Introduction to High Resolution Mass Spectrometry for Qualitative and Quantitative Analysis: A Summary

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Fundamental Concepts for HRMS

- Mass Terminology: nominal, average, accurate, exact, monoisotopic
- Mass Separation: resolution, resolving power
- Mass Measurement: centroid, profile, peak top, millimass units vs. parts per million
- Mass Defect: the story in the decimal places
- Isotopes
Instrument Types and Concepts

Major operating principles and challenges in
• Time of Flight MS: delayed extraction, reflection, orthogonal acceleration
• Fourier Transform Ion Cyclotron Resonance (FTICR) MS: Magnet, cell types, pressure/vacuum requirements, ultrahigh resolution
• Orbitrap MS: ion storage and injection techniques, MS/MS options, scan speed vs. resolution
• Quadrupole and Quadrupole-based ion traps: resolution capability, impact on hybrid MS/MS systems
MS/MS

• Major options
  – Resolution in each mass analyzer
  – Scan speed compatibility (full scan and MS/MS switching, parallel processing options)

• Fragmentation options: CAD, ETD, etc.
Qualitative Analysis

• HRMS based options for metabolite/ degradant/ unknown structural elucidation
• Interpretation of elemental composition
  - Mass Defect Filter
  - Nitrogen Rule
  - Ring double bond
• Interpretation of MS/MS data-Narrowing the site of metabolic modification
• Utility of Hydrogen-Deuterium Exchange for structural elucidation
Quantitative Analysis

• Key variables in HRMS quantitation:
  – Operating resolution during acquisition
  – Selection of m/z for quantitation: peak summing; multiply-charged analytes; resolved isotopic envelopes
  – Data processing peak widths
    • Processing of centroid vs. profile data

• Data-mining for metabolites, biomarkers and other non-targeted, non-anticipated analytes
  – Next day, next year
Qual-Quan Workflows

• Instrument requirements and options
  – Strengths and weaknesses
• Major decisions prior to data acquisition
  – Role, limitations of MS and MS/MS in alternating scans
• Example workflows and data sets.
• Application to small molecules and peptides
• On-the-fly vs post acquisition options for data mining