PharmacoLearn

MPG101T Modern Pharmaceutical Analytical Techniques, M.Pharm Semester 1

Unit 3: Mass Spectroscopy

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3.1 Principle and theory

Mass spectroscopy is a technique in which the sample is first converted into ions in the gas phase, the ions are separated according to their mass to charge ratio (m/z), and each ion type is counted. The record is a graph of relative abundance against m/z, called a mass spectrum. It is not a true absorption spectroscopy: no radiation is absorbed by the sample. No other single technique gives the molecular weight so directly, and, from the fragmentation pattern, strong clues to the structure.

Three results are read from almost every spectrum:

- The **molecular ion peak (M+.)**: the ion formed by removal of one electron from the whole molecule without breaking it apart. Its m/z gives the molecular weight of the compound.
- The **base peak**: the tallest peak of the spectrum, assigned a relative abundance of 100 percent. It is usually the most stable fragment ion, not necessarily the molecular ion.
- **Fragment ions and neutral losses**: smaller peaks formed when the molecular ion breaks at its weaker bonds. The mass differences between peaks correspond to lost neutral fragments, for example loss of 15 (CH3), 18 (H2O), 28 (CO), 29 (C2H5 or CHO) and 31 (OCH3).

The **nitrogen rule** is a quick check: a molecule with an odd nominal molecular weight contains an odd number of nitrogen atoms, and a molecule with an even molecular weight contains zero or an even number of nitrogen atoms.

3.2 Instrumentation of mass spectrometer

The working flow of the instrument is:

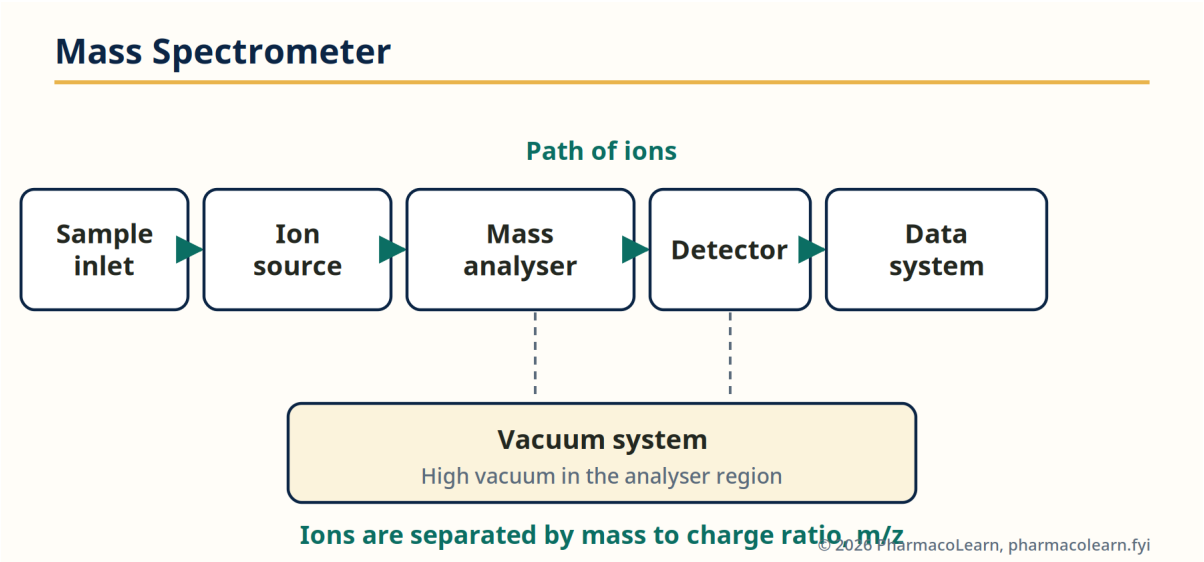

Figure: Main parts of a mass spectrometer along the path of the ions.

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Sample inlet -> Ion source (ionisation chamber) -> Accelerating plates
-> Mass analyser (separates ions by m/z) -> Detector -> Amplifier -> Recorder or computer
        (the whole ion path is kept under high vacuum)
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- **Sample inlet:** introduces the vaporised sample at a very low, steady pressure. For GC-MS and LC-MS the chromatograph itself is the inlet.
- **Ion source:** ionises the molecules by the chosen method (Section 3.3).
- **Accelerator:** charged plates give every ion the same kinetic energy, so that afterwards velocity depends only on mass.
- **Analyser:** separates the ions by m/z (Section 3.4).
- **Detector:** an electron multiplier converts each arriving ion into an amplified electrical pulse.
- **Vacuum system:** the ion path is kept at about 10^-5 to 10^-7 torr so that ions do not collide with air molecules and lose their path or energy.

3.3 Types of ionisation

Ionisation methods divide into hard methods, which fragment the molecule extensively, and soft methods, which give mainly the molecular ion or a simple adduct ion. Soft methods made mass spectroscopy usable for large, polar and heat sensitive drug molecules and biomolecules.

Method	How ions are formed	Type	Best suited for
Electron impact (EI)	A beam of high energy electrons (usually 70 eV) knocks an electron out of the molecule	Hard	Volatile, thermally stable small molecules, GC-MS, gives rich fragmentation for structure work
Chemical ionisation (CI)	A reagent gas such as methane is ionised first, and its ions transfer a proton gently to the sample	Soft	Molecular weight confirmation, strong (M+H)+ peak, little fragmentation
Field ionisation and field desorption (FI, FD)	A very strong electric field at a sharp emitter removes an electron, or desorbs ions directly from the emitter	Soft	Non volatile and thermally labile compounds, very little fragmentation
FAB (fast atom bombardment)	A beam of fast argon or xenon atoms strikes the sample dissolved in a viscous matrix such as glycerol	Soft	Polar, non volatile molecules of moderate mass, peptides
MALDI (matrix assisted laser desorption ionisation)	The sample in a UV absorbing matrix is hit by a laser pulse, the matrix absorbs the energy and desorbs sample ions	Soft	Very large molecules, proteins, polymers, usually with a time of flight analyser
ESI (electrospray ionisation)	The liquid sample is sprayed through a charged capillary, droplets evaporate and leave multiply charged ions	Soft	LC-MS workhorse, polar and large molecules, proteins give a series of multiply charged peaks
APCI (atmospheric pressure chemical ionisation)	The LC effluent is vaporised by heat and ionised by a corona discharge at atmospheric pressure	Soft	Less polar, moderately sized molecules that respond poorly in ESI
APPI (atmospheric pressure photoionisation)	UV photons from a lamp ionise the vaporised sample, often with a dopant	Soft	Non polar compounds that neither ESI nor APCI ionises well

3.4 Mass analysers: quadrupole and time of flight

An analyser separates ions of different m/z and passes one m/z at a time, or measures all of them by a physical property linked to mass.

Quadrupole analyser: four parallel metal rods carry combined direct current and radiofrequency voltages. For a given voltage setting, only ions of one m/z follow a stable oscillating path through the rods and reach

the detector; all others strike the rods and are lost. Scanning the voltages scans the mass range. Quadrupoles are compact, relatively cheap, fast scanning and tolerant of higher pressure, which is why they dominate routine GC-MS, LC-MS and the triple quadrupole instruments used for drug quantification in plasma.

Time of flight (TOF) analyser: all ions are accelerated by the same voltage, so lighter ions move faster than heavier ones. There are no scanning parts: the time taken to drift down a field free tube to the detector gives the m/z directly. TOF has, in principle, an unlimited mass range and very high resolution in reflectron designs, and it pairs naturally with MALDI, where ions are made in pulses.

Other analysers to name in an exam answer: magnetic sector (the classical high resolution instrument, deflects ions in a magnetic field according to momentum), ion trap (stores ions in a three dimensional quadrupole field and ejects them by mass) and Fourier transform ion cyclotron resonance (highest resolution, measures the orbital frequency of ions in a magnetic field).

3.5 Mass fragmentation and its rules

Fragmentation is not random. The molecular ion breaks where the resulting cation is most stable, and the standard rules are:

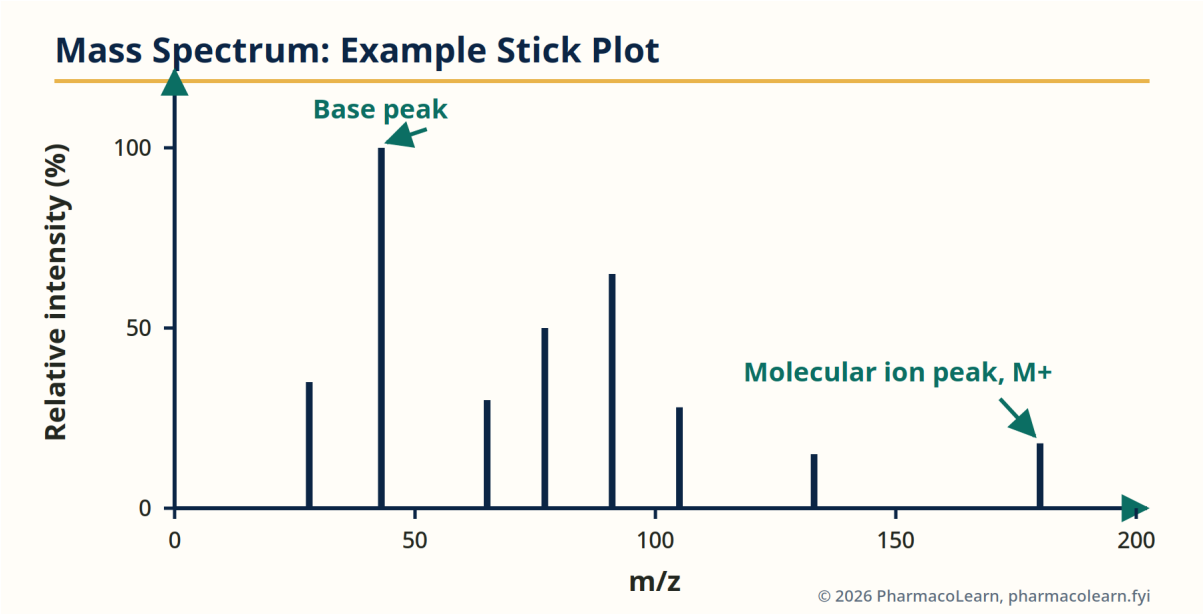

Figure: Example mass spectrum, the tallest stick is the base peak and the highest mass stick shown is the molecular ion peak.

- Cleavage is favoured at branched carbons, because tertiary carbocations are more stable than secondary, which are more stable than primary.
- Bonds next to a heteroatom (alpha cleavage) break readily because the heteroatom lone pair stabilises the resulting cation by resonance, as in alcohols, ethers and amines.
- Allylic and benzylic cleavage is favoured because the cation is resonance stabilised.
- Double bond systems and carbonyl compounds undergo characteristic rearrangements. The most famous is the **McLafferty rearrangement**: a carbonyl compound with a hydrogen on the gamma carbon transfers that hydrogen through a six membered transition state and cleaves, giving a characteristic enol radical cation peak, classically at m/z 58 for simple ketones and at m/z 60 for methyl esters.
- Loss of stable neutral molecules such as H2O (alcohols), CO (phenols, ketones), CO2 (acids) and small alkenes is common and shows as a peak at M minus that mass.

- Rings and double bonds increase the stability of the molecular ion, so aromatic and cyclic compounds show a strong M+. peak, while long straight chain alcohols may show almost no molecular ion.

3.6 Metastable ions and isotopic peaks

Metastable ions are ions that survive the ion source but fragment during flight, in the field free region. They appear as broad, weak, diffuse peaks at non integral m/z values given by m star = (m2 squared) divided by m1, where m1 is the parent ion mass and m2 is the daughter ion mass. A metastable peak is proof that a particular daughter ion came directly from a particular parent ion in one step.

Isotopic peaks arise because elements occur as isotope mixtures. Chlorine is about 3:1 of mass 35 to 37, so a compound with one chlorine shows M and M+2 peaks in a 3:1 ratio; bromine is about 1:1 of mass 79 to 81, so one bromine gives M and M+2 of nearly equal height; sulphur gives a small M+2 (about 4.4 percent of M); carbon-13 gives an M+1 peak whose height relative to M is about 1.1 percent per carbon atom, which is used to estimate the number of carbons in the molecule. Isotope patterns are the fastest way to detect chlorine or bromine in an unknown drug or impurity.

3.7 Applications of mass spectroscopy

Determination of molecular weight and molecular formula (with high resolution data), structure elucidation from fragmentation, identification of metabolites and impurities at trace level, quantitative bioanalysis by LC-MS/MS and GC-MS (the gold standard for pharmacokinetic studies), confirmation of identity in quality control, isotope ratio work, and proteomics and polymer characterisation by MALDI and ESI. Hyphenated use with chromatography is covered in Unit 4: the chromatograph separates, the mass spectrometer identifies and quantifies each peak.

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