Isotope Peaks

The peaks from the isotopes.

The intensity ratios (relative intensities) in the isotope patterns arises from the natural abundance of the isotopes, thus are valuable to ascertain the atomic composition of ions.

M+1 peaks are primarily due the presence of $^{13}C$ in the sample.

M+2 peaks useful for N, O calculation (< seven C cpds)

M+2, M+4, .. indicative of presence of Br, Cl, S;

$(^{79}Br : ^{81}Br \approx 1:1, ^{35}Cl : ^{37}Cl \approx 3:1)$

Isotope peak abundance depends on the molecular constitution. Example, halogens Cl, Br.

Calculating isotope peak abundances (%) to confirm fragment and parent peaks.

<table>
<thead>
<tr>
<th>Element</th>
<th>Name</th>
<th>Mass</th>
<th>Abundance %</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>Carbon</td>
<td>12</td>
<td>0.012n</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen</td>
<td>1</td>
<td>1.08n</td>
</tr>
<tr>
<td>O</td>
<td>Oxygen</td>
<td>16</td>
<td>0.369n</td>
</tr>
<tr>
<td>Si</td>
<td>Silicon</td>
<td>32</td>
<td>0.801n</td>
</tr>
<tr>
<td>S</td>
<td>Sulfur</td>
<td>34</td>
<td>0.038n</td>
</tr>
<tr>
<td>Cl</td>
<td>Chlorine</td>
<td>35</td>
<td>5.08n</td>
</tr>
<tr>
<td>Br</td>
<td>Bromine</td>
<td>80</td>
<td>4.52n</td>
</tr>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X&gt;M</td>
</tr>
</tbody>
</table>

Parent/Molecular Peak M: an ion that has not lost/gained atoms (odd electron fragment, U=integer; reverse not true).

Parent Peak leads to molecular formula.
Molecular formula leads to the structural (partial) features of the possible molecular structure.

Unsaturation can be calculated from the molecular formula of the parent ion.

$$U = 2R + 2DB + 2TB = c - h / 2 + n / 2 + 1$$

$c = \#C & \#Si$

$h = \#H & \#halogens$

$n = \#N, \#P, \#As.$

Nominal mass, M = 100%

%M+1 = 0.012n$_H$+1.08n$_H$+0.369n$_O$+0.038n$_S$+5.08n$_S$+0.801n$_S$

%M+2 = 0.005n$_C$$_{(n-1)}$+0.205n$_C$+3.35n$_O$+4.52n$_S$+32.0n$_O$+97.3n$_S$
\[ U = 1 + \frac{1}{2} \sum_{i=1}^{i_{\text{max}}} N_i(V_i - 2) \]

where \( i_{\text{max}} \) is the total number of different elements in the compound, \( N_i \) the number of atoms of element \( i \), and \( V_i \) is the valence of atom \( i \).

If \( U \) is not a whole number – then it’s not a molecular ion. It’s an even electron ion.

Any reasonable molecular ion formula should lead to a whole number \( U \); OE ion.

3. If needed - find the formula with \( n_O \) atoms of O for the same \( M \);

New formula = Base formula + \( n_O \) O – \( n_O \) C – 4\( n_O \) H
which changes \( U \) to \( U+n_O \);

4. If needed - find the formula with \( n_N \) atoms of N for the same \( M \),

New formula = Base formula + \( n_N \) N – \( n_N \) C – 2\( n_N \) H
and recalculate \( U \); Fractional \( U \)’s – unlikely formula.

\( U < 0 \) is an impossible combination of atoms and indicates likely presence of O and N.

**Rule of Thirteen:**

Used to generate the possible formula for a given nominal molecular mass.

1. Generate a base formula consisting of C and H, not necessarily the actual formula;

\[ \frac{M}{13} = n + \frac{r}{13} \quad \text{C}_n \text{H}_{r} \]

2. Calculate the index of deficiency, \( U \), for the base formula.

\[ U = \frac{n - r + 2}{2} \]

**Useful Links**

- [http://www.colby.edu/chemistry/NMR/NMR.html](http://www.colby.edu/chemistry/NMR/NMR.html)
- [http://www.colby.edu/chemistry/PChem/Fragment.html](http://www.colby.edu/chemistry/PChem/Fragment.html)
- [http://www.chem.uni-potsdam.de/tools/index.html](http://www.chem.uni-potsdam.de/tools/index.html)
- [http://www.chemcalc.org/](http://www.chemcalc.org/)

**Example 1**

<table>
<thead>
<tr>
<th>m/z</th>
<th>Relative abundance (normalized)</th>
</tr>
</thead>
<tbody>
<tr>
<td>64</td>
<td>100.0</td>
</tr>
<tr>
<td>65</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>66</td>
<td>5.0±0.5</td>
</tr>
</tbody>
</table>

**Nitrogen Rule:**

Many peaks can be ruled out as impossible simply on the grounds of reasonable structure requirements.

Molecule of even nominal mass must contain zero or even number of \( N \) atoms. An odd numbered nominal mass requires an odd number of \( N \).

\((M+1)/M\) ratio is particularly useful to estimate the \#C in the species.

\[ \#C \leq \frac{\%M + 1 \text{ peak}}{\%M \text{ peak}} \times 100 \]

**Example 1**

only peaks in the MS

<table>
<thead>
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</thead>
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</tr>
<tr>
<td>66</td>
<td>5.0±0.5</td>
</tr>
</tbody>
</table>

[http://www.colby.edu/chemistry/PChem/Fragment.html](http://www.colby.edu/chemistry/PChem/Fragment.html)
%M+1 = 0.7 – 1.1    one C and no Si
%M+2 = 4.5 – 5.5    no Cl, Br

64 - 12 = 52; mass of 52 cannot come solely from H

Probably one S (from M+2), if so no C present; M+1 √
Tentatively; one S present.

64 – 32 = 32; two O

SO₂

Nominal mass, M = 100%  
%M+1 = 0.012n₁ + 1.08n₁ + 0.369n₂ + 0.038n₃ + 5.08n₁ + 0.801n₄
%M+2 = 0.005n₁(n₁ - 1) + 0.205n₂ + 3.35n₃ + 4.52n₄ + 32.0n₁(n₁ + 97.3n₄)

Example 2 Organic compound

m/z Relative abundance Normalized
58 12.0 100.0
59 0.5±0.2 4.2±1.7
60 0.0±0.2 0.0±0.2

http://www.colby.edu/chemistry/PChem/Fragment.html

M+1 = 59; 2.5-5.9% makes #C = 3, 4 or 5

M = 58 possibilities

C  O  no odd # of N present (even M)
3  0
4  0 (may be)
5  0 - not possible

X+1 = 44 ; 3-3.6% makes #C = 3,
leaves 43-36 = 7 mass units in the fragment

C₃H₇;  U = 0.5  EE ion

Molar mass: 58

<table>
<thead>
<tr>
<th>Formula</th>
<th>M+1</th>
<th>M+2</th>
<th>MM</th>
<th>e/o</th>
<th>db = U</th>
</tr>
</thead>
<tbody>
<tr>
<td>N3O</td>
<td>1.15</td>
<td>0.20</td>
<td>58.0042</td>
<td>e</td>
<td>2.5</td>
</tr>
<tr>
<td>H2N4</td>
<td>1.51</td>
<td>0.00</td>
<td>58.0281</td>
<td>o</td>
<td>2</td>
</tr>
<tr>
<td>CNO2</td>
<td>1.54</td>
<td>0.40</td>
<td>57.9929</td>
<td>e</td>
<td>2.5</td>
</tr>
<tr>
<td>CH2N2O</td>
<td>1.91</td>
<td>0.21</td>
<td>58.0168</td>
<td>o</td>
<td>2</td>
</tr>
<tr>
<td>C2H2O2</td>
<td>2.30</td>
<td>0.41</td>
<td>58.0055</td>
<td>o</td>
<td>2</td>
</tr>
<tr>
<td>C2H6N2</td>
<td>3.03</td>
<td>0.03</td>
<td>58.0532</td>
<td>o</td>
<td>1</td>
</tr>
<tr>
<td>C3H6O</td>
<td>3.43</td>
<td>0.24</td>
<td>58.0419</td>
<td>o</td>
<td>1</td>
</tr>
<tr>
<td>C4H10</td>
<td>4.55</td>
<td>0.08</td>
<td>58.0783</td>
<td>o</td>
<td>0</td>
</tr>
</tbody>
</table>

Base peak
Aromatic parent ions – large abundance.

**Fragmentation:**
Fragmentation leads to smaller ions by the cleaving of parts of the molecule.

**Unreasonable losses from molecular ion:**
M - [3→14] and M - [21→26] are unreasonable losses. (next slide)

**Reasonable losses from molecular ion**
Neutral fragments expelled by simple cleavage
\[ \text{OE}^{+} \rightarrow \text{EE}^{+} + \text{OE} \]
Neutral fragments expelled by multi-centered fragments
\[ \text{OE}^{+} \rightarrow \text{OE}^{+} + \text{EE} \]
See handout

**Fragmentation pattern:**
From the fragment losses the parent peak may be predicted by working back.

**Notation:**
\[ \text{C}_2\text{H}_5\text{C}_6\text{H}_{13}^{85.1017 \text{ Da}} \]
\[ \text{C}_3\text{H}_7^{43.0548 \text{ Da}} \]
\[ \text{C}_7\text{H}_{15}^{99.1174 \text{ Da}} \]
\[ \text{C}_2\text{H}_5^{29.0391 \text{ Da}} \]

Unique isotope peak patterns are useful for the analysis.

**One electron movement**
\[ \text{C}_2\text{H}_5\text{C}_6\text{H}_{13} \rightarrow \text{CH}_3\text{C}_7\text{H}_{15} \]

**Two electron movement**
\[ \text{C}_2\text{H}_5\text{C}_6\text{H}_{13} \rightarrow \text{CH}_3\text{C}_7\text{H}_{15} \]

138 - (81 + 14) - 29 = 14
136 - (79 + 14) - 29 = 14
Unique isotope peak patterns are useful for the analysis.

Fragmentation:

Nominal mass of parent ion containing C, H, O, S, Si, P and halogens is even.

- odd m/z ion
  - $R \rightarrow R^+$ (homolytic) cleavage
- even m/z, no N
  - neutral species, e.g. CO, water, ...
- even m/z ion

Fig. E3

<table>
<thead>
<tr>
<th>M = odd</th>
<th>17</th>
<th>one N</th>
</tr>
</thead>
<tbody>
<tr>
<td>$M+1$</td>
<td>0.4%</td>
<td>no C</td>
</tr>
<tr>
<td>Consistent with 1N</td>
<td>$\text{NH}_3$</td>
<td></td>
</tr>
</tbody>
</table>

Nominal mass
- 17
- $14 - 1N$
- $3 \rightarrow 3\text{H}$

PE1

Fig. E4

Molar mass of parent ion 170 1.8 100 3

Relative abundance

100-29 = $M-29$

Structural isomers differentiation

m/z = 100

peptide
PE1

M = even

M+1 1.1% 1 C

Nominal mass 16

12 4

1C 4H

CH₄

PE2

1 O present from M+2 peak

2 H present consistent with M+1 peak

M+1, M+2 peaks confirm H₂O.

Nominal mass, M = 100%

%M+1 = 0.012n₁+1.08n₂+0.369n₃+0.038n₄+5.08n₅+0.81n₆

%M+2 = 0.005n₁(n₁-1)+0.205n₂+3.35n₃+4.52n₄+32.0n₅+97.3n₆

Data: Contains 3 elements, one is F.

PE3

Nominal mass

34

12 1 C

22

19 1 F

3 3 H

CH₃F

Further 34-15=19

Fragment intensities depend on the stability of the ion and the probability of formation.
Electron repelling methyl group stabilizes the carbocation.

Four ways to form carbocation:

1. Alkenes frequently undergo fragmentations that yield allylic cations

2. Carbon–carbon bonds next to an atom with an unshared electron pair usually break readily because the resulting cation is resonance stabilized.

3. Carbon–carbon bonds next to the carbonyl group of an aldehyde or ketone break readily because resonance-stabilized ions called acylium ions are produced.

Z = N, O, or S; R may also be H.
3. Carbon-carbon bonds next to the carbonyl group of an aldehyde or ketone break readily because resonance-stabilized ions called acylium ions are produced.

\[ R' - \text{C}=\text{O}^+ + R \]

4. Alkyl-substituted benzenes undergo loss of a hydrogen atom or methyl group to yield the relatively stable tropylium ion. This fragmentation gives a prominent peak (sometimes the base peak) at m/e 91.

\[ \text{CH}_3 - \text{C}=\text{O}^+ \]

5. Substituted benzenes also lose their substituent and yield a phenyl cation at m/e 77.

\[ \text{C}_3\text{H}_7\text{NO} \rightarrow \text{C}_3\text{H}_7^+ + \text{NO}^- \]

Y = halogen, -NO₂, -Keto group, R, etc.

### INTENSITY (AS PERCENT OF BASE PEAK)

<table>
<thead>
<tr>
<th>m/e</th>
<th>14</th>
<th>15</th>
<th>18</th>
<th>28</th>
<th>29</th>
<th>42</th>
<th>43</th>
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<tbody>
<tr>
<td>PE7</td>
<td>8.0</td>
<td>38.6</td>
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<td>39.7</td>
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**Recalculating intensities to normalize**

<table>
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<th>m/e</th>
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<th>74</th>
<th>75</th>
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<tr>
<td>PE7</td>
<td>86.1</td>
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Mass spectrum for Problem E. 7.

**Recalculating intensities to normalize**

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**Confirming MF**

\[ \text{C}_3\text{H}_7\text{NO} \]

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Mass spectrum for Problem E. 7.
No halogens

Probably 1 O; (M+2)
4 C; M+1

Nominal mass
72
48 - 4 C
24
16 - 1 O
8 8 H

72 - 44 = 28 loss of 28 from M to form base peak

No straightforward cleavage possible; probably a rearrangement occurs before cleavage.

Cleavage of Two bonds:

1. Alcohols frequently show a prominent peak at M - 18.
This corresponds to the loss of a molecule of water.

2. Cycloalkenes can undergo a retro-Diels-Alder reaction that produces an alkene and an alkadienyl radical cation.

3. Carbonyl compounds with a hydrogen on its α-carbon undergo a fragmentation called the Mclafferty rearrangement.

Y may be R, H, OR, OH, etc.

In addition to these reactions, are present peaks that result from the elimination of other small stable neutral molecules, e.g. N2, NH3, CO, HCN, H2S, alcohols, and alkenes.

The compound is butanal. m/z = 44 arises from the Mclafferty rearrangement.

m/z=29 arises from acylium ion.
GC-MS, LC-MS are hyphenated analytical techniques. Two techniques are combined to form a single method for analyzing mixtures of chemicals. Chromatography separates the components and MS detects and characterizes each of the components. Combination of the techniques allows both qualitative and quantitative evaluation of analytical samples.

The MS spectrometer can be highly selective for analyte of interest.

http://www.shsu.edu/~chm_tgc/sounds/flashfiles/GC-MS.swf
http://www.shsu.edu/~chm_tgc/sounds/flashfiles/SIM.swf
Each analyte’s MS produced

Total ion current (TIC) Chromatography

Total current of all ions of all masses from a GC peak is detected above a selected value of time (solvent peak) for a separated analyte.

http://www.gmu.edu/departments/SRIF/tutorial/gcd/gc-ms2.htm

GC, LC/MS takes data in three dimensions simultaneously, recording the number of ions created along with their masses over time.

This information is generally represented by examining the total ion chromatogram (TIC) and ‘slicing’ along the third dimension (m/z) of a given peak to look at the mass spectrum at a chosen time.

MS requires high vacuum $\sim 10^{-3} - 10^{-6}$ torr. The eluate from a chromatographic system has much more carrier (gas/eluent) than the analyte. If eluate from the chromatograph is allowed to enter directly into the MS it would overwhelm the vacuum system (especially in LC – liquid vaporizes into a large volume).

To alleviate the strain on the system, different strategies are employed at the sample introduction/ionization stage.
Electrospray interface:

Coaxial flow

For positive ion MS

Strong electric field + nebulization; ions vaporize

Low collisional dissociation; minimal fragmentation.

Positive ions accelerate and collide with $N_2$ molecules; few fragments form. Changing skimmer voltage to larger $-$ would produce more fragmentation.

Atmospheric Pressure Chemical Ionization API-CI

Electrons formed at corona, injects into aerosol.

This technique produces single charged ions. Little fragmentation, less structural information.

$N_2 + e \rightarrow N_2^+ + 2e$

$N_2^+ + 2N_2 \rightarrow N_2^+ + N_2$

$N_4^+ + H_2O \rightarrow 2N_2 + H_2O^+$

$H_2O^+ + H_2O \rightarrow OH^+ + H_3O^+$

$H_3O^+ + nH_2O \rightarrow H_3O^+(H_2O)_n$

$H_3O^+(H_2O)_n + M \rightarrow MH^+ + (n+1)H_2O$

Selected Ion Monitoring

SIM describes the operation of the mass spectrometer where the intensities of one or several specific ion beams (Multiple ion monitoring) are recorded rather than the entire mass spectrum.

The SIM more sensitive than the full scan experiment because the mass spectrometer can dwell for a longer time over a smaller mass range; reduces background.

Selected ion monitoring possible with the quadrupole mass analyzer with appropriate tuning.

SIM leads to selective detection of analytes.
Selected Reaction Monitoring

SRM delivers a unique fragment ion that can be monitored and quantified in the midst of a very complicated matrix. Plots are simple, usually a single peak. Therefore the SRM plot is ideal for sensitive and specific quantitation.

The SRM experiment is accomplished by specifying the parent mass of the compound for MS/MS fragmentation and then specifically monitoring for a single fragment ion. Same as the SIM of a fragment ion. The specific experiment in known as a "transition" and can be written (parent mass → fragment mass) For example 534→375.

Selectivity and S/N ratio increased markedly with QqQ triple quad arrangement.
Quantitative analysis with MS can be done with the calibration curve method.

Internal standard method gives better results; a fixed amount of an IS is incorporated to both samples and calibration standards. The ratio of the peak intensity of the analyte standard to that of the IS, is plotted as a function of standard analyte concentration. Main advantage of the use of IS, is the minimization of uncertainties arising at the sample preparation and introduction stage.

A suitable IS would be a stable, isotopically labeled analog of the analyte; where one or more atoms of 2-H, 13-C or 15-N have been incorporated.

It is reasonable to assume that in the mass spectrometer the labeled molecules would be subjected to same process as do the unlabeled analyte molecules.
Lasers are used to deliver a focused high density of monochromatic radiation to a sample target, which is vaporized and ionized.

The yield of ions is often increased by using a secondary ion source or a matrix.

Reflex Time-of-Flight Mass Spectrometry

Not all ions gets desorbed at the same time and in exactly the same location, leads to slightly different velocities for identical ions. Degrades the resolution due to peak broadening (lower resolution).

Reflex can detect post source decay.

Mass Spectroscopy

Bioanalytical Perspective
MALDI is incompatible with continuous operation. It is a pulsed technique. Not for tandem methods as GC-MS, LC-MS and CE-MS.

Peaks such as [M+2H]^{2+}, [M+H]^+, [M+Na]^+ is typical.

Used for analysis of proteins, peptides, fragments of peptides and their mixtures (molecular weights with practically no fragmentation).

Fast, mixtures handled, more powerful than electrophoresis, chromatography.

MALDI tolerates moderate concentrations of buffers and salts.

Identification of proteins via the "peptide fingerprint".

Protein enzyme \rightarrow products from cleavage at specific places of protein.

\[ \text{MALDI-TOF/MS} \]

Spectrum = "MS fingerprint"

---

Electrospray Ionization

Electrospray ionization is a versatile ionization method, capable of analyzing compounds directly from aqueous or aqueous/organic solutions, mol wt ~200,000Da; peptides, proteins, carbohydrates, DNA fragments and lipids.

It is compatible with continuous operation.

Analyte molecules in solution reach the end of a stainless steel needle which is held at a high voltage in relation to the counter electrode.

A fine spray of micro droplets is formed when the analyte leaves the needle. This spray is highly charged due to the high potential of the needle.

On traveling to the counter electrode, the highly charged droplets lose the solvent molecules, the micro droplets shrinks due to solvent evaporation.

The region between the needle and the counter electrode is held at atmospheric pressure, whilst the other side of the counter electrode is in vacuum.

Evaporation of analyte from the micro droplet surface takes place continuously. The diameter of the droplet decreases whilst the total amount of charge remains the same.

Coulombic forces are exerted and induce instability on the droplet surface, and as a result the droplet breaks up into smaller droplets.

This process leads to very small droplets containing only an analyte ion with a lot of solvent around it.

When the diameter becomes less than 10nm the remaining solvent leaves the analyte ion, and free ions is formed.

The resulting increase in charge density of the droplet, forces the charged analyte ion out of the solution, leaving highly charged analyte molecules.

The charge on the species due to the protons bonded.

\([\text{M}+n\text{H}^+]\) type ions produces for a given analyte; \(n\sim+50\).

Electrospray ionisation produces multiply charged ions, (up to +30 to +50 charges). Therefore high molecular weights can be measured using mass analyzers with a limited weight range.

The number of charges an ion can depends strongly upon its 3D dimensional structure.

When a protein assumes a tight conformation, only surface sites are available for protonation making the observed charge states are relatively low.

The electrospray process produces a distribution of charge states, used in the interpretation of the spectra.
The m/z values can be expressed as follows:

Because z number of H$^+$ ions are added to the molecule

$$m/z = (MW + zm_p)/z$$

where m/z = the mass-to-charge ratio (from plot)

MW = the molecular mass of the sample

z = the integer number of charges on the ions

comes from the attached protons

$m_p$ = the mass of a proton = 1.008 Da.

But we do not know z!!

For example, if the ions appearing at m/z 1431.6 in the lysozyme spectrum have "z" charges, then the ions at m/z 1301.4 will have "z+1" charges.

For these two consecutive peaks using:

$$m/z = (MW + zm_p)/z$$

$$m/z_1 = 1431.6 = (MW + zm_p)/z$$

$$m/z_2 = 1301.4 = [(MW + (z+1)m_p)/(z+1)]$$

Solve; MW = 14,305.9 Da and z = 10

Based on two consecutive peaks

In general the following relationships can be used:

$$m_i = \text{the value for the peak on the m/z-axis}$$

MW can be calculated from

$$MW = \frac{m_i - m_j}{z_i} = \frac{(m_i - m_j)}{z_i}$$

requires $z_i$

$m/z$ values on x-axis: $m_i = \frac{MW + zm_p}{z_i}$ and $m_j = \frac{MW + zjm_p}{z_j}$

i.e. $MW = M$

$$MW = M = m_iz_i - z_im_p = mz_i - zjm_p$$ where $m_p$ = mass of H$^+$

and $z_j = z_i + j$

$$z_i = \frac{j(m_j - m_p)}{(m_i - m_j)}$$
Based on two consecutive peaks

The result corresponds to the average molecular weight of the protein.

Identifying one charge state effectively identifies them all, as they increase or decrease by one by going left or right respectively.

Charges can only be integers.