Friday afternoon – Abstracts WRM 226 - 266

WRM 226

Best practice of ultra-high pressure liquid chromatography (UHPLC) in pharmaceutical analysis

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In the last four decades, the performance of HPLC separations has been bounded by a system pressure limit of 6000 psi - effectively limiting separation performance for routine analysis to column efficiency (N) of ~20,000 plates or a peak capacity (Pc) of ~ 200. The advent of ultra-high-pressure liquid chromatography (UHPLC) and its successful commercialization coupled with the use of sub-2um-particle columns has set a new HPLC performance benchmark. It is now possible to perform high-resolution separation of very complex mixtures approaching the efficiency of capillary GC (Pc = 600 - 1,000) or very fast ICH compliant impurity analysis using broad gradients in a few minutes. Most major HPLC manufacturers have product UHPLC offerings capable of pressure limits in the range of 15,000 to 19,000 psi with reduced system dispersion and dwell volume characteristics as well as improved precision and sensitivity performance. In the last few years, UHPLC has evolved from a scientific curiosity for research and high-throughput screening into a modern standard HPLC platform for routine testing and quality control.

This paper focuses on the benefits, perspectives and current status of UHPLC in pharmaceutical analysis, highlighting instrumental designs tradeoffs (system dispersion, dwell volume, autosampler mechanism, and flow cell volume) which can affect columns compatibility, peak area precision, and detection sensitivity. Potential issues in QC implementation are described together with discussions on method transfer.

WRM 227

Utilizing Design of Experiments (DOE) for Method Robustness Optimization

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Robustness of liquid chromatographic methods can often be difficult to asses.

Traditional approaches which involve one factor at a time may not predict real world scenarios. Optimizing a method to avoid these robustness pitfalls is therefore one of the major challenges method developers face.

This talk will discuss an approach to method development which not only effectively assesses robustness, but also optimizes the method for the best performance possible.

The approach is a multivariate one, whereby Design of Experiments (DOE) software manages the study. Full knowledge of the method's behavior within its design parameters is possible. This lends awareness to how the method may fail so that effective system suitability criteria can be set appropriately.

WRM 228

Adaption of Retention Models to Allow Optimization of Peptide and Protein Separations

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Retention modelling has successfully been used for the optimization of analytical scale separations of small molecules for approximately 30 years. Consequently various commercial software packages for such modelling are available to guide experimentation. However, it was realized when attempting to define a method development strategy for peptides and proteins

involving retention modelling that these software are not typically capable of accurately modelling the retention of proteins.
The set of isocratic relationships described by Snyder and Dolan [1] are applied in relating the retention factor (ln k) as a function of the fraction of the strong solvent for predicting the retention of proteins in gradient elution. Since individual relations don't adequately predict protein retention, combinations of reversed phase (RPC) and hydrophobic interaction chromatography (HIC) or ion exchange chromatography (IEC) and hydrophilic interaction chromatography (HILIC) were considered for calculating times.
For small molecules simultaneously modelling gradient shape and temperature has been found to be very effective. However, the linear temperature relationship normally used was found to be insufficient for proteins. In order to accurately account for the observed retention it was necessary to add a higher order term:
$\ln k = f + g / T + h / T^2$ (1)
Protein structure is taken into consideration to propose a potential explanation for this behaviour. This presentation presents the adaption and validation of the retention models necessary in order to accurately model and optimise RPC and IEC separations of peptides and proteins, analytes with increasing importance for the pharmaceutical industry.
[1] L.R. Snyder, J.W. Dolan, High-Performance Gradient Elution: The Practical Application of the Linear-Solvent-Strength Model, Wiley, Hoboken, NJ, 2007.

WRM 229
Genotoxic impurities analysis and control in pharmaceutical development
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Genotoxic or carcinogenic impurities belong to a class of compounds that interact with DNA causing mutations. Regulatory guidelines for the control of these impurities in pharmaceutical compounds pose a significant challenge. These limits are well below the ICH Q3A reporting levels and trace level analytical methods are needed to analyze these impurities.
Effective analytical strategies are required to develop selective and sensitive trace level methods and physical properties of the GTIs need to be considered such as volatility, presence of UV chromophore, ionizable groups, presence of derivatizable functional groups. API method may be used with high column loading strategy if the analyte has a strong UV chromphore. However, this poses its own challenge due to interference with the matrix and presence of other process related impurities at a comparatively higher level. Some well known genotoxic impurities such as alkyl chlorides, sulfonic acid esters and hydrazines lack UV chromophores and achieving the required quantitation limits can be challenging. Derivatization/LC-UV approach may be followed or using GC-MS (SIM) can increase sensitivity. A number of case studies utilizing various analytical techniques such as high column loading, derivatization/LC-UV, GC-SIM, GC-FID, CAD in determining trace level GTIs and the control strategies will be presented.

WRM 230
Mixed-Mode Chromatography and Its Uses in Pharmaceutical Analysis
***Xiaodong Liu**, xiaodong.liu@thermofisher.com, Chris Pohl. Thermo Fisher Scientific, Sunnyvale, CA 94088, United States*
Selectivity, mainly governed by column chemistry, is the key in HPLC separation. Although reversed-phase columns (e.g. C18) are most commonly used in pharmaceutical applications, they often fail to retain highly polar molecules (e.g. counter ions), and offer limited selectivities. Mixed-mode chromatography provides a viable solution to these challenges by using both reversed phase and ion-exchange retention mechanisms. One major advantage of this approach is that column selectivity can easily be modified by adjusting mobile phase ionic strength, pH and/or organic solvent concentration. As the result, not only is the selectivity of a mixed-mode column complementary to that of reversed-phase columns, but it also allows for the development of multiple complementary selectivities on a given column under different appropriate conditions. Mixed-mode chromatography is also well-suited to retaining ionic analytes, hydrophobic (e.g. Naproxen) or hydrophilic (e.g. Na^+ and Cl^- ions), and requires no ion-pairing agents in the method, significantly improving MS compatibility.

This presentation will address the following aspects:

1. An overview of state-of-the-art mixed-mode chromatography technology
2. Discussion on method development/optimization strategy using reversed-phase/ion-exchange columns in terms of mobile phase organic solvent, pH and buffer concentration.
3. Examples of pharmaceutical applications by mixed-mode chromatography, such as API and counter ion, surfactants, glycans, and etc.

WRM 231

Applications of two-dimensional liquid chromatography mass spectrometry in pharmaceutical analysis

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Co-elution of chemical components is an age-old problem since the advent of chromatography with continuous efforts being made to enhance efficiency and resolution. In the past couple of decades, significant advancements have been made in area of two-dimensional chromatography. Most of the applications are focused on analyzing complex sample mixtures with minimal efforts made on extending these techniques to simple but yet difficult separations.

One of the major challenges encountered in pharmaceutical industry is the analysis of potentially co-eluting impurities in the midst of active pharmaceutical ingredients (API). Currently, the pharmaceutical industry relies on HPLC with diode array detection (DAD) and mass spectrometric (MS) detection to address this problem. However, similarities in structures of closely eluting components and or presence of isomers limit the applicability of DAD and MS. Analysis of potential stereoisomers from desired enantiomers is another area of concern and challenge that gets compounded with increase in number of chiral centers. The advent of sub-two micron chromatography, improvements in column technology have helped alleviate these problems to some extent. However, irrespective of these challenges, the industry is yet to embrace/explore 2D-LC as an alternative to conventional separations. Possible explanations includes, complexity in filing, compliance, inability to generate high speed, high efficiency separations in secondary dimension, baseline noise resulting from repeated valve switching limiting the accessible area and hence peak capacity.

The presentation will primarily focus on the design and application of an automated 2D-LC-MS system in small molecules analysis. Analytical strategies for selective, comprehensive 2D-LC-MS analysis for potential co-eluting impurities in the midst of API at trace levels will be discussed using similar and complementary phases in the two dimensions. Also application of 2D-LC in simultaneous, sequential, achiral-chiral analysis including quantitation of undesired enantiomer will be presented. The presentation will highlight the importance of 2D-LC-MS in pharmaceutical analysis.

WRM 232

Improving the Prediction of Drug Disposition in the Brain with Mechanistic Models of Brain Penetration Characteristics

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Ability to cross the blood-brain barrier is one of the key ADME characteristics of all drug candidates regardless of their target location in the body. Despite a high demand of computational methods for estimating brain transport early in drug discovery, achieving good prediction accuracy still remains a challenging task. A recently published overview [Expert Opin Drug Metab Toxicol. 2013, 9, 473-86] of various measures employed to quantify brain delivery and recent advances in QSAR approaches for predicting these properties from the compound's structure forms the basis of this presentation.

The particular properties of focus are brain penetration rate expressed by permeability-surface area product (log *PS*) and the extent of brain penetration represented by steady-state brain/blood distribution ratios (log *BB*). Consideration is given to the classification models attempting to

distinguish between permeable and impermeable chemicals on the basis of predicted quantitative parameter values. The outlined mechanistic models predict both log PS and log BB with residual error under 0.5 log units and a simple combination of those models achieves 90% accuracy in classification of access to CNS.
Recent research in the field of brain penetration modeling has shown an increasing understanding of the processes involved in drug disposition. Mechanistic knowledge is a prerequisite to guide rational drug design efforts. Together, good performance of predictive models with their simple physicochemical interpretation can be key factors providing a clear route to design viable CNS drugs.

WRM 233

Understanding the Electronic Structure of Cobalt-Dithiolene H_2 Evolution Catalysts

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One of the main challenges that mankind has to face is the reduction of greenhouse gas emissions. Therefore, it is crucial to develop alternative renewable energy sources. One potential route passes through the splitting of water to release oxygen and hydrogen. Metal-dithiolene complexes have been demonstrated to be an effective class of electrocatalysts for the hydrogen evolution reaction (HER). Dithiolene ligands feature properties that include non-innocent behavior for electron storage, S-centers poised for H^+ shuttling, and functionality that can be tuned for catalysis.
We investigated the mechanism for the electrocatalytic generation of hydrogen from weak acids using cobalt-dithiolene complexes. Density functional theory calculations suggest that electrocatalysis occurs via initial formation of a cobalt(III)-hydride intermediate from which further reduction accompanied by protonation of the sulfur atom releases H_2. A transition state for this last step was located and suggests that H_2 elimination proceeds through the formation of a cobalt dihydrogen species.

WRM 234

Interactive ab initio molecular dynamics

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Graphical processing units have proven their utility in extending quantum chemistry calculations to systems of tremendous size. Today electronic structure calculations on whole proteins have actually become routine. Less attention has been given to the potential impact of GPU computing on small molecular systems. In this talk we discuss recent work to accelerate ab initio electronic structure calculations for systems up to a few dozen atoms. These performance gains allow ab initio calculations to be performed essentially in real time, opening the door to novel and exciting applications. As an example, we demonstrate interactive ab initio molecular dynamics, a virtual molecular modeling kit that promises unique insight into chemical phenomena.

WRM 235

Predicting drug secondary pharmacology and mechanistic targets

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Chemically similar drugs often bind to multiple molecular targets that are unrelated by sequence or by protein structure. Systems pharmacology networks organizing drugs and their targets by ligand structure could reveal links that molecular biology methods might otherwise miss. To investigate this assumption, we developed the Similarity Ensemble Approach (SEA), which

compares known drugs against more than two thousand therapeutic targets. Using it, hundreds of novel off-target drug activities consistent with adverse events, mechanisms of action, and new uses were predicted and confirmed in pharmacological assays. We have since extended these methods to prioritize testing for adverse drug reactions, and to predict the mechanism-of-action targets of compounds found to be active in whole-organism phenotypic screens. The chemical similarity approach used here is systematic and may find use for illuminating unexpected effects of approved and new drugs.

WRM 236

Proteus among proteins: A collection of short stories about conformational changes in GPCRs and Kinases

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Mechanistic understanding of the large scale conformational transformations coupled with activation of key signaling proteins, G-Protein Coupled Receptors and Kinases are of enormous importance for treating diseases such as cancer, asthma, cardiovascular disorders etc. We used cloud computing to simulate the β2 adrenergic receptor - a major drug target G protein-coupled receptor (GPCR) and c-Src kinase - a key enzyme involved in uncontrolled cell growth and differentiation in cancerous cells. Markov state models (MSMs) are used for aggregating independent simulations into a single statistical model. Our models provide the first atomistic description of the activation of a GPCR, revealing multiple activation pathways. Similarly, MSMs of c-src tyrosine kinase capture the thermodynamics and kinetics of kinase activation for the first time, and help identify key intermediates along the activation pathway. We also predict the presence of a novel allosteric site in c-src intermediate state, which could be potentially utilized for drug design. Furthermore, these model results are validated by previous computational and experimental observations. The implications of this work are far-reaching and will likely have broad impact and significance even further beyond the application proposed here. Cloud computing is opening up a promising new avenue to tackle challenging questions in science more routinely, leaving the complexity of the underlying infrastructure hidden and enabling a larger proportion of time spent on science.

WRM 237

Automated Catalytic Site Detection

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As a component of the a proposed Protein Function Prediction Platform, we present a catalytic site identification procedure.

It uses a template-matching algorithm and a scoring procedure that allows for rapid, scalable protein-to-template matching for catalytic sites from a catalog of binding sites. We develop the procedure using the Catalytic Site Atlas (CSA) of Thornton.

The Catalytic Site Identification web server provides the innovative capability to find structural matches to a user-specified catalytic site among all Protein Data Bank (PDB) proteins very rapidly (in less than a minute). The server also can examine a user-specified protein structure or model to identify structural matches to a library of catalytic sites. Finally, the server provides a database of pre-calculated matches between all PDB proteins and the library of catalytic sites. The database has been used to derive a set of hypothesized novel enzymatic function annotations. In all cases matches and putative binding sites (protein structure and surfaces) can be visualized interactively online. The website can be accessed at http://catsid.llnl.gov/. We will go through some online examples.

WRM 238

Innovation, Innovation Ecosystems, and Incubators: Bridging the Innovation Gap

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The Life Sciences industry is simultaneously facing significant challenges from lack of productivity in discovery research and cost pressures exacerbated by the shifting health care reimbursement outlook in the U.S. and globally. The challenge is Innovation and the answer is Innovation. The industry is going to its roots in biochemistry, molecular medicine, and systems biology and is developing innovative approaches to transform health care.
It may be Change, but is it Innovation? Some of these approaches encompass collaboration and environments that foster innovation. A number of Disruptive Innovation Frameworks are discussed, including open innovation, ecosystems for innovation, and incubation models. Deloitte conducted research uncovered emerging trends within the incubator / innovation center market. Peer analysis showed that incubators provided multiple offerings but no incubator offered the whole spectrum of services. These models are examined and some implications for the future are discussed.

WRM 239
Creating an Innovation Ecosystem
***Kara Bortone**, kbortone@its.jnj.com. Company Sourcing, Janssen Labs, San Diego, California 92121, United States*
This presentation will be an overview of several distinct initiatives ranging from incubators, EIR programs, direct investing, challenge grants, commercialization curriculum and much more. Session participants will walk-away with an overview of how Johnson & Johnson has created a rich ecosystem to foster innovation.

WRM 240
Enabling Awesome-QB3's Life Science Incubators
***Douglas Crawford**, douglas.crawford@qb3.org.QB3, California Institute for Quantitative Biosciences, United States*
Despite extraordinary advances in biological knowledge and research tools, the biopharmaceutical industry has found it increasingly hard to turn these discoveries into profitable products. QB3 believes that the solution to this challenge is to empower entrepreneurial scientists to create cool companies based on their insights. Given the right environment, entrepreneurial scientists can change the world, but the right environment is critical. Since 2006, QB3 has been developing innovative incubators to allow startups to go further on less: renting as little as a single bench and taking advantage of the millions of dollars worth of University research resources. The program has worked – QB3's incubators have been home to 84 companies in the program and they have gone on to raise $380 million and create over 400 jobs. We think this is a model that should be copied in every university.

WRM 241
Bayer's new approaches to building connections for partnered research
***Christopher Haskell**, christopher.haskell@bayer.com.U.S. Science Hub, Bayer HealthCare LLC, San Francisco, California 94158, United States*
Bayer strongly values partnerships as a means to advance innovative new therapies that will impact human health. A key success factor to these partnerships is a high level of engagement between the partner scientists. Recognizing this, Bayer has developed the CoLaborator; an incubator in Mission Bay, San Francisco for startup companies working in areas of interest to Bayer R&D. The concept revolves around bringing emerging innovation in close proximity with our research groups, fostering interaction and collaboration. This model is also being replicated at our Berlin R&D site, creating an innovation hot-spot directly in proximity with one of our German research centers. However, as ideas can come from anywhere, Bayer also uses the internet and crowd-sourcing platforms to reach inventors around the world. Our Grants4Targets and Grants4Leads programs provide funds for early ideas, and set the stage to build relationships around new discovery. As an innovator company, Bayer believes that creativity and experimentation are also required in partnering models, and these are but two approaches currently in place to advance innovation.

WRM 242

Creating a multidimensional, laboratory intensive, support system

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MMRI is an independent, nonprofit research organization founded in 1995. The Institute supports entrepreneurial scientists in achieving early development of therapeutic and diagnostic goals within a working multidimensional laboratory environment. MMRI provides medical researchers transitioning to company formation the space, tools, and infrastructure for immediate experimentation at a substantially lower cost than independent laboratory formation. Barriers to success are lessened scientifically and financially at the critical juncture required to add value and attract capital. The same day affiliates join the Institute they can begin advancing the understanding of the molecular and cellular basis of their target disease, with the goal of translating scientific findings into direct patient care.

This unique model has proven to be successful. In our 18-year history, affiliate researchers have founded 60 companies, brought new drugs and diagnostics to late stage clinical trials, produced investor liquidity events including corporate acquisition and alliances, mergers, and IPOs. The longer individuals and organizations conduct research within this network of accomplished and diverse scientists clustered at MMRI, the more fruitful their synergy.

WRM 243

The Science and Politics of Hydraulic Fracturing in California

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Despite being used frequently as a well-stimulation technique in California for over sixty years, hydraulic fracturing has attracted enormous public attention following the distribution of the docu-drama, *Gasland* in 2010. The policy discussions in California around hydraulic fracturing began in earnest in the California Legislature during the budget hearings for the Department of Conservation in March 2012. Prior to that period, hydraulic fracturing was considered by the Division of Oil, Gas and Geothermal Resources (DOGGR) to be one among several well stimulation techniques used regularly in California's oil and gas operations. And hydraulic fracturing as a regulatory issue was one among several key issues being addressed by the Division, including carbon capture and storage, H2S gas disposal, cyclic steaming in shallow diatomite, CEQA and land use challenges and other matters. Currently, the Department is working through a rulemaking process on hydraulic fracturing, and expects to complete this process sometime mid-year 2014. This keynote will discuss the scientific and technical challenges involved in the rulemaking process, and address some of the policy and political dynamics that have developed over the past two years with regard to hydraulic fracturing and other oil and gas production issues.

WRM 244

Advocating for safe oil and natural gas extraction policies; an overview of FracTracker as a mechanism for overcoming the barriers to scientific advocacy and community engagement

***Kyle J. Ferrar**[1,2], Ferrar@FracTracker.org, Samantha L. Malone[1], Matthew T. Kelso[1], Brook Lenker[1]. (1) California State Coordinator, FracTracker Alliance, Berkeley, California 94704, United States (2) Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania, United States*

The inability to translate data to scientific information that can readily be incorporated by citizens into the public arena is an obstacle for science–based advocacy. This issue is particularly poignant for shale oil and natural gas development via hydraulic fracturing, as the issue has become highly politicized. Barriers to engaging in policy debate are different but highly related for community members and scientists. For citizens and interest groups, barriers including accessibility, public awareness and data presentation limit the motivation for community involvement in political interactions. To overcome such barriers, social researchers call for public engagement to move upstream and many call for a broad engagement of scientists in science-based advocacy. Furthermore surveys have shown that citizens, interest groups, and decision-

makers share a broad desire for scientists to engage in environmental policy development. Regardless, scientists face a number of perceived barriers, with academics expressing the most resistance to overcoming the tension created by adherence to the scientific method and the need to engage with the broader society, described by Schneider (1990) as the “double ethical bind”. For the scientific community the appeal of public dissemination of information beyond the scope of academic journals is limited for a number of reasons. Barriers include preservation of credibility, peer attitudes, training, and career trajectory. The result is a lack of translated information available to the public. This presentation will provide an explanation of the FracTracker platform and a discussion of the features that allow for public engagement and will show how FracTracker can be used as an outlet for scientific researchers to engage with citizens. An analysis of web traffic will also provide insight into what information the public is most interested in concerning hydraulic fracturing and unconventional oil and natural gas development.

WRM 245

Hydraulic Fracking and The Battle Over Beneficial Water Use in California, The Next Phase of Fracking Litigation in California

***Earl Hagström**, Earl.Hagstrom@sedgwicklaw.com, Meegan Brooks.Sedgwick LLP, San Francisco, CA, United States*

The competition between agriculture, energy, domestic and environmental interests for water in California is fierce and is about to become more contentious. In a “wet year” the demand for water from these interests may exceed the ability to deliver sufficient volumes of water to the end user. In a drought period the demand for water may exceed the available volume. When demand exceeds supply the issue of what constitutes the beneficial use that best serves the people of the State will need to be decided. That decision will likely be made in the California Courts.

Under current state law Counties have the authority to regulate groundwater usage. Although counties can choose which interests to prioritize for their laws, it is worth noting how the state has balanced competing “beneficial uses” in the past. In acting upon applications to appropriate water, a board shall consider the relative benefit to be derived from (1) all beneficial uses of the water concerned including, but not limited to, use for domestic, irrigation, municipal, industrial, preservation and enhancement of fish and wildlife, recreational, mining and power purposes, and any uses specified to be protected in any relevant water quality control plan, and (2) the reuse or reclamation of the water sought to be appropriated, as proposed by the applicant. A board may subject such appropriations to such terms and conditions as in its judgment will best develop, conserve, and utilize in the public interest, the water sought to be appropriated.

When balancing the “public benefit” conferred the competing interests of the agricultural, energy, domestic and environmental communities will be weighed. In any such consideration preemption, Home Rule and the difference between withdrawal and consumption will be of major importance.

WRM 246

How API's Shale Gas Standards and Best Practices support Sustainable Shale Gas Development

***David Miller**, Miller@api.org. Standards, American Petroleum Institute, Washington, DC 20005, United States*

Founded in 1919, the American Petroleum Institute (API) has a long history in the development of industry standards, starting with the first standards published in 1925 on drilling thread specifications to ensure safety in the production of oil and natural gas. Continuing on for nearly 90 years, API has led the development of petroleum, petrochemical and natural gas equipment and operating standards. These standards represent the industry's collective wisdom on subjects ranging from drill bits to environmental protection and embrace proven, sound engineering and operating practices and safe, interchangeable equipment and materials. API maintains more than 650 standards, many of which have been incorporated into state and federal regulations. API standards are also the most widely referenced oil industry standards cited by the international regulatory community.

API ensures the technical relevance of its standards by maintaining its status as an American National Standards Institute (ANSI) accredited standards developing organization. As an

accredited body, API's standards program undergoes regular program audits to ensure it meets ANSI's “Essential Requirements” for openness, balance, consensus and due process.
API has also been the leader in the development of standards supporting sustainable shale gas development. API published its first of five specific shale gas standards in 2009 on well integrity, and subsequently developed standards on water resource management, environmental practices, and well cementing technology. This presentation will focus on the status of updates to these standards as well as a new standard on Community Engagement, being developed to ensure well operators, drilling and well servicing companies and the communities in where the shale gas is being developed fully understand the important aspects of operations taking place in and around their neighborhoods, towns, and cities.

WRM 247
How environmental research can lead to more sustainable food production systems
James N. Seiber, *jnseiber@ucdavis.edu. Department of Environmental Toxicology, UC Davis, Davis, CA 95616, United States*
There is great interest in 'sustainability' these days, including in the production and use of foods and beverages. In food production, sustainability can refer to foods that optimize health, safety, quality, and consumer appeal, as well as use of less inputs at the farm or processing plants of energy, fertilizers, pesticides and water. It can include minimizing waste generation, from the food itself or its packaging, reducing emissions, lowered carbon footprint, humane treatment of farm animals, recycling waste for energy recovery, capturing and using wastewater and rainwater, and pest control with biopesticides rather than synthetics. This presentation will focus on research into the environmental benefits of using reduced-risk biopesticides or even chemical-free pest control in sustainable systems of food production.

WRM 248
Targeted unknown UHPLC-(ESI+)-Q/TOF MS approaches for understanding flavonoid bioavailability and metabolism.
Alyson E Mitchell, *aemitchell@ucdavis.edu.Department of Food Science and Technology, University of California, Davis, Davis, California 95618, United States*
The bioactive flavonoid quercetin occurs as a range of glycosides in foods. The composition and levels of glycosides depend on the species and cultivar. Primary dietary sources include apple peel and onion. Upon absorption, quercetin is subject to extensive Phase II metabolism (biotransformation), which results in a range of complex conjugated metabolites, including glucuronides, sulfates and methylated forms of quercetin. The bioactivity of quercetin metabolites depends on the types and position of the conjugate group on the metabolites as shown in *in vitro* studies. Herein, quercetin metabolite profiles are described in the plasma of 16 human subjects after the consumption of a quercetin-free applesauce enriched with micronized apple peel or onion delivering ~100 mg quercetin equivalents. Qualitative analysis of extracts was performed by UHPLC (ESI)/Quadrupole Time-of-Flight (Q-TOF) mass spectroscopy (MS). Quercetin metabolites were identified based on their exact mass and isotope spacing. Identified metabolites were further characterized by UHPLC-Q/TOF MS/MS. Sixteen quercetin metabolites, including glucuronide, sulfate, methyl and glutathione conjugates and mixed conjugates, were identified. Consumption of apple peel resulted in glucuronide-related metabolites such as quercetin monoglucuronide and quercetin diglucuronide, whereas consumption of onion resulted in sulfate-related metabolites, such as quercetin sulfate and quercetin monoglucuronide sulfate.

WRM 249
Determination of Volatile Carbonyls in Olive Oil using Ultra Performance Liquid Chromatography and Gas Chromatography-Electron Ionization Mass Spectrometry
Hanjiang Zhu, *nnzhu@ucdavis.edu Selina Wang, Charles O. Shoemaker. Food Science & Tech., UC Davis, Davis, CA 95616, United States*
Many volatile carbonyl compounds produced though lipoxygenase pathway and oxidation process contribute to the complex flavor of olive oil; some of the carbonyls, such as hexanal and

nonanal, have been widely considered as indicators of lipid oxidation. An sensitive Ultra Performance Liquid Chromatography (UPLC) method following dynamic headspace sampling and 2,4-dinitrophenylhididrazine (2,4-DNPH) derivatization was established to determine the volatile carbonyls in olive oil. Quantification of 8 characteristic carbonyls (hexanal, E-2-hexenal, octanal, E-2-octenal, nonanal, E-2-nonenal, E,E-2,4-decadienal, and E,E-2,4-nonadienal) was achieved by using isobutyl acetate as internal standard. To assist the identification of unknown compounds, Gas Chromatograph-Electron Ionization Mass Spectrometry (GC-MS) was employed to exam the same samples of derivatized carbonyls (carbonyl (2,4-DNPH) hydrazones), and the peak assignment was performed on the basis of relative retention time and percentage peak area. New harvested extra virgin olive oil and aged unrefined olive oil were tested to have a better understanding on the changes in the volatile carbonyls during room temperature oxidation.

WRM 250

Tissue concentrations of β-cryptoxanthin in Mongolian gerbils increase in parallel with its dietary concentration

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β-Cryptoxanthin is a provitamin A carotenoid with antioxidant activity. There is a lack of knowledge of its absorption and tissue storage. We investigated the whole body distribution of β-cryptoxanthin in the Mongolian gerbil (*Meriones unguiculatus*), an appropriate small animal model for human provitamin A carotenoid metabolism. After a 5 day carotenoid depletion period, 5 gerbils were euthanized to determine baseline values. The remaining gerbils were put into 3 weight-matched treatment groups (n=8). Group CX20 was fed 20 μg/d β-cryptoxanthin from tangerine concentrate. Groups CX40 and CX60 received the tangerine concentrate plus 20 and 40 μg/d of pure β-cryptoxanthin, respectively, for 21 days. Fourteen tissues, including liver, kidney, adipose and intestines were surgically removed and analyzed by reversed-phase HPLC. β-Cryptoxanthin was detected in 12 of the 14 tissues analyzed and in blood. Most β-cryptoxanthin was stored in the liver, as is common for carotenoids. Liver concentrations in the CX60 and CX40 groups were significantly greater (P = 0.004, 0.006) than the CX20 group: 17.8 ± 0.7, 16.2 ± 0.9, and 13.3 ± 0.4 μg/organ, respectively. Most tissues had a similar response to increased concentrations, but β-cryptoxanthin in intestinal tissue increased only for the CX60 group. β-Cryptoxanthin appeared to maintain vitamin A concentrations. No other β-cryptoxanthin metabolites (apocarotenoids, β-ionone, 3-hydroxyretinol) were detected, likely due to their low concentrations or transitory nature. These results show that β-cryptoxanthin is stored in many tissues, and that concentrations in most tissues increase in parallel with greater β-cryptoxanthin intake; potentially suggesting its functions are widespread.

WRM 251

The physicochemical stability and in vitro bioaccessibility of beta-carotene in oil-in- water sodium caseinate emulsions

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Beta-carotene (BC), the most important dietary source of provitamin A, is necessary for optimum human health. BC is insoluble or only slightly soluble in most liquids but its bioavailability improves when ingested with fat. Therefore lipid emulsions are ideal matrices for BC delivery. BC (0.1%) in corn oil, the dispersed phase (5 or 10%), was homogenized with 2% sodium caseinate solution in a microfluidizer. Homogenization at different pressures produced droplet diameters, Dz=368-124 nm. Nanoemulsions (r<200 nm) were prepared at 100 MPa. The sodium caseinate emulsions were generally very stable to coalescence or flocculation over 30 days and the slow rate of volume increase was found to be related to the square of the initial droplet radius following Stokes velocity of settling equation. BC stability towards oxidation was lower but the rate of lipolysis in an in vitro system was higher with decrease of droplet diameter. Bioaccessibility, as defined by the amount of BC recovered in the aqueous phase after ultracentrifugation, was linearly related to smaller emulsion droplet diameter. These results show that sodium caseinate, a

food grade emulsifier, can be used to prepare stable emulsions of food oils carrying beta-carotene.

WRM 252

Hydrocarbon Renewable and Synthetic Diesel Fuel Blendstocks: Composition and Properties

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We examined the chemical composition and properties of several diesel fuels and blendstocks derived from Fischer−Tropsch (FT) synthesis, hydroisomerization of lipids, and fermentation of sugar via the terpenoid metabolic pathway. Comprehensive two-dimensional gas chromatographic analysis with nonpolar and polar columns, ^{13}C NMR, GC-MS, and elemental analysis were used to assess fuel chemistry. Performance properties included density, heat of combustion, cetane number, and cloud point, as well as other properties. The fuels consisted almost entirely of normal and iso-paraffins. Three samples contained residual oxygen below 0.1 mass %. All of the renewable and synthetic diesel fuels have significantly lower density than is typical for a petroleum-derived diesel fuel. As a result, they have slightly higher net heat of combustion on a mass basis (2%−3% higher), but lower heat of combustion on a volume basis (3%−7% lower). Two critical diesel performance properties, cetane number and cloud point, were correlated with iso-paraffin content and chain length. The results confirm that properties of hydroisomerized fats and oils, as well as FT diesel, can be tuned by increasing the degree of isomerization to lower cloud point which also lowers the cetane number. In spite of this trade-off between cloud point and cetane number, the cetane numbers were still over 70 for fuels with cloud points as low as −27 °C. The terpenoid biofuel exhibited a cloud point below −70 °C and a cetane number of 58.

WRM 253

Rapid and Sensitive Determination of Biofuel Sugars by Ion Chromatography

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Biofuels have emerged as an attractive alternative to fossil fuels; however, the development of robust analytical methods remains a challenge. It is critical to determine carbohydrates during biofuel alcohol production to ensure process yield and product quality. The carbohydrates in biomass samples are often quantified by high-performance liquid chromatography (HPLC) using refractive index detection. The refractive-index-based method is not specific, and could have interferences from other compounds in the complex biomass matrices. Using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD), the key carbohydrates can be separated in under 10 min with minimal sample pretreatment. The method is robust and can be used in the highly concentrated samples encountered in biofuel processing.

WRM 254

Cold Flow and Phase Transition of Transparent and Opaque Oils and Fuels

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The determination of the onset of wax formation is an important consideration in the handling, pumpability and operability of oils and fuels typically in cold conditions. This paper examines recent development in testing of cloud point, freezing point, as well as wax appearance temperature in fuels and oils. Both transparent and opaque samples are discussed. Applications to other chemicals, surfactants, and food oils will also be presented

WRM 255

Quantitation of Low Levels of FAME, Fatty Acid Methyl Esters, in Fuel and Waste Water

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Measuring biodiesel/fatty acids methyl esters in either petroleum products or water is necessary in petroleum arena. For example, there is a very tight restriction on the presence of biodiesel in jet fuel. These compounds [FAME] in waste water also pose difficulties in treatment, and concentration measurements are of interest. We have developed a method to measure FAME typically found in biodiesel and petroleum Diesel by the use of deuterium-labeled compounds, with or without a pre-concentration step. This study by GC-MS and FTIR demonstrates the method detection limit [MDL] is 1 ppm per compound.

WRM 256
Elemental Analysis of Fuels Using Monochromatic X-Ray
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Advances in x-ray fluorescence technology has enabled significant reductions in detection limits for critical elements in hydrocarbon samples. These advances have led to the introduction of process and lab based XRF systems capable of achieving detection limits historically associates with wet chemistry type systems. Quantification and ppb levels of key elements such as Ni, V, Fe, Zn and Ca as well as light elements such as S, Cl, Si and P can now be achieved with optic enabled XRF systems. The optic enables xrf technologies will be discussed and data from lab and process systems will be reviewed.

WRM 257
Solid Phase Microextraction GCMS of Organics in Water
***James D Hudson**, jameshudson@chevron.com, Michael T Cheng. Analytical, Chevron, Richmond, CA 94802, United States*
Solid Phase Microextraction (SPME) was introduced in 1993 and provides a fast and cost-effective extraction method for analysis of organic compounds in aqueous matrices by GC/MS. Specific hydrocarbon compounds in refinery wastewater are routinely measured by isotope dilution mass spectrometry using benzene-d_6 and decane-d_{22}as internal standards in conjunction with solid phase microextraction. Refinery wastewater contains many organic compounds with a wide range of polarity. In order to monitor the concentration of these compounds in the wastewater, analytes are adsorbed directly onto a fused-silica fiber coated with a stationary phase. After adsorption, the analytes are thermally desorbed from the fiber into the GC injection port. The concentration of the compounds in refinery wastewater is often in the PPM range, and direct analysis would be difficult without the concentrating capability of SPME. SPME/GC/MS provides an easier way to analyze for organic compounds in aqueous solution because no solvent is used and less sample is required. When used in conjunction with labeled internal standards, quantitative data for specific compounds can be obtained by isotope dilution mass spectrometry.

WRM 258
Increased Use of Spectrographic Methods for Fuels Analysis
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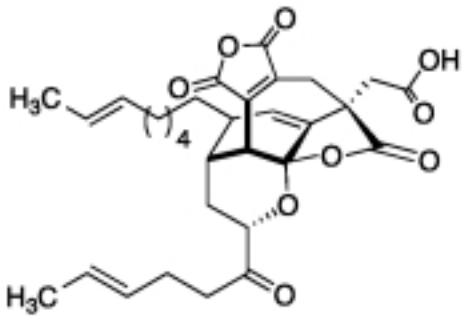
Fuel specifications require several analysis to ensure all the required properties are tested and are within acceptable tolerance. Many of these are very time consuming and expensive to perform. Some are not too accurate but are still required for historical reasons or lack of other methods. There is a growing trend to use spectroscopic methods as many properties can be measure in one non-destructive test. This trend will be discussed with several examples as well as advantages and disadvantages.

WRM 259
Chemical synthesis of secondary metabolites
***Ryan A Shenvi**, rshenvi@scripps.edu. Chemistry, The Scripps Research Institute, La Jolla, CA 92037, United States*

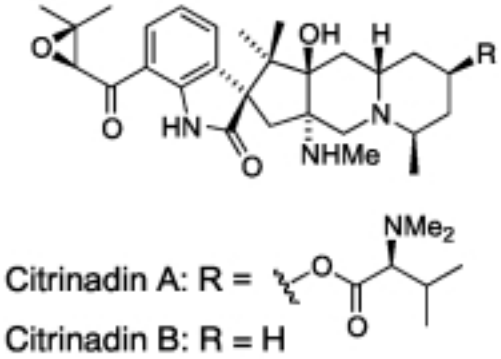
The majority of small molecule therapeutics approved by the FDA over the last twenty years are derived in structure or function from secondary metabolites, which can therefore be viewed as privileged leads in drug discovery. Recent advances in the chemical synthesis of secondary metabolites and associated methods developed in our laboratory will be presented.

WRM 260
Synthesis of natural products with conformational chirality
Christopher Beaudry, *christopher.beaudry@oregonstate.edu. Department of Chemistry, Oregon State University, Corvallis, OR 97331, United States*
The identification of molecular chirality in molecules that lack sp3 hybridized stereogenic atoms is not straightforward. We have synthesized a variety of natural products that are chiral by virtue of restricted rotations of single bonds. Experimental techniques are used to determine the propensity of such molecules to racemize.

WRM 261
Recent progress in the synthesis of complex natural products
John Wood. *Chemistry, Baylor University, Waco, TX 76706, United States*
Recent efforts in our laboratories have focused on the synthesis of several complex natural products. The evolution of synthetic strategies directed toward the phomoidrides and citrinadins will be discussed.



Phomoidride B



WRM 262
Natural Product Synthesis: A Platform for Chemical Discovery
Sarah E. Reisman, *reisman@caltech.edu. Chemistry & Chemical Engineering, California Institute of Technology, Pasadena, CA 91108, United States*
Our laboratory has ongoing research programs targeting the chemical syntheses of several natural products, including members of the epidithiodiketopiperazines, the ent-kauranoids, and the acutumine alkaloids. The densely packed arrays of heteroatoms and stereogenic centers that constitute these polycyclic targets challenge the limits of current synthetic methodology. This seminar will describe our latest progress in both our methodological and target-directed synthesis endeavors.

WRM 263
Understanding the Interplay of Physicochemical Properties in the Drug Development Process
Konstantin Tsinman, *ktsinman@PION-INC.com Science and Research, Pion, Inc., Billerica, MA 01821, United States*
A key task for pre-clinical research is the prediction of human pharmacokinetics for compounds entering clinical studies. The ability to profile pharmaceutical properties of candidate compounds can culminate in improved *in vivo* performance predictions for lead generation,

optimization, and selection. This presentation will cover the development of physicochemical profiles of new drug candidates using solubility, permeability, ionization and dissolution measurements. The interpretation of this data can result in more meaningful in vitro – in vivo correlations during the drug development phase.

WRM 264

Pharmaceutical API properties modulation using cocrystals

Patricia Andres, *patricia.andres@aptuit.com. Department of Chemistry, SSCI, a division of Aptuit, West Lafayette, IN 47906, United States*

Over 50% of active pharmaceutical ingredients (API) on the market are currently administered as salts. Salt formation is commonly used in drug development both for purification purposes and as a method to improve the solid state properties of the API. The development of an increasing number of non-ionizable and poorly soluble compounds requires new approaches to replace the void caused by the inability or limited possibilities to form salts of these new chemical entities. Physical properties of solid forms are affected by the supramolecular organization of the components in the solid. The formation of cocrystals provides an alternative solution to the need for purification and property modulation of an API, giving an opportunity to modify critical attributes of an API as well as improve the performance of a drug. A review of similarities and differences between salt and cocrystal forms will be provided, with examples of property modulation and uses of cocrystals for pharmaceutical development, to demonstrate the usefulness and somewhat untapped potential of cocrystals in this field.

WRM 265

Solid-state challenges during drug development: Processing induced phase transformations and their impact on pharmaceutical product quality

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The importance of suitable form selection for the lead candidate is commonly recognized as an exercise of paramount importance since the solid-state form has a significant impact on the stability and performance of the final drug product. However, it may so happen that the appropriate form selected may not be retained in the final pharmaceutical product owing to inadvertent phase transformations caused by stresses (thermal, mechanical, moisture) commonly encountered by the active during drug product manufacturing. In such cases, the quality of the drug product will be influenced by both the extent of conversion and the properties of the product (transformed) phases. It is therefore important to detect, characterize (qualitative and if possible quantitative) and understand the implications of such phase transformations on product performance. In addition, it is important that the physical form of the active be monitored *during* processing since there is a potential for multiple phase transformations to occur during the sequence of pharmaceutical unit operations. A thorough understanding of the effect of every processing step on the physical form can be utilized to optimize formulation and process variables in order to achieve rigorous control over the solid form, during manufacturing and throughout the product shelf life. The objective of the talk will be to focus on case studies exemplifying solid-state phase transformations brought about by pharmaceutical processing, their detection and characterization and finally, their potential impact on the quality and performance of the dosage form.

WRM 266

Solubility & Dissolution Enhancement of Weakly Basic Compounds – Understanding Precipitation & Supersaturation

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Purpose

The aim of this study was to evaluate the supersaturation and precipitation behaviour of poorly soluble ionizable compounds in the presence of cellulose polymers.

Methods

The CheqSol solubility method was used to monitor the crystallization tendency of ionizable drugs when titrated towards precipitation of the free acid or free base form of the compound. CheqSol uses principles of mass and charge balance to determine the solution concentration of the neutral species and can be used to study supersaturation and crystallisation behaviour of compounds during precipitation events. Seven compounds were studied: piroxicam, metoclopramide, rosiglitazone, paliperidone, dipyridamole, aripiprazole and tolbutamide. Measurements were made in the presence of hydroxypropylcellulose (HPC, Klucel® EF) and hydroxypropylmethylcellulose (HPMC, Pharmacoat® 603) to investigate the influence on solubility enhancement and precipitation behaviour.

Results

At 1:1 cellulose:drug ratio, rosiglitazone precipitated in an amorphous form (solubility 110μM), which was more soluble than the crystalline form found without cellulose (5.5μM). The amorphous form endured for approx. 30mins. The solubility of dipyridamole, tolbutamide, aripiprazole and paliperidone only marginally increased with cellulose, and crystallization could not be prevented. A high solubility form of metoclopramide was achieved with cellulose (1.6mM) and endured for longer time periods with increasing cellulose content. A slow conversion to a low solubility form (0.33mM), was then observed. Peak supersaturation levels of piroxicam were proportional to polymer content; Klucel achieved a 5-fold increase at 1:1 cellulose:drug ratio and a 4-fold increase at 1:4. Pharmacoat also performed well with a 3-fold increase at 1:1 cellulose:drug ratio and a 2-fold increase at 1:4.

Conclusion

The precipitation behaviour of various poorly soluble drugs and the solubility enhancement property using cellulose polymers, Klucel and Pharmacoat, have been studied. Klucel and Pharmacoat were proven to be efficient in increasing the extent and/or duration of supersaturation of several of the studied compounds.