Alturas Analytics, Inc.
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The LC/MS Experts™

LC-MS Regulated Bioanalysis for Large and Small Molecules: Methods, Practices, Technologies and Instrumentation

This course gives an overview of the methods, practices, technologies and instrumentation used to perform LC-MS in the regulated bioanalytical laboratory. Along with an introduction of LC-MS fundamentals, the course will give step-wise method development and validation schemes for small and large molecule LC-MS method development. Current validation and development expectations from the latest white papers will be presented for discussion. Sample preparation techniques such precipitation, SPE, SLE and LLE will be discussed. For large molecule LC-MS/MS bioanalysis, a "bottom-up" digestion approach will be presented. Specific examples on how to avoid the pitfalls of ion-suppression will be presented. This course will feature a dynamic format where the latest technology in MFLC, UPLC and the advantages of the latest LC-MS instruments will be highlighted. A portion of the course will be devoted to, "validating LC-MS instruments for "21 CFR Part 11" compliance. At the end of the course, a method development tutorial for particular molecules will be presented to the participants.

Shane Needham, Alturas Analytics, Inc., AM: "History and Application of LC-MS in the Bioanalytical Laboratory" PM Part 1: "MFLC-MS/MS in the Bioanalytical Laboratory" PM Part 2: "Method Development Tutorial"
Jim Shen, BMS, "LC-MS Bioanalysis Method Development and Validation for Small and Large Molecules"
Dave Abramowitz, AB SCIEX, "Validation of LC-MS Instruments for 21 CFR Part 11 Compliance"

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Course Title: LC-MS Regulated Bioanalysis for Large and Small Molecules: Methods, Practices, Technologies and Instrumentation

Lead Instructor(s): Shane Needham, Ph.D. and Jim Shen, Ph.D. BMS
Associate Instructor: Dave Abramowitz, AB SCIEX
Type of Instruction: Short Course
Course Length: One Day
8:30 AM

1. **Introduction-Needham**
   - 1.1. Brief History of Coupling HPLC and Mass Spectrometry
     - 1.1.1. Early Pitfalls of LC/MS/MS without Good HPLC Separations
     - 1.1.2. Why is a separation important with the selectivity of MS?
       - 1.1.2.1. Ionization effects in the source of the MS
       - 1.1.2.2. Separation of labile molecules from parent
       - 1.1.2.3. Separation of isobaric drugs or metabolites
2. **Interfacing HPLC with Atmospheric Pressure Ionization-Needham**
   - 2.1. Electrospray Ionization (ESI)
     - 2.1.1. Optimum Operating Conditions
       - 2.1.1.1. Apparent Concentration Sensitivity
   - 2.2. Atmospheric Pressure Chemical Ionization (APCI)
     - 2.2.1. Optimum Operating Conditions

10-10:15 AM Break

10:15 AM

3. **LC-MS Method Development and Validation for Small Molecules-Shen**
   - 3.1. Small molecule method development concepts and approaches
     - 3.1.1. Extraction approaches
       - 3.1.1.1. Traditional: protein precipitation, liquid/liquid, solid phase
       - 3.1.1.2. Novel: SLE, online SPE, hybrids SPE.
     - 3.1.2. Separation approaches
       - 3.1.2.1. Column phase, mobile phase, modifier selections
       - 3.1.2.2. Using sub 2 micron and fused core HPLC columns in bioanalysis

3.1.3. Instrumentation approaches

3.1.3.1. Triple quads vs. high resolution instrumentations

3.2. Ion suppressions

3.2.1. Theory, background.

3.2.2. Measurement

3.2.3. Remediation

3.3. Method development concepts

3.3.1. Fundamental troubleshooting skills

3.3.2. 2D experiments

12-1:00 PM Lunch

4. <u>LC-MS Method Development and Validation for Large Molecules-Shen</u>

4.1. Large molecule method development and concepts

4.1.1. Basic concepts

4.1.1.1.1.1. Protein therapeutics

4.1.1.1.1.2. Monoclonal antibodies (mAb), chimeric antibodies, humanized antibodies.

4.1.1.1.1.3. Antidrug conjugates (ADC).

4.1.1.1.1.4. Immunogenicity.

4.1.2. Analytical Approaches

4.1.2.1.1.1. Overview: Quantitation of protein therapeutics via signature peptide

4.1.2.1.1.2. Digestions

4.1.2.1.1.3. Clean up

4.1.2.1.1.3.1. Pellet digestion

4.1.2.1.1.3.2. Protein precipitation

4.1.2.1.1.3.3. Excess protein removal

4.1.2.1.1.3.4. Immunocapture

4.1.2.1.1.4. Separation approaches

4.1.2.1.1.4.1. MicroLC

4.1.2.1.1.4.2. 2D HPLC

4.1.2.1.1.5. Detection approaches

4.1.2.1.1.5.1. Triple quads vs. high resolution instrumentations

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4.1.2.1.1.6. Other unconventional and cutting edge approaches.

4.1.3. Examples of successful protein quantitation via LC-MS/MS or LC-MS.

4.2. Method Validations

4.2.1. Modular method development approaches

4.2.2. Management vs. method developer expectations

4.2.3. Method development and validation "gotcha"

4.2.4. Method validation guidance

3-3:15 PM Break

3:15 PM

5. Validation of LC-MS Instruments for 21 CFR Part 11 Compliance-Abramowitz

4:15 PM

6. MFLC-MS/MS in the Bioanalytical Laboratory-Needham

7. Method Development Tutorial in the Bioanalytical Laboratory-Needham

8. Questions and Answers

4:30 PM - Surveys and Course Completion