Short Course 1 - Sunday July 29 @ 9:00am-4:00pm

Two-dimensional Liquid Chromatography: Principles, Instrumentation, Method Development, and Applications

Instructors: Dwight R. Stoll, Gustavus Adolphus College;
Mark R. Schure, Kroungold Analytical, Inc.; Kelly Zhang, Genentech, Inc.

In 2DLC, sample components are fractionated by two different columns utilizing different retention mechanisms. To achieve successful 2D resolution of complex sample components, dissimilar (orthogonal) retention mechanisms are required to effectively spread the peaks throughout the available separation space.

In this course we will discuss in detail:

- Theory of 2DLC, from a practical point of view
- Instrumentation, including commercially available instruments
- Method development across a wide variety of sample types
- Applications to lipids, peptides, proteins, small and large molecule pharmaceuticals, biological extracts, industrial polymers, and surfactants
- Helpful insights that are important for achieving good results in the lab

Students are expected to be familiar with HPLC.

Those who take this course will learn background information essential to understanding the technique and achieve useful results with commercial instrumentation. Many aspects of 2DLC are shared with one-dimensional HPLC such as column technologies, pumps, solvent systems, and matching the detector. However, 2DLC has some issues which are not present in one-dimensional HPLC, and these will be explained in detail so that course participants will have this knowledge prior to starting method development. We will also explore the suitable instrumentation for 2DLC, and how to process data external to the acquisition software. Applications of comprehensive 2DLC will be shown for complex industrial and biological samples, as well as simpler applications such as column switching, target peak purity investigation, and biopolymer analysis using commercial two-dimensional chromatographic instruments.

Instructor Bios:

Mark Schure has worked in separation science for over 35 years in industry and academics. He is presently the Chief Technology Officer at Kroungold Analytical, a consulting firm he started in 2012. Dr. Schure has been Adjunct Professor in the Department of Chemical and Biomolecular Engineering at the University of Delaware for over 20 years. He has published over 110 papers, has 4 patents and recently edited the book *"Multidimensional Liquid Chromatography."* His scientific interests include the fundamental separation science of complex molecules, polymers and colloids, colloid chemistry, computational materials science and all aspects of solving large-scale chemical and physical problems with computers. He has received many awards including the Arthur Doolittle award from the American Chemical Society, the Northeastern University Distinguished Alumni Lecture award, the Douglas Leng award from The Dow Chemical Company, the Eastern Analytical Symposium award in separation science, the L. S. Palmer award from the Minnesota Chromatography Forum and in 2015 he received the Stephen Dal Nogare award and the Uwe D. Neue award.

Dwight Stoll is currently Associate Professor at Gustavus Adolphus College, where he teaches quantitative and instrumental analysis courses and is currently co-chair of the Chemistry Department. Active research projects in his laboratory touch upon most aspects of multi-dimensional separation methodologies, including optimization strategies, characterization of selectivity in reversed-phase HPLC, instrument development, and applications in biopharmaceutical analysis. Dwight is the author or co-author of 51 peer-reviewed publications and three book chapters in the area of separation science, is a named co-inventor on four patents, and has instructed numerous short courses in two-dimensional liquid chromatography. In 2011 he was the recipient of *LCGC's Emerging Leader in Chromatography Award*. In 2014 he was named to *The Analytical Scientist's* list of 'Top 40 Under 40' analytical scientists. In 2017 he received the *Georges Guiochon Faculty Fellowship*, and was recognized with an *Agilent Technologies Thought Leader Award*, which will support research in his laboratory on the development of 2DLC methodologies for biopharmaceutical analysis.

Kelly Zhang is a Principal Scientist and a Group Leader at Genentech (a member of the Roche group) in South San Francisco, California. Prior to joining Genentech, she worked at Allergan and Pfizer. She has over 15 years of pharmaceutical industry experience from drug discovery to commercial launch. She has worked on therapies of oncology such as Cotellic™, immunology, ophthalmology and metabolic diseases. She has contributed to regulatory filings of over 15 new chemical entities. She received her Ph.D. in Analytical Chemistry from Wuhan University, China, in 1999. She has published over 40 scientific papers in the areas of 2DLC, chiral separation, impurity profiling etc. She was named one of the top 50 most influential women in analytical science by *The Analytical Scientist*.

Short Course 2, Sunday, July 29 @ 9:00am-4:00pm

Chromatography in the Analysis and Characterization of Protein Therapeutic Drugs

Instructor: C. David Carr, Director of Training, Bioanalytical Technologies

This course explains the properties of proteins that must be characterized in the course of developing a protein therapeutic drug and monitored during production and lot release. It then describes the theory and practice of a number of chromatographic separation techniques that play key roles in the analysis and characterization of protein therapeutic drugs. These include reversed-phase HPLC, ion exchange, size exclusion and several less well-known techniques in chromatography. Examples of how these are used in the development and release of protein therapeutic drugs are shown.

Course Outline

- Protein properties that must be analyzed and characterized in protein therapeutic drugs are discussed. These include deamidation, oxidation, glycosylation, charge state variants, aggregation and pegylation
- Reversed-Phase HPLC and its role in protein therapeutic analysis
    - Typical operating conditions for protein/peptide analysis
    - Column characteristics best suited for protein and peptide analysis
    - Optimum mobile phase conditions and the effect of gradients and temperature on peptide separations
- How reversed-phase HPLC is used to characterize and analyze protein therapeutics for degradation products, disulfide bonds, glycosylation, and other modifications
- Other Types of Liquid Chromatography and their role in protein therapeutic drug analysis
    - Ion Exchange chromatography
    - High pH Anion Exchange Chromatography
    - Normal Phase Liquid Chromatography
    - Hydrophobic Interaction Chromatography
    - Size Exclusion Liquid Chromatography

Instructor Bio:

David Carr has been involved in High-Performance Liquid Chromatography for more than thirty-five years. He has worked with the biotechnology industry for many years in the characterization and analysis of protein therapeutics. He is the author of the popular booklet *"The Handbook of Analysis and Purification of Proteins and Peptides by Reversed-Phase HPLC*" and is very experienced with the uses of chromatography, electrophoresis and mass spectrometry for the analysis of proteins and peptides. For the past ten years he is the principal instructor for Bioanalytical Technologies (www.bioanalyticaltech.com), teaching a class on the Analysis and Characterization of Protein Therapeutic Drugs. He has taught this class to scientists from most of the major biotechnology firms such as Amgen, Genentech, Biogen Idec and Genzyme as well as members of the staff of a great many smaller biotech companies.

Short Course 3, Sunday, July 29 @ 9:00am-4:00pm

LC-MS and LC-MS/MS of Small Molecules

Instructor: Perry Wang, LC-MS Technical Expert

This course offers practical training for intermediate-level scientists and focus on LC-MS/MS method development. It will take the participants step-by-step through the concepts and techniques to develop LC-MS/MS methods. The emphasis is on practical issues associated with developing LC-MS/MS methods for small molecules. It also emphasizes problem-solving skills with examples encountered in the pharmaceutical industry and other fields. This course will provide the participants with an updated overview and a solid working knowledge of LC-MS/MS. The participants will learn useful theoretical concepts, instrumental fundamentals and operating principles. After this course, the participants will be able to independently develop their own LC-MS/MS methods. New technologies and techniques, such as monolithic chromatography and hydrophilic interaction liquid chromatography (HILIC) will be presented. Since some of the participants are from the pharmaceutical industry, which is regulated by GLP and GMP, some regulation and validation concepts will be introduced.

Instructor Bio:

Perry G. Wang is a research chemist in the Office of Regulatory Science, CFSAN, the U.S. Food and Drug Administration (U.S. FDA). His expertise focuses on analytical method development and validation for drugs and constituents of foods, dietary supplements and cosmetic products using GC-MS/MS and LC-MS/MS. His interests include GLP regulations and high-throughput bioanalytical method development and validation for the pharmaceutical industry by LC-MS/MS. He received his B.S. degree in chemistry from Shandong University. He earned his M.S. and Ph.D. degrees from Oregon State University.

In addition to over twenty peer-reviewed scientific papers, Dr. Wang has recently published five monographs:

High Throughput Analysis for Food Safety (ISBN-10: 1118396308), John Wiley & Sons Inc., 2014.
Counterfeit Medicines (ISBN: 978-1-906799-08-3) ILM Publications, 2012.
Hydrophilic Interaction Liquid Chromatography and Advanced Applications (ISBN: 978-1-4398-0753-8) CRC Press, 2011.
Monolithic Chromatography and Its Modern Applications (ISBN: 978-1-906799-03- 8) ILM Publications, 2010;.
High-Throughput Analysis in the Pharmaceutical Industry (ISBN: 978-1-4200-5953- 3) CRC Press, 2008.

Short Course 4, Sunday, July 29 @ 9:00am-12:00pm

HPLC/UHPLC Method Development

Instructor: Michael Dong, MWD Consulting

This half-day workshop provides an overview of the tradition strategy for the development of stability-indicating HPLC method, augmented by simpler 3-pronged template approach and the use of a modern universal generic gradient method(s) for multiple new chemical entities. The use of a QbD/DoE software platform for the development of more robust methods for complex samples will be described.

Instructor Bio:

Michael W. Dong is a principal consultant in MWD Consulting focusing on consulting and training services on HPLC/UHPLC method development, pharmaceutical analysis and drug quality. He was formerly Senior Scientist at Genentech, Research Fellow at Purdue Pharma, and Senior Staff Scientist at Applied Biosystems / Perkin-Elmer. He holds a Ph.D. in Analytical Chemistry from the City University of New York, and has 100+ publications including a bestselling book on chromatography. He is an editorial advisory board member of *LCGC* magazine and *American Pharmaceutical*.

Short Course 5, Sunday, July 29 @ 9:00am-12:00pm

Contributions of LC and LC/MS to Characterize Protein Glycosylation

Instructor: Ron Orlando, University of Georgia, Athens, GA and GlycoScientific, LLC

Glycosylation is one of the most common post-translational protein modifications in eukaryotic systems, with estimates that 60-90% of all mammalian proteins are glycosylated at some point during their existence and virtually all membrane and secreted proteins are glycosylated. For many years, observations of abnormal glycosylation in virtually all types of human cancers have identified the potential of using glycan markers in either a diagnostic or a prognostic manner. The glycosylation on recombinant protein therapeutics is also known to have significant effects on pharmacokinetics, impact on pathways of immune stimulation, and to have direct effects on glycoprotein structure and biophysical properties. Hence, quantification of glycoprotein glycans plays important roles from the discovery of new diagnostic/prognostic markers to the development of therapeutic agents, to basic understanding of cellular physiological controls. High resolution separations methods are central to the analysis of glycoproteins and their glycans. This workshop will focus on biochemical and analytical approaches to define glycan structures, and to measure the quantities of such glycans. Emphasis will be placed on the use of liquid phase methods, particularly HPLC methods, which have emerged as popular tools for analysis of glycoproteins, at the levels of intact glycoproteins, proteolytic fragments (glycopeptides), and released glycans. The Lecturer will share examples from the literature and from his personal experience on the use of the variety of methods required to address glycan changes on both native and recombinant glycoproteins.

Instructor Bio:

Ron Orlando received his B.S. in natural science in 1983 from St. Mary's College of Maryland, his Ph.D. in chemistry in 1988 from the University of Delaware, and served as a post-doctoral fellow at the University of Maryland Baltimore County from 1988 until 1990. After serving for two years as a senior scientist at the Suntory Institute of BioOrganic Research (Osaka, Japan), he joined the faculty of the Complex Carbohydrate Research Center at the University of Georgia in January 1993, where he is currently a Professor of Biochemistry & Molecular Biology and Chemistry. Ron Orlando has over 30 years of experience with mass spectrometry, 26 of these years focused on the identification, characterization, and quantitation of proteins and their post-translational modifications. He has co-authored over 140 publications in peer reviewed journals, has given over 100 invited lectures at various conferences, and has served on over 40 NIH review panels. Dr. Orlando is the current Editor-in-Chief for the *Journal of Biomolecular Techniques* (JBT), and serves on a number of editorial boards. He has organized and taught short-courses on the Analysis of Glycoproteins by Mass Spectrometry at meetings of the American Society for Mass Spectrometry (ASMS), the Association of Biomolecular Resource Facilities (ABRF) and the International Symposium and Exhibit on the Separation of Proteins, Peptides & Polynucleotides (ISPPP), and is the founder and past-chair of the ABRF Glycoprotein Research Group. Dr. Orlando is also a serial entrepreneur having founded 3 spin-off/start-up companies: BioInquire in 2007, GlycoScientific in 2009 and Photochem Technologies in 2012. He was given the award of "Entrepreneur of the Year" from the Georgia BioBusiness Center, and was named a leader of tomorrow by Spectroscopy magazine.

Short Course 6, Sunday, July 29 @ 9:00am-12:00pm

Introduction to Capillary Liquid Chromatography

Instructors: Justin Godinho, Advanced Materials Technology, Inc.; James Grinias, Rowan University

This course is designed to introduce those familiar with analytical scale HPLC to capillary (or "nano") liquid chromatography. Although both techniques are based on the same fundamental principles, capillary LC has a number of distinct advantages and challenges that will be detailed. Commercial instrument options, as well as the basics of preparing your own capillary LC columns, will be described. Because one of the most prominent uses of capillary LC is its coupling to mass spectrometry for complex biological sample analysis, special attention will be given to this important area. Both academic and industrial researchers will be able to apply the information gained through this course to overcome the challenges faced when using this essential technique.

Course Highlights:
After completing this course, participants will be able to:

- Understand the differences between analytical and capillary scale LC
- Describe the fundamentals of capillary LC column preparation
- Determine the best detection modes for a given application
- Explain the advantages of coupling capillary LC with mass spectrometry and how to approach method development using capillary LC-MS
- Identify best practices for the use of capillary LC to solve analytical challenges

Instructor Bios:

James Grinias is Assistant Professor in the Department of Chemistry & Biochemistry at Rowan University in Glassboro, NJ. Dr. Grinias has a number of research interests focused on chemical separations and microfluidics, both at the fundamental level and for the analysis of biological systems. He was previously granted fellowships from the National Science Foundation for his graduate work at the University of North Carolina at Chapel Hill (where he was also a member of the Royster Society of Fellows) and from the National Institutes of Health for his postdoctoral research at the University of Michigan. Dr. Grinias' graduate research on liquid chromatography also led to him being named a Csaba Horváth Young Scientist award winner at the 2013 HPLC Conference, a top award for young researchers in the field. At Rowan, he teaches courses in general, analytical, and bioanalytical chemistry while also conducting research on UHPLC column performance and instrument miniaturization.

Justin Godinho is a research scientist at Advanced Materials Technology, Inc. in Wilmington, Delaware. Dr. Godinho's research interests largely focus on the fundamentals of chromatographic separations in capillary ultrahigh pressure liquid chromatography columns. His research has explored methods of capillary column packing, column characterization and column implementation. He has been involved in collaborations studying the microstructure of the packed bed and how it relates to column performance. During his postdoctoral research at the University of North Carolina at Chapel Hill he studied electrophoretic separations in microfluidic devices. These devices were coupled with mass spectrometry for analyte detection. Currently, Dr. Godinho is researching chromatographic materials and methods at Advanced Materials Technology.

Short Course 7, Sunday, July 29 @ 1:00pm-4:00pm

HPLC Operation, Maintenance and Troubleshooting

Instructor: Michael Dong, MWD Consulting

This half-day workshop provides the attendees with an overview of the best practices (standard operating procedures) in HPLC/UHPLC operation including mobile phase and sample preparation for pharmaceutical analysis. Common HPLC and UHPLC maintenance procedures are described together with HPLC troubleshooting strategies illustrated with practical case studies.

Instructor Bio:

Michael W. Dong is a principal consultant in MWD Consulting focusing on consulting and training services on HPLC/UHPLC method development, pharmaceutical analysis and drug quality. He was formerly Senior Scientist at Genentech, Research Fellow at Purdue Pharma, and Senior Staff Scientist at Applied Biosystems / Perkin-Elmer. He holds a Ph.D. in Analytical Chemistry from the City University of New York, and has 100+ publications including a bestselling book on chromatography. He is an editorial advisory board member of *LCGC* magazine and *American Pharmaceutical*.

Short Course 8, Sunday, July 29 @ 1:00pm-4:00pm

The Essential Roles of Separation Science in Mass Spectrometry-based Metabolomics for Biomarker Discovery in Clinical Research

Instructor: Philip Britz-McKibbin, McMaster University

This short course is aimed at presenting major technical hurdles associated with biomarker discovery in clinical metabolomics research when using high resolution, accurate mass spectrometry when coupled to high efficiency separation techniques based on chromatography, electrophoresis and ion mobility. The history of biomarkers and their applications in clinical medicine will first be described, including criteria needed to be satisfied for their qualification and translation to improve patient outcomes. Recent advances in metabolomics for the discovery of new biomarkers of clinical significance will next be discussed, including recommended strategies within a study design, data workflow and statistical approaches to reduce false discoveries while incorporating quality control/quality assurance practices. The important roles of orthogonal separation platforms in metabolomics will also be presented, including recent breakthroughs to enhance sample throughput, expand metabolome coverage, and identify unknown compounds of clinical consequence. Biomarkers not only provide important tools for improved decision making in the screening, prognosis or risk assessment of treatable human diseases, but also shed new insights into the mechanisms of disease pathophysiology, as well as individual responses to lifestyle and/or pharmacological interventions. In this context, rigorously validated separation methods coupled to mass spectrometry play important roles in both the discovery and routine analysis of biomarkers in clinical medicine.

Instructor Bio:

Philip Britz-McKibbin is a Professor at the Department of Chemistry and Chemical Biology and Cystic Fibrosis Canada Researcher at McMaster University in Hamilton, Canada. Dr. Britz-McKibbin obtained his B.Sc. in Chemistry (U. Toronto, 1994), and Ph.D. in Analytical Chemistry (UBC, 2000) and a Japan Society for Promotion of Science PDF position in Japan (Himeji Institute of Technology, 2001-2003) prior to starting his academic position at McMaster. His research interests in bio-analytical chemistry, separation science, mass spectrometry and metabolomics include the design of novel analytical strategies to quantify and identify metabolites of clinical significance in biological samples, as well as characterization of their interactions with protein. Philip's laboratory aims to discover new biomarkers that support early detection and treatment of human diseases relevant to population health and preventative medicine with emphasis on inherited metabolic disorders and chronic human diseases.

Short Course 9, Sunday, July 29 @ 1:00pm-4:00pm

Cannabis Analysis

Instructors: Jeffrey Dahl, Vikki Johnson, Craig Young, Shimadzu Scientific Instruments

Cannabis products for medical and recreational use are enjoying an unprecedented surge in popularity. Accurate cannabis analysis is required in order to ensure customers get what they pay for. And since cannabis is often used as medicine, additional analysis is required to protect consumers from potentially harmful microbiological contamination, and chemical residues such as pesticides. This short course will cover the basics of cannabis and the industry, as well as the state-of-the-art testing methods currently available.

Part I. Cannabis and cannabis industry background. The cannabis plant and its use and cultivation will be presented. The plant's medical use, growing techniques, and final product formulation will be briefly introduced. The history and current legal status of cannabis will be summarized. The testing lab industry and the problems which have led to the need for testing will be emphasized.

Part II. Basic testing for potency, moisture, and microbiology. Accurate cannabis potency testing is of the utmost importance for establishing safe and effective dosages, optimizing growing conditions, and preventing fraud in the cannabis industry. High accuracy and high precision methods of cannabinoid determination will be discussed, including HPLC-UV analysis. Since poor moisture control of cannabis often leads to mold and bacteria growth, testing for moisture and microbes are essential, and will be briefly discussed as well.

Part III. Advanced testing. Terpenes are responsible for the unique aroma of each cannabis strain. Both gas and liquid chromatographic techniques are available for terpene analysis, and the basic techniques will be described. Pesticide and chemical residue analysis is one of the more challenging requirements for cannabis labs, and relies on liquid and gas chromatography with mass spectrometry. High speed and robust methods for analysis of these substances will be discussed in detail.

Instructor Bios:

Jeff Dahl is an application scientist with Shimadzu, and specializes in Mass Spectrometry. Jeff received his Ph.D. in Medicinal Chemistry at the University of Illinois at Chicago in 2010. His research, among other things, includes small molecule quantitation, pesticides and other chemical residues, cannabis analysis, and metabolomics using high resolution MS.

Vikki Johnson received her Master's in Chemistry from San Diego State University in 2011 with a focus on small molecule organic synthesis. She has been working with Shimadzu Scientific Instruments for the last five years as a Field Technical Support Specialist covering the vast range of analytical instruments. She has recently moved into the mass spectrometry technical support role focusing on Shimadzu's LCMS and biotech product lines.

Craig S. Young received a Master's Degree in Organometallic Chemistry from the University of Utah Department of Chemistry, where he then held a position as organic chemistry lecturer for ten years. He has spent many years as a field-based LC/LCMS Product Specialist for several companies and now serves as HPLC Product Manager for Shimadzu Scientific Instruments in Columbia, Maryland. He has authored numerous papers and technical notes in the field of analytical HPLC.