the detection sensitivity. In this presentation we discuss various configurations of the electrolytic suppressor with the goal of lowering the operational noise when operated with carbonate and/or bicarbonate eluents. Additionally we discuss a new ERS 500e carbonate suppressor design that allowed low noise performance with carbonate and/or bicarbonate eluents. We show results from comparing the performance of the conventional membrane suppressor design with the performance of the new design. In addition we will also discuss a new design of a consumable accessory that allows continuous operation of the carbonate removal device without any external reagents.

108 Solving Problems in the Pharmaceutical Industry with Chromatography
Sut Ahuja, Ahuja Consulting, 1061 Rutledge Ct., Calabas, NC 28467
New drug development may take as many as nine years and requires inputs from medicinal chemistry, analytical chemistry, chemical development, pharmaceutics, pharmacology, toxicology, manufacturing, marketing, and regulatory departments. The inputs from analytical research and development are important for all of these operations because they can make the difference between slow and fast development. Drug discovery is generally initiated with the synthesis of a new chemical entity (NCE) based on combinatorial chemistry, or drugs are based on recombinant products. The molecular structure, including chirality, has to be confirmed. It is necessary to demonstrate the absence of any undesirable impurities, including enantiomers that may exhibit unusual pharmacologic or toxicologic activities. Finally, it is necessary to select an optimum dosage form, based on therapeutic and marketing needs. The selected dosage form has to meet good manufacturing practice and good laboratory practice requirements. Discussion focuses on how chromatography can help solve a variety of problems encountered in the pharmaceutical industry.

References:

109 Sharing Passion for Analytical Chemistry with Sandy
Janusz Pawlizyn, University of Waterloo, Department of Chemistry, Waterloo, ON N2L 3G1, Canada
The presentation focuses on the research directions shared with Sandy interests. It covers early work on use of light-emitting diodes (LEDs), laser diodes and communication technology in developing microfluidic flow injection analysis detectors. It also describes evolution of whole column imaging technology and invention of fluorescence evanescent wave capillary isoelectric focusing detector. Finally, selected projects related to sample preparation related to single drop extraction and total and free concentration determination based on solid phase microextraction and needle trap technologies used jointly are emphasized.

110 What the Instrument Sees
Franklin E. Barton II, LLS Instruments, 165 Sunnybrook Dr., Athens, GA 30605
The basic principle of a spectral instrument has not changed since Galileo invented the telescope in 1604. There is a source of light (in this case the stars), an optical system to focus the light for the selected wavelengths (visible) onto a detector (the human eye). In the case of the telescope the sample is also the source of light. Things progressed over the next two centuries until Herschel discovered a spectral region above the visible in 1800. From then on the instrument had one more component, a device to produce and separate light into monochromatic wavelengths. Instruments whether dispersive, interferometric, filter, photo diode array all have been designed to take a spectrum by putting source radiation on the sample, capturing the light transmitted or reflected, separating the wavelengths and focusing the results on the detector/detectors. In most cases the results are optimized by how the sample is presented to the instrument. How much of the sample is really analyzed or seen will be discussed in terms of speed of data acquisition, spot size, optics efficiency and detector area. The sample and the desired information will dictate the configuration of the instrument to give the best results. Best is a moving target and will depend on the nature of the assay and how the information can and will be used.

111 How the Parts Influence the Results
James A. de Haseth, LLS Instruments, Inc., 165 Sunnybrook Dr., Athens, GA 30605, Franklin E Barton
There has been a longstanding practice to match all the components of a spectrometer so that each component is consistent with the projected use of the instrument. In many ways the design starts with the sample, that is, the size of the sample, its characteristics and the information to be derived from the spectrum of the sample. Care must be taken to assure the spectrometer is designed to fit the constraints of the sample. This includes, but is not limited to, the source, the size and throughput of the optics, the detector, electronics, software processing, as well as thermal and environmental conditions. It has always been the case that engineers and spectroscopists are restricted to available components, or technology, to modify or redesign components. New technologies not only yield additional opportunities to extend or improve instrumentation, but also in some ways redirect or even restrict the development of new instrumentation. Computing has also led to many advantages to the extraction of information from spectra, but all too often algorithms are used to compensate for poor spectral performance. The goal of this presentation is raise questions about how we are looking at spectrometry today and question if shortcuts are being taken at the expense of appropriate data. Are we losing information because spurious information or invalid assumptions are being made about the spectra as a consequence of spectrometer design?

112 Withdrawn by the author.

113 Inter- and Intra-Cultural Competence Skills for Managing a Majority-Minority Laboratory
Ephraim M. Govere, Pennsylvania State University, 116 Agricultural Sciences & Industries Building, University Park, PA 16802
The United States of America (US) is becoming a ‘plurality’ nation of racial and ethnic groups. Currently there are four majority-minority states in the US: California, Hawaii, New Mexico and Texas. The number of majority-minority states will keep increasing because the minority population is expected to rise to 56 percent of the total population in 2060, compared with 38 percent in 2014. Likewise, majority-minority laboratories will increase making it imperative for laboratory managers to acquire inter- and intra-cultural competence skills in order for them to manage successfully. According to the United Nations Educational, Scientific and Cultural Organization (UNESCO), an intercultural competent person is one that has adequate relevant knowledge about particular cultures, as well as general knowledge about the sorts of issues arising when members of different cultures interact, holds receptive attitudes that encourage establishing and maintaining contact with diverse others, and has the skills required to draw upon both knowledge and attitudes when interacting with others from different cultures. An intracultural competent person is culturally competent among members of his or her cultural group. For a laboratory manager to competently manage a majority-minority laboratory, she or he needs both intercultural and intracultural skills. This presentation highlights the inter- and intra-cultural competence skills for managing a majority-minority laboratory.

114 Planning for the Future: Succession Planning and Knowledge Transfer
Scott Hanton, Intertek, 7201 Hamilton Blvd., MS: RD1, Dock5, Allentown, PA 18195
While the future is difficult to predict, we can take actions to prepare ourselves, our business/department, and our staff for the changes that the future will bring. Even in the best of situations, we all experience turnover in staff from retirements, voluntary and involuntary exits from the group. Planning for succession can help drive appropriate hiring and development of existing staff to smooth the disruptions caused by staff turnover. In addition, we can use knowledge transfer tools to help retain and archive critical knowledge to enable the people who are new to the roles to be successful after the changes. This kind of planning helps remind us that people are the most important component of any business, and the key responsibility of people managers.
Leading a Safer and Healthier Diverse Organization
Jim A. Kaufman, Laboratory Safety Institute, 192 Worcester St., Natick, MA 01760, Rajeev Santhappa
What does it take to create a safer and healthier organization? This presentation describes the key ingredients in a more effective lab safety program. You will learn simple and inexpensive ideas and techniques that 95% of all employer fail to use. You will receive a 33 element checklist that you can use to audit your program.

Communicating Across Cultures
Patreece Ingram, Pennsylvania State University, 230 Agricultural Administration Building, University Park, PA 16802
Working in a lab is very often a collaborative effort. Oftentimes, people work in teams and groups. And since we work with people from different cultures who may have different communication styles it may cause some of the differences. It is almost impossible to send a message that does not have at least some cultural content, and it is not possible to receive a message without passing it through our own cultural filters. This presentation helps us to take a look at some of the ways that both verbal and nonverbal communication styles differ across cultures. An increased understanding can help us avoid misunderstandings and misinterpretations of the behavior of our colleagues, and can lead to more effective communication within our laboratory settings.

Meeting the Changing Expectations for Container Closure Integrity Testing of Parenterals on Stability
Steven E. Klohr, Bristol-Myers Squibb, MS: B105/2451D, One Squibb Dr., New Brunswick, NJ 08903, Casey Tyrell-Pawlowicz, Chris Knutsen, Nikiun Vasoya, Antonio Fernandez
Ensuring container closure integrity of parenteral pharmaceutical products is necessary to provide protection and maintain quality throughout the shelf life of a sterile drug product. Container Closure Integrity Testing (CCIT) has gained industry attention due to increased regulatory agency scrutiny regarding the analytical rigor of CCIT methods and expectations to use CCIT in lieu of sterility tests in stability programs. The 2008 United States Food and Drug Administration Guidance for Industry on container closure system integrity testing in lieu of sterility provides recommendations for using methods other than sterility testing to confirm container and closure system integrity as a part of stability protocols for sterile products. The recent revision to USP <1207> provides a comprehensive overview of available methods for container closure testing and describes many important elements to consider for a successful CCIT program. However, it also infers that dye ingress methods are inferior to newer, deterministic methods. Here we demonstrate an analytically rigorous fluorescein dye CCIT method with visual detection that can reliably detect a 5 μm breach in a variety of drug product filled vials and syringes. These methods are robust and have been successfully used to test numerous drug products on stability in many quality control labs. While it is true that some traditional CCIT dye methods do not meet current expectations, this work demonstrates that rather than focusing on the technique, emphasis should be placed on qualifying and validating an appropriate CCIT method, irrespective of the technology.

Analytical Method Development for Long-Acting Parenteral (LAP) Suspensions
Donna Carroll, Merck & Co., MS: R80T-B164, 126 E. Lincoln Ave., Rahway, NJ 07065, William Forrest, Claudia Neri, Kevin G. Reuter
Over the past decade, long-acting or extended release parenteral dosage forms have attracted extensive attention due to their ability to maintain therapeutic drug behavior. The development of both quality control and predictive analytical methods are not as straightforward as for more traditional dosage forms. Discussed herein are the results from the investigation into dissolution methods for LAPs which are formulated as crystalline suspensions or nano-suspensions. Several methodologies, including compendial United States Pharmacopeia (USP) II and USP IV dissolution with ad-hoc modifications, were evaluated for their potential to discriminate between suspensions with different quality attributes as well as their potential to help to predict the in-vivo behavior of LAP suspension formulations.

Analytical Method Evolution during Biological Product Life Cycle
Paula Lei, National Institutes of Health Vaccine Production Program, Department of Laboratory Development, 9 West Watkins Mill Rd., Room 162, Gaithersburg, MD 20878, KC Cheng, Dick Schwartz
Methods evolve as project evolves: Fit-for-purpose evaluation, analytical target profile establishment, method change in response of vendor changes, method bridging at different development stages, continuous method optimization and trending. Strategy for guideline of method life cycle management is important to help streamline product development, to provide quality measurements, and to deliver quality product. We present some case studies in establishing method life cycle management.

Use of Electrochemical Methods in the Assay of Critical Components in an Oxygen Sensitive Drug Product
Charles C. Van Kirk, Lantheus Medical Imaging, MS B200-1, 331 Treble Cove Rd., Billerica, MA 01862
In the pharmaceutical realm, various detection systems are available to characterize components of the final drug product. As such, an analytical chemist has a plethora of tools at their disposal when attempting to quantify the potency of an analyte of interest or its related degradation products. Such analyses are commonly performed using high-performance liquid chromatography (HPLC) separation coupled with ultraviolet, charged aerosol, or refractive index detectors. Another modality that should not be overlooked are the electrochemical detectors. Electrochemistry is a powerful, precise analytical tool that can be used to determine the mass of species to part per billion (or per trillion) scale. At its heart, the electrochemical detector is based on the fundamentals of electron charge transfer. This talk reviews the electrochemical behavior of a particular analyte (stannous) that was not amendable to quantification from spectroscopic techniques nor from the non-specific universal detectors. Instead its electrochemical nature (i.e., its oxidation-reduction potential) was used in establishing the quantitative control method. In order to establish the electrochemical and chemical behavior of the stannous analyte, two distinct analytical techniques were utilized. The first technique (cyclic voltammetry or CV) was used in a development fashion to determine the underlying mechanistic pathway of electrochemical and chemical processes. The second was a differential pulse technique (differential pulse anodic stripping voltammetry) which was developed and validated as an analytical control method. The validation of this method and the development approaches taken are summarized in the presentation.

Things You Don't “SEE” Everyday: Unique Trace Evidence
Richard S. Brown, MVA Scientific Consultants, 3300 Breckinridge Blvd., Ste. 400, Duluth, GA 30096
One of the fundamentals of trace evidence examination is the identification of small particles, especially small particles that would be considered unusual, uncommon or rare in a given situation. The first case involves a misguided bank robber who poured ketchup on his shirt, then wrapped his head with the shirt claiming he was injured. When staff came to his aid...he robbed the bank! We were tasked with determining if the stain was blood and if not, “what was it?” The second case involves the King brothers, convicted of killing their father with a baseball bat, then burning down the house. An investigator testified that lumps of grey metal found near the deceased were probably from the aluminum window frames. Our analysis showed that the melted lumps of metal were a 7000 series aluminum-zinc alloy used in sports equipment and for aircraft struts. The third case involved the Shannon Melendi kidnapping and murder in 1994 that was not prosecuted until 10 years later. MVA scientists (not the FBI as reported by the media) determined that trace metallic particles adhering to masking tape rolls found in the suspect’s car were consistent with materials, specifically super alloys, used in the manufacture and repair of jet engines. The suspect was a local jet engine repair facility. Finally, a brief look at Takata airbag death # 9. The current Takata airbag recall affects every driver. The steel airbag inflator, located on the steering wheel, can rupture sending metal parts through the driver.

TraceEvidenceApplications of Cold-Cathode Cathodoluminescence Microspectrophotometry
Jo Ann Buscaglia, FBI Laboratory, MS: CFSRU, 2501 Investigation Pkwy., Quantico, VA 22135, Christopher S. Palenik, Sarah A. Brokus, Danielle K. Silletti, Dyanne E. Carpenter, Dale K. Purcell, Graham F. Peasee
Cathodoluminescence (CL) microscopy and spectroscopy can aid in comparison, authentication, and provenance determinations of forensic geologic materials. The CL emission is characteristic of either the geological environment of formation of the mineral or, for a synthetic luminescent material, the manufacturing process. CL is observed in many materials routinely encountered as trace evidence, including sediment and rocks, building materials, glass, and polymers. CL is a relatively nondestructive method, which is particularly important for trace evidence analysis due to limited sample size and restrictions on the destruction, alteration, or consumption of evidence. Many of the most abundant minerals (e.g., quartz, feldspar, and carbonate minerals) are cathodoluminescent. The variation in luminescence within a given mineral type can provide additional source discrimination beyond commonly used methods and offers the potential for improving the significance of geological evidence and evidence containing geologically derived additives. Research has demonstrated that cold-cathode CL with light microscopy provides a relatively fast method to screen soil samples through visual identification of luminescent minerals, the ability to determine if multiple populations of a given mineral exist, and a means to estimate the relative abundances of luminescent minerals in a sample. Applications of CL microspectrophotometry with image processing for forensic trace evidence examination are presented. A novel application of CL spectroscopy to detect neutron irradiation damage as a potential nuclear forensics tool is introduced.
Rapid Shear of Garment Fibers – An Indication of a Ballistic Event

The phenomenon of rapid shear can be very helpful in distinguishing between mechanical damage and bullet passage in garments made from certain classes of synthetic fibers. When conventional methods for the detection of a bullet hole fail or are not applicable, such as the presence of propellant residue or the presence of bullet wiper surrounding the hole, a microscopic approach may be more fruitful. Microscopic examination of the fibers surrounding the suspected bullet hole, provided the garment is constructed with thermoplastic synthetic fibers, may provide useful information about the possible causes for the hole. The fibers can take on a melted appearance, which is due to the internal heating from the rapid passage of the bullet through the garment. This short duration event causes sufficient friction within the polymer bonds to create enough heat to melt the ends of those fibers that came in contact with the bullet. In addition to the bulbous appearance of the fiber ends, often visible with stereomicroscopy, examination with the polarized light microscope will reveal a decrease in retardation of the bulbous portion. The orderly polymer structure of the undamaged fiber shaft has a higher retardation than the disrupted orientation of the portion damaged by a ballistic event. Attempts at plugging objects by hand through garments made of thermoplastic synthetic fibers do not exhibit this phenomenon.

The Evolution of Forensic House Hold Dust Analysis during the Last Hundred Years

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In the late 19th Century, Hans Gross, a German magistrate, speculated in his writings that dust is a representation of our environment in miniature. Gross proposed that the constituents composing a particular dust sample could be used to help solve crimes. Gross’s writings inspired many a scientific detective study dust in criminal investigations. One such individual was Dr. Edmond Locard. Locard established a police laboratory in Lyon, France, where his ability to solve crimes by analyzing dust became known throughout the world. In his work, Locard showed that studying trace evidence in criminal investigations could be used to solve crimes. In the past few years, papers have been presented which demonstrate that household dust specimens may in fact be unique to a given location. Data collected in these studies was tabulated on a data sheet specifically designed for this study. The acquired data study was combined and subjected to rigorous statistical analysis. A number of interesting trends were found, extensively studied, and reported. Currently, a new study is underway that combines the data from the prior studies with the acquired human DNA data and new data collected from the mitochondrial DNA study of the human hair present in each dust specimen. It is believed that the combination of these different approaches will greatly enhance the discriminating power and the probabilistic value of household dust and well enabling one to not only identify a location, but also to identify its habitual occupant(s).

EAS & CoSMoS Method Development Olympics Competition Introduction

Karen Alasante, CoSMoS/Pfizer, Eastern Point Rd., Groton, CT 06340

An increasingly popular event yearly at the Conference on Small Molecule Science (CoSMoS) has been the Method Development Olympics. The program was initiated in 2011 and has evolved each year because a changing committee, often involving past winners, has lent its expertise to the next year’s challenge. This year’s committee consisted of Karen Alasante, Ruchi Mehta, Michael Roy and William Farrell, all of Pfizer, along with Kevin Gauger of Catalent Pharma Solutions. The committee designed a sample that met a variety of requirements: It had to be nontoxic, be able to be sent to candidates, and remain stable. And of course, the sample had to be intriguing for those keen to challenge their analytical expertise. In this session, we will tee up this year’s challenge. This year’s medalists will present their award winning research effort and we will conclude with the actual results with an inside look at this year’s challenge from the two lead designers, Ruchi Mehta and Michael Roy from Pfizer.

Presentation by the MDO Silver Medal Award Winner

Stephen Weber, University of Pittsburgh, Department of Chemistry, Pittsburgh, PA 15260, Stephen R. Groskreutz, Rachael Wilson, Klang Ngo, Michael Retrick

Solvent-based, on-column focusing is a simple, widely applied, and effective way to increase concentration sensitivity and reduce precolumn dispersion. While it can be practiced at any scale, it becomes more important as column size decreases. We have developed two separate approaches for improved focusing: one applicable to non-metal, capillary columns, and one to larger analytical- or microbore-scale columns. In the former case, we cool the head of a capillary column during injection to improve focusing. In the latter case, we use 1-mm ID precolumn in front of a 2.1 mm analytical column and it is the precolumn that is cooled during injection. The instrumentation required to do this is simple, robust, and very reproducible. We employ thermoelectric coolers (Peltier device, or TECs) and control their temperature with a simple LabVIEW program. We have developed a sound theoretical basis for understanding the use of temperature control on separations. This has allowed us to extend the idea to achieve things that are not possible, or that would be very complex, with solvent composition alone. Focusing can be achieved multiple times on a single capillary column if it has multiple, spatially separate cooling/heating zones. The approach has been applied to gradients as well as isocratic separations; to peptides as well as neurotransmitters (using reversed phase (RP)/RP-ion pair, resp.).

Capillary-Channeled Polymer (C-CP) Phases for Very High-Throughput Protein Analytics and Downstream Processing

R. Kenneth Marcus, Clemson University, Department of Chemistry, Clemson, SC 29634, Lwiwei Jiang, Hung K. Trang, Lei Wang

At the end of the day, the quality of any chromatographic separation comes down to simple kinetics and thermodynamics. The kinetics of the processes dictate the extent of band broadening as well as the overall throughput of the process. The thermodynamic aspects dictate the selectivity of the separation and the ultimate purity of the isolated components. In the case of protein separations, there is no such thing as “simple kinetics and thermodynamics,” as exceedingly slow diffusion-transport and multiphase surface interaction modalities greatly complicate the situation. This laboratory has developed capillary-channeled polymer (C-CP) fiber support/stationary phases explicitly for protein separations on both the analytical and preparative scales. The on-column arrangement of the fibers affects thousands of parallel channels of 1-5 microns diameter, which extend the length of the columns. High permeability and exceedingly efficient solute mass transfer couple to allow separations of viscous samples such as cell lysates at linear velocities of up to 100 mm/s. While the native fiber (polyester, polypropylene, and nylon 6) provide very diverse surface chemistries, far higher levels of selectivity and capacity can be affected through a number of simple modifications. We present our most recent efforts in combining the high mass transport efficiency and novel chemistries of C-CP phases for applications in analytical and preparative separations of proteins. While versatile as stand-alone columns, very early efforts in the use of C-CP fibers in the final stage of two-dimensional liquid chromatography (2D- LC) of proteins indicate a great deal of potential.

Sol-Gel Organic-Inorganic Hybrid Materials in Separation Science

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Sol-gel chemistry provides an effective pathway to material synthesis and offer new opportunities to chemically integrate organic and inorganic components, allowing facile creation of hybrid materials under remarkably mild reaction conditions. This attribute of sol-gel chemistry has been effectively exploited in the area of separations science by developing sol-gel stationary phases and microextraction media. The sol-gel chemistry allows the preparation of separation and extraction media in different formats (thin film, monolithic bed, particles, etc.) An important feature of the sol-gel approach is that the sol-gel stationary phases and microextraction media get chemically bonded to the substrate (e.g., fused silica capillary walls, solid-phase microextraction fiber surface, etc.) in the course of the in-situ creation. So far, silica-based sol-gel separation and extraction media have received most attention. However, silica-based materials inherently possess low stability under acidic and basic conditions. To address this issue, transition metal/metalloid-based sol-gel media have been developed. In this presentation, we provide an overview of our work in the area of sol-gel separation and microextraction media developed through hydrolytic as well as non-hydrolytic routes.

References:

131 Incursions into Material Chemistry for Chromatography
Luis A. Colón, University at Buffalo, State University of New York, Department of Chemistry, Natural Sciences Complex, Buffalo, NY 14260, Karina M. Tirado-González, Zuqin Xue, Amaris C. Borges-Muñoz, Joseph R. Ezzo, Josmely Vélez-González

Separation media for column technology in liquid phase separations depend on advances in material chemistry to provide stable and affordable chromatographic phases. The availability of the stationary phase can dictate, for example, the opportunity to tune selectivity by means of pH and/or temperature, which in turn can provide a wider range of capabilities when designing a separation method with a given stationary phase. Through the years, our research group has been investigating new approaches to chromatographic materials for liquid phase separations. This has led to the synthesis of stable hybrid silicas in the monolithic and particulate formats, studies of submicron hybrid particles for separations under ultra-high-pressure liquid chromatography (UHPLC) and capillary electrophchromatography modes, as well as exploring non-silica materials. In continuing our efforts to investigate new approaches to column chemistries for HPLC, more recently, we have attached layers of nanodiamonds on silica particles to study their chromatographic behavior. We have also synthesized a carbonaceous layer on the surface of superporous silica particles to study their chromatographic properties on these relatively new type of particles. After material characterization and chromatographic testing, these materials have shown their suitability for liquid phase separations. We take this opportunity to provide an overview of our incursions into material chemistry to produce chromatographic media. In particular, we focus on our recent silica modification approaches to produce chromatographic phases for liquid phase separations.

132 Potential Applicability of FPA-FTIR Spectroscopy for Rapid Identification of Foodborne Pathogens
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Focal plane array (FPA) transform infrared (FPI-FTIR) spectrometers are capable of acquiring several orders of magnitude more data than conventional spectroscopy necessitating the use of novel analysis techniques to exploit the information-rich nature of FPA detectors. These techniques are demonstrated in the context of bacteria identification by FPA-FTIR spectroscopy. Initially, an examination is made of the within-image and between-image fidelity of multiple FPA-FTIR instruments which demonstrates the high variability that is encountered with this technology (as compared to what is seen with conventional FTIR spectroscopy). This is followed by a description of the pixel filtration routines which allow for the extraction of the most relevant data from non-uniform samples. A genetic algorithm (GA) approach is introduced for determining the relevancy of spectral features and is compared to other forms of classifier optimizations. A proof-of-concept study is conducted on the potential for infrared imaging to detect samples having originated from a non-pure culture of bacteria and demonstrating feasible mixtures consisting of two types of bacteria. Finally, the overall methodology involving the combination of the methods, including additional approaches towards the development, maintenance, and validation of databases based on infrared imaging data are presented. This methodology is presented with an emphasis on accessibility by implementing the elements of an expert system which allows for this technology to be employed by a non-technical user.

133 Single- and Multi-Laboratory Evaluations of Mycotoxin Analysis in Foods by Liquid Chromatography-Mass Spectrometry
Kai Zhang, United States Food and Drug Administration, Center for Food Safety and Applied Nutrition, Office of Regulatory Science, MS: HFS-717, 5001 Campus Dr., College Park, MD 20740
United States Food and Drug Administration (FDA) laboratories have been using liquid chromatography ultraviolet (LC-UV)/fluorescence methods to monitor regulatory methods that targets aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, HT-2 toxin, T-2 toxin, and zearalenone in corn, peanut butter, and wheat flour. These newly validated methods can identify and quantitate mycotoxins in different matrices using a single sample preparation procedure and LC-MS analysis. If used for routine regulatory monitoring and surveillance, these methods could improve the efficiency of mycotoxin determinations. It is worth noting that there are still challenges related to LC-MS-based multi-mycotoxin analysis methods such as availability, stability, and traceability of the standards; automation of sample preparation; matrix effects; appropriate identification criteria; and harmonization of existing analytical procedures.

134 A GMO Testing Primer- Introduction to GMO Testing
W. Jeffrey Hurst, PO Box 872, Mt. Gretna, PA 17064
Recently the United States Congress massed a genetically modified organism (GMO) Labeling Bill that will be implemented over the next few years. This development and others throughout the world point to the increasing importance of GMO testing. This presentation provides an introduction to GMOs, types of testing including protein and DNA. Finally it also describes the issues in setting up and running such a laboratory.

135 Gluten Testing: Variables and Standardization
Q. Julia Zhao, Bowen Bioscience, LLC, 4 California Ave., Framingham, MA 01701
Accurate and sensitive gluten detection is of utmost important for gluten sensitive individuals and food industry. Gluten is a complex protein found in wheat, rye, barley, and oats. Celiac disease (CD) is an autoimmune enteropathy triggered by gluten; wheat allergy an allergic reaction of the immune system to gluten; and wheat indolence a reaction of gastrointestinal system to gluten. Following Codex Alimentarius’ recommendation, the United States Food and Drug Administration (FDA) passed a final rule in 2013 and set the limit of food with less than 20 ppm of gluten be labeled as “gluten-free.” However, studies found over 20% foods labeled as “gluten free” in the supermarkets of the United States contain higher than 20 ppm gluten. Unexpected presence of gluten in these foods pose serious health risks to wheat-allergic and celiac patients. The purpose of the study is to analyze issues that may affect gluten testing with the mostly employed enzyme-linked immunosorbent assay (ELISA) method from the perspective of independent testing agency. Several grains: wheat, rye, barley, oats and corn, were used to evaluate hydrolysis efficiency. Gliadin/gluten of different grains react differently to the same extraction and hydrolysis methods. Differences in grading level and fineness of samples create great differences in extraction efficiency. Extraction reagents, heating temperature, reference materials, sensitivity and specificity of antibodies used, different kits from commercial available vendors are all areas of variations. This talk intends to address the major challenges in gluten detection, including gluten extraction, standardization, antibody development, comparison of current and emerging detection methods, and their practical applications.

136 Development and Validation of a Cleanability Test Device
Jing Mi, Center for Pharmaceutical Cleaning Innovation, Bldg 1, Unit 8, Hillsborough, NJ 08844, Andrew Walsh
This presentation reviews the development of an automated, high throughput device for measuring the cleanability of compounds or products and the validation of a method for it using Gage R&R and design of experiments. The device’s application to pharmaceutical cleaning validation for cleaning agent selection, determining the “time-to-clean” and optimizing cleaning procedures using statistical techniques are discussed.

137 Modified SDS-PAGE for Protein Degradation Studies
Dylan Wang, Dartmouth College, 12 Ralston Ln., Lebanon, NH 03784
This talk presents a quantitative method to quantify the degradation of biopharma- ceutical protein products during the cleaning process. With the use of this method, the degradation of proteins can be measured and proven instead of assumed. Based on the existing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method, we have developed a highly accurate, low cost and time saving technique to analyze protein degradation. The time of exposure, degradation concentration and temperature can be accurately controlled to mimic cleaning pro- cesses. Furthermore, with the design of experiments (DOE) platform, the presentation will statistically analyze the importance of each cleaning factor as well as their interactions, providing additional information to help manufacturers optimize their cleaning processes.

138 Application of At-Line TOC - Case Study
Andrew Walsh, Center for Pharmaceutical Cleaning Innovation, 2 Ilene Ct., Bldg. 1, Unit 4, Hillsborough, NJ 08844
This presentation reviews total organic carbon (TOC) analysis for use in pharma- ceutical cleaning validation and how it can be used as an “at-line” process analytical
technology (PAT) application. The time and cost savings using this approach are presented through a value stream map.

139 Cleaning Limits and Visual Inspection from an Analytical Perspectives
Mariani Neverovitch, Bristol-Myers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903, Antonio Fernandez and Elizabeth McMonigal
Visual inspection following equipment cleaning is a mandatory step in the cleaning verification workflow for pharmaceutical equipment. Equipment must pass visual inspection before swab sampling for analysis can be performed. However, since a significant number of low risk compounds are visible well below established safety levels, it is possible to justify equipment as “visually clean” without performing swabbing analysis. Internal studies performed at Bristol-Myers Squibb showed that over 90% of participants could identify residual product at a level of ~2 ppm without preliminary training. The implementation of a robust visual inspection qualification program and clear “Visually Clean” inspection parameters can enable visual inspec-
tion to be used to qualify equipment in lieu of swab analysis for low risk products.

140 A Review of the Testing and Development of Incralac Lacquer
Rosie A. Grayburn, Getty Conservation Institute, 1200 Getty Centre Dr., Ste. 700, Los Angeles, CA 90049, Julie Wolfe
Incralac was developed by the International Copper Development Association (IN-CRA) in the early 1960s as a clear coating for copper alloys and it remains to this day a common coating for outdoor sculpture. However, since its initial development the formulation has undergone many changes, most recently to adapt to environ-
mental regulations, and reports of its performance are inconsistent. In collaboration with sculpture conservators at the J. Paul Getty Museum a comprehensive review of the history of INCRALAC development, resulting formulations and more recent weathering studies has been conducted. This detailed study of previous formula-
tions has also allowed the deconstruction of the recipe in order to replicate Incralac with less toxic solvents. A wide-ranging analytical study was performed to compare Incralac with home-made imitations, which showed that the home-made mixtures perform comparably with the proprietary product. This research has shown that Inc-
ralac is still a viable option for outdoor sculpture conservation. The role of various components in the coating has been elucidated and it has been demonstrated just how it is possible for conservators to take control over the coating formulation, and prepare the lacquer in-house.

141 Reversible Aqueous Coatings for Outdoor Painted Surfaces
Anthony F. Lagalante, Villanova University, Department of Chemistry, Villanova, PA 19085, Richard C. Wolbers
The engineering of appropriate protective coatings for public art is especially chal-
lenging given the need for reversibility from extremely sensitive surfaces. Murals created from acrylic paints for instance are sensitive to both aqueous and solvent systems that would be normally be necessary for application or removal of highly weather resistant protective coatings on general building or monument surfaces. In particular, in the city of Philadelphia, approximately 3500 building-sized murals have been created under the auspices of the Mural Arts Program from commercially available water-based acrylics. The wide range of building surfaces types for these murals further challenge the engineering of an appropriate protective coating sys-
tem. High moisture vapor transmission rate (MVTR) and mechanical and physical properties that match both artist’s paints and building substrates alike are critical design factors. City, state and federal requirements for increasingly lower volatile organic compound (VOC) application and removal methods generally dictate that any coating be applied and removed by aqueous methods. While many protec-
tive coatings presently exist in water dispersions for application, few, if any, can be safely removed without solvent use and severe mechanical damage to the artist’s paints. Our efforts have developed a coating system of exceptionally high durability, but one that can be both a “water-on” and “water-off” type of system over artist’s acrylic paints in particular. The coating represents a novel blending of stable acrylic and poly acrylic acid monomers to tailor the physical and chemical properties of the coating that includes MVTR and pH reversibility.

142 Coatings: Art and Science: The Formulation of Low Solar Absorbing Polyurethane Coatings for Outdoor Sculptures
John A. Escarsega, United States Army Research Laboratory, 4600 Deer Creek Loop, APG, MD 21005
This work highlights the formulation and development of low gloss coatings, coatings used not only on military assets, yet also used to paint Alexander Calder, Tony Smith and Louise Nevelson sculptures. For the sculptures the coatings are being formulated to enhanced durability while maintaining and preserving the original intent of the artist in regards to aesthetics. While the DOD drivers are sup-
porting survivability, environmental and application elements as well as durability. Unique aspects that are being implemented into the coatings are the use of low molecular weight resins compared to current resin systems, this enables the reduc-
tion of solvents and permits easier application due to less viscous properties with the resins themselves. The incorporation of low solar absorbing pigmentation into the coatings, these pigments are near equivalent visually to existing pigmentation, though provide a higher reflection. This composition “shields” the resin/binder sys-
tem from potentially harmful photons that typically degrade the polymer system or the coating itself. The hopeful outcome and results of this research effort will be a unique coating system that will provide enhanced durability and maintain the original coating traits for an extended period of years for all users. Numerous works of art have already been coated with the first generation coating and this paper introduces the next generation of coatings for both the DOD and our National Treasures of Art.

143 Art & Industry: A Collaborative Approach to Solving Technical Challenges in the Field of Art Conservation
Melinda H. Keefe, The Dow Chemical Company, 3140 Woodland Rd., Willow Grove, PA 19090, Bronwyn Ormsby, Tom Learner, Alan Phenix
This presentation reviews the technical collaboration between The Dow Chemical Company and the art conservation community designed to leverage industrial coating expertise and resources to solve technical challenges in the field of art con-
servation. This journey began with a three-way collaborative effort between Dow, Tate, and the Getty Conservation Institute focused on identifying improved cleaning systems for unvarnished modern painted surfaces. Over the years this effort has expanded to address other technical challenges in the field of art conservation. This talk reviews this unique partnership highlighting select areas of research.

144 Application of PAT on a Pharmaceutical Coating Process: Prediction of Coating Thickness Using In-Line Spectroscopic Techniques
James K. Drennen III, Duquesne University, 600 Forbes Ave., Pittsburgh, PA 15282, Hanzhou Feng, Yuxiang Zhao, Carl A. Anderson
Coating thickness is a critical quality attribute governing drug release and thera-
peutic performance of pharmaceutical dosage forms. It is necessary to develop an effective quality control approach to ensure the desired coating thickness has been achieved in a coating process. In recent years, process analytical technologies and associated multivariate modeling strategies have been used to predict the coating thickness in real time. However, the variability from incoming materials and manu-
facturing process, such as particle size, shape or moisture content, may affect the performance of the developed analytical models. In this work, the impact of variabil-
ity in particle size on the coating thickness model was investigated. The objective was to develop an efficient and robust calibration model that provides accurate pre-
diction of coating thickness in a dynamic coating process.

145 Process Analytical Technology Methods as Part of a Comprehensive Control Strategy for Pharmaceutical Manufacturing
Kirby Amsopah-Chan, GlaxoSmithKline, 709 Swedeland Rd., King of Prussia, PA 19406
Process analytical technology (PAT) applications continue to increase for both phar-
aceutical drug substance and drug product development and manufacturing. In GlaxoSmithKline (GSK), the deployment of PAT methods is based on the expec-
tation that the information and knowledge gained from development studies and manufacturing processes can provide scientific understanding to support the es-
tablishment of the device specifications, and manufacturing controls. In this presentation, some PAT applications to drug product manufacturing within GSK are presented. The use of PAT as part of a comprehensive control strategy to support key manufacturing activities from the pilot plants to the commercial manufactur-
facilities is discussed. Specifically, an approach to better understand process flow through different unit operations encompassing granulation, drying and milling, blending, compression and film coating are highlighted. Technical, operational and administrative challenges of deploying PAT methods to support drug product manu-
facturing are emphasized.

146 Fit-For-Purpose Methods to Support Drug Product Design
Gary McGeorge, Bristol-Myers Squibb, MS: 109-A158K, 1 Squibb Dr., New Brunswick, NJ 08903, Boyong Wan, Dongsheng Bu
The landscape to deliver quality medicines to patients has never been more competitive and significant pressure is on innovators to develop medicines as rapidly as feasible. While some of the time is defined by regulatory requirements and clinical studies, significant time is still take to design robust formulations and effective and efficient processes. Spectroscopic methods are used throughout development to support a variety of applications, but are often under applied due to perceptions that only the most accurate, precise methods are suitable. This presentation presents ideas and options on how to leverage alternate spectroscopic methods from the early stages of developing a commercial dosage form through to filing a registrational method. Examples include discussions around typical tablet manufacturing; for blend analy-
is, ribbon attribute and tablet measurements.
For the preparation of spray-dried solid dispersions, it is important to ensure the complete dissolution of all components (i.e., active pharmaceutical ingredient (API), polymer, and compatibilizers if used) prior to initiating the spraying process. Complete dissolution of both API and compatibilizers, such as surfactants, is required to mitigate risks associated with phase separation, both at time of release and on storage. A near-infrared (NIR) method was developed to monitor dissolution endpoint for these components in the mixed solvent system prior to spray drying. This in-line method obviates the need for offline sampling to establish the minimum mixing time as part of the control strategy. In addition NIR was employed to determine the end of spray dried intermediate (SDI) drying in the convective secondary drying process (post spray drying). A NIR method can be used to detect −residual solvent and moisture, which are in-process controls (ICPs) on the spray dried intermediate. This NIR method for secondary drying was also utilized to optimize a convective drying process and utilized to study the effect of agitation rate, jacket temperature, and drying gas (N2) sweep rate on drying cycle time. The online NIR methods have demonstrated and utilized to study the effect of agitation rate, jacket temperature, and drying gas (N2) sweep rate on drying cycle time. The online NIR methods have demonstrated the utility to call the endpoint of secondary drying, minimizing off-line sample handling and analytical testing.

As the United States Pharmacopeia and International Conference on Harmonization prepare to finalize chapters that dictate testing standards for elements of toxicological relevance, collectively termed Elemental Impurities, a great deal of emphasis has been focused on the various atomic spectroscopy techniques that will be employed to satisfy these impending regulations. Certainly, the low threshold limits that must be demonstrated for toxic elements such as arsenic, cadmium, mercury and lead will require pharmaceutical atomic spectroscopy laboratories to invest in and develop various technologies with which they may have limited experience, including inductively coupled plasma mass spectrometry (ICP-MS) and ICP- atomic emission spectroscopy (AES). However, the utility of such instrumentation, as well as those that contribute to a comprehensive atomic spectroscopy toolbox (Laser ablation-ICP-MS (LA-ICP-MS), X-ray florescence, laser-induced breakdown spectroscopy (LIBS)) is not relegated to just Elemental Impurities. Indeed, data generated from atomic spectroscopy methods can be used to support various stages of the entire pharmaceutical development process, from early stage research development, to clinical scale up and finally throughout life-cycle management. The atomic spectroscopy lab at Bristol-Myers Squibb has a long history of employing a wide variety of techniques to provide knowledge that impacts numerous stages of development. While often referred to as the ‘metals lab,’ this label does not adequately describe the capabilities, including comprehensive studies in vessel/equipment leaching (including glass, plastic and solder), forensic foreign matter investigations, raw material comparisons, equipment and consumer integrity assessments and expedient formulation homogeneity.

Using Single Particle ICP-MS and Capillary Electrophoresis-ICP-MS

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Single particle inductively coupled plasma mass spectrometry (sp-ICP-MS) can provide rapid (< 3 minutes/sample) measurement of nanoparticle size distributions and number concentration. In sp-ICP-MS, signals (200 to 1000 µs wide) from individual nanoparticles are detected and measured. sp-ICP-MS can provide element specific detection (using a quadrupole or sector field MS) or major element composition of each nanoparticle (using a time of flight MS). The number of signal peaks/s depends on the number of nanoparticles per mL while the signal intensity depends on the mass of the element detected in each nanoparticle. We discuss the calibration of nanoparticle number concentration and nanoparticle mass (size) using nanoparticle standards or solution standards and potential sources of error. The minimum detectable nanoparticle size (typically >10 to 20 nm for metal nanoparticles) depends on the mass of the analyte element in the nanoparticle, the ion transmission efficiency from the ICP to the MS detector and signal due to dissolved analyte and any spectral overlap ions. Surfactant assisted capillary electrophoresis-ICP-MS (CE-ICP-MS) can separate nanoparticles based on the charge on nanoparticle/surfactant assemblies in order to measure nanoparticle size distributions and number concentrations. Ideally, the number of surfactant molecules associated with each nanoparticle depends linearly on the nanoparticle radius. Because many nanoparticles of the same size enter the plasma simultaneously, CE-ICP-MS can measure smaller nanoparticles (< 5 nm) than sp-ICP-MS. We discuss the capabilities of CE-ICP-MS and variables, such as the nanoparticle surface chemistry, that could affect the number of surfactant molecules associated with each nanoparticle.

An Assessment of the Model Used to Estimate the Origin of Radial Spatter Patterns

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The bloodstain pattern analysis (BPA) model used to estimate the trajectory of an airborne drop from its resultant stain dimensions is currently based on the oblique projection of a sphere. This model is used to estimate the incident angle of a blood drop trajectory, by calculating the arcossine of the width-to-length ratio of an elliptical bloodstain. Despite using various geometric and mathematical principles, inaccuracies in the calculated result arise from a number of assumptions. With modern investments in computer software designed for BPA which boost higher accuracy in these estimates, it is important to determine if such investments are justified, particularly since many are based on the same underlying mathematical model. This project aims to assess the accuracy and precision of the model as well as to determine if the error margin is significant. Additionally, it seeks to validate the claim that computer-generated calculations provide better estimates. Bloodstains of fresh defibrinated ovine blood were created on white, letter-size copy paper as well as various glass plates using varying heights, gauge sizes, and incidence angles. Resultant stains were measured in two ways: physically and using Microsoft® Excel to find the best-fit ellipse as described in other studies. The BPA model was then applied to the measurements to obtain the estimated incident angle. Results of both measurement techniques were compared to actual incident angles using a two sample t-test to assess if differences were statistically significant.
153 Using APCI-MS-MS and Syringe Liquid Injection for the Screening of Ignitable Liquids in Fire Debris Samples
Clare Fried, Cedar Crest College, 100 College Dr., Allentown, PA 18104, Thomas Pritchett, Thomas Brettell
An atmospheric pressure chemical ionization tandem mass spectrometry (APCI-MS/MS) method has been developed to screen fire debris samples for ignitable liquids. A carbon disulfide extract was injected into the source of a tandem mass spectrometer, incorporating methylene chloride, gasoline, dichloromethane, and APCI solvent. The use of a high-intensity, tunable-wavelength light source may excite some GSR resulting in fluorescence, thereby increasing contrast between the fur and GSR. Infrared (IR) light can also be used to enhance GSR patterns and radiography can be employed to detect the presence of radiopaque metallic particles. Once all methods of visual enhancement are complete, the Modified Griess test (MGT) can be used to detect the presence of nitriles. Cow and rabbit hides were shot from a range of distances with jacketed and unjacketed ammunition using multiple handguns. Visualization with white light and IR light shows patterns increasing in size as muzzle-to-target distance increases. In some instances, the presence of a radiopaque ring, presumably metallic lead, around the entrance hole was detected with radiography. As expected, both observable features begin to fade with increasing muzzle-to-target distance. The Modified Griess Test was conducted using filter paper rather than photographic paper. Results of the MGT are consistent with the visual methods, as the patterns increase in size and become more discernible with increased firing distance.

154 A Three-Year Study of Synthetic Cannabinoid Formulations in Pennsylvania
Kacee J Rizzo, West Chester University of PA, Department of Chemistry, 700 S High St., West Chester, PA 19383, Monica Joshi, David Scott VanGorder
In recent years, a class of synthetic substances purported to mimic the effects of naturally occurring cannabinoids have become very prominent. These substances are collectively called synthetic cannabinoids and have been the bulk of the novel psychoactive substances seized in correctional facilities across Pennsylvania. Their effects are severe, dangerous and unpredictable. This presentation covers a comprehensive study of over 250 confiscated synthetic cannabinoid formulations. Trends in synthetic cannabinoids in the various formulations were determined by studying the extracts of the herbal materials by gas chromatography-mass spectrometry (GC-MS). The results demonstrate the extent of changes in chemical structure that have happened in rapid succession. Preventing the use and entry of these substances into correctional facilities is vital to maintaining the safety of inmates, visitors and correctional personnel. In this presentation we discuss the development and validation of an ion mobility spectrometry (IMS) method to detect contact traces of synthetic cannabinoids. Canines have been used successfully for many years to detect concealed drugs. However, there are very few vapor phase studies conducted for synthetic cannabinoids. This presentation discusses the headspace analysis of different formulations using solid phase microextraction (SPME) to aid in canine training for detection of concealed synthetic cannabinoids. All these studies have significantly improved the efforts to stem the spread and application of an ion mobility spectrometry (IMS) method to detect contact traces of synthetic cannabinoids. All these studies have significantly improved the efforts to stem the spread and use of these substances into correctional facilities.

155 Stability of Synthetic Cathinones in Biological and Non-Biological Matrices
Heather L. Ciallella, Arcadia University, 450 S. Easton Rd., Glenside, PA 19038, Lorna A. Nisbet, Karen S. Scott
Synthetic cathinones, current designer alternatives to stimulants such as amphetamines, have chemical structures derived from cathinone from the shrub Khat (Catha edulis) found in the Middle East and East Africa. Although restrictions exist in some countries, they persist in forensic casework. Therefore, it is critical to understand their stabilities in both working solutions and biological matrices as concentrations detected will impact any subsequent interpretations. This research investigated the stability of mephedrone and naphyrone, two Schedule I synthetic cathinones, in methanol, acetonitrile, human blood and urine, at three storage temperatures to assist in the interpretation of toxicological samples. Solutions (1 mL/L) of each drug in each of the four matrices were stored in aliquots (100 µL, 1.2 mL, blood and urine) at 21 °C (room temperature), 4 °C (fridge), and -20 °C (freezer) before undergoing solid phase extraction and gas chromatography-mass spectrometry analysis on days 0, 3, 7, 14, and 30. Results show that mephedrone and naphyrone are unstable in all four matrices depending on the storage temperatures. Biological and solvent samples containing mephedrone and naphyrone showed the least degradation when stored at -20 °C, indicating that freezer storage of samples discourages degradation. However, even at this temperature, there is over a 20% loss of mephedrone in methanol and human blood over a 30-day period. This data emphasizes the need to process samples suspected to contain mephedrone as quickly as possible.

156 Simultaneous Quantitative Analysis of Prescription Opioids and Cannabis in Wastewater Samples
Alethea Jacox, CUNY John Jay College of Criminal Justice, 2910 Wallace Ave., Apt. 4F, Bronx, NY 10467, Jillian Wetzel, Shu-Yuan Cheng, Marta Concheiro-Guisan
Wastewater-based epidemiology is an innovative and promising approach that provides information about exposure to drugs in defined population groups by the analysis of human excretion products in wastewater. Marijuana is the most popular illegal drug in the United States, and the misuse of prescription opioids has increased dramatically in recent years. We developed and validated an analytical methodology for the simultaneous determination of prescription opioids (morphine, oxycodone, hydrocodone, oxymorphone, and hydromorphone), and delta-9-tetrahydrocannabinol (THC), and its metabolites, 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH) and 11-nor-9-carboxy-tetrahydrocannabinol-glucuronide (THCCOOH-Glu) in wastewater samples by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) with dual atmospheric pressure-electrospray ionization (API-ESI) source. The samples were cleaned by filtration and cation exchange solid phase extraction. Method validation included linearity (5-1,000 ng/mL for opioids, and 10-1000 ng/mL for THC and its metabolites), imprecision (<20%), accuracy (80-120%), and effect (from -30 to 7% for opioids, from -8 to 15% for cannabinoids), and extraction efficiency (75-84% for opioids, 25-67% for cannabinoinds), limit of detection (1.5 ng/mL) and quantification (5-10 ng/mL), interferences, and auto-sampler stability (no loss detected). River and wastewater samples were collected in triplicate from locations in New York City into Environmental Protection Agency certified sample containers and stored at -20 °C until analysis. Water from the sewage overflow location tested positive for morphine 10.7 ng/mL and oxycodone.
5 ng/L, and wastewater samples tested positive for morphine, hydrocodone and THC/COO in concentrations between 6–1,526 ng/L. We developed a sensitive and specific method for the simultaneous determination of prescription opioids and marijuana in wastewater samples.

159 Where is the Charge Located in Multifunctional Gaseous Ions? Andrew B. Attygalle, Stevens Institute of Technology, Department of Chemistry, Hoboken, NJ 07030

The preferred charge sites of protonated or deprotonated molecules in the gas phase has been a topic of extensive research. In fact, the exact location of the initial charge site of the precursor ion is requisite that should be addressed before any pragmatic interpretation of a fragmentation spectrum is attempted. Although the challenge appears seemingly simple, in reality it is not at all a trivial problem. One often assumes that the charge location can be independently assigned by analyzing the gas-phase acidity or basicity of various groups present in a polyfunctional molecule. However, in reality this a very challenging problem because generalizations valid for solution-based chemistry cannot be extended directly to gas-phase phenomena. For example, there is sufficient experimental evidence to demonstrate that deprotonated p-hydroxybenzoic acid exists in gas phase as a mixture of carboxylate and phenoxide forms. Analogously, protonated aniline exists as mixture of nitrogen- or ring-protonated forms. Ion-mobility mass spectrometry (IM-MS) provides a way to determine relative population ratios of protoners or deprotoners that coexist under mass spectrometric ion generation conditions. Employing IM-MS separation, we demonstrate that mass spectrometric source conditions used for gas-phase ion generation play an important role on the relative ratios of isomeric protoners and deprotoners that coexist under a specific set of experimental conditions.

160 The Use of HRMS for Antibody Drug Conjugate Payload Quantification and Characterization in Biological Samples Joseph Tweed, Pfizer, Eastern Point Rd., Groton, CT 06340

No abstract submitted by the author.

161 Effect of Flow Rate on Column Re-Equilibration after Gradient Elution for One- and Two-Dimensional Liquid Chromatography Joe P. Foley, Drexel University, Department of Chemistry, 3141 Chestnut St., Philadelphia, PA 19104, Michael R. Fletcher

In ultra- and high-performance liquid chromatography (U/HPLC), column re-equilibration (CR) after an analysis performed with gradient elution is necessary to prepare a column for subsequent experiments. During CR the final mobile phase in the column is replaced with the initial mobile phase for the next separation. The replacement of mobile phase occurs in four regions within the column: 1) between the particles (intraparticle); 2) in the ‘bulk’ volume within the pores of the particles (intraparticle); 3) in the interfacial region between the mobile phase and stationary phase; and 4) between the chains/ligands of the bonded phase. We hypothesize that, with typical flow rates, the rate of column re-equilibration is limited by the rate of diffusive mass transfer within the latter three regions (2-4). Under such rate-limiting conditions, the volume of mobile phase required for CR can be expected to increase linearly with increasing flow rate. A related prediction is that at lower flow rates where diffusive mass transfer (steps 2-4) is no longer rate-limiting, the volume of mobile phase required for CR will reach a minimum value that is independent of flow rate. To test this hypothesis we conducted experiments with MeOH/H2O and ACN/H2O mobile phases and numerous bonded-phase columns (C18, C8, PFP, etc.) over a range of flow rates from fast (e.g., 0.40 mL/min for a 2.1-mm i.d. column) to very slow (0.02 mL/min). The results we obtained strongly supported our hypothesis, i.e., both predictions were confirmed. Other observations (TBD) can also be explained by our kinetic model.

162 Understanding the Impact of Pore Exclusion on Reversed-Phase HPLC Column Performance Richard A. Henry, Consultant, 983 Greenbriar Dr., State College, PA 16801, Stephanie A. Schuster

Porous and superficially-porous silica gels have become the workhorse particles in high-performance liquid chromatography (HPLC) column methods. It has become increasingly important in bioseparations to understand how pore geometry can impact column performance if molecules become sufficiently large to experience partial or complete exclusion from the particle interior. Modern silica particles for HPLC typically exhibit a broad distribution of pores, which have been divided (International Union of Pure and Applied Chemistry) into categories: 1) micropores of <2 nm; 2) mesopores of 2-50 nm, and 3) macropores of >50 nm. Small molecule separations normally take place in the silica mesopore region (20-500 Å) because it contains the vast majority of bonded stationary phase. Molecules up to about 20 Å in diameter may be assumed to have full access to the mesopore region and stationary phase. Above 20 Å, however, solutes may experience partial or complete exclusion from pores and deliver unsatisfactory separation. It may therefore be important to have accurate knowledge about particle pore geometry before including any HPLC column in a modern method for biomolecules. Guidelines will be offered for selecting columns with pores that are large enough to retain and resolve all solutes that may be present in the sample. Sources for obtaining solute size data will be discussed. Performing size exclusion chromatography (SEC) during HPLC method development may be useful for gaining critical knowledge about size of unknown solutes. While examples will focus on silica reversed-phase columns, the principles can be applied to other HPLC retention modes such as hydrophilic interaction liquid chromatography (HLIC), ion-exchange, and others.

163 Factors Affecting Selectivity of Solid-Core Particles Lavelay O. Kizekai, Waters, 5 Technology Dr., Milford, MA 01757, Bonnie Alden, Cheryl Boisiel, Babajide Okanadeji, Jacob Fairchild

With the exceptionally broad applicability of reversed-phase chromatography, it can be challenging to choose a column with the required selectivity for a new separation method. As the use of solid-core particle packed columns continues to grow, the range of stationary phases available on those particles has also grown to provide a range of chromatographic selectivities. In this presentation, we relate the stationary phase and mobile phase properties to describe the tool set for driving separations. The chemical properties and surface concentration of the stationary phase are important factors creating specific interactions and determining the mobile phase compatibility. The use of ionic ligands, aromatic moieties and careful choice of surfactant concentration have been used to alter selectivity. We found that methanol when used with phenyl bonded phases can drive pi-pi interactions, while optimization of the C18 surface concentration and particle pore diameter will achieve sufficient and stable retention in highly aqueous (>90% aqueous) mobile phases. These concepts as well as a brief description of the significant improvements offered by solid-core particles to both column efficiency and back pressure are discussed. Ultimately, selectivity is necessary to achieve a separation, which is afforded by the stationary phase chemical properties and the chosen chromatographic conditions.


Systematic approaches to chromatographic method development with emphasis on knowledge gathering and method control provide robust separation methods with fewer analysis failures. The chromatographic modeling tools capable of multi-factor optimization can reduce the amount of experimental work needed and simultaneously deliver globally optimum method conditions. This work presents the development of a robust ultra-performance liquid chromatography (UPLC) method using multi-factorial optimization approaches for analyzing the purity and potency of a pharmaceutical dosage form. Commercially available chromatography modeling software (such as DryLab, Design Expert) were employed to determine the final method conditions and evaluate the method robustness.

165 Hydrophilic Interaction Liquid Chromatography: Comparison of Selectivity Differences Observed on Polar Silica and Zwitterionic Stationary Phases Daniel Shollenberger, MilliPoreSigma, 595 N. Harrison Rd., Bellefonte, PA 16823, Dave Bell, Patrik Appelblad, Craig Aurand

Hydrophilic interaction liquid chromatography (HILIC) is a unique system that involves a variety of molecular interactions including hydrophilic partitioning, polar, and ion-exchange interactions. There has been increased interest in this chromatographic mode given the complementary nature to reversed-phase analysis and compatibility with mass spectrometry. Given the growing number of stationary phase chemical properties and the chosen chromatographic conditions. Both polar silaceous based phases and zwitterionic chemistries are evaluated. Fundamental studies of selectivity are used to provide practical insight for method development and the use of mass spectrometry for quantitative and structural elucidation.

166 Wide Pore Monolithic Silica of Various Functionalization: Protein A, C18, C8 and CN, in High-Performance Liquid Chromatography (HPLC) for Large Molecule Separations Egidius Machtjevases, Merck KGaA, Frankfurt St 250, Darnstadt 63182, Germany

In the last years the pharmaceutical market has change dramatically from small molecules to protein-based drugs. The development of biological entities is strong increasing because biotechnological processes now enable a production with reasonable costs. That implies a demand of suitable analytical methods for process monitoring and quality control of biomolecules with therapeutic purposes. Especially the HPLC is the mostly used analysis method. Most important for the HPLC analysis are the properties of the column. For bigger peptides, proteins or antibodies a new type of columns is needed which provide a good permeability, better mass transfer
and a better selectivity. In contrast to conventional packed-column columns, wide pore (300 Å) monolithic silica columns are made of a single continuous-bed rod of high purity porous silica that is then bonded with C18, C8, CN and Protein A. Monolithic columns remove backpressure as the primary consideration in method development and give back the flexibility of choices in flow rates for much higher throughput. Because they have no individual particles to shift or break, column performance is very consistent over much longer lifetimes, making them ideal for relatively "dirty/matrix rich" sample analysis. Their high permeability also makes them very forgiving of shortcuts and time saving in sample preparation as well as easier to aggressively flush out to re-equilibrate. This presentation guides you through the world of wide pore monolithic silica materials. Benefits are demonstrated with many application examples including pharmaceutical and bioanalysis separations (proteins, antibodies, etc.), calibration curves, recovery calculations, and method robustness overviews.

167 Stable Isotope Ratio Mass Spectrometry: An Old Technology Gets a 21st Century Upgrade
Arthur Kasson, Elemental Americas Inc., 520 Fellowship Rd., Mount Laurel, NJ 08054

Isotope ratio mass spectrometers (IRMS) were developed in the 1950s and were utilized mainly for geological and geochemistry applications for 30+ years. In the 1980s, with the development of continuous flow technologies or the ability to interface inlets like elemental analyzers and gas chromatographs to the IRMS, the range of applications were expanded to include environmental and ecological measurements. Here in 2016, we have seen this technology expand once again to research across a wide range of applications including forensic, pharmaceutical, biomedical, and food testing. The technology is no longer limited to university research and government agencies, but is now being widely used by several companies who benefit from the technology. These expansions involve smaller, more flexible instruments with the capacity to give high precision measurements on varying sample matrices and the development of software to operate the instrument configuration and process data reliably. Additionally, these instruments are now equipped with smart features that reduce consumption of operating gases and consumables, perform functions that were historically done by a laboratory operator or manager, and reproduce data at an alarmingly effective speed. This has significantly reduced operator hours spent on these tedious tasks and has made stable isotope analysis into a turnkey way of performing mass spectrometry across several avenues of research and testing.

168 Mass Spectrometry-Based Protein Biomarker Discovery in Autism Spectrum Disorder (ASD)
Kelly L. Wormwood, Clarkson University, 8 Clarkson Ave., Box 5810, Potsdam, NY 13699, Laci Charette, Jeanne P. Ryan, Alisa G. Woods, Costel C. Darie

ASD affects approximately 1/68 children in the United States and is characterized by repetitive behaviors, communication deficits and impairments in social interactions. Males are affected approximately 4.5 times more than females and diagnosis typically occurs when a child is about 3 years old. There is currently no biologically based diagnosis or clear, consistent cause of ASD. There are also no approved medications that treat any of the core symptoms of ASD. Here, saliva samples from people with ASD and matched controls were analyzed using a combination of gel electrophoresis (SDS-PAGE), in gel digestion or in solution digestion and nanoliquid chromatography-tandem mass spectrometry (nanoLC-MS/MS) to investigate differences between the proteomes of people with ASD and matched controls. Several protein differences were identified between ASD and control samples. These differences may lead to potential biomarkers for diagnosis, possible therapeutic targets and an altogether better understanding of the disorder.

169 Analytical Challenges in the Detection and Quantitation of Asbestos in Non-Building Materials from an International Perspective: A Case Study
Shane G. Cone, International Asbestos Testing Laboratory, 9000 Commerce Pkwy, Ste. B, Mount Laurel, NJ 08054, Thomas Barkley

In the United States, bulk asbestos analytical methods center around the use of polarized light microscopy (PLM) and transmission electron microscopy (TEM) for identifying and quantifying 6 regulated asbestiform minerals in building materials at or below a concentration of 1%. Despite the extensive use of these asbestos analytical methods in US laboratories, standard methodology is lacking for the full range of non-building materials that the average asbestos testing laboratory may encounter. In this case study, we discuss preparatory and analytical challenges as they pertain to the analysis of vehicle brake pads as well as other industrial machinery materials. Compounding these challenges is the fact that asbestos is a global problem and it is not uncommon for non-building materials that are formulated and manufactured in foreign countries to be examined by US laboratories. When analytical requests for brake pads or other machinery come from foreign countries that do not recognize, for example, the validity of quantitation by polarized light microscopy (PLM) which is so widely used in the US, choosing a preparatory and analytical method can be challenging. How do we adjust and/or compare our standard operation procedures and practices to provide meaningful and quality results through the lens of foreign regulatory statutes? How do we compare our analytical results to those of another country when the methods differ so greatly, or when methods do not exist at all?

170 Peak Capacity and the Probability of Success in Capillary and Microchip Electrophoresis
Joe P. Foley, Drexel University, Department of Chemistry, 3141 Chestnut St., Philadelphia, PA 19104, Erin J. Ennis

Although the dependence of the probability of a successful separation on the peak capacity has been studied thoroughly from the perspective of one- and two-dimensional chromatography, from the viewpoint of capillary electrophoresis (CE) and microchip electrophoresis (MCE), it has remained a matter of debate as to whether the probability of a successful separation is so widely used in the US, choosing a preparatory and analytical method can be challenging. How do we adjust and/or compare our standard operation procedures and practices to provide meaningful and quality results through the lens of foreign regulatory statutes? How do we compare our analytical results to those of another country when the methods differ so greatly, or when methods do not exist at all?

171 Mid-Infrared Quantum Cascade Optical Coherence Tomography (MIR-OCT) System for Spectroscopy and Imaging
Deborah Varnell, Princeton University, Equad, Olden St., Princeton NJ 08544, Mei Chai Zheng, Claire Gmachl

We present a new mid-infrared optical coherence tomography (MIR-OCT) system for simultaneous spectroscopy and three-dimensional (3D) imaging. Utilizing a novel mid-infrared superluminescent emitter, our MIR-OCT system can perform high resolution, non-invasive imaging. Using mid-infrared light allows our system to chemically analyze samples due to unique “fingerprints” of molecules in this wave-length region. Thus, our system can remove the need for a biopsy by performing in-vivo chemical analysis. Tests on our system show an accuracy comparable to commercial Fourier transform infrared spectrometer. Possible applications include biomedical imaging, industrial process monitoring and art conservation.

172 Laser Ablation Direct Analysis in Real Time Mass Spectrometry—A New Approach to Imaging the Spatial Distributions of Small Molecules in Complex Matrices
Rabi A. Musah, State University of New York - Albany, Department of Chemistry, Albany, NY 12222, Kristen L. Fowble

In recent years, imaging mass spectrometry techniques that are used to determine the spatial distribution of small molecules in several types of matrices, have expanded to include ambient ionization methods. These approaches often require a solvent, application of a matrix, the need for high vacuum, and a variety of sample pre-treatment steps. We demonstrate here that the spatial distribution of small molecules in a variety of matrices can be mapped by ablating the sample with an ultraviolet (UV) laser coupled to a direct analysis in real-time ion source, interfaced with a time-of-flight mass spectrometry (TOF-MS). Samples were either embedded in putty or affixed to carbon tape, and mounted on an x-y stage that was rastered while the laser was applied to create an ablation plume. The resulting plume was directed through a tube to the open air space between the direct analysis in real-time (DART) ion source and the MS inlet. The method was applied to map the differential distribution of dye molecules in images, secondary metabolites in biosynthetic cascades in plant tissue, and small molecules in insect tissue. Samples were analyzed in their native form and no pre-treatment steps were necessary.

173 Determination of 2-Methylisoborneol and Geosmin in Water Using Solid Phase Microextraction
Anne Jurek, EST Analytical, 503 Commercial Dr., Fairfield, OH 45014, Wade Stephenson, Kelly Cravenor

The compounds 2-Methylisoborneol (2-MIB) and Geosmin are the primary source of the foul odor found in drinking water. Algal contamination is the principal cause of the formation of these compounds. Since Geosmin and 2-MIB have such a low
odor threshold, even the slightest amount of them can produce an unpleasant odor and taste in drinking water. In order to detect 2-MIB and Geosmin at these low levels, the sampling and analysis of the water has to be optimized. Standard Method 6040D describes a procedure for the detection of 2-MIB and Geosmin using solid phase micro extraction (SPME) coupled with a gas chromatograph (GC) and mass spectrometer (MS). Selective ion monitoring (SIM) is used for compound detection down to part per trillion (ppt) levels. This examination optimizes the sampling and detection of 2-MIB and Geosmin.

174 Rapid Extraction of Polycyclic Aromatic Hydrocarbons from Avian Red Blood Cells and Plasma and Subsequent Analysis by UPLC-UV
Steven L. Kolakowski, Center for Environmental Science and Engineering, 3107 Horsemab Hill Rd., Storrs, CT 06269, Anthony A. Provatas, James D. Stuart, Christopher R. Perkins

Many groups of organic pollutants, such as petroleum oils and products produced from combustion, have received increasing attention of the scientific community due to their lipophilic nature, analysis of PAHs in biological tissue matrices, such as blood and tissue, have received particular interest. However, preparation of these samples has been proven to be problematic because of the high lipids and protein content of these matrices. Our developed methodology provides a unique, rapid preparation of PAHs from red blood cells as well as plasma and subsequent analysis by ultra-PLC-ultra-violet that is efficient, yet retains analyte sensitivity. Samples are prepared by few steps of vortexing and centrifugation, requiring only the most common laboratory equipment and reagents.

175 The Future of Environmental Analysis Utilizing Exposomics to Assess the Effects of Environmental Stressors on the Biota and Human Health
Emmanuel O. Omiari, University of Connecticut - Center for Environmental Science and Engineering, 3107 Horsemab Hill Rd., Storrs, CT 06269, Anthony Provatas, James Stuart, Sniguelu Stapcinskaite, Christopher Perkins

Our understanding of chronic diseases such as cardiovascular disease, neurodegenerative diseases, and cancer have evolved extensively over the past several decades by in large due to our understanding of the human genome and the ability to map the human genome. However, only about 17% of chronic diseases are linked to genes, the remaining 83% can be attributed to both internal and external exposures. Internal exposures consist of the foods and drugs that are consumed, while external exposures consist of environmental dangers such as chemicals and pollutants. External exposure assessments measure environmental stressors such as polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated biphenyls (PCBs), and heavy metals utilizing sensitive and selective analytical instrumentation. Historically the Organics and Metals laboratories of the University of Connecticut's Center for Environmental Sciences and Engineering have been geared toward solely identifying these environmental stressors and utilizing refined techniques to accelerate the process of detection. By utilizing previous projects that were undertaken, we have realized the labs capacity to expand upon its current niche and collaborate in the effort to contribute to the understanding of the exposome, and its effects on the biota by assessing environmental stressors that the lab is able to detect. We hold high conviction in the idea that environmental analysis as a field, will encompass not only the detection and quantification of environmental stressors, but also assessments on the effects which these stressors have on human health.

176 Optimizing the Analysis of Semi-Volatiles by EPA Method 8270
David Steinger, Thermo Fisher Scientific, 2215 Grand Avenue Pkwy, Austin, TX 78728, Tommaso Albertini, Richard Law, Lori A. Dolata

The Thermo Scientific 8270 D Analyzer Kit can ensure method requirements, sensitivity, robustness and sustainability using a start to proven workflow. This kit allows labs to capitalize on analyzing more samples per unit of time with significant cost savings while meeting method requirements. These smart innovations enrich the laboratory's gas chromatography mass spectrometry semivolatile organic compounds (GC-MS SVOC) workflow and provides improved performance and productivity. The results of this study show how the Thermo Scientific ISQ™ Series Single Quadrupole GC-MS system can meet United States Environmental Protection Agency (US EPA) 8270D Method requirements. Thanks to the extend-
and ExpoCast). The available data and searches provide a valuable path to structure identification using high-resolution mass spectrometry data. Standard approaches for both mass and formula lookup are available but the dashboard delivers a novel approach for hit ranking based on functional use of the chemicals. The focus on high-quality data, novel ranking approaches and integration to other resources of value to mass spectrometrists makes the CompTox Dashboard a valuable resource for the identification of environmental chemicals. This abstract does not reflect US EPA policy.

180 Metal Analysis of Potable Water Sources: An Educational Activity to Introduce Toxicology to Undergraduate Students

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Early introduction to applied science and research is pivotal to kindling interest in fields such as toxicology and environmental health science. Of environment contaminants, heavy metals have been relevant historically, but recently resurfaced in the United States as an interest to human health. To increase scientific literacy of toxicology and promote team-based learning, students in the Summer Undergraduate Research Fellowship (SURF) Program participated in an educational study to assess metal contamination of drinking water in NJ. This activity was part of a 10-week research program that included didactic sessions on the neurotoxicity of lead and assessment of metal exposure. Students were divided into teams to determine a list of sampling sites. Students were provided with materials and instructions on how to collect specimens. Analysis of metal concentrations was performed using indubitably coupled plasma-mass spectrometry (ICP-MS). Teams were provided with their data and asked to compare their results to the NJ Department of Environmental Protection Drinking Water Quality Standards for real-world context and regulatory application. Additional hypotheses about potential contributions, including naturally-occurring and anthropogenic sources, water system sources, and electronic waste (e-waste) were assessed. There were no trends indicating aggregate metal locations, differences in concentrations between initial draws and following a purge, or that e-waste contributed to a positive correlation between metals commonly used in the same products. Of the topics and activities included in the SURF program, the team-based water sampling exercise, which coupled toxicology, exposure, and environmental health science, was rated favorably amongst participants and fostered collaboration and networking.

181 Study on Dissolved Oxygen and Water Quality Using Numerical and Computational Simulations

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In order to protect the survival of aquatic life, there must be a minimum concentration of dissolved oxygen present. Besides extinction of aquatic species, an effect of low dissolved oxygen is degradation, which occurs when bacteria demand dissolved oxygen (DO) by decomposing the material in an aquatic environment with a waste. As degradation continues, the biological oxygen demand (BOD) increases, resulting in the decrease in DO available in the aquatic environment. In order to restore balance, the process of reaeration occurs, in which oxygen is added to the decreased DO amount. To analyze bio-degradation, the Streeter-Phelps equation, a rate of deoxygenation became the same as the rate of reaeration. Depending on the range of reaeration constants, the DO and BOD of the bodies converge to equilibrium in different ways.

182 Structure Identification Using High Resolution Mass Spectrometry Data and the EPA’s CompTox Dashboard

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The ability to identify body fluid traces at crime scenes, and preserve any DNA present, is critically important in forensic science. Identification can be difficult because many of the current techniques are specific to one body fluid, and typical biochemical methods are destructive-preventing any further analysis. To develop a universal, confirmatory, nondestructive, approach that can be used to differentiate and identify body fluids, we combined the specificity of Raman spectroscopy with the analytical power of statistical modeling. Raman spectra were collected from 75 body fluid samples, including peripheral blood, saliva, semen, sweat, and vaginal fluid. After preprocessing the experimental spectra, the samples were split into calibration and validation datasets. Several chemometric analysis techniques were trained and tested to find the best model. By exploring so many different combinations of classification algorithms and variable selection methods, we were able to study patterns in the data, the effects of various modeling parameters, and ultimately determine the most robust method for differentiation. The final model was a support vector machine discriminant analysis model built on a dataset rendered by genetic algorithm. This model accurately predicted the identity of 99.9% of the spectra from the calibration dataset, after cross-validation. More importantly, it correctly predicted the identity of 100% of the spectra in the external validation dataset. All five body fluids 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.

183 Forensic Body Fluid Identification and Differentiation by Raman Spectroscopy and Chemometrics

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The emergence of counterfeit prescription drugs has become a major concern for the pharmaceutical industry and for law enforcement. Counterfeit drugs are drugs that are fraudulently manufactured and/or mislabeled to appear genuine. These drugs may contain no active pharmaceutical ingredient (API), or may contain ingredients that are highly potent or dangerous. Recently, Xanax laced with the highly potent opioid fentanyl has accounted for several overdoses in the United States. Due to the prevalence of these potentially dangerous counterfeit drugs, it is necessary to develop a technique that can quickly confirm the identity of a suspected drug. Because the active ingredient in pharmaceuticals used in Xanax and other similar drugs is alprazolam, a power of SERS to discriminate the API in Xanax from the inactive ingredients in the pill and from fentanyl, allowing law enforcement to rapidly confirm the identity of a suspected substance. In this study we develop a surface-enhanced Raman spectroscopy (SERS)-based approach to identify a low-dose of alprazolam (the API in Xanax) using a handheld Raman spectrometer. A correlation coefficient algorithm was used to match sample spectra to a library spectrum. The method demonstrates the power of SERS to discriminate the API in Xanax from the inactive ingredients in the pill and from fentanyl, allowing law enforcement to rapidly confirm the identity of a suspected substance.

184 Rapid Surface-Enhanced Raman Anti-Counterfeit Detection of a Low-Dose of Alprazolam in Xanax

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Rapid detection of counterfeit alprazolam, or Xanax, has become an urgent need due to the current illicit drug trade and increased availability of counterfeit drugs. Due to the prevalence of these potentially dangerous counterfeit drugs, it is necessary to develop a technique that can quickly confirm the identity of a suspected drug. An additional challenge is comparing counterfeit tablets to a single active ingredient in the pill. Due to the prevalence of these potentially dangerous counterfeit drugs, it is necessary to develop a technique that can quickly confirm the identity of a suspected drug. An additional challenge is comparing counterfeit tablets to a single active ingredient in the pill. Because of the low concentrations of APIs in pharmaceutical drugs, normal Raman spectroscopy is typically not sensitive enough to detect an API directly from the surface of a pill, which contains several inactive excipients. In this study we develop a surface-enhanced Raman spectroscopy (SERS)-based approach to identify a low-dose of alprazolam (the API in Xanax) using a handheld Raman spectrometer. A correlation coefficient algorithm was used to match sample spectra to a library spectrum. The method demonstrates the power of SERS to discriminate the API in Xanax from the inactive ingredients in the pill and from fentanyl, allowing law enforcement to rapidly confirm the identity of a suspected substance.

185 LC-MS-MS Method Development Challenges for the Analysis of 43 Anxiety Medications and Metabolites

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Anxiety medications are used to treat a variety of conditions and are often abused in conjunction with other drugs. The presence of isomers and the need to collect data in positive and negative ion modes can present significant chromatographic challenges. An extensive assay was developed for analysis of medications used to treat anxiety including benzoazapines, muscle relaxants, hypnotics, sedatives, z-drugs, and barbiturates on the Raptor™ Biphenyl column. Anxiety medications were divided into six mixtures containing a total of 43 drugs and metabolites. Mixtures were diluted in water and injected into a Shimadzu Nexera UHPLC equipped with a SCIEX API 4500™ MS-MS. Detection was performed using electrospray ionization in positive and negative ion modes with multiple reaction monitoring. Multiple mobile phase combinations and additives were investigated and comparisons were made.
made using scouting gradients to evaluate retention, resolution, and sensitivity. Using the Biphenyl column, a combined analysis of anti-anxiety drugs and barbiturates was achieved in under 8 minutes with polarity switching. Alternatively, near baseline resolution (~95%) of the positional isomers, amobarbital and pentobarbital, could be accomplished by analyzing the barbiturates separately on the Raptor™ Biphenyl column in 5.5 minutes. The final optimized methods utilized water and methanol mobile phases modified with 0.1% formic under gradient conditions on Restek Raptor™ Biphenyl and Restek Raptor™ C18 2.7-μm, 100 x 2.1mm columns. Both columns were equipped with EXP® 2.7-μm, 5 x 2.1mm guard columns of the same phase.

186 Development of a Liquid Chromatography Tandem Mass Spectrometry Method for Analysis of Stimulants in Dried Blood Spots
Emily A. Williamson, Cedar Crest College, 100 College Dr., Allentown, PA 18104, Thomas A. Brintell
In this study, a liquid chromatographic electrospray-ionization tandem mass spectrometry (LC-ESI-MS-MS) method was developed in order to analyze various stimulant type drugs that have been extracted from dried blood spots (DBS). FTA DMPK-C blood cards were used as the medium to collect and store the spotted blood samples. The extracts from 30 μL of blood deposited on blood cards were analyzed using a Shimadzu LC system connected to an ABI Sciex 3200 QTRAP triple quadrupole mass spectrometer operating in positive-ion mode. Using a Restek Ultra2 C18 column (5.0 cm x 2.1 mm, 3.0 μm), the liquid chromatographic separa-
tion of the compounds was completed and optimized. The high-performance liquid chromatography (HPLC) method used a gradient mobile phase system consisting of 0.1% formic acid in water weak phase and 0.1% formic acid in acetonitrile strong phase with a total run time of 6.5 minutes. A retention time versus column tempera-
ture optimization study provided the most favorable separation conditions for the compounds at 25 °C. Optimum mass spectrometry (MS) conditions (Q1 and Q3 ions, collision energy, declustering potential) were determined for each of the com-
ounds as well as their internal standards. The extraction procedure for the DBS was optimized through testing various extraction solvents, mixing techniques, blood spot sizes, and drying down techniques. The most favorable extraction conditions involved the use of a 1:1 ratio of methanol and acetonitrile as the extraction solvent.

187 Forensic Drug Identification, Confirmation and Quantitation Using a Fully Integrated GC-FT-IR-MS
Adam C. Lanzarotta, FDA Forensic Chemistry Center, 6751 Steger Dr., Cincinnati, OH 45427, Lisa Lorenz, Sarah E. Voeker, JaCinta S. Batson
While gas chromatography with mass spectrometric detection (GC-MS) is the most widely employed analytical tool in forensic laboratories for identifying volatile and semi-volatile drug compounds, it often has difficulty identifying analytes with non-specific and/or nearly identical fragmentation patterns (e.g., positional isomers, diastereomers, etc.), especially if they co-elute. Gas chromatography with Fourier transform infrared (GC-FT-IR) detection can be employed to overcome some of these limitations but it is usually done so with a significant sacrifice in sensitivity, which is why a directly-linked GC-FT-IR-MS is an attractive alternative. The first fully integrated GC-FT-IR-MS instrument was reported in the early 1980s, but there has been only a moderate demand for its use until just recently, due to the increased prevalence of designer drugs that are difficult to identify using GC-MS but are easily distinguished using IR. GC-FT-IR-MS was employed to differentiate co-eluting syn-
ergetic classes and samples. These findings further demonstrate great poten-
tial for applying Raman spectroscopy to the field of forensic serology, especially for species identification of a suspected bloodstain.

188 Analysis of Carbamate and Organophosphate Pesticides in Animal Poisoning Cases by Gas Chromatography-Mass Spectrometry
Jessica M. Greene, Arcadia University, 450 S. Easton Rd., Glenside, PA 19038, Stephen Donovan, Alana Balogh, Karen S. Scott, Barry K. Logan Organophosphate (OP) and carbamate pesticides are widely available in many un-
derdeveloped countries and have been implicated in the poisoning of endangered species. These poisonings are a violation of local and international laws protecting these animals, and represent a further risk to other species in the animal and human food chains as the deceased animals that ate the poisoned bait are liable to be eaten by other scavenging animals such as vultures or opportunistically by humans. Lack of available analytical resources in these countries makes local verification of poisoning cases difficult. International shipping of biological materials or potentially toxic substances creates challenges in getting the samples to a capable laboratory. A method is described using basic, readily available supplies for a field extraction of biological samples, including meat baits, gastric contents and tissue samples from suspected poisoned animals, and then further preparation of these crude extracts for analysis by gas chromatography-mass spectrometry (GC-MS). This method is also used to identify neet pesticide formula to combat forgeries which are rampant in Africa. An in-field acetone extraction was used to isolate possible pesticide residue from stomach contents, baits or tissue of animals suspected of being poisoned. The acetone fraction is filtered and evaporated onto a paper towel. The dried residue is shipped according to local and international regulations to the laboratory where the residue is reconstituted, extracted using an acid-base extraction and analyzed by GC-MS. The method is capable of the analysis of OP and carbamate pesticides including aldicarb, carbofuran, and malathion.

189 Human and Animal Blood Differentiation Using Raman Spectroscopy and Chemometrics
Kyle C. Doty, University at Albany, SUNY, 1400 Washington Ave., MS: LSRB 1114, Albany, NY 12222, Gregory McLaughlin, Igor K. Lednev
The species identification of a bloodstain is an important and immediate challenge for forensic science, veterinary purposes, and wildlife preservation. In particular, de-
termining the origin of a bloodstain is a critical, yet overlooked, step in establishing its relevance to the crime. The current methods used to identify the species of origin of a bloodstain are limited in scope and destructive to the sample. We have previ-
ously demonstrated that Raman spectroscopy can reliably differentiate blood traces from three species: human, cat, and dog. The research presented here demon-
strates that multivariate statistical analysis of near infrared Raman spectroscopic data can be effectively applied as a nondestructive technique for differentiating hu-
man blood from animal blood of eleven different species; both in a binary (human vs. animal) format as well as classifying individual species. The developed approach does not require the knowledge of a specific (bio)chemical marker for each species class but rather relies on a spectroscopic statistical differentiation of various compo-
unds. Several performance measures, including a blind test and external validation, confirmed the discriminatory performance of the chemometric models developed. This approach results in remarkable classification ability even with intrinsically het-
rogenous classes and samples. These findings further demonstrate great poten-
tial for identifying Fentanyl and other Synthetic Opiates Using Ambient Ionization High Resolution Time-of-Flight Mass Spectrometry
Jamie Foss, PerkinElmer, 710 Bridgeport Ave., Shelton, CT 06484, Amanda Moore, Sabra Botch-Jones, Frank Kero
Fentanyl analogs and designer opioid drugs are a hot topic in the news right now contributing to numerous fatal overdoses. These drugs elicit analgesic effects simi-
lar to heroin making them desirable drugs to abuse. Fentanyl analogs and designer opioid drugs are expected to be more prominent in forensic casework in the near fu-
ture. Fentanyl analogs and designer opioid drugs can be seen in forensic casework either alone or can be mixed with other drugs of abuse such as heroin. It is therefore necessary to have an efficient methodology to identify these compounds. Currently, gas chromatography-mass spectrometry (GC-MS) is used to identify drugs of abuse and is considered the “gold standard” in forensic casework. However, analysis times can often run from 15-60 minutes in length. Another drawback to GC-MS is need for spectra library matching, giving the need for analytical reference materials for identification leading to an inability to identify new designer drugs before a refer-
ence material is available. In this study, direct sample analysis time-of-flight mass spectrometry (D-TOFMS) was utilized to provide rapid identification of fentanyl and related synthetic opiates. DSA is a direct ambient ionization source, requiring no chromatography and minimal sample preparation. High resolution time-of-flight mass spectrometry generates empirical formula information bypassing the need for a reference material, and in-source collisionally induced dissociation (CID) produc-
es additional structural information for confirmation. An overview of the instrumenta-
tion and use of DSA-TOFMS to rapidly generate exact mass data and fragmentation data from in-source CID for the identification of synthetic opiates is presented. Anal-
alytes explored include: heroin, 6-monoacetamorphine, morphine, fentanyl, acetyl fentanyl, butyln fentanyl, furanyl fentanyl, U-47700, and W-18.

191 Optimizing Mobile Phase Solvents for the LC/MS Analysis of Oligonucleotides
Haibo Wang, Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95114, Stephen C. Hansen
Trace amounts of metal ion in the organic solvents of mobile phases can have a significant impact on the formation of sodium and potassium adducts of proteins and oligonucleotides during LC-MS analyses.[1] In this work, different grades (purity levels) of organic solvent were investigated for the formation of sodium and potas-
sium adducts of oligonucleotides using instrument systems with high resolution and excellent mass accuracy. Fisher OptimaTM LC-MS and UHPLC-MS grade solvents (acetonitrile, methanol, and water) provide low ppb level of metal ion content in
192 Evaluation of Formulation-Induced Aggregation in Peptide Drug Products by IMS-MS

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Rapidly rising interest in therapeutic peptide drug products within the pharmaceutical industry requires novel formulation and analytical characterization strategies to enable successful development. Sterile peptide formulations are often multi-dose, requiring additives to inhibit microbial growth. These excipients can induce peptide aggregation; however, the mechanism is poorly understood. Additionally, the aggregation process may be aggravated by the conjugation of the peptide active pharmaceutical ingredient (API) with polyethylene glycol (PEG) or fatty acid chains to improve pharmacokinetic performance. Peptide aggregation and fibrilization pose several risks to drug product development, including immunogenic response, reduced bioavailability, and limited shelf life. A representation of possible aggregation events is shown. Current approaches used for the characterization of the wide size range of aggregates include analytical ultracentrifugation, size-exclusion chromatography (SEC), turbidity, and light scattering. We have observed higher-order oligomers formed during SEC using UV and multi-angle light scattering (MALS) detection after incubation in a potential drug product vehicle at low temperatures over the course of hours to days. In this study, ion mobility spectrometry–mass spectrometry (IMS-MS) is utilized to explore the landscape of lower-order peptide oligomers formed during incubation of therapeutic peptides with phenol, a common antimicrobial additive, to elucidate information on the mechanism of reversible, excipient-induced peptide aggregation.

193 Characterization of a Biologic Therapeutic: Reversed-Phase Analysis of Protein and Excipients

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Purpose: To develop a method for the simultaneous separation of therapeutic proteins and amino acid excipients. Methods: A two-dimensional (2D) approach for the separation of protein therapeutics and underivatized amino acid excipients. An integrated ultra-performance liquid chromatography (UHPLC) system with a UV and universal charged aerosol detection offering multi-mode detection for the simultaneous analysis of both non-chromophore and chromophore compounds was employed. Results: A method for the determination of label free amino acids and proteins from a commercial therapeutic protein formulation using multi-modal UV and charged aerosol detection is described. Multi-modal UV and charged aerosol detection in an integrated system provides a suitable means for the analysis of analytes consisting of both chromophore and non-chromophore species. The detectors are orthogonal and complimentary in nature so that more compounds in the sample can be detected.

194 Exploring Possible Biomarkers for Smith-Lemli-Opitz Syndrome (SLOS) Using a Mass Spectrometry-Based Proteomic Investigation

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Smith-Lemli-Opitz syndrome (SLOS) is a rare neurodevelopmental disorder that affects 1/20,000 children. SLOS occurs due to a deficiency in the enzyme DHCR7, which leads to a high level of toxic cholesterol precursors and an inefficient level of cholesterol. Symptoms of this disorder include growth failure, mental deficiency, altered muscle tone, facial dysmorphism, and delayed milestones. Almost all children with SLOS also show symptoms of Autism spectrum disorder (ASD). It is thought that these conditions may have comorbidity due to the dysregulated cholesterol system and a shared pathophysiological pathway. SLOS can be diagnosed by measuring levels of the cholesterol precursor, 7-dehydrocholesterol (7DHC) in the blood. There are currently no biological diagnostic procedures for ASD. In this proteomic investigation, saliva samples from people with SLOS and matched controls were analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis, in-gel tyrosin digestion and nanoliquid chromatography-polymer-tandem mass spectrometry (nanoLC-MS-MS). By analyzing these results and comparing to previous results of proteomic investigation of ASD saliva samples, it may be possible to identify biomarkers that would help with a diagnosis for ASD and treatment targets for both ASD and SLOS.

195 Proteomics Investigation of Induced Obstructive Sleep Apnea (OSA) in Rat Atria Using Mass Spectrometry

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Obstructive sleep apnea (OSA) affects up to 24% of the adult population and is associated with several atrial diseases. Despite the association between OSA and cardiac disease, the specific molecular mechanisms remain unclear. We have initiated a recently developed rat model which closely recapitulates the characteristics of OSA, to study OSA-induced cardiac changes. Male Sprague Dawley rats, aged 50-70 days, received surgically implanted tracheal balloons which were inflated to cause transient airway obstructions. Apnea groups experienced 60 apneas per hour of either 13 seconds (moderate apnea) or 23 seconds (severe apnea) for 2 weeks and 8 hours per day. Control rats received surgeries but no inflations. Proteomics analysis was done on the rat atria homogenates to identify dysregulated proteins in moderate and severe apnea when compared to control. Sodium dodecyl suflate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the homogenates to separate the proteins and the gel bands were trypsin digested to obtain the peptide mixtures. The peptides were analyzed by a Nano Acquity ultra-performance liquid chromatography (UPLC) coupled with Xevo G2 Mass Spectrometer. Data analysis was done using ProteinLynx Global Server (PLGS 2.4), Mascot server and Scaffold 4.1 software. The proteomics analysis revealed that 3 of the 9 enzymes in glycolysis and 2 proteins related to oxidative phosphorylation were down-regulated in the severe apnea group. In contrast, several structural and pro-hypertrophic proteins were up-regulated with chronic OSA. The data suggests the chronic OSA causes proteins changes which lead to cessation of glycolysis, a diminished capacity to generate reducing equivalents (i.e., nicotinamide adenine dinucleotide) as well as promotion of cardiac hypertrophy.

196 Structural Differences Associated with DNA Binding of p53 Family Member Proteins

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p53 family member proteins, which consist of p53, p63, and p73, possess significant structural homology and have long been presumed to function as tumor suppressors. Growing evidence shows that p63 and p73 initiate unique pathways, which play larger roles in development. This study seeks to further highlight the structural differences between the DNA-binding regions of the three proteins and how they may translate into functional differences. Structures of the DNA binding domain of each protein were obtained from the RCSB Protein Data Bank. The final structures used for p53, p63 and p73 were PDB ID 2ADY, 3USO and 4G82 respectively. MOE 2013 was utilized to visualize the structures of each DNA binding domain and was then used to perform a sequence and structural alignment of the three proteins. The structures were compared at the atomic level, and the amino acids in contact with the DNA were selected for further analysis. Out of six regions of interest, which were all in contact with DNA, five were verifications of information previously published. Further analysis was then conducted on a conserved arginine with a noticeably different orientation and an equally unique electrostatic map in all three proteins. The presence of a conserved arginine within the p53 family does not guarantee proteins will interact with DNA in the same manner. This is highlighted by the unique orientation observed in thearginine of each family member protein and may contribute to the diversity in their ability to initiate the activation of various proteins.

197 Lifetime Study of a Protein-A Affinity Column for the Analysis of IgG1 Derived from Multiple Sources

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Protein-A affinity chromatography column is used for the rapid separation of IgG from impurities and for tier determination. Lifetime and robustness of a Protein A column is important for the analyst. Here in this study we have used a TSKgel Protein-A-SPW column, containing recombinant protein-A ligand which is a code-modified hexamer of the C domain. The column is packed with hydroxylated methacrylic polymer beads with a high degree of crosslinking which allows high flow rate for chromatography while still maintaining chromatographic efficiency, peak width and resolution. The study shows that TSKgel Protein-A-SPW column quantitatively analyzed the pure mAb in less than 2 minutes per injection at the flow rate of 2 mL/min for more than 2,000 injections without regeneration or cleaning. Preliminary results indicate the column remained highly stable with the clarified cell culture supernatant or feedstock. We also report the usefulness of this column for the high-throughput analysis of IgG1 from a number of different sources such as rat, rabbit, goat, and chicken. Overall this column with the low level of Protein A leaching, excellent base stability in 0.1 mol/L NaOH and excellent lifetime is very useful for high-throughput analysis of IgG1 from multiple sources.
198 Biophysical Investigation of a Transient but Essential Protein-Ligand Complex
May Poh Lai, CUNY-City College of New York & CUNY-The Graduate Center, Advanced Science Research Center/Center for Discovery & Innovative, 85 Saint Nicholas Ter., New York, NY 10031, Cédric Bernard, Ruth Stark

Fatty acid ethanolamide (FAE) belongs to a class of important lipid-signaling molecules biosynthesized “on demand” inside the mammalian cell. These lipids are known to provoke a multitude of physiological responses including satiety, pain and inflammation. However these molecules have limited solubility and short lifetimes in solution. Protein transporters such as the fatty acid binding proteins (FABP) were previously proposed to facilitate intracellular distribution of these lipids, but so far there is limited evidence that supports the existence of such protein transporters. The biophysics behind the protein-ligand interaction is being investigated using robust magnetic resonance (NMR), isothermal titration calorimetry (ITC) and native mass spectrometry (native MS). Elucidating our fundamental understanding of the mechanism behind FAE transport can enhance our knowledge for this class of lipid molecules. The latest results on this protein-ligand system are presented in detail.

199 Determination of Tissue Specific Cancer of Chemical/Metabolites Based on Sequence Specific DNA Damage across the Exons of P53 Gene Fragments Using LC-MS-MS and Magnetic Bio-Colloid Technology
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Throughout magnetic bio-colloid technology is being developed to screen drugs, chemicals, environmental pollutants and their reactive metabolites that have the potential to covalently bind to DNA fragments and possibly cause mutations. P53 gene fragments are specifically used in this study. P53 is tumor suppressor gene and mutations with P53 gene are known in more than 50% cancer. Most mutations with P53 gene extend from exon 5-8. High-throughput magnetic bio-colloid technology is coupled with liquid chromatography tandem mass spectrometry (LC-MS-MS) to determine the codon specific chemical-nucleobase adduction and this information is translated to organ specific cancer based on databases available. Chemicals under study include Benzo[a]pyrene and Affatuxin B1 and are known to be associated with lung, liver cancer respectively. Exon fragments under consideration are codon 242 to 253 exon 7. Potential hot spots are codon 248 for lung cancer and 249 for liver cancer.

200 Luminescence Sensing Characteristics of Novel Os(II) and Ru(II) Complexes to DNA and Other Polyanions
Enju Wang, St. John’s University, Dept. of Chemistry, 8000 Utopia Pkwy., Jamaica, NY 11439, Mehrun Uddin, Karen Chen, Stacey Wong, Cody Piotrowski, Armando Seitllari, Elise Meghees

DNA detection is important to many biological processes. DNA biosensors are increasingly used in hybridization reactions, mutation detection, genomic sequencing, and identification of pathogens. The macromolecular polysaccharide-based polyanions, including heparin salts and carrageenan have unique properties and functions in physiology and food technology. The quantity of polyanion reflecting the exact number of charges in samples administered in biological procedures has to be strictly controlled. Thus the detection of these polyanions in clinical or commercial samples is key in the diagnostic and quality control processes of related fields. A series of Os(II) carbonyl complexes with two phenanthroline and either a 4-phenyl pyridine or phenyl imidazole group exhibit moderate emission intensity in the visible region. Our recent results show that the luminescence intensity of these osmium complexes can be significantly reduced or enhanced by different DNA strands. Ruthenium(II) complexes were evaluated for their luminescence responses to DNA and other polyanions. This presentation compares the luminescence response of the Os(II) and Ru(II) complexes to various DNA samples and other polyanions, in the effort of developing DNA detection markers and/or binding agents for biological applications.

201 Thermodynamic Analysis of Compounds in the Antiaging Components and the Reactive Oxygen Species (ROS)
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This paper research uses computational analysis to figure out the thermodynamic stability of various compounds used in anti-aging antioxidants. Primary method to determine stability include finding the optimal shape based on stereosensitivity. Reactive oxidation species (ROS) causes a shift in cell structure as it allows highly reactive chemicals to bond to the nucleus of cells. The results of such changes lead to cell aging. In this paper, we investigate the stability and the capacity of the components used in vitamin E such as α-, β-, γ- and δ-tocopherol and α-, β-, γ- and δ-tocotrienol, which reduce the level of ROS of our body cells. This is achieved via studying the stereosensitivity of the compounds by using computational thermodynamic analysis and force optimizations. Molecules that we examined include a few isomers of fullerene. Density Functional Theory (DFT), a computational chemistry technique, is used in order to model the electron properties of the compound. The research validates that chemical compounds that have lower optimization energy are more safe and effective to be used in anti-aging products.

202 DNA Interaction with Artemisinin: Chromatographic Analysis of Reaction Products
Ebenzer O. Newton, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Geoffrey Kamau, Amos Mugwenu

Abstract Artemisinin is of current major interest among many researchers trying to understand the nature and properties of this drug. New literature suggest the drug could be used for treatment of various forms of cancer. We are investigating possible interaction between Artemisinin and the Deoxyribonucleic acid (DNA) bases (adenine, cytosine, guanine, and thymine) under various conditions. High performance liquid chromatography (HPLC) was employed as an analytical tool to monitor and establish this possible interaction. Standard samples of the individual DNA bases were run separately using HPLC for comparison. The results of DNA interaction with other artemisinin derivatives are also discussed.

203 Withdrawn by the author.

204 Exploring Low-Volume Particle Detection Methods for Monitoring the Effectiveness of Amino Acids as Excipients for Mitigating Particle Formation
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United States Pharmacopeia (USP) General Chapter <787>, “Subvisible Particulate Matter in Therapeutic Protein Injections” is an alternative procedure to USP General Chapter <788>, “Particulate Matter in Injections”, as the latter requires large volume of samples. Low sample volume particle counting methods specified in USP General Chapter <787> are desired particularly in therapeutic biologics drug products with high concentration and limited availability of material. In this study, we utilized two particle counting instruments, AccuSizer 780 FX Nano SIS and reconfigured small-volume HIAC, to characterize the effectiveness of amino acids as excipients for mitigating particle formation in a therapeutic monoclonal antibody drug product. Both instruments were capable of measuring particles ≥ 2 µm using low sample volumes, and the results obtained were consistent to one another. In addition, these results indicated that inclusion of the amino acids in the formulation reduced particle formation. Furthermore, we utilized an additional light scattering feature of AccuSiz er to count and size particles as low as 0.150 µm, which helped to further understand the availability and limitation of AccuSizer for submicron particle detection in biologics drug product development.

205 Determination of 3-chloropropionic Acid Residue in an Active Pharmaceutical Ingredient by UHPLC Following Derivatization with Benzylamine
Ruchi P. Mehta, Pfizer, 550 Eastern Point Rd., Groton, CT 06340

Alkyl Halides are a class of compounds where a halogen atom or atoms are bound to a sp3 orbital of an alkyl group. Due to their high electronegativity, alkyl halides are used in various substitution and/or elimination reactions. This reactivity makes alkyl halides very popular chemical reagents used in the synthesis of many active pharmaceutical ingredients. However, due to the known mutagenicity of alkyl halides, it is imperative to control the levels of this class of compounds at or below the threshold of toxicological concern (TTC) in active drug substances. Due to the nature of the molecules that belong to this class of compounds, they are often difficult to detect in their native form by common analytical techniques such as liquid chromatography, mass spectrometry, gas chromatography, etc. This paper outlines the method developed for the low level detection and quantitation of 3-chloropropionic acid (an alkylating agent used in the final step of the synthesis of an active pharmaceutical ingredient) in the drug substance. The method involves derivatization followed by ultra-high-performance liquid chromatography – diode array detection (UHPLC-DAD) of 3-chloropropionic acid in an active pharmaceutical ingredient. Benzyl amine was chosen as the derivatizing agent and Propane phosphonic acid anhydride (T3P) was used as the coupling agent to form the derivatized product (N-benzyl-3-chloropropionamide) which was then amenable to detection by liquid chromatography. Good linearity and mean recoveries were achieved at low limits of quantitation. The proposed method was successfully used for the quantification of 3-chloropropionic acid in the active drug substance.
206 Direct Analysis of Free Drug in Antibody-Drug Conjugate by Reversed-Phase HPLC Using a Shielded Hydrophobic Phase Column
Marie-France Morissette, Genentech, 1 DNA Way, South San Francisco, CA 94080, Christine Gu, Yi Li, Colin Medley, David Russell

An important quality attribute shared by all antibody-drug conjugates (ADCs) is the quantity of free drug, which may increase the side effects and adverse events in patients. A reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed on a shielded hydrophobic phase (SHP) column to quantify free drug in ADC directly without time-consuming sample preparation. Various parameters have been optimized to allow a better separation of the three free drug species including the free drug, the linker drug, and the NAC linker drug complex. For example, pH, ionic strength, modifier, column size, and injection volume have been studied. Several approaches were also evaluated in order to improve the recovery of the linker drug in the presence of the protein, including lowering the autosampler temperature or adding n-ethylmaleimide to cap the residual reactive groups on the ADC. In addition to the liquid chromatography ultra violet (LC-UV) method, a LC-mass spectrometry (MS) method was also investigated to improve the detection limits. The SHP column is not suitable for MS detection, however, because polyethylene oxide bleeds from SHP packing material. To overcome this problem we investigated a new approach to couple the SHP column with LC-MS by employing differential ion mobility spectrometry (DMS) to separate the column bleed species from the free drug. The approach presented here provides a practical leap forward to attain quantitative information on free drug in ADC without the time-consuming sample cleanup step. The technology can be applied to ADC manufacturing and also has the potential to analyze protein-drug conjugates in biological matrices.

207 Supercritical Fluid Chromatography for Compound Purification in Drug Discovery
Dawn Sun, Bristol-Myers Squibb, PO Box 4000, Princeton, NJ 08543, Dauh-Ruung Wu, Peng Li, Henry Yip, Arvind Mathur

Supercritical fluid chromatography (SFC) has been demonstrated to be a great tool for chiral separation and achiral purification in drug discovery. Achromatic columns – Cyanopropylene, and achiral columns – OD, OJ, AD and AS, play an important role in achiral purification. This study shows more than 90% of achiral purification could be accomplished by these columns.

208 Electrochemical and Chromatographic Characteristics of Artemisinin: Chromatographic Analysis of Reduction Products
Zahilis Mazzocchi, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Geoffrey Kamau, Amos Musquero

Artemisinin is a plant extract used for treatment of many diseases including malaria. It is a naturally occurring sesquiterpene lactone with an endoperoxide bond capable of generating free radicals. The drug is also being investigated as a potential anti-cancer agent. We are investigating the reduction of this drug on an electrode surface, and using tetrabutyl ammonium bromide as charge transfer agent. Chromatographic methods for analysis of the reduction products have been developed. Preliminary results using liquid chromatography mass spectrometry (LC-MS) indicate that electrochemical reduction of artemisinin generates mainly dihydro-artemisinin.

209 Exploring the Capabilities of Capillary Electrophoresis in Solving Difficult Separations for Small and Large Molecules
Van Truong, Merck & Co., 126 E. Lincoln Ave., Rahway, NJ 07065, Bing Mao

While the application of capillary electrophoresis (CE) in pharmaceutical analysis is not as common as high-performance liquid chromatography (HPLC) or supercritical fluid chromatography (SFC), CE in fact offers many advantages and has proven to be an orthogonal technique complementing both HPLC and SFC. This study focuses on exploring the advantages and capabilities of CE and applying it to solve difficult separations for both small and large molecules. One example of a challenging chiral separation is discussed, in which the enantiomers of a small chiral compound failed to separate on multiple chiral reversed-phase-LC, normal phase-LC and SFC methods in chiral screening experiments, while CE successfully resolved the pair of enantiomers. The application of CE in biomolecules separation is also included in this study. In one example, CE could efficiently separate all components of a mixture of insulin and related compounds while this mixture proved difficult to separate by traditional reverse-phase (RP)-LC techniques. CE hyphenated techniques, such as CE coupled with mass spectrometry detector (CE-MS), can be useful for the detection and separation of small polar compounds with no chromophores and for impurity identification of biomolecules. Therefore, the capability of the CE-MS technique has been evaluated for this purpose. Initial data from a CE-MS demonstration showed good separation and MS sensitivity for two polar positional isomers that differ in the location of the phosphate group, 1’ and 5’ phosphate. These isomers were difficult to separate and detect by an LC-UV method. The CE methods development and optimization and the selection of electrolyte additives and buffer pH needed for the separation selectivity are discussed.

210 Determination of Degradation Products with an Acquity UPLC System and an Acquity QDa Mass Detector
Honggen Zhang, Merck & Co., 124 E. Lincoln Ave., Rahway, NJ 07065, Zhenyu Wang

A method was developed for determination of degradation products utilizing an Acquity UPLC System with Photodiode Array (PDA) and Quantitative Detection Analyzer (QDa). The stability of drug products is critical to patient health by limiting the exposure to potential toxic degradants. Therefore, it is important to determine forced degradation products under various environmental conditions (such as light, heat, humidity et al). A review of the literature indicates that the available analytical methods could not detect all possible forced degradation components of a drug, particular for those with no chromophore. The ACQUITY QDa is a bench top mass spec detector consolidated into a ultra-performance liquid chromatography (UPLC) system with the ability to track degradation products. The ion source used is electrospray ionization (ESI). Figure 1 shows an example of light degradation components detected by a PDA and QDa. The PDA detected eight components, but the QDa showed at least 14 peaks, including 2 co-elution components and 4 weak UV response peaks. The new mass analysis window arranged all analyte mass spectra (Da) and UV maximum absorbance (λ), along with its retention time (RT), in one chromatogram. The developed method is a simple, cost effective and robust method for tracking the identities of forced degradation products. A wide molecular weight range from 80 to 800 Da could be detected successfully.

Reference:

211 Guidelines for Method Transfer and Optimization of the Corona Veo Charged Aerosol Detector
Daniel Kutscher, Thermo Fisher Scientific, 1230 York Ave., New York, NY 10065

Purpose: To exhibit the process of transferring a high-performance liquid chromatography (HPLC) method from a Thermo Scientific™ Dionex™ Corona™ ultra RS charged aerosol detector (CAD) to a Thermo Scientific™ Dionex™ Corona™ Veo™ RS detector. Methods: An optimized HPLC method for theophylline and caffeine was created and optimized on the Corona ultra RS and transferred to the Corona Veo RS charged aerosol detector. A second method, using alkaline mobile phase, was optimized for the Corona Veo RS detector. Results: The optimum detector settings for the Corona Veo RS detector, for the conditions used with the Corona ultra RS CAD method, were an evaporation temperature of 30 °C, and a filter setting of 3.6 seconds.

212 Examining Superficially Porous Phenyl Phase Selectivity for Pharmaceutical Mixtures

Superficially porous alkyl phases are widely used for reversed-phase high-performance liquid chromatography (HPLC). These columns show comparable efficiency to sub 2 micron particles with less sample cleanup needed. However, analysts often encounter difficult separations for which selectivity, ruggedness or reproducibility is not easily obtained under initial method development conditions. In many cases Phenyl bonded phases are an excellent choice to consider when a C18 column does not achieve sufficient resolution of desired compounds. A generic gradient is run with a wide range of pharmaceutical compounds and used to determine the degree of difference in chromatographic selectivity between methods. Mobile phase used in this work is limited to Ammonium Formate/Formic Acid Water and Methanol Formic Acid. The effect of bonded phase is examined as various phenyl superficially porous columns are compared to C18 phases using regression data to measure orthogonality. Tanaka testing is also carried out, with the results compared to help explain differences between phases. When large numbers of analytes are examined using liquid chromatography triple quadrupole (LC QQQ), resolution of all compounds becomes less important than specific isobaric interference pairs within the expected sample set. For the most part C18 columns deliver excellent separation and high reproducibility. Phenyl columns are in general more retentive than C18 columns under the conditions studied. Using these gradient conditions studied specific peak pair examples can be found for each column that might make one column choice more desirable.

213 Reproducible Size Exclusion Chromatographic Analysis of Composite Polymers
Reza Farasat, Tosoh Bioscience, 3604 Horizon Dr., Suite 100, King of Prussia, PA 19406

Almost all blend of polybutylene terephthalate (PBT) and polycarbonate (PC), present excellent impact and chemical resistance even at low temperatures. Also they demonstrate great heat resistance, along of exceptional aesthetics and flow char-
teristics. These specifications introduce them as a novel topic for study. A system consisting of an EcoSEC GPC System (HLS-8320) equipped with a dual flow refractive index detector (RI) (Tooshibioscience LLC), and coupled in series to a Viscostar II (Wyatt Technology Corporation) was used to analyze the polymers. Two studied samples were a composite of 30% PC and 70% PBT. The mobile phase and solvent in this work was Hexafluorobispropanol (HFIP) with flow rate of 0.5 mL/min. A universal calibration curve was created at 40 °C by using narrow molecular weight Poly (methyl methacrylate) (PMMA) standards with molecular weight ranging from 6,270 to 1,99E+5 g/mol. The number, weight, and z-average molar mass values (Mn, Mw, and Mz), and intrinsic viscosity were calculated for the multiple injections of both of the samples. The results indicate stability and reproducibility of the RI detector housed within the EcoSEC GPC System. Almost no variation in the sample retention and superb baseline stability is observed for the sample. A stable RI detector baseline is essential as it directly impacts the accuracy and reproducibility of the molecular weight data extrapolated from the GPC elution profile.

214 Sequential Elution Liquid Chromatography Using a Wide-Range Mass Spectrometry Compatible pH Gradient
Catherine Kla, Drexel University, Department of Chemistry, 3141 Chestnut St., Philadelphia, PA 19104, Joe P. Foley Sequential elution liquid chromatography (SE-LC) is a novel approach for the liquid chromatographic separation of samples comprised of analytes that can be sub-divided into two or more groups, through the use of multiple, class-selective elution modes. Previous work showed a higher probability of a successful separation when utilizing SE-LC compared to conventional high-performance liquid chromatography. By employing a wide-range pH gradient, weak acids and bases in a sample can be separated both by group and within group (i.e., from each other). In this work, a mass spectrometry compatible pH gradient based on a wide-range buffer system is employed for the sequential elution separation of ionizable compounds by liquid chromatography mass spectrometry.

Reference:

215 Differentiate δ9-THC from δ8-THC by Reversed-Phase Core-SHELL HPLC
Ken Tseng, Nacalai USA, 10225 Barnes Canyon Rd., Ste. A103, San Diego, CA 92121, Toshi Ono, Tsunehisa Hirose Of the roughly 80 cannabinoids found in cannabis plants, delta-9-tetrahydrocannabinol (δ9-THC) is the primary psychoactive molecule. In the first part of this study, delta-8-tetrahydrocannabinol (δ8-THC) and δ9-THC are baseline separated using a core-shell reversed-phase high-performance liquid chromatography (HPLC) column with UV detection. The Cholestery HPLC column is compared with C18 under LC-mass spectrometry (MS)-compatible mobile phase. Δ9-THC is an isobaric isomer of δ9-THC that differs by the position of a double bond. It has lower psychoactive potency, more chemically stable, and potentially better medicinal properties than Δ9-THC. In the second part of this study, Δ9-THC and its metabolite hydroxy-Δ9-THC and 11-nor-9-carboxy-Δ9-THC are detected using a simple HPLC gradient.

216 Development and Application of a HPLC-CAD Method to Quantitate HP-β-CD During Formulation Development
Alexandra L. Esposito, Merck & Co., MS: B164, 126 E. Lincoln Ave., Rahway, NJ 07065, Jameson R. Bothe, Paul L. Walsh, Yogita Krishnamachari An excipient screen focused on improving physical stability of a synthetic peptide solution determined that 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD) was able to improve the physical stability of the active pharmaceutical ingredient in a concentration dependent manner (increasing stability with increasing concentration). HP-β-CD is a cyclic oligosaccharide with a lipophilic central cavity and hydrophilic outer surface. As a complicating agent, HP-β-CD can be used to increase the water solubility of poorly soluble drugs, and is known to impact the physical stability of proteins. From an analytical standpoint, HP-β-CD does not contain an ultraviolet chromophore, and therefore alternative analytical techniques were explored including using a charged aerosol detector (CAD). In order to determine the amount of HP-β-CD in formulated samples we developed a high-performance liquid chromatography (HPLC)-CAD method to characterize the HP-β-CD in the formulations used for stability studies. To further investigate the use of HP-β-CD as an excipient, a filter absorption study was performed due to the possibility of binding during the sterile filtration process. If the HP-β-CD binds to the filter, there could be a significant loss in concentration within the final solution, and therefore negatively impact the physical stability of the active pharmaceutical ingredient (API) and reduce the predictability of formulation stability. This HP-β-CD analytical method will help future formulation development by providing insight into sources for variability observed during stability studies.

217 Evaluation and Implementation of Ultrafast Chromatographic Separations in Support of Synthetic Chemistry Reaction Screening
Wendy Wang, Pfizer, Eastern Point Rd., Groton, CT 06475, Angel Diaz, Frank Riley During the development cycle of Pharmaceutical Drugs multiple synthetic steps may be proposed to increase efficiency, productivity and decrease impurities. To this end the synthetic organic chemist can apply modern instrumentation/robotics to aid in the reaction screening. One of the challenges that need to be addressed is the large amount of samples generated by these screens for analysis. Once the screening has been accomplished, one of the tools that can be employed to assess the best reaction conditions is chromatographic separations. In the past couple of years we have seen the advent of columns packed with supercritically porous sub 2 micron particles (SPP). The advantage of using these columns is a gain in separation efficiency and throughput when compared to columns packed with sub 2 micron fully porous particles. In our work we present method conditions and experimental results from three different column chemistry to illustrate the utility and potential advantages of supercritically porous sub 2 micron particles (SPP) in high-resolution separations, particularly for high throughput environment.

218 Evaluation of 5 Kinds of 2-μm and Sub-2-μm C18 Columns Based on Separation Behavior
Norikazu Nagae, ChromaNik Technologies, 6-3-1 Namiyoke, Minato-ku, Osaka 552-0001, Japan, Shun Kojima, Tomoyasu Tsukamoto A column packed with 2.5-μm or 2.7-μm supercritically porous particle has been widely used on high-performance liquid chromatography (HPLC) and ultra-HPLC, because it showed not only excellent column efficiency but also lower back pressure than sub-2 μm column. Recently 2.0-μm and less than 2.0-μm supercritically porous C18 columns were developed and have been available. In this study, 3 kinds of 2.0-μm and 1.7-μm supercritically porous C18 and one totally porous hybrid C18, one totally porous monodisperse C18 were evaluated regarding efficiency, hydrogen bonding capacity, hydrophobicity, steric selectivity as well as peak shape of acidic, basic and metal chelating compounds. Compared C18 columns were SunShell C18 2-μm, Ascentis Express C18 2-μm, Kinetex C18 1.7-μm, Acquity BEH C18 1.7-μm and Titan C18 1.9-μm. Furthermore, efficiency loss due to frictional heat which yielded under high pressure and at high flow rate was observed. This efficiency loss was larger for a totally porous C18 than a supercritically porous C18. Especially totally porous hybrid C18 showed the largest efficiency loss because of the lowest thermal conductivity.

219 Evaluation of C30 Phase Bonded on Superficially Porous Silica
Norikazu Nagae, ChromaNik Technologies, 6-3-1 Namiyoke, Minato-ku, Osaka 552-0001, Japan, Shun Kojima, Tomoyasu Tsukamoto A long alky group like C30 (triacetyl group) phase has been known to be more suitable than a conventional C18 phase for separation of hydrophobic structurally related isomers such as vitamin E or vitamin K1. In this study, separation factor of beta-tocopherol and gamma-tocopherol which were structurally related isomers was evaluated to vary both a pore diameter of the superficially porous silica and a ligand density of the C30 group. Regarding a pore diameter, 12 nm showed the largest separation factor of beta and gamma-tocopherol among 10nm, 12 nm and 16 nm. Regarding a ligand density, the higher a ligand density, the larger a separation factor of beta and gamma-tocopherol. However, when a ligand density was too high, much high back pressure was caused due to the increasing ligand density. The most suitable ligand density existed for the highest resolution. Final separation of cis and trans-vitamin K1 was compared and the same result as separation of beta and gamma-tocopherol was obtained.

220 RP-HPLC Method for Simultaneous Determination of Cinatapride and Pantoprazole in Bulk and Capsule Dosage Form
Pareshkumar U. Patel, Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Mehsana-Gozariya Highway, Ganpat Vidyanagar, Mehsana 384012, India A simple, accurate and rapid reversed phase high performance liquid chromatographic method has been developed and validated for the simultaneous estimation of Cinatapride (CNT) and Pantoprazole (PTZ) in bulk and capsule dosage form. The chromatographic separation was achieved on Purospher® Star C18 column (250 mm x 4.6 mm i.d., 5 μm), using mobile phase comprised of acetonitrile: water (90:10, v/v), at a flow rate of 1.5 mL/min. The determination was carried out at 266 nm wavelength by PDA detector. The linearity was obtained in the concentration range of 1.5 – 9.0 and 20 – 120 μg/mL for CNT and PTZ, respectively. The retention times for CNT and PTZ were found to be 3.02 min and 1.72 min, respectively. Results of analysis were validated statistically and by recovery studies. The recovery was obtained in the range of 98.79 – 102.0 and 98.84 – 102.0 for CNT and PTZ, respectively. The limit of detection (LOD) was found to be 0.11 μg/ml and 0.27 μg/ml, respectively. The limit of quantification (LOQ) was 0.34 μg/ml and 0.82 μg/ml for CNT and PTZ respectively. The proposed RP-HPLC method was successfully applied for determination of CNT and PTZ in their combined capsule dosage form. The percentage of CNT and PTZ were found to be satisfactory; which was comparable.
with the corresponding label claim. The proposed method was found to be simple, accurate, precise, sensitive and robust. Hence, it can be used successfully for the routine analysis of CNT and PTZ in pharmaceutical dosage form.

221 Extraction of Active Ingredients in Moringa Leaves: Chromatographic Analysis of Extraction Products
Suzanne A. Caussennean, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Amos Mugwenu
Moringa oleifera plant is widely known to be rich in nutrients and with many health benefits. In some parts of Africa and Asia, the plant is used as an anti-diabetic among other ailments. The objective of this work was to extract the active Ingredients in the leaves of this plant. The leaves underwent reflux techniques using different solvents (both polar and non-polar as well as volatile and non-volatile), during a period of time varying from 2 to 7 hours. By gas chromatography mass spectrometry (GC-MS) and high-pressure liquid chromatography (HPLC) were used to analyze both the volatile and non-volatile active ingredients.

222 Creation of a 113Sn/113m In Generator System Utilizing Polymeric Solid Supports
Christopher Caroff, Westminster College, 319 South Market St., New Wilmington, PA 16172, Jonathan Fitzsimmons, All Younes
Radioisotope generators of 113m In have long been established as an efficient system for the separation of the 113In daughter isotope from its parent isotope 113Sn. The 113Sn isotope was widely used in medicine during the 70’s and 80’s. In addition, this isotope is used as a teaching tool for secular equilibrium in the ACS sponsored Nuclear Chemistry Summer School. The separation of this isotope pair is commonly accomplished through the use of simple ion exchange resins such as a quaternary amine resin anion exchange resin. By functionalizing a solid support polymer with equivalent quaternary amines, a 113Sn/113m In generator system was established for direct comparison with a generator system utilizing a standard anion exchange resin. Optimization of this separation could be added to an increasing list of direct applications for functionalized solid support polymers in nuclear chemistry. High purity germanium detectors were used to measure generator yield and parent breakthrough over the span of one and a half months. Preliminary results from the solid support polymer generator system showed promise with lower elution volumes and comparable yield, but significant parent breakthrough was also observed.

223 Elastic Variable Selection Approach for Calibration
Cannon Giglio, University of Delaware, D 163 Department of Chemistry and Biochemistry, 163 The Green, Newark, DE 19716, Steven D. Brown
A wide range of methods for selecting variables for chemometric models have been reported. Most focus on an examination of the results of partial least squares (PLS) regression to find which variables in the model can be removed. However, if there are variables in the data that are corrupted by noise or by other effects, the resulting PLS model can often be affected unfavorably by the noisy variables. Using that model to decide on what variables are most useful is often unreliably at best. Elastic net regression has been reported as an approach that blends a lasso-based approach to variable shrinkage with ridge regression. It is designed to reduce the number of variables in the data that are included when making predictions, subject to the constraint of minimizing prediction error. By itself, elastic net regression is not competitive with PLS for predictive modeling, however. We report use of elastic net regression to find a subset of the variables in the data that are highly suited to predictive modeling. When PLS modeling is performed on that subset, results are superior to those obtained by using PLS modeling on the whole set or those from PLS modeling done on variable subsets found using other approaches that have recently been reported in the chemometrics literature. The methodology and results from the use of the method on several well-characterized data sets are illustrated.

224 High-Resolution Mass Spectrometry in NOM Characterization and DBP Formation Prediction
Dominika Houserova, University of South Alabama, CHEM 231, 6040 USA South Dr., Mobile, AL 36688, Alexandra C. Stenson, Jimmie McGehee, Benjamin Jackson, Bradford Harris, Taylor A. Brown, Logan C. Karajewski, Andrew J. Whelton
When subjected to chlorinating agents, Fulvic Acid (FA)– a complex mixture of acid-soluble compounds of natural organic matter (NOM)– creates a broad spectrum of potentially harmful disinfection by-products (DBPs). Long-term exposure to DBPs in treated water has been linked to bladder cancer. Because NOM is highly complex and often composed of chemically similar molecules, it is difficult to determine the exact structures. This, in turn, makes removal/extraction method development difficult. In this study, Suwanee River FA (SRFA) was chromatographically separated into fractions, which significantly reduced its complexity and allowed for an easier attribution of observed changes induced by chlorination to specific components. The fractions were treated with 0.1-0.4 g of Cl (in the form of NaClO) per gram of C, allowed a 3-day reaction period, and subsequently analyzed with electrospray-ionization mass spectrometry (ESI-MS). Results revealed that NOM with low O/C ratios (late eluting fractions) reacted almost exclusively with one Cl molecule and mostly retained its humic-like composition, whereas highly oxidized material (early eluting fractions) tended to be far more reactive- undergoing drastic transformations, producing more diverse Cl-containing DBPs, and often incorporating several molecules of Cl. Similarly to less oxidized NOM, N-containing and lipid-like materials underwent less chemical change, whereas the material with low H/C ratios created more DBPs. Despite the variable composition of NOM, it is clear that certain universal trends can be observed regarding reactivity and DBPs formation.

225 Investigating the Antioxidant Activity of Fe(II)-Binding Thione and Selone Complexes Utilizing Mass Spectrometry, Gel Electrophoresis, Polymerase Chain Reaction and HPLC
Emily A. Kurfman, Furman University, Department of Chemistry, Greenville, SC 29613, Julia L. Brumaghim, Sandra K. Wheeler, John F. Wheeler
Oxidative DNA damage is one of the leading causes of cancer, and reactive oxygen species (ROS) (e.g., hydroxyl radical) are well associated with the formation of DNA lesions. One avenue to reduce the concentration of ROS and/or prevent the formation thereof is through the local availability of antioxidants. We are currently exploring the application of thione and selone complexes as potential antioxidants through mechanisms of action that have previously not been fully elucidated. The best known mechanism of protection is radical scavenging; however, we are investigating a mechanism of protection in which the target antioxidant (i.e., a S or Se-containing compound) may coordinate with a physiologically relevant metal of interest (e.g., Fe2+), thereby preventing the initial formation of ROS. As a model system for study, we are investigating compounds N,N-dimethylhydrazine thione (dmt) and selone (dms), as well as methmethylole (Metm). Results from gel electrophoresis and polymerase chain reaction demonstrate that these three compounds partially protect DNA from oxidative damage, as evidenced by the continued successful replication of DNA after exposure to ROS. Further, by direct comparison, these species are found to protect DNA at lower concentrations than the biologically significant antioxidant glutathione, and demonstrate evidence of their efficacy through metal binding. Preliminary liquid chromatography data likewise shows evidence of partial protection of DNA by dmt, with additional compounds under investigation. To explore how these compounds work through metal binding, the compound Fe(dmt)-;Cl; was synthesized and analyzed via electrospray ionisation mass spectrometry (ESI-MS) pre- and post-oxidation. Results show evidence for a possible sacrificial oxidation mechanism.

226 Surface Modification of Silicon Nitride Based Sensors for Enhanced Sensing and Low Cost Diagnostics
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A nanopore—a molecular scale channel with dimensions less than 100 nm in all directions—is a widely used tool for single molecule sensing applications. Silicon nitride—a nanofabrication compatible material with favorable physical and chemical properties—is the most widely used material to fabricate solid state nanopores. Modification of the surface, either by electroless gold deposition or by tailored organic monolayer formation is an integral part of our work. Simulated time-dependent conductance data resulting from such surface modifications have been used by us to build a framework to determine the size and shape of these custom nanopores. In-situ fabrication of nanopores in its native sensing environment by dielectric breakdown of thin silicon nitride films using off-the-shelf components have made nanopore fabrication both cost and time effective, and has rendered the method widely accessible and user-friendly. Combination of the two surface modification methods, on a planar silicon nitride surface, have allowed us to create micron-scale conductive patterns with high degrees of infilling and spatial selectivity. The grain size, in particular, of the electrolessly gold plated substrates has made them useful for collecting surface-enhanced Raman (SERS) spectra, and plated SERS substrates readily qualify as low-cost when the plating is done on materials such as paper. Forming a polymer on the silicon nitride surface prior to electroless plating, modified the gold grain shape and distribution, and improved the SERS sensing performance.

Scott E. Crawford, University of Pittsburgh, Department of Chemistry, Pittsburgh, PA 15260, Christopher M. Andolina, Ashley M. Smith, Lauren E. Marbella, Kathryn A. Johnston, Patrick J. Straney, Michael J. Hartmann, Jill E. Millstone
Small gold nanoparticles (~1.4–2.2 nm core diameters) exist at an exciting interface between molecular and metallic electronic structures. These particles have the potential to elucidate fundamental physical principles driving nanoscopic phenomena and to be useful in a wide range of applications. Here, we study the optoelectronic properties of aqueous, phosphine-terminated gold nanoparticles (core diameter = 1.7 ± 0.4 nm) after ligand exchange with a variety of sulfur-containing molecules.

228 Dielectric Breakdown of Thin Silicon Nitride Films Using Off-The-Shelf Components
Annie M. Girven, University of South Alabama, CHEM 231, 6040 USA South Dr., Mobile, AL 36688, Alexandra C. Stenson, Jimmie McGehee, Benjamin Jackson, Bradford Harris, Taylor A. Brown, Logan C. Karajewski, Andrew J. Whelton
Breakdown of thin silicon nitride films using off-the-shelf components have made the fabrication of nanopores in its native sensing environment by dielectric breakdown of thin silicon nitride films using off-the-shelf components have made nanopore fabrication both cost and time effective, and has rendered the method widely accessible and user-friendly. Combination of the two surface modification methods, on a planar silicon nitride surface, have allowed us to create micron-scale conductive patterns with high degrees of infilling and spatial selectivity. The grain size, in particular, of the electrolessly gold plated substrates has made them useful for collecting surface-enhanced Raman (SERR) spectra, and plated SERS substrates readily qualify as low-cost when the plating is done on materials such as paper. Forming a polymer on the silicon nitride surface prior to electroless plating, modified the gold grain shape and distribution, and improved the SERS sensing performance.
No emission is observed from these particles prior to ligand exchange, however the introduction of sulfur-containing ligands initiates photoluminescence. Further, small changes in sulfur substituents produce significant changes in nanoparticle photoluminescence features including quantum yield, which ranges from 0.13 to 3.65% depending on substituent. Interestingly, smaller ligands produce the most intense, highest energy, narrowest, and longest-lived emissions. Radiative lifetime measurements for these gold nanoparticle conjugates range from 59 to 2590 µs, indicating that even minor changes to the ligand substituent fundamental frequency affect the electronic properties of the luminesphore itself. These results isolate the critical role of surface chemistry in the photoluminescence of small metal nanoparticles and largely rule out other mechanisms such as discrete (Au(I))—S—Rₙ, impurities, differences in ligand densities, and/or core diameters. Taken together, these experiments provide important mechanistic insight into the relationship between gold nanoparticle near-infrared emission and pendant ligand architectures, as well as demonstrate the pivotal role of metal nanoparticle surface chemistry in tuning and optimizing emergent optoelectronic features from these nanostructures. Additionally, steady state and time-resolved photoluminescence spectroscopic techniques are demonstrated to be a powerful analytical probe of nanoscopic electronic properties.

228 Raman Microspectroscopic Mapping with Multivariate Curve Resolution-Alternating Least Squares (MCR-ALS) Applied to the High-Pressure, α-PbO₂-Structured Polyform of Titanium Dioxide, TiO₂-II
Joseph P. Smith, University of Delaware, 004 Larmont du Pont Laboratory, Newark, DE 19716, Frank C. Smith, Billy P. Glass, Karl S. Borchers, and Holly M. Currie
The high-pressure, α-PbO₂-structured polyform of titanium dioxide, termed TiO₂-II, was recently identified in micrometer-sized grains recovered from four Neoarchean spherule layers using Raman microspectroscopy. This discovery has eluded researchers for nearly 30 years until now and ultimately helps illustrate that the Earth underwent an ancient heavy bombardment by large impactors. Detailed characterization of the distribution of TiO₂-II within these grains can help globally correlate the spherule layers and elucidate the origin of both the spherule layers and the grains containing TiO₂-II. In this study, grains from four spherule layers were investigated using multivariate curve resolution-alternating least squares (MCR-ALS) applied to Raman microspectroscopic mapping. Raman spectra provide evidence of grains dominated by rutile and TiO₂-II, as shown by Raman bands at 174 cm⁻¹ (TiO₂-II), 428 cm⁻¹ (TiO₂-II), 443 cm⁻¹ (rutile), and 610 cm⁻¹ (rutile). Principal component analysis yielded a predominantly three phase system comprised of rutile, TiO₂-II, and substrate-adhesive epoxy. Scanning electron microscopy suggests heterogeneous grains containing polycrystalline microcrater and submicrometer-sized particles. MCR-ALS applied to Raman microspectroscopic mapping yielded five distinct chemical species - rutile, TiO₂-II, anatase, quartz, and substrate-adhesive epoxy - in which respective spectral profiles and spatially-resolved chemical maps were generated. The spatial resolution of the Raman microspectroscopic maps was enhanced in comparable, cost-effective analysis times by limiting spectral resolution and optimizing spectral acquisition parameters. Using the resolved spectrum of TiO₂-II generated from MCR-ALS analysis, a Raman spectrum of pure TiO₂-II was estimated to further facilitate its identification within complex materials.

229 Development of Electrochemical Microsensors for In-Vivo Neurotransmitter Detection
Cheng Yang, University of Virginia, Department of Chemistry, McCormick Rd., Charlottesville, VA 22904, Christopher B. Jacobs, B. Jill Venton
Carbon nanomaterials (CNs) have promising electrochemical properties for real-time and sensitive in vivo neurotransmitter sensing. The use of CNs in fabricating large electrodes has been widely demonstrated but the reproducible production of small, sensitive CNs-based sensors is not as well studied. The complicated fabrication process and low reproducibility of electrodes fabricated by dip coating/drop casting method limit its future applications. Our lab has investigated several different techniques for CNs-based microelectrodes fabrication and modifications, and investigated their response using fast-scan cyclic voltammetry, which is the most popular electrochemical technique for in vivo detection of neurotransmitters in vivo. Our group has successfully grown vertically-aligned CNTs on cylindrical metal substrate and niobium for the first time. The alignment of CNTs and the inherent high conductivity of metal substrates determine the sensitivity of the microelectrodes for nanomolar in vivo detection of neurotransmitters. We also applied carbon nanospikes, a novel CNs, for their first bio-sensing application. CNT fiber/yarn as CNs macro-structure fabricated by spinning CNTs draw more attention due to their well-aligned CNTs and simple fabrication process in a similar manner to carbon fiber microelectrodes. Moreover, the different adsorption/desorption properties for dopamine at CNT fiber/yarn allow them to be used with higher temporal resolution without a decrease in sensitivity. In addition, two novel electrode fabrication methods based on CNs for neurotransmitter detection will be introduced. Overall, this presentation will explore the development of CNs based microelectrodes for in vivo neurotransmitters detection, their correlation between electrochemical performance and surface properties, as well as novel electrodes design.

230 Surface Enhanced Raman Spectroscopy for Rapid Detection of Engineered Nanomaterials
Lili He, University of Massachusetts-Amherst, 102 Holdsworth Way, Amherst, MA 01003
The increasing use of engineered nanomaterials (ENMs) has resulted in environmental, agricultural, and food contamination. However, there is no effective technique for rapidly detecting these ENMs in complex matrices. Here we aim to explore surface enhanced Raman spectroscopy (SERS) for rapid detection of engineered nanomaterials, i.e., silver nanoparticles (Ag NPs) and titanium nanoparticles (TiO₂ NPs) in various matrices. Upon adding a surfactant ligand which can bind and replace the ligands on the NPs, we were able to extract these modified NPs using the hydrophobization-mediated extraction and followed by Raman measurement. For Ag NPs, 4-mercaptobenzoic acid (4-MBA) and tetracyclammoniumbromide (TOAB) were used to modify the NP surface for extraction, and the detection is based on the 4-MBA signals. For TiO₂ NPs, myristin, a nature flavonoid was used for surface hydrophobization and extraction. The extracted TiO₂ NPs can be quantified based on the intrinsic Raman signals of TiO₂ NPs. We showed the capability of the SERS methods for detecting as low as 0.1 ppb Ag NPs and 200 ppb TiO₂ NPs in water within 1 hour and also validated the methods in various matrices. In conclusion, our study demonstrates the great potential of establishing SERS as a rapid, simple, and effective method for detecting ENMs in environmental, agriculture, and food matrices.

231 Whole Organism Metabolomics in Support of Nanotoxicology Research
Bryan C. Nelson, NIST - Material Measurement Laboratory, 100 Bureau Dr., Gaithersburg, MD 20899, Monique E. Johnson, Christopher M. Sims
The rapid growth of nanotechnology increases the likelihood that engineered nanomaterials (ENMs) and/or nano-enabled products will come into contact with humans and the environment in both the near and far future. Sensitive measurement tools, robust metrology, and tools/metrics for achieving measurement assurance are urgently needed for nanosafety risk assessments and regulatory decision making. There is an evolving movement, spurred by legislation in Europe that is now finding increasing application in the United States for the development of alternative testing models that reduce the use of animals (i.e., rodents) in nanotoxicology research. Alternative model organisms such as Daphnia magna, Danio rerio, Caenorhabditis elegans, Drosophila melanogaster and Arabidopsis thaliana, etc., are increasingly utilized to study the potential uptake, bioaccumulation, translocation, transformation and toxicity of ENMs in the environment. Thus far, there exist few recognized protocols or standard practices for evaluating the uptake and toxic potential of ENMs in whole organisms. In this presentation, I describe our most recent efforts focused on the development of standard protocols utilizing single particle inductively coupled plasma-mass spectrometry (ICP-MS) and electron microscopy imaging such as transmission electron microscopy (TEM), scanning electron microscope - energy-dispersive spectroscopy (SEM-EDS), and focused ion beam (FIB)-SEM for evaluating the uptake and toxic potential of ENMs in Caenorhabditis elegans. Sensitivitve measurements tools, robust metrology, and quantifiable metrics for achieving measurement assurance are critical for nanosafety risk assessments and regulatory decision making.

232 Bioavailability and Toxicity of Nanomaterials in Biosolids
Jason Urrine, University of Kentucky, 1100 S. Limestone St., Lexington, KY 40546, Jon Judy, Chun Chen, David McNear, Olga Tsyusko, Elma Lahive, Claus Svendsen, Jieren Li, Rui Ma, Gregory V. Lowry, Steve Loffs
We examined the effects of amending soil with biosolids produced from a pilot-scale wastewater treatment plant containing a mixture of metal-based engineered nanomaterials (ENMs) on the growth of Medicago truncatula, its symbiosis with the n-trogen-fixing bacteria Sinorhizobium melliloti, on earthworms and on soil microbial community structure. Treatments consisted of soils amended with biosolids generated with (1) Ag, ZnO, and TiO₂ ENMs introduced into the influent wastewater (ENM biosolids), (2) AgNO₃, Zn(SO₄)₂, and micron-sized TiO₂ (dissolved/bulk metal; hereafter bulk metal) introduced into the influent wastewater stream, or (3) no metal added to influent wastewater (control). Tissue Zn concentrations were significantly higher in the plants grown in the ENM treatment compared to those from the bulk treatment. Large reductions in nodulation frequency and plant growth as well as, in the soil abundance of fungi, actinomycetes and gram-negative bacteria were observed in the ENM treatment compared to the bulk metal treatment. We also observed greater toxicity in earthworms exposed to the ENM treatment as compared to the bulk treatment. This is despite identical extractability and solid state X-ray absorption spectroscopy-based speciation of the metals in the bulk soil. This demonstrates that current methods for characterization of metal speciation and disposition in soil are missing important characteristics that
influence metal bioavailability. We describe the use of a new hard X-ray nanoprobe, which may prove useful to elucidate how the metal distribution on the nanoscale influences bioavailability. Although not readily accessible, this technique is bound to provide new insights into how the nanoscale distribution of metals in soils influences their bioavailability.

233 Applications of Synchrotron X-Ray Methods for Determining the Environmental Fate of Nanomaterials
Gregory V. Lowry, Carnegie Mellon University, 5000 Forbes Ave., Pittsburgh, PA 15213
Understanding the environmental implications of engineered nanomaterials requires knowledge of their environmental transformations, fate and distribution. Synchrotron based X-ray methods are ideal for analytical analysis of metal and metal oxide nanomaterials in environmental and biological samples because they can be applied directly to wet (and living) tissues and samples. They are also ideal because they provide detailed information on metal speciation and crystal structure, which yields information on chemical transformations that may have occurred. Some methods such as bulk X-ray absorption spectroscopy (XAS) provide spatially averaged information about metal speciation in samples, whereas other methods such as X-ray fluorescence imaging and X-ray absorption near edge structure (XANES) mapping can provide highly spatially resolved information on metal distribution and speciation in samples, e.g., plant roots or leaves. Together, this suite of tools in an essential analytical tool for assessing the fate and effects of engineered nanomaterials in natural systems. This presentation includes relevant examples of these techniques applied to important engineered nanomaterials in laboratory, microcosm, mesocosm, and natural samples to understand the fate processes affecting them.

Dana Spence, Michigan State University, Department of Chemistry, Department of Cell & Molecular Biology, 578 S. Shaw Ln., East Lansing, MI 48824
The Spence group, housed in the Department of Chemistry at Michigan State University, currently has 4 core projects under investigation. Broadly defined, these projects are in the fields of (1) diabetes, (2) multiple sclerosis (MS), (3) blood banking, and (4) drug discovery. In this presentation, I try to convince those in attendance that the red blood cells (RBC) are a key determinant in diabetic complications, especially its role with C-peptide, the 31 amino acid peptide that is co-secreted with insulin from pancreatic -cells. While a number of measurement schemes (classic scintillation counting, microscopy, mass spec) and methods (enzyme-linked immunoassortant assay (ELISA), cell culture, separation science) are used to investigate these problems, it has been our ability to fabricate three dimensional (3D) printed devices that has advanced our understanding of the molecular level events important to each project. Various enabling technologies from the 3D printer are shown throughout the talk, with an emphasis on 3D-printed, in-vitro devices that contain membranes. These devices enable pharmacokinetics (PK) / pharmacodynamic (PD) profiling in parallel format, cell to cell communication studies that mimic in-vivo events, and are now helping our group measure binding constants between proteins and peptides that are determinants in delivery of key molecules and ions to cells.

235 Single Molecule Redox Cycling in Recessed Dual Ring Electrode Zero-Mode Waveguide Structures
Kaiyu Fu, University of Notre Dame, 325 Sirston Remick Hall, Notre Dame, IN 46556, Donghoom Han, Chaoxiong Ma, Paul W. Bohn
Arrows of nanoscale-recessed dual ring electrodes fabricated using layer-by-layer deposition coupled with focused ion beam etching can function both as working generator-collector electrode pairs and also as zero-mode waveguide (ZMW) arrays. The dual functionality makes it possible to perform single molecule spectroelectrochemical measurements under redox cycling conditions – both when the upper electrode is potential-controlled and self-induced redox cycling. In these experiments, the redox cycling behavior of flavin mononucleotide (FMN), is explored. FMN contains an isoalloxazine chromophore which is fluorescent in the oxidized state, while the reduced state, FMNH2, exhibits a substantially lower quantum efficiency, thus permitting the redox state of single FMN molecules to be followed by observing their fluorescence behavior. Because the ~100 zeptoliter volumes of these nanopores dictate very short residence times, evidence for single molecule redox cycling is obtained from the fluorescence dynamics. Freely diffusing species exhibit characteristic behavior in which the probability of observing single reduced molecules increases as the potential is scanned to more negative values. Conversely, single molecule cycling behavior is evidenced by the distribution of on- and off-times, which are altered relatively to freely diffusing FMN/FMNH2. Comparisons are made between capture efficiencies with the upper ring electrode floating vs. potential controlled as well as the propensity for the dual ring structure to stabilize the intermediate redox species which are assigned tentatively to semiquinone species.

236 Microporous Membranes for Protein Isolation and Digestion
Merlin Bruening, University of Notre Dame, Department of Chemistry, Notre Dame, IN 46556
Capillaries with inside diameters <5 microns are attractive for fast separations and catalysis because flow rapidly brings analytes to binding or catalytic sites. Moreover, microporous membranes present an array of such capillaries to enable macroscale catalysis or separations, and variation of flow rates through membranes affords milliseconds to never reside in solutions to manipulate reactions. Our research focuses on functionalizing microporous membranes with (a) affinity groups that selectively bind proteins and (b) enzymes that digest proteins for mass spectrometry analysis. Simple adsorption of poly(acrylic acid) in the pores of nylon membranes allows coupling of nitrocellulose-Ni(II) complexes to the pore surface. These Ni(II) complexes isolate polyhistidine-tagged proteins directly from cell extracts, and binding capacities are twice that of commercial beads. Remarkably, entire purification process requires <5 minutes. Small peptides immobilized in membrane pores capture specific therapeutic antibodies and may facilitate the determination of antibody concentrations in blood. Finally, immobilization of enzymes such as trypsin and pepsin in membranes enables controlled protein digestion. Rapid flow of antibody solutions through enzyme-containing membrane pores leads to large proteolytic peptides along with high amino acid sequence coverages and rapid identification of post-translational modifications in subsequent mass spectrometry analyses. The combination of the large and small peptides that result from varying the flow rate through the membrane should enhance de novo antibody sequencing. These applications clearly demonstrate the versatility and utility of functionalized microporous membranes.

237 Quantitative Electrochemical Detection of Antibodies at Sub-Picolomolar Levels Using a Simple Paper Sensor
Richard M. Crooks, University of Texas-Austin, MS: A5300, 105 E. 24th St., Austin, TX 78712, Josephine C. Cunningham, Paul DeGregory
The objective of the project described in this presentation is the creation of new, low-cost, appropriately sensitive paper universal diagnostic devices for the electrochemical detection of analytes ranging from biological weapons to DNA to biomarkers. We have developed a simple device using a magnetic microbead-supported silver nanoparticle metallomunnoassay. The sensor integrates picomolar affinity antibodies (or DNA) with an easily handled, but sophisticated, electrochemical detection platform: the NoSlip. This device exhibits quantitative detection of analytes present at sub-picomolar levels using non-enzymatic signal amplification. Total assay time is <5 min and matrices include urine and blood plasma. The cost is ~US$0.30/sensor (not including reagents).

238 Smart Chemical Imaging Sensors: Current and Emerging Strategies for Automated Detection of Hazardous Materials
In order to combat the continued terrorist threats associated with improvised explosive devices (IEDs) and the growing threat of chemical warfare agents, smart sensor systems that provide the operator with real-time, high confidence, autonomous results. Chemimage Sensor Systems (CISS) has developed a wide array of devices for the detection of chemical warfare agents, and nanomaterials present at sub-picomolar levels using non-enzymatic signal amplification. The potential is scanned to more negative values. Conversely, single molecule behavior in which the probability of observing single reduced molecules increases as the potential is scanned to more negative values. Conversely, single molecule cycling behavior is evidenced by the distribution of on- and off-times, which are altered relatively to freely diffusing FMN/FMNH2. Comparisons are made between capture efficiencies with the upper ring electrode floating vs. potential controlled as well as the propensity for the dual ring structure to stabilize the intermediate redox species which are assigned tentatively to semiquinone species.

239 The Use of Spatially-Offset Raman Spectroscopy (SORS) to Identify Unknown Threats Through Opaque Containers
Eric G. Roy, Cobalt Light Systems, 11951 Freedom Dr., Reston, VA 20190
Handheld Raman-based chemical detectors have been used for more than a decade by hazmat, military, and security personnel to identify unknown chemicals in the field. However, the scope of previous generation handheld Raman systems is limited to materials that are either unpackaged or contained in thin, transparent
containers (e.g., baggies, thin-clear bottles). When opaque containers (envelopes, opaque plastics) are encountered by these operators in the field, the package had to be opened and manually sampled prior to analysis, which decreases operational efficiency and exposes the operator to the potentially hazardous substance. Spatially offset Raman spectroscopy (SORS) is a new variant of Raman spectroscopy capable of determining the Raman spectrum of a material contained within opaque containers such as: colored and opaque plastics, paper, fabric and dark glass. The SORS technique collects two spectra (zero and offset points) and processes the information into a representative Raman spectrum of the contained material. Co-balt Light Systems has productized the SORS technique into a handheld system (Resolve	extsuperscript{TM}), which is purpose-built for security personnel. In this presentation, we present various real-world scenarios where handheld SORS has been used to identify chemical targets (e.g., explosives, toxic materials, “white powders”) through opaque containers that cannot be addressed using traditional Raman-based detection systems. The results of these trials are discussed in context of the operational relevance (capabilities & limitations) to missions encountered in the security applications.

240 Advancements in Chemical Biological and Explosive Detection at the United States Army Edgewood Chemical Biological Center

Jason Guicheteau, United States Army Research, Development and Engineering Command, Edgewood Chemical Biological Center, Attn: RDCB-DRI-S/E5560, 5183 Blackhawk Rd., APO, MD 21010, Augustus W. Fountain III, Steven Christesen, Ashish Tripathi, Erik Emmon

The Spectroscopy Branch at the United States Army Edgewood Chemical Biological Center (ECBC) has an active research program on the application of Raman spectroscopy for the detection of hazardous materials including chemical, biological, and explosives materials. This talk introduces ECBC’s mission and focus on various aspects of advancing Raman spectroscopy for military relevant detection scenarios and forensic attribution.

241 How Security Threats Have Pushed FTIR Technology Development

David W. Schiering, Cztek, 6 Finance Dr., Danbury, CT 06810

The terrorist events of 2001 and the continuing conflicts in the Middle East have heightened the need for in-field detection of threat substances. Soldiers, police, and military and civilian responders require sensitive, selective, low-cost, and compact detectors for fast indication of threat or benign materials. Fourier transform infrared (FTIR) spectrometers have found widespread use in military and civilian operations for the identification of potential threats including chemicals, white powders, explosives, and narcotics. This talk focuses on the challenges faced by responders and how these challenges have driven FTIR instrumentation development. The miniaturization of FTIR is of significant importance and the technological elements that have enabled miniaturization are discussed. Speculation regarding future improvements and performance trade-offs are also presented.

242 Forensic Aspects of Homemade Explosives Part I: Scene Considerations

Michelle Evans, Bureau of Alcohol, Tobacco, Firearms and Explosives, 6000 Ammendale Rd., Ammendale, MD 20705

Homemade explosives (HME) have become more prevalent in the United States in recent years due to the availability of the precursor materials and the popularity and accessibility of the internet. First responders, investigators, and forensic chemists need to be aware of the variety of types of products that can be found in an HME laboratory and the hazards associated with these scenes. This presentation provides a brief overview of the classes of HME, including nitrate-based mixtures, chlorate/perchlorate explosives and peroxides, as well as how to recognize the chemicals used in the manufacturing process.

243 Forensic Aspects of Homemade Explosives Part II: Laboratory Analysis

Robert F. Mothershead II, Federal Bureau of Investigation Laboratory, 2501 Investigation Pkwy, MS: EU, Rm 4140, Quantico, VA 22135

Homemade explosives (HME) have become more prevalent in the United States in recent years due to the availability of the precursor materials and the popularity and accessibility of the internet. Forensic chemists need to be aware of the variety of chemicals that can be found in an HME laboratory and the laboratory analytical procedures available to identify them. This presentation provides a brief overview of the analysis of various HME, including nitrate-based mixtures, chlorate/perchlorate explosives and peroxides, as well as precursor chemicals used in the manufacturing process.

244 Polarized Light Microscopy, Raman and FTIR Micro-Spectroscopy Analysis of Explosives

Andrew Bowen, United States Postal Inspection Service, 22433 Randolph Dr., Dulles, VA 20104

Explosive materials often consist of mixtures or both inorganic and organic phases. Some of these mixtures can pose analytical challenges to the forensic chemist, especially in post-blast samples. Polarized light microscopy (PLM) offers benefits for the analysis of certain types of explosive mixtures. This is particularly true when PLM is used in a coordinated manner with Raman and/or Fourier transform infrared spectroscopy (FTIR) micro-spectroscopy. This presentation provides a brief overview of PLM and how it can be used in a complementary manner with these micro-spectroscopy techniques. The process involves recognizing the presence of microscopically distinct phases in a mixture followed by spectroscopic analysis of individual particles of each observed phase. Several case examples illustrate the potential of this approach.

245 Improvised Explosive Devices from the Postal Perspective

Vincent J. Desiderio, United States Postal Inspection Service, Forensic Laboratory Services, 22433 Randolph Dr., Dulles, VA 20104

The United States Postal Inspection Service is a Federal Law Enforcement Agency that is tasked with protecting postal employees, customers, and infrastructure and enforcing criminal laws and civil statutes that protect employees and preserve public trust in the United States Postal Service. As a critical part of this agency, Forensic Laboratory Services exists to provide scientific support to its investigative efforts. This presentation provides a brief introduction to the services and activities of Forensic Laboratory Services and discuss the methods that are employed for the analysis of improvised explosive devices. The presentation culminates with a case study highlighting the multi-disciplinary laboratory approach that was applied to provide support to the investigation of an improvised explosive device addressed to a high profile individual that was placed in a rural mailbox in Flagstaff, Arizona.

246 Microfading and Other Tools for Tailoring Preservation of Individual Objects

Paul M. Whitmore, Yale University, PO Box 27393, West Haven, CT 06516

When the microfading technique was first developed, management of light fading risks to colored materials was primarily guided by collection management principles: grouping objects by type or material composition and assuming a “typical” stability as learned from experience. Microfading added another possibility, allowing rapid screening to determine approximate light stabilities (and thus exhibition guidance) for individual objects. In addition to enabling preservation more suited to an individual object’s needs, this insight into material stability also has opened gateways to understanding collection objects and groups in an unprecedented way, particularly important for objects with which there is little prior experience. Some examples of recent microfading research are discussed in this lecture. That research includes further examinations of the technique and the interpretation of the test results, as well as recent study of collection materials using microfading as a nondestructive probe. Microfading is one example of the interrogation of individual objects that has been enabled by technology advances. Further efforts to develop new monitors of changes and risks to individual collection objects will also be described.

247 Integrating Micro-Fade-Testing with Non-Invasive Change Detection Technologies


Predictive testing for cultural heritage has become increasingly important as institutions move towards longer exhibition periods for heritage materials, often those with light-sensitive components. Long-term exhibits are becoming increasingly common – from five to thirty years to permanent – often due to donor requirements for specific collections. While the term “fading” is commonly used to describe change due to light, the reality is that the term “color change” is more descriptive, since depending on the specific materials involved, the change in observed color can move anywhere within the prescribed color-space, light-dark, yellow-blue, or red-green deviations from the original color. Further, in addition to light-sensitivity, some fugitive media can also change color due to thermal and other degradative mechanisms, many of affects of these degradation components being intertwined. Linking non-invasive spectral imaging with micro-fade testing (MFT) allowed accelerated and natural aging change detection to be linked and provide a more accurate assessment of actual change on collection and reference material items. These analytical techniques were applied to historic and modern (sixteenth, eighteenth and twentieth century) materials to assess the impact of display environments on light-sensitive and fugitive media. For modern fugitive media, reference materials were exposed to light, dark and cold storage conditions after inks had their component dyes separated by thin layer chromatography (TLC) and then assessed after successive periods of time by MFT and spectral imaging. Historic materials were tested before and after display, where non-visible change could be detected before visible by the unaided eye, enabling better collection care decisions.
Preserving collection item, thus obtaining information on its light behavior that is more accurate it becomes possible for conservators to routinely test light sensitivity of a particular the collection item. With the development of portable and less expensive devices, the quantitative data obtained from local MFtesting into corresponding categories software and graphical user interface (GUI) that could help conservators translating vulnerable colors and quantification of the light dose associated with the indispensable frame in which the quantitative data obtained with a microfader (MFT) is presented:

Implementing Lighting Policy for Vulnerable Collection Items by Using a Microfader Christel Pesme, Independent Researcher- MFTesting Provider, Mittiere Strasse 105, Basel 4056, Switzerland

Light exposure constitutes an important risk for cultural heritage. Implementing lighting policy is of considerable importance for cultural institutions as it supports institution’s responsibilities to make collections available while keeping long-term preservation in mind. The methodology to set a Preservation Target (PT) for lighting policy is presented: PT defines specific light exposure conditions in a given timeframe under which access to values of a collection item is optimized, while the value loss induced by its exposure to light is minimized. It will be shown that PT provides the indispensable frame in which the quantitative data obtained with a microfader (MFT) should be used. MFT allows for the assessment of relative fading rates of collection items, providing key data informing lighting decisions: identification of the most vulnerable colors and quantification of the light dose associated with the PT for the collection item. With the development of portable and less expensive devices, it becomes possible for conservators to routinely test light sensitivity of a particular collection item, thus obtaining information on its light behavior that is more accurate than is available from the literature. Finally, the project ExPres – Exhibiting while Preserving, currently in progress at the Haute Ecole Arc Conservation-restauration (HE-ARC CR), is briefly discussed: it focuses on development of an open-source software and graphical user interface (GUI) that could help conservators translating the quantitative data obtained from local MFTesting into corresponding categories related to the loss of cultural value.

Properties of Deep-Eutectic Solvents and Their Use in Chemical Extractions Douglas E. Raynie, South Dakota State University, Department of Chemistry and Biochemistry, Box 2202, Brookings, SD 57070, Ganesh Kasare, University of Waterloo, Waterloo, ON N2L 3G1, Canada

Deep eutectic solvents (DES) result from the strong intermolecular attraction between, for example, a quaternary ammonium salt and a hydrogen-bond donor, resulting in a highly significant melting point lowering. The classic example is choline chloride/urea. Choline chloride decomposes at 302 deg. C, while urea melts at 133 deg. C. When mixed in a 1:2 molar ratio, the resulting eutectic melts at 12 deg. C. These solvents act in some respect as ionic liquids. Starting with choline chloride or acetylcholine chloride and urea, glycol, or water, we measured physical properties such as Kow, melting point, viscosity, pH, and Kamlet-Taft solubility parameters. Using this information, properties were measured for the halides choline chloride, bromide, and iodide mixed with water, methanol, or ethanol. Thermodynamic modeling studies confirmed the results. Finally, armed with knowledge of the solvent properties, we have begun exploring analytical extractions of lignin from switchgrass, caffeine from coffee and tea, and essential oils from natural products.

Innovative Sample Preparation Technology for Improved Analytical Performance in Multi-Residue Analyses Bruce Richter, Agilent Technologies, 2850 Centerville Rd., Wilmington, DE 19808, Deepak Lucas, David Long, Lin Zhao

Advanced modern analytical instrument detection systems such as liquid chromatography tandem mass spectrometry (LC-MS-MS) and gas chromatography (GC) MS-MS have been used widely for trace-level contaminant analysis in complex sample matrices. These instruments provide excellent detection sensitivity and selectivity, which allow using relatively easy sample preparation techniques to extract analytes from sample matrices, such as QuEChERs (quick, easy, cheap, effective, rugged, and safe), protein precipitation, dilute and shoot, etc. However, these simple sample preparation techniques do not remove large amounts of the matrix interferences from samples that contain high levels of lipids. Therefore, the injection of these complex samples can add more pressure on chromatographic columns and detection systems and results in more instrument downtime and frequent replacement of chromatographic columns and other instrument consumables. In addition, the presence of these interferences can cause ion suppression/enhancement, interference with the ionization of analytes of interest, and contribute to unreliable quantitative and qualitative results. Agilent Bond Elut EMR-Lipid is the next generation of sample preparation product and is implemented in a convenient dispersive solid phase extraction (DSPE) format for highly selective matrix removal, especially for high fat samples, without negatively impacting analyte recovery. We discuss the benefits of using EMR-Lipid cleanup that dramatically reduces matrix co-extractives while maintaining acceptable quantitation accuracy and precision. Data demonstrates the impact of superior cleanliness when conducting multi-residue, multi-class analysis in complex sample matrices using LC-MS-MS and GC-MS-MS. The ease of use, time and cost savings, minimal method development, and dramatically cleaner samples make this an attractive cleanup option for laboratories conducting trace analysis, especially in complex matrices.

Direct coupling of Blade Spray Solid Phase Microextraction to Mass Spectrometry Janusz Pawlizyn, University of Waterloo, Department of Chemistry, Waterloo, ON N2L 3G1, Canada

Coated blades spray (CBS) is a novel technology based on solid-phase microextraction (SPME) that efficiently integrates collection of analytes from complex matrices and direct ionization of target analytes in ambient mass spectrometry conditions. Essentially, the device consists of a stainless steel sheet cut as a “sword” and coated with a biocompatible polymer (e.g., HLB-PAN). As a sample preparation method, the ultra-thin SPME coating simultaneously isolates and enriches small molecules present in the matrix, and allows for clean-up of undesirable artefacts that might provide ion suppression or enhancement. Whereas as an ambient ionization technique, CBS performs as a solid-substrate electrospray ionization source, where ions of the extracted analytes are generated by applying a high electric field to a device pre-wetted with a desorption solution. Analyte-enrichment and sample-clean up is performed in exceedingly short times (i.e., 1 min or less), such that the total analysis time does not exceed 3 minutes. Limits of detection at the low pg mL-1 levels, great accuracy, and outstanding reproducibility can be achieved for a broad range on analytes in complex matrices of clinical, forensic, and environmental relevance. Given the structural configuration of the apparatus, these can be used to perform extractions independently of the sample complexity (e.g., plasma) or its dimensions (i.e., few µL up to liters when doing on-site analysis). In addition to the quantitation of target analytes in key matrices such blood and urine, this study presents a full characterization of CBS devices in terms of blade geometry and coating characteristics.

Recent Advances in Biocompatible Solid Phase Microextraction (BioSPME) Sara E. Smith, MilliporeSigma, 595 N. Harrison Rd., Bellefonte, PA 16823, Emily Barney, Craig Aurand, Candace Price

Since its first report in 1990, solid phase microextraction (SPME) has become one of the most rapidly growing sample preparation methods. Although considered an exceptional technique, its use has traditionally been somewhat focused on gas chromatography (GC)-amenable analytes, as desorption was often accomplished through thermal means. More recently, an expansion of this technique known as biocompatible SPME (BioSPME) has been developed, allowing the sample preparation methodology to evolve into a useful tool for a broader range of applications. BioSPME devices can be used to perform immune extraction and solvent desorption, thus allowing for the utilization of liquid chromatography (LC) based analyses. This greatly expands the utility of the technique, as an extensive variety of analytes can be extracted and analyzed using highly sensitive and specific LC-tandem mass spectrometry methods. Extractions can also be directly performed from complex biological matrices such as plasma, serum, whole blood, or urine, without fouling of the extraction material or significant co-extraction of interferences. Specifically, BioSPME fibers have allowed for the development of simple, rapid, high-throughput amenable methods that have been implemented within many fields, including clinical chemistry, forensic toxicology, pharmaceutical science, and food safety. This presentation highlights some of these recent advancements in the technology, and provides a glimpse of the future for microextraction.

Networking Tips to Enhance Your Career Search Bill Suits, New Jersey American Chemical Society, 30 Meadow Lakes 10, East Windsor, NJ 08520 A great network of friends and coworkers is the most common link to that next great job. Yet, how does that happen. First they must know what you are looking for. It is not just a job, but rather a role where your skills can make a difference. Those
that you must know what you are looking for and want to help. Rather than asking for a job, ask for advice that will lead to a hiring manager. We discuss how to gain your networks support improving your resume and guidance locating a key manager. Discover how to scout the competition by assuming the role of a manager looking for a person like you. After alerting your friends, move on to those that were critical of your past performance as well. Next, you want to be visible in groups that hiring managers participate. Live courses that managers use to advance their skills are helpful. Finally, building your own board of directors and maintaining contact in the right way will open many doors.

255 Academics to Industry or There and Back Again
Thomas Twardowski, Integra LifeSciences, 311 Enterprise Dr., Plainsboro, NJ 08536
Opportunities to re-assess our career are a modern employment reality. In an era of shrinking enrollments, vanishing government funding, a changing tenure environment, and high worldwide production rates of qualified, aspiring professorial candidates, the industrial sector can seem an appealing option if not an outright necessity. Common knowledge tells tales that salaries outside the academic walls are higher and that work is actually put away at night, but that intellectual rigor will continue to live together. It is particularly prevalent when one or both people standing the different expectations of these pathways allows for the possibility of a smooth transition between them. Demands within the industrial segment are often guided by business needs. Compliance to government guidelines and adherence to workplace safety per OSHA are also critical aspects of the work environment. Job performance is based on accomplishments. Teaching is a primary expectation in academia. Traditional lectures are augmented by high impact practices (HIPs). Student reviews often play a significant role in the evaluation of faculty. Research also plays a role in academia, with an expected outcome of multiple publications. Acquiring sponsors to support student research and obtain instrumentation and materials is another important aspect. In addition to teaching and research responsibilities, faculty members are also expected to be involved in academic citizenship. The attainment of tenure is a multi-year process required of academicians if they wish to retain their position. Having worked initially in industry and presently in academia, insights into each of these professional areas will be provided.

256 Industry to Academia - A Quantum Leap or One Small Step
Shirley Fischer-Drowos, Widener University, One University Pl., Chester, PA 19013
Industry and academia are employment options available to scientists. Understanding the different expectations of these pathways allows for the possibility of a smooth transition between them. Demands within the industrial segment are often guided by business needs. Compliance to government guidelines and adherence to workplace safety per OSHA are also critical aspects of the work environment. Job performance is based on accomplishments. Teaching is a primary expectation in academia. Traditional lectures are augmented by high impact practices (HIPs). Student reviews often play a significant role in the evaluation of faculty. Research also plays a role in academia, with an expected outcome of multiple publications. Acquiring sponsors to support student research and obtain instrumentation and materials is another important aspect. In addition to teaching and research responsibilities, faculty members are also expected to be involved in academic citizenship. The attainment of tenure is a multi-year process required of academicians if they wish to retain their position. Having worked initially in industry and presently in academia, insights into each of these professional areas will be provided.

257 Two Body Problems and Job Transitions
Amber F. Charlebois, SUNY-Geneseo, 4320 S. Livonia Rd., Livonia, NY 14478
The “two-body problem,” is an inelegant term describing the dilemma that couples have in finding good jobs for both people that are geographically close enough that they can continue to live together. It is particularly prevalent when one or both people in the relationship are in academia. Given the shortage of full-time academic jobs, couples are often put in a position where they have to choose between serious underemployment for one of them and living separately. It is not limited to academics, however, but it does seem to effect couples/partners who are both specifically and highly trained. In this presentation I share my personal journey with respect to the two-body problem and provide additional insight into this prevalent phenomenon.

258 Characterization of Active Matrix Modulation – A New Approach to Improve Detection Sensitivity in Two-Dimensional Liquid Chromatography
Dwight Stoll, Gustavus Adolphus College, 800 West College Ave., St. Peter, MN 56082, Ray Sajulga, Tyler Brau, Eli Larsen, Sarah Rutan, Peter W. Carr, Konstantin Shoykhet, Stephan Buckenmaier
Much of the overall performance of a two-dimensional liquid chromatography (2D-LC) separation depends strongly on the characteristics of the second of the two dimensions of separation. The overall detection sensitivity of a method (i.e., as measured by detection limits) is heavily influenced by the characteristics of each second dimension of separation. Improving the detection sensitivity in the second dimension is currently a very active area of research by a number of groups, including our own. In this presentation we first give an overview of the different approaches that are being explored by the community, and then describe a new approach developed recently by us, which we refer to as Active Matrix Modulation (AMM). The AMM approach involves novel valve technology that enables modulation of the sample matrix that contains analytes being transferred from the first to the second dimension column. Specifically, AMM enables weakening of the sample matrix to promote focusing of the analyte band as it enters the second dimension column. This in turn allows injection of relatively large volumes into small second dimension columns, which increases detection sensitivity without sacrificing resolving power. We first demonstrate the effectiveness of the AMM approach using simple small molecule probes, and compare performance with different degrees of matrix weakening. Finally, we show examples of how AMM can be used to improve detection sensitivity in real 2D-LC applications.

259 Two-Dimensional-LC as an On-Line Desalting Tool Allowing Peptide Identification Directly from MS Unfriendly HPLC Methods
Hao Luo, Merck & Co., 126 E. Lincoln Ave., MS: RY818-B221, Rahway, NJ 07065, Wendy Zhong, Jiong Yang, Ping Zhuang, Fanyu Meng, Bing Mao, Christopher Welch
The increasing interest in peptides and proteins in pharmaceutical research and development has led to many new opportunities for the researchers tasked with characterizing and analyzing these bigger molecules. Due to the more complicated impurity profile of peptides and proteins, multiple liquid chromatography techniques are often needed to achieve comprehensive analysis. However, many of these separation conditions require buffers, salts or additives that render them incompatible with mass spectrometry (MS) detection. In this paper, an Agilent heart-cutting two-dimensional liquid chromatography (2D-LC) system was connected with an Agilent Q-TOF (quadrupole time-of-flight) mass spectrometer to build a 2D-LC-MS system to solve this frequently encountered analytical challenge. On this 2D-LC-MS system, fractions containing the peaks of interest are separated by the first dimension using a MS incompatible mobile phase, then sent to a second dimension high-performance liquid chromatography (HPLC) method where fast desalting using an MS compatible mobile phase is performed prior to MS analysis. The system allows for fast and direct collection of MS information for chromatographic peaks eluted in MS incompatible mobile phases, without requiring difficult, time consuming and error prone translation of chromatographic methods from MS incompatible to MS compatible eluents. Several examples showing the application of the approach to complex mixtures containing Peptides with impurities and positional isomers are presented.

260 Ultimate Limits of Peak Capacity in Gradient Elution Liquid Chromatography
Peter W. Carr, University of Minnesota, Department of Chemistry, Smith Hall, 207 Pleasant St. SE, Minneapolis, MN 55455
Peak capacity is the chief metric of resolving power in both one- and two-dimensional gradient elution chromatography. It has been known since the early 1970s that the ultimate limit of resolving power (plate number) in one-dimensional isocratic liquid chromatography is established by the so-called “Knox-Saleem line.” This limit is defined by answering the question: “How many plates can be generated in a given time at a fixed maximum system pressure when the particle size, column length and flow rate are simultaneously optimized?” In this work we will show how Knox-Saleem optimization can be adopted to the theory of gradient elution chromatography in one-dimensional gradient liquid chromatography and use it to define its ultimate theoretical limits. It is shown that under optimum conditions the peak capacity increases in proportion to the 1/4th root of both the column length and gradient time (tG) and is directly proportional to the solute’s solvent sensitivity coefficient (S in linear solvent strength theory) as well as the range in solvent volume fraction position. We also show the existence of an optimum dimensionless gradient slope.

Lu Bai, Dow Chemical Company, 400 Arcola Rd., Collegeville, PA 19426
No abstract submitted by the author.

262 Comparison of Two Multiplexed LC-MS-MS Platforms for High-Throughput Bioanalytical Support of ADME Assays
Jun Zhang, Bristol-Myers Squibb, 5 Research Pkwy, Wallingford, CT 06429
No abstract submitted by the author.

263 High-Throughput Clinical Mass Spectrometry: An Analytical Approach to Tackle the Breadth of Biology
Matthew Crawford, LabCorp, 1447 York Ct., Burlington, NC 27215, Chris Shuford, Russell Grant
In the realm of clinical mass spectrometry, ensuring accuracy in the biological space is key; however, that does not mean we must sacrifice throughput. We have implemented the high-throughput mentality within our laboratory from the minute samples come through the door through data review. This presentation describes practical realization of multiple islands of automation that enhance throughput capabilities in the clinical laboratory. Operation of multiplexed 4-channel LC systems in a staggered parallel manner coupled to one triple quadruple mass spectrometer has enabled > 1 samples/minute capacity. From small molecule steroids to exogenous toxicological agents to larger peptides our laboratory has implemented multiple


### 2016 EAS Abstracts November 2016

**264 Identification of TarA Fragment Inhibitors by High Concentration Mass Spectrometry Screening Coupled with an EPIC-Based Aggregation Assay**

Juncal Meng, Merck & Co., 150 Wissahickon Ave., North Wales, PA 19454

The emergence of drug resistance associated with methicillin-resistant Staphylococcus aureus (MRSA) has made the identification of novel antibiotics an imperative. It is known that the inhibition of the first two steps of the biosynthesis pathway (TarO or TarA) of wall teichoic acid resists MRSA to beta-lactam antibiotics, such as Imipenem, thereby restoring the efficacy of this widely used class of antibiotics against MRSA. We report here the development of a label-free, high-throughput screening mass spectrometry assay for the identification of potential inhibitors of TarA. A library of ~1,500 fragments, which provides a good diversity with a relatively low number of compounds, was screened at high compound concentrations in dose titration on a RapidFire platform for quick identification of TarA fragment inhibitors. A Corning EPIC-based high-throughput aggregation assay was paired with the mass spectrometry-based screen to identify potential aggregators in the screen, resulting in the removal of about 25% of the fragment hits. Finally, five fragments with IC50 values ranging from 40 μM to 250 μM were identified as potential TarA inhibitors. The results indicate that the combination of these two technologies is an efficient approach for the identification of TarA fragment inhibitors.

**265 The Quest for a Mass Spectrometry-Based Plate Reader: Evaluating Laser Diode Thermal Desorption (LDTD) Coupled with Nanoliter Dispensing for HTS and HT-ADME Applications**

Andrew Wagner, Bristol-Myers Squibb, 5 Research Pkwy., Wallingford, CT 06492, Kingsley Appiah, David Harden, Zuzana Haarhoff, Jefferson Chin, Tatyana Zvyaga, Wilson Shou

Improvements in mass spectrometry (MS)-based analytical throughput have been achieved using laser desorption ionization (LDI) techniques and have increased interest in MS-based screening amongst research groups supporting early drug discovery efforts. In particular, discovery teams that perform high-throughput screening (HTS) assays intended to identify active compounds against therapeutic targets of interest have used this technology as an orthogonal screening approach to well-established fluorescence-based assays. Furthermore, optimization of the “hits” identified in such LDI mass spectrometry based HTS (LDI-HTS) trials requires additional in vitro studies to assess absorption, distribution, metabolism, and elimination (ADME) properties by liability profiling groups who prefer to use native, clinically-relevant substrates. Since HTS and HT-ADME groups can generate thousands of samples on a daily basis, it is necessary to have high-throughput analytical platforms capable of processing these demands. Assays that utilize mass spectrometry are sensitive and selective while offering the ability to provide label-free detection of physiologically relevant substrates. MS-based methodologies, however, have traditionally relied upon liquid chromatography (LC) or on-line solid phase extraction (SPE) as front-end sample delivery mechanisms. Because of this, they typically have slower cycle times that aren’t amenable to high throughput screening efforts. Using laser desorption techniques to directly introduce samples into the mass spectrometer, it is possible to achieve throughput that approaches those of plate-readers. Here we investigated the approach of coupling nanoliter sample deposition with laser diode thermal desorption (LDTD) - tandem mass spectrometry (MS/MS) and evaluated its utility in providing an ultra-high-throughput, label-free detection method for various applications in HTS and HT-ADME groups.

**266 Improving the Sensitivity of the 19F-13C HSQC Experiment by Use of BURBOP and BIP Pulses in 19F**

Alexander A. Marchione, Chemours, Experimental Station, 200 Powder Mill Rd., Wilmington, DE 19803, Breanna Conklin

19F-13C scalar coupling correlation nuclear magnetic resonance (NMR) experiments are important tools for the structural elucidations of polyfluorinated organic species, yet their sensitivity (particularly that of the key heteronuclear single quantum coherence (HSQC) experiment) is poor compared to that of their 1H-13C analogues, even after correcting for the difference in magnetogyric ratios of the observed nuclides. This is a result, in no small part, to the difficulties in uniform excitation of the widely dispersive 19F spectral window. We have discovered that the use of universal rotation (BURBOP) inversion pulses for 19F in the INEPT and reverse-INEPT transfer segments of the HSQC markedly improves the sensitivity of the experiment over a broad spectral window, even relative to previously-reported frequency-swept adiabatic pulses such as CHIRP. Benefits were also observed in the use of broadband inversion pulses (BIP) during the T1 evolution period. In different systems, improvements in S/N from 25% to 450% have been obtained. It is expected that the use of BURBOP and BIP pulses can improve the HSQC experiment.

**267 PEA-15 Phosphorylation at the C-Terminal Tail Modulates Conformation and Binding Specificity at the Death Effector Domain**

Yingying Wei, Northeastern University, Department of Chemistry, 309 John F. Kennedy Blvd., Jersey City, NJ 07305, Victor Leon, Chanel Wright

PEA-15 (phosphoprotein enriched in astrocytes, 15 kD) is a small, non-catalytic, death-effector domain (DED) containing protein, that is widely expressed in different tissues and highly conserved among mammals. PEA-15 has been found to interact with several protein targets in various pathways, particularly Fas associated with a death domain (FADD) and procaspase-8 (apoptosis) and ERK1/2 (cell cycle entry). The switch of the binding specificity of PEA-15 from ERK1/2 to FADD is regulated by phosphorylation of two C-terminal serine residues, S104 and S116, and the phosphorylation changes PEA-15 from a tumor suppressor to a tumor promotor. Our previous nuclear magnetic resonance (NMR) studies of unphosphorylated PEA-15 interaction with ERK2 showed that PEA-15 displays exceptionally complicated backbone dynamics within the DED and is only weakly bound to ERK2. In contrast, while the NMR studies of PEA-15 with FADD revealed well-folded DED. Particularly, helices α1, α5, and α6 are tightly bound to ERK2, while helices α2, α3, and α4 show complex motions and tumble at a much higher rate with significant conformational change. Recently, our NMR studies indicated that PEA-15 DED may adopt additional conformations with rearranged relative helical orientations among the six helices in S104D and S116D mutant (PEA-15DD), mimicking the doubly phosphorylated state of PEA-15. The charge-triad residues, D19-R72-D74L, located at a strategic hinge position between two dynamically distinct segments, mediate necessary conformational changes which are crucial for its binding specificity. The interactions between the FADD DED and PEA-15DD DED are mostly electrostatic, and the binding site of PEA-15 on FADD coincide with the binding site of procaspase-8, and thereby blocking the recruitment and activation of procaspase-8 at the DISC.

**268 Atropisomerization of 8-membered Dibenzo[ac]loctan: Experimental NMR and Theoretical DFT Study**

Alexei V. Buevich, Merck & Co., 2015 Galloping Hill Rd., Kenilworth, NJ 07033, Biaryl moieties remain one of the most captivating structural motifs. They are frequently encountered in both pharmacologically active natural products and synthetic drugs. Axial chirality of biaryl systems has been widely employed in synthetic organic chemistry where they are used as chiral auxiliaries and catalysts to transfer chirality. When biaryls have bulky substituents in ortho positions they are prone to atropisomerism, a property of stereoisomerism defined by the high (>25 kcal/mol) barrier of rotation. Despite considerable experimental data on cyclic biaryls, the mechanism of their atropisomerization remains unknown. Our NMR studies indicated that the atropisomerization mechanism of the 8-membered dibenzo[ac]loctan will be presented. Experimental Gibbs free activation energy, activation enthalpy, and activation entropy were established by temperature-dependent kinetic nuclear magnetic resonance (NMR) experiments. Theoretical analysis utilized density functional theory (DFT) calculations at the B3LYP/6-31G(d) level of theory. Twelve energy minima and seventeen transition states associated with five different atropisomer interconversion pathways were determined by DFT calculations. Among the five pathways the lowest Gibbs free activation energy 25.8 kcal/mol was in close agreement with the experimentally determined value of 26.8 kcal/mol. Theoretical activation entropies and enthalpies were also consistent with experimental data. The mechanism of atropisomer interconversion is a rotation of the eclipsed endo-endo conformational coordinate in a clockwise or counterclockwise direction along the ring axis.

**269 Dynamic Nuclear Polarization 35Cl Solid-state NMR for the Analysis of Active Pharmaceutical Ingredients**

Robert W. Schurko, University of Windsor, 401 Sunset Ave., Windsor, ON N9B3P4, Canada, David A. Hirsh, Aaron J. Rossini, Lyndon Emsley

The majority of solid active pharmaceutical ingredients (APIs) have one or more distinct phases (e.g., polymorphs, hydrates, and solvates), which can have very different physicochemical properties, such as bioavailability, stability, and/or solubility. In addition, each form represents unique intellectual property. As such, molecular-level structural characterization of APIs is of enormous importance to the pharmaceutical developers. The development of a high-throughput technique for the analysis of APIs has been a significant challenge. Dynamic nuclear polarization (DNP) 35Cl solid-state NMR offers a unique approach to the characterization of APIs, as it is capable of obtaining high-resolution spectra of complex systems at a much faster rate than traditional solid-state NMR. In this study, we have utilized the principles of DNP 35Cl solid-state NMR to demonstrate its utility in the analysis of APIs. We have shown that DNP 35Cl solid-state NMR can be used to obtain high-resolution spectra of APIs, which can provide valuable information regarding the structural properties of these compounds.
industry. Two of the most common methods for characterizing APIs are X-ray diffraction (XRD) and 13C solid-state nuclear magnetic resonance (SSNMR). While both are exceptional for characterizing pure, bulk forms of APIs, they are limited for probing dosage forms, due to interfering signals from excipient matrices (e.g., sugars, starches, polymers, and other binding ingredients and fillers). Since more than 50% of solid APIs are produced as HCl salts, we have proposed the use of 35Cl NMR for their structural characterization. Each API has a unique powder pattern influenced by quadrupolar and anisotropic chemical shift interactions, which acts as a spectral fingerprint. However, 35Cl powder patterns tend to be quite broad; therefore, their spectra often have poor signal-to-noise (S/N). Hence, methods are needed to enhance S/N in 35Cl NMR spectra. I present recent developments in 1) the design of broadband pulse sequences for rapid acquisition of 35Cl SSNMR spectra of APIs, and 2) the use of 35Cl SSNMR under conditions of dynamic nuclear polarization (DNP). The combination of these methods permits the study of dosage forms with low wt-% API contents. Applications to dosage forms including polymorph identification, NMR crystallography, impurity detection, and high-throughput analysis are discussed.

270 Quantitative Component Analysis of Solid Mixtures by Analyzing Time-Domain 1H and 19F T1 Saturation Recovery Curves
Active pharmaceutical ingredients (APIs) often exhibit extensive polymorphism and the tendency to form solvates and hydrates. In addition, the interaction of the desired API lead form with excipients in formulations during processing or during long-term storage may lead to form change and/or amorphization. Consequently, API and formulation characterization early in development often contain solvates, amorphous material, and excipients. The ability to characterize and quantify relevant API forms in these complex mixtures in the presence of each other and excipients is crucial in the early development process because polymorphs often exhibit distinct physical properties that may alter the dissolution and bioavailability performance, processability, and/or chemical stability of formulated drug product. In recent years, high-field and high-resolution solid-state NMR (ssNMR) has emerged as an invaluable tool for analyzing API and formulated pharmaceutical materials in the solid state. The method proposed here, QSRC, represents a new and very efficient approach for quantifying the components in solid mixtures. It utilizes 1H and 19F T1 saturation recovery curves (SRCs) measured on a Bruker Minispec mq20 benchtop time domain nuclear magnetic resonance (TD-NMR) instrument. For the analysis of a given mixture, the SRCs for the relevant pure components, as well as for the mixture itself, are measured. The relative amounts of the mixture components are obtained from a fit of the mixture SRC with a linear combination of weighted pure component SRCs.

271 Benchtop NMR Pot Luck: A Case Study Analysis of Practical Applications
Marcel Lachenmann, Oxford Instruments, 300 Baker Ave., Ste. 150, Concord, MA 01742, David Williamson
While benchtop nuclear magnetic resonance (NMR) instrumentation has been available for two decades, early incarnations were limited to low-resolution time-domain applications. In recent years, advances in technology have made possible the production of benchtop Fourier Transform NMR spectrometers. These instruments, generally operating at 60 MHz (1.4 T), offer higher resolution and stronger performance than their earlier benchtop counterparts, but are less powerful than larger, more expensive, systems based on superconducting magnets. Because NMR has been dominated by high-field systems for decades, most applications have been designed for those systems, even in cases where a lower-field instrument would excel. While benchtop instruments have the potential to expand the reach of NMR into general laboratory and production settings, the identification of practical, real-world applications for the benchtop is ongoing. This talk explores several case studies using simple benchtop NMR techniques to obtain relevant results for industrial and regulatory application. Common characteristics of successful benchtop applications are also considered. Finally, data processing and analysis techniques for successful extraction of information from benchtop NMR spectrums will be discussed.

272 Ultra-High Resolution Ion Mobility Separations based upon Long Path Length Structures for Lossless Ion Manipulations (SLIM)
Richard D. Smith, Pacific Northwest Laboratory, Box 999, Richland, WA 99352
Ion mobility-based separations are of increasing importance in conjunction with mass spectrometry (MS). Ion mobility separations not only provide additional structure-related information in terms of collision cross sections, but potentially enable more complete analysis of complex samples that include detection of lower level constituents, and much greater speeds than feasible with liquid phase separations. The benefits of mobility-based separations generally increase as separation power increases, but high resolution mobility separations have only been achievable to date in conjunction with significant ion losses and over very limited ranges of ion mobilities, substantially limiting their practicality and range of applications. This presentation describes progress in the development of new approaches capable of achieving ultrahigh resolution ion mobility separations based upon the use of electric traveling waves in very long serpentine path length structures for lossless ion manipulations (SLIM). The simplest SLIM are fabricated from two printed circuit boards spaced by ~3 mm and each having arrays of electrode to which radio frequency voltages are used to create pseudopotentials that collapse ions between the surfaces, effectively eliminating ion losses during separations. This presentation describes initial studies using long serpentine paths (~10 m) SLIM modules, as well as additional SLIM-based approaches for further increasing resolution as well as sensitivity and dynamic range of measurements, and also provide initial demonstrations of their applications enabled by these new capabilities.

273 Molecular Phenomics in Systems, Synthetic, and Chemical Biology
John A. McLean, Vanderbilt University, 7330 Stevenson Center, Nashville, TN 37235
One of the predominant challenges in systems-wide analyses is the broad-scale characterization of the molecular inventory in cells, tissues, and biological fluids. Advances in computational systems biology rely heavily on the experimental capacity to make omics measurements, i.e., integrated metabolomics, proteomics, lipiddomics, glycomics, etc., accompanied with fast minimal sample preparation, fast measurements, high concentration dynamic range, low limits of detection, and high selectivity. This confluence of figures-of-merit place demanding challenges on analytical platforms for system-wide studies. Ion mobility-mass spectrometry (IM-MS) provides rapid (ms) gas-phase electrophoretic separations on the basis of molecular structure and is well suited for integration with rapid (us) mass spectrometry detection techniques. Furthermore, the timescales of this multi-dimensional separation are well suited for combination with fast condensed-phase separations such as gas chromatography, supercritical fluid chromatography, and ultra-high-performance liquid chromatography (HPLC) for enhanced separation selectivity as the sample complexity becomes ever more challenging. This report describes recent advances in IM-MS integrated omics measurement strategies in the analyses of complex biological samples of interest in systems, synthetic, and chemical biology. New advances in bioinformatics and biostatistics are also described to approach biological queries from an unbiased and untargeted perspective and to quickly mine the data gathered to provide targeted and actionable information.

274 Pathways and Thermodynamics of Polyproline Helix Formation in Solution from Measurements of Ions in the Gas Phase
David E. Clemmer, Indiana University, 800 E. Kirkwood Ave., Bloomington, IN 47405
The measurement of an ion’s mobility through an inert gas can be compared with calculated mobilities for trial geometries to obtain insight about the abundances and overall shapes of specific ions. In these studies, energy can be added to induce structural transitions and follow changes in the abundances of different conformations that are favored before and after activation. Here, we extend this idea as a means of following structures and transitions in solution, examining the well-studied model system, polyproline. Our approach is to vary the solution composition from which ions are electrosprayed. For different systems, solution phase structures are more or less preserved in the gas phase; in some cases, new structures are favored in the gas phase—but, these can be mapped back in order to obtain insight about populations of states that were favored in solution. The polyproline system provides a chance to study such transitions in detail. When in relatively non-polar solvents such as propanol, polyproline forms a compact type PPII helix; when placed in water the polymer undergoes a series of cis-trans interconversions to produce a type PPII helix, in which water molecules intercalate along the peptide backbone, stabilizing a much more extended structure in solution. We find that although the PPII helix collapses in the gas phase, ion-mobility spectrometry—mass spectrometry (IMS-MS) techniques provide an ideal means of studying the step-by-step transitions that connect these different structures.

275 Increasing Molecular Coverage in Complex Biological and Environmental Samples Using IMS-MS
Erin Baker, Pacific Northwest National Laboratory, PO Box 999, MSIN KB-98, 902 Battelle Blvd., Richland, WA 99352, Xueyun Zheng, Kristin Bloomum-Johnson, Daniel Orton, Jennifer Kyle, Young Mo Kim, Yehia Ibrahim, Matthew Monroe, Ryan Renso, Dennis Thomas, Thomas Metz, Justin Teegarden, Richard Smith
Mass spectrometry (MS)-based technologies are playing a growing role in the analysis of complex samples. Despite significant advances in MS technology, currently it is difficult to obtain measurements of both high throughputs and high sensitivity for samples with great dynamic ranges such as plasma and serum. This problem
ultimately results in the inability to effectively account for variation among sample conditions and/or biodiversity leading to inconsequential findings for samples which have great variation. To address this challenge, we have coupled an ion mobility separation (IMS) with MS to afford greatly improved measurement throughput, sensitivity, robustness, and quantitative capabilities for rapid analysis of complex samples. The benefits we have observed in omic studies with IMS-MS are summarized in this presentation.

276 Cannabis Use in Adolescents: Potential Adverse Effects
Donald E. Greydanus, Western Michigan University, Homer Stryker MD School of Medicine, 1000 Oakland Dr., Kalamazoo, MI 49008

The issue of adolescents smoking cannabis becomes important in view of the current climate of legal adult recreational cannabis use and legalized medicinal marijuana in the United States. Recent research has identified that effects of recreational cannabis legalization include increase in both heavy cannabis smoking and number of new cannabis users. Advocates for medical cannabis in youth must consider known potential adverse effects from recreational smoking of this plant. Those who drive a motor vehicle under the influence of cannabis have two times an accident risk. Research notes that cannabis smoking during pregnancy can lead to offspring with increased risks for decreased fetal growth, thicker frontal cortex, aggression, attention deficits, executive function dysfunction, and memory processing dysfunction. DSM-5 Cannabis-related disorders as identified by the American Psychiatric Association include cannabis use disorder, cannabis intoxication, cannabis withdrawal, and other cannabis-induced disorders. Heavy cannabis use increases risks of schizophrenia in youth by two to three times. Heavy cannabis use by young teenagers (<15 years of age) increases risks for lower cognitive dysfunction, multiple central nervous system changes, increased consumption of illicit drugs, increased neuropsychiatric disorders, and increased cannabis dependence. Potential medical complications of smoking cannabis include adverse dental effects (increased caries and periodontal disease), chronic bronchitis, coronary artery vasospasm, acute coronary syndrome, arrhythmias, arthritis, and cannabis hyperemesis syndrome. Smoking cannabis by athletes leads to decreased exercise test duration with maximal exercise and thus, reduced overall athletic performance.

277 Analytical Prospects and Challenges to Detection of the Medicinal Use of Cannabis
Dayong Lee, Houston Forensic Science Center, 1301 Fannin St., Ste. 170, Houston, TX 77002

Cannabis is the most widely abused illicit substance nationally and internationally. Consequently, it is among the most frequently detected drugs in workplace, sports, clinical, and legal settings. Growing interest in the therapeutic applications of cannabis has complicated the regulation of recreational cannabis use and interpretation of test results. Medical cannabis products can be administered via oral formulation (e.g., dronabinol), oromucosal spray (nabiximols), smoking, and vaporizing. Whether medicinal use can be differentiated from illicit use remains to be seen and may depend on the route of administration and the cannabinoid composition of the drug. The cannabis plant contains more than 100 cannabinoinds, including delta9-tetrahydrocannabinol (THC), the primary psychoactive constituent. Toxicology analysis for cannabinoinds typically detects THC and its major metabolites, 11-hydroxy-THC and 11-nor-9-carboxy-THC, in biological matrices such as blood, urine, and hair. Some testing also detects minor cannabinoinds such as cannabidiol or cannabinoinds. Immunoassay, gas chromatography-mass spectrometry, and liquid chromatography-mass spectrometry have been utilized for cannabinoinds’ analysis. In this presentation, attendees learn about the recent advances in analytical technologies that have led to more sensitive and comprehensive methods for detection and quantification of cannabinoinds in biological specimens. The analytical progress also allows the use of oral fluid as an alternative biological matrix for clinical and forensic drug testing, which offers non-invasive, simple, and directly observable sample collection. Additionally, this presentation covers limitations of current drug testing for cannabinoinds in relation to sample collection, storage condition, pharmacokinetic properties, detection windows, and correlation with observed impairment.

278 Phytofacts: A Novel Approach to Characterizing and Classifying Cannabis Medicines
Mark Lewis, NaPro Research, 2146 Queens Chapel Rd. NE, Washington, DC 20018

A broad survey of California medical cannabis cultivars was carried out over five years. Several endpoints were sought employing a validated and published analytical chemistry method.1 First, analytical chemotype data was collected and compared from a pool of 400 different lots, found to represent sixty different archetypes. This analysis was then compared to complementary data produced from seed propagated hybrids in an attempt to demonstrate a new paradigm for commercial cannabis propagation will be based on production from seed rather than asexual propagation.

279 Analytical Testing for the Cannabis Industry: Consumer Safety vs. Regulatory Requirements
Christopher Hudalla, ProVerde Laboratories, 420 Fortune Blvd., Milford, MA 01757

The cannabis industry is currently one of the fastest growing industries in the United States, with 25 states permitting medicinal use, as well as 4 states that permit adult-use. This growth is fueled by recent revelations of the benefits of cannabinoind therapies for many health conditions. One challenge that has emerged is the ability to ensure consumer safety, providing accurate dosing and products that are free from potential contaminants. Analytical testing is a necessary component to ensure patient/consumer safety for products that are being consumed both medicinally as well as recreationally. Yet, many states have minimal or no regulations in place require analytical testing. For the states that do mandate testing, there is little synchronicity between requirements from state to state. In an effort to address this, several organizations have begun to develop and validate methods that can be used as a basis for this testing. These methods not only encompass testing for the active psychochemical constituents (cannabinoids and terpenes), but also for potential contaminants including heavy metals, residual solvents (VOCs), pesticides, mycotoxins, and microbiological contaminants. The methodologies that are being used to meet these testing requirements include a wide variety of chromatographic techniques in addition to mass spectrometry and variety of approaches to address microbiological contaminants. Standardization of these methods for the industry will give regulators the resources they need to include sensible requirements for regulation and legislation that is being crafted to monitor and control the use of cannabis within the US medical and adult-use markets.

280 Analytical Challenges During Development of Biosimilars
Nanda Subbarao, Biologics Consulting Inc., 400 N. Washington St., Ste. 100, Alexandria, VA 22314

Analytics plays a critical role in biosimilar development projects and the success of the project hinges on a scientifically sound analytical package that meets the current regulatory requirements. Over the past few years, the industry and regulatory agencies have continued to evaluate the testing required to assure that a biological product is “highly similar” to the reference product. The result evolving and clarification of the regulatory requirements has caused a gradual increase in the number of analytical methods that need to be included in the biosimilars testing and characterization package. The development of a comprehensive testing methodology is also at least partly driven by newer/better technologies and techniques that have become available for implementation in the quality control and development laboratories. There is an increasing need for more precise methods sensitive enough to evaluate the differences between the reference product and the biosimilar as well as between the lead biosimilar candidates. All this testing has to be performed in the very compressed biosimilars development timeline along with the extensive comparability testing. This talk discusses the steps in developing an analytical package for a biosimilar regulatory submission.

281 Analytical Challenges with Development of Generic Products
Linda Ng, Fresenius-Kabi, Three Corporate Dr., Lake Zurich, IL 60047

The development of analytical methods for generic products is challenging. Since these are not new, never marketed products in the United States, the time and cost to develop the methods are expected to be less. The wish of an analytical method developer in a generic firm is to hope the dosage form is part of a pharmacopeia so that all the methods listed in the monograph can be adopted, and are easy to duplicate. This presentation is to share some of the challenges, and how sometimes, the amount of time verifying a method is not any different than for an innovator method developer. Chemical and microbiological analytical procedures are discussed.
282 Analytical Challenges Developing Stability Indicating Methods for Generic Combination Products
Jennifer C. Lewis, FreeThink Technologies Inc., 35 Northeast Industrial Rd., 2nd Fl., Branford, CT 06405
Fixed-dose combination drug products (FDCs), where two or more active ingredients are provided in a single dosage form, offer advantages such as enhanced efficacy, improved patient compliance, decreased potential for abuse, reduced prescription cost, and better tolerability. Though advantageous, FDCs present significant analytical challenges particularly in terms of developing stability-indicating methods necessary for required formal stability studies. For the analytical chemist developing the method, a dramatic increase in the level of complexity is often encountered moving from a single active ingredient to multiple actives. Fundamental challenges arise when the actives have different chemical and physical properties such as their water solubility profile, and pH-dependence. Among the challenges created are assuring extraction of all actives in a single solvent system, then being able to quantitate both the actives and degradation products chromatographically. In some cases, multiple methods may be necessary for the product. In addition, FDCs often have complex formulations and large dose disparities (a low dose component in combination with a high dose component). Such disparities can have a major impact on whether or not the product passes its specification limit for an unknown impurity depending upon which active ingredient is used to calculate the level of the unknown in the stability sample. This presentation describes a process and give case study examples for stability-indicating method development for a combination products.

283 Developing Dissolution Methods for Generics
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No abstract submitted by the author.

284 Microfading Testing: A Multifunctional Tool for Collection Care, Materials Research and Conservation Training
Jacob Thomas, Gothenburg University, Department of Conservation, Göteborg 40530, Sweden, Ellen Heimdal
Microfading testing (MFT) is a multipurpose analytical technique. It can be used as a survey tool, analytical instrumentation and as a pedagogic aid in the training of conservators. As a survey tool, MFT is seeing increasing application in heritage institutions worldwide. It has the advantage of being able to provide information on light sensitivities of presumed light sensitive materials as well as the limitations of the technique and the interpretation of the results. With respect to use as analytical instrumentation, MFT instrumentation is a long-working-distance fiber optic reflectance spectrometer with an intense light source. The retroreflective instrument design lends itself to integration with other analytical instrumentation (micro Raman, reflectance spectroscopy, as well as x-ray methods, etc.) for operando spectroscopy study of materials. In terms of conservation training, the development of MFT instrumentation, evaluation of the technique and application to specific objects in a collection have been the focus of several PhD, masters and undergraduate research theses. The simple, exposed optical components of a MFT, and its application as a tool to measure color change in real time make it an ideal tool for teaching the underlying fundamentals of UV-Vis spectroscopy, color science and photochemistry in an applied and cross-disciplinary manner. A grasp of these concepts is necessary to understand the limitations of MFT and interpret the results in a meaningful way. For MFT to become as widely used as it has the potential to be end users should have confidence to interpret MFT results and integrate MFT results into their workflows.

285 Assessment of Microfading as a Tool for Accelerated Photodegradation of Polymer Based Rapid Prototyping Materials.
Carolien Coon, University College London, Institute for Sustainable Heritage, Central House, 14 Upper Woburn Place, London WC1H 0NN, United Kingdom, Jacob L. Thomas, Matija Strlic
Additive manufactured objects and artworks printed by means of Rapid Prototype (RP) techniques are experiencing intense degradation problems that have also been adopted for use in conservation. Initially RP technologies were designed to produce temporary prototypes and little is known about their long term stability. A significant concern is that due to post-processing or digital preservation issues, re-printing may not be possible. Rapid Prototypes are extremely complex objects due to unknown processing parameters and chemical compositions. Fast developments in the field mean that new systems and materials continuously enter the market, not leaving enough time for in-depth study. A tool to quickly assess the photo-stability of individual RP artworks as they become part of collections would be beneficial, informing best practice for the care, storage and display of such artworks. Microfading (MFT) has successfully been used to identify light sensitive objects in museum collections. If applicable to RP materials, MFT would be an excellent method to use as it is micro-destructive and provides quick responses. It was therefore assessed as a tool for rapid identification of photosensitive RP artworks. Initial results when microfading RP polymers indicated that reciprocity did not hold compared to an 8-month natural daylight ageing experiment, possibly due to limited oxygen diffusion. Results of this study are discussed as well as the issues related to fading RP polymers and steps taken to overcome these.

286 The Interpretation of Microfading of Unvarnished Modern Paint and the Practical Issues Involved
Joyce H. Townshend, Tate Britain, Conservation Dept., Millbank, London SW1P 4RG, Great Britain
Microfaders can give information on the lightfastness of materials suspected of being very light-sensitive, but are not typically applied to large paintings on canvas, or to chemically complex systems such as artists’ paint. Both categories are generally much more resistant to fading to benefit from the technique, but there are exceptions, initially highlighted by conservators who are specifying appropriate illumination and display. In the author’s institution these exceptions have been created exclusively from the mid and later twentieth century, and all were suspected of including paints and colorants of lower quality, on the grounds of visual or documentary evidence. Whether microfading can make a useful contribution to assessing the lightfastness of a range of modern paint types and/or large canvas paintings is addressed with four case studies. Vibration of the canvas support is an issue that has to be mitigated for large paintings, and the handling with precision as well as safety presents practical problems. Within the context of a technical study and/or a study of reconstructions made from similar paints, microfading was found to give very useful information in such cases, but applied in isolation it could give rise to predictions that would not relate closely to normal display and storage conditions. These case studies will be contrasted with several examples of works of art on paper from the same period, whose microfading and its interpretation were much simpler to carry out.

287 A Discussion of Some Uncertainties in Microfading
Andrew Lernvill, University of the Bahamas, Grand Bahama Highway, Freeport, Bahama, Christel Pesme, Vincent Beiralan, Jim Družik
Two investigations of the validity of microfading spectroscopy to predict the fading behavior of a diversity of colorants at lower light levels is presented. In both experiments standard lightfastness testing apparatus (light box aging) was compared to microfading, and samples received similar lux-hours exposure. ISO Blue Wool Standards and 15 dyed papers were tested as were ten different aniline dyes. Results from both experiments illustrate a positive correlation between the compared light sensivity to MFT methods. This leads to the conclusion that fugitive colorants can be reliably highlighted by the microfading technique. In both experiments a lower value of induced color difference was observed when using microfading compared to standard lightfastness testing apparatus. This indicates that the quantitative prediction of color change from real illumination in lower illumination conditions is not secure. Making reference to separate experiments, analysis and these results, a discussion surrounding the potential sources of uncertainty associated with light accelerated aging and more specifically microfading spectroscopy follows. Commonly cited concerns including biphotonic events, diffusion-limited photo-oxidation reaction rates, dehydration and heating of the sample, the choice of different color difference units and statistical methods applied etc. is discussed.

288 Microfade as a Precision Lab Instrument versus In-Situ Application
Haida Liang, Nottingham Trent University, College of Arts & Science, Burton St., Nottingham NG1 4BU, United Kingdom
No abstract submitted by the author.

289 Applications of DNP MAS NMR in Structural Biology and Pharmaceutical Formulations
QingZhe Ni, Massachusetts Institute of Technology, MS: 3rd Fl., NW 14 Bitter Lab, 170 Albany St., Cambridge, MA 02139, Fengyuan Yang, Thach Can, Ivan Sergeyev, Maya Lipert, Sudheer Jawla, Yongjun Li, Wei Xu, Anthony Leone, Robert Griffin, Yongchao Su
Solid phase characterizations are critical in the preformulation and formulation development of drug products. Solid-state nuclear magnetic resonance (ssNMR) spectroscopy has proven to be an indispensable tool to investigating solid-state pharmaceutical systems. However, due to the low natural abundance of pharmaceuticals and low sensitivity in ssNMR measurement, it has become a technical challenge for pharmaceutical applications. Recent advances of dynamic nuclear polarization (DNP) boosts ssNMR signals by factors of 10^2 to 10^3. This gain in sensitivity allows for mechanistic studies of drug release and bioavailability of monoclonal antibodies or peptide drugs. In particular, the L intermediate, which is known to play a crucial role in β2’s pumping mechanism. Our studies also aim to utilize DNP-enhanced ssNMR in the practical development of pharmaceutical formulations. Amorphous solid dispersions are successfully prepared by incorporating polarizing agents in the formulation processes of spray drying (SD) and hot-melt extrusion (HME). A few active pharmaceutical ingredients (APIs) and the excipient copovidone are utilized for binary solid dispersion to investigate the DNP enhancement. We first investigate the dependence of enhancement from the NMR intensity on the radical concentration. The spray
dried API exhibits an optimum enhancement at a TOTAPOL radical concentration of 1% (w/w). By deuterating the excipient, it gains an additional factor of 3 in enhancement. To further optimize the DNP sample of solid dosages, AMUPol is used and compared. The optimal conditions exhibit an enhancement factor ~25, giving a reduction of NMR measurement time by 600. This largely boosted intensity facilitates the two-dimensional homonuclear and heteronuclear correlation experiments, allowing the characterization of the solid dispersion samples.

290 Characterization of Pure and Formulated Active Pharmaceutical Ingredients by DNP Enhanced Solid-State NMR
Aarón J. Rossini, Iowa State University, Department of Chemistry, 2438 Pammel Dr., Ames, IA 50011, Lyndon Emsley
Solid-state nuclear magnetic resonance (NMR) spectroscopy is an ideal probe of structure and crystal phase for active pharmaceutical ingredients (APIs) in both pure and dosage forms. However, solid-state NMR experiments on many APIs suffer from poor sensitivity because proton longitudinal relaxation times are very long in crystalline solids (T1(1H) = 2-700 s). I describe how dynamic nuclear polarization (DNP) enhanced solid-state NMR can be applied to ordinary micro-particulate organic solids (such as APIs) to improve sensitivity by two orders of magnitude. In the “remote DNP” approach, finely ground micro-particulate solids are impregnated with a minimal amount of biradical solution. The liquid for the impregnation step is chosen to be a non-solvent for the organic solid. DNP enhanced proton polarization is then transported from the surface into the interior of the particles by proton spin diffusion. Sensitivity gains of one to two orders of magnitude can be obtained for micro-particulate APIs. With DNP it is now straightforward to obtain natural abundance 13C-13C INADEQUATE, 1H-15N HETCOR, 14N overtone, 35Cl, and natural abundance 2H solid-state NMR spectra. DNP can also be applied to formulations with low API loading to rapidly obtain high quality one-dimensional and two-dimensional natural abundance 13C, 15N and 35Cl solid-state NMR spectra of the API. This enables the API phase to be assessed and interactions between the API and excipients can be probed. Analysis of the DNP enhancements enables the macroscopic side of the API particles to be measured, which is a critical parameter for drug release.

291 Experimental Insights into Dynamic Nuclear Polarization and its Application in Biological Contexts
Joanna R. Long, University of Florida, Box 100245, Gainesville, FL 32610, Bimala Lama, Adam N. Smith, James H.P. Collins, Daniel M. Downes, Wenzing Tang, Johannes Mckay, Stephen Hill, Thierry Dubroca
Dynamic nuclear polarization (DNP) as a means to enhance nuclear magnetic resonance (NMR) sensitivity has made significant strides in the past twenty five years, in particular due to the development of hardware, sample preparation strategies, and efficient polarization agents which have demonstrated its promise. The advent of commercially available high frequency microwave sources and auxiliary hardware compatible with high field NMR and magnetic resonance imaging (MRI) instruments has led to the transition of DNP-based research from proof of principle experiments to applications enabling spectroscopic investigation of samples previously beyond the reach of NMR. Key experiments measuring polarization enhancements in various field and temperature regimes coupled with electron paramagnetic resonance (EPR) measurements have yielded a rough topological map for mechanisms of DNP and how they might be exploited to enable new applications in biologic NMR and real time in vivo magnetic resonance spectroscopic imaging (MRSI) of metabolism. Nonetheless, our recent work highlights the need to continue to develop DNP approaches and a framework for polarization of “real world” systems. In particular, biologically relevant systems are both highly aqueous and naturally highly heterogeneous. Freezing samples for DNP adds another level of complexity. I primarily focus on our recent efforts examining electron spin dynamics and nuclear spin polarization at 5 T and 1.2 K for dissolution DNP applications as well as provide a few updates on DNP at 14.1 T and ~100 K for biomolecular magic angle spinning (MAS) solid-state NMR applications. In both cases, pragmatic considerations of spin distributions, there coupling to other spins and how they may be manipulated can improve observed polarization.

292 High-Throughput Structure Determination of Supramolecular Assemblies by Dynamic Nuclear Polarization Enhanced NMR
Frédéric Blanc, University of Liverpool, Department of Chemistry, Crown St., Liverpool L69 7ZD, Great Britain
Nuclear magnetic resonance (NMR) is the most powerful atomic scale structure determination technique of powdered solids as the frequency resonance signal depends on the chemical environment surrounding the atom and is therefore able to identify materials in all phases of matter. NMR provides an additional advantage over diffraction-based methods which are limited to crystalline solids only and is therefore widely used in polymer science. However, the main limitation of NMR is its extremely low sensitivity and hinders fast acquisition of the NMR signals in seconds. Dynamic nuclear polarization (DNP) is the most dramatic approach for signal enhancement in NMR®, enabling an increased in sensitivity by multiple orders of magnitude, and is currently revolutionizing the atomic scale structure elucidation of an increasing number of always more complex range of materials. We present how this could be used to quickly detect the carbon-13 and nitrogen-15 NMR signals of supramolecular assemblies allowing high throughput characterization of libraries of polymers. We illustrate the approach and potential in a series of functionalized microporous organic polymers, hyper-cross linked polymers and conjugated microporous polymers, including photocatalysis, revealing unprecedented details in the molecular structure of the polymeric networks and their three-dimensional structures.

References:

293 Getting the Spectroscopist out of Raman Spectroscopy: Challenges and Opportunities in Life Science, Biomedical and Process Raman Spectroscopy
Karen Esmonde-White, Kaiser Optical Systems, 371 Parkland Plaza, Ann Arbor, MI 48104, Maryann Cuellar, Alexander Pitters, Sean Gilliam, Carsten Uerpmann, David Strachan, Bruno Lenain, Ian Lewis
Raman spectroscopy is an established tool in research analytical laboratories because of its sampling versatility, minimal sample preparation, and compatibility with aqueous systems. Advances in instrumentation technology have enabled application of Raman spectroscopy in fields such as life sciences, biomedical specimens and process control in manufacturing environments. In these fields, the end goal is to make Raman spectroscopy a reliable analytical tool for non-specialists that is easy to use and with a low cost of ownership. Translating Raman spectroscopy for each of these fields has its own set of unique challenges, but there are common technological and logistical requirements. For the past 20 years, we have applied the measurement principles of Raman spectroscopy in a manufacturing environment for understanding, monitoring, and controlling continuous processes or unit operations. Raman spectroscopy in life sciences, biomedical or process environments requires an integrated approach, with careful attention to the reliability of the analyzer and sampling probe optics and transferability of the data analysis methodology. Some environments have additional environmental or regulatory requirements, which can impact technology development and product manufacturing. We broadly discuss these requirements in the context of customer applications and provide examples that illustrate the challenges, successes and benefits of Raman spectroscopy in life sciences, biomedical and manufacturing environments.

294 Raman Depth Profiles of Skin to Measure the Effectiveness of Topical Products Designed to Improve Hydration
Fran Adar, HORIBA Scientific, 3880 Park Ave., Edison, NJ 08820, Catalina David, Vincent Larat
Raman depth profile measurements have been used for more than 10 years to assess hydration of the skin as a function of depth below the surface. We show how the hydration depth profiles evolve as a function of time after the application of products such as glycerin, and several compounded skin care products. We will also explore the possibility of measuring the migration of identifiable ingredients through the various layers of the skin as a function of time. Note that these same techniques can also be used to monitor migration of active pharmaceutical ingredients applied with various drug delivery schemes through the skin.

295 Real-Time and In-Situ Monitoring of Pesticide Penetration in Edible Leaves by SERS Mapping
Tianxi Yang, University of Massachusetts-Amherst, Department of Food Science, 102 Holdsworth Way, Amherst, MA 01003, Lili He
Understanding the penetration behaviors of pesticides in fresh produce is of great significance for effectively applying pesticides and minimizing pesticide residues in food. There is lack, however, of an effective method that can measure pesticide penetration. Herein, we developed a new method for real-time and in-situ monitoring of pesticide penetration behaviors in spinach leaves based on surface-enhanced Raman scattering (SERS) mapping. Taking advantage of penetrative gold nanoparticles (AuNPs) as probes to enhance the internalized pesticide signals in-situ, we have successfully obtained the internal signals from thiamidazole, a systemic pes-
ticide, following its penetration into spinach leaves after removing surface pesticide residues. Comparatively, ferbam, a non-systemic pesticide, did not show internal signals after removing surface pesticide residues, demonstrating its non-systemic behavior. In both cases, if the surface pesticides were not removed, co-penetration of both AuNPs and pesticides was observed. These results demonstrate a successful application of SERS as an effective method for measuring pesticides penetration in fresh produce in-situ. The information obtained could provide useful guidance for effective and safe applications of pesticides on plants.

296 Study of Higher Order Structure of Stressed Monoclonal Antibody by Deep UV Resonance Raman Spectroscopy

Sergey Archzantsev, United States Food and Drug Administration, 645 S. Newstead Ave., Saint Louis, MO 63110, Chen Qu

The higher order structures of proteins can be disturbed by external stress and stress-induced changes may affect therapeutic activity of proteins. Deep ultraviolet resonance Raman (DUVRR) spectroscopy is capable to characterize the higher order structure of proteins in the presence of excipients in aqueous media with minimal sample preparation. DUVRR spectroscopy was applied to compare the higher order structure of formulated monoclonal antibodies, Rituxan and Avastin. The formulated products were degraded with chemical and thermal stress and the degradation products were analyzed using DUVRR spectroscopy. Chemical stresses included acidification and addition of surfactant to the formulated product. Changes were observed in the DUVRR spectra with each level of stress, and multivariate statistical models were applied to identify trends within the data. The similarity and differences between monoclonal antibodies under the stressed condition detected by DUVRR spectroscopy are discussed in this presentation.

297 Application of Raman and LIB Spectroscopy to Support Root Cause Investigation of Particulate Matter in Parenterals

Olga Laskina, rap.id Inc., Princeton Corporate Plaza, 11 Deer Park Dr., Ste. 201, Monmouth Junction, NJ 08852, Olivier Valet, Markus Lanterns The United States Pharmacoevea (USP)1787 chapter “Measurement of Sub-visible Particulate Matter in Therapeutic Protein Injections” defines different particle types: “extrinsic”, unexpected foreign material, “intrinsic”, from the production environment or primary packaging, and “inherent”, from the formulation itself. It is important that inherent particles are distinguished from the other two types and that the extrinsic and inherent particles sources are found and eventually eliminated. The revised USP<787> states that membrane microscopy method cannot visualize translucent particles and therefore is primarily suited for particles other than inherent. Micro-flow imaging technique allows quantification and characterization of sub-visible particles based on the morphology, but lacks chemical identification. Single Particle Explorer (SPE) instrument (rap.ID GmbH) can count, measure size, determine shape and chemical composition of all types of particles, such as organic, inorganic, metallic and glass. The source of the particles can be easily tracked based on the determined composition. We show that inherent, intrinsic and extrinsic particles can be analyzed in a single analysis with the use of one instrument. More than 200 particles per hour were analyzed using this method enabling large sample size statistical results for particulate matter identification. Extrinsic, intrinsic and inherent particles can be easily distinguished. Custom database allows quick and reliable analysis and eventually elimination the sources of contamination. Additionally analyzed, inherent particles is possible for better understanding the formulation properties. Additionally, in-situ analysis of some common extrinsic particles was successfully performed which allows avoiding time consuming sample preparation that can lead to the loss of particles and cross contamination.

298 Instrument Response Correction for Raman Spectral Library Searching

Jun Zhao, B&W Tek, 19 Shea Way, Newark, DE 19713, Jack Zhou

Raman spectroscopy is gaining widespread acceptance as a chemically specific identification tool, as manifested by the rapid growth of handheld analyzers in the past decade. A commonly used method to identify unknown material is searching through standardized spectral libraries by means of hit quality index (HQI). Due to variations in instrument response, Raman spectra of the same material acquired on different instruments are not directly comparable. To be useful, commercial Raman libraries are always corrected for Instrument response, typically using white light standards or National Institute of Standards and Technology supplied luminous glass. To match the standardized library spectra, the sample spectrum is also corrected for the instrument response. This practice, however widely adopted, has a serious drawback when used with dispersive instruments, as the correction magnifies the contribution to the HQI from spectral regions of lower responsivity, resulting in reduced specificity. This effect is particularly prominent in handheld analyzers using 785 nm excitation and charge-coupled device (CCD) detectors, where the instrument response varies greatly across the spectral range. To circumvent this problem, we propose a different way of treating the spectra, which results in significantly improved searching performance.

299 Using SPME GC/ITMS to Evaluate Drinking Water Removal Efficacy for Unregulated Organic Contaminants

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Steps involved in evaluating a source water contamination issue within the United States include a health assessment to determine criteria levels of concern for the chemical identified in the environment. This is often accomplished on an individual chemical by chemical basis even though the individual chemical is only one of a whole class of chemicals with similar formulations. State regulations stipulate a risk level of one in a million as the lifetime exposure threshold. This process generates very low human health criteria levels (part-per-billion or part-per-trillion) for quantification of the chemical of interest. Once the health based level is established, identification of the compound in finished-drinking water is the next step and many conventional methods of analysis cannot achieve these lower limits. Gas chromatography mass spectrometry (GC-MS) methods, employing solid phase microextraction (SPME) were used on the screen raw and finished water samples from sources in NJ. This methodology was capable of detecting compounds to the tens of ppb for some compounds and deemed a sufficient screening tool for the presence of multiple classes of organic contaminants not detected using other methods. Once detected the compounds had to be identified using mass spectral libraries, sorted based on raw water processing stage, subtracted for background and then characterized for potential risk. This presentation focuses on the non-standard SPME/GC/ITMS method developed to survey raw and finished water sources within the state to evaluate clean-up efficiency for unregulated organic contaminants. Data evaluation tools are also discussed.

300 Micro-Gas Chromatography Systems for Photochemical Assessment Monitoring Stations

Douglas R. Adkins, Defiant Technologies, 6814 Academy Pkwy West NE, Ste. A, Albuquerque, NM 87109, Patrick R. Lewis

In 2014 RTI International in cooperation with the Environmental Protection Agency undertook a study to evaluate eight auto-sampling gas chromatography (GC) systems for possible deployment in Photochemical Assessment Monitoring Stations (PAMS). These stations are designed to monitor ozone precursors in areas across the US where ozone levels routinely exceed acceptable levels. The precursors include NO and 3 carbonyls. Defiant Technologies made a last-minute entry into the competition and offered a truly portable system based on microfabrication technology. While the system made a respectable showing, it was a challenge to separate and detect the large number of analytes on a 4-meter column. Since the time of this test, however, Defiant Technologies has continued developing technology to meet the challenge. This new system includes a 13-meter long GC column with an integrated thermoelectric cooler for sub-ambient temperature operation. The GC can be ramped to multiple set-point temperatures for enhanced separations. It uses ambient air as the carrier gas and operates on less than 30 watts of power. The system also includes orthogonal detectors to sense a wider range of compounds. While the initial investigation addressed air quality, the resulting system can address a wide variety of applications including monitoring chemicals in pharmaceutical manufacturing, industrial processing, and food packaging. The construction of this new portable system and recent test results are the subject of this presentation.

301 Practical Guidance to Increase Productivity, Reproducibility, and Efficiency with Microwave Extraction for Environmental Labs

Anit Joshi, Milestone, Inc., 25 Controls Dr., Shelton, CT 06484, Tim Michel

Microwave solvent extraction (MSE) is an excellent addition to any contract environmental lab to compliment or replace existing Soxhlet, sonication, or accelerated solvent extraction technologies. Due to MSE’s high-throughput capabilities (48 samples/hr) and low solvent consumption (20-30ml/sample), labs can achieve significantly higher productivity and drastically reduce operation costs. United States Environmental Protection Agency (EPA) method 3546 for microwave extraction is applicable, to the extraction of semi-volatile organic compounds, pesticides, herbicides, phenols, polychlorinated biphenyls (PCB’s), and Polychlorinated dibenzodioxins / dibenzofurans (PCDD/f’s/PCDF’s). Closed-vessel microwave extraction allows for higher temperatures to be reached, accelerating the rate of extraction with complete control of instrument parameters allowing for great reproducibility between runs. Milestone’s Ethos UP for microwave extraction is utilized by a growing number of environmental labs. It will significantly increase productivity, reproducibility, and extraction efficiency while reducing everyday costs.
Surface-Enhanced Raman Scattering for Monitoring the Formation and Transport of Noble Metal Nanoparticles in Plant-Associated Systems

Huiyuan Guo, Stockbridge School of Agriculture, University of Massachusetts-Amherst, Paige Lab Room 413, Amherst, MA 01003, Baoshan Xing, Lili He

Noble metal nanoparticles (NMNs) attract particular attention from both the industry and the research community due to their unique properties and the concern regarding their biological behavior and toxicity. However, tracking their exposure and transport pathways in biological systems has been a challenge because of a shortage of effective techniques. Surface-enhanced Raman scattering (SERS) is a powerful technique that is able to detect NMNs based on the adsorbed ligands. In this study, we employed SERS-based strategies to monitor the formation and transport of NMNs in different plant-associated systems including growth matrices (soil or hydroponic system), root surface, and plant tissues (inside). Different SERS methods were used based on the characteristics of the sites. The enhanced spectra of SERS indicators suggest silver or gold nanoparticles (AgNPs or AuNPs) can be naturally formed within 6-24 h in wheat-grown soils and hydroponic systems, once contaminated by corresponding ions (0.1-1 mM). They were also detected on root surface and inside plant tissues. To investigate whether the detected NPs were transported to root surface and plant tissues or in-situ formed, we used citrate coated AuNPs (60 nm) as a model to treat live plants in hydroponic system and tracked the movement using SERS after extraction and in-situ SERS mapping without out-extraction. The data prove that AuNPs can transport into plant roots and shoots. Overall, this work demonstrates the dynamic formation and transport of NMNs in plant-associated systems by using SERS, which facilitates our understanding of the behavior and fate of NMNs in agricultural systems.

Comprehensive Real-Time Environmental Air Analysis Using Dual-Polarity SIFT-MS

Bary J. Prince, SyfT Technologies, 3 Craft Pl., Christchurch 8024, New Zealand, Daniel B. Milligan, Vaughan S. Langford, Murray J. McEwan, Chuck Renner

This paper introduces a multi-purpose analytical technique for atmosphere research and routine atmospheric monitoring applications: selected ion flow tube mass spectrometry (SIFT-MS). SIFT-MS is a real-time analytical technique that rapidly analyses air to ultra-trace levels for volatile organic compounds (VOCs) and inorganic compounds (Prince et al., 2010). SIFT-MS utilizes three positively charged reagent ions (Mg2+, Na+ and O2+) and five negatively charged reagent ions (OH-, O, O2-, NO2- and NO3-) to provide very selective analysis via gas-phase chemical separation coupled with mass spectrometric detection. The reagent ions are created from a microwave discharge through moist or dry air and subsequently mass-selected using a quadrupole mass spectrometer. The mass-selected reagent ions are then injected into the flow tube, where they react with the air sample introduced directly and continuously through the sample inlet. Reagent ions used for gas analysis are produced using a second mass spectrometer and detected with a particle multiplier. SIFT-MS provides a unique ability to conduct highly selective analysis in real-time, by applying multiple rapidly switchable reagent ions and mass spectrometric detection. The outcome is that gaseous pollutants in air are typically monitored at part-per-trillion levels within one second. We present data that demonstrate the ability of SIFT-MS to monitor a diverse range of VOCs and inorganic gases. Data illustrates the flexibility of the technique, including its ability to be used in full scan mode to identify and quantify non-targeted species.

Reference:

Advantages of Silica Hydride HPLC Stationary Phases for Food and Beverage Applications

Joshua E. Young, MicroSolv Technology Corporation, PO Box 4, South New Berlin, NY 1384, Joseph J. Pesek, Maria T. Matyska

Some of the problematic obstacles currently encountered in high-performance liquid chromatography (HPLC) analysis of ingredients in foodstuffs are investigated using HPLC columns based on silica hydride. Examples pertinent to the food and beverage industry include the difficulty in adequately retaining hydrophilic sugars by conventional chromatographic methods, low sensitivity/poor specificity of trans-resveratrol present in red wine, and time-consuming sample preparation steps typically required in analysis of folic acid in cereals. Silica hydride-based separation media using HPLC columns based on silica hydride have an array of unique properties that can be used to address these kinds of issues, both as analytical columns and as solid phase extraction sorbents. These characteristics include the ability to retain polar compounds by a separation mode known as reversed normal phase (ANP), more efficient removal of matrix interferents in sample preparation strategies, and liquid chromatography mass spectrometry compatible mobile phase conditions. This behavior is the result of the surface properties of silica hydride stationary phases, which have Si-OH groups replaced with Si-H and consequently do not strongly associate water as typical silica-based materials do. Emphasis is placed on applicability of methodologies to real-world samples containing the analytes of interest.

Method Development in the Use of Solid Phase Microextraction for the GC-MS Analysis of Pesticide Residues in Baby Food

Katherine K. Staszewski, MassHDX Harrison Rd., Bellefonte, PA 16823, Robert Shirley, Leonard M. Sidsky, Craig Aurand

Solid phase microextraction (SPME) is a technique that can be used for the analysis of a wide variety of analytes in many different sample matrices. It offers flexibility in that extraction can be done by direct immersion, or from sample headspace. Headspace is the preferred approach, especially for high background samples. However analytes with low volatility, such as pesticides, cannot be extracted from headspace. As a result, SPME is often used in combination with the sample. High background samples, such as many foods, pose a challenge with this approach due the presence of fats, sugars, pigments and other macromolecules. These can stick to the fiber and reduce its usable life and/or be transferred to the gas chromatography (GC), where they may interfere with chromatographic analysis. In this work, we present data illustrating the development of an SPME method for the GC-mass spectrometry (MS) analysis of pesticide residues from a heavy background food sample—baby food. Specifically, the advantages of using an overcoated vs. a standard SPME fiber are presented. Ruggedness of the SPME method was found to be improved through the use of the overcoated SPME fiber in combination with a post-extraction wash step. In addition, optimization of parameters in the SPME method such as sample pH adjustment, salt addition, sample dilution, and extraction temperature are described. Results are presented showing the final SPME method applied to samples of pureed prunes baby food spiked with pesticides included on the list described as part of EU directive 2006/125/EC.

Interaction between TiO2 Nanoparticles and Quercetin and Its Impact on Uptake of TiO2 Nanoparticles by Intestinal Epithelial Cells

Xiaojong Cao, University of Massachusetts-Amherst, 431 Chenoweth Laboratory, 100 Holdsworth Way, Amherst, MA 01003, Lili He, Hang Xiao

TiO2 nanoparticles are commonly presented in many food products as part of food additives (E171), and they have been associated with potential adverse effects on health. However, little knowledge is available regarding the interaction between TiO2 nanoparticles and food components such as flavonoids. In this study, we aimed to characterize the molecular interactions between TiO2 anatase nanoparticles and quercetin using surface-enhanced Raman spectroscopy (SERS). The SERS results clearly demonstrated that quercetin could bind TiO2 nanoparticles and the binding was due to hydroxy groups at 4’ and 5’ position of the quercetin structure. Dynamic light scattering analysis showed that TiO2 nanoparticles aggregated to form much bigger particles after interaction with quercetin, presumably due to the hydrophobic property of quercetin. The impact of interaction with quercetin on the cellular uptake of TiO2 nanoparticles was determined in the intestinal epithelium monolayers. As the particle size increased after interaction with quercetin, the uptake of TiO2 nanoparticles decreased. These results provide insights into the interactions between TiO2 nanoparticles and important food components and its implication on the intestinal fate of the TiO2 nanoparticles, which is critical for better understanding of potential adverse effects of TiO2 nanoparticles on human health.

Analysis of Mycotoxins in Various Food Matrices via SFE-SFC-MS

Todd M. Anderson, Shimadzu Scientific, 7102 Riverwood Dr., Columbia, MD 21046, Kenichiro Tanaka, Taizo Ogura

Mycotoxins are a metabolite of certain fungi that are produced throughout the harvest, transport and storage process. These toxins can therefore be found in everyday food products and can exhibit significant toxicity to humans and animals. Our objective is to develop a methodology by which we can detect these toxins from food products in a single instrument. Typically analysis consists of sample homogenization, some form of extraction technique followed by derivatization and finally analytical analysis. This talk discusses the means by which we utilize a single instrument to perform all these functions, except for the sample homogenization. We utilize samples of commercial commodities as a source for the mycotoxins and analyze for several mycotoxins using supercritical fluid chromatography (SFC) CO2 extraction, and SFC-mass spectrometry for analysis.
metallic nano-substrate. Herein, we developed an innovative SERS sandwich assay which is based on 3-mercaptopropionylglycine acid (3-MPBA) or aptamer as a capturer, and 3-MPBA and silver nanoparticles (AgNPs) as the reporter for non-selective and selective detection of bacteria. Both optical images and chemical images (SERS mapping) were used as mechanisms for detection and quantification. Using Salmonella enterica as a model bacterium, we have identified a unique bacterial SERS signal upon the interaction between the captured bacteria, 3-MPBA and Ag NPs, which was used as the base for reliable detection of bacteria using SERS mapping. Using either 3-MPBA or aptamer, we achieved sensitive detection and quantification of as low as 10^2 CFU/mL for nonselective detection of Salmonella. The total analytical time is less than 40 minutes. Our assay represents an innovative platform for rapid, sensitive and reliable detection of total bacteria and selective bacteria for an array of industrial applications. Future work will focus on evaluating this assay for detection of bacteria in real food matrices.

309 Rapid Quantitative NMR Analysis of Edible Oils to Combat Oil Adulteration
Sue (Shumiei) Wang, Industrial Technology Research Institute, 804 Grand Champion Dr., Rockville, MD 20850, Pei-Chen Chen
A food safety crisis has exploded in Asia recently. In 2000-2014, gutter oils and adulterated edible oils have affected more than 1000 businesses in China, Hong Kong and Taiwan. Traditionally, gas chromatography (GC) or GC-mass spectrometry (MS) have been used to analyze edible oil. Chromatographic methods take a long time and require chemical modification. Recently some researchers have used NMR (nuclear magnetic resonance) spectroscopy to determine the compositions of edible oils. Most of the NMR studies focused on the authentication of virgin olive oil throughout Europe and United States. In Asia, several high-priced specialty oils, tea seed oils and sesame oils, have also been adulterated with cheaper oils. We have collected and analyzed more than 80 edible oils consumed by Asians, including imported oils, Asian specialty oils and freshly-made lards. We have developed a rapid quantitative 1H NMR method to determine the fatty components in common edible oils, including linolenic, linoleic, oleic, and saturated fatty acid. The fatty compositions obtained from our NMR method correlate very well with the fatty compositions analyzed using official AOAC-996-06 GC method. In addition to 1H NMR and 13C NMR, several two-dimensional NMR techniques were also employed to identify minor components in sesame oils. Autodigestion of tea seed oil was detected during stability study. We also analyzed waste oils collected from restaurants. Minor signals associated with hydrolysis, oxidation and degradation were easily detected by 1H NMR. We have demonstrated that our NMR can identify virgin oils from blended or adulterated oils, and can also differentiate fresh edible oils from recycled waste oils.

310 Methods to Control Silicone-Induced Protein Agglomeration
Olgak Laskina, rap.id Inc., Princeton Corporate Plaza, 11 Deer Park Dr., Ste. 201, Monmouth Junction, NJ 08852, Oliver Valet, Markus Lankers
Silicone induced protein agglomeration is a common pathway of protein formulation separation. Silicone oils are often applied to the inner surface of syringes to form lubricating films. The uniform distribution of the silicone layer in prefilled syringe guarantees the consistent injection of the drug. However, protein based therapeutics can have strong interactions with the silicone oils. Excess of silicone or its non-homogeneous distribution can induce protein aggregation in formulations. The formation of protein aggregates is highly undesirable as it can cause undesirable immune responses resulting in reduced efficacy and even life threateningautoimmunity. In this work we investigated the relationship between silicone oil layer distribution and sub-visible particle formation. Layer Explorer (LE) instrument was used to characterize the silicone layer in syringes periodically during the storage period. LE is an automated device that measures layer thickness within the inner layer of a syringe and generates a map of the silicone oil. Single particle Explorer (SPE) instrument was then used to count and analyze the silicone particles as well the particles that were induced by heterogeneity of the silicone layer. The analysis of silicone layer distribution in syringes together with analysis of particles found in the formulation shows that heterogeneity of the silicone layer in prefilled syringes during storage causes the formation of protein aggregates. The study demonstrates the importance of maintaining the silicone oil layer homogeneity to insure the quality of protein based formulations in prefilled syringes.

311 Risk-Based Assessment to Determine Criticality of Reagents & Solvents and Extent of Analytical Methodology Validation
Andrew S. Marriott, Bristol-Myers Squibb, Reeds Lane, Moreton CH46 7AG, United Kingdom, James Chadwick, Ekong Bruce, Jing Kong
The reagents and solvents used in the synthesis of an active pharmaceutical ingredient (API) are commercially available and utilized regularly by multiple industries. These materials typically do not contribute significantly to structural elements of the regulatory starting materials, intermediates or API. In many instances reagents and solvents are commodity chemicals used to create simple compound derivatives or as reaction and crystallization solutions. A collaborative risk-based assessment process was developed to focus the analytical method development, validation and testing activities towards critical quality attributes of a material thereby ensuring that acceptable process efficiency, product quality and regulatory acceptance are maintained for the manufacturing of intermediates and APIs. The recommended reagent or solvent risk release test(s) are derived from process development knowledge, vendor material assessments, use tests and fate and tolerance (F&T) studies. This presentation outlines the risk-based assessment workflow and how reagent/solvent criticality and extent of analytical method validation is defined.

312 Analytical Investigation of an Unexpected Dehydration Behavior and its Impact on Material Properties
Roxana Schlam, Bristol-Myers Squibb, 1 Squbbl Dr., New Brunswick, NJ 08903, Matthew Haley
The selected form of a drug is a monohydrate and was found to display unusual dehydration behavior. A detailed characterization using several analytical techniques are presented to discuss the discovery, elucidation, and understanding of this phenomenon at the molecular level, in the bulk properties, and its impact on active pharmaceutical ingredient processing and drug product performance. The knowledge gathered from this work has implications beyond this project and may be relevant to other hydrated pharmaceutical solids.

313 Utilizing Predictive In-Vitro Methodologies to Guide Successful Development of Gastro-Retentive Drug Delivery Systems
Sanjaykumar Patel, Merck & Co., 126 East Lincoln Ave., MS: 80T-A168, Rahway, NJ 07204, Pranav Gupta, Hong Xu, Gerard Bredael, Evan Friedman
Gastro-retentive (GR) controlled release technology is often used to prolong absorption for drugs such as Raltegravir that is primarily restricted to the upper GI tract. Single and dual swell polymeric GR formulations were developed to achieve C24 trough concentrations comparable to the BID immediate release (IR) formulation. Given lack of good preclinical animal models for GR systems makes it imperative to rely on predictive in-vitro analytical tools for optimal pharmacokinetic (PK) performance. Various in-vitro analytical methodologies such as disintegration modified USP and USPIII apparatus were used to investigate drug release mechanism and swelling/erosion profiles of the GR formulations under fasted/fed state. Formulations robustness was confirmed by performing alcohol induced dose dumping in SFG/FASSIF. The performance of GR formulations were evaluated in a flexible clinical study design paradigm for determination of PK and in-vivo gastric retention times via scintigraphic imaging. GR formulations were determined to swell to at least twice their original size within an hour followed by continued swelling to at least four times their original size over 9-12 hrs. Based on erosions studies and USPIII analysis, drug release from formulations was determined to be via an erosion mediated mechanism. No significant difference in drug release was observed between modified USP and USPIII dissolution. Furthermore, the formulations were found to be robust to alcohol induced dose dumping. Preliminary proof-of-concept clinical data demonstrated prolonged gastric retention of up to ~14-16 hours with dual swell polymeric GR formulations with C24 trough concentrations comparable to the IR formulation.

314 Analytical Challenges in Extractable Studies of IV Bags
Dujian Lu, SGS Passaic Ave., Fairfield, NJ 07004, Kenneth Wong, Jing Kong, Danny Hower, Kate Comstock, Ekong Bassey
Intravenous (IV) therapy is commonly used in hospitals. Solutions in IV bags, such as IV drugs, blood-based products and parenteral nutrition, are infused directly into the veins of the patients in significant amounts. Most of the IV bags are made of plastics and those plastics additives and degradants may easily leach out into the IV solutions. As part of safety risk assessment, it is very important to identify and quantify those extractables and leachables (E&L) as they may pose safety risks to patients and/or change the efficacy of the medical products. An extractable study was performed on IV bags by using different solvent systems, such as acidified water, alkaline water, PBS buffer, and organic/aqueous solvent mixtures to bracket and mimic pH values, ionic strength and hydrophobicity of common IV solutions. In order to obtain a comprehensive extractable profile, multiple analytical techniques were used to identify and quantify the extractables, including headspace gas chromatography mass spectrometry flame ionization detection (HS-GC-MS-FID) analysis for volatile organic compounds, GC-MS-FID analysis for semi-volatile organic compounds, liquid chromatography mass spectrometry ultraviolet (LC-MS-UV) analysis for non-volatile organic compounds, and inductively coupled plasma optical emission spectrometry (ICP-OES) analysis for trace elements. During E&L analysis, analysts encountered unexpected analytical challenges. For instance, during this study, there was one unexpected finding that the vapor of the volatile organic solvents in the IV bags could migrate into the solvents in other bags. Modified experiments were then applied to investigate the root cause and effectively eliminated the problem. In addition, the diversified extractable profiles were compared and discussed for different extraction solvents. The results also demonstrate that high resolution accurate mass (HRAM) data facilitate confident compound identification and unknown compound structure elucidation.
315 Determining pH of Maximum Stability to Enable Development of Liquid Formulation
Nicole Ferreira, Merck & Co., 126 E. Lincoln Ave., Rahway, NJ 07065, Tiffany Jarrell, Alexander Chin, Lila Low-Beinart, Brian Regler, Alfred Rumondor, Chaitanya Wannere
In order to inform the drug product development of a robust liquid formulation, a study was designed to assess the energy of activation of the major degradation products as a function of pH. The active pharmaceutical ingredient (API) was subjected to hydrolysis in various pH buffers ranging from pH 2 to pH 10 under elevated temperature stress conditions between 30°C and 90°C. Aliquots were analyzed for up to 48 hours (and, in some cases up to 144 hours) to determine reaction rates for six major degradants. These degradants demonstrated a remarkable correlation for pseudo zero-order kinetics rate (% degradant versus time). Arrhenius plots (ln(rate) versus temperature-1) were used to derive the activation energies at each pH for each degradation product. Results indicate that a pH range of 4 to 8 will provide most stable environment for an aqueous formulation, as the activation energies for most of the degradants were relatively high in this pH range.

316 Method to Improve the Recovery of pH Labile Anti-Drug Antibodies during Acid Dissociation and Extraction for Immunogenicity Testing
Weifeng Xu, Bristol-Myers Squibb, MS: L14-08, Rte. 206 and Province Line Rd., Princeton, NJ 08543
When large amount of biotherapeutics drug is present in the clinical samples, these drugs have to be dissociated and removed from anti-drug antibodies (ADA) so that ADAs can be detected by either ligand-binding assay or cell-based bioassay. By screening a panel of more than 20 ADA positive control (PC) Abs, we found that currently widely used acid dissociation followed by biotinylated-drug extraction led to low recovery of more than 40% of these ADA PCs, due to sensitivity to low pH and denaturation. Here we discuss the alternative methods for ADA extraction so that pH labile species can be maximally recovered. This will increase the sensitivity of immunogenicity testing.

Costel C. Darie, Clarkson University, 8 Clarkson Ave., Potsdam, NY 13699, Roshanak Aslebagh, Bruce A. Pfeffer, Steven J. Fliessler
Modification of proteins by 4-hydroxy-2-nonalen (HNE), a reactive by-product of ω polyunsaturated fatty acid oxidation, on specific amino acid residues is considered a biomarker for oxidative stress, as occurs in many metabolic, hereditary, and age-related diseases. HNE modification of amino acids can occur either by Michael addition or by formation of Schiff-base adducts. These modifications typically occur on cysteine (Cys), histidine (His) and/or lysine (Lys) residues, resulting in an increase of 156 Da (Michael addition) or 138 Da (Schiff-base adducts), respectively, in the mass of the residue. Here, we employed biochemical and mass spectrometry (MS) approaches to determine the MS “signatures” of HNE-modified amino acids, using lysozyme and bovine serum albumin (BSA) as model proteins. Using direct infusion of unmodified and HNE-modified lysozyme into an electrospray quadrupole time-of-flight mass spectrometer, we were able to detect seven HNE modifications per molecule of lysozyme. Using nanoliquid chromatography tandem mass spectrometry, we found that, in addition to N-terminal amino acids, Cys, His, and Lys residues, HNE modification of arginine (Arg), threonine (Thr), tryptophan (Trp) and histidine (His) residues also can occur. These sensitive and specific methods can be applied to the study of oxidative stress to evaluate HNE modification of proteins in complex mixtures from cells and tissues under diseased vs. normal conditions.

318 Reduction of Anthelmintic Drug (Methyl [1]carbamate- albendazole) on Electrodes Electrode Surfaces and Analysis of Chromatographic Reduction Products
Amos M. Mugweru, Rowan University, 201 Mullica Hill Rd., Glassboro, NJ 08028, Zahalis Mazzochette, Geoffrey Kamau
Methyl [1]carbamate- albendazole, is a widely used anthelmintic drug for the treatment of parasitic worms and other diseases. The electrochemical reduction of albendazole (ABZ) was carried out in ammonium bromide- acetonitrile solution under stirring conditions. The reduction of albendazole at gold electrode gave three non-reversible waves corresponding to three major products. The products of electrochemical reduction were characterized using liquid chromatography- mass spectrometry. The observed results suggested that the second and third reduction steps occurred at the sulfur functional group of albendazole.

319 The Optimization of Protein Buffers
Shawn A. Clark, Delta™ Technologies, 65 Main St., Ste. 3101, Potsdam, NY 13676, Jeff A. Signor
Proteins comprise an extremely diverse class of biological macromolecules. They are often unstable when not in their native environments, which can vary considerably. If specific buffer conditions are not maintained proteins may lose activity, aggregate or fall into proteolysis. For this reason, it is essential to nominate specific factors that increase the physical and chemical stability of a given target protein. Delta™ Technologies has developed a method based on the thermal stability assay (TSA) to rapidly and efficiently nominate factors that increase target stability. These screens define buffers to optimize: protein purification and kinetic buffers, cryopreservatives, quality control measures and crystallization conditions. Our smart research and development solutions encourage basic and applied research by characterizing a stable, consistent source of proteomic materials. This promotes efficiency by increasing experimental accuracy and product longevity which translates to reduced cost and collateral waste.

320 Nod2 Directly Binds Muramyl Dipeptide through Its Leucine Rich Repeat Domains
Mackenzie L. Lauer, University of Delaware, 32 Wenark Dr., Apt. 3, Newark, DE 19713, Brian Bahnson, Catherine L. Grimes
Crohn’s disease (CD) is a debilitating, inflammatory bowel disorder that is proposed to arise from an atypical reaction to commensal bacteria. CD patients suffer from a complex host of dysregulated interactions between their innate immune system and microbiome. The most predominant link to the onset of CD is a genetic mutation in the innate immune receptor Nucleotide-binding Oligomerization Domain-containing 2, (Nod2). Nod2 responds to the presence of bacteria, specifically a fragment of the bacterial cell wall, muramyl dipeptide (MDP). CD mutants of Nod2 have an aberrant signaling when triggered by MDP that leads to uncontrolled inflammation, and the basis of this interaction is not understood at the molecular level. By investigating the molecular interactions between Nod2 and MDP, we can gain insights into the mechanism of CD. A functional Nod2 construct has been successfully purified in high yield by E. coli expression. This construct includes the leucine rich repeat (LRR) domain, a common motif for protein interaction with ligands. Using surface plasmon resonance (SPR), we determined that the LRR domain is sufficient for binding MDP with high affinity. A proposed binding region of this domain was also determined by protein modelling and ligand docking using Autodock 4.2. Alanine scanning mutagenesis of these critical residues is being used to determine the effects on binding and activation. Current work also aims to confirm the critical portions of the LRR for binding using solid state NMR experiments with isoptide labeled protein.

321 Building a Protein Biomarker Signature for Early Detection of Breast Cancer Using Discovery Proteomics
Roshanak Aslebagh, Clarkson University, 8 Clarkson Ave., CU Box 5810, Potsdam, NY 13699, Costel C. Darie, Kathleen F. Arcaro
Early detection of breast cancer (BC) is critical due to the lower 5-years relative survival rate in late stages of BC. Different types of bodily fluids can be analyzed for identification of BC biomarkers, such as blood/plasma/serum, nipple aspirate fluid and ductal lavage fluid, tears, urine, saliva and breast milk. We analyze human breast milk as an appropriate cancer microenvironment for BC biomarkers discovery using physical and chemical stability of protein (one- or two-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)) and in-solution digestion of human breast milk samples coupled with nanoliquid chromatography tandem mass spectrometry (nanoLC-MS-MS) followed by data processing, data base search and statistical analysis. We were able to identify the differences in the proteins expressed in the breast milk of the donors with BC, as compared to controls. These alterations, such as downregulation and upregulation in protein expression could be related to BC. These comparisons could help us in building a biomarker signature. We also used mass spectrometry to identify a biomarker signature that will allow us to predict the onset of BC. We did so by analysis of the breast milk samples that were donated 1-2 years prior the donors were diagnosed with BC by biopsy test. This is an ongoing investigation and currently we are analyzing more milk samples from BC and control donors which will allow us to build a strong and robust statistical-backed protein biomarker signature.

322 Investigation of NIR Spectral Shifting Due to Sample Temperature Variation
Herman He, Thermo Fisher Scientific, 4410 Lotsford Vista Rd., Latham, NY 12110
Near-infrared spectroscopy (NIR), due to its fasts scanning speed, non-destructive, and the ability of using optic fibers and probe, makes it an excellent for process analytical technology (PAT) applications. NIR measures the intensities and vibration frequencies of function groups inside molecular compounds and uses chemometrics to correlate sample spectral info to chemical concentrations. NIR is a very sensitive tool but like other spectroscopic techniques, it can be affected by many other physical and environmental parameters, like sample particle size distribution, tempera-
tune, and pH value. In this presentation, DI water and vegetable oil were selected because their popularity, and the investigation includes the degree of shifting in both wavelength and absorption peak intensity due to sample temperature variation. The discussion also includes the effectiveness of a few spectral pre-processing techniques to compensate the sample temperature variations in model predictions.

323 Studying RNA-Protein Binding Using Paramagnetic Relaxation Enhancement for In-Cell NMR
In-cell nuclear magnetic resonance (NMR) spectroscopy is a powerful technique to study proteins in their native cellular environments. Advanced NMR experiments have given rise to individual resonance frequencies which provide a spectrum of an isolated protein. This spectrum can provide a wealth of information about a protein’s structure, conformational state, and interactions. The capabilities of these advanced NMR experiments have resulted in an emerging interest in expanding the scope of in-cell NMR to study intracellular RNA-protein interactions. This is a more complex type of NMR experiment, as it would require isolating labeling the RNA in addition to the protein. This work involves the development of an alternative labeling method involving paramagnetic transition metal ion-based probes which can be covalently attached to RNA in the vicinity of the protein’s binding site. These paramagnetic probes enhance the relaxation of spins from nuclei in close proximity, and because the extent of relaxation is directly related to the probe’s proximity we are able to construct a protein contact surface map to visualize the RNA-protein interaction. “Proof-of-principal” in-vitro studies have been completed using a model system. Preliminary in-vivo studies are currently underway.

324 Observation of Pitfalls of 2Jcc and 3Jcc Correlations in 1,1-ADEQUATE Spectra
Hai-Young Kim, Merck & Co., MS: 3-148, 33 Avenue Louis Pasteur, Boston, MA 02115, Josep Sauri, Ryan D. Cohen, Gary Martin
1,1-ADEQUATE is a powerful nuclear magnetic resonance (NMR) method which has been used to establish one bond carbon-carbon correlations with modest sample quantities when cryogenic probe technology is available. Albeit this advantage, potential drawbacks such as weak or missing 1JCC correlations in strongly coupled 13C-13C AB spin systems and unexpected cross-peaks from unusually large multi-bond (nJCC) correlations for particular functional groups are not widely investigated. These unexpected cross-peaks can sometimes complicate 1,1-ADEQUATE spectra with rather strong intensity leading to misinterpretation in structural connectivity. The presence of these anomalous correlations is intensified when the homodecoupled version of the experiment (HD-ADEQUATE) is utilized. Several examples showing large nJCC in 1,1-ADEQUATE spectra are illustrated, with the results supported by the measurement of the 2JCC coupling constants in question using J-modulated ADEQUATE and DFT calculations.

325 Practical Considerations for Use of Either 1H-13N or 13C-15N Coupling Constant Measurements for Geometric Assignment
Dawn Pierce, DuPont Crop Protection, MS: S315/1347B, PO Box 30, Newark, DE 19714, Michael Kline, Steve Cheatham
In addition to correlation data, 1H-13N and 13C-15N coupling constants are a powerful tool for structure determination. The relationships between structure and coupling constants can be complex, however, and successful application requires selection of the appropriate experiment. The best experiment to utilize can be determined based on the complexity of H-1H homonuclear splitting, sample availability, and solubility. In this poster we detail a simple “decision tree” for structure determination using 1H-13N and 13C-15N coupling constants and provide typical sample conditions required to obtain the 1H-13N or 13C-15N coupling constants.

326 Mapping Bacteria on Filter Membranes, an Innovative SERS Approach
Siuyi Gao, University of Massachusetts-Amherst, 102 Holdsworth Way, Amherst, MA 01002
The existence of pathogenic bacteria in drinking water has been a threat to the safety of human well-being. Traditional methods to detect bacteria are standard plate counts or enzyme-linked immunosorbent assay (ELISA) and protein coupled receptor (PCR). However, those methods can be time-consuming or involve complicated procedures. Filtration of bacterial cells on a membrane and followed by culturing the membrane has been applied for water quality control. In this study, we applied surface enhanced Raman spectroscopy (SERS) to rapid screening of bacterial cells before membrane culture. After collecting bacteria cells onto a membrane, we incubated the membrane with 4-mercaptopropionic acid (4-mpba) for 30 minutes so that the diol group of the molecule can specifically bind to the surface of the bacteria. Then we wash the membrane to eliminate excess 4-mpba, leaving only the 4-mpba molecules that are bound to bacteria. With the addition of gold nanoparticle probes, 4-mpba gives characteristic SERS signal, indicating the presence of bacteria. The developed method can detect E. Coli down to 10^4 CFU/ml in 80 minutes using 1 ml of water sample, which has been validated with membrane culture down to 10^2 CFU/ml. In addition, Salmonella and Listeria have been successfully detected at 7.27*10^2 CFU/ml and at 8.37*10^1 CFU/ml, respectively. The developed SERS mapping method offers a rapid screening and estimation of total bacterial level in water samples.

327 Using NMR and Partial Least Squares to Predict Biomass Pyrolysis Oil Properties and Elemental Composition
Gary D. Strahan, United States Department of Agriculture, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, Charles A. Mullen, Akwasi A. Boateng
Interest in the pyrolysis of biomass has grown with increasing demand for renewable resources for fuel production. Current methods for pyrolysis often produce highly heterogeneous organic materials that require separation as separate fractions and hydrotreatment for deoxygenation before they can be used as suitable fuels or for petroleum blends. The resulting pyrolysis oils are complex and variable mixtures, often with unique properties due to changing feedstocks and processes. Consequently, the chemical and elemental analysis of these biofuels is often arduous, requiring many different and customized analytical steps. In this work we utilized nuclear magnetic resonance (NMR) spectroscopy, which can characterize an entire sample in one step without prior treatment, to construct several partial least squares (PLS) models capable of predicting their elemental compositions and several important chemical properties. These models were built using the 1H and 13C NMR spectra of 56 different pyrolysis oils from various sources (crude and intermediate products), as well as fossil fuels (gasoline and diesel) and several small molecule standards. The intensities of the NMR spectra these samples were binned and subjected to partial least squares analysis to correlate them to their fractional mass content of H, C, O, and N, as well as to their values of higher heating value (HHV), total acid number (TAN), and concentrations of phenols and cresols. Two models based exclusively on 13C-NMR data demonstrated the best over-all ability to predict these same characteristics for an unknown sample. These models are expected to also be useful for a wider range of applications.

328 Towards Minimum Reporting Standards for Microfading Tests
Betty Sacher, University College London, Institute for Sustainable Heritage, Central House, 14 Upper Woburn Pl., London, WC1H 0NN, United Kingdom, Jacob L. Thomas, Stefania Signorello, Matija Strlic
Defining the light sensitivity of objects in heritage collections is crucial for their long-term preservation. Microfading tests are currently underway. A sub-millimeter area of an object is exposed to light of high intensity and the change of reflectance measured simultaneously by a spectrophotometer. The test is virtually non-destructive and relatively quick, and is used on diverse materials such as graphic media, textiles, photographs etc. However, the technique has not been standardized and there are no generally agreed test protocols to ensure reproducibility and comparability. In this project, the impact of material characteristics such as texture and roughness on microfading data is investigated, taking aspects such as directionality and the measuring background into consideration. The role of the instrument design is assessed by comparing two different instruments: the Newport Apex Microfading Tester and a retro-reflective system by Townshend & Thomas LLP with variable probe head position. Two different light sources (LED and xenon) are used in this protocol. The best protocol would be developed with the view to achieve reproducible and comparative data, which may facilitate inter-laboratory exchange of fading data in the future.

329 Overcoming Challenges in Solid Dosage Form Reflectance IR Microscopy Imaging
Ronald L. Rubinovitz, Thermo Fisher Scientific, 4410 Lottsford Vista Rd., Lanham, MD 20803
Through the use of automated sample stages and array detectors, it is not uncommon to collect maps containing thousands of spectra using infrared (IR) microscopy. In order to efficiently analyze these large spectral data sets, a number of methods have become common software tools for extracting chemical distribution information from maps. However, these methods generally require that the spectrum of any particular chemical component remain constant across the surface of the map. Normally this is a straight-forward consequence of transmission and attenuated total reflectance (ATR) measurements; however the effects of particle size and crystallinity can introduce significant variation in the spectra of powders when they are measured in reflectance mode, offsetting the speed and minimal sample preparation advantages of non-contact reflectance mode compared to ATR and transmission (LED and xenon) are used. The aim is to develop a test protocol with the view to achieve reproducible and comparative data, which may facilitate inter-laboratory exchange of fading data in the future.
spectral variation, thus producing more accurate composition maps. Results are shown from a number of solid dosage form cross sections in which active materials are present in varying concentrations, as well tablets containing multiple actives.

330 Scanning Electron Microscopy and Its Capability in the Analysis of Collagen-Based Devices
Hao Fu, Integra LifeSciences, 315 Enterprise Dr., Plainsboro, NJ 08536.
Timothy Brockman, Thomas Twardowski
Scanning electron microscopy (SEM) is considered an essential tool for a broad range of industrial investigations, including biomedical, biological, forensic science, and pharmaceuticals, providing information about the surface topography and chemical composition. SEM is able to produce images with high resolution, at a wide range of magnification levels, with exceptional depth of field as well as versatility and convenience of use. Modern SEM can be used to generate high quality images. However, maximizing the capabilities of the SEM to obtain optimal image information from fragile, non-conductive and often wet samples such as collagen-based medical devices can require understanding many different parameters as well as different modes of detection available. This poster discusses a number of different techniques in scanning electron microscopy for analyzing advanced collagen devices, collagen dispersions, and other fragile biomaterials. Some techniques discussed include reaching high magnification (10,000 x and higher), charge management, beam deceleration for surface sensitive samples, analysis of wet, slurry, moist, or dynamic samples, contamination investigation using energy-dispersive X-ray, and image analysis.

331 USP Monograph Modernization - Development and Validation of a Microwave Assisted Acid Digestion ICP-OES Method for Selenium Quantification in Selenium Sulfide Shampoo and Lotions
Michael G. Truchan, United States Food and Drug Administration, 300 River Place Dr., Ste. 6700, Detroit, MI 48207. Weiwei Kuo, Geneve M. Maxwell, Aurora M. Trifanof, Philip A. Klimkiewicz, Bruce D. Harris, Ian P. Meyers, Susan H. Moini, Michael Chang, Alan R. Potts
The United States Food and Drug Administration (FDA) in collaboration with United States Pharmacopeia (USP) is engaged in modernizing existing compendial monographs to ensure product quality and safety. The current USP selenium sulfide topical suspension monograph uses an open digestion followed by a non-specific titration procedure for assay. USP and FDA recognize the need to update the selenium sulfide topical suspension compendial monograph with up-to-date technology to deliver improved specificity and sensitivity. Due to inherent sample matrix difficulties of topical suspensions, a microwave assisted acid digestion with inductively coupled plasma-optical emission spectrometry (ICP-OES) method is a modern replacement to the titrimetric assay and colorimetric identification of selenium (Se). This current study aimed to develop a method to quantify Se by ICP-OES after microwave acid-assisted digestion, and the method was validated according to USP General Chapter 1222 for 1 % selenium sulfide (SeS2) shampoo and 2.5 % SeS2 lotion. Method validation characteristics, specificity, linearity, system suitability, solution stability, robustness, precision, accuracy, and intermediate precision were evaluated for their respective preset acceptance criteria and the results were well within the acceptance limits. We developed and successfully validated a two-step microwave assisted acid digestion method with ICP-OES method to determine levels of SeS2 in topical suspensions of various therapeutic strengths. Our method utilized microwave assisted acid digestion and achieved greater laboratory safety with a closed digestion process. Incorporating current ICP technologies, our newly proposed method delivery specific and accurate quantification of Se to meet the public health standard.

332 A New Algorithm for the Identification of Spectra of Components in Mixtures
Richard Jackson, Galaxy Scientific, 14 Celina Ave., #17, Nashua, NH 03063, Qian Wang
Infrared, near-infrared, and Raman spectroscopy are well established techniques for the identification of unknown materials, because comparison of the spectrum of the material under investigation to a reference spectrum is often a very reliable indicator of identity. There are numerous metrics used for such spectral comparisons, such as Euclidean distance or correlation coefficient. These metrics may perform extremely poorly; however, when comparing the sample spectrum of a mixture to a reference spectrum of a pure component contained in that mixture. It should be noted that in this context “mixture” does not necessarily mean that the components are physically mixed, but rather that they all contribute to the spectrum. If the spectra of the components in the mixture are known, then in principle spectral subtraction can be used to remove all the spectral contributions except the one that is of interest. In practice however, this is difficult, and may not be feasible at all if the spectra of some components (e.g., a natural product) exhibit variability between samples. We have developed a new algorithm that can extract the spectrum of the component of interest from the spectrum of the mixture, so that the extracted spectrum can be compared to the reference spectrum, even in the case where the spectrum of one or more components exhibits variability. Details of the algorithm, and several examples of its application are presented.

333 Melamine Detection Using Surfaced Enhanced Raman Spectroscopy (SERS) and 1064 nm Handheld Raman Spectrophotometer
Joseph M. Stoltz III, MS, Joseph J. Fizer, MS: MS8156-048, Eastern Point Rd., Groton, CT 06475, Jonas E. Sacco, Thomas MacNeil, Suzanne Schreyer
One approach for facilitating the identification of melamine adulteration is to develop a portable method for on-site rapid testing. We describe the detection of melamine using surface enhanced Raman spectroscopy (SERS) in conjunction with a 1064 nm hand held, portable Raman spectrophotometer with minimal sample preapration. Several SERS techniques are explored and results presented.

334 Faster and Improved Ease-of-Use Assays of Citrate and Phosphate in Pharmaceutical Formulations Using Ion Chromatography with Suppressed Conductivity
Hua Yang, Thermo Fisher Scientific, 1214 Oakmead Pkwy, Sunnyvale, CA 94085, Parul Angrish
Citrate and phosphate are important counter ions in pharmaceutical formulations. The purity of counter ions affects the active pharmaceutical ingredient (API) solubility and subsequently impacts the efficacy and toxicity, it is therefore important to accurately assay counter ions. The benefits of using ion chromatography methods to determine counterions was previously demonstrated and validated. The method has been adopted by United States Pharmacopeia (USP) in General Chapter <345>; however, there have been many technological advancements since then. The previous monograph specified an L61 column. Here, however, we demonstrate that a higher porosity, higher capacity, and smaller particle size L81 column with similar selectivity to L61 has much higher efficiencies, capacities, and sensitivities, and extended linear range with reduced run times. Additionally, these analyses are facilitated by a high-pressure capable compact in chromatography system with many features that increase ease-of-use, including low void volume universal fittings, separate compartment for tighter temperature control, longer consumables life, and consumables tracking to meet device monitoring requirements.

335 Withdrawn by the author.

336 Development of Novel Non-conventional Dissolution Method to Monitor Amorphous Conversion of API in Low Drug Loading Solid Dosage Forms
Steven Wang, Merck & Co., 126 E. Lincoln Ave., Rahway, NJ 07065
Physical stability of an active pharmaceutical ingredient (API) plays a significant role in solid dosage form development. Undesired form change could have significant impact on bioavailability and safety of the drug product. Amorphous conversion is one of the form changes that could have significant impact on both solubility and stability of the API. Among many well-established techniques such as X-ray powder diffraction (XRPD), Raman, thermal analysis, and solid state nuclear magnetic resonance (ssNMR) are routinely used to monitor form change in the solid dosage formulations, monitoring amorphous conversion in low drug load (DL) dosage forms continues to be a significant challenge due to insufficient sensitivity or low throughput. Therefore, developing an alternative sensitive method to monitor form change in low drug loading products is highly desirable to fulfill this unmet business need. Potential amorphization and disproportionation has been demonstrated for Merck & Co. compound A in drug product. In this study, a novel non-conventional dissolution method was developed to monitor the amorphous conversion in low drug loading drug product (DL <0.3%). This novel method takes advantages of significant solubility differences (> 20x) between amorphous and crystalline API in selected organic solvents, such as toluene and 80/20 heptane/ethyl acetate. This organic solvent based non-conventional dissolution method successfully detected dissolution rate changes of Merck & Co. compound A during stability studies. In contrast, similar aqueous based conventional dissolution failed to discriminate amorphous and crystalline forms in the drug product. This study demonstrated for the first time that organic solvent based dissolution can be used to detect undesired form change in low drug loading product.

337 Using Analysis of Variance (ANOVA) to Optimize the Standard Preparation of Amorphous Drug Substances
John S. Lena, Merck & Co., MS: PYRO7-8164, 126 E. Lincoln Ave., P.O. Box 2000, Rahway, NJ 07065, Paul L. Walsh, Jennifer Drake, David J. Lavrich
In analytical analysis, one of the greatest challenges can often be the drug substance itself. Proteins and more specifically peptides are typically amorphous and hygroscopic with a low bulk density. These properties can lead to variability in preparation standards, a process that needs to be reliable and reproducible, especially in a quality control environment. One way to mitigate potential reproducibility issues is to employ analysis of variance (ANOVA) testing. ANOVA testing uses statistical
an analysis to measure the difference between selected key components that can be attributed to potential sources of variation. A process flow diagram was created to determine the areas with the greatest potential for variance in a standard preparation procedure. For the purpose of this experiment, several key easily controllable factors were tested by designing a fully nested ANOVA. Identified variances include the type of balance used, the absolute amount of drug substance weighed, the composition of the prepared solution used, and the solution made with glassware. Each of these factors contributes to the total variance, and by controlling each factor independently, we were able to deliver a standard preparation procedure that was easily reproducible with a low relative standard deviation.

338 Dissolution Method Development of a Delayed Release Compound
Margaret Roeder, Merck & Co., MS: RY 807-B168, 126 East Scott Ave., Rahway NJ 07065, Zheng (Jen) Zhao, Sanjaykumar Patel, Justin Pennington

Dissolution testing measures the rate of drug substance release into solution, impacting the extent of drug absorption. Drug manufacturers and regulatory agencies use dissolution testing to identify drug products that do not meet the desired clinical performance. Formulation composition and manufacturing processes can impact the material attributes of a drug product, which directly affect the dissolution mechanism. The dissolution method must be robust and sensitive in order to accurately measure these attributes. The establishment of dissolution specifications involves the creation of a test method as well as acceptance criteria driven by regulatory and internal expectations. Here, we present the dissolution method development of a solid dispersion comprised of active pharmaceutical ingredient (API) and polymer. The drug product is a delayed release dosage form, designed to resist drug release in the stomach using a pH sensitive polymer in the solid dispersion. For such dosage form, dissolution testing should adhere to United States Pharmacopeia (USP) <711> Dissolution for Delayed-Release Dosage Forms, which utilizes 0.1N HCl (pH 1) as the first stage acid medium, unless there is evidence to use a more appropriate method to evaluate dissolution performance. Early dissolution method development utilized 0.01N HCl (pH 2) as the acid medium based on consistent drug release obtained in buffer stage. Continued dissolution method development of this compound focusing on the impact of acid medium, pH, particle size, and optimization of surfactant level is presented. This development work provides justification for selecting the appropriate dissolution method for further analytical development and commercialization of this drug product.

Development of an Atypical Dissolution Method for a Modified Release Solution
Raghini Narla, Merck & Co., 126 East Lincoln Ave., Rahway NJ 07065, John Hayes, Keith Freehauf, Tiffany Jarrell, Brian Regler

An in-vitro dissolution method has been developed for a subcutaneous long acting parenteral solution-based drug product containing a poorly water soluble active pharmaceutical ingredient (API). Center for Veterinary Medicine (CVM) expects data submitted in the New Animal Drug Application (NADA) to demonstrate that the formulation and procedures used in manufacturing will ensure release of the active ingredients of the drug at a safe and effective rate and that these characteristics will be maintained until the drug’s expiration date. During field studies of this non-aqueous injectable formulation, a depot of API was observed at the injection site. The dissolution method was developed that would exhibit this behavior. Following Guidance for Industry #238, a stepwise approach to method development was followed, including assessment of API solubility in various media, evaluation of United States Pharmacopeia (USP) Apparatus II and Apparatus IV, and demonstration of the discriminatory power of the dissolution method. The final method was performed on USP Apparatus II, with sample introduction by an electronic pipette. Upon addition of the sample, a bolus of product formed at the bottom of the vessel, mimicking the in-vivo performance. Following a ten minute delay, paddles were rotated at 50 rpm, with samples withdrawn over 48 hours. The method was evaluated for its ability to discriminate among formulation variations and stressed drug product.

Automated Release Control System (ARCS) for Long Term In-Vitro Release Testing of Extended Release Dosage Forms
Ye Tian, Merck & Co., MS: RY80M-213, 126 E. Lincoln Ave., Rahway, NJ 07065, Parul Kadakia, Zhentian Wang

In-vitro release (IVR) rate is a key test in pharmaceutical product development. It measures the quantity of drug active(s) released into a highly defined solution system in a certain time interval, and reflects the in-vivo behavior of drug(s). For polymeric dosage forms, e.g., vaginal rings, implants, intra-uterine system, etc., it requires daily drug release determination for extended duration (3 weeks - 5 years). It is very challenging for United States Pharmacopeia (USP) apparatus to support such long term release with continuous measurement. Therefore, an automated re-lease control system (ARCS) technology was developed to support the IVR testing of polymeric dosage forms. This system consists of a liquid handler, a stirring unit, a test tube rack of 48/96 vessels, a high-performance liquid chromatography (HPLC) vial rack, and a water bath with thermostat. The system is also connected to photo detectors, i.e., Spectrorfluorometer and UV Spectrophotometer, which are capable to measure daily release rates in real-time. The system is being used to develop a labor alleviating IVR method for an implant product. A. The product was first investigated by a manual IVR method which requires daily sampling and medium refill. The automated solution handling and sample acquisition system of ARCS significantly reduces human interference but adds more precise volume and time control. The method on ARCS generates IVR profiles with good correlation with the manual method, and has sufficient discrimination power on different formulations. Besides, the solution volume can also be adjusted between 50 mL and 300 mL to support various extended release dosage forms with different dose ranges.

Preparing Your Laboratory for USP 232/233
Tina A. Restivo, CEM Corporation, PO Box 200, Matthews, NC 28104, Sam Heckle, Michael Howe

With harmonization having been completed the next phase for United States Pharmacopeia (USP) 232 & 233 will be implantation. With the January 1, 2018 deadline rapidly approaching now is the time to make sure that your laboratory will be up and running and validated by this date. Sample preparation plays a prominent role in the analysis of the heavy metals. Laboratories will have several options to meet the guidelines listed by USP. In addition, USP is looking to vendors to propose conditions and acid or acid mixtures to be used in order to be provide solutions ready for analysis. Closed vessel microwave digestion offers the best option for samples that either are not aqueous or become aqueous in an aqueous solution. CEM pioneering the industry of Microwave Digestion and is the worldwide leader in this field. In addition CEM was the only microwave vendor to have attended USP 232/233 meet-ups over the past several years to help with the methods needed to obtain proper results. In this poster we show digestion schemes and results for a variety of active ingredients and excipients as prepared using an automated microwave digestion system. This easy to use system not only meets the requirements of the new USP methods but provides a unique platform that allows users to load an autosampler and then walk away to attend to other tasks. The system also provides full documentation of every sample making compliance fast and easy.

Leveraging Abbreviated Impaction (AIM) to Streamline Development of an Inhalation Product
Tina Masuik, Merck & Co., MS: RY80M-213, 128 E. Lincoln Ave., Rahway, NJ 07065, Buchilingam Bupathi, Parul Kadakia, Josephine Bermudez, Adrian Goodyear

Andersen Cascade Impaction (ACI) testing is a critical quality test that has been employed for the aerodynamic particle size distribution (APSD) characterization of inhalation products such as metered dose inhalers (MDI) and dry powder inhalers (DPI). It is a rigorous test requiring significant amounts of time, analyst labor and solvent consumption generating volumes of hazardous waste. In recent years, efforts have focused on developing abbreviated impaction (AIM) strategies as suitable alternatives to ACI testing. Although AIM methods inherently yield less detailed characterization of APSDs, they have been demonstrated to be at least as sensitive to changes in formulation as the conventional ACI methods. Moreover, AIM can serve as a valuable tool throughout the product lifecycle, with different configurations employed at different stages of development, according to program needs. Early in development, for example, a screening configuration yielding only essential metrics can streamline efforts to identify the right formulation and device combination. Alternatively, post-approval, a quality control (QC) configuration sensitive to changes in the APSD can be relied upon for batch release decisions. An early phase development program was selected for development of an AIM method. With implementation of AIM in the QC setting as the ultimate goal, here we demonstrate feasibility of the AIM method for assessment of a new inhalation product, significantly reducing analyst and resource consumption.

Fast and Reliable Quality Control of Nutraceutical Products with Near-IR Spectroscopy
Adam J. Hopkins, Metrohm USA, 6555 Pelican Creek Cir., Riverview, FL 33578, Kyle A Hollister

The supplement industry is the subject of increasing regulatory scrutiny due to its rapid growth and some news reports showing products with incorrect labeling. Because many supplements are based on natural extracts, which are often similar, the identity of raw materials is hard to measure by traditional means. However, these extracts often have varying numbers of hydroxyl and aromatic groups, both of which are strongly active in the near-infrared (NIR). In this poster, we demonstrate two applications of NIR spectroscopy: identification of fish oils and blends; and quantification of vitamin E blends. The results show that NIR can be a fast, non-destructive method for analyzing products in the nutraceutical and supplement industry to ensure consistent product quality.
344 Rethink the Approaches for Evaluation of Chromatographic Method Robustness
Jianhua Li, Bayer Healthcare, 36 Columbia Rd., Morrisatown, NJ 07960, Lucy Zhao, Kangping Xiao
Both the International Conference on Harmonization and the United States Pharmacopoeia guidelines define the robustness of an analytical procedure as a measure of its capacity to remain unaffected by small but deliberate variations in procedural parameters during normal use. Based on that, the robustness of a procedure is often demonstrated by deliberately changing parameters such as column temperature, gradient, flowrate, and/or mobile phase compositions. However, given the modern chromatography technologies and tight regulations imposed on pharmaceutical industry, chromatography systems are calibrated and well maintained at optimal working conditions during normal use. From the authors’ point of view, changing those parameters to demonstrate method robustness is becoming less meaningful. Moreover, human errors, such as mixing organic modifier at an amount deviated from the test procedure to the mobile phases should never be allowed for routine analysis. Rather, real variations in a method performance can be seen on different chromatographic instrumentation used in different laboratories. The variations between quaternary pumps (low pressure mixing) vs. binary pumps (high pressure mixing); or Agilent HPLCs vs. Waters HPLCs, have much more profound impact on the method performance if the procedures are followed as written. Moreover, most of the analytical out-of-specification investigations usually point to the incorrect sample preparations (whether inherently in the method or by analyst error) as the root causes. In this poster, we designed some experiments to illustrate the importance of looking into the sample preparation robustness and versatility of the method on various instruments than the common robustness evaluation practice.

345 Enhanced Assessment of Nanoparticle Colloidal Stability viaFFF-PALS
Vincent Hsieh, Wyatt Technology, 6300 Hollister Ave., Santa Barbara, CA 93117, Daniel Some, Eric Seymour, Bob Collins
Zeta potential (ζP), a measure of net charge in solution, is widely utilized to optimize formulations of nanoparticle drug delivery systems for colloidal stability. Standard technologies for determining ζP such as phase analysis light scattering (PALS) typically provide a ζP value that is averaged over the entire ensemble. Nanotrack analysis (NTA), a more recent development, can provide a low-resolution distribution of ζP vs. size. However, a more general and high-resolution method for characterizing ζP and other properties of nanoparticles has been lacking. We demonstrate a novel technique, field flow fractionation - phase analysis light scattering (FFF-PALS), which combines high-resolution, size-based separation of nanoparticles with downstream massively parallel (MP)-PALS detection. FFF-PALS quantifies high-resolution distributions of nanoparticles by size and ζP with the potential for adding additional characterization techniques in-line such as spectroscopic, conformational or thermal analysis.

346 DOE Method Development for Metered Dose Inhaler Shake and Fire Performance Testing
Carlos M. Santos, Merck & Co., MS: RY80M-213, 126 E. Lincoln Ave., Rahway, NJ 07065, Adrian P. Goodey, Kuriakose Jacob, Buchilingam Bupathi
Metered Dose inhaler (MDI) performance tests are ripe candidates for automation given the variability inherent to manual shake and fire techniques. The InnovaSystems Actuation Collection Station (ACS) allows users to define automated MDI shake and fire routines via 13 independent parameters. However, this versatility depends upon whether it was a polished or an etched crystal and also whether the material or thermal analysis.

347 Secure and Compliant Data Integrity Made Simple with LabX Software
Christoph Jansen, Mettler Toledo, Sonnenbergstrasse 74, Schwerzenbach 8603 Switzerland, Mari Lynne Gentry
As companies face increasing and ever changing electronic data regulations, such as recent United States Food and Drug Administration (FDA) warning letters, ‘data integrity’ becomes a more prevalent concern every day. Not only does converting from manual data recording to electronic offer its challenges, keeping a system compliant with ever changing regulations is becoming more of a challenge. The gains from electronic data collection and high integrity far outweigh the challenges. Increased efficiency, security, ‘greener’ operations as well as reduced transcription errors are just some of the gains from a ‘paperless’ lab. Mettler Toledo can lead a company into a more efficient and secure data collection process with LabX® Software. LabX® offers full compliance by meeting FDA 21 CFR Part 11 requirements, accompanying validation manuals, and quality support. LabX can network to up to 30 balances, titrators, UV/Vis spectrophotometers, pH, density, refractive index meters and more without requiring a computer in the lab. On average, this single software can connect to 40% of a typical lab’s equipment! LabX also increases efficiency as it bi-directionally communicates to LIMS and ERP systems and is a central system to manage results, methods and operators. Mettler Toledo can guide you to a more secure, efficient and compliant lab with LabX software. Consolidate your instruments to a seamless combined network for faster operation, learning, and management with our professional support staff to assist you to a ‘paperless’ lab.

348 Direct Method for Accurate Heat Capacity Measurements by DSC on a Range of Materials
Andrei Levenenko, TA Instruments, 159 Lukens Dr., New Castle, DE 19720
A differential scanning calorimetry (DSC) three-run method described in an American Society for Testing and Materials standard E1269 has been traditionally used for heat capacity measurements. The three-run method requires three ramp experiments (blank, reference and sample) with isothermal segments on both end of the ramp. Assumptions are made with respect to the DSC cell baseline being absolutely repeatable. For a direct Cp method the DSC is designed and calibrated in such a way that repetitive blank and reference runs are unnecessary. In this paper we demonstrate the benefits and accuracy of the direct method on a wide range of materials which may not be ideal for a traditional DSC.

349 Unraveling the Solid State of MK-8970: A Racemic Acetal Carbonate Prodrug of Raltegravir
Nancy Tsou, Merck & Co., MS: RY818-8112, 126 E. Lincoln Ave., Rahway, NJ 07065, Scott Shultz
MK-8970 is an acetal carbonate prodrug of raltegravir (Isentress). This work presents the Merck & Co. team’s investigations into the polymorphism of MK-8970, the thermodynamic relationship between the discovered crystalline forms, and implementation of that knowledge toward solving key processing challenges. MK-8970 was found to exist in two enantiotropic polymorphs, with a crossover temperature of approximately 117 °C, as determined from solubility data. Form 2 of MK-8970, the stable form at ambient temperature, was confirmed to be a true racemical crystal form and not a conglomerate on the basis of single-crystal X-ray structure data. In preparation for scale-up of MK-8970, form control was established by mapping out solubility curves for the relevant crystalline forms in ethyl acetate as a function of temperature. Lastly an investigation of the relative solubility of MK-8970 and a troublesome imidate impurity identified improved solvent systems for maximizing rejection of this impurity while avoiding significant yield losses.

350 Interpretation of the Frequency Transients Accompanying the Immersion of a QCM Quartz Crystal in Water
Ho Yeon Yoo, Stanley Bruckenstein Chemical Consulting and Services, 115 Foxpoint W., Williamsville, NY 14221, Stanley Bruckenstein
10 MHz qcm crystals, uncoated or coated with films of poly-methyl methacrylate (PMMA) or poly-ethylene oxide (PEO), were rapidly submerged into water. The change in these crystals’ resonant frequencies were recorded. In all cases after a few seconds a crystal’s resonant frequency became constant at a value that depended upon whether it was a polished or an etched crystal and also whether the viscosity of water was changed adding ethylene glycol. An uncoated polished crystal’s frequency changed more on submersion in water if it was etched. The frequency of an etched crystal in air or water on being coated with a PMMA film exhibited the same frequency change in air as when it was submerged in water. A blended polymer film composed of PMMA and PEO when coated on qcm crystals and then submerged in water shows a rapid frequency transient corresponding to the removal of PEO from the film. A film leached of PEO by water was dried, weighed and again submerged into water. It gained weight which we explain in terms of water entering a porous structure of PMMA that was created by the leaching of PEO with water in the previous step. Finally, the influence of viscosity on polished and etched qcm crystals in aqueous media was determined by adding up to 50% ethylene glycol. The ratio
of the crystals’ frequency decrease on submerging an etched crystal in water com-
pared to a polished crystal was greatest for the etched crystal.

351 Importance of Small but Essential Details in Proper Laboratory Practice
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Rodlin, Vladimir Ioffe
Most of the work in modern analytical methods is performed by sophisticated ins-
truments (spectrophotometers, high-performance liquid chromatography (HPLC),
gas chromatography, mass spectrometers). However, one should not neglect the
importance of “manipulations” which should be performed properly. This “hand-
made” work is a key to success of analysis and reliability of analytical result. Most
critical cases depend on manual operations are in the field of inter-laboratory
activities, such as “Cross-Testing,” “Intermediate Precision” chapters in validation
of analytical methods or “Tech. Transfer” of analytical methods. Difference in work
habits of “originating” and “adopting” laboratories may be a root cause for failures
of inter-laboratory operations and compliance issues with preset specifications lim-
its and acceptance criteria. Real examples of such cases are presented: - Proper
volumetric adjustments, especially, with formation of foam - Accurate weighing of
liquid standards and samples - Proper performance of mechanical operations (stir-
ring, mixing, shaking, sonicating, etc.), to ensure reliable sample extraction (recov-
ery) - Using low volume volumetric pipettes for dilution - Liquid-liquid extraction for
sample preparation - Difference between chromatographic equipment in different
laboratories: “seemingly similar” requiring method adjustment, especially for gra-
dient HPLC methods - Use of polymer labware instead of glassware. Only face-
to-face meetings between originating and adopting laboratories staff, inspection of
differences in analytical equipment and examination of work habits can solve the
problems of inter-laboratory work. Conclusions: Every questionable case should be
treated individually; all the definitions of manual operations should be provided in
details in analytical methods and protocols to ensure that in all laboratories involved
in an inter-laboratory study, all the operations will be performed in identical manner.

352 Imaging of Lead in Latent Prints by Laser Desorption-Ionization Mass Spectrometry (LDI-MS)
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Pavlov, Athula B. Attygalle
In the past, Chrome Yellow (lead chromate, PbCrO4) has been one of the most
widely used inorganic pigments in the production of paints, coatings, and plastics.
For example, the yellow color of Van Gogh’s paintings is based on Chrome Yellow. We
demonstrate that laser desorption-ionization mass spectrometry (LDI-MS) is a
powerful tool for imaging lead distribution in latent prints. For example, a latent
print obtained by impressing the laterally cut surface of an old pencil on an acet-
nitrite-moistened paper, was successfully imaged for both lead and chromate using a
Synapt G2 HDMS instrument (Waters Corp., Manchester, UK). After rastering the
print with a 355 nm laser beam and recording positive and negative ion mass spec-
tra over the range m/z 50-1200, we generated a false-color “heat maps” for Pb++
(m/z 207.98) and for Cr2O6-• (m/z 199.85). The heat maps matched closely with the
visual images of the pencil imprint. Our results confirmed that LDI-MS can be used in
the pigment coating of old pencils. Evidently, LDI-MS is a powerful tool for detecting
and imaging many heavy metals and their compounds present on surfaces.

353 Structural Characterization of Spider Peptide Phα1β by Mass Spectrometry
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Costel C. Darie
Phα1β is a peptide purified from the venom of the armed spider Phoneutris ni-
griventer that has been shown to have an extensive analgesic effect with less side
effects than ω-conotoxin. Because of this it has been suggested that Phα1β may
have potential to be used as a therapeutic for controlling persistent pathological
effects than ω-conotoxin. Because of this it has been suggested that Phα1β may
be a root cause for failures of inter-laboratory operations and compliance issues with preset specifications limit-
its and acceptance criteria. Real examples of such cases are presented: - Proper
volumetric adjustments, especially, with formation of foam - Accurate weighing of
liquid standards and samples - Proper performance of mechanical operations (stir-
ring, mixing, shaking, sonicating, etc.), to ensure reliable sample extraction (recov-
ery) - Using low volume volumetric pipettes for dilution - Liquid-liquid extraction for
sample preparation - Difference between chromatographic equipment in different
laboratories: “seemingly similar” requiring method adjustment, especially for gra-
dient HPLC methods - Use of polymer labware instead of glassware. Only face-
to-face meetings between originating and adopting laboratories staff, inspection of
differences in analytical equipment and examination of work habits can solve the
problems of inter-laboratory work. Conclusions: Every questionable case should be
treated individually; all the definitions of manual operations should be provided in
details in analytical methods and protocols to ensure that in all laboratories involved
in an inter-laboratory study, all the operations will be performed in identical manner.

354 Trace Analysis of Impurities in Pharmaceutical Substances by Cold Electron Ionization LC-MS (Ei-LC-MS) with Supersonic Molecular Beams
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07065, Ryan Cohen, Renee K. Dermendjian
Mass spectrometry (MS) is a powerful technique for analysis of low level impurities in
drug development samples due in part to its sensitivity, speed and chromatog-
graphic compatibility. With the advent of electrospray ionization (ESI), liquid chro-
matography combined with MS analysis has become a vital tool in analytical work-
flows for drug development. However, many small molecule, non-polar impurities
cannot be ionized by ESI, which inhibits identification and quantitation. Recently, an
electron ionization liquid chromatography (LC-MS) system was developed by Aviv
Analytical which is capable of ionizing nonpolar molecules. This instrument gener-
ates reproducible Ei fragment spectra which can be searched against the Na-
tional Institute of Standards and Technology (NIST) library database for compound
identification. Additionally the cold electron ionization (Ei) mass spectra produced by
this instrument often show enhanced molecular ion signal, which can aid in the
identification of compounds that are not present in the NIST library. Unlike LC-UV
and LC-MS with ESI, the cold Ei-LC-MS has uniform response over a wide range of
analytes, enabling easier quantification without internal standards. In this work we
examine the performance of this technology for various figures of merit affect-
ing quantification of low level impurities. A case study using the cold Ei-LC-MS to
detect low level impurities of dapsone (i.e., intermediates in the synthesis of the
active pharmaceutical ingredient) will be used to demonstrate the capability of
the technology.

355 Withdrawn by the author.

356 Withdrawn by the author.

357 The Analysis of Evolved Gasses by TG-GC-MS
Thomas Mancuso, Perkin Elmer, 1 Albertson Ave., Blairstown, NJ
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The use of thermogravimetric analysis (TGA) measures the minute change in the
weight of a sample as a function of temperature. The results of a TGA cannot give
conclusive information about the compounds lost at a specific temperature. The
analysis of gases evolved during a TG-GC-MS experiment by gas chromatography
mass spectrometry (GC/MS) provides laboratories with a way to identify the com-
 pound or groups of compounds evolved during a specific weight-loss event in a TGA
analysis. This paper examines a polymer sample and show the instruments ability
to see a variety of compounds in the evolved gas.

358 Withdrawn by the author.

359 Vacuum Chromatography: Column Behavior
Kurikose T. Joseph, Coconut Associates, 755 Magee Ave.,
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The discovery of vacuum chromatography (VC) has recently been reported. It is
very similar to gas chromatography (GC) and is a phaseless chromatography tech-
nique where no mobile phase (neither gas nor liquid) or stationary phase (neither
solid nor liquid coating) is needed. Instead a vacuum is applied at the outlet to pull
the compounds in vapor state through a blank capillary column. The lighter mole-
cules travel through the column faster than heavy molecules and elute primarily in
the reverse order of molecular weight. However, boiling point, structure, functional
group, etc., have high influence in the order of elution. Compounds with low boiling
points elute earlier and some isomers appear as well separated peaks. Relative
elution order changes between columns of different dimensions and as the oven
temperature programming conditions are varied, however, reproducibility appears to
be good under same conditions. Compounds elute at relatively lower temperatures
than GC and so advantages of vacuum distillation are applicable to VC also. As no
stationary phase is needed, the columns are cheap and VC could be employed to
very high temperatures, especially without column bleeding and could be cleaned
by baking out or rinsing with solvents. The possibilities of using metal columns also
exist. Overall, VC could be amenable to far more compound than GC. Several ex-
amples of analyses on various columns of different dimensions have obtained so far.

360 Profiling of Ocimum Tenuiflorum (Holy Basil) Using both Headspace and Liquid Autosampler GC-MS to Discover Pharmacological Activity
Bindya Verma, Kean University, 542 Bloy St., Hillside, NJ 07205, Mima
Giron, Dil Ramanathan
Ocimum tenuiflorum (Tulsi) has been used in India and many other countries for its
therapeutic and medicinal benefits. It can be easily accessed, grown, and prepared
providing ample health benefits. The objective of this study is to discover which key
compounds exist in Tulsi first, and then further analyze those for pharmacological
activity. The varying Tulsi samples analyzed ranged from powder, fresh leaves, oven dried leaves at varying temperatures and lastly sun dried leaves. The various samples of Tulsi were extracted in Dichloromethane (DCM) and hexane and analyzed using the Perkin-Elmer GC Clarus 680 and MS Clarus SQ BT. Headspace analysis is used for detecting volatile compounds while the other is a liquid autosampler. The results of this study are further used to test each significant compound for antibacterial and other medicinal properties.

361 The Effect of Draw-Out Lens Diameter on Sensitivity of GC-MS Analysis
Ed Connor, Peak Scientific, Fountain Ave., Inchninnan PA4 9RE, United Kingdom, Carlos Fidelis
Increases in helium price combined with temporary shortages of the gas have recently prompted many gas chromatography (GC) users to look to alternative carrier gases for their GC and GC-mass spectroscopy (MS) analysis. Hydrogen is a cost-effective, viable alternative to helium with potential for improved chromatography and decreased analysis time, however there are concerns about hydrogen carrier gas for GC-MS because of some sensitivity effects when using hydrogen in place of helium. This study investigated the effect of hydrogen and helium carrier gas across a range of column flow rates (1.0 – 2.0 cc/min), with a different draw-out lenses (3mm vs 6mm) and selected ion monitoring (SIM) vs full scan detection for essential oil analysis. Results showed that flow rate did affect resolution and signal-to-noise ratios, with results corresponding to the theoretical changes in carrier gas efficiency according to the van Deemter equation. The 6mm draw-out plate orifice did increase sensitivity compared to the standard 3mm draw-out plate when using hydrogen carrier gas, especially at higher flow rates. SIM detection, in combination with flow rate, also improved sensitivity, with hydrogen carrier gas eliciting a similar response to helium carrier gas when using SIM detection at higher flow rates. This study demonstrates some simple adjustments to the GC-MS system can enable analysts to mitigate some of the negative effects that hydrogen can have on GC-MS detection and obtain like-for-like results.

362 Analysis of PAH Metabolites in Fish Oil Using HS-SPME Coupled to GC-MS-MS
Anumeha P. Muthal, Seton Hall University, Chemistry Department, 400 South Orange Ave., South Orange, NJ 07079, Nicholas H. Snow
Polycyclic aromatic hydrocarbons (PAHs) are carcinogens which are released to the environment due to human activities and from disasters such as oil spills. This study aims at determining the trace levels of PAHs and metabolites, which are generally mono-hydroxy derivatives present in fish oil and food using solid-phase micro-extraction coupled to gas chromatography with two-dimensional mass spectrometric detection (SPME-GC-MS-MS). The combination of the concentrating power and ease of automation of SPME with the selective and sensitive detection afforded by MS-MS provides very low detection limits with a straightforward analysis. PAHs were detected in aquatic life after the Deepwater Horizon oil spill of 2010. In previous work using headspace SPME with single quadrupole GC-MS, the limits of detection and quantitation varied from 0.1 to 50 ppb. This work extends detection to GC-MS-MS using a triple quadrupole mass spectrometer to increase the selectivity for the PAHs and also to test simultaneously for the mono-hydroxy metabolites of PAHs which are produced by fish as the PAHs are injected. The PAHs were extracted from samples of essential oil fish capsule with the standard EPA 610 mixture and standard PAH metabolites and extracted using a polydimethylsiloxane fiber and further analysed using GC-MS-MS with multiple reaction monitoring. Limits of detection and quantitation ranged from 10-2000 parts-per-trillion.

363 Quantitation of PAH Metabolites by GC-VUV
Adigun Ajayi, Seton Hall University, Chemistry Department, 400 South Orange Ave., South Orange, NJ 07079, Anumeha P. Muthal, Nicholas H. Snow
This study focuses on the performance of a new Vacuum Ultra-violet detector (VUV) for analyzing the metabolites of Polycyclic aromatic hydrocarbons (PAHs). These metabolites are found in aquatic life such as fish which have been exposed to oil spills or industrial effluent. A VUV detector (VGA-100) coupled to a gas chromatograph (GC) can test gas phase absorption in the UV range from 125-240 nm. PAH metabolites readily absorb in this UV region and were detected using VUV detector. A quantitative analysis was performed on the PAH metabolites to confirm the analytical figures of merit. Limits of detection (LOD) and quantitation in the low-ppm range for five monohydroxy PAH derivatives that are representative of PAH metabolites in fish were obtained using simple splitless injection of 1 microlitre samples and extracts. GC-VUV shows a high potential for gas chromatographic analysis, complimentary to mass spectrometry. There is high potential for selectivity in the analysis of isomers and compounds such as PAHs that do not fragment substantially in traditional electron ionization mass spectrometry.
that demonstrate the feasibility of microalgae as embedded environmental sensors. Furthermore, it has been observed that microalgal biomass chemically adapts to concentrations of large quantities into oceans where it serves as nutrient for microalgae. On the other hand, a large number of biologically relevant compounds are determined by their chemical environment. The objective of this research project is to develop novel analytical methodologies for investigating the impacts of inorganic compounds on microorganisms. In order to acquire the chemical signature of life microalgae in marine environments, attenuated total reflection Fourier transform infrared spectroscopy has been employed as it is sensitive to a large number of biologically relevant compounds. However, preliminary studies have shown that chemical conditions in the cells' environment have a highly nonlinear impact on their biomass and therefore conventional, linear chemometrics is not applicable. To overcome this, a nonlinear calibration technique will be presented that derives a nonlinear model coined 'Predictor Surface' that expresses an unknown concentration-spectra relation by means of a multi-variate Taylor approximation. Nanochloropsis oculata cultures have been exposed to concentration series of the two most relevant algae nutrients, i.e., bicarbonate originating from atmospheric CO2 and nitrate. Experimental results are presented that demonstrate the feasibility of microalgae as embedded environmental sensors. The Predictor Surfaces themselves sheds light on the biological consequences of chemical modifications of a marine ecosystem.

The detection of airborne chemicals is a key capability in a variety of environmental monitoring scenarios. For these applications, passive infrared remote sensors collect infrared emissions from natural and manmade sources such as the radiant emission from the earth or emissions from the stacks of a chemical plant. Chemical compounds absorb or emit infrared energy at characteristic wavelengths, and the profile of these absorption or emission signatures can be used to identify a chemical and to estimate the amount present. Passive infrared remote sensors can be implemented in either imaging or non-imaging configurations and can be constructed to acquire infrared emission data in either multispectral or hyperspectral modes. Implementing these measurements successfully requires the construction of rugged and portable instruments capable of being mounted on platforms such as moving aircraft or ground vehicles. In addition, sophisticated computer processing techniques must be designed to allow the automated analysis of the large quantities of data acquired by these sensors. The research presented here describes the development of novel signal processing and pattern recognition methodology for application to multispectral imaging data and to non-imaging data acquired with a hyperspectral instrument. Remote sensing data were collected with these instruments mounted on an aircraft platform as part of the United States Environmental Protection Agency’s ASPECT emergency response program. Remote measurements collected during several field responses are used to validate the analysis methodology and to assess the strengths and weaknesses of the imaging and non-imaging remote sensing approaches.
Photography exhibition allowed for the comparison of pre-exhibition MFT results at the Getty Research Institute and the J. Paul Getty Museum focusing on a 19th century material response when exposed to comparable light exposures in the two studies. Additionally we have added a modelling component to predict reactor solvent concentration based on distillate monitoring. We describe our method development and implementation techniques that have increased process efficiency, green chemistry score and data capture.

Use of PAT for In-Process Control: Enhance Robustness and Increase Productivity of Sitaglitin Manufacturing


While implementing quality-by-design (QbD) methodology, risk assessment and corresponding design on experiment (DOE) studies showed that the final active pharmaceutical ingredient particle size distribution (API PSD) strongly depended on the seeding-point temperature which in turn relied on the solution composition. Thus, it is imperative to accurately measure this composition in real-time for a through process stream, enabling a robust crystalization process within the process design space as well as reduction of cycle time. A process near-infrared (NIR) equipped with a flow-through cell was used to collect spectra of simulated process streams, upon which NIR methods were then developed for API, water, dimethylsulfoxide (DMSO) and isopropanol (IPA), respectively. After incorporating additional samples of process streams at production, these NIR models were updated and performed robustly over a wide range of temperature regardless of variable amount of co-existing residual species from upstream. After they were regulatory-approved and used for production several years ago, these NIR models have been played an important role in the Sitaglitin manufacturing. In addition, learnings and experiences on maintaining the performance of this process analytical technology (PAT) application are discussed.

Development and Implementation of Spectroscopy Methods for Quantitative Analysis of Pharmaceutical Reagents

Bernard Agyei, Bristol-Meyers Squibb, 1 Squibb Dr., New Brunswick, NJ 08903, John Wasylyk, Robert Wethman, Ming Huang

Off-line spectroscopy is a prevalent technique used for rapid evaluation of pharmaceutical raw materials and is suited for qualitative as well as quantitative purposes. Qualitative analysis is generally used for rapid material confirmation by comparing the Raman or near-infrared (NIR) spectra to that of an established reference spectra. Quantitative methods are applied to both incoming reagents as well as prepared product solutions where a specific concentration is required by process requirements. The type of analysis and the level of which the method must be qualified in order to meet each goal depends on the level of sensitivity required. Specificity, linearity, precision and accuracy must be established regardless of the spectroscopic technique used for quantification. Furthermore, robustness testing adds an additional level of reliability to the method when analyzing varying grades of reagents from multiple sources. Issues concerning cross-contamination from either the source of the material or from sampling techniques can impact the results and can often times be identified by the spectroscopy method. This study demonstrates the applicability of the off-line spectroscopy as a tool for quantification, as well as challenges faced, when developing methods for rapid analysis of pharmaceutical raw materials and reagents.

Microfading at the Getty Conservation Institute: From Scientist to Conservator

Vincent L. Beltran, Getty Conservation Institute, 1200 Getty Center Dr., Ste. 700, Los Angeles, CA 90049, Jim Druzik, Christel Pesme, Andrew Lerwill, Mark Benson, Sarah Freeman, Jane Bassett, Nancy Turner

The microfade-tester (MFT) represents an important tool in understanding the in-situ light sensitivity of an artifact and aids in guiding exhibition light exposure policies. Introduced at the Getty Conservation Institute (GCI) in 2002, the MFT has been employed across the Getty campus by scientists and conservators alike. This talk describes selected collaborative microfading activities. While object-specific assessments remain common, GCI scientists have also employed the MFT to examine preventive conservation topics such as the effect of anoxia on light-induced color changes. Testing samples similar to those exposed in a pre-exhibition photography exhibition allowed for the comparison of pre-exhibition MFT results with in-situ color measurements during the show. Similar to the anoxia experiment, this study represented an occasion in which accelerated ageing induced by micro-fading could be evaluated against natural ageing in the gallery. Lastly, though use of the original Whitemore-designed MFT by Getty conservators has been facilitated by training and instrument transport to galleries and storage areas, the development of a simplified contact MFT by GCI scientists using an LED light source and a ball lens has provided conservators an alternate instrument that can serve as a routine screening tool for collection management.

Microfademeter Designed for Conservators - Presentation of a New Instrument

Tomasz Lojewski, AGH University of Science and Technology, Faculty of Materials Science and Ceramics, Mickiewicza 30, 883/36, Krakow 30-059, Poland

Microfademeter (MFT) measurements performed using self-made set-ups (as it is usually the case) often challenge numerous difficulties which may result in poor reproducibility of results. To worsen the situation, due to submillimeter scale of microfading tests many samples turn out to be heterogeneous even in areas where we expect their homogeneity. A new MFT instrument has been designed where the problems of poor focusing, drift of the lamp power output or extent of the aperture opening have been solved. Certain tasks (focusing, opening and closing the aperture, calibration on white standard, dark noise measurement, reciprocity testing) have been automated which has given rise to significant improvement of the comfort and speed of work. A user can specify at which point the test should complete, by selecting time, a dose or a maximum color change of a tested spot. The device is equipped with an optically stabilized xenon lamp and optical system (retrofocus type), with light output of 12 MLx focused on the 0.5 mm point. The reflected light goes through an optical fiber to the spectrophotometer, which can be easily detached from the instrument and used for other purposes. Color coordinates (CIE L*a*b*) and color changes (dE1976 and dE2000) are calculated and displayed on-the-fly. Advanced users have an option to store reflectance spectra as a time series with a specified interval and number of averages. Simplicity of operation and pre-processing of the collected data by the instrument software allows for its use by an operator . . . without engineering skills.

Beyond Lightfastness: Further Roles for Micro-Fade Testers


Expanded applications of the micro-fade tester (MFT) used at the Library of Congress have exploited its “secret identity” as a micro-scale ultraviolet-visible (UV-VIS) spectrometer, often coupled with other analytical techniques as part of an integrated multi-instrument analytical approach. This integrated approach utilizes specific features of the MFT (spot-size, etc.) that assist our knowledge of materials and complement other analytical techniques, providing conservators and scientists with additional tools for preservation of paper-based materials and media. The analysis of collection materials through micro-scale fiber-optic reflectance spectroscopy (micro-FORS) has been effectively applied for colorant identification on tinted prints. The MFT provides an advantage by avoiding interference from underlying printing inks in areas of color too small for conventional spectrometers. This approach permits very fine spatial resolution combined with high quality spectral resolution. The assessment of conservation treatments is an ongoing initiative at the Library of Congress. Non-destructively collected MFT hyperspectral reflectance data have been applied for the analysis of hue and intensity shifts in thin lines of iron gall ink after experimental conservation treatments. Color space measurements – light-dark, red-green, and blue-yellow (L*a*b*) values – were extracted from single spectra and used to calculate the total color space difference (delta E) from the original state resulting from the various treatments. In the same manner, each treated specimen’s color evolution during accelerated aging was measured. This informs knowledge of both immediate and long-term effects prior to treatments being applied.

Paint Alteration as a Function of Pigment-Binder Interactions Studied by Spectroscopic Techniques

Marcie B. Wiggins, University of Delaware, 163 The Green, Newark, DE 19716, Kristin deGhetaldi, Joshua Ottaway, Joseph P. Smith, Brian Baade, Thomas P. Beebe, Karl S. Booksh

Paints in many historical objects have been known to discolor and flake away over their lifetime. As paint is a mixture of organic or inorganic pigments with organic binders, some of these degradation mechanisms are believed to be the result of pigment-binder interactions. This promotes a need for a better understanding of these degradation processes for the preservation of cultural heritage objects. For the purpose of this study, several inorganic pigments, such as copper-based azurite and mercury-based vermilion, were mixed with various ratios of two common binders, egg tempera and cold-pressed linseed oil. These mixtures were compared to control samples of raw pigments and binder gradients without pigment. Additionally, a set of these pigment-binder mixtures were light aged to study the degradation products with respect to the different combination types. Changes in the oxidative states of the pigments appear to be the result of the differing binder combinations.
Furthermore, though Fourier transform infrared (FTIR) and Raman spectroscopy and principal component analysis (PCA), the light aged samples are chemically different depending on the pigment and binders prompting further analysis into these degradation processes.

382 An Inexpensive System High Performance Programmable Measurement and Control System
Scott D. Abbott, Phoenix First Response, 1419 Alverado St., Pittsburgh, PA 15216, Ryan I. Taylor
We developed a complete data logging and remote control system (called DAQ2GO®) for our gas chromatography (GC) and other lab instrumentation (gel permeation chromatography, high-performance liquid chromatography, Viscometers). This system takes the place of $50K of vendor software at less than 1/10th the cost. The system has analog resolution, analog inputs and outputs, digital inputs and outputs, RS485 and network communications. It uses a combination of high performance electronics and software based in Excel®. This system can also be used in many different ways, from a working as a networked datalogger to being a complete instrument data system with user interface, data taking, data storage, data post processing and automation. Several applications are described. This approach gives many labs the freedom from expense, dedicated software packages, and makes it easy to revive old instruments (e.g., with broken controllers or lost software) or make new measurements.

383 HPAE-PAD Applications for Biosimilar Development Processes: Monosaccharide and Sialic Acid Determinations
Hua Yang, Thermo Fisher Scientific, 1214 Oakmead Pkwy, Sunnyvale, CA 94085, Parul Angrish
Biosimilar development is increasing because of expiring patents. The United States Food and Drug Administration (US FDA) recently recommended that biosimilar sponsors use a stepwise approach to develop the evidence needed to demonstrate biosimilarity. The stepwise approach starts with extensive structural and functional characterization of both the proposed product and the reference product. This regulatory guidance for biosimilar development is driving demand for monosaccharide and sialic acid analyses, which is used for monitoring biosimilar glycosylation. High-performance anion-exchange with pulsed amperometric detection (HPAE-PAD) is a direct analysis method for monosaccharides, sialic acids, and other carbohydrates. It is sensitive and selective for a large variety of carbohydrates. Here HPAE-PAD is used to directly determine the carbohydrates present in glyco-proteins, without additional labeling steps that are often needed for other analysis methods, saving time and reagent costs.

384 Gas Cluster Ion Sputtering: Topographic and Chemical Changes to Polymer Surfaces
Christopher M. Goodwin, University of Delaware, 163 The Green, Room 023 LDL, Newark, DE 19716, Zachary Voras, Thomas P. Beebe Jr.
The development and application of gas cluster ion sputtering (GCIS) of soft materials opens the ability to analyze clean polymer surfaces that are not prepared in vacuum with little damage to the sample. GCIS allows for depth profiling and sample cleaning in vacuum, without loss of chemical information. Irgonax 1010 has been used as a soft sputtering standard to understand the topological effects of GCIS with atomic force microscopy (AFM) while chemical composition was monitored with X-ray photoelectron spectroscopy (XPS). In addition to Irganox 1010, polyaniline was studied because it is a conductive plastic, this allowing for many applications such as a solar cells, antistatic and corrosion-resistant coatings, and superconductors. Depth profiling was done with an Argon GCIS in thin films of polyaniline, AFM showed significant topological changes and an overall chemical change was observed with XPS. Our interest is in exploring how these changes caused by GCIS affect the electronic band structure and conductivity of polymers.

385 Separation of Cellulosic Polymers by Interaction Polymer Chromatography
Guanglou Cheng, Teva Pharmaceuticals, 223 Quaker Rd., Pomona, NY 10970
Size exclusion chromatography (SEC) has been widely used to determine the molecular weight distribution (MWD) of polymers in many industries. However, separation is largely based on physical properties, and not chemical properties. On the other hand, interaction polymer chromatography (IPC), though still unfamiliar to many chemists despite an increasing number of useful applications allows leveraging of the chemical properties. In this presentation, IPC separation of various cellulosic polymers is reported. The pH of the mobile phase was shown to have significant impact on enthalpic interactions between some cellulose polymers and stationary phase, but little effect on others. The contribution of entropic interactions or the size exclusion effect between cellulose polymers and stationary phase during IPC separation was found to be insignificant.
of protein in just a few minutes. The enhanced resolution of µSEC-MALS permits the identification of impurities and degradands including aggregates and fragments, as demonstrated for an IgG sample. Finally, we demonstrate a promising new application, at-line process monitoring, which is made possible by the high-throughput and information-rich µSEC-MALS measurements.

390 Noninvasive Glucose Sensing in Skin Based on Mid-Infrared Laser Spectroscopy
Alexandra Werth, Princeton University, Department of Electrical Engineering, Engineering Quadrangle, Olden St., Princeton, NJ 08544, Sabbir Liakat, Anqi Dong, Yezezi Zhang, Claire Gmachl

Diabetes is a growing problem worldwide. For many diabetics it is imperative to regularly monitor their glucose concentration; currently the most common way of doing this involves drawing blood to obtain a finger pricked glucose concentration. This can be painful. Therefore, a noninvasive glucose sensor is highly desired. We have implemented a noninvasive, mobile glucose sensor based on mid-infrared quantum cascade (QC) laser spectroscopy utilizing wavelengths from 8-10µm. This wavelength range contains unique spectral absorption features of glucose, particularly the C-O stretching modes at 8.8 and 9µm. The light from the QC laser penetrates into the dermis layer of the skin where glucose molecules are present in the interstitial fluid (ISF). The light is either scattered or absorbed by molecules in the skin. The backscattered light, which is collected by a miniature 1° gold coated integrating sphere, contains information about the absorption features of the molecules in the skin. In order to relate these absorption features to a glucose concentration we employ a genetic algorithm. The genetic algorithm uses a training set to generate an expression which can map the glucose absorption spectra to glucose concentrations.

391 Near Infrared Spectrometry of Temperate Earth-sized Planets Orbiting a Nearby Ultracool Dwarf Star
Robert A. Lodder, University of Kentucky, 192 Timberlane Ct., Nicholasville, KY 40356, Anne Brooks

Thirty-nine light years away from earth, in the constellation Aquarius, three Earth-size planets are orbiting a red dwarf star (TRAPPIST-1, or 2MASS J23062928-3922038). These planets have been found planets around a red dwarf star (TRAPPIST-1, or 2MASS J23062928-3922038). Thirty-nine light years away from earth, in the constellation Aquarius, three Earth-size planets are orbiting a red dwarf star (TRAPPIST-1, or 2MASS J23062928-3922038). Thirty-nine light years away from earth, in the constellation Aquarius, three Earth-size planets are orbiting a red dwarf star (TRAPPIST-1, or 2MASS J23062928-3922038). Thirty-nine light years away from earth, in the constellation Aquarius, three Earth-size planets are orbiting a red dwarf star (TRAPPIST-1, or 2MASS J23062928-3922038). Thirty-nine light years away from earth, in the constellation Aquarius, three Earth-size planets are orbiting a red dwarf star (TRAPPIST-1, or 2MASS J23062928-3922038). Thirty-nine light years away from earth, in the constellation Aquarius, three Earth-size planets are orbiting a red dwarf star (TRAPPIST-1, or 2MASS J23062928-3922038).

392 Improved Performance InGaAs Linear Arrays and New 1.45-µm Cutoff Version for Handheld Raman Spectroscopy
Douglas S. Malchoff, UTC Aerospace Systems, 330 Carter Rd, Ste. 100, Princeton, NJ 08540

A report on development of improved processing for Sensors Unlimited InGaAs photodetectors arrays, resulting in lower dark current and better uniformity for 1.7, 2.2 and 2.6 micron wavelength cutoff linear arrays. A new wavelength option with 1.45-µm cutoff is introduced for providing even lower dark current for Raman spectroscopy which utilizes 1 micron wavelength lasers for avoiding organic fluorescence. Comparative dark current and wavelength range data is provided.

393 Multivariate Exploratory Methods Applied to Raman Microspectroscopic Mapping of Titanium Dioxide Polymorphs
Joseph P. Smith, University of Delaware, 504 Lammot du Pont Laboratory, Newark, DE 19716, Frank C. Smith, Billy P. Glass, Karl L. Booksh

Titanium dioxide (TiO₂) is an extensively researched semiconductor oxide due to its proven capability in environmental purification, photocatalysis, and photovoltaics. Identification and differentiation of TiO₂ polymorphs is thus of considerable interest in many applications. Specifically, the α- and β-structured polymorph, termed ΤΙΟ-ΙΙ, is formed under extreme pressures and temperatures—4-2.6 GPa and 500-1200 °C for static high-pressure formation—and is used as a key diagnostic for impact events on Earth. In this work, we report the use of multivariate exploratory methods, mainly principal component analysis (PCA) and multivariate curve resolution-alternating least squares (MCR-ALS) methods, with Raman microspectroscopic mapping to investigate polymeric of TiO₂. Micron-sized grains containing ΤΙΟ-ΙΙ recovered from Archaean (>2.5 billion years old) spherule layers were utilized in this study. Raman spectra of these grains displayed bands at 174 cm⁻¹ (ΤΙΟ-ΙΙ), 426 cm⁻¹ (ΤΙΟ-ΙΙ), 443 cm⁻¹ (rutile), and 610 cm⁻¹ (rutile), highlighting the presence of ΤΙΟ-ΙΙ and rutile in these grains. PCA and backscattered electron imaging (BSE) demonstrate the grains are comprised primarily of rutile, ΤΙΟ-ΙΙ, and substrate-adhesive epoxy, and contain heterogeneous, polydispersed micron and submicron-sized particles. MCR-ALS applied to Raman microspectroscopic mapping revealed two additional chemical components, anatease and quartz, and produced spatially-resolved chemical maps with corresponding resolved Raman spectra of the chemical components. By minimizing spectral resolution and maximizing spectral acquisition density, the spatial resolution of the MCR-ALS generated chemical maps was increased. A Raman spectrum for pure ΤΙΟ-ΙΙ, which can assist in the identification of ΤΙΟ-ΙΙ within heterogeneous materials, was estimated using MCR-ALS.
396  **Application of Laser Induced Breakdown Spectroscopy to Forensic Science**
Olga Laskina, rap.ID Inc., Princeton Corporate Plaza, 11 Deer Park Dr., Ste. 201, Monmouth Junction, NJ 08852, Oliver Valet, Markus Lankers, Lin Bui

Laser induced breakdown spectroscopy (LIBS) is a fast and robust technique for elemental analysis. Elemental information is obtained in seconds without sample preparation. This technique covers wide range of elements from hydrogen to uranium. Trace amounts of inclusions and impurities can be determined as well as composition of alloys. LIBS removes the top microns of the sample. This property can be used to study the depth profile of the layered samples or as a sample preparation method when the top layer needs to be removed. In this work we will show how LIBS can be used for elemental analysis of gunshot residue. The presence of ammunition particles is an indicator that a firearm has been used. We also cover the advantage of using LIBS for glass analysis. Glass samples are often found on crime scenes, and therefore glass origin determination is a standard task in forensic laboratories. Finally we will show how non-metallic inclusions formed during steel production can be identified. LIBS can be used in many fields: material science, automotive, oil, and metallurgical industries. Archeologists and mineralogists will benefit from ability to determine soil origin, detect minerals and trace elements immediately in the field. LIBS is of service to law enforcement as many samples of trace evidence including firearm residue, soil, glass, pigments can be determined fast and reliably with this technique. LIBS is an ideal technique for forensic examiners because it is capable of quickly identifying large number of samples using virtually no sample preparation.

397  **Green Subcritical Water Extraction of Medicinal Herbs**
Yu Yang, East Carolina University, Chemistry Department, Greenville, NC 27858, Ninad Doctor, Janete Amreola

Due to effective remedial achievements in the medical field, wide availability, and low side effects and costs, the use of herbal medicine has been multiplied all around the world in recent years. The traditional way of consuming medicinal herbs is to cook them with boiling water. However, this is not the most effective method in removing active pharmaceutical ingredients (APIs) from herbs. We have developed a method to extract the APIs from medicinal herbs such as Salvia miltiorrhiza and Isatidis indigotica using subcritical water. The herbs were extracted at four different temperatures, 75 °C, 100 °C, 125 °C and 150 °C for 30 min at each temperature. The herbal extracts were then analyzed using high-performance liquid chromatography. Our results have revealed that sub-critical water extraction is much more efficient than the traditional boiling water extraction.

398  **Methamphetamine, Amphetamine, and Norephedrine Levels in Dermestid Beetle Frass after Consumption of Dosed, Buried Rat Remains**
Meaghan P. Drumm, Arcadia University, 450 South Easton Rd., Glenside, PA 19038, Kimberlee S. Moran, Karen S. Scott, M. Lee Goff

The Schedule II drug, methamphetamine, (MA), is associated with high death rates in users who abuse this substance. In traditional post mortem cases, blood and urine are used to determine the presence of drugs. However, in cases where a body is badly decomposed, alternative matrices, such as insects, may be required. There are multiple studies detecting various drugs within blowflies (order Diptera), but few studies on detecting drugs within other insects such as Dermestid beetles (order Coleoptera). Rats dosed with 5 mg/kg (n=4), 3 mg/kg (n=4), 2.5 mg/kg (n=2), 1.5 mg/kg (n=2), 0.5 mg/kg (n=4), and 0.25 mg/kg (n=2) methamphetamine were euthanized and buried. Control rats injected with saline were buried (n=3) or un-injected and not buried (n=2). Buried specimens were exhumed at different decomposition stages (89, 182, 395, and 819 accumulated degree days (ADDs)) to determine effects of decomposition on the detection of the drugs using gas chromatography mass spectrometry (GC-MS). The rats were exhumed, dissected, pelted, dried, and fed to Dermestid beetles (Dermestidae maculatus) until skeletonized. Frass was collected, incubated and then extracted using solid phase extraction. Methamphetamine, and metabolites could be detected in the frass of Dermestid beetles. Methamphetamine concentrations ranged from <0.001 to 1.07 ng/mg frass, amphetamine concentrations ranged from <0.001 to 0.465 ng/mg, and norephedrine concentrations ranged from <0.001 ng/mg to 0.025 ng/mg. All un-drugged controls were found to be negative. No correlation was observed between rats receiving the same dose at different ADD’s or rats receiving increasing doses at the same exhumation time.
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