SFC 2019 Oral Abstracts

Monday, September 30

8:40 am

Footsteps of a Giant - Intellectual Travels with Larry T. Taylor, J. David Pinkston, Ph.D., Archer Daniels Midland Company, Decatur, IL USA

Larry T. Taylor, Professor Emeritus at Virginia Tech University, is indeed a giant in the worlds of supercritical chromatography, extraction, and processing. The Green Chemistry Group recognizes Larry for his contributions in these fields, both in scientific advances and in encouraging scientific communication. Not only was he on the Scientific Organizing Committee for the current series of international conferences on SFC that began in 2007, but he was Co-Organizer for the series of International Symposia on SFE/SFC, which spanned 1992 through 2004. He has published well over 400 peer-reviewed publications, book chapters, and government reports during his long and productive career, and mentored 68 successful Masters and Ph.D. students. He is well known as an engaging teacher, having taught for many years at Virginia Tech, as well as teaching dozens of short courses on SFC and SFE.

This presentation will describe some of the more memorable research contributions from Larry's laboratory. He has been one of the leaders in applying SFC to polar and ionic materials, such as polypeptides, ionic surfactants, and mixtures of biological importance. He has also explored the use of more polar additives in SFC, including the use of water as an additive, and expanded the understanding of the mechanisms by which these polar additives affect retention in SFC. Larry and his colleagues made great contributions in the area of detection in SFC, including FTIR, mass spectrometry, electrochemical detection, ELSD, sulfur and nitrogen chemiluminescent detection, and electron capture detection.

Larry is also known for his contributions in furthering the understand and application of SFE and supercritical fluid processing. He helped quantify the impact of dynamic vs. static extraction in SFE, as well as the influence of additives, pressure, and temperature on extraction in a wide variety of matrices. Finally, Larry's laboratory made great strides in supercritical fluid impregnation, specifically in the area of metallization of polymers.

Larry T. Taylor is indeed a Scientific Giant in SFC, SFE, and SF processing. We are grateful to follow in his footsteps.

9:10 am

SFC-PDA-ESIMS as a framework for impurity fate mapping in the development and manufacture of drug substances, Erik L. Regalado, Gregory F. Pirrone1, Rose M. Mathew, Alexey A. Makarov, Frank Bernardoni, Artis Klapars, Robert Hartman, John Limanto, Merck Research Laboratories, Rahway, NJ, USA

Impurity fate and purge studies are critical in order to establish an effective impurity control strategy for approval of the commercial filing applications of new medicines. Reversed phase liquid chromatography-diode array-mass spectrometry (RPLC-DAD-MS) has traditionally been the preferred tool for impurity mapping in fate and purge. However, separation of some reaction mixtures by LC can be very problematic, requiring combination LC-UV for area % analysis and a different LC-MS method for peak identification. In addition, some synthetic intermediates might be chemically susceptible to the aqueous conditions used in RPLC separations. In this presentation, the use of SFC-PDA-ESI-MS for fate and purge of specified impurities from starting materials used in the synthesis of uprifosbuvir is illustrated. Readily available SFC instrumentation with a Chiralpak IC column (4.6×150 mm, 3 μm) and ethanol: carbon dioxide based mobile phase eluent enabled the separation of closely related components from complex reaction mixtures where RLPC failed to deliver optimal chromatographic performance. These results illustrate how SFC combined with PDA and ESI-MS detection can become a powerful tool for direct impurity fate mapping across multiple reaction steps.

9:35 am

Active Flow Technology Improves Peak Efficiency in SFC, Bill Farrell, Pfizer, La Jolla, CA, USA

Recent advances in alternative column design and construction techniques (e.g. 3D printing) are expanding the applicability of traditional columns while allowing for different chromatographic approaches to be applied. One such approach, which was introduced in 2012, is Active Flow Technology (AFT). AFT columns are designed such that the radial flow through the center of the column is segmented from the flow along the walls to create virtual column without a barrier. This design has shown to maximize peak efficiency in high performance liquid chromatography (HPLC) applications while demonstrating sensitivity gains using variants of AFT. Recently, the application of this technology to supercritical fluid chromatography (SFC) has been applied with some surprising and counterintuitive results. This presentation will cover these aspects with a focus on elucidating the source of band broadening and propose a potential solution to improve peaks shape and resolution.

10:45 am

New Approaches to SFC Separations Using Water as a Stationary Phase, Kevin B. Thurbide, Andrea F. Scott, Jackson J. Frantz, and Matthew T. Saowapon, Department of Chemistry, University of Calgary, Calgary, Alberta, Canada.

Stationary phase development is an important area in SFC due to the impact it can have on separation selectivity. In recent years we have been exploring a novel SFC separation method that employs water as a stationary phase. The SFC system utilizes carbon dioxide as the mobile phase, and it displays a normal phase retention pattern as well as compatibility with the universal flame ionization detector. The ability of the system to separate analyte mixtures of varying polarity (e.g. alcohols, carboxylic acids, etc.) without organic modifiers has also been demonstrated, along with some unique features that can simplify the determination of such compounds in certain complex matrices. Here results will be presented that show how the properties of the water stationary phase can be altered and controlled externally in order to manipulate SFC separation properties. Further, it will be illustrated how customizing the composition of this phase can greatly impact analyte retention and selectivity in different SFC applications.

11:10 am

Determination of Drugs of Abuse in Human Hair by On-line Supercritical Fluid Extraction – Supercritical Fluid Chromatography - Mass Spectrometry, Blair Berger[1], A. Paige Wicker[1], Tairo Ogura[2], Kenichiro Tanaka[2], Masayuki Nishimura[3], Vivian Chen[3], William Hedgepeth[3], Kevin A. Schug[1] 1) Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, TX 76109, USA, 2) Shimadzu Corporation, Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan, 3) Shimadzu Scientific Instruments, Inc, Innovation Center, 7102 Riverwood Drive, Columbia, MD 21046, USA

The detection of drugs of abuse (DoA) in hair; being a convenient and noninvasive technique for the determination of controlled substances, is important in forensics and toxicology. On-line supercritical fluid extraction – supercritical fluid chromatography (SFE-SFC) coupled with mass spectrometry (MS/MS) is a quickly developing technique for the extraction, separation, detection, and quantification of analytes in a single analysis. SFE-SFC-MS/MS being ideal for DoA determination in complex matrices, provides a highly specific and sensitive chemical analysis while limiting the need for extensive manual sample preparation.

SFC-MS. Chromatographic separation of sixteen DoAs (representing a 10-panel drug test) was investigated using carbon dioxide (CO_2) with 5 mM ammonium formate in methanol at 3.00 ml/min on a HILIC-Si column [150 mm x 4.6 mm, 2.7 µm (Restek Corp.)]. Selectivity changes were observed due to changes in column temperature, and ultimately 55 °C was chosen for optimal chromatographic resolution at below 30% modifier concentration. Detection of compounds was made via DUIS-MS analysis on a triple quadrupole mass spectrometer in positive and negative mode by Multiple Reaction Monitoring (MRM).

SFE-SFC-MS. Currently, significant challenges for hyphenated method development exist due to the complexity of the system, number of variables that require optimization, and the lack of a clear set of starting extraction parameters.

Supercritical fluid extraction of sixteen DoAs from human hair was performed on-line using carbon dioxide (CO_2) with 5 mM ammonium formate in methanol. Dual, variable, back pressure regulators (BPRs), pre- and post-column, were used to 'trap' extractant at the column head. Pre-column backpressure was increased directing flow at 3.0 ml/min for chromatographic separation on a HILIC-Si column [150 mm x 4.6 mm, 2.7 µm (Restek Corp.)] at 55 °C.

Optimal starting extraction parameters for extracting five of the sixteen drugs of abuse were predicted using models developed in a previous work by response surface methodology. Multivariate analysis, was used to optimize extraction parameters, including time (static and dynamic), modifier concentration, flow rate and pressure, and indicated, optimal extraction would occur using 30% modifier at 5.00 mL/min with 30 °C vessel temperature with dual backpressures of 10.0 MPa. Extraction performance using these starting conditions, gave the highest (between 56-100%) recovery of spiked standards, but proved to ineffectively trap early eluting compounds at the column head. Alternatively lower modifier concentration (as predicted) proved to be insufficient in extracting the more polar, later eluting analytes, quickly lowering extraction performance below 15% modifier concentration. In order to optimize the method for a wider range of polarity, It was determined that a compromise in modifier concentrations was needed between static and dynamic extractions. Overall extraction and analysis for all analytes was ultimately achieved using a higher modifier concentration during dynamic extraction (in order to allow sufficient extraction of the more polar analytes) while a much lower modifier concentration was needed during static extraction (to assist formation and retention of the sample plug at the column head). Optimal conditions were found to be 15% modifier for an 8 minute static extraction which was then followed by a 15 minute dynamic extraction using 2% modifier. Method validation is currently underway to ensure detailed quantitative analysis for hair samples.

11:30 am

Could SFC-MS/MS be considered as a viable alternative to LC-MS/MS in the routine anti-doping analysis of prohibited substances in urine? *Gioacchino Luca Losacco[1], Raul Nicoli[2], Tiia Kuuranne[2], Jean-Luc Veuthey[1], Davy Guillarme[1]*, [1]*School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU – Rue Michel Servet 1, 1211 Geneva 4, Switzerland.* [2]*Swiss Laboratory for Doping Analyses, University Centre of Legal Medicine Lausanne-Geneva, Centre Hospitalier Universitaire Vaudois, University of Lausanne, Chemin des Croisettes 22, 1066 Epalinges, Switzerland.*

Supercritical fluid chromatography (SFC) has witnessed a tremendous growth over the last decade in terms of interest and developed applications. The possible combination of SFC with different technologies, such as mass spectrometry (MS) and ultraviolet (UV) detectors has enabled this technique to be successfully used in various analytical areas. Particular examples of SFC applications are e.g. the analysis of chiral and achiral impurities analyses of pharmaceutical

formulations, as well as the detection of compounds in different complex biological matrices, such as plant extract or biofluids, such as animal and human urine and plasma [1-3].

Nonetheless, the implementation of SFC in routine anti-doping laboratories is still not comparable to that of liquid chromatography (LC). SFC, unlike LC, is not yet approved by the World Anti-Doping Agency (WADA) for doping control analysis. The generally high compressibility of a SFC mobile phase might cause significant shifts in retention times, affecting negatively on the robustness of developed SFC methods. However, there has been recently some efforts in demonstrating that SFC can provide similar performance to that of LC in routine laboratories [4]. Initial results have been obtained on standards and pharmaceutical formulations, but very little has been done on biological matrices. Consequently, the next step will be the evaluation of SFC-MS/MS for routine anti-doping analyses and the comparison with LC-separation.

The aim of this presentation is to determine the robustness of a generic SFC-MS/MS method in routine screening and confirmation analyses for anti-doping purposes, using standard mixtures as well as biological matrices. An evaluation of retention time stability of 53 compounds, as standards and spiked urine samples, belonging to different classes of doping substances (i.e. stimulants, narcotics, diuretics, steroids, etc.), with three representative SFC column chemistries (bare silica, 2-PIC and C18) was performed. Dilute-and-shoot (DS) was chosen as the sample treatment procedure for urine. In the first part of this work, two sets of columns *per* stationary phase, belonging to the same production lot, were used to assess the intra-day, inter-days, inter-weeks and inter-months stability of retention times for each compound, as standards and spiked in urine, respectively. In the second stage of this study, the inter-batch variability of SFC columns was determined. For that, three columns from different batches for each chemistry were evaluated, focusing on retention times of spiked substances in urine. The presentation will discuss the results of these two stages of research.

References

[1] Pilařová V., Plachká K., Khalikova M., Svec F. Nováková L., *Trends Anal Chem* **2019**, *112*, 212-225
[2] Harps L., Joseph J., Parr M., *J Pharm Biomed Anal* **2019**, *162*, 47-59
[3] Nováková L., Rentsch M., Grand-Guillaume Perrenoud A., Nicoli R., Saugy M., Veuthey J.-L., Guillarme D., *Analytica Chimica Acta* **2015**, *853*, 647-659
[4] Dispas A., Marini R., Desfontaine V., et al., *J Pharm Biomed Anal* **2018**, 414-424

2:00 pm

Expanding the reach of SFC to biomolecules at analytical and preparative scale: evaluation of global conformational changes in peptides and proteins, Raffeal Bennett (presenter), Alexey A. Makarov, Erik L. Regalado, Merck Research Laboratories, Rahway, NJ, USA

Supercritical fluid chromatography (SFC) has historically been very effective for exacting difficult chiral separations, with an unmatched power in terms of enabling highly efficient enantioselective chromatography at both analytical and preparative scale. In recent years, the pharmaceutical industry has shifted focus towards new challenging chemistries including therapeutic modalities that can generate multitudes of closely related species, overwhelming the

ability of today's platform separation tools. Recent SFC investigations across both pharmaceutical and industrial sectors have clearly demonstrated the applicability of SFC beyond separation of enantiomers. Biomolecules including peptides and proteins remain a challenging problem in SFC as well as other forms of chromatography. Furthermore, for protein-type pharmaceutical targets, it is important to not only separate and purify the target, but often to interrogate and preserve the original structural conformation. The inclusion of aqueous and polar additives has made SFC more amenable to biomolecule separations in the analytical space, but little is shown regarding the impact of SFC mobile phases on non-reversible conformational changes after preparative purifications. In addition to showcasing several potential SFC applications for bioanalytical testing and preparative separation of hydrophilic compounds, the work presented herein illustrates an analytical procedure introduced by our group consisting of size exclusion chromatography coupled with hydrogen–deuterium exchange (SEC-HDX) methodology and circular dichroism (CD) spectroscopy to probe the global conformational structure of model peptides and proteins following purification by preparative SFC. The structures of ubiquitin and myoglobin were significantly modified after exposure to SFC mobile phases, but insulin proved to be a successful purification candidate, effectively retaining its higher order structure. In summary, not all proteins can assume their original conformational state after purification by SFC, but the described workflow enables the rapid identification of peptides and small proteins compatible with an SFC purification method.

2:20 pm

A new strategy for improved method transfer and scale up in SFC, Martin Enmark[1], Marek Szymanski[2], Jörgen Samuelsson[1] and Torgny Fornstedt[1]**.** [1] Department of Engineering and Chemical Sciences, Karlstad University, SE-651 88 Karlstad, Sweden**,** [2] Department of Mathematics, School of Science and Technology, Örebro University, Örebro, Sweden

A new and alternate strategy for retention matching due to the, usually unavoidable, varying pressure drop under the SFC run, will be presented. This strategy involves adjusting the mass fraction co-solvent in such a way that the apparent retention factor, obtained under varying conditions of local pressure/density in the column, exactly matches the retention factor of the reference system.

We developed a simple simulation software based on empirical retention modelling using data from twelve different solutes eluting under varying experimental conditions. With these models we can calculate the apparent retention factor for SFC systems using any amount of co-solvent, pressure and temperature. The simulations accounted as well for axial density and linear velocity gradients.

The pressure drop over the column can increase or decrease under a chromatographic run. The former can be the case, under method transfer in order to increase resolution in an analytical separation problem, for example scaling down (to smaller particles sizes) from SFC to UHPSFC.

The is the case for a standard scale up of an analytical SFC system to a larger particle-size preparative system.

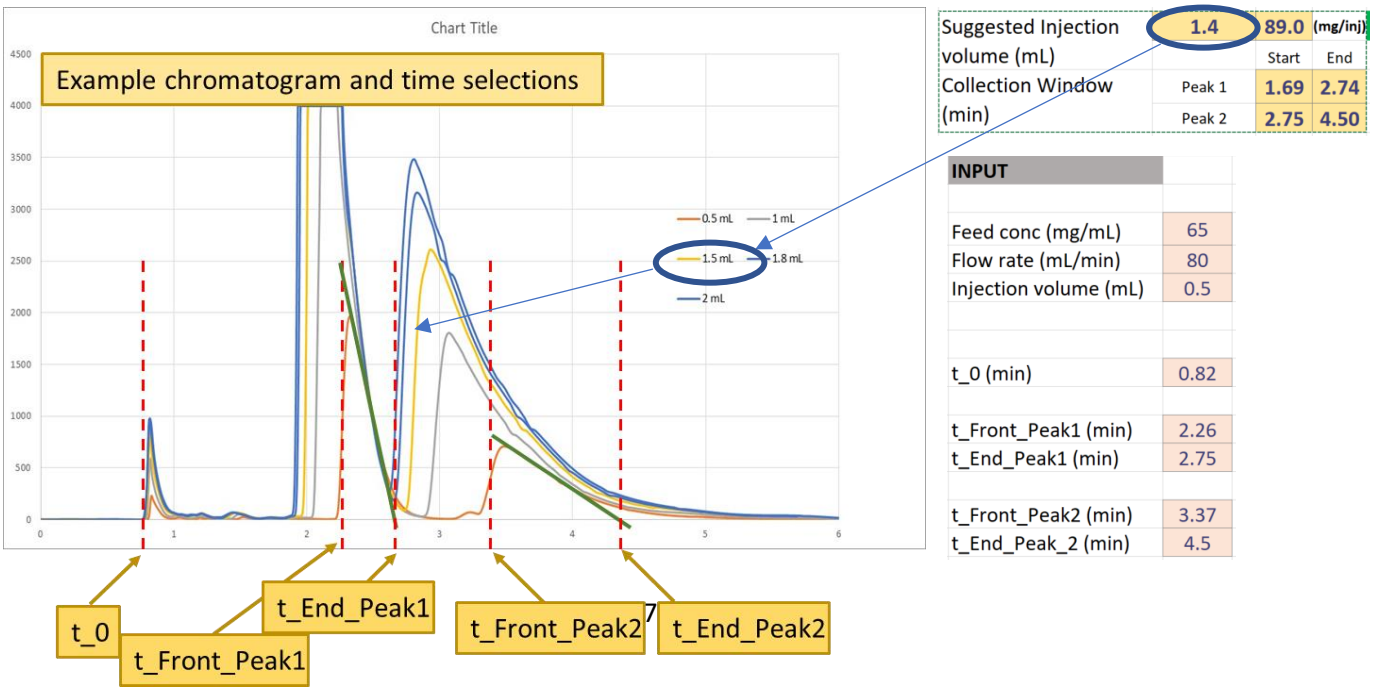
The strategy will be demonstrated by firm theory, but we also derive a rule of thumb to empirically utilize the strategy without the need for complex calculations. The new approach was shown to only marginally affect the selectivity and does not require any adjustments of the back-pressure.

2:40

Estimation of maximum injection volume from minimum experimental data for chiral separation, Abhijit Tarafder, Tom Kazarian, Martin Beres, Yinong Zhang, Yue Lei, Larry Miller, Amgen Inc, Cambridge MA, USA

Estimation of maximum volume of sample that can be injected during a prep separation can be challenging. Generally chemists try to maximize injection volume while achieving target purity (e.g. enantiomeric excess) and not sacrificing on product recovery. The Standard approach is to inject increasing volumes of sample mixture until the two enantiomeric peaks reach baseline. Separation scientists may spend considerable time to find the right injection volume that will minimize total runtime while satisfying these other criteria.

The Current presentation describes a mathematical formula that can be used for a quick estimation of maximum injection volume based on data available from experimental chromatograms of one or two initial trials. The formula is simple enough to be implemented in an Excel spreadsheet, but offers a reasonable estimate of the maximum injection volume. A description on the fundament basis of the formula will be presented along with experimental results that demonstrate the utility of this approach in practical situations. The figure below shows a snap-shot of an Excel-file based calculator and its predictive capability.



3:00

Separation and Purification by supercritical fluid chromatography from 1mg to several kg in Pharmaceutical R&D, Pierre Billemont, Antoni Severino, Didier Senechal, Rudi Jacqmin
UCB Biopharma, Chemin du Foriest, 1420 Braine-l'Alleud, Belgium

Pharmaceutical R&D departments have been successfully coupling chemistry and chromatography for a long time. HPLC platforms allow efficient purification, generally by Reverse Phase chromatography, of either single samples or compound libraries. Nevertheless, over the last decade, the proportion of products purified by supercritical fluid chromatography (SFC) has been increasing and this is particularly so for chiral separations. The low proportion of solvent (mainly alcohols) used, the smaller collection volumes, faster separation times, more concentrated fractions and the orthogonality to HPLC explains this trend.

At UCB, SFC is now used daily at all different scales for all research projects but also to support process development for manufacturing. Some case studies will be presented to highlight the great efficiency of separation and purification.

For small scale, compound libraries or isolated MedChem compounds, HPLC remains, for the moment, the most used technique. This is explained by the fact that a generic achiral stationary phase (C18) is sufficient to perform most purifications. On the contrary, SFC requires method development to determine the best stationary phase to perform the separation. We will present some examples where SFC has been compared to HPLC, and where SFC provided advantages where there were instability issues when using HPLC. Some other cases will also be highlighted in which SFC was used to perform purifications not possible by HPLC.

At larger scales, SFC is increasingly used in place of HPLC. HPLC is only used if the separation is simple and can be performed in a few injections and more quickly than SFC (evaporation included). Liquid Normal Phase purification is generally only considered for crude samples or as a pre-purification clean-up step. Some examples and statistics about the utility of different stationary phase will be shown.

In terms of chiral separation, SFC is now the most commonly used technique for separations on scales of tens of mgs to hundreds of grams and sometimes even kilograms. However, HPLC remains the preferred technique for quality control as the signal to noise ratio is often better. Some examples where the productivity and solvent consumption has been compared to HPLC will be presented. We will also discuss large scale separations for early stage development projects.

4:10 pm

Strategies for Separations of Highly Hydrophobic Materials: Supercritical Fluid Chromatography of Synthetic Sebum Oil, Robert M. Campbell, Aslin Izmitli, Eric Wasserman and Tobias Sidle, Dow, Inc., 1897 Building, Office D-38, Midland, MI, USA

Highly hydrophobic components can be difficult to separate and determine, especially when found in complex mixtures. In reversed phase liquid chromatography (LC), hydrophobic components are strongly retained and columns with bonded hydrocarbon stationary phases leave few options for optimizing selectivity. By normal phase LC, the hydrophobic components are poorly retained, even with the weakest of mobile phases such as hexane or isooctane, making separations of them extremely challenging. By gas chromatography, many hydrophobic materials lack sufficient volatility and selectivity choices are limited among the columns which are also compatible with high temperatures.

Sebum oil is naturally occurring on human skin and is of great interest in the personal and home care industries. Synthetic versions of this complex mixture have been developed for use in studies to develop products in the personal and home care market place. To better understand product performance, it is advantageous to determine individual species in the complex mixture which includes paraffin wax, squalene, fatty esters, fatty acids and cholesterol.

In this study, packed column supercritical fluid chromatographic (SFC) conditions were developed to separate and determine the components of synthetic sebum oil as part of a product development effort. Various columns and mobile phases were trialed to obtain an optimum separation. LC and GC techniques were briefly evaluated, but SFC was found to give the best separation of sebum oil components. Extraction conditions were developed to remove the oils from home care matrices and allowed determination of the individual species by SFC. Details of the development effort will be shown.

4:30 pm

Limits of Kinetic Performance in Supercritical Fluid Chromatography, Terry A Berger, PhD, DIC**,** SFC Solutions, Inc., USA

Modern supercritical fluid chromatography (SFC) started with Dennis Gere's use of 3µm particles in 1979, which was state of the art in high performance liquid chromatography (HPLC) at the time. While HPLC has undergone dramatic development since, SFC instrumentation has not kept up. Current commercial SFC instruments are plumbed for use with 4.6mm ID columns and 5µm particles. HPLC has progressed to the widespread use of sub-2µm fully porous particles, and sub-3µm superficially porous particles, often in 2.1mm or 3mm ID columns. Several groups have attempted to modify their SFC instrumentation for use with such particles

and columns. In most cases, the results have been disappointing, and confusing. Some of the reasons are explained.

Recent SFC results produced a reduced plate height of 1.65 using a 3x20mm column packed with 1.8µm fully porous particles. This required modifications producing extra-column dispersion of less than $2\mu L^2$, along with a surprisingly well packed short column. Such extra-column dispersion is similar to ultra high dispersion LC. One conclusion is that most columns packed with polar (normal phase or HILIC) stationary phases with small (sub-2µm, sub-3µm) particles are poorly packed. It appears that several other aspects separate SFC from HPLC that have not been obvious, or well characterized, which will be discussed.

4:50 pm

A systematic approach for the optimization and validation of on-line supercritical fluid extraction – supercritical fluid chromatography – mass spectrometry for polyaromatic hydrocarbons in soil, A. Paige Wicker[1], Kenichiro Tanaka[2], Masayuki Nishimura[3], Vivian Chen[3], William Hedgepeth[3], Tairo Ogura[3], Kevin A. Schug[1]**,** 1) Department of Chemistry and Biochemistry, The University of Texas at Arlington, Arlington, TX 76109, USA**,** 2) Shimadzu Corporation, Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto 604-8511, Japan**,** 3) Shimadzu Scientific Instruments, Inc., Innovation Center, Columbia, MD 21046, USA

Coupling supercritical fluid extraction (SFE) with supercritical fluid chromatography (SFC) is a relatively new technique in the analytical toolbox. On-line SFE-SFC provides a platform for analyte extraction, separation, and detection in a single analysis, while limiting sample preparation, loss or contamination, and significantly decreasing total analysis time. Here, we systematically evaluate the parameters of on-line SFE-SFC-MS to validate the quantification of polycyclic aromatic hydrocarbons (PAHs) in soil.

Extraction of sixteen environmentally important PAHs from soil was performed using supercritical CO_2 with acetonitrile modifier on-line to a 250 mm x 4.6 mm, 5 µm Cholester column for SFC at 50 °C. Detection of compounds was made via APCI-MS analysis on a triple quadrupole mass spectrometer in positive mode by MRM. For on-line SFE-SFC, both extraction and separation parameters were considered simultaneously for method optimization as SFE conditions had extreme effects on separation. Parameters optimized included chromatography modifier, make up solvent flow, extraction time (both static and dynamic), extraction modifier, and back pressure regulator split ratio.

MRM transitions were optimized for each compound. Five sets of isomers were indistinguishable by their respective MRMs; thus, good chromatographic separation was required. Both $[M]^{+\cdot}$ and $[M+H]^+$ precursor ions were monitored. Charge transfer dominated ion formation in APCI source.

Extraction conditions were evaluated to improve retention, peak shape, and intensity. Optimal extraction occurred at 10% acetonitrile modifier with static extraction of 7 minutes followed by

5 minutes dynamic extraction at 40 °C. Two back pressure regulators (BPRs), one pre-column and one post-column, were used to control trapping extractant onto the head of the column. BPRs are held at 15.0 MPa throughout extraction to reduce extract volume loaded onto column. Following extraction, post-column BPR is held at 15.0 MPa, while pre-column BPR increased to 40.0 MPa to direct flow to the column where analytes are trapped.

Calibration curves (n=3) were created for 16 PAHs with 7 calibration points in the range of 10–1500 ng per gram of soil in CRM sediment, sand, and clay. Linearity of $R^2 \geq 0.99$ achieved. Empirically determined LODs ranged 5–20 ng/g and LOQs ranged 10–40 ng/g.

5:10 pm

Method Development for Isomer Interconversion Profiling in Human Plasma by Supercritical Fluid Chromatography Coupled with Mass Spectrometry, Tony Q.Yan, Frank Riley**,** Pharmaceutical Pfizer Global R&D, Pfizer Inc., Groton, CT, USA

Definitive isomeric interconversion in human plasma has recently attracted the attention of regulatory agencies beyond that of historic, paper, justification and evolving into required experimental justification during applicant filings. Chromatographic analysis of isomer interconversion in human plasma often presents analytical challenges due to structure similarities and limited material available in the sample matrix. A quantitative examination of isomerization as a metabolic fate in humans will require development of a chiral assay to first assess the propensity of the parent compound towards isomerization. In this presentation, the isomeric interconversion has been studied using supercritical fluid chromatography coupled with mass spectrometry. The first study discusses a Pfizer research compound containing four diastereomers, SFC chiral method development and testing for chiral interconversion. The second study also represents four diastereomers and related SFC chiral method development coupled with MS/MS detection. The third study involves with reverse phase HPLC chiral method development strategy. The detailed analytical method developments and detection methods are discussed along with detection sensitivity evaluation for both techniques in this presentation.

Tuesday, October 1

Critical Fluid Extraction and Chromatography - Application to Cannabis/Hemp Processing, [1]King, Jerry; [2]Montanes, Fernando; [2]Moreno, Teresa; [2]Mitchell, Kevin; and [2]Tallon, Stephen. [1]CFS, Fayetteville, AR, 72701, USA, [2]Callaghan Innovation, Lower Hutt, 5040 New Zealand

Supercritical fluid chromatography (SFC) and supercritical extraction (SFE) have mutually developed and complimented one another over the past 50 years. This has provided a "green" separations platform using mainly carbon dioxide (CO_2) at pressures typically between 10 and 100 MPa with accrued benefits of low solvent viscosity, fast mass transfer and minor organic solvent usage. The current fusion between cannabis science/technology and extraction-chromatography-based separation platforms continues to develop as the cannabis field matures into a "pharmaceutical", GMP-level industry. Chromatography has the potential to address major issues plaquing hemp/cannabis processing such as the separation of THC from CBD, isolation of the terpene fraction vital to the "entourage" effect, and removal of unwanted contaminants, such as pesticides, etc. With regard to "contamination", SFC methodology is ideally suited for the characterization of non-cannabinoids and -terpene constituents such as lipids, waxes, carotenoids, sterols, and flavonoids - based on a plethora of demonstrated separations in the food, pharmaceutical, and natural product areas. Other modes of chromatography are being applied for the enrichment and purification of cannabinoids that do not employ sub- or super-critical fluids, but coupled with SFE and traditional condensed phase solvent extraction fluids, such as butane and ethanol. It will be demonstrated that activity coefficient and solubility parameter correlations can be used to rationalize and optimize the extraction and purification of cannabinoids and terpenes in various media. In this regard, the use of various chromatographic techniques can provide valuable physicochemical data in support of these efforts. Sorbent-based technologies particularly at the analytical level of application have proven useful in the separation of contaminants such as pesticides found in cannabis extracts - and are extrapolatable to larger-scale processing operations. Specifically, clays, carbonaceous sorbents, silica, MIPS, and diatomaceous earths have been employed for fractionation after executing the primary mode of extraction to provide an improved extract with respect to color, terpene and higher cannabinoid concentrations. A Waters UPC^2 analytical SFC system coupled with a Waters QTOF high resolution mass spectrometer has been applied to characterize different sources of hemp seed oil products, using both scan and selective ion monitoring modes, due to the complex elation and low concentrations of some compounds. Similarly, this mode of chromatography and detection has been applied to characterize hemp extracts containing cannabinoids such as CBDA and trace levels of THCA. Semi-preparative SFC has been used to fractionate cannabinoid-containing extracts obtained from hemp flower using CO_2-SFE at 40^{o}C and pressures from 100-300 bar. Preliminary SFC fractionation was accomplished using CO_2 at 100 bar, 60^{o}C, with 10% ethanol co-solvent and various ES Industries columns. Currently several modes of "continuous chromatography" are being applied in the cannabis field. These include countercurrent chromatography (CC), centrifugal partition

chromatography (CPC), and simulated moving bed chromatography (SMB). For example, CPC can be applied potentially at ton-throughput scale for cannabinoid purification and pesticide or THC removal as demonstrated using the Rotachrom at feed rates 1-2 kg/hr. system. An example of using analytical SFC for the analysis of a hemp-based feedstock for further purification via CPC will be illustrated and translation of these smaller-scale chromatographic conditions to production-scale using a Novasep-SuperSep 1000 system featuring 50 mm i.d. columns will be discussed. Finally, transferring of critical fluid approaches and technology developed for food and nutraceutical application as potential replacements for currently-used vacuum distillation and solvent-based phase separation methods used in the cannabis industry will be noted.

9:15 am

Enhanced-Fluidity Liquid Chromatography Analysis for Disease Diagnostics, Susan Olesik, The Ohio State University, USA

Recent advances in analysis of proteins have increased the demand for more efficient techniques to separate intact proteins. Enhanced-fluidity liquid chromatography (EFLC) involves the addition of liquefied CO_2 to conventional liquid mobile phases. The addition of liquid carbon dioxide enhances diffusivity and decreases viscosity while maintaining mixture polarity, which typically results in reduced time of analysis.

EFLC will be described for the separation of proteins under both hydrophilic interaction, as well as, hydrophobic interaction liquid chromatography conditions.

EFLC mobile phase are readily compatible with electrospray ionization. The sensitivity of detecting proteins increases using EFLC-MS compared to conventional LC-MS. Furthermore, the charge distribution of the measured protein can be readily shifted to lower or high average charge states based on operating conditions.

The application of this hyphenated method will be illustrate for the detection of proteins that are biomarker to characterize a number of diseases.

10:45 am

EPSA: When SFC Enables Drug Design, Gilles Goetz, Pfizer, Groton, CT, USA

Applications of a new chromatographic method using SFC technology developed recently at Pfizer are described here. The EPSA method, as readout of polarity, correlates retention on a specific stationary phase with the exposed polarity of a molecule. Changes in retention can be interpreted by changes in polarity induced by the presence of Intra-Molecular Hydrogen Bonding (IMHB): indeed, IMHBs tend to impact molecular conformation, inducing hidden polarity that results in a decrease in analyte retention on the EPSA support. We demonstrate here the impact of this method on multiple Pfizer Beyond Rule of 5 projects (NS5A, Oxytocin Receptor, CXCR7 Modulator, others) as well as select examples gathered across the pharmaceutical industry. Given that conformational changes (induced and/or stabilized by the formation of IMHB)

increases potential for membrane permeability, we show here that EPSA, and the EPSA prediction model, have significant impact in peptide drug design.

11:10 am

Impact of EPSA measurements in drug design, R Romero, J Kingston, T Leek, Astra Zenca, Waltham, MA, USA

The polarity of a compound is an important parameter in drug design as an understanding of compound polarity allows both membrane permeability and bioavailability to be predicted. In practice the polar surface area (PSA) is typically used and although fast 2-dimensional in-silico models are available and generally work well for small, rule-of-five (Ro5) compliant chemical series, these models do not consider 3-dimensional molecular conformation. A better tool is therefore required to accurately assess exposed polar surface area for the wide range of small molecules and beyond rule-of-five (bRo5) chemical space included within modern drug discovery portfolios.

A supercritical fluid chromatographic (SFC) method for directly measuring exposed polar surface area, EPSA, has been developed recently (Goetz et al 2014). This method uses compound retention on a polar stationary phase with a non-aqueous SFC mobile phase to determine exposed polar surface area and predict *in-vivo* behaviour (Goetz and Shalaeva, ADMET and DMPK, 2018). At AstraZeneca, we have developed, validated and implemented an accelerated SFC method at our R&D sites to more rapidly gather EPSA chromatographic data in a global database for our drug design projects.

This presentation describes how we have adopted SFC as a tool for measuring physico-chemical parameters within AstraZeneca. We describe the successful adoption of chromatographic EPSA within drug design assays where it provides a straightforward and robust technique for directly measuring the polar surface area of discovery compounds. We compare EPSA data derived from our SFC assay with that derived from 2-dimensional polar surface area models. We describe how this data has been used to understand and predict the *in-vivo* behaviour of both small molecule and bRo5 discovery programmes. The predictive value of directly measured EPSA data is quantitatively and critically assessed.

11:30

The Development of Stationary Phase Chemistries for Optimized SFC Separations of Natural Products, Matthew Przybyceil, ES Industries, NJ, USA

In recent years, a number of commercial available stationary phases have been developed specifically for SFC applications. However, not all of these columns are ideally suited for specific applications. Unfortunately, there is not yet “universal” stationary phase chemistry for SFC and as consequent a number of stationary phase chemistries have been introduce for a variety of separations. However, a column selected for a particular separation may not represent completely optimized separation method. It is the goal of this presentation to

explore various natural product separations and to provide guidance to optimal stationary phase selection. The selection of a particular stationary phase for specific separation or application will take into account several factors such as overall separation performance, peak shape, modifier use/amount, additive use, chromatographic cycle time, scalability, temperature and robustness. Applications/separations area will focus on stationary phase selection for natural products including cannabinoids, quinine bark, tea extracts and natural oils. We will demonstrate how these SFC columns can provide for the high performance separations over a wide variety flow rate conditions and mobile compositions.

11:50

Characterization of 14 SFC stationary phases and application for the separation of pharmaceutics and natural compounds,

Q. Gros[1,2], J. Molineau[1], A. Noireau[1], T. Bamba[3], J. Duval[2], E. Lesellier[1], C. West[1]., [1]: *University of Orleans, ICOA, CNRS UMR 7311 ; Pôle de chimie rue de Chartres - BP 6759 45067, Orléans Cedex 2, France*. [2]: *Shimadzu France, Le luzard 2, Bat A, Bd Salvador Allende Noisiel, 77448 Marne-la-Vallée, France*, [3]: *Kyushu University, Division of Metabolomics, Medical Institute of Bioregulation, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

With the hyphenation to mass spectrometry and in view of its ecological aspect, Supercritical Fluid Chromatography (SFC) has showed an important regain of interest in academic laboratories and in industries [1]. Furthermore, over the last decade, major instrument manufacturers such as Agilent, Waters and Shimadzu developed their own Ultra-High-Performance SFC (UHPSFC) systems and specific components such as columns dedicated to SFC, regarding this increased concern.

SFC columns stationary phases have been largely investigated in the last two decades by West & Lesellier, based on the use of linear solvation energy relationships (LSER)[2]. By using the LSER method, the columns were plotted on a seven-axis graph, each representing the different interaction parameters. This classification can be used as a first tool for the screening of the most suitable column for the separation of analytes, before going deeper on the system optimization.

In this context, the characterization of the 14 columns from the Shim-pack UC series (Shimadzu, Japan) dedicated to SFC and more broadly to unified chromatography (UC) was performed, using the same methodology. These different columns are largely scattered in the selectivity space, reflecting various retention and separation properties. Based on these observations, sample applications will be demonstrated with the separation of pharmaceutics and natural compounds by SFC-MS on the Nexera UC system (Shimadzu, Japan).

Keywords: Supercritical fluid chromatography, unified chromatography, hyphenation, stationary phase.

[1]: West, C. (2018). Current trends in supercritical fluid chromatography. Analytical and Bioanalytical Chemistry, 410(25), pp.6441-6457.

[2]: West, C., Lemasson, E., Bertin, S., Hennig, P. and Lesellier, E. (2016). An improved classification of stationary phases for ultra-high performance supercritical fluid chromatography.

2:40 pm

The use of Supercritical Fluid Extraction and Chromatography in the Food and Nutritional Supplement Quality Control Laboratory: Selected Applications, Daniel Hengst, Eurofins, USA

Although Supercritical Fluid Chromatography is routinely used for product analysis in many industries, there has not been a corresponding widespread implementation in food analysis laboratories. However, as manufacturers release next generation supercritical fluid instruments and stationary phases, food chemists are revisiting this technology. Advantages seen in sample throughput, reductions in solvent usage and unique chromatographic separations have sparked a renewed interest in this analytical technique. An application that has specifically generated interest is coupled Super Critical Fluid Extraction and Chromatography (SFE-SFC). This technique largely automates the sample extraction procedure, reducing laboratory staff workloads from hours to minutes. Several SFE-SFC and offline extraction-SFC methods have been recently developed and validated at Eurofins Food Integrity and Innovation for the Fat Soluble Vitamins, including Vitamins K1, K3, D2, D3, A Acetate and A Palmitate. The successful implementation of this technique on these limited applications demonstrates the potential of this instrumentation for a vast array of additional analytes.

3:00 pm

Green approaches to the analysis of cannabinoids in natural products, Lucie Nováková[1], Kateřina Pražáková[2], Veronika Pilařová[1], [1] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic, [2] První soukromé jazykové gymnázium Hradec Králové, Brandlova 875, Hradec Králové, Czech Republic

Cannabis is a complex plant containing over 400 different chemical compounds. Cannabinoids with major compounds including delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are responsible for different pharmacological effects in humans. CBD is considered to have various health-beneficial effects, such neuroprotective, anti-inflammatory, and antipsychotic, while THC is responsible for psychoactive activity. Moreover, these effects are strongly dependent on the chemical composition, which may vary substantially among different *Cannabis* varieties. The legalization of *Cannabis* in many countries has stimulated marijuana cultivators to optimize the chemical profile of *Cannabis* to eliminate the presence of THC and manufacturers to explore new types of products. Therefore, development of new and effective quality control approaches is highly desired.

In our study we focused on the use of supercritical fluids in both, separation and extraction steps. To develop ultra-high performance supercritical fluid chromatography method, stationary and mobile phase screening was carried out to enable the separation of structurally close compounds, such as THC and CBD, their acids, and other minor cannabinoids, such as cannabigerol, its acid, cannabicyclol, and others. Hybrid silica stationary phase with 2-EP functionality and CO_2 with methanol + 0.1% NH_4OH + 2% water in the mobile phase were finally chosen for separation using gradient elution. Mass spectrometry was used for detection to allow adequate selectivity in complex plant matrices. Extraction method to isolate cannabinoids from

plant and dietary supplement products was optimized using design of experiment. In this regard, supercritical fluid extraction (SFE) with pure CO_2, SFE with modifier, CO_2 expanded liquid extraction, and pressurized liquid extraction can be set-up based on the content of CO_2 and organic modifier. The developed methods were finally used for analysis of various *Cannabis* products to control their quality, especially with emphasis on the content of THC and CBD.

This work was supported by the project EFSA-CDN (No. CZ.02.1.01/0.0/0.0/16_019/0000841) co-funded by ERDF.

3:20 pm

Development of polar metabolite profiling method by supercritical fluid chromatography/mass spectrometry, Yutaka Konya, Yoshihiro Izumi, Takeshi Bamba**,** Medical Institute of Bioregulation, Kyushu University

Supercritical fluid chromatography (SFC) has hardly been applied to hydrophilic metabolites, because supercritical carbon dioxide ($scCO_2$), the main fluid of the mobile phase in SFC, is non-polar, and SFC technique was not considered to be suitable for hydrophilic metabolites. However, by improving the modifier and additives, the solubility of the hydrophilic metabolite in the mobile phase of SFC is increased.

In this study, it was examined whether SFC/MS technique was applicable to the analysis of hydrophilic metabolites. First, 11 types of columns having different stationary phases were compared using peak shapes and separations of 20 proteinnogenic amino acids as indexes. As a result, CROWNPAK CR-I (+) column showed the best peak shape and separation, when adding water and trifluoroacetic acid were added to the modifier. Using the optimized condition, it was possible to detect about 100 cation metabolites in standard solutions including 20 proteinogenic amino acids. Furthermore, 39 metabolites among the 100 cation metabolites were detected in rat or mouse serum.

These results showed that the SFC/MS/MS method is applicable to simultaneous analysis of hydrophilic metabolites.

3:40 pm

Supercritical fluid extraction and chromatography for in situ planetary science applications, Victor Abrahamsson, Bryana L. Henderson, Fang Zhong, Ying Lin and Isik Kanik**,** Jet Propulsion Laboratory, California Institute of Technology, Pasadena, CA 91109, USA

Detection of biomarkers in situ on planetary bodies in the solar systm requires robust and low risk instrumentation with low detection limits. Chromatographic techniques hyphenated with mass spectrometry offer low detection limits and the high resolving power and is suitable for non-targeted analysis. Such an approach has to date only been performed in situ at Mars by employing gas chromatography-mass spectrometry on the Vikings, Phoenix and Curiosity landers. Sample introduction and sample preparation was then performed using various combinations of pyrolysis of dry extraterrestrial material and thermochemolysis using a one-pot extraction/derivatization solution [1-3].

These robotic missions have so far been entangled by complications including undesired reactions and potentially complete combustion of organic molecules during pyrolysis due to the presence of inorganic salts in an oxidative sample matrix [2, 4]. Attempts to circumvent extreme degradation and to enable analysis of amino acids and fatty acids by using a one-pot liquid extraction and thermochemolysis, instead led to contamination of the lander due to leaking of solvent and derivatization reagent [5].

We have been developing instrumentation and methodology that is completely free of organic additives for on-line analysis using supercritical fluid extraction (SFE) and chromatography (SFC) for both aqueous and solid samples [6]. This approach offers some distinct benefits:

- Reduces ion suppression during MS analysis since inorganic salts are not soluble in the $scCO_2$ phase.
- Non-volatile analytes such as free fatty acids can be analyzed without derivatization as opposed to gas chromatography-based methods.
- Native analytes are detected, rather than fragments generated by pyrolysis.
- Heat sensitive analytes such as pigments (carotenoids) can be analyzed.
- Minimizes risk of contamination.

Addressed practical and theoretical considerations will be presented regarding extraction from aqueous samples at for example Europa (under reduced gravity), including coupling of SFE and SFC and performing packed column SFC with water-saturated $scCO_2$ without any organic modifiers or additives. Applications and validation data will also be presented to support that SFE-SFC is fit for the purpose of detecting biomarkers.

Acknowledgments: This research was funded by the NASA's Concepts for Ocean Worlds Life Detection Technology (COLDTech). The work was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration.

References:

1. Mahaffy, P.R., et al., 2012. **170**: p. 401-478.
2. Navarro-González, R., et al., Journal of Geophysical Research: Planets, 2010. **115**.
3. Toner, J.D., D.C. Catling, and B. Light, Geochimica et Cosmochimica Acta, 2014. **136**: p. 142-168.
4. Glavin, D.P., et al., Journal of Geophysical Research E: Planets, 2013. **118**: p. 1955-1973.
5. Eigenbrode, J.L., et al., Science, 2018. **360**: p. 1096-1101.
6. McCaig, H.C., et al., Astrobiology, 2016. **16**: p. 703-714.