LECTURE 6

High Resolution Accurate Mass Spectrometry (HRAMS)

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HRMS Contents

• Fundamentals
• Mass Defects
• Isotopic patterns
• Mass axis calibration
• Types of HRMS systems
• Qual/Quan Analysis
• Data mining processes
• Future directions
Definition of Mass Resolution

On Tof’s and quads, the peak width is measured at half height. On sector instruments, the peak width is often measured at the 5% point.

FWHM = 0.310 Da

Half Height Width

Peak Center = 633.2 Da

5% Width

Mass Resolution is 633.2 / 0.310 = 2040

‘m’ is the center of the peak and Δm is the peak width.
Background Definitions

- Quadrupoles are common and do not provide high mass resolution
  - Peak width at half hight usually about 0.5-0.7 Da
  - This provides ‘unit mass resolution which provides nominal mass accuracy; e.g. m/z 301 vs. m/z 302

- HRMS provides high selectivity with accurate mass determination
  - Measures small mass differences; often 0.001 Da

- HRMS provides refined mass resolution
  - Beneficial when coupled with accurate mass measurement
  - High accuracy when coupled with careful operation and calibration of mass scale.
Information provided by HRMS?

• Extracted ion current of accurate masses provides unparalleled selectivity
  – Extract an exact mass from the full-scan HRMS acquisition

• Qual/Quan
  – Qualitative analysis: what is this chemical?
    • Provides reliable elemental compositions
  – Quantitative analysis: How much of this chemical is present?
    • When/how does HRMS replace triple quad data?
Definition of Terms

• Mass defect
• Isotopes
  – Isotope ratios
• Monoisotopic, exact, ppm, mDa., etc.
• Resolving power vs. resolution?
• Calibration
  – Internal, external, lock mass...
• Mass measurement
  – Centroiding vs. mass peak apex
Mass Units

• Unified atomic mass unit: “u”
  – 1/12th of mass of carbon $^{12}\text{C}$.
  – This “u” is also known as a dalton (Da) or thomson (Th)
    • Notice in each case the name is lower case and the abbreviation is capitalized

• Previously the definition was based upon oxygen
  – E.g. 1/16th of the mass of $^{16}\text{O}$

• The use of the term ‘amu’ is no longer accepted
  – Some manufacturers still incorrectly use this term for the mass axis scale.
Mass Terminology

• Elemental Composition:
  – Molecular formula showing the exact number of atoms of each element in the molecule.

• Nominal Molecular Weight: 291
  – Sum of nominal masses of the elements

• Average Molecular Weight: 291.3654 (Do not use this value for mass spectrometry)
  – Calculated using the average atomic weight of each element present in a compound

• Exact Mass: 291.0925
  – Exact molecular mass of the structure, where atomic masses of each atom are based on the most common isotope for the element.
<table>
<thead>
<tr>
<th>Element</th>
<th>Symbol</th>
<th>Exact Mass*</th>
<th>Abundance</th>
<th>$X + 1$ Factor†</th>
<th>$X + 2$ Factor†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen</td>
<td>H</td>
<td>1.007825</td>
<td>99.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[D or $^2$H]</td>
<td>2.014102</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>$^{12}$C</td>
<td>12.000000</td>
<td>98.9</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>$^{13}$C</td>
<td>13.003354</td>
<td>1.1</td>
<td>$1.1n_c$</td>
<td>$0.0060n_c^2$</td>
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<tr>
<td>Nitrogen</td>
<td>$^{14}$N</td>
<td>14.003074</td>
<td>99.6</td>
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<tr>
<td></td>
<td>$^{15}$N</td>
<td>15.000108</td>
<td>0.4</td>
<td>$0.37n_N$</td>
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<tr>
<td>Oxygen</td>
<td>$^{16}$O</td>
<td>15.994915</td>
<td>99.76</td>
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<td></td>
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<tr>
<td></td>
<td>$^{17}$O</td>
<td>16.999133</td>
<td>0.04</td>
<td>$0.04n_O$</td>
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<td></td>
<td>$^{18}$O</td>
<td>17.999160</td>
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<td>$0.20n_O$</td>
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<tr>
<td>Fluorine</td>
<td>F</td>
<td>18.998405</td>
<td>100</td>
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<tr>
<td>Silicon</td>
<td>$^{28}$Si</td>
<td>28.976927</td>
<td>92.2</td>
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<td></td>
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<tr>
<td></td>
<td>$^{29}$Si</td>
<td>28.976913</td>
<td>4.7</td>
<td>$5.1n_Si$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{30}$Si</td>
<td>29.973861</td>
<td>3.1</td>
<td>$3.4n_{Si}$</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>P</td>
<td>30.973763</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfur</td>
<td>$^{32}$S</td>
<td>35.97704</td>
<td>95.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$^{33}$S</td>
<td>35.97453</td>
<td>0.76</td>
<td>$0.8n_S$</td>
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</tr>
<tr>
<td></td>
<td>$^{34}$S</td>
<td>33.967665</td>
<td>4.22</td>
<td>$4.4n_S$</td>
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<tr>
<td>Chlorine</td>
<td>$^{35}$Cl</td>
<td>35.453855</td>
<td>75.77</td>
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<tr>
<td></td>
<td>$^{37}$Cl</td>
<td>36.965736</td>
<td>24.23</td>
<td>$32.5n_{Cl}$</td>
<td></td>
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<tr>
<td>Iodine</td>
<td>I</td>
<td>12.540352</td>
<td>100</td>
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</tr>
</tbody>
</table>


†Assume $X = 100\%$; $X$ represents the relative intensity of the first peak in a cluster of isotope peaks.

The factor is multiplied by the number (n) of atoms present to determine the magnitude of the abundance contribution for a given isotope. For example, the contribution at $X = 1$ for $^{14}$N for an ion containing three nitrogens would be $0.37 \times 3 = 1.11$ relative to 100 at $X$.

\[ C_{15}H_{17}NO_3S \]

- Atomic weight of Carbon = 12
- Atomic weight of Carbon = 13
- Atomic weight of Hydrogen = 1
- Atomic weight of Deuterium = 2
- Atomic weight of Nitrogen = 14
- Atomic weight of Oxygen = 16
- Atomic weight of Sulfur = 32

\[ C_{15} = 180 \]
\[ H_{17} = 17 \]
\[ N_{1} = 14 \]
\[ O_{3} = 48 \]
\[ S_{1} = 32 \]
\[ MW = 291 \]

But is this the correct mass?
### Nominal vs. Exact Masses for MW Determination

<table>
<thead>
<tr>
<th>Nominal Masses</th>
<th>Exact Masses</th>
<th>‘Actual’ Masses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atomic weight of Hydrogen = 1</strong></td>
<td>H17 = 17</td>
<td>Atomic weight of Hydrogen = 1.0078</td>
</tr>
<tr>
<td><strong>Atomic weight of Deuterium = 2</strong></td>
<td></td>
<td>Atomic weight of Deuterium = 2.0141</td>
</tr>
<tr>
<td><strong>Atomic weight of Carbon = 12</strong></td>
<td>C15 = 180</td>
<td>Atomic weight of Carbon = 12.0000</td>
</tr>
<tr>
<td><strong>Atomic weight of Carbon = 13</strong></td>
<td></td>
<td>Atomic weight of Carbon = 13.0033</td>
</tr>
<tr>
<td><strong>Atomic weight of Nitrogen = 14</strong></td>
<td>N 1 = 14</td>
<td>Atomic weight of Nitrogen = 14.0030</td>
</tr>
<tr>
<td><strong>Atomic weight of Oxygen = 16</strong></td>
<td>O 3 = 48</td>
<td>Atomic weight of Oxygen = 15.9949</td>
</tr>
<tr>
<td><strong>Atomic weight of Sulfur = 32</strong></td>
<td>S 1 = 32</td>
<td>Atomic weight of Sulfur = 31.9720</td>
</tr>
<tr>
<td></td>
<td>MW = 291 (Nominal)</td>
<td>Exact MW = 291.0925</td>
</tr>
</tbody>
</table>

- **Exact Mass:** 291.0925  
  \( C_{15}H_{17}NO_3S \)  
  - Exact molecular mass of the structure, where atomic masses of each atom are based on the most common isotope for the element.
Definitions of Small Mass Differences

• PPM: Parts-per-million
  – A relative measure analogous to percent
    • Measured mass – Theoretical mass \( \times 10^6 = \text{ppm} \)

• Millimass units (mmu or mDa)
  – An absolute measure
  – 1 mmu = 0.001 u
## Relationship Between ppm and mmu

<table>
<thead>
<tr>
<th>m/z</th>
<th>ppm</th>
<th>u</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>2</td>
<td>0.0004</td>
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<tr>
<td></td>
<td>5</td>
<td>0.001</td>
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<tr>
<td></td>
<td>10</td>
<td>0.002</td>
</tr>
<tr>
<td>600</td>
<td>2</td>
<td>0.001</td>
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<tr>
<td></td>
<td>5</td>
<td>0.003</td>
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<tr>
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<td>10</td>
<td>0.006</td>
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<tr>
<td>1000</td>
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<td>0.002</td>
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<td></td>
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<td>0.005</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.010</td>
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</table>
Using Accepted Mass Terminology

• Resolving power vs. Mass resolution
  – **Mass Resolving Power** is defined as m/dm
    • Where m designates the mass and dm the peak width necessary for separation at mass m. A specific m/z value and also the method like 10% valley or 50% valley or full width at half maximum (FWHM) must be given.
    • **Mass Resolution** usually refers to the ability of separating two narrow mass spectral peaks

• Refer to good reference books
  – IUPAC Gold Book
  – ASMS definition of terms
  – MS terms Wiki
  – Mass Spec Desk Reference by O. David Sparkman
Representative Examples of Resolving Power

<table>
<thead>
<tr>
<th>Type</th>
<th>Resolving Power (FWHM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT-ICR-MS</td>
<td>1,000,000</td>
</tr>
<tr>
<td>FT-Orbitrap</td>
<td>100,000</td>
</tr>
<tr>
<td>High-Res-TOF</td>
<td>60,000</td>
</tr>
<tr>
<td>TOF</td>
<td>10,000</td>
</tr>
<tr>
<td>Quadrupole / IonTrap in UltraZoom mode</td>
<td>10,000</td>
</tr>
<tr>
<td>Quadrupole / Iontrap</td>
<td>1,000</td>
</tr>
</tbody>
</table>
Mass Defect

• $^{12}\text{C}$ is the only isotope with an integer exact mass: 12.00000.
  – Used to set mass scale for all other elements, isotopes

• All others have a “Mass Defect”.
  – Difference between actual exact mass and integer mass
Mass Defect Details

- **Positive mass defect**: Hydrogen 1.0078
  - gain almost 0.008 Da per hydrogen
  - typically, many hydrogen present: 40 hydrogen in molecule yield positive mass defect of about +0.3 Da.

- **Negative mass defect**: Cl, Br, S, O
  - lose 0.031 Da per chlorine
  - Factor in HRMS of environmental analytes
Mass Defect: Positive and Negative

• Difference between the exact mass and the integer mass of the nuclide (the element’s atoms)
  – Mass defect of an ion is the sum of all the mass defects within that ion’s nuclides (atoms).

• Carbon-12 is the reference element and has no mass defect: C=12.0000
  – Masses of the other nuclides all have a mass defect relative to carbon
  – **Positive mass defect**: atomic weight greater than nominal mass
    • H, N
  – **Negative mass defect**: atomic weight less than nominal mass
    • F, Cl, Br, I, O, Si, P, Na, K

![Pos Mass Defect](image1)

**C_{12}H_{18}NO_{3}**
Exact Mass: 224.1287

![Neg Mass Defect](image2)

**C_{12}H_{9}Cl_{5}**
Exact Mass: 351.9147

**DDT**
Mass Defect Isotopes
Impact of Mass Defect

• The magnitude of the mass defect depends upon elements present and their abundance
  – Mass defect is an indication of:
    • Low mass defect may indicate halogens (look for their unique isotopic pattern) or rings plus double bonds
      – Unsaturated, few hydrogens
    • High mass defect may indicate many hydrogens (highly saturated) or very little unsaturation or rings present.

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Nominal</th>
<th>Accurate</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl Stearate</td>
<td>C₁₉H₃₈O₂</td>
<td>298</td>
<td>298.2872</td>
<td>298.5114</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>C₃₇₈H₆₃₀N₁₀₅O₁₁₈S</td>
<td>8556</td>
<td>8560.6254</td>
<td>8565.873</td>
</tr>
</tbody>
</table>
Using Negative Mass Defects to Locate Chemical Alterations

Figure 1: Base peak chromatograms of 10mM Irinotecan rat hepatocyte incubation: (a) Original; (b) After single Mass Defect Filter; (c) After Multiple Mass Defect Filters (MMDF).
Mass Defect Filtering

Mass Defect in Metabolism

- [Chemical Structure 1] 308.1637 Da
- [Chemical Structure 2] 312.1141 Da
- [Chemical Structure 3] 326.1298 Da
- [Chemical Structure 4] 340.1091 Da
- [Chemical Structure 5] 342.1247 Da
Types of Metabolites May be Distinguished

Mass Defect Distribution – Clozapine Metabolites

Real-Time Multiple Mass Defect Triggered IDA (Information-Dependent Acquisition)

- Looks at TOF MS scan to identify ions matching the specified mass defect ranges
- Gives priority to matching ions over other ions, even if they are more intense
- Two modes
  - Non-exclusive mode: Priority given to matching ions. Other ions are still triggered if no matching ions take priority
  - Exclusive mode: Only matching ions are triggered. Other ions ignored even if there are no matching ions
- Significantly improves MS/MS triggering of metabolites in difficult matrices e.g. bile and protein precipitated plasma
- Marginal advantage for high concentrations in clean matrix e.g. 10 μM incubation in microsomes
- Best results obtained when MDF and DBS combined