# HPLC 2016 Short Courses

Two days of Educational Short Courses will be offered the Sunday and Monday preceding the conference. The courses are taught by leading academic and industrial scientists and cover both fundamentals as well as real-world application examples. HPLC 2016 reserves the right, without notice, to modify the short course material or schedules, as well as to amend the roster of short course presenters. Short course notes will be emailed to each course participant who should download and bring a copy of the short course notes with them to the short course. There will be no printed copies available. **Short course registration is either within a bundled registration fee package or may be purchased separately by conference participants with a paid/purchased conference meeting registration. Space is limited, and details regarding fees are posted at http://hplc2016.org/reg.html**

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|---|---|
| Course 1: | HPLC and UHPLC Troubleshooting: A Performance Qualification Approach |
| Course 2: | LC/MS/MS Strategies for Identification and Quantitation |
| Course 3: | Two-Dimensional Liquid Chromatography: Principals, Instrumentation, Method Development, and Applications |
| Course 4: | The Role of Chromatography in the Analysis and Characterization of Protein Therapeutic Drugs |
| Course 5: | Capillary Electrophoresis Mass Spectrometry CE-MS, An Easy to Operate and Information Rich Technology |
| Course 6: | Troubleshooting HPLC and UHPLC Separations |
| Course 7: | Chiral Separations |
| Course 8: | Measuring Glycosylation of Proteins by HPLC/Mass Spectrometry |
| Course 9: | HPLC/UHPLC Method Development for Small Molecules Made Easy |

## Course #1 - Sunday, June 19, 9:00 AM - 4:00 PM

# HPLC and UHPLC Troubleshooting: A Performance Qualification Approach

### A comprehensive short course in the isolation, correction, and prevention of liquid chromatographic problems.

**Expert Instructor: John Dolan, LC Resources**

**Who should take this course?**
This course is designed for anyone who works with HPLC or UHPLC. No previous experience with HPLC or UHPLC systems is assumed; however, much of the course will appeal to the more experienced worker. This is one of our most popular classes – one that students say is a “must take” for everyone who uses HPLC and UHPLC. Students are encouraged to bring examples of problems they have in the laboratory for discussion in the class.

**What does it cover?**
"HPLC & UHPLC Troubleshooting: A Performance Qualification Approach" is an intensive one-day course that teaches you the ins and outs of solving problems that occur with your LC methods. You will learn how to qualify the performance of your HPLC using specific tests that also can be used for isolating existing problems. More importantly, you'll learn how to prevent many of these problems from happening in the first place. Here's what the course covers:

- The operating principles of each module in an HPLC and UHPLC system
- A review of basic HPLC theory as it applies to troubleshooting and instrument maintenance
- Why performance qualification (PQ) is so important to ensure the reliable operation of your HPLC and UHPLC and improve the quality of your results
- Proven techniques for systematic problem-solving and instrument maintenance
- The most effective, timesaving, money saving approaches to preventing common hardware problems and method failures.

**What will I get from this course?**

- What you learn will demystify your instrument.
- You’ll discover that understanding how each instrument module works will help you to diagnose and correct problems quickly.
- You'll find that all of the perplexing and frustrating problems your experience have simple and logical solutions.
- You'll learn how to prevent most problems.
- You’ll be equipped with testing techniques to help evaluate instrument performance and to isolate problems when they occur.
- You’ll find how tell the difference between equipment and separation problems.
- You’ll learn how to use the appearance of the chromatogram to help diagnose the problem source and how to correct the problem.
- You’ll have access to one of the world’s experts in HPLC troubleshooting to help solve your specific problems.

**Lecture topics**

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| Section 1. | Principles of HPLC & UHPLC Troubleshooting |
| Section 2. | Performance Qualification, Part 1: Pump & Detector |
| Section 3. | PQ, Part 2: On-Line Mixing |
| Section 4. | PQ, Part 3: Chromatographic Checks |
| Section 5. | The Separation: Physical Problems with Columns |
| Section 6. | The Separation: Chemical Problems with Columns |
| Section 7. | Problems with Quantification |

## Course #2 - Sunday, June 19, 9:00 AM - 4:00 PM

# LC/MS/MS Strategies for Identification and Quantitation

**Expert Instructor: Mike Lee, Milestone Development Services**

This course will focus on simple and easy to understand LC/MS and LC/MS/MS approaches for structure identification and quantitation. Practical advantages of LC/MS-based methods and MS/MS techniques are highlighted with straightforward tips for spectral interpretation and method development for quantitative analysis. LC/MS/MS applications for the structure analysis of metabolites, impurities, natural products, and biomolecules are reviewed in logical and stepwise detail. Real world examples will be highlighted with case studies that involve the analysis of Paclitaxel, Buspirone, Sotolol, and Butorphanol. Instrumentation, strategies, and methods will be presented with specific focus on: sample preparation, chromatography, ionization, and mass spectrometry. Emerging trends for high performance quantitation that involve the use ultra high performance liquid chromatography, nanospray, and accurate mass analysis techniques will be discussed.

**Course Outline**

**Lecture 1. Structure Analysis Matrix**
- Definitions
- Tools
- Industry Trends

**Lecture 2. LC/MS Overview**
- Chromatography Considerations
- Method Development
- Ionization
- Mass Analysis

**Lecture 3. Interpretation Roadmap**
- Retention Time
- Molecular Weight
- Molecular Formula
- Substructures

**Lecture 4. Template Strategy**
- C-N and C-O Bonds
- Tabulate Formulas and Fragments
- Create Substructure Template
- Identify Modified Substructures

**Lecture 6. Structure Refinement**
- Increased Structural Detail
- Retention Time and Polarity
- Chemical/Biological Knowledge

**Lecture 7. Structure Databases**
- Substructure Analysis
- Substructure Searches
- MS Assignment & Prediction
- Archiving Data
- Technology Transfer

**Lecture 8. LC/MS/MS Method Development for Quantitation– A Reversed Engineered Solution**
- Sample Preparation
- Analytical Chromoatography
- Ionization
- Carry Over
- Matrix Effects
- Industry Perspectives

**Lecture 9. Case Studies**

- Impurities in Drug Substance - TAXOL®
- Degradant Analysis - Sotalol®
- Degradant in Stability Study - Butorphanol®
- Predictive Models
- Qual/Quan Approaches

**Target Audience**

The course is ideal for either a beginner who is relatively new to the field or an expert/manager who is in need of an updated perspective on current strategies and analytical technologies. The purpose is to share a foundation of knowledge and practical skills leading to the generation of reliable information for research, development, manufacturing, and/or marketing.

The running title for the short course is "LC/MS/MS Strategies for Identification and Quantitation." This one day course will be intended for a beginner who is new to the field or an expert who is in need of an updated perspective on current strategies, new methods, and emerging technologies.

**Course materials will be derived from several books. See below:**

- LC/MS Applications in Drug Development
- Mass Spectrometry in Drug Metabolism and Disposition: Basic Principles and Applications
- Characterization of Impurities and Degradants Using Mass Spectrometry
- Integrated Strategies for Drug Discovery Using Mass Spectrometry
- Mass Spectrometry Handbook

## Course #3 - Monday, June 20, 9:00 AM - 4:00 PM

# Two-dimensional liquid chromatography: Principles, instrumentation, method development, and applications

**Expert Instructors: Dwight R. Stoll, Gustavus Adolphus College; Robert E. Murphy, Kroungold Analytical, Inc.; and Mark R. Schure, Kroungold Analytical, 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 practically 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.

# Course #4 - Monday, June 20, 9:00 AM - 4:00 PM

## The Role of Chromatography in the Analysis and Characterization of Protein Therapeutic Drugs

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

**Course Description**
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
    - Hydrophobic Interaction Chromatography
    - Size Exclusion Liquid Chromatography
    - Chromatography for the analysis of glycans

**Instructor**
David Carr has been involved in High-Performance Liquid Chromatography for more than forty 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.

## Course #5 - Monday, June 20, 9:00 AM - 12:00 PM

# Capillary Electrophoresis Mass Spectrometry, an easy to operate and information rich technology

**Expert Instructor: David Chen, University of British Columbia**

- From fundamental theory to hands-on experience
- From simplest applications, such as routine test of peptides purity to more complex operations, such as capillary isoelectric focusing coupled to mass spectrometry
- From small organic molecules to large glycoproteins
- CE-MS: method development made simple

The short course will be divided into three parts. In the first part, the theme is “where is my analyte”. The principles of CE as well as separation science will be discussed, in conjunction with the practical aspects of CE specifically, to address why it is difficult for some beginners to find where their analytes are in such a simple separation system. Practical ways of avoiding such problems and to ensure a quick start of using the CE technology will be discussed. Part Two will focus the different ways of interfacing CE and MS. The pros and cons of the currently available CE-MS interfaces, and different combinations of the CE and MS systems will be discussed. Depending on the specific application, one may choose the most appropriate CE-MS system. Different CE-MS applications will be presented in Part Three of this short course. Strategies for CE-MS of small molecules, including neutral, acidic, basic, as well as zwitterionic ones, and biological molecules such as proteins and complex glycans, will be discussed.

## Course #6 - Monday, June 20, 1:00 PM - 4:00 PM

# Troubleshooting HPLC and UHPLC Separations

## A half-day course in isolation, correction, and prevention of liquid chromatographic separation problems.

**Expert Instructor: John Dolan, LC Resources**

**Who should take this course?**
This course is designed for anyone who works with HPLC or UHPLC. No previous experience with HPLC or UHPLC systems is assumed; however, much of the course will appeal to the more experienced worker. This is a half-day version of one of our most popular classes – one that students say is a “must take” for everyone who uses HPLC and UHPLC. Students are encouraged to bring examples of problems they have in the laboratory for discussion in the class.

**What does it cover?**
"Troubleshooting HPLC and UHPLC Separations" is a half-day course that teaches you the ins and outs of solving problems that occur with your LC separations. More importantly, you'll learn how to prevent many of these problems from happening in the first place. Here's what the course covers:

- A review of basic HPLC theory as it applies to troubleshooting
- How to determine whether a problem with a chromatogram is most likely due to physical or chemical problems
- Tricks and techniques to both correct existing problems and prevent them in the future
- A chance to pick the brain of one of the world’s leading experts in HPLC troubleshooting to help solve your specific problems

**Lecture topics**

| | |
|---|---|
| Section 1. | Principles of HPLC & UHPLC Troubleshooting |
| Section 2. | The Separation: Physical Problems with Columns |
| Section 3. | The Separation: Chemical Problems with Columns |
| Section 4. | Problems Specific to Gradient Elution |

## Course #7 - Monday, June 20, 1:00 PM - 4:00 PM

# Chiral Separations

**Expert Instructors: Zachary Breitbach and Daniel Armstrong, University of Texas at Arlington**

**Course Description**
The goal of the “chiral separations” short course is to familiarize the participants with the best available options when confronted with the need for chiral separations. The course format allows for the dissemination of a considerable amount of information in an interesting and informal manner. Attendees can expect to gain an in-depth knowledge of chirality, the different means to separate enantiomers, and when / how to apply different state-of-the-art techniques. There will be a question and answer session at the conclusion of the course and participants are encouraged to ask any question related to the course as well as discuss current chiral separations challenges in their laboratory. The material is broadly applicable and useful for researchers in industry (e.g. pharmaceutical, agrochemical, etc…), as well as, academic researchers. Participants should have a basic knowledge of chromatography and experience with routine achiral HPLC.

**Course Highlights**

- Review of chirality and theory behind enantiomeric separations.
- Summary of all HPLC chiral separations techniques.
- New HPLC chiral selectors described.
- Recent developments in chiral column technology discussed.
- Supercritical fluid chromatographic (SFC) enantiomeric separations.

## Course #8 - Monday, June 20, 9:00 AM - 12:00 PM

# Measuring Glycosylation of Proteins by HPLC/Mass Spectrometry

**Expert Instructors:** Barry Boyes, Advanced Materials Technology and Glycoscientific; Ron Orlando, University of Georgia, Athens and Glycoscientific; and Yehia Mechref, Texas Tech University

**Course Description**
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. Transient glycosylation has also emerged as an important cellular physiology control mechanism, and a complex arrangement of mechanisms have identified such events as epigenetic markers in normal cells.

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 protein glycans. This short course will focus on biochemical and analytical approaches to define glycoconjugate structures, and to measure the quantities and heterogeneity of glycans and glycoproteins.

This short course will specifically

- Describe the analysis of glycoproteins at the levels of intact glycoproteins, subunits and proteolytic fragments (domains and glycopeptides), and enzymatically-released glycans.
- Examine workflows for determining N- and O-linked glycan structures on specific proteins and protein mixtures; compare the use of permethylated, terminus labeled, and native glycan analytical strategies
- Explain alternative quantitative approaches to measure quantities and compositions of glycans, using direct and isotopic referenced methods
- Show examples from the literature and from the instructor’s labs on the use of methods which address glycomic and glycoproteomic research problems

## Course #9 - Monday, June 20, 9:00 AM - 12:00 PM

# HPLC/UHPLC Method Development for Small Molecules Made Easy

**Expert Instructor:** Dr. Michael W. Dong, MWD Consulting

**Course Description**

This half-day HPLC/UHPLC method development course will be conducted at an intermediate level. The course reviews best practices, short cuts and tricks-of-the-trade to help the attendees to become more successful in developing HPLC methods (for potency, purity and ICH-compliant stability-indicating assays). The focus is on pharmaceutical analysis using UV detection for small molecule drugs though the approach is useful to other applications or sample types. One particularly simple and exciting idea is the use of a universal generic method(s) that appears to work well for multiple new chemical entities (NCE) for cleaning verification and serves well as a starting point for a stability-indicating method(s).

**Target audience**

This course is intended for analysts, managers, and researchers using HPLC/UHPLC in the pharmaceutical or other laboratories. A fundamental understanding of HPLC is assumed and some practical hands-on HPLC experience is highly recommended.

**Course Agenda**

**1. The Traditional Method Development Approach for stability-indicating assays**

- Defining method types/goals and gathering pertinent sample / analyte information
- Scouting gradient and getting the first chromatogram
- Method fine-tuning and optimization (Solvent strength/type, pH, buffer/additive, F, T, $t_G$)
- Demonstrating method specificity and stability-indicating capability (how to conduct rapid forced degradation studies); Development of orthogonal methods
- Use of software and automated systems to facilitate screening and method optimization
- Case studies for NCEs, complex formulations and drug products with multiple active ingredients

**2. The 3-Pronged Template Approach for Rapid Method Development and case studies**

- Isocratic fast LC methods for potency or performance assessment
- Generic broad-gradient methods for high-throughput screening, in-process testing and purity assays
  (including the use of a universal generic method(s) for multiple NCEs in cleaning verification and other applications)
- Multi-segment gradient methods for ICH compliant stability-indicating assays of complex molecules

**Instructor**

Dr. Michael W. Dong is a principal consultant in MWD Consulting which provides expert consulting and training services in HPLC/UHPLC, pharmaceutical analysis and drug quality. He was formerly Senior Scientist in Analytical Chemistry and Quality Control at Genentech, Research Director at Synomics Pharma, 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 a certificate in Biotechnology from U. C. Santa Cruz. He has 100+ publications including a bestselling book on chromatography (Modern HPLC for Practicing Scientists). He is an editorial advisory board member of LCGC magazine and American Pharmaceutical Review.