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-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