ME 330.804: Mass Spectrometry in an “Omics” World

Lecture 1
MON 22 OCT, 2012
R Cotter
Introduction to mass spectrometry

ME 330.804: Mass Spectrometry in an “Omics” World
Beginning October 22, 2012, Mon & Wed 10-11 AM, WBSB Abel Library

Oct 22
Introduction to mass spectrometry. Components of a mass spectrometer, mass spectrum; nominal, exact and average mass; mass resolution and accuracy, fragmentation, MALDI and electrospray

Oct 24
Mass analysis and MS/MS. Time-of-flight, quads, traps and Orbitraps, tandem mass spectrometry, product ion and selected ion scans

Oct 29
Introduction to proteomics. Bottom-up versus top-down methods, proteases, peptide fragmentation and sequence information, post-translational modifications; de novo sequencing

Oct 31
Basic sample preparation. DOs and DON'Ts of MALDI and ESI sample preparation; matrix selection and purification, buffer selection and concentration; various methods for sample clean up; tissue preparation for drugs, lipids and protein imaging

Nov 5
Basic chromatography. Basic principles of reverse phase and ion exchange high pressure liquid chromatography. How to choose the appropriate buffers, ion pairing agents and organic modifiers. Principles of capillary electrophoresis

Nov 7
Global and discovery proteomics. Sample preparation for on-line HPLC/MS; instrumentation, data-dependent acquisition, bioinformatics tools (Mascot, Sequest, Protein Center, etc.)

Nov 12
Advanced Separations methods. HPLC vs UPLC, nanoflow vs ESI, HILIC; when to use each method; laser capture microdissection

Nov 14
Quantitative proteomics. iTRAQ, SILAC, O-18 labelling, label-free methods (spectral counting)

Nov 19
EXAM

Nov 26
Selected reaction monitoring, instrumentation (triple quads, Q-trap), SRM, using Skyline to optimize transitions and read quantitative data

Nov 28
Glycoproteomics. Mass spectrometry of carbohydrates, methods for isolating the glycoproteome

Dec 3
Lipidomics. Including quantitative (MRM) methods, mass spectral libraries for lipids, and considerations for the quantitative analysis of clinical samples

Dec 5
Metabolomics. Metabolomics mass spectral libraries, quantitation, isotope labeling

Dec 10
Clinical studies and basic biostatistics. Coefficient of variance (cv), T-test and ANOVA

Dec 12
Advanced bioinformatics. Prosign, Masspectras, trans-proteome pipeline (TPP), combining omics data

Dec 17
Imaging and cell sorter mass spectrometry. Imaging microprobes vs microscopes; tissue preparation; CyTOF mass spectrometry

Dec 19
EXAM (Presentations)

The mass spectrometer is a balance for weighing molecules

Neutral molecules are converted to charged particles, i.e. ions
Ions are separated according to mass by electric and/or magnetic fields, or time
Ions impact a detector surface or are sensed by induced currents

Sample inlet system or interface to GC or LC
Vacuum pumps
Vacuum chamber

Ion sources

Gas phase ionization

*Electron impact* (EI) is used for volatile compounds and GCMS analysis
*Chemical ionization* (CI) is a softer ionization method for increasing molecular ion production

Desorption methods

*Matrix-assisted laser desorption* (MALDI) and *Electrospray ionization* (ESI) are used for the analysis of proteins, peptides, carbohydrates and other biological macromolecules
**Most commonly used mass analyzers**

**Mass analyzers (MS):**
- Time-of-flight (TOF)
- Quadrupole (Q)
- Quadrupole ion trap (GCQ or LCQ)
- Linear quadrupole (LTQ)
- Fourier transform mass spectrometer (FTMS)
- Orbitrap

**Tandem and hybrid mass spectrometers (MS/MS):**
- Triple Quadrupole (QqQ)
- Quadrupole/time-of-flight (QTOF and QSTAR)
- Tandem time-of-flight (TOF-TOF or TOF²)
- LTQ/orbitrap

**Vacuum: the “mean free path”**

<table>
<thead>
<tr>
<th></th>
<th>Oxygen</th>
<th>Actinomycin</th>
<th>Chymotrypsin</th>
<th>Aspartate transcarbamoylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter¹</td>
<td>3.61 Å</td>
<td>20 Å (σ=11.8)</td>
<td>40 Å (σ=21.8)</td>
<td>70 Å (σ=36.8)</td>
</tr>
<tr>
<td>10⁻³ torr</td>
<td>5.3 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁴ torr</td>
<td>53 cm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁵ torr</td>
<td>5.3 meters</td>
<td>0.49 meters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁶ torr</td>
<td>53 meters</td>
<td>4.9 meters</td>
<td>1.4 meters</td>
<td>0.51 meters</td>
</tr>
<tr>
<td>10⁻⁷ torr</td>
<td>530 meters</td>
<td>49 meters</td>
<td>14 meters</td>
<td>5.1 meters</td>
</tr>
</tbody>
</table>

TOF mass analyzer: 10⁻⁷ torr
Orbitrap mass analyzer: 10⁻⁷ torr
Quadrupole ion trap: 10⁻³ torr (1 millitorr)
Electron impact ionization source: 10⁻⁴ torr
Chemical ionization source: 1-4 torr
Electrospray ion source: 1 atmosphere
Mass is an intrinsic property of a molecule

The molecular weight is determined from the mass of a molecular ion that appears in the spectrum, and is directly related to the elemental composition of the compound.

Fragment ions are produced whose masses are directly related to structure.

It is therefore often possible to identify or determine the structure of a molecule without the need for a reference spectrum.

For small molecules we refer to this as an interpretive approach.

For peptides we refer to this as de novo sequencing.

Old fashion mass spectrometry: electron ionization

Positive ion formation:

\[
e^{-} + AB \rightarrow \left[AB^{+}\right] + 2e^{-} \quad \text{ionization}
\]

\[
\left[AB^{+}\right] \rightarrow A + B^{+} \quad \text{dissociation}
\]

[\left[AB^{+}\right] \text{ is an odd electron ion carrying excess internal energy}]

Molecules have different ionization potentials (IP), but their ionization efficiencies are relatively flat around 70 ev.
**Old fashion mass spectrometry: electron ionization**

**Negative ion formation:**
- negative EI is not as useful, since electron capture is resonant at different energies for each compound
- useful for electrophores, compounds used as labels that are ionized at different energies

![Diagram of electron ionization](image)

**Ionization usually occurs at “heteroatoms”**

**Heteroatoms** are atoms that contain non-bonding electrons.

**Ionization order:** S>N>O

In **electron impact (EI)** ionization, ions are formed by removal of a non-bonding electron to form a **radical ion**:

\[
\begin{align*}
\text{e} & + \text{H}_3\text{C}^\cdots\text{C}^\cdots\text{O}^\cdots\text{CH}_2\text{CH}_3 & \rightarrow & \text{H}_3\text{C}^\cdots\text{C}^\cdots\text{O}^\cdots\text{CH}_2\text{CH}_3 + 2\text{e} \\
\end{align*}
\]

Can calculate: \(4\text{C} + 2\text{O} + 8\text{H} = 48 + 32 + 8 = 88\)

Molecular ions observed with a mass/charge: \(m/z = 88\)
**Ionization directs the fragmentation**

Rearrangement of electrons leads to a stable, even-electron fragment ion:

\[
\begin{align*}
\text{H}_3\text{C} & \text{C} \text{O} \xrightarrow{\text{H}_3\text{C} \text{C} \equiv \text{O}} \text{H}_3\text{C} \equiv \text{O}^- + \cdot\text{OCH}_2\text{CH}_3 \\
\text{m/z} & = 43
\end{align*}
\]

followed by loss of a small neutral molecule:

\[
\begin{align*}
\text{H}_3\text{C} \equiv \text{O}^- & \xrightarrow{-\text{CO}} \text{CH}_3^- \\
\text{m/z} & = 15
\end{align*}
\]

Note that all these masses can be calculated from the structure.

---

**Isotopes contribute to the mass spectrum**

<table>
<thead>
<tr>
<th>Element</th>
<th>Nominal mass</th>
<th>Exact mass</th>
<th>Abundance</th>
<th>Average mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>12, 13</td>
<td>12.00000, 13.00335</td>
<td>98.95%, 1.1%</td>
<td>12.01115</td>
</tr>
<tr>
<td>H</td>
<td>1, 2 (D)</td>
<td>1.00783, 2.0140</td>
<td>99.98%, 0.025%</td>
<td>1.008665</td>
</tr>
<tr>
<td>O</td>
<td>16, 18</td>
<td>15.99491, 17.9992</td>
<td>99.8%, 0.2%</td>
<td>15.994</td>
</tr>
<tr>
<td>N</td>
<td>14, 15</td>
<td>14.00307, 15.00011</td>
<td>99.63%, 0.37%</td>
<td>14.0067</td>
</tr>
<tr>
<td>S</td>
<td>32, 33, 34</td>
<td>31.97207, 32.97146, 33.96786</td>
<td>95.0%, 0.76%, 4.22%</td>
<td>32.064</td>
</tr>
</tbody>
</table>
How this might all look in a mass spectrum

\[
\begin{align*}
&\text{H}_3\text{C} = \text{C} \rightarrow \text{O} \rightarrow \text{CH}_2\text{CH}_3 \\
m/z &= 88
\end{align*}
\]

\[
\begin{align*}
&\text{H}_3\text{C} \rightarrow \text{C} \rightarrow \text{O} \\
m/z &= 43
\end{align*}
\]

\[
\begin{align*}
&\text{H}_3\text{C} \rightarrow \text{C} \rightarrow \text{O} \\
m/z &= 15
\end{align*}
\]

Note: mass is an intrinsic property; relative abundance is not!

The “monoisotopic peak” and some definitions

**Ethyl acetate**

Empirical formula: \( \text{C}_4\text{H}_8\text{O}_2 \)

Nominal mass = 88, where C=12, H=1 and O=16 isotopes

\[
\begin{align*}
\text{now:} & \quad 4 \text{ C} \times 12.00000 = 48.00000 \\
& \quad 8 \text{ H} \times 1.00783 = 8.06264 \\
& \quad 2 \text{ O} \times 15.99491 = 31.98982 \\
\text{Monoisotopic mass} &= 88.05246 \\
\text{Exact mass} &= 88.05246 \\
\text{Mass defect} &= 0.05 \text{ (or 0.05246)}
\end{align*}
\]
**The molecular ion distribution**

**Monoisotopic peak (100%)**

- $4\ C^{12}$: 4 x 12.0000 = 48.0000
- $8\ H^1$: 8 x 1.0078 = 8.0624
- $2\ O^{16}$: 2 x 15.9949 = 31.9898
- **Monoisotopic mass**: 88.0522

**The second peak (4.56%)**

- $3\ C^{12}$: 3 x 12.0000 = 36.0000
- $1\ C^{13}$: 1 x 13.0033 = 13.0033
- $8\ H^1$: 8 x 1.0078 = 8.0624
- $2\ O^{16}$: 2 x 15.9949 = 31.9898
- (1.1% x 4 = 4.4%)

- $4\ C^{12}$: 4 x 12.0000 = 48.0000
- $7\ H^1$: 7 x 1.0078 = 7.0546
- $1\ H^2$: 1 x 2.0140 = 2.0140
- $2\ O^{16}$: 2 x 15.9949 = 31.9898
- (0.25 X 8 = 0.16%)

**Mass resolution**

Mass resolution is the ability to distinguish two adjacent peaks in a mass spectrum.

$$R = \frac{m}{\Delta m}$$

where

- $\Delta m$ = peak width (FWHM definition)
- $\Delta m$ = mass difference between two peaks (valley definition)

**What mass resolution is required to separate m/z 88 and 89?**

$$m/\Delta m = 88/1 = 88$$

**What mass resolution is required to separate the peaks at m/z 89?**

$$m/\Delta m = 89/0.0029 > 30,000$$
How fragmentation distinguishes structures

![Diagram of molecular structures and fragmentation reactions]

Homework problems

1. Predict the molecular and fragment ion structures and the masses that would be observed in the electron impact (EI) mass spectra of:
   - amine: CH₃CH₂NHCH₂CH₃
   - ketone: CH₃CH₂(CO)CH₂CH₃
   - ether: CH₃CH₂O-CH₂CH₃

2. What resolution is required to distinguish CO⁺ and N₂⁺?

3. The mass spectrum of methane has a molecular ion CH₄⁺ at m/z 16, with an isotopic peak at m/z 17. What resolution is needed to distinguish the second peak from that of the monoisotopic peak of an ammonium ion NH₃⁺ contaminant?
**Making ions softly: chemical ionization**

**Chemical ionization reactions of methane**

\[
e^{-} + CH_{4} \rightarrow CH_{4}^{**} + 2e^{-} \quad \text{electron ionization (IP = 9.95 ev)}
\]

\[
CH_{4}^{**} + CH_{4} \rightarrow CH_{5}^{+} + \cdot CH_{3} \quad \text{in 1–4 torr methane (AP = 14.4 ev)}
\]

\[
CH_{5}^{+} + M \rightarrow CH_{4} + MH^{+} \quad \text{protonated molecular ion}
\]

\[
\Delta H_{\text{reaction}} = PA (CH_{4}) - PA (M)
\]

- electrons are not removed; protons are transferred
- protonation reaction is *mildly* exothermic (\(\Delta H_{\text{reaction}}\) is negative)
- ionization is “soft”; i.e. there is little fragmentation

**Reagent gases and their proton affinities**

<table>
<thead>
<tr>
<th>Gas</th>
<th>Proton Affinity (Kcal/mole)</th>
<th>Softness</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH(_3)</td>
<td>207</td>
<td>softest</td>
</tr>
<tr>
<td>isobutene</td>
<td>195</td>
<td></td>
</tr>
<tr>
<td>H(_2)O</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td>CH(_4)</td>
<td>126</td>
<td></td>
</tr>
<tr>
<td>H(_2)</td>
<td>101</td>
<td>hardest</td>
</tr>
</tbody>
</table>

A large reagent gas proton affinity means that the reagent ion does not easily transfer its proton to the sample; ionization is soft.

\[
e^{-} + NH_{3} \rightarrow NH_{3}^{**} + 2e^{-} \quad \text{electron ionization}
\]

\[
NH_{3}^{**} + NH_{3} \rightarrow NH_{4}^{+} + \cdot NH_{2} \quad \text{generation of reagent ion}
\]

\[
NH_{4}^{+} + M \rightarrow NH_{3} + MH^{+} \quad \text{protonated molecular ion}
\]

\[
\Delta H_{\text{reaction}} = PA (NH_{3}) - PA (M)
\]
Comparison of EI and CI and the importance of closed shell ions

Electron impact: m/z 73 (radical ion) m/z 58 (even electron ion)

Chemical ionization: m/z 74 (even electron ion) m/z 58 (even electron ion)

Proton affinities of target molecules

<table>
<thead>
<tr>
<th>Molecule Type</th>
<th>Formula</th>
<th>Proton Affinity (Kcal/mole)</th>
<th>Electron Affinity (ev)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertiary amines</td>
<td>(CH₃)₃N</td>
<td>225</td>
<td>9.8</td>
</tr>
<tr>
<td>Secondary amines</td>
<td>(CH₃)₂NH</td>
<td>220</td>
<td>9.5</td>
</tr>
<tr>
<td>Primary amine</td>
<td>CH₃NH₂</td>
<td>214</td>
<td>9.3</td>
</tr>
<tr>
<td>Amide</td>
<td>CH₃CONH₂</td>
<td>210</td>
<td>9.1</td>
</tr>
<tr>
<td>Ammonia</td>
<td>NH₃</td>
<td>207</td>
<td>9.0</td>
</tr>
<tr>
<td>Acids</td>
<td>CH₃COOH</td>
<td>196</td>
<td>8.5</td>
</tr>
<tr>
<td>Alcohols</td>
<td>CH₃OH</td>
<td>189</td>
<td>8.2</td>
</tr>
</tbody>
</table>

A higher proton affinity means a more likely site for protonation, but may also result in more fragmentation.

Carboxylic acids

\[
\text{R-COH} + \text{H}^+ \rightarrow \text{R-COH} \rightarrow \text{R-CO}^+ \text{OH}^{-}
\]
Protons attach to non-bonding electrons

Esters

\[ R_1-C-OR_2 + H^+ \rightarrow R_1-C-OR_2 \]

Ethers and sugars

\[ R_1-O-R_2 + H^+ \rightarrow R_1-O-R_2 \]

An amino acid will protonate at the amine nitrogen:

\[ R-NHH + H^+ \rightarrow R-NH^+ \]

Acetamide: a model for an amide (peptide) bond

Acetamide may have two protonated forms:

\[ H_3C-C-NH_2 + H^+ \rightarrow H_3C-C-NH_3 \]

\[ H_3C-C-NH_2 + H^+ \rightarrow H_3C-C-NH_2 \]

Order of protonation:

Basic side chain (Arg, Lys) > amide > (amine) N > O
Losses of small neutral molecules may involve rearrangements

- **loss of side chain**

\[
\begin{align*}
\text{H}_3\text{N} & \text{H} \quad \text{C} \quad \text{CO} \quad \text{H} \\
\text{C}_4\text{H}_9 & \quad \text{- C}_4\text{H}_{10}
\end{align*}
\]

\[\text{m/z 132} \quad \text{y-ion}\]

\[
\begin{align*}
\text{H}_2\text{N} & \text{H} \quad \text{C} \quad \text{CO} \quad \text{H} \\
\text{H} & \quad \text{- C}_4\text{H}_9
\end{align*}
\]

\[\text{m/z 74} \quad \text{v-ion}\]

- **loss of HCOOH**

\[
\begin{align*}
\text{H}_2\text{N} & \text{C} \quad \text{H}_9 \\
\text{C} & \quad \text{O} \\
\text{H} & \quad \text{OH}
\end{align*}
\]

\[\text{m/z 86} \quad \text{a-ion}\]

Protonation of a dipeptide results in cleavage of the amide bond. The charge may be retained on the N-terminal amino acid:

- **heterolytic cleavage**

\[
\begin{align*}
\text{H}_2\text{N} & \text{C} \quad \text{C} \quad \text{N} \quad \text{H} \quad \text{C} \quad \text{CO} \quad \text{H} \\
\text{CH}_3 & \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3
\end{align*}
\]

\[\text{carbonium ion}\]

\[
\begin{align*}
\text{H}_2\text{N} & \text{C} \quad \text{C} \quad \text{N} \quad \text{H} \quad \text{C} \quad \text{CO} \quad \text{H} \\
\text{CH}_3 & \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3
\end{align*}
\]

\[\text{b-ion}\]

\[
\begin{align*}
\text{H}_2\text{N} & \text{C} \quad \text{C} \quad \text{O} \quad \text{H} \\
\text{CH}_3 & \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3
\end{align*}
\]

\[\text{acylium ion}\]

\[
\begin{align*}
\text{H}_2\text{N} & \text{H} \quad \text{C} \quad \text{N} \quad \text{H} \\
\text{CH}_3 & \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3
\end{align*}
\]

\[\text{leaving group is a stable neutral amine}\]

\[
\begin{align*}
\text{H}_2\text{N} & \text{C} \quad \text{C} \quad \text{N} \quad \text{H} \quad \text{C} \quad \text{CO} \quad \text{H} \\
\text{CH}_3 & \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3 \quad \text{CH}_3
\end{align*}
\]

\[\text{a-ion}\]
Alternatively, the charge may be retained on the C-terminal amino acid:

The z-ion might be formed more directly by a simple cleavage of the N-C bond:

Can we do negative ion CI?

**Chlorine ion attachment:**
\[ [R+Cl^-] + M \rightarrow R + [M+Cl^-] \]

**Thermal electrons:**
\[ e^- (70ev) + CH_4 \rightarrow CH_4^{**} + 2e^- \text{ (thermal, 0.1 ev)} \]
\[ CH_4^{**} + CH_4 \rightarrow CH_5^* + \cdot CH_3 \]
\[ CH_5^* + M \rightarrow CH_4 + MH^+ \]
\[ e^- \text{ (thermal)} + M \rightarrow M^- \]

**PPNICI**

pulsed positive negative ion chemical ionization
generally carried out on a quadrupole mass spectrometer
How are EI and CI used today?

The most common configuration is combined gas chromatography and mass spectrometry, or GCMS

http://en.wikipedia.org/wiki/Gas_chromatography-mass_spectrometry

MARS ORGANIC MOLECULE ANALYZER: PERFORMANCE OF A MINIATURE MASS SPECTROMETER FOR IN SITU DETECTION OF MARTIAN ORGANICS.

V. T. Pinnick¹, F. H. W. van Amerom², R. M. Danell³, A. Buch⁴, M. Atanassova¹, R. Arevalo¹, L. Hovmand¹, W. B. Brinckerhoff¹, P. R. Mahaffy¹, R. J. Cotter⁵, and the MOMA Team¹-⁸

(1) NASA Goddard Space Flight Center, USA (veronica.t.pinnick@nasa.gov), (2) SRI International, USA, (3) Danell Consulting, Inc., USA, (4) LPGM, Ecole Centrale Paris, Châtenay-Malabry, France, (5) Johns Hopkins University, School of Medicine, USA, (6) Max Planck Institut für Sonnensystemforschung, Germany, (7) LISA, LATMOS, Guyancourt, France, (8) Univ. Paris-Est, Créteil, France.
Matrix-assisted laser desorption/ionization

1. Sample (A) is mixed with excess matrix (M) and dried on a MALDI plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules are ionized by proton transfer from matrix:
   $$\text{MH}^+ + A \rightarrow M + AH^+$$. 

1. The matrix absorbs UV or IR energy from the pulsed laser
2. The matrix ionizes and dissociates; it undergoes a phase change to a supercompressed gas; charge is passed to some of the analyte molecules
3. Matrix expands at supersonic velocity; additional analyte ions are formed in the gas phase; ions are entrained in the expanding plume

Matrices

- nicotinic acid (NA)
- caffeic acid (CA)
- sinapinic acid (SA)
- gentisic acid (DHBA)
- 3-hydroxyxpycolinic acid (HPA)
- α-cyano-4-hydroxycinnamic acid (CHCA)

http://www.hopkinsmedicine.org/mams/
Matrix-assisted laser desorption (MALDI) mass spectrum on a time-of flight (TOF) MS

Glucagon-like peptide
R=13,370

Angiotensin II
R=1,990

Mass resolution for macromolecular ions

glucagon  \( \text{C}_{153}\text{H}_{225}\text{N}_{42}\text{O}_{50}\text{S} \)  nominal mass = 3,481
monoisotopic mass: 3,482.61
average mass: 3,484.75

What does the isotopic distribution look like?

first peak:  \( ^{12}\text{C}_{153}^{1}\text{H}_{225}^{14}\text{N}_{42}^{16}\text{O}_{50}^{32}\text{S} \)  100%

second peak:  \( ^{12}\text{C} \rightarrow ^{13}\text{C} \) 153 x 1.1% 170%
\( \text{H} \rightarrow \text{D} \) 225 x 0.02% 4.5%
\( ^{14}\text{N} \rightarrow ^{15}\text{N} \) 42 x 0.37% 15.5%
190%

third peak: six isoforms (multiplicity) since \(^{18}\text{O}\) and \(^{34}\text{S}\) are possible
Mass resolution for macromolecular ions

Resolution = 1/3,000  Resolution = 1/500

Monoisotopic mass: 3,482.61  Average mass: 3,484.75

Very large molecular ions may never be resolved

How much resolution do you need?

Theoretical peak shapes for a protein with an elemental composition of
C_{900}H_{1323}O_{294}N_{247}S_{6}
with a molecular weight = 20,488.5

When does high mass resolution matter?

Even 1 million resolution will not improve this
Answer: when one wants to distinguish peptides with the same nominal mass

Mass resolution to resolve these two monoisotopic ions if they appear in the same spectrum:

\[
\begin{align*}
\text{KSAPSTGGVK(CH}_3\text{)}_3\text{KPHR} & \quad (1491.8760) \\
\text{KSAPSTGGVK(Ac)KPHR} & \quad (1491.8396)
\end{align*}
\]

\[
\frac{m}{\Delta m} = 1492/0.0364 = 41,000
\]

**Mass accuracy**

Mass accuracy is a measure of how close we are to the actual mass

If the peptide KSAPSTGGVK(CH₃)₃KPHR agrees within 0.01 mass units, then

\[
\text{mass accuracy} = \frac{0.01}{1492} \approx 7 \text{ ppm}
\]

---

**High mass resolution: acetylated histone H3 K₇⁹ observed using an LC-LTQ-Orbitrap**

\[\text{R} = 30,600 \quad \text{R} = 33,770\]

**High mass accuracy: acetylated histone H3 K79 for the double mutant**

<table>
<thead>
<tr>
<th>ΔDot1 ΔSir2</th>
<th>Structure</th>
<th>Calc MW</th>
<th>Calc m/z</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>EIAQDFKAcTDLR</td>
<td>1376.6936</td>
<td>459.9057</td>
<td>1.1ppm</td>
<td></td>
</tr>
<tr>
<td>EIAQDFKMe3TDLR</td>
<td>1376.7300</td>
<td>459.9178</td>
<td>17.0 ppm</td>
<td></td>
</tr>
</tbody>
</table>

**Electrospray Ionization (ESI)**

ESI produces an abundance of multiply-charged molecular ions. M.wt. = ~16951 Da

M.E. 330.804 MS2012
Mass Spectrometry in an "Omics" World
http://www.hopkinsmedicine.org/mams/
What is the mechanism?

1. Initial droplet with excess positive charge
2. Evaporation
3. Rayleigh limit
4. Ion ejection; favors high charge state
5. Charged droplet continues to evaporate until it again reaches the Rayleigh limit
6. Rayleigh limit
7. Continued ion ejection

A distribution of multiply-charged ions

Homework problem: use any two peaks to determine the molecular mass of myoglobin. Try another set to see how accurate this is.

Note that m/z values are generally centered at m/z 1,000

How can I use the masses of multiply-charged ions to determine the molecular weight?

Calculation using any two peaks and simultaneous equations:

Every peak has the formula: \((M+nH)^n\)

Solve the equations: 
\[
\begin{align*}
\frac{M + n}{n} &= m_n & \text{any peak} \\
\frac{M + n + 1}{n+1} &= m_{n+1} & \text{the peak just below it}
\end{align*}
\]

Deconvolution:

There are any number of commercial programs or instrument manufacturer software that convert any multiply-charge ion mass spectrum into a singly charge spectral readout. One example is ProMass Deconvolution 2.5 from Thermo. Because these programs use multiple peaks (not just two) they can achieve very accurate mass measurements, but they can be fooled by complex mixtures.

Why do electrospray mass spectra show such high charge state?

THERMOSPAY:
charge state distribution of zwitterionic peptides when there is no electric field

ELECTROSPRAY:
+ve electric field produces highly charged droplets that shift ions to higher positive charge states

QUADRUPOLE ION TRAPS have a low mass/charge range, thereby favoring higher charge states*

ION EJECTION process favors the ejection of the highest charge states

* as long as they are above the low mass cutoff
What kind of mass spectrometers are used with electrospray?

**Quadrupoles** were the first. Their limited mass range could accommodate high charge states, and they work best with continuous sources.

**Quadrupole ion traps** were next, but required an RF-only quadrupole ion guide to transport the ions from atmospheric pressure into the trapping field. ESI is an atmospheric technique.

Other quadrupole-based instruments, such as **linear ion traps** (LTQ) and **quadrupole /TOF** instruments.

**Time-of-flight** mass spectrometers required ion guides, ion storage (since TOF is a pulsed technique) and orthogonal extraction.

**Fourier transform mass spectrometers** and **Orbitraps**, with an LTQ or quadrupole as MS1.

---

Resolution and accuracy of mass analyzers

<table>
<thead>
<tr>
<th>Mass Analyzer</th>
<th>Mass Resolution</th>
<th>Mass Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time-of-flight (TOF)</td>
<td>10,000 to 20,000</td>
<td>10 – 100 PPM</td>
</tr>
<tr>
<td>Quadrupole (Q)</td>
<td>2,000</td>
<td>100 - 1,000 PPM</td>
</tr>
<tr>
<td>Quadrupole ion trap (GCQ or LCQ)</td>
<td>20,000</td>
<td>100 – 1,000 PPM</td>
</tr>
<tr>
<td>Linear quadrupole trap (LTQ)</td>
<td>20,000</td>
<td>100 – 1,000 PPM</td>
</tr>
<tr>
<td>Fourier transform mass spectrometer (FTMS)</td>
<td>100,000 to 1M</td>
<td>0.1 – 1 PPM</td>
</tr>
<tr>
<td>Orbitrap</td>
<td>30,000 to 60,000</td>
<td>0.1 – 1 PPM</td>
</tr>
</tbody>
</table>
How accurate should the mass measurement be?

Number of peptides (positive and false positive) identified as a function of mass accuracy

FURTHER READING


