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, reflectron, 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
• HRMS options for Drug-to-Antibody (DAR) ratio determination
• HRMS for biomarker discovery
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 and non-anticipated components

• ADC Payload Quantitation
Qual-Quant 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.
• Applications to small molecules, peptides, ADC payloads, and biomarkers
• On-the-fly MS/MS vs post-acquisition data mining