Practical LC-MS/MS Method Development

Instructor

People attending this course might also be interested in attending *LC-MS: Practical Bioanalytical Method Validation by LC-MS/MS*, so we bundled these two one-day courses as a two-day course (there is a cost savings in purchasing one two-day course versus two one-day courses). The bundled two-day course is listed as *LC-MS: Practical Method Development AND Bioanalytical Method Validation* under the two-day course section.

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LC-MS Technical Expert

Course Description

This LC-MS/MS short course is designed to offer practical training for the practicing scientists in the analytical field. This course will provide the participants with an updated overview and a solid working knowledge of LC-MS/MS. 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 LC-MS/MS method development. It also focuses on problem-solving skills with examples encountered in the analytical fields. The participants will learn useful theoretical concepts, practical fundamentals and operating principles. After this course, the participants will be able to independently develop their own LC-MS/MS methods to use in their laboratories. New technologies and techniques, such as hydrophilic interaction liquid chromatography (HILIC), monolithic chromatography, and core-shell particles for HPLC will be presented.

Intended Audience

This course will benefit analytical chemists and researcher, lab supervisors and lab managers using LC-MS/MS. It will benefit the scientists ranging from college graduates to professionals in the analytical field.

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

1. What you need to know to develop a successful LC-MS/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
   - How to eliminate and compensate matrix effects of MS
   - Validation consideration

2. Operating Parameters and Column Selection

   - Flow rate
   - Gradient time
   - Column temperature (T)
   - Packed columns (support type, dimensions, particle size and pore size)
   - Monolithic columns
   - HILIC columns

3. Mass spectrometer (MS)

   - Fundamental – charged species, mass resolution and mass accuracy
   - What kind of ionization should be selected – ESI, APPI or APCI
   - Single and Triple quadropole, TOF, Ion trap and Q-exactive
   - How to develop an MS and MS-MS methods
   - How to perform a qualitative and quantitative analysis

4. Method Development Approaches

   - Finding or estimating pK_a or pK_b of the analytes
   - Defining method type (reversed phase or normal phase or HILIC)
   - Estimating buffer pH
   - Scouting gradient to get the first chromatogram

- Fine-tuning and optimizing the method – solvent type and strength

5. Special Topics

- Monolithic chromatography
- Hydrophilic interaction liquid chromatography
- Core-shell technology

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:

1. *High-Throughput Analysis in the Pharmaceutical Industry*
2. *Monolithic Chromatography and Its Modern Applications*
3. *Hydrophilic Interaction Liquid Chromatography (HILIC) and Advanced Applications*
4. *Counterfeit Medicines*
5. *High-Throughput Analysis for Food Safety*

His expertise focuses on high throughput analysis and validation by LC-MS/MS. He has been invited to teach this course at *Eastern Analytical Symposium* (*EAS*) since 2014, at *HPLC* since 2012, at *American Chemistry Society* (*ACS*) since 2009 and at *PittCon* since 2007. He teaches these courses in his own capacity as a scientist, but not as an employee of the FDA.