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

Matt Blatnik, Cong Wei and Graham West

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: HCD, CID, CAD, ETD, etc

Qualitative Analysis

- HRMS based options for metabolite/ degradant/ unknown identification and structural elucidation
  - Utility of MS-based methods for protein/polymer structural elucidation
  - Utility of MS- and MS/MS-based methods for unknown structural elucidation
- Interpretation of elemental composition
  - Mass Defect Filter
  - Nitrogen Rule
  - Ring Double Bond
  - Isotopes
- Data mining for metabolites and other non-targeted and non-anticipated components
- HRMS options for ADC Drug-to-Antibody (DAR) ratio determination

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
  - Dynamic range and isotopes

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, proteins and antibody-drug conjugates (ADC)
- On-the-fly MS/MS vs post-acquisition data mining
- Protein quantitation, label-free and labeling (e.g. isobaric tags & SILAC)