Mass Spectroscopy

Mass spectrometry is the study of systems generating the formation of gaseous ions, with or without fragmentation; which are then characterized by their mass to charge ratios (m/z) and relative abundances.

In MS compounds are ionized, the ionized molecule decomposes into smaller ions/radicals/radical-ions/neutrals. One way to ionize molecules is to extract electrons from a molecule by high energy collisions.

The positively charged species thus produced are separated, based on their mass/charge (m/z) ratio.

In ionization and fragmentation, the molecular ion decomposes into smaller ions/radicals/radical-ions/neutrals. Parent ion/daughter ions, radicals, neutral, ...

Most of the ions has $z=+1 \Rightarrow m/z = \text{mass of the fragment}$. A plot of relative abundance of ions vs. m/z of the charged particles is presented as the Mass Spectrum.

A plot of relative abundance of ions vs. m/z of the charged particles is presented as a histogram.

Fragmentation:

\[
\text{M}^+ \quad \rightarrow \quad \text{M}_1^+ + \text{N}_1^+ \\
\text{M}^+ \quad \rightarrow \quad \text{M}_2^+ + \text{N}_2^+
\]

\[
\text{M}^+ \quad \rightarrow \quad \text{M}_1^+ \rightarrow \text{M}_3^+ + \text{N}_4
\]

\[
\text{M}^+ \quad \rightarrow \quad \text{Radical ion (odd e)} \\
\text{N}^+ \quad \rightarrow \quad \text{Neutral radical (odd e)} \\
\text{N} \quad \rightarrow \quad \text{Neutral (even e)}
\]

\[
\text{M}^+ \quad \text{(even e) would not break up into a radical ion.}
\]

Actual signal has peaks with a line width.
Isotope peaks:
In mass spectroscopy the actual mass of the fragments generated are determined. Therefore fragments with different isotopes are distinguished, e.g. Lead metal.

For molecular fragments, the isotope peak abundance is dependent on the molecular constitution and the natural isotope abundance of the constituent elements.

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[Diagram of isotope peaks]

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Mass Spectrometer:

Mass spectrometer has devices for each of the following:

Sample Introduction
Create gas-phase ions of the sample
Separate ions in space or time based on m/z ratio; accomplished by mass analyzers
Detect the of ion current from each m/z ratio ion

Ionization: If a quantity of energy is supplied to a molecule greater than the ionization energy of the molecule, a molecular ion is formed \( M^+ \).

Electron Ionization (Electron Impact, EI)
Chemical Ionization (CI)
Matrix Assisted Laser Desorption Ionization (MALDI)
Atmospheric Pressure Chemical Ionization (APCI)
Atmospheric Pressure Laser Ionization (APLI)
Fast Atom Bombardment (FAB)
Inductively Coupled Plasma (ICP)

Mass Analyzers:
Magnetic Sector Mass Analyzer (Single/Double Focusing)
Quadrupole Mass Filters
Time-of-flight (TOF) Mass Analyzer
Ion Trap Mass Analyzer
Fourier-Transform Mass Spectrometry (FTMS)

Ion Detection:
Faraday Cup
Electron Multiplier
Photomultiplier Conversion Dynode

The interior of the mass spectrometer must be evacuated. The ion source, mass filter/analyzer, and detector are under vacuum so that the ions would move from the ion source to the detector without colliding with other ions and molecules. *The mean-free path of a charged particle should be greater than the distance between ionization and detection regions.*

A high vacuum is created with two pumps where a low-vacuum pump is connected to the output of a high-vacuum pump.

Diffusion pump(s) + rotary-vane rough pump
Turbo-molecular pump(s) + rotary-vane rough pump

<table>
<thead>
<tr>
<th>Pressure (Torr)</th>
<th>Mean Free Path (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>760</td>
<td>6.0x10^{-8}</td>
</tr>
<tr>
<td>1</td>
<td>4.5x10^{-5}</td>
</tr>
<tr>
<td>10^{-3}</td>
<td>4.5x10^{-2}</td>
</tr>
<tr>
<td>10^{-5}</td>
<td>4.5</td>
</tr>
<tr>
<td>10^{-7}</td>
<td>4.5x10^{2}</td>
</tr>
<tr>
<td>10^{-9}</td>
<td>4.5x10^{4}</td>
</tr>
</tbody>
</table>
Volatilized compound is ionized by electron impact. An electron beam is generated by accelerating the electrons from a heated filament through an applied voltage.

The electron energy is defined by the potential difference between the filament and the source housing and is usually set to 70 eV.

A field keeps the electron beam focused across the ion source.

Upon impact with a 70 eV electron, the gaseous molecule may lose one of its electrons to become a positively charged radical ion, daughter ions, etc.

All ions are subsequently accelerated out of the ion source by an electric field produced by the potential difference applied to the ion source and a grounded electrode, V.

A ‘repeller’ serves to define the field within the ion source.

Depending on the lifetime of the excited state, fragmentation will either take place in the ion source giving rise to stable fragment ions, or on the way to the detector, producing metastable ions.

Each m/z beam follows its own path (r) for a given B and V in the magnetic sector (60°/90°).

\[
\frac{m}{z} = \frac{eB^2}{2V}
\]

For specific V and B ions of unique m/z pass through the magnetic sector and reaches the stationary detector. Variations of V and/or B causes fragments of different m/z value to reach the detector.

Usually B is scanned to allow different m/z’s to reach the detector sequentially generating the complete mass spectrum keeping V constant.

The m/z ratio of the ions that reach the detector can be varied by scanning either the magnetic field (B) or the applied voltage of the ion optics (V).

i.e. by varying the voltage or magnetic field of the magnetic-sector analyzer, the individual ion beams are separable spatially, radius of curvature is held constant.
The distribution of a given mass by way of energy distribution of kinetic energy.

\[
e(V + \Delta V) = e(V) + e\Delta V
\]

\[
eV = \frac{mv^2}{r} \quad \text{and} \quad zeV = \frac{mv^2}{2}
\]

\[
r = \frac{2V}{E} \quad \text{r independent of } \frac{m}{z} \text{ in } E; \text{ focussing!}
\]

Sample introduction/ionization method:

<table>
<thead>
<tr>
<th>Ionization Technique</th>
<th>Typical Analytes</th>
<th>Sample Introduction</th>
<th>Mass Range</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron Impact (EI)</td>
<td>Relatively small volatile</td>
<td>GC or liquid/solid probe</td>
<td>upto 1,000 Datons</td>
<td>Hard method variable provides structure info</td>
</tr>
<tr>
<td>Chemical Ionization (CI)</td>
<td>Relatively small volatile</td>
<td>GC or liquid/solid probe</td>
<td>upto 1,000 Datons</td>
<td>Soft method molecular ion peak [M+H]+ recommended; then the resolving power is defined as [\frac{m_1}{\Delta m}].</td>
</tr>
<tr>
<td>Electrospray (ESI)</td>
<td>Peptides, Proteins, nonvolatile</td>
<td>Liquid Chromatography or direct injection</td>
<td>upto 200,000 Datons</td>
<td>Soft method ions often multiply charged</td>
</tr>
<tr>
<td>Fast Atom Bombardment (FAB)</td>
<td>Carbohydrates, Organometallics, Peptides</td>
<td>Sample mixed in viscous matrix</td>
<td>upto 6,000 Datons</td>
<td>Soft method but harder than EI or MALDI</td>
</tr>
<tr>
<td>Matrix Assisted Laser Desorption (MALDI)</td>
<td>Peptides, Proteins, Nucleotides</td>
<td>Sample mixed in solid matrix</td>
<td>upto 500,000 Datons</td>
<td>Soft method very high mass</td>
</tr>
</tbody>
</table>

Actual signal has peaks with a line width.

Imposes a limitation on the resolvability of consecutive peaks

Resolving Power

1. Defined in terms of the overlap (or 'valley') between two peaks. For two peaks of equal height, masses \(m_1\) and \(m_2\), when there is overlap between the two peaks to a \textit{stated percentage} of either peak height (10% is recommended), then the resolving power is defined as \[\frac{m_1}{\Delta m}\].

The percentage overlap (or 'valley') concerned must always be stated.

Minimum resolution necessary to separate \(N_2^+\) and \(CO^-\) peaks?

Exact masses:
\(N_2^+ = 28.006158 \text{ amu}\)
\(CO^- = 27.994915 \text{ amu}\)

\[
R = \frac{M}{\Delta M} = \frac{28}{0.011241} = 2490
\]

\[
\frac{M}{\Delta M} = 500
\]
2. Resolving power \( \frac{m_{\text{ip}}}{m_{x}} = 1040 \)

State the method of calculation when expressing resolving power, and the position of the lower peak.

Example

The molecular ion (dominant) is formed by the removal of the least tightly bound electron.

Ionization EI:

The molecular ion (dominant) is formed by the removal of the least tightly bound electron.

Chemical ionization (CI):

Interaction of the molecule M with a reactive ionized reagent species (gaseous Bronsted acids), e.g., EI of methane, generates \( \text{CH}_4^+ \) which then reacts to give the Bronsted acid \( \text{CH}_5^+ \):

\[
\text{CH}_4^+ + \text{CH}_4 \rightarrow \text{CH}_5^+ + \text{CH}_3
\]

If M in the source has a higher proton affinity than \( \text{CH}_4 \), the protonated species \( \text{MH}^+ \) will be formed by the exothermic reaction.

\[
\text{M} + \text{CH}_5^+ \rightarrow \text{MH}^+ + \text{CH}_3
\]

CI is a softer ionization process.

CI is a lower energy process than EI, results in less fragmentation and therefore a simpler spectrum with the parent/molecular ion intact.

\( \text{M}^+ \) nearly nonexistent.

Abundant \( \text{M}^+ \)
Fast Atom Bombardment Ionization:

The sample droplet is bombarded with energetic atoms (Ar, Xe) of 8-10 keV kinetic energy. Ions (e.g., Cs+) can be used as the bombarding particle in a similar technique termed liquid secondary ion mass spectrometry (LSIMS).

Beam collides with the sample and matrix molecules, producing positive and negative sample-related ions that can be accelerated into the mass spectrometer.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Analyte Index</th>
<th>Analyte Description</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2</td>
<td>H2</td>
<td>H2</td>
<td></td>
</tr>
<tr>
<td>CH3</td>
<td>CH3</td>
<td>CH3</td>
<td></td>
</tr>
<tr>
<td>CH3CN</td>
<td>CH3CN</td>
<td>CH3CN</td>
<td></td>
</tr>
<tr>
<td>CH3COOH</td>
<td>CH3COOH</td>
<td>CH3COOH</td>
<td></td>
</tr>
<tr>
<td>CH3CHO</td>
<td>CH3CHO</td>
<td>CH3CHO</td>
<td></td>
</tr>
<tr>
<td>CH3Cl</td>
<td>CH3Cl</td>
<td>CH3Cl</td>
<td></td>
</tr>
<tr>
<td>CH3COCH3</td>
<td>CH3COCH3</td>
<td>CH3COCH3</td>
<td></td>
</tr>
<tr>
<td>CH3CN</td>
<td>CH3CN</td>
<td>CH3CN</td>
<td></td>
</tr>
<tr>
<td>CH3COOH</td>
<td>CH3COOH</td>
<td>CH3COOH</td>
<td></td>
</tr>
<tr>
<td>CH3CHO</td>
<td>CH3CHO</td>
<td>CH3CHO</td>
<td></td>
</tr>
<tr>
<td>CH3Cl</td>
<td>CH3Cl</td>
<td>CH3Cl</td>
<td></td>
</tr>
</tbody>
</table>

Fast Atom Bombardment

Used for polar organic compounds, acidic and basic functional groups.
Basic groups run well in positive ionization mode and acidic groups run well in negative ionization mode.
FAB analytes: peptides, proteins, fatty acids, organometallics, surfactants, carbohydrates, antibiotics, and gangliosides.

Behavior of electrical charges in electric fields.
The path of charged particles depends on size/mass differences.
Larger masses have a higher inertia than a small mass.

Quadrupole Mass Analyzer (spectrometer):

Diagonal electrodes have potentials of the same sign
U= DC voltage, V=AC voltage and \( \omega \) = angular velocity of alternating voltage in RF range.

Parameters affecting motion: m/z, U, V, r₀ and ω.

Apply a DC; make it + on two diagonal rods.
Superimpose an AC, V sin ωt with an amplitude V and a frequency ω.

Lighter ions spirals out of the quadrupole (filters out).

Apply a DC; make it + on two diagonal rods.
Superimpose an AC, V sin ωt with an amplitude V and a frequency ω.

Ions repelled from rods, AC component act on small m/z more effectively.
Heavier ions travel straight to the detector because of their high inertia.

For a given U and V there will be m/z below which ions would not pass through the rods.

Ions attracted from rods, AC component act on large m/z more effectively.
Heavier ions spirals out of the quadrupole (filters out).
Lighter ions travel straight to the detector.

Quadrupole Mass Analyzer (Spectrometer):

Ions oscillate under the influence of the variable fields.

Combined DC and RF potentials on the quadrupole rods create a stable linear path and passes only a selected m/z ratio (resonant ion) at a time. All other m/z ions acquire unstable paths and spirals out.

The mass spectrum is obtained by varying the voltages on the rods and monitoring which ions pass through the quadrupole rods.

Quadrupole mass (QM) analyzer is a "mass filter".

The solution of equations of motion of ions traveling through a QM analyzer shows that for an ion with a particular m/z to pass through at certain combinations of U and V must be obtained.

Varying rod voltages (scanning the spectrum):

a. scan \( \omega \) while holding U and V constant
b. scan U and V but keep the ratio U/V fixed

If U and V are scanned such that U/V = constant, with V>U then successive detection of ions of different m/z is achieved.

Two functions \( a \) and \( q \) define a stable trajectory for which ions do not collide with the rods across a range of values of U and V.

\[
a = -\frac{4zeU}{m^2r_0^2\omega^2} \quad q = -\frac{2zeV}{m^2r_0^2\omega^2}
\]

In principle QM analyzer can be operated for a range of U and V values.

\[
\frac{a}{q} = \frac{2U}{V}
\]

Stability curve for an ion - m/z

Of all possible U,V coordinates there exist combinations where the ion path through the quad is stable (ions reaches the detector); other combinations are unstable.

A given m/z ion travels thro' the quadrupole if the values of U and V are in a segment of the operating line and bounded by the stability curve.
Graphically, e.g., the three stability curves represent values of $U$ and $V$ for which the masses $m_1$, $m_2$ and $m_3$ have stable trajectories through the quadrupole.

Only those mass values on the operating line transmit.

The resolution is determined by the magnitude of $U/V$ ratio, i.e. the slope.

Resolution of the mass analyzer can be increased by increasing the slope of the line, $U/V$ (= held const.), and that if $U = 0$ then ions of all $m/z$ are transmitted.

Because quadrupoles operate at lower voltages, they can be scanned at faster rates (~1000 a.m.u./s) than magnet based spectrometers. QMs are better detectors for LC-MS and GC-MS implementation.

Holding $U$, $V$ allows ions of only one $m/z$ ion move down to the detector. Simultaneous ramping of both $U$, $V$ fields (with the suitable ratio $U/V$ held constant); ions of various $m/z$ ions passes through the mass filter to the detector sequentially.

SCANNING!!
DC fields tend to focus positive ions in the positive plane and defocus them in the negative plane. Ions oscillate with increasing amplitudes until they collide with the electrodes. The lighter the ion in mass, the smaller the number of cycles before it is annihilated.

**MS/MS**

Scan MS1 (Q1 only) turned on – entire MS spectrum.

Set (e.g. proper U and V) MS1 to filter fragment of interest, dissociate further in q (Q2) the fragment selected in MS1 by collision with Ar or N$_2$. Mass analyze the products from Q2 by scanning in MS2 (Q3).

**Time-of-Flight Mass Analyzers (spectrometer):**

TOF measures the mass-dependent time required for ions of different masses to move from the ion source to the detector.

This requires that at the starting time t=0, (time ions leaves the ion source) to be well-defined.

Ions are created by a *pulsed method* (MALDI), or by rapid electric field switching that serves as a ‘gate’ to release the ions from the ion source in a very short time.
**Time-of-Flight Mass Spectrometry, Principle**

\[ t_{\text{total}} = t_{\text{source}} + t_{\text{tof}} \]

**Ion Source - MALDI**

- **Metal plate**
- **Laser**
- **ion signals**

**Time-of-Flight Mass Spectrometers,**

**Reflex MALDI TOF Mass Spectrometer**

**Reflection time-of-flight mass spectrometer**
Parent/Molecular Peak $M$:

An molecular ion that has not lost/gained atoms. The nominal mass of which is calculated with the mass numbers of the predominant isotopes of atoms.

Base peak:

Base peak is the peak from the most abundant ion, which is often the most stable ion.

High Resolution MS:

Using mass number for isotopes of atoms is approximate. Actual mass of a given isotope deviates from this integer by a small but unique amount ($\Delta E = \Delta mc^2$). Relative to $^{12}$C at 12.0000000, the isotopic mass of $^{16}$O is 15.9949146 a.m.u., etc.

High resolution mass spectrometers that can determine m/z values accurately to four/more decimal places, making it possible to distinguish different molecular formulas having the same nominal mass.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Accurate Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-H</td>
<td>1.007825</td>
</tr>
<tr>
<td>2-H</td>
<td>2.014102</td>
</tr>
<tr>
<td>12-C</td>
<td>12.000000000</td>
</tr>
<tr>
<td>13-C</td>
<td>13.003548</td>
</tr>
<tr>
<td>14-N</td>
<td>14.00000000</td>
</tr>
<tr>
<td>15-N</td>
<td>15.00000000</td>
</tr>
<tr>
<td>16-O</td>
<td>15.9949146</td>
</tr>
<tr>
<td>17-O</td>
<td>16.9991306</td>
</tr>
<tr>
<td>18-O</td>
<td>17.9991594</td>
</tr>
</tbody>
</table>

Very short list.

$$ m/z=74 $$

<table>
<thead>
<tr>
<th>MF</th>
<th>Unsaturation</th>
<th>Exact Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{C}_2\text{H}_2\text{O}_3$</td>
<td>2.0</td>
<td>74.00040</td>
</tr>
<tr>
<td>$\text{CH}_3\text{N}_2\text{O}_2$</td>
<td>2.0</td>
<td>74.01163</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_2$</td>
<td>6.0</td>
<td>74.01565</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_4\text{O}_2$</td>
<td>2.0</td>
<td>74.01679</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_4\text{F}_2$</td>
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<tr>
<td>$\text{C}_2\text{H}_6\text{O}_2$</td>
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<tr>
<td>$\text{C}_2\text{H}_6\text{N}_2$</td>
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<td>74.04801</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_4$</td>
<td>1.0</td>
<td>74.05318</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_6\text{O}_2$</td>
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<td>74.07316</td>
</tr>
<tr>
<td>$\text{C}_2\text{H}_6\text{N}_2$</td>
<td>0.0</td>
<td>74.08440</td>
</tr>
</tbody>
</table>

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 $\cong$ 1:1, $^{35}$Cl : $^{37}$Cl $\cong$ 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>Mass (amu)</th>
<th>Abundance Relative %</th>
<th>Mass (amu)</th>
<th>Abundance Relative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>1.007825</td>
<td>0.801</td>
<td>Si</td>
<td>24.031508</td>
</tr>
<tr>
<td>C</td>
<td>12.010700</td>
<td>0.012</td>
<td>Br</td>
<td>79.904000</td>
</tr>
<tr>
<td>N</td>
<td>14.006700</td>
<td>1.080</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>15.999400</td>
<td>0.369</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>32.065500</td>
<td>5.080</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>35.452600</td>
<td>4.520</td>
<td>Br</td>
<td>79.904000</td>
</tr>
</tbody>
</table>

Nominal mass, M = 100%

\[ %M+1 = 0.012n_H + 1.080n_C + 0.369n_N + 5.080n_S + 4.520n_{Cl,Br} \]

\[ \text{Combination of the techniques allows both qualitative and quantitative evaluation of analytical samples.} \]

Chromatography – Mass Spectroscopy

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 (the analytes in the effluent) and characterizes each of the components.

The MS spectrometer is highly selective of analyte of interest.

http://www.shsu.edu/~chm_tgc/sounds/flashfiles/GC-MS.swf
http://www.shsu.edu/~chm_tgc/sounds/flashfiles/SIM.swf
**Total ion current (TIC) Chromatogram**

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.

GC-MS, 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.

http://www.gmu.edu/departments/SRIF/tutorial/gcd/gc-ms2.htm
MS requires high vacuum ~$10^{-3} - 10^{-6}$ 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.

**GC-MS; EI**

- Quadrupole mass spectrometer
- Electron ionization
- High voltage power supply
- One of the softest ionization techniques.

**LCMS - ESI: Electrospray interface**

- Electrospray ionization
- Low collisional dissociation; minimal fragmentation.
- Positive ions accelerate and collide with $N_2$ molecules; few fragments form. Changing skimmer voltage to larger $-ve$ would produce more fragmentation.
Atmospheric Pressure Chemical Ionization APCI (API)

Coaxial flow

Electrons formed at corona, injects into aerosol.

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

\[
N_2 + e \rightarrow N_2^+ + 2e \\
N_2^+ + 2N_2 \rightarrow N_4^+ + N_2 \\
N_4^+ + H_2O \rightarrow 2N_2 + H_2O^+ \\
H_2O^+ + H_2O \rightarrow OH^+ + H_2O \\
H_2O^+ + nH_2O \rightarrow H_2O^{+\bullet}(H_2O)_n \\
H_2O^{+\bullet}(H_2O)_n + M \rightarrow (MH)^+ + (n + 1)H_2O
\]

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 monitored 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 highly selective detection of analytes.
Selectivity and S/N ratio increased markedly with QqQ triple quad arrangement.

Selected Reaction Monitoring (SRM)

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. Similar to the SIM of a fragment ion. The specific experiment is known as a "transition" and can be written (parent mass → fragment mass) For example 534 → 375.

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 get desorbed at the same time and in exactly the same location, leading to slightly different velocities for identical ions. Degrades the resolution due to peak broadening (lower resolution).

Reflex can detect post source decay.

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.

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.

$[M+nH]^+$ type ions produces for a given analyte; $n=+50$.

Electrospray ionization 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 depend 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.
ESI-MS Spectrum of a biomolecule: Monoisotopic Mass
peaks considered here

z increases, m/z decreases for the same molecule

http://www.chm.bris.ac.uk/ms/theory/esi-ionsaltion.html

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)
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:

\[ MW = M = m_{i}z_{i} - z_{i}m_{p} = m_{i}z_{2} - z_{i}m_{p} \]

where \( m_{i} \) is mass of \( H^{+} \) and \( z_{i} = z_{1} + j \)

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.
Rapid acting insulins: Humalog LisPro
mol wt = 5807

Mass Spectral Information:
Human Insulin