Introduction to Quantitative Analysis Using LC/MS/MS with Triple Quadrupoles (QqQ)

1-Day Short Course

The course – catered to analysts new to mass spectrometry - will provide an introduction to quantitative analysis using liquid chromatography/ tandem mass spectrometry (LC/MS/MS), specifically the use of multiple reaction monitoring (MRM) on triple quadrupole tandem mass spectrometers (QqQ). The course will review the principal components of LC/MS instruments, will describe the methods of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) and will summarize the operating principles of QqQ's. The principals and advantages of tandem MS approaches will be presented, followed by the steps involved in first setting-up and then analyzing the data acquired using a MRM LC/MS/MS method.

MORNING

9:00 – 10:15 a.m.

LC/MS and LC/MS/MS INTRUMENTS
Overview
Overview of LC/MS Instruments ?
- Why use a Mass Spec Detector for Quantitation?
- Extracted Mass Chromatograms

Components of a Mass Spectrum
- Full-scan Mass Spectra and Types of Ions

Selected Reaction Monitoring/Multiple Reaction Monitoring
- What is Selectivity?
- Signal/Noise Ratio (S/N)
- Why use MS/MS for Quantitation

A Closer Look at "Mass"
- Isotopes of the Elements
- Why are Isotopes Important in Quantitation
- Calculating Molecular Mass
- Units of Mass
- Mass Defect

Overview of Sample Preparation

10:15 – 10:30 a.m. COFFEE BREAK

10:30 a.m. – 12:00 p.m.

LC/MS Instruments
Vacuum System
Sample Inlet
LC/MS Interface Region
- Nebulization, Desolvation, Analyte Ion Enrichment

Ion Sources
- Atmospheric Pressure Ionization (API)
  - Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI)

Ion Optics
Mass Analyzers
Ion Detector (Multipliers)

A Closer Look at API Sources
Electrospray Ionization (ESI)
- How are Ions Formed?
- Types of Ions Former

Ionization Efficiency
ESI-Active vs. ESI-Inactive Analytes
Analyte Ion Suppression/Matrix Effects
Tuning ESI Source Parameters

12:00 – 1:00 p.m. LUNCH

AFTERNOON

1:00 – 2:30 p.m.

Atmospheric Pressure Chemical Ionization (APCI)
How are Ions Formed?
Types of Ions Formed
Ionization Efficiency
Tuning APCI Source Parameters

Choosing Between ESI and APCI
API Probe Arrangements

A Closer Look at Quadrupole Mass Analyzers (Q's)
Operating Principles
Mass Resolution
Why is Mass Resolution Important in Quantitation
Selected Ion Monitoring (SIM)

Tandem Mass Spectrometry (MS/MS) with Triple Quadrupoles (QqQ)
QqQ Instrument Configuration
Scan Modes
Precursor (Parent) and Product (Daughter) Ions
Product (Daughter) Ion Scans
Selected Reaction Monitoring (SRM), also called Multiple Reaction Monitoring (MRM)

Rf-Only Collision Cells (q)
Collision-Induced Dissociation (CID)

2:30 – 2:45 a.m. COFFEE BREAK

2:45 – 4:00 p.m.

SETTING UP AN LC/API/SRM METHOD ON a QqQ
Measurement of Signals
Peak Height vs. Peak Area

Setting Up an MRM Method on a QqQ
LC Considerations When a MS Is Used as a Detector
Optimizing API/MS/MS with MRM
Tuning the API Source
SRM/MRM Scan Parameters
Precursor/Product Ion Pairs
Mass Resolution of Q1and Q3
Collision Conditions
Multiplier Gain
Scan Time

Integrating MRM Data
Peak Detection and Integration Algorithms
Smoothing
Asymmetric Peaks
Manual Integration

APPENDIX – Time Permitting

Internal Standards (IS)
Types

Matrix Effects
How to address

Validation Figures of Merit
Accuracy, Precision, Linearity, Sensitivity and Dynamic Range
Limits of Detection (LOD) and Quantitation (LOQ)

LC Factors Affecting Chromatographic Peak Separation (R)
Retention, Selectivity, Column Plate Number, Sample Size
Optimizing Reverse Phase (RP)/LC
Columns
Mobile Phase
Gradients
Additives (Buffers, Ion Pairing Agents)

Trouble-Shooting TIC Peaks
Loss of Chromatographic Resolution
Change in Retention Times
Decrease in S/N
Baseline Drift
Erratic Baselines
Asymmetric Peaks

REFERENCES

R.K. Boyd, C. Basic, R.A. Bethem, **Trace Quantitative Analysis By Mass Spectrometry**, John Wiley and Sons, New York, New York, April 2008.

M.W. Duncan, P.J. Gale, A.L. Yergy, The **Principles of Quantitative Mass Spectrometry**, Rockpool Productions, Denver, Colorado, 2006.

P.R. Loconto, **Trace Environmental Quantitative Analysis: Principles, Techniques and Applications,** CRC Taylor & Francis, Boca Raton, Florida 2006.

W.M.A. Niessen, **Liquid Chromatography-Mass Spectrometry**, 2nd ed., Marcel Dekker, New York, 1999.

L.R. Snyder, J.J. Kirkland and J.L. Glajch, **Practical HPLC Method Development**, 2nd ed., John Wiley and Sons, 1997.

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THE INSTRUCTOR

Dr. Cecilia Basic received her Ph.D. in analytical mass spectrometry with Dr. Richard A. Yost at the University of Florida and her M.Sc. degree in mass spectrometry with Dr. Alex G. Harrison at the University of Toronto. She has over 15 years of experience in mass spectrometry fundamentals and applications and has authored numerous research articles, book chapters and is co-author of "Trace Quantitative Analysis by Mass Spectrometry", John Wiley, 2008.

Dr. Basic has been conducting training courses on the fundamentals and applications of mass spectrometry for 10 years with over 300 scientists in the pharmaceutical, agriculture and biotech industries attending her courses. Clients have included: Bristol-Myers Squibb, Merck, Amgen, Quest Diagnostics, FMC Corporation, Johnson and Johnson, DuPont, Sigma Aldrich, Abbott Laboratories, Thermo Fisher Scientific, and Waters Corp.

She is a past member of the American Society for Mass Spectrometry's (ASMS) Education Committee and is was co-instructor of the ASMS annual Short-Course "Quantitative Mass Spectrometry" for 6 years. In addition, she is a past instructor/moderator of Lehigh University's satellite Distance Education Course "Mass Spectrometry in the Pharmaceutical Industry" and a past professor at Villanova University. Dr. Basic has worked as a Visiting Scientist at the Beckman Research Institute at the City of Hope and as a scientist at NASA's Jet Propulsion Lab.