Free Tutorials

open to all conferees, first-come seating

The tutorial track is part of the educational mission of HPLC 2016. Experts are asked to give 45-90 minute presentations on a topic with more background than might be found in a typical 20 minute talk. The goal is to make the topic more accessible to those less expert in the area. In some cases, discussion and other interactive activities may be used.

TUTORIALS		
Tuesday, June 21	**Tutorial Title**	**Tutorial Presenter**
9:20-10:05 am	Understanding Separations in HILIC Chromatography: Theory to Practice	David Bell, MilliporeSigma
11:15 am-12:00 pm	PROTEOFORMS: The New Challenge In Protein Separations	Fred Regnier, Purdue University
1:30-3:00 pm	Preparing Your Manuscript and Publishing it from an Editor's Perspective	Jonathan Sweedler, University of Illinois at Urbana-Champaign
4:40-5:25 pm	Method Development in HPLC	Paul Haddad, University of Tasmania
TUTORIALS		
Wednesday, June 22	**Tutorial Title**	**Tutorial Presenter**
9:20-10:05 am	New Directions in Sample Preparation for Chromatographic Analysis	Ronald Majors, LCGC
11:15 am-12:00pm	Fundamental and Instrumental Aspects of UHPLC	Monika Dittmann, Agilent Technologies
2:20-3:05 pm	The Importance of Small Molecule Analysis in Biopharma	Holly Shackman, Bristol-Myers Squibb
4:40-5:25 pm	Introduction to Fundamental Separation Theory for Better Understanding the Recent High Pressure Trend and for Improved Quality Control Methods	Torgny Fornstedt, Karlstad University
TUTORIALS		
Thursday, June 23	**Tutorial Title**	**Tutorial Presenter**
9:20-10:05 am	Ion Mobility Spectrometry for Separations	Gerard Hopfgartner, University of Geneva
11:15 am-12:00 pm	Analysis of Small Molecules by LC/MS	Michal Holcapek, University of Pardubice
2:20-3:05 pm	Speed and Efficiency Optimization	Gert Desmet, Vrije Universiteit Brussel
4:40-5:25 pm	Comprehensive Two-dimensional Liquid Chromatography (LC×LC) – Why, When and How?	Peter Schoenmakers, University of Amsterdam
TUTORIALS		
Friday, June 24	**Tutorial Title**	**Tutorial Presenter**
9:20-10:05 am	Impurity Profiling in Pharmaceuticals	Kelly Zhang, Genentech
11:15 am-12:00 pm	Capillary Electrophoresis-Mass Spectrometry for Metabolomics	Rawi Ramautar, Leiden University
2:20-3:05 pm	Modern SFC – What the Analytical Chemists Should Know About It?	Abhijit Tarafder, Waters Corporation

Tuesday Tutorial:
Understanding Separations in HILIC Chromatography: Theory to Practice

David Bell, MilliporeSigma, Bellefonte, PA, USA

Abstract: HILIC chromatography is a complex system involving partition, polar and ion-exchange interactions. In this tutorial we will first explain the present theories regarding the dominant retention mechanisms at play in HILIC. We will then use that knowledge to discuss method development practices that can be greatly facilitated by understanding these interactions. We will explore and contrast different HILIC stationary phases that are available in terms of the mechanisms of interaction they provide and practical examples applying this knowledge to the various separation tasks will be discussed. Finally we'll explore and attempt to explain problems often associated with HILIC methods such as irreproducibility and lengthy equilibration times. Attendees are expected to leave with a more firm understanding of HILIC mechanisms; improved understanding of the stationary phases utilized in HILIC and armed to minimize issues commonly associated with the practical use of HILIC.

Tuesday Tutorial:
PROTEOFORMS: The New Challenge In Protein Separations

Fred Regnier, Purdue University, West Lafayette, IN, USA

Abstract: HPLC of proteins and peptides has been widely practiced for more than 40 years. With that level of experience it would seem there would be little left to learn about protein separations; actually not. Genomics and proteomics have taught us that there are probably 10-50 times more proteins in cells than the genes from which they arise. Bottom-up proteomics has shown that splice variations, more than 200 types of post-translational modifications (PTMs), and mutations lead cells to produce large numbers of primary structure variants. We know this because multiple tryptic peptides bearing a PTM, amino acid substitution(s), and/or splice variations from specific proteins have been found by mass spectrometry based sequencing. Both modified and un-modified versions of peptides are found as well. With phosphorylation and glycosylation there can be 10 to 50 different modifications of a protein. The great problem with this is that following trypsin digestion we don't know whether these structure alterations were all contained within a single protein or many. A problem with current methods and the proteomics literature is that few of the predicted proteoforms have been isolated and characterized. Structure predictions are being based on fragment ions seen in a mass spectrometer. What the world is doing about this, and proposals of how to deal with proteoform issues will be the basis for this "Tutorial Lecture". The lecture will begin with a discussion of what we know about proteoforms, proteoform separation and identification issues in diagnostics versus those with biopharmaceuticals and biosimilars, the differing needs of the CRO versus the point-of-care and process monitoring communities, and FDA concerns and mandates. The lecture will proceed on to discussions of retention mechanisms in the various modes of chromatography along with why proteoform resolution can be difficult, the role of bottom-up and top-down mass spectrometry in proteoform recognition, methods by which proteoform identification can be achieved, preparative versus analytical methods of proteoform recognition and resolution, higher order structure variations, and proteoform issues in quality control. The lecture will conclude with an examination of potential ways to address these issues including increases in resolution versus selectivity, the value of decreasing and/or simplifying the number of separation dimensions, separation cost, the need to "keep it simple", and point-of-care devices.

Tuesday Tutorial:
Preparing Your Manuscript and Publishing it from an Editor's Perspective

Jonathan Sweedler, University of Illinois at Urbana-Champaign, Urbana, IL, USA

Abstract: This tutorial is aimed at graduate students, postdoctoral associates, early stage professors, or anyone with limited experience publishing. The mechanics of preparing and publishing your work, including selecting potential reviewers and responding to reviewer comments are covered. After an overview presentation, a panel of editors will answer any questions related to the publishing and reviewing process. Lastly, the workshop will end with a FAST PITCH networking event where editors will separate to different tables and potential authors will have a few minutes to "pitch" their research to an editor to see if their research is something that would make it in their journal; the editors will also offer advice to aid in getting the work published. This is a great chance to learn about the mechanics of publication, make connections with editors, and hopefully gain useful advice on publishing a masterpiece.

Tuesday Tutorial:
Method Development in HPLC

Paul Haddad, University of Tasmania

Abstract: This tutorial is aimed at analytical scientists in industry, academia and students who want to increase their understanding and skills in chromatographic method development. Chromatographic methods often underwrite quality of products as well as safety of consumers and therefore analytical methods used to assess quality and safety must be adequately robust and suitable for the generation of reliable results. In this tutorial we will initially focus on factors affecting chromatographic resolution. We will describe how chromatographic columns are selected and how temperature, pH, organic content of mobile phase, and mobile phase additives affect resolution and how these factors can be used to achieve a robust separation. Next we will demonstrate how dedicated software tools for HPLC method , such as DryLab or LC Simulator, can reduce the number of experiments required to optimize these factors. We will also show how this software can be used to assess method robustness using Design of Experiments approach and Quality-by-Design principles. Finally, we will show how a Quantitative Structure-Retention Relationships (QSRR) approach based only on structural features of analytes can be used to predict retention times in a range of HPLC methods, such as reversed-phase HPLC, hydrophilic interaction liquid chromatography (HILIC) and ion-exchange chromatography. Use of QSRR to assess chromatographic space in-silico and to identify areas suitable for experimentation which will lead to robust methodology will also be discussed.

Wednesday Tutorial:
New Directions in Sample Preparation for Chromatographic Analysis

Ronald Majors, LCGC, West Chester, PA, USA

Abstract: For many years, classical sample preparation techniques such as Soxhlet-, liquid-liquid- and solid-phase-extraction (SPE) have been widely used for chromatographic analysis. With the development of improved chromatographic separation columns and detection techniques such as LC-MS/MS, the principle of "Just Enough" sample preparation, where selectivity can be achieved anywhere in the sample preparation-chromatography-detection process, simpler and faster sample prep is now possible. For example, QuEChERS, which consists of a simple extraction and dispersive SPE process, is now used for analytes and matrices well beyond pesticides in fruits and vegetables such as biofluids and certain environmental samples. Enhanced matrix removal systems are now being used to simplify multi-component samples by removing the interfering matrix rather than focusing on isolating the analytes of interest from the sample. A case in point is the removal of lipids, that can foul columns and cause ion suppression in MS, from high-fat samples leaving analytes behind in solution. The results are better sensitivity and analyte recovery. There has been a renaissance in sample preparation techniques that are scaled down to work with smaller samples and are simpler to perform. Miniaturization techniques which

can be readily automated, such as the use of 96 well plates, have revolutionized high-throughput sample prep so that many samples can be processed in a single day that used to require multiple days. Not only are smaller samples used but reduced reagent consumption and less manual sample handling are also benefits. Automation allows the improvement in quantitation and reproducibility by minimizing the manual transfer of samples. The use of 96-well SPE plates and support liquid extraction plates have allowed these classical techniques to receive more attention as part of the sample preparation arsenal. Protein precipitation plates have also provided improved results in the automation of the analysis of drugs in biological fluids such as plasma. Even improvements in simple filtration are achieved by the use of 96-well filtration plates. In this tutorial, attendees will learn about some of the newer techniques for sample preparation that are faster, provide lower detection limits, better recovery, and less manual labor. The presentation will be available in electronic format for those interested.

Wednesday Tutorial:
Fundamental and Instrumental Aspects of UHPLC

Monika Dittmann, Agilent Technologies, Waldbronn, Germany

Abstract: Over the past 10 years the increasing need for higher speed and resolution in analytical separations has driven the trend towards the use of higher pressures (> 1000 bar) in liquid chromatography (UHPLC). This tutorial will cover the basic principles of UHPLC and discuss various aspects that need to be considered to successfully apply this technology in method development and routine work. The first part of the tutorial will deal with optimization strategies for UHPLC separations for different types of applications, based on mass transfer theory and kinetic optimization. It will be shown how the kinetic plot model (also known as Poppe plot) combines column efficiency with column permeability to determine the optimum conditions for a desired separation. The kinetic plot model, which is a much more useful measure for separation efficiency than plate number alone, will be explained in detail and examples for different column types and applications will be shown. The second part of the tutorial will focus on instrumental aspects of UHPLC such as the impact of dwell volume and system dispersion on the separation performance. The requirements for UHPLC instruments are much more stringent compared to those used for HPLC, mainly due to the smaller column diameters used in UHPLC. The impact of extra-column volume on the apparent efficiency for small bore columns will be demonstrated for isocratic and gradient separations. Finally the factors that influence transfer of methods between HPLC and UHPLC instruments will be discussed as differences in system properties (dwell volume and mixing behavior) can lead to significant changes in retention times and selectivity in gradient separations.

Wednesday Tutorial:
The Importance of Small Molecule Analysis in Biopharma

Holly Shackman, Bristol-Myers Squibb, New Brunswick, NJ, USA

Abstract: Over the past decade, a trend has emerged with traditionally small molecule-based pharmaceutical companies embracing larger biological compounds as potential therapeutic agents, giving rise to the emergence of today's biopharma. Biologic therapeutics provide supplementary pathways to address the myriad of serious diseases, but also bring additional challenges in their development and analysis. Though much focus is centered around the different techniques required to appropriately characterize and quantitate these molecules, numerous analytical complexities still exist that require small molecule analysis to support the development and manufacture of biologics.

One such example occurred during the manufacturing of a biologic drug substance at an external vendor when it was observed that 100% benzyl alcohol, which was used to prepare the Protein A column storage solution, had been filtered through a polyethersulfone (PES) membrane prior to being diluted with water. As the correct process utilized PES filtration of 2% benzyl alcohol, this deviation in procedure triggered an investigational study to assess the potential impact on the Protein A column and the drug substance

manufactured using this column. The filter manufacturer had not previously conducted a compatibility assessment when using 100% benzyl alcohol with their product, therefore BMS was tasked with determining if any potential leachables posed either a product quality or patient safety concern. Based on previous extractables and leachables studies performed by the vendor and in-house, a target analyte list was generated which contained slip agents, polyethersulfones, cyclic ether sulfones (CES) as well as commonly observed extractables. LC-MS was selected for this investigation due to its ability to perform a rapid and sensitive qualitative and quantitative analysis of non-volatile compounds. When comparing the components detected in filtered vs. unfiltered benzyl alcohol, several oligomers of CES were observed to originate from the PES membranes. As tricyclic ether sulfone had the greatest response, it was chosen for subsequent monitoring. In order to develop an LC-MS limit test specific for tricyclic ether sulfone, several obstacles needed to be overcome, with one such example being the rapid generation of an in-house reference material by extraction, isolation, preparative LC, and quantitative NMR as this material was not commercially available. The final method was able to utilize LC-MS for a limit test of tricyclic ether sulfone at 0.9 ng/mL.

Wednesday Tutorial:
Introduction to Fundamental Separation Theory for Better Understanding the Recent High Pressure Trend and for Improved Quality Control Methods.
Torgny Fornstedt, Karlstad University, Karlstad, Sweden

Abstract: The purpose of this tutorial is to demonstrate how fundamental separation theory can be used to gain a better understanding of the separation process. We aim at presenting the theory in an accessible way, with graphics and practical examples, so that even without a deep understanding of the mathematical models, the participant should obtain a better feeling for the chromatographic processes. We will focus on modern UHPLC method development with sub 2 µm porous/fused core particles. The understanding of pressure and temperature effects in UHPLC is particularly important in method transfer from HPLC to UHPLC and for developing methods that needs regulatory approval in the pharmaceutical industry. In this tutorial we will incorporate our most recent findings regarding pressure and temperature effects on separation performance. We demonstrate how different types of analytes behave and explain the differences using a two-site adsorption model. We will also compare the advantages/disadvantages of porous vs. semi porous particles with a firm theoretical basis. For example, the impact of frictional heating on the column efficiency is quite different in porous and semi porous particles; here also the heating mode (water or air) is important to consider. We will show how the heat conductivity of the solid core plays an important role in understanding the differences. Finally, we will focus on how fundamental separation theory can benefit Quality Control (QC) method development in the pharmaceutical industry. QC-methods play an important role in the overall control strategy for drug manufacturing. However, efficient life-cycle management and continual improvement are hindered due to a variety of post-approval variation legislations across territories and a lack of harmonization of the requirements. In cooperation with AstraZeneca we have been outlining a QC method enhancement concept where fundamental science, extended robustness testing and clear quality attributes gives the pharmaceutical industry flexibility built on scientific understanding. Strong and well explained scientific based arguments with a proper regulatory risk assessment can realize efficient life-cycle management, e.g. switching from HPLC to UHPLC. This work was supported by a grant (Nr 2015/18/M/ST8/00349) from National Science Centre, Poland and by a grant (nr 15/497) from ÅForsk Foundation in Sweden).

Thursday Tutorial:
Ion Mobility Spectrometry for Separations
Gerard Hopfgartner, University of Geneva, Geneva, Switzerland

Abstract: Since several decades ion mobility spectrometry (IMS), which separates ions according to their size-to-charge ratio has been used as a standalone technique for the detection of chemical warfare

agents, explosives or narcotics. IMS is also widely hyphenated to mass spectrometry (IMS-MS) where the orthogonality of both techniques allows to separate isomeric compounds. IMS can be separated into two classes: the first one includes dispersive approaches where all ions are separated in a single analysis while the second includes scanning approaches where a specific parameter needs to be scanned to obtain a full spectrum. They are several methods of performing IMS including: field-asymmetric waveform ion mobility spectrometry (FAIMS), also known as differential ion mobility spectrometry (DMS), the traditional drift tube (DTIMS), the traveling-wave devices (TWIMS), the trapped IMS (TIMS) or differential mobility analyzers (DMA). Beside the use as standalone device IMS is very attractive for hyphenation with separation sciences such as gas or liquid chromatography. IMS can either be used to add an additional selectivity step or it can be applied as an additional separation step for LC-IMS-MS. The present tutorial will describes the current state-of the art in ion mobility with the focus as an alternative for liquid chromatography for ambient ionization or as an additional step chromatography step, in multidimensional separation for the analysis of low molecular weight compounds or peptides.

Thursday Tutorial:
Analysis of Small Molecules by LC/MS

Michal Holcapek, University of Pardubice, Pardubice, Czech Republic

Abstract: This tutorial deals with the qualitative and quantitative analysis of small molecules using the hyphenation of liquid chromatography and mass spectrometry (LC/MS). LC offers various chromatographic modes with a different selectivity, which can be tuned to achieve the required type of separation, e.g., reversed-phase LC for the isomeric resolution, hydrophilic liquid chromatography (HILIC) for the class separation based on differences in polar parts of molecules, normal-phase LC for the class separation of nonpolar molecules, etc. Ultrahigh-performance liquid chromatography (UHPLC) or LC with core-shell particles offers better efficiencies and high throughput, which is beneficial also for MS coupling, but it requires higher scanning speeds. Mass spectrometers in LC/MS are typically based on atmospheric pressure ionization techniques, mainly electrospray ionization suitable for polar molecules, atmospheric pressure ionization or atmospheric pressure photoionization better suited for less polar small molecules. Different configurations of mass spectrometers can applied for the detection of small molecules starting from simple low-resolution instruments, quadrupoles (Q), ion traps and linear ion traps (LIT), or high-resolution tandem mass analyzers, such as time-of-flight (TOF) based configurations or Fourier transform mass spectrometers (orbitrap and ion cyclotron resonance, ICR) providing unique resolving power and high mass accuracy but also slower scanning speeds on the other hand, therefore there are not typical selected for coupling with fast UHPLC methods. Triple quadrupoles (QqQ) or quadrupole – linear ion traps (Q-LIT) are typical configurations for the quantitative analysis using dedicated scans (selected reaction monitoring, precursor ion and neutral loss scans) and isotopically labelled internal standards. The structural analysis of small molecules is based on high-resolution analyzers, such as TOF, orbitrap or ICR coupled with Q/LIT as the first mass spectrometric analyzer. Full-scan and MS/MS spectra measured in both polarity modes (if possible) are important for the structural elucidation together with the knowledge of fragmentation behavior characteristic for individual functional groups, software tools for the spectra interpretation and in specific cases also MS library for particular compound groups. Examples will be given for individual approaches described here for the real analysis of various types of small molecules, for example drugs, dyes, drug metabolites and lipids. This work was supported by ERC CZ project No. LL1302 (MSMT, Czech Republic).

Thursday Tutorial:
Speed and Efficiency Optimization

Gert Desmet, Vrije Universiteit Brussel, Brussels, Belgium

Abstract: In the past decade, researchers and scientists using chromatographic separations in their analytical work have been witnessing tremendous shifts in the performance and speed offered by the newest generations of (U)HPLC instruments and columns. This progress was achieved by developing columns packed with sub-2µm particles (now down to 1.3 µm in a commercial format) and by introducing

a new generation of superficially porous particles. In addition, also a quantum leap in the inlet pressure available on the instruments has been made, increasing the available inlet pressure with a factor of nearly four, increasing from the 400 bar that was typically available in 2005 until the 1500 bar available today on the newest commercial instruments. In the present tutorial, these advances will be put in perspective, both from a theoretical and a practical point of view. Emphasis will be put on showing for which applications the move to an ultra-high pressure system is really beneficial, whereas for some other applications the move to just another particle type or a different column length would already be sufficient. In addition, the importance of a proper system configuration will be accentuated. This is indispensable, because the newest ultra-small particle columns produce so little band broadening that the quality of the instrument (and more specifically its own contribution to the over-all band broadening) is prone to become the limiting factor. For this purpose, the most important factors affecting the instrument contribution (tubing length and diameter, injection volume, detector size) on the observed column performance will be reviewed and illustrated with results obtained using state-of-the-art UHPLC instruments together with the most efficient columns that are available today.

Thursday Tutorial:
Comprehensive Two-dimensional Liquid Chromatography (LC×LC) – Why, When and How?

Peter Schoenmakers, University of Amsterdam, Amsterdam, The Netherlands

Abstract: During the last decade comprehensive two-dimensional liquid chromatography (LC×LC) has become increasingly used. Until recently, it was largely the domain of academic researchers, but now that commercial instrumentation is improving rapidly and increasingly available, there now is a strong proliferation of LC×LC in industry. LC×LC is advantageous for several reasons. It may provide high peak capacities in a much shorter time than one-dimensional LC. It may also provide structured separations for samples that are complicated, but have a low "sample dimensionality". In some cases, LC×LC may augment mass-spectrometric (MS) analysis. One such example is the analysis of complex mixtures of peptides. In some cases, for example for many high-molecular-weight synthetic polymers, MS is not feasible and LC×LC is the best approach. Using contemporary instrumentation LC×LC separations can be performed in reasonable time (typically 20 to 60 min). Method development requires considerable knowledge and skills, or specialist software that is currently being developed. In this tutorial the principles of LC×LC are briefly reviewed and some recent developments are highlighted. Practical examples will be used to illustrate the advantages of the technique.

Friday Tutorial:
Impurity Profiling in Pharmaceuticals

Kelly Zhang, Genentech, South San Francisco, CA, USA

Abstract: Pharmaceutical impurity profiling is extremely important for drug research and development as well as for drug safety and efficacy. Impurities can come from process by-products, residues carried from raw materials, starting materials and intermediates, degradation products, contaminations, extractables/leachables, etc. As we don't know exactly how many impurities we should expect for real world pharmaceutical samples, complete impurity profiling heavily relies on the analytical technologies we use. Impurities may have distinct physiochemical properties that one method may not able to capture the whole impurity profile. For instance, reversed-phase HPLC method may not retain polar impurities and some impurities may not have UV chromophores, so these impurities could be easily overlooked by traditional HPLC-UV methods. On the other hand, some impurities (e.g. isomers) could have similar physicochemical properties as the active pharmaceutical ingredient (API), so that they could co-elute. Method specificity and stability indicating features are required to be demonstrated during method development. This tutorial will start with regulatory requirements for pharmaceutical impurities in drug substances and drug products. We will discuss the technologies and challenges for pharmaceutical impurity profiling, focusing on separation and detection technologies. We will discuss targeted and non-

targeted impurities. We will cover impurities include but not limited to inorganic impurities, organic impurities, residual solvents, metals, genotoxic impurities, etc.

Friday Tutorial:
Capillary Electrophoresis-Mass Spectrometry for Metabolomics

Rawi Ramautar, Leiden University, Leiden, The Netherlands

Abstract: In the field of metabolomics, capillary electrophoresis-mass spectrometry (CE-MS) has become a very useful analytical technique for the profiling of highly polar and charged metabolites in complex biological samples. In this tutorial, a comprehensive overview of recent developments in CE-MS for metabolic profiling studies is provided. Topics that will be covered include CE separation modes, capillary coatings and recent interfacing designs for hyphenating CE to MS. In this context, special attention will be devoted to the recently developed sheathless porous tip interface allowing in-depth metabolic profiling studies by CE-MS. Throughout the discussion attention will be paid to practical aspects used for CE-MS-based metabolomics. The potential of this analytical technique for large-scale and quantitative clinical metabolomics studies is also addressed. Finally, the utility of CE-MS for metabolomics is demonstrated for selected clinical and biological problems, especially for volume-limited samples. Conclusions and perspectives on this unique analytical strategy are presented.

Friday Tutorial:
Modern SFC – What the Analytical Chemists Should Know About It?

Abhijit Tarafder, Waters Corporation, Milford, MA, USA

Abstract: Outside a limited group of analytical chemists, SFC (often expanded as Supercritical Fluid Chromatography) is generally considered as a chromatographic technique that uses supercritical fluids as mobile phase. The main advantage, as perceived widely, is the ability to manipulate chromatographic behavior through pressure or density gradients. Such ideas portray the technique as a very different, unusual form of chromatography, especially when compared with more established techniques like reversed-phase liquid chromatography (RPLC). While these definitions were appropriate in the past, modern-SFC (or just SFC) has evolved with a very different concept. SFC is no longer about working in supercritical conditions; actually the expansion - Supercritical Fluid Chromatography is a misnomer now. SFC rarely works with pressure or density gradients anymore. The modus operandi of SFC is rather close to RPLC, and the capabilities often comparable, sometimes more than what offered by RPLC. In SFC CO2 is used as the principal solvent, as opposed to water in RPLC. Due to the wide miscibility range between CO2 and polar organic co-solvents, e.g. methanol, ethanol, isopropanol, acetonitrile etc., application of RPLC-like solvent gradients for faster separation is the norm in SFC. Organic additives, e.g. TFA, IPAmine etc., like in RPLC, are used for improving peak shapes. Because CO2+ modifiers can seamlessly work with both normal-phase and reversed-phase columns, RPLC-like solvent gradient techniques can be now used with normal-phase columns as well. Chiral and achiral columns can be used in tandem, bringing some unique selectivities. SFC has also brought some special capabilities in chromatographic separation, previously not witnessed either in LC or GC techniques. Many separations which normally need multiple methods (e.g. LC+GC, or RP+HILIC, or RP+NP) can be done in a single SFC run. Operationally the main difference between SFC and RPLC is that in the former an elevated pressure needs to be maintained at the system outlet. While addressing some of the existing myths around SFC, the main objective of the session is to explain the unique capabilities of modern SFC, detailing upon the areas of applications where it has advantages over LC and GC techniques.
