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
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
• 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-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.

• Applications to small molecules, peptides, ADC payloads, and biomarkers

• On-the-fly MS/MS vs post-acquisition data mining