Practical LC-MS Method Development and Bioanalytical Method Validation

(Formerly "**Introduction to GLP Regulations and Bioanalytical Method Validation by LC-MS/MS**")

Instructor

LC-MS: Practical Method Development (Course 20) AND Bioanalytical Method Validation (Course 25).

These two 1-day courses are related so we bundled them as a 2-day course. There is a cost savings in purchasing a 2-day course versus two 1-day courses. If you are interested in attending just one of the days, please register for the 1-day course.

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LC-MS Technical Expert

Course Description

Both LC-MS method development and bioanalytical method validation play a crucial role for successfully conducting regulatory studies including, but not limited to, nonclinical, biopharmaceutics, and clinical pharmacology studies. This short course offers practical training for practicing scientists in the analytical field. It focuses on practical LC-MS method development and bioanalytical method validation. It takes participants step-by-step through the concepts and techniques to develop and validate bioanalytical methods. The emphasis is on day-to-day issues associated with LC-MS method development and validation. Participants will learn the essentials of method development and validation. After this course, participants will be able to independently develop and validate their own LC-MS methods. How to apply the validated methods for routine assays and typical case studies will be presented.

Intended Audience

This course will benefit analytical chemists and researcher using LC-MS, lab supervisors, QA/QC managers, regulators, GLP auditors, and CRO consultants who work in GLP-regulated labs and the pharmaceutical industry. This course will also benefit all levels of management as a refresher course for staying current with the LC-MS techniques and GLP regulations.

Detailed Outline

1. Key Concepts

   - Retention time (tR),
   - Retention factor (k')
   - Separation factor (α)
   - Column efficiency (N)
   - Chromatographic resolution (R)
   - pK_a/pK_b of analytes

- van Deemter Equation
- Fundamentals of mass spectrometry
- Atmospheric pressure ionization (API) in mass spectrometry
- Common ionization modes: ESI, APCI and APPI
- Mass analyzers: quadrupole, time of flight, ion trap and orbitrap
- Mass resolution and mass accuracy
- Matrix effects

2. What you need to know to develop a successful LC-MS method

   - What kind of columns should be selected
   - How column physical property affects the resolution
   - How column chemical property affects the resolution
   - How pH affects the separation
   - How to transfer HPLC methods to UHPLC/UPLC methods
   - Which mode should be selected – isocratic or gradient
   - How to select the best solvents for LC-MS
   - How to optimize a gradient profile
   - Separation mechanism: reversed-phase or HILIC or normal-phase
   - Mobile phase selection and organic modifiers
   - How pK_a/pK_b affect separation
   - MS Fundamental – charged species, mass resolution and mass accuracy
   - What kind of ionization should be selected – ESI, APPI or APCI
   - Mass analyzer and their functions: single/triple quadrupole, TOF, ion trap, Q-exactive and hybrid MS-MS
   - How to build up LC-MS and LC-MS/MS methods
   - How to eliminate and compensate for matrix effects of MS

3. Validation guidance/guidelines

   - ICH
   - AOAC
   - IUPAC
   - Eurachem
   - EMA
   - FDA
   - SANCO
   - Nordtest guide for measurement uncertainty

4. What questions do the validated methods answer?

   - Does the method measure the intended analyte?
   - Does anything interfere with the measurement?
   - Is the method specific or selective for the analyte?
   - What variability is associated with these measurements? - What are the accuracy and precision of each method?

- What is the LLOQ? What is the ULOQ?
- How do sample collection, handling, and storage affect the reliability of the data?
- Do the samples need to be frozen during shipping?
- What temperatures are required to store the samples, and for how long can the samples be stored?

5. Bioanalytical method validation

   - How to design an accuracy & precision (A&P) run
   - What are the acceptance criteria for an A&P run?
   - Requirements for accuracy, precision, recovery, selectivity, and specificity
   - How to design a calibration curve - LLOQ, ULOQ, and weighting factor
   - How to prepare quality control (QC) samples
   - Acceptance criteria for standards and QCs
   - How to design selectivity and specificity tests
   - How to design matrix effect and recovery tests
   - How to design dilution effect tests
   - How to design stability tests for autosampler, benchtop, extracted samples, stock solutions, and long-term storage
   - When do I need partial or cross-validation?
   - How to report bioanalytical method validation

6. How to apply the validated methods for routine bioanalysis

   - Recommendations for routine drug analysis
   - Design an analytical run/batch
   - How to arrange samples - by subject or by period?
   - Evaluation of LLOQ, ULOQ, and QCs
   - Criteria for approving or rejecting results
   - Evaluation of unknown study samples
   - Deviations and remedial actions
   - Re-assay and incurred sample reanalysis (ISR)
   - How to select re-assay results
   - How to report bioanalytical data

7. Case studies and GXP discussion

   - Validation bottlenecks and challenges
   - How to prepare for bioanalytical inspections
   - How to measure and minimize matrix effects
   - How to harmonize the various global bioanalytical guidance documents
   - How to deal with urine samples
   - How to improve throughput

Instructor Qualifications and Experience

Dr. Perry G. Wang has been a chemist at US FDA since 2008. Prior to joining FDA, he worked in the pharmaceutical and medical-device industry for more than 10 years. He received his Ph.D. from Oregon State University. In addition to over 30 peer-reviewed publications, he has edited or co-edited five books: "*High-Throughput Analysis in the Pharmaceutical Industry*"; "*Monolithic Chromatography and Its Modern Applications*"; "*Hydrophilic Interaction Liquid Chromatography (HILIC) and Advanced Applications*"; "*Counterfeit Medicines*"; and "*High-Throughput Analysis for Food Safety*". He specializes in LC-MS method development and validation for drugs, cosmetics, foods, and dietary supplements. He has been invited to teach this course at the American Society for Mass Spectrometry (ASMS) since 2015. He has also taught courses on LC-MS method development for the American Chemical Society (ACS), Pittsburgh Conference (PittCon), Eastern Analytical Symposium (EAS) and HPLC for more than 10 years. He teaches these courses in his own capacity as a scientist, but not as an employee of the FDA.