Monday, October 16, 8:45 am

# When one column is not enough - Column coupling in SFC

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Unlike HPLC, column coupling with carbon dioxide – based mobile phases is facilitated by the low viscosity of supercritical fluids. Therefore, by keeping identical column and particle diameters, the use of very long column lengths is possible. 30 years ago, Terry Berger achieved a separation with two meters of packed columns.

While increasing column length provides the obvious advantage of improving column efficiency (N), different stationary phases providing complementary selectivities (achiral-achiral, chiral-chiral or achiral-chiral) can also be coupled.

However, coupling columns with compressible fluids is not straightforward as internal pressure will vary when column length changes. As a consequence, the elution strength of the fluid is affected by column length, thereby impacting separation quality and analysis time. In the case of coupling different stationary phases, the prediction of retention factors and selectivity can be disappointing.

Besides, a column classification for packed column SFC was designed in our laboratory, based on the solvation parameter model, which currently comprises data for about seventy different columns. This classification is very helpful in a first intention to select stationary phases achieving complementary or orthogonal separations.

Several examples will be provided to illustrate the effects of particulate and monolithic stationary phases, for achiral and chiral separations, for analytical and preparative purposes.

Monday, October 16, 9:10 am

# Applicability of SFC-MS to metabolomics: which kind of analytes can be successfully analyzed?

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Metabolomics represents the comprehensive study of the metabolites, which are the intermediates of biochemical processes in living organisms. Any pathophysiological mechanism caused by disease will inevitably lead to related changes in the concentrations of specific metabolites. In consequence, metabolomics offers a promising tool for the discovery of potential diagnostic biomarkers.

In metabolomics, the main analytical challenge is related to the great diversity of physico-chemical properties. Then, various analytical methods have to be combined: Reversed Phase Liquid Chromatography (RPLC) has been widely used for the analysis of lipophilic compounds, while Hydrophilic Interaction Chromatography (HILIC) is employed for the analysis of more polar metabolites. However, no method is currently satisfactory to evaluate all these substances within a single injection.

SFC (Supercritical Fluid Chromatography, or in the present case Separation Favored by Carbon dioxide, an acronym more suitable as the fluid is not anymore supercritical at very high proportion of co-solvent) has been scarcely evaluated in the field of Metabolomics. Still, it is able to retain both polar and apolar compounds, and might be an interesting complement to RPLC and HILIC methods to increase the metabolome coverage.

The goal of the present study was to prove that a wide variety of compounds, ranging from very apolar (lipids, liposoluble vitamins, steroids…) to highly polar metabolites (sugars, amino acids, nucleotides…) can be successfully analyzed using generic mobile phase conditions. For this purpose, various aspects were evaluated: (i) the possibility of eluting the most polar substances by increasing the mobile phase composition up to very high proportion of co-solvent (up to 100% MeOH), (ii) the kinetic performance of SFC when using important amount of co-solvent, (iii) the selection of the most suitable stationary phases chemistries and dimensions to analyze simultaneously lipophilic and hydrophilic substances, (iv) the compatibility with MS detection and behavior of the SFC-MS interface under these conditions.

Monday, October 16, 10:35 am

# Application of Different Chromatography and Mass Spectrometry Solutions to the Analysis of Fuels and Fuel Matrices

Professor G John Langley

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The technical revolution in engine design and fuel delivery has been matched by similar advances in chromatographic and mass spectrometric instrumentation. Within the petrochemical industry, GC-MS is the technique of choice for analysis of fuels (gasoline, diesel and AVTUR fuel), *e.g*. readily differentiating aliphatics from aromatics and easily identifying and quantifying rapeseed methyl ester biodiesel. Whilst effective at detecting these volatile species GC-MS is limited in detecting higher molecular weight and thermally labile species, *e.g*. surfactants, detergents, lubricity agents.

The advent of atmospheric pressure ionisation techniques in the 1980s together with advances in chromatography *e.g.* ultrahigh pressure liquid chromatography (UHPLC), ultrahigh pressure supercritical fluid chromatography (UHPSFC) and ultrahigh resolution mass spectrometry, now affords the tools to address the analysis of these higher molecular weight species.

This presentation will discuss the different approaches required to unravel the complexity of these fuels, highlight the need for and success of UHPSFC-MS approaches to fully characterise and understand modern fuel packages.

Monday, October 16, 11:00 am

# Development and Comparison of Quantitative Methods Using Orthogonal Chromatographic Techniques for the Analysis of Potential Mutagenic Impurities

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Purpose: There are many steps during the manufacturing process of an active pharmaceutical ingredient (API) where impurities can be introduced, whether as reagents, byproducts, intermediates, etc. Some of these impurities may be mutagenic, or have the potential to interact with DNA and ultimately cause carcinogenicity. Methodologies associated with monitoring API purity levels are often HPLC-UV based, which frequently do not provide the sensitivity levels needed to detect potentially mutagenic impurities (PMIs) at the levels required by regulatory agencies. For example, ondansetron is a pharmaceutical used in the prevention of nausea and vomiting and may contain one potential mutagenic impurity, 2-methylimidazole, as well as a second impurity very closely related in structure, imidazole.

Similar to 2-methylimidazole and imidazole, many mutagenic impurities are small, highly polar compounds that are poorly retained under typical reversed phase liquid chromatography (RPLC) conditions. Alternate forms of chromatography, such as hydrophilic interaction chromatography (HILIC), or the use of ion-pairing reagents can be employed, but these often result in tedious method development or non-MS friendly mobile phases. Supercritical fluid chromatography (SFC) is known to be orthogonal to RPLC, and employs reagents which are suitable for MS detection. In this study, methods for the analysis of ondansetron and five organic impurities were developed using both liquid and supercritical fluid chromatographic methods.

Methods: Quantitative methods for the analysis of ondansetron and five process impurities, including the potential mutagenic impurity 2-methylimidazole and imidazole, were developed using two orthogonal chromatographic methods- reversed phase liquid chromatography and supercritical fluid chromatography. Both chromatographic methods were interfaced with a tandem quadrupole mass spectrometer for detection and MS source conditions were optimized separately for the LC and SFC experiments.

Results: Using liquid chromatography, it was necessary to develop two methods for the analysis of the five ondansetron impurities- a RPLC method for the less polar impurities, and a HILIC method for the two polar impurities. However, by using SFC, it was possible to generate a single method for the quantitation of all five ondansetron impurities. Method parameters, such as limit of quantitation, linearity, and run time will be compared between the two orthogonal chromatographic methods to determine the benefits of each technique in the analysis of ondansetron and its potentially mutagenic impurities.

Conclusion: Both chromatographic techniques generated high sensitivity methods that met the required limits of detection and both techniques showed good accuracy and reproducibility. The use of liquid chromatography required the development of two separate methods for quantification of all five impurities while supercritical fluid chromatography was able to analyze and quantify all impurities in a single method.

Monday, October 16, 2:00 pm

## Ultrafast chiral separation of pharmaceutical drugs and synthetic intermediates: SFC leading the way

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Recent developments in fast chromatographic enantioseparations now make high throughput analysis of enantiopurity on the order of a few seconds achievable. Nevertheless, routine chromatographic determinations of enantiopurity to support stereochemical investigations in pharmaceutical research and development, synthetic chemistry and bioanalysis are still typically performed on the 5–20 min timescale, with many practitioners believing that sub-minute enantioseparations are not representative of the molecules encountered in day to day research. In this presentation, we illustrate ultrafast chromatographic enantioseparations for a variety of pharmaceutically-related drugs and intermediates, showing that sub-minute resolutions are now possible in the vast majority of cases by SFC. Examples of high-speed chiral RPLC vs SFC separations are provided illustrating how such methods can be routinely developed and used for ultrafast high throughput analysis to support enantioselective synthesis investigations.

Monday, October 16, 2:25 pm

## How supercritical fluids can be useful for extraction, separation, and impregnation in the field of cosmetics.

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In the field of cosmetics or neutraceuticals, the variety of samples and compounds encountered requires a versatile analytical method, suitable to achieve very different separations. For instance, the cosmetic products are mainly made of lipids and/or polymers as basis of body creams or gels. They also contain minor amounts of some active or bioactive compounds, which are often secondary metabolites issued from plant extracts. Moreover, in formulations, these bioactive compounds can be adsorbed onto/into porous solids to ensure delayed contact with the skin.

For each of these steps, extraction of bioactive molecules from plants, separation of plants extracts or complex formulation, impregnation of bioactive compounds onto/into porous particles, $CO_2$-based supercritical fluids provide varied relevant responses. It is due in part to the addition of solvents to $CO_2$ that tune the fluid polarity, i.e. the compound solubility at each steps described previously. For the separation step, it is related to the use of very different stationary phases that cover the whole polarity range of adsorbents. And finally for impregnation, it is due to the change in fluid properties through fluid density changes.

Several examples of such approaches will be described: method development for quantification of compounds in cosmetic formulations, studies on separation of plant extracts or raw materials, descriptions of extraction and on-line extraction/impregnation processes.

Monday, October 16, 2:50 pm

## Life Cycle Analyses of Preparative-Scale Supercritical Fluid Chromatography and High-Performance Liquid Chromatography

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Within the last fifteen years, supercritical fluid chromatography (SFC) has overtaken normal phase high-performance liquid chromatography (HPLC) as the method of choice for preparative chiral purifications in the pharmaceutical industry. This is due to the numerous advantages of SFC over HPLC that are often touted by experienced SFC users: increased separation efficiency, reduced purification times, higher productivity, reduced solvent consumption (and cost), and improved environmental sustainability. As a result of these inherent advantages, SFC continues to expand outside its original niche in chiral purifications. While the first four of these advantages have been thoroughly examined and validated by numerous studies, the advantage of increased environmental stability is often based more on perception or intuition than on actual experimental analysis. Few in-depth studies exist to assess or quantify the environmental friendliness, or "green" character, of SFC relative to HPLC. The few studies that do exist often take too narrow of an approach and/or ignore energy considerations. Given the ever-increasing importance of "green" processes in today's world, definitive evidence of SFC's environmental advantages over HPLC would continue to encourage researchers to evaluate SFC as an alternative to HPLC in their work, particularly when performance is not compromised when switching from HPLC to SFC. A comprehensive life cycle analysis of both preparative SFC and preparative HPLC was conducted to evaluate the true environmental cost of each method, including both material and energy costs.

Monday, October 16, 4:10 pm

# The Effect of Modifier Concentration and Column Outlet Pressure on Efficiency and Optimum Velocity in Supercritical Fluid Chromatography

Terry A Berger
SFC Solutions, Inc.

With increasing use of sub-2µm packings, column head pressures can often exceed 400 bar, especially at higher % modifier, and flow rates. There also appears to be interest in longer columns using such smaller particles. Since >1000bar pumping systems are typical in UHPLC, it is likely that similar systems will appear in SFC.

It was recently noticed [1] that the optimum linear velocity of the mobile phase changed significantly when the modifier was varied between 5% and 40%, with an outlet pressure of 100 or 150 bar. These shifts are most likely due to decreasing diffusion coefficients. The pumping pressure increased significantly with modifier concentration. Experiments were designed to determine the effect of higher column outlet pressures on efficiency and $\mu_{opt}$.

Three achiral stationary phases were evaluated: RX-Sil, $NH_2$, and CN. The columns were 4.6x150mm packed with 5µm particles, chosen since they had relatively low pressure drops. vanDeemter like plots were generated with the backpressure set to 100, 150, 200, and 300 bar, with methanol concentrations of 5%, 10%, 20%, and 40%. At 100 bar and 5% modifier, $\mu_{opt}$ sometimes exceeded 3.25mL-min$^{-1}$. However with 300 bar outlet and 40% methanol, $\mu_{opt} \approx 1.0$mL-min$^{-1}$. Modifier % seemed to be more important than the outlet pressure. There were significant differences in the performance of the columns.

1.] TA Berger, "Kinetic performance of a 50mm long 1.8µm chiral column in supercritical fluid chromatography", J. Chromatogr.A, 1459, 2016, 136-144.

Monday, October 16, 4:35 pm

# Solvent gradient modeling in SFC - Effects of simultaneous variation of composition and pressure/density

Abhijit Tarafder and Jason Hill

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Modern SFC widely uses solvent gradient chromatography (SGC) to achieve intended separations. Along with composition variation during a gradient method, pressure variation also takes place due to changing viscosity of the mobile-phase. Being compressible, even with addition of significant volume of modifier, pressure variation causes mobile-phase density in SFC to vary. So, a gradient of composition variation in SFC imparts simultaneous variation of two fundamental factors that can affect chromatographic retention - the composition as well as the density. A clear understanding on the underlying mechanisms of SFC retention during SGC should take into account both these factors.

Currently available SGC retention models were developed mostly focused on the retention behavior of liquid chromatography, especially for reversed-phase liquid chromatography (RPLC) [1]. The main variant of all these models is the volume fraction of the mobile-phase. Although the HPLC models may not be directly applicable for SFC, the fundament retention model can be adopted for SFC, when the additional factor of pressure/density variation is included in the model.

In this direction, we developed a model that includes pressure as an additional parameter along with the co-solvent composition, so that the effect of simultaneous variation of pressure-profile and composition-profile inside the column can be simulated. Pressure was considered in place of density because pressure-variation as a function of composition-variation could be represented by a simpler equation. The resultant differential equation was numerically solved.

In this presentation we will describe this model and use the model to predict retention times under different gradient conditions. Experimental results, which will be presented after the theory, will verify applicability of the model over wide ranges of experimental conditions.

[1] High-Performance Gradient Elution, L R Snyder, J W Dolan, John Wiley & Sons Inc., 2007, Hoboken, NJ, USA.

Monday, August 16, 5:00 pm

# Understanding and avoiding peak distortions in SFC separations of basic compounds/pharmaceuticals

Emelie Glenne[1], Jörgen Samuelsson[1], Hanna Leek[2], Magnus Klarqvist[2], Patrik Forssén[1], Torgny Fornstedt[1]

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Most pharmaceuticals today are basic compounds, and the SFC analysis of these is considerably more complicated as compared as neutral/acidic drug compounds. To obtain acceptable retention, and peak shapes, for these molecules in Supercritical Fluid Chromatography (SFC), complex mobile phases consisting of co-solvent as well as of a basic additive, e.g. diethylamine (DEA), are generally required.

In this presentation we will discuss the role of the mobile phase components and their impact on retention and peak shape. To distinguish the effects from each other, we will initially use an experimental design approach. Three β-blockers are selected as model compounds: metoprolol, alprenolol and propranolol. The retention and peak shape of three β-blockers as models are investigated by varying co-solvent fraction and DEA content in the mobile phase. Generally, we then found that the co-solvent mainly influences the retention, whereas the additive more strongly affects the peak shape. The deformations of the elution profiles likely belong to two categories: (i) tag-along effects on analytes eluting early and close to the additive and (ii) more complicated deformations on analytes eluting late. Both these effects are strongly affected by the mobile phase composition. To understand the underlying reason for the later deformation, advanced adsorption isotherm computer estimation and mechanistic modeling, based on solid physicochemical theory, will be presented.

Tuesday, October 17, 8:30 am

# Expanding the Reach of Enhanced Fluidity Liquid Chromatography using Selectivity Gradients

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The value of exploring selectivity and solvent strength ternary gradients in enhanced fluidity liquid chromatography (EFLC) is demonstrated. A complex sample such as fructans from chicory was used as a model system for analysis. Commercial binary pump systems for supercritical fluid chromatography only allow for the implementation of ternary solvent strength gradients which can be restrictive for the separation of polar polymeric analytes. In this work, a custom system was designed to extend the capability of EFLC to allow tuning of selectivity in a ternary gradient. Selectivity gradients provided the separation of more analytes over time. Furhter evaluation of the gradients by the Berridge function showed favor to selectivity gradients with comparable peak capacity to that of solvent strength gradients. EFLC with hydrophilic interaction chromatography, HILIC, separation mode was successfully employed to separate up to 47 fructooligosaccharides in less than 25 minutes using a selectivity gradient.

Tuesday, October 17, 8:55 am

# Achiral SFC: No C18 Equivalent, No Problem

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For years, a large amount of research has been conducted in the search for a universal achiral SFC column. This universal column would be the SFC equivalent to the reverse phase C18 column. These efforts have evaluated many forms of column chemistry, long lists of probes and employed sophisticated statistical treatment of screening data. However, to date there is still no magic universal column for SFC.

A universal column for achiral SFC applications would be convenient but does SFC need to have a universal column? Chiral chromatography in the pharmaceutical industry can be credited with shaping the entire SFC industry into its current position. Chiral chromatography does not have a universal column. SFC has excelled in this separation science niche because it is particularly effective at screening multiple columns and different eluent compositions. The same approach can be applied to achiral chromatography. SFC can be effectively applied to achiral applications when a limited set of columns and cosolvents are known to be applicable for the diverse range of compounds suitable for SFC.

The work presented here will discuss a three-step process for the development of achiral SFC applications. This process will be described and demonstrated with examples relevant to the pharmaceutical and cannabis industries. Columns typically utilized for normal phase, reverse phase and chiral chromatography will be evaluated for their applicability in achiral SFC applications. Changes in achiral selectivity that are observed with the different columns will be highlighted. Experimental evaluation of cosolvents and modifiers will also be addressed.

Tuesday, October 17, 9:20 am

# The Analysis of Steroidal Compounds using UHPSFC-MS

Julie M Herniman
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Steroids are a family of lipid molecules that include cholesterol, steroid hormones and bile salts. Traditionally gas chromatography mass spectrometry (GC-MS) has been used for analysis following derivatisation to increase volatility and stability. More recently high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) has been utilised due to its improved specificity and selectivity. Whilst reversed phase HPLC-MS is successful for the analysis of many steroid compounds, some however are over-retained and/or separation of mixtures is difficult to achieve.

UHPSFC-MS methods have previously been developed for the analysis of lipids at Southampton and this approach has now been extended to a different steroidal compound classes. A Waters Acquity UPC$^2$ is coupled to a TQD mass spectrometer and different organic make-up solvents are used to promote ionisation for ESI, APCI and APPI. Separation has been achieved for mixtures of steroid compounds, such as cholanic acids/esters and steroid derivatives using different column phases (Torus Diol and Torus 1-AA) and different modifier solvent gradients. The methods used for standard compounds are subsequently embedded into an open access environment to facilitate the analysis of archaeological, biological, chemical, and medical research samples.

Tuesday, October 17, 10:45 am

# Investigating Novel Substituted Polysaccharide Phases for Chiral Separations by SFC

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While a majority of enantioseparations are successfully obtained using a few traditional chiral stationary phases (CSPs), the remainder requires significant method development on unique phases or alternative approaches to achieve separation. This is especially true as the incorporation of multiple chiral centers within biologically active small molecules in pharmaceutical drug discovery has become more prevalent. Efforts to find new and unique polysaccharide CSPs included revisiting several phases that were created and tested in the 1990s but never commercialized. As a result, CSPs were found which exploited the effect of fluorophilic retention mechanisms on the enantioseparation of halogenated compounds. Since the initial results were positive, additional prototype phases were prepared on cellulose and evaluated by SFC using drug-like compounds. This presentation will highlight the evaluation of these new phases, including the comparison of 4-chloro-3-trifluoromethylphenyl carbamate and its corresponding fluoro- and trifluoromethyl counterparts. Additionally, the effects of replacing the methyl group with trifluoromethyl will be presented. The use of pyridine as a replacement of the phenyl carbamate is currently being evaluated to determine its suitability for chiral (or achiral) separations.

Tuesday, October 17, 11:10 am

# The Utility of Ultra High Performance Supercritical Fluid Chromatography and Ultraviolet Detection for the Analysis of Synthetic Cathinones

Stephanie Carnes [1], Stacey O'Brien[1], Angelica Szewczak[1], Jonathan Smuts[3], Lauriane Tremeau-Cayel[1], Walter F. Rowe[1], Ioan Marginean[1], Bruce McCord[2], Ira S. Lurie[1]*

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Synthetic cathinones are frequently thought of as "legal highs" and may be sold with the label "bath salts" or "plant food" and annotated with "not for human consumption". Structural changes are regularly introduced during synthesis to circumvent controlled substances laws. A comparison of ultra high performance supercritical fluid chromatography (UHPSFC), ultrahigh performance liquid chromatography (UHPLC), and gas chromatography (GC) for the separation of synthetic cathinones has been performed. Thirty-five different synthetic cathinones were analyzed, including a general set of controlled synthetic cathinones as well as several sets of positional isomers. For UHPSFC, separations were performed using a series of achiral Torus and chiral Trefoil columns with either ammonium formate or ammonium hydroxide as additives, and organic solvents as modifiers. UHPLC separations were performed in both reversed phase and hydrophilic interaction chromatographic modes using SPP C18 and SPP HILIC columns. GC separations were carried out using an Elite-5MS capillary column. The orthogonality of UHPSFC, UHPLC and GC was examined using principal component analysis. For the best overall separation of synthetic cathinones, the use of UHPSFC in combination with GC is recommended. Significantly improved overall resolution of synthetic cathinones was obtained using serial coupled columns with different stationary phases. The utility of ultraviolet (UV) detection for enabling the identification of synthetic cathinones, including different subclasses and positional isomers is presented. For UHPSFC and UHPLC, unique UV spectra are obtained for sub-classes, where position and type of substitution on benzene rings give rise to differences in UV maxima and relative intensity of the spectral bands. In contrast, GC with vacuum UV spectra can also distinguish between positional isomers where differences occur on the aliphatic portion of the molecule. UV detection provides complementary information to electron ionization mass spectrometry, which for the latter technique can lack specificity, especially when substitution occurs on the benzene ring. For UHPSFC versus UHPLC, there was at least a 10 nm blue shift in UV maximum, which high- lights the importance of taking into account the effect of mobile phase on the UV maximum when performing method development in UHPSFC.

Tuesday, October 17, 2:00 pm

# Assessing “in-vivo” Inter-conversion of Chiral Drug Molecules by 2D LC-SFC-MS

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The assessment of “in-vivo” inter-conversion of chiral drugs is a FDA requirement in preclinical and clinical studies. The analysis can be quite challenging due to the low abundance of the active pharmaceutical ingredient (API) and its potential metabolites. Not only is a high degree of sensitivity required to detect the API and its enantiomer, but additional sensitivity is required for the analysis of chiral metabolites as well. This analysis will require the separation of the API and the potential metabolites from the matrix components and from each other. Further chiral separation will be needed to separate each component from its corresponding enantiomer.
Two dimensional Reversed Phase Liquid Chromatography-Supercritical Fluid Chromatography-Mass Spectrometry (2D LC-SF-MS) was used to perform this analysis. The first LC dimension can be used as a desalting step and can efficiently separate the API and potential metabolites from the matrix components. The API and its metabolites can be trapped on small columns and then transferred into the second SFC dimension for chiral separation. The sensitivity of this system was optimized in order to achieve extremely low detection limits (ng/ml). In this work, we have studied different system parameters to enhance the sensitivity. The different parameters investigated include: 1D and 2D column dimensions, trapping column stationary phase, system tubing ID, and detection techniques (UV and MS). The optimized system’s limit of detection in the second dimension was determined to be 10ng/ml.

Tuesday, October 17, 2:25 pm

# High-throughput analysis of vitamin E by means of UHPSFC-MS and evaluation of matrix effects in plasma and in urine

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Vitamin E comprises two different groups of compounds, tocopherols and tocotrienols, both consisting of four isoforms (α-; β-; γ- and δ-). Being structurally similar fat-soluble vitamins, their analysis remains very challenging despite the use of highly efficient liquid chromatographic approaches. Ultra-high performance supercritical chromatography (UHPSFC) coupled to mass spectrometry (MS) emerged as an important technique in the field of bioanalysis only recently. Due to many advantages, such as high separation efficiency, reduced analysis time, high sensitivity, and above all, different selectivity, UHPSFC-MS offers a complementary approach to HPLC.

This study aimed at developing UHPSFC-MS method for high-throughput analysis of all eight derivatives of vitamin E including the sample preparation approaches and a thorough evaluation of the matrix effects. The UHPSFC method optimization involved the selection of stationary and mobile phases, and fine-tuning of parameters such as flow-rate, temperature, and pressure controlled by back-pressure regulator (BPR). The coupling of UHPSFC and MS was accomplished via the pre-BPR flow splitting and addition of make-up solvent. The single quadrupole mass spectrometer operating in ESI positive ion mode was used for detection in selected ion monitoring mode. Thorough optimization of sample preparation was necessary for both types of biological matrices, i.e. urine and plasma. Non-selective methods such as protein precipitation and "dilute and shoot" as well as more selective approaches including solid phase extraction, liquid-liquid extraction, and supported liquid extraction were individually optimized for each matrix. Evaluation of the sample preparation approaches was based on the results of the method recovery, precision, matrix effects, and practical aspects of the sample pre-treatment. While UHPSFC-MS analysis of urine samples was straightforward while using most of the sample preparation approaches, analysis of plasma was more challenging due to the strong analyte binding to proteins and due to the presence of lipophilic interferences in plasma.

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Tuesday, October 17, 2:50 pm

# Bioanalysis of stereoisomers by chiral supercritical fluid chromatography – tandem mass spectrometry: Application to pharmacokinetics

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The stereoisomers of a chiral drug could exhibit great differences in the pharmacodynamic and pharmacokinetic properties. Chiral bioanalytical assays are necessary to measure the profiles of individual enantiomers and/or chiral metabolites in biological samples in order to assess the absorption, distribution, metabolism, excretion and toxicity of the individual enantiomers. In addition, chiral assays are required to evaluate potential chiral inversion caused by the biotransformation process. While normal phase or reversed phase chromatography remains the common choices for chiral bioanalytical assays, supercritical fluid chromatography (SFC) demonstrated faster analysis time without affecting separation efficiency, due to the lower viscosity and faster mass transfer properties of supercritical or subcritical carbon dioxide than traditionally used mobile phases.

As one of the most recent chromatographic techniques used in pharmaceutical industry, SFC has been implemented in various application areas for small pharmaceutical molecules: impurity profiling, degradation products, fast screening, and preparative scale purifications. However, SFC chiral separation in the area of bioanalysis has yet to be widely applied. Compared with conventional liquid chromatography (reversed-phase and normal-phase), higher efficiencies, improved resolution and the 'green' aspect of using environmentally friendly carbon dioxide are the major driving force of implementing SFC in the bioanalysis where high throughput assays are desirable. In recent years, the introduction of innovative SFC instrumentation which adapt well to analytical scale applications greatly benefit the use of SFC chiral separation for biological samples. Furthermore, low dead volume backpressure regulators and splitless connection allow the SFC outlet to readily couple with mass spectrometry without sacrificing sensitivity.

In this oral presentation, we will present examples of developing chiral SFC-multiple reaction monitoring (MRM) assays to address different aspects of pharmacokinetic measurement: i) qualitative and quantitative determination of the chiral inversion in vivo; ii) determination of the individual enantiomer PK profiles and iii) measurement of racemates and their metabolites in a high throughput assay. We will further discuss the applicability of chiral bioanalytical assays in the drug development process and highlight some practical considerations of chiral SFC assays, specifically dynamic range, internal standard, matrix effect, and column selection.

Tuesday, October 17, 3:15 pm

# Physical Properties by SFC: Determination of $CO_2$-$H_2O$ Partition Coefficients of Surfactants

Robert M. Campbell, Adam Grzesiak, Jacob Crosthwaite, Margaret Covington, Arne Ulbrich, Troy Knight, Pramod Patil, Amit Katiyar
The Dow Chemical Company

The structure and composition of surfactant materials has a direct effect on physical properties and performance in end-use applications. One property that has a large effect on performance is the $CO_2$-$H_2O$ partition coefficient, K. However, direct measurement methods of this property are time-consuming and subject to large experimental error. A procedure to rapidly determine the partition coefficient for a large number of samples was needed.

Retention in gradient elution supercritical fluid chromatography (SFC) has been modeled using the Hildebrand solubility parameter. More recently, linear solvation energy relations (LSER) with Abraham descriptors have been used to describe SFC retention. Empirically, retention can be modeled as a simple linear relationship to the mole fraction of the B mobile phase of the gradient. In both models, fundamentally, the partitioning of the analyte between the stationary phase and mobile phase determines retention. If the stationary phase is polar and a gradient from non-polar to polar mobile phase is used, we hypothesize that the resulting retention times will correlate to carbon dioxide/water partition coefficients for surfactant materials.

In this study, the $CO_2$/water partition coefficients of a set of 14 surfactant materials were determined directly at 2000 psi and 40°C. These data, expressed as log K, were then linearly correlated to their SFC retention times in a gradient elution system. The retention time of the first quartile of the surfactant distribution was found to correlate most strongly to the partition coefficient, log K, giving a correlation coefficient ($R^2$) of 0.79. Adjusting the model to include the additional parameters improved the correlation to 0.88. The resulting model was used to predict the partition coefficients for an additional 26 surfactants.

Tuesday, October 17, 3:40 pm

# Effects of acidic and basic additives on retention mechanisms on a teicoplanin aglycon stationary phase

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Supercritical fluid chromatography (SFC) is well-known for chiral applications but more and more achiral ones are emerging. Usually, most of studies in SFC are focused on small molecules, but the study of bigger ones is increasing. The goal of this study is to investigate how amino acids (or peptides) behave in SFC. But it is interesting to start with the analyze of small molecules behavior on a stationary phase coated with a peptide. The stationary phase used is the teicoplanin aglycon, known as Chirobiotic TAG, commercialized by Merck. The teicoplanin is a glycopeptide used as an antibiotic, but the silica-bonded molecule in the TAG stationary phase does not contain the glycon part so it is just a cyclic peptide.

Two additives (trifluoroacetic acid and isopropylamine) were tested at different levels of concentration to understand how the stationary phase evolves with the nature and the concentration of additive in order to broaden the knowledge about the behavior of amino acids or peptides in SFC.

The retention mechanism provided by this stationary phase was determined, based on a set of 54 achiral and 24 chiral compounds. For achiral compounds, a modified version of the Linear Solvation Energy Relationships (LSER) was established to take account of ionic interactions, using the $D^+$ et $D^-$ descriptors in addition to the classical Abraham descriptors (E, S, A, B, V). This study evidenced the change in retention mechanism (hydrogen bonding and ionic interactions) as a function of concentration and nature of additive in the mobile phase as (i) acidic and basic additives modify the mobile phase acidity, (ii) they may affect the ionization state of stationary phase functional groups and (iii) they adsorb on the stationary phase. For chiral compounds, enantiomers separation was monitored to evaluate the changes in interaction between molecules and the chiral selector of the stationary phase.