

Topic 37 – Analytical Chemistry

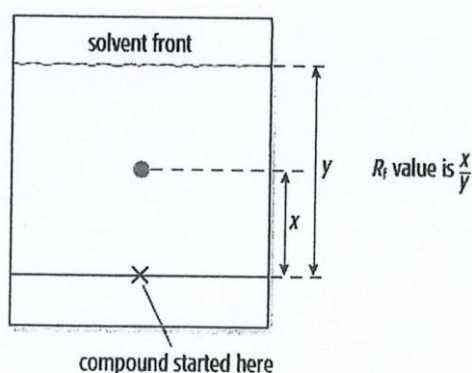
37.1 Thin layer chromatography

Reasons we use it:

- To separate the component of a mixture
- To determine purity
- To determine the identity of components of the mixture (usually by comparison with known compounds)
- To determine the amounts of the various components present in a mixture

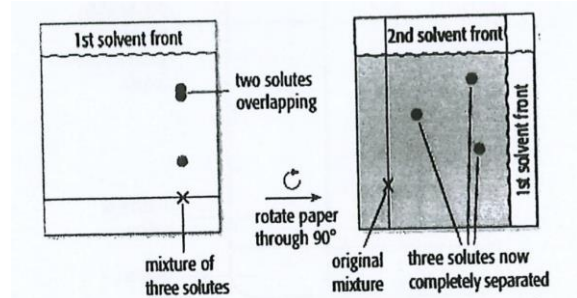
The Mechanism of separation of a mixture

- All Chromatographic techniques involve a stationary phase a mobile phase
- The components in a mixture are separated because of their difference in affinity for the stationary and mobile phases
- This is made by either adsorption and partition
- The R_f values (retardation factors) of substances are calculated
- The condition must be identical to those quotes in the R_f data table
- Coloured substances can be seen directly on the paper but others are sprayed with a chemical that forms colours on the chromatogram (such as amino acids)



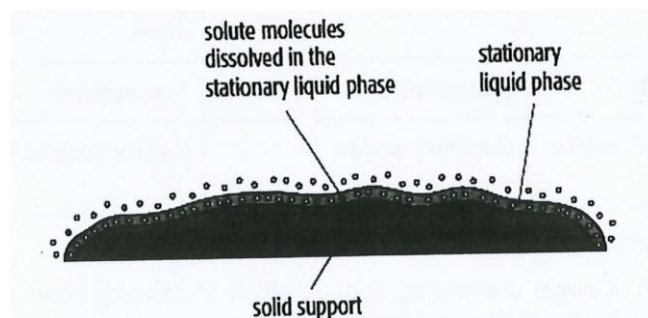
Two way chromatography

- Sometimes two or more components in a mixture can have similar R_f values in a particular solvent
- This means that their spots on the paper chromatogram will overlap and separation will be poor
- This can happen when we hydrolyse a protein and try to identify the amino acid residues present
- This is when two-way chromatography is useful
- The chromatography is done normal but after obtaining the results the chromatogram is rotated by 90 degrees and re-run in a different solvent
- It is unlikely that the R_f values will coincide in two different solvents, so the separation takes place



Partition coefficients in chromatography

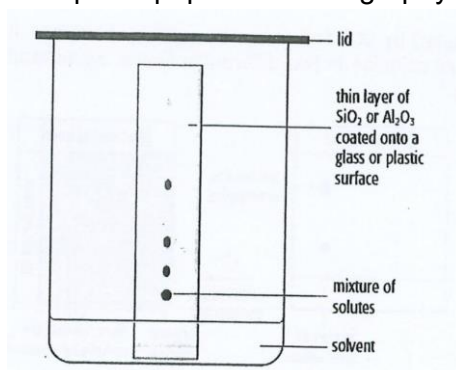
- Partition coefficient is the ratio of the concentration of a solute in the two immiscible solvents at equilibrium
- In paper chromatography the different partition coefficients of the components in a mixture correspond to their relative solubilities in the two solvents
- The mobile phase is the solvent chosen
- the other solvent is the water trapped in the paper's structure, which is the stationary phase
- The solutes in the mixture being separated are partitioned to different extents between the solvents in the mobile and stationary phase
- The greater the relative solubility in the mobile phase, the faster the rate of movement as the mobile phase passes over the stationary phase



- Partition chromatography, the mobile phase moves over the stationary liquid phase, carrying solute particles in it
- the filter paper is the solid support

Thin Layer Chromatography (TLC)

- Stationary Phase: Silica mounted on a glass phase
- Mobile phase: Suitable organic solvent
- Separation: Similar technique to paper chromatography



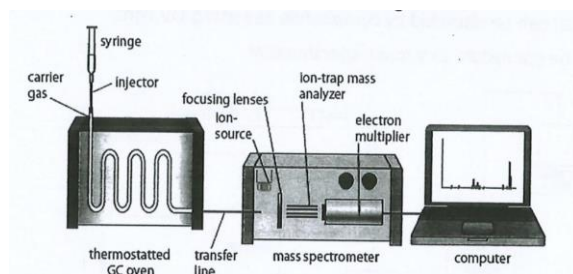
- TLC is quicker than paper chromatography and can be used on smaller samples
- TLC is used for the analysis of a substance that is suspected to be cannabis
- The stationary phase is silica sprayed with silver nitrate solution, which is then dried.
- The mobile phase is methylbenzene

	Paper chromatography	Thin layer chromatography
Method of separation	Partition	Adsorption
Stationary phase	Water on the fibers of the paper	solid particles (silica/ silica gel or alumina) coating the plate
Mobile phase	The solvent	The solvent
Movement of mobile phase	Capillary action	Capillary action

Gas liquid Chromatography (GLC)

- Stationary phase: Liquid absorbed on an inert solid / solid on solid support
- Mobile Phase: Gas

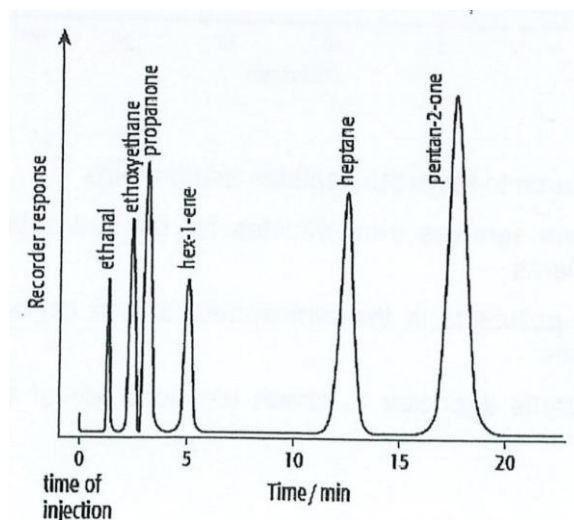
This method is used with gases, liquids and volatile solids (as they must be in the form of vapour)



- A very small amount of a sample is injected into a machine using a syringe
- The injector is contained in an oven
- The sample boils and is carried into a long thin column by an inert carrier gas
- The time a compound takes to travel through the tube will depend on how much time is spent moving with the gas rather than being attached to the liquid

As in all chromatography, the conditions must be controlled in order to make comparison with published databases

- The chromatogram must be obtained using the same carrier gas, flow rate, stationary phase and temperature that were used when the standard data was obtained



Determination of the percentage composition of a mixture by GLC:

- For quantitative analysis, the components peaks are first identified and then the area of each is measured
- The peaks are roughly triangular in shape hence their areas are:

$0.5 \times \text{base} \times \text{height}$

- The GLC machine usually measures the area of the peak automatically and can print the results with the chromatogram
- If the peaks are very narrow or have similar base widths, then the peak height may be used instead of peak area to estimate the proportions of components in a mixture

For this method

- The chromatogram must show peaks for all components in the mixture
- All the components of the mixture must be separated
- The detector must respond equally to the different components so that peak area is directly proportional to the component concentration

The amount of each component in a mixture is found by expressing it as a percentage of the sum of areas under all peaks

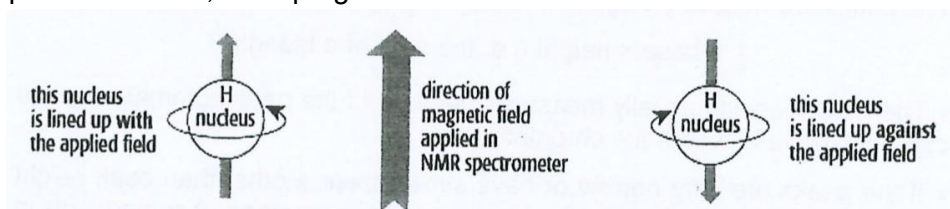
$$(\text{approx.}) \% \text{ of ester A} = \frac{\text{peak area (or height) of A}}{\text{sum of the areas (or heights) of A, B and C}} \times 100$$

GLC is used for:

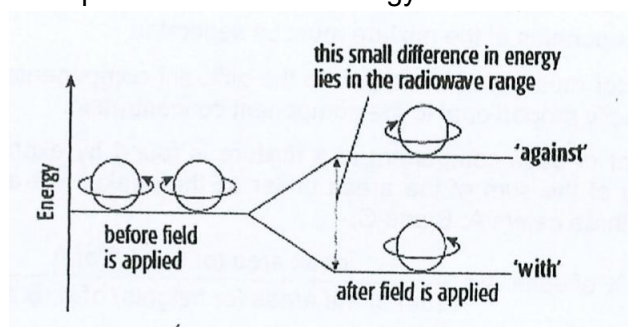
- In testing for steroids in athletes
- testing fuels in F1 races
- Medical diagnosis in blood samples
- determine the percentage of dissolved oxygen, nitrogen, carbon dioxide, and carbon monoxide in blood samples

Proton Nuclear Magnetic Resonance (^1H NMR)

- NMR is based on the fact that the nucleus of each hydrogen atom in an organic molecule behaves like a tiny magnet
- The nucleus of a hydrogen atom consists of a single proton
- This proton can spin
- The spinning motion of the positively charged proton causes a very small magnetic field to be set up
- In NMR we put the sample to be analysed in a magnetic field
- The hydrogen nuclei (proton) either line up with the field or, by spinning in the opposite direction, line up against it

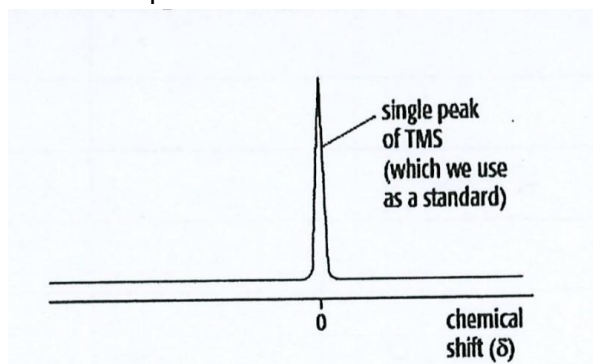


- There is a small difference in energy between the oppositely spinning ^1H nuclei
- This difference corresponds to the energy carried by waves on the radio wave range of the electromagnetic radiation spectrum
- In NMR the nuclei flip between the two energy levels



- Only atoms whose mass number is an odd number like ^1H or ^{13}C absorb energy in the range of frequencies that are analysed
- The size of the gap between the nuclear energy levels varies slightly depending on the other atoms in the molecule
- Therefore NMR can be used to identify ^1H atoms in different parts of a molecule
- e.g. Methanol
 - There is a H in the CH_3 and an H in OH
 - The energy absorbed by the CH_3 H atoms are different from the energy absorbed by the H atoms in the OH
- In NMR, we vary the magnetic field as that is easier than varying the wavelength of radio waves
- As the magnetic field is varied, the ^1H nuclei in different molecular environments flip at different field strengths
- The different field strengths are measured relative to a reference compound, which is given a value of zero
- The standard compound chosen is tetramethylsilane (TMS)

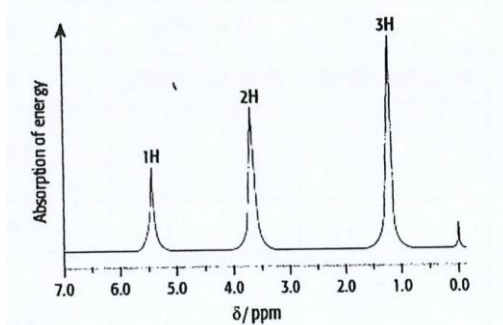
- TMS is an inert volatile liquid that mixes well with most organic compounds
- It has a formula of: $\text{Si}(\text{CH}_3)_4$ so all its H atoms are equivalent (same molecular environment)
- TMS only gives one, sharp absorption, called a peak, this peak is at a higher frequency than most other protons



- All other absorptions are measured by their shift away from the TMS line on the NMR spectrum
- This is called chemical shift and is measured in units per million (ppm)

Low resolution NMR

- shows a single peak for each non equivalent hydrogen atom

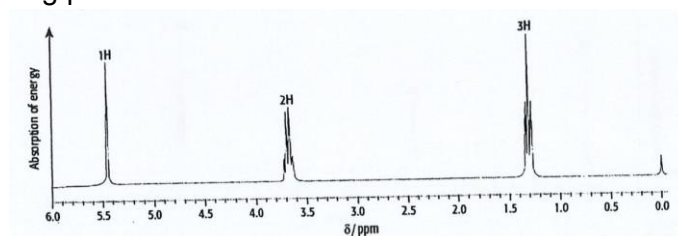


- Note how the zero point on the x axis, chemical shift is on the right of the spectrum and the shift increases in value going left
- There are three peaks on ethanol's low-resolution NMR spectrum. These correspond to the ^1H atoms -OH -CH₂ and -CH₃. Note how the heights of the peaks vary
- The area under each peak tells us the relative number of equivalent ^1H atoms responsible for that particular chemical shift
- The largest peak will be from the -CH₃ hydrogen atoms
- The middle peak from the -CH₂ hydrogen atoms
- The smallest peak from -OH hydrogen
- The relative areas under the peaks are shown on the NMR spectrum by the labels 1H, 2H, 3H
- The type of H atom present can be checked against tables of data

Type of proton			Chemical shift, δ / ppm
TMS			0
R-CH ₃			0.7-1.6
N-H	R-OH		1.0-5.5 ^(a)
R-CH ₂ -R			1.2-1.4
			2.0-2.9
			2.3-2.7
N-CH ₃	N-CH ₂ R	N-CH ₂ R ₂	2.3-2.9
O-CH ₃	O-CH ₂ R	O-CH ₂ R ₂	3.3-4.3
Br or Cl-CH ₃	Br or Cl-CH ₂ R	Br or Cl-CH ₂ R ₂	3.0-4.2
			4.5-10.0 ^(a)
-HC=CH-			4.5-6.0
			5.0-12.0 ^(a)
			6.5-8.0
			9.0-10.0
			11.0-12.0

High resolution NMR

- High resolution NMR gives us more information to interpret
- Peaks that appear as one 'signal' on a low resolution NMR spectrum are often revealed to be made up of a cluster of closely grouped peaks
- This is because the magnetic fields generated by spinning nuc.ei interfere slightly with those neighbouring nuclei
- This inference is called spin-spin coupling
- This exact splitting pattern of a peak depends on the number of hydrogen atoms on the adjacent carbon atom or atoms
- The number of signals peak split equals $n+1$ where n is the number of ^1H atoms on the adjacent carbon atom
- The high resolution NMR spectrum of ethanol illustrates this $n+1$ rule used to interpret splitting patterns



- The $-\text{CH}_3$ peak is split into three because there are two ^1H atoms on the adjacent $-\text{CH}_2$ group. $n + 1 = 3$ (as $n = 2$); this is called a **triplet**.
- The $-\text{CH}_2$ peak is split into four because there are three ^1H atoms on the adjacent $-\text{CH}_3$ group. $n + 1 = 4$ (as $n = 3$); this is called a **quartet**.
- The $-\text{OH}$ peak is not usually split as its ^1H atom is constantly being exchanged with the ^1H atoms of other ethanol molecules and any water present. This results in one average peak being produced.

Table below shows the relative intensities and distribution of the splitting patterns you are likely to meet.

Number of adjacent ^1H atoms	Using the $n + 1$ rule, the peak will be split into ...	Relative intensities in the splitting pattern	Observed on the NMR spectrum as ...
0	1 peak, called a singlet	1	
1	2 peaks, called a doublet	1 : 1	
2	3 peaks, called a triplet	1 : 2 : 1	
3	4 peaks, called a quartet	1 : 3 : 3 : 1	

Different OH and NH protons	Chemical shift (δ) / ppm
in alcohols, R-OH protons	1.0–5.5
in phenols, arene -OH protons	6.5–7.0
in carboxylic acids, R-COOH protons	11.0–11.7
in amines, -NH ₂ / -NH-	1.0–5.5
in aryl amines, arene -NH ₂	3.0–6.0
in amides, -CONH ₂ , -CONH-	5.0–12.0

Carbon 13 Spectroscopy

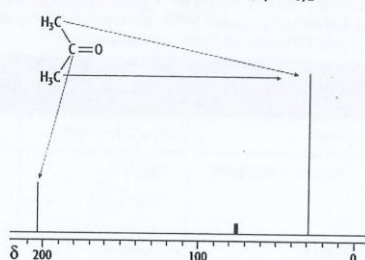
- Carbon 13 NMR produces a spectrum with different chemical shifts for non-equivalent carbon atoms in a molecule
- In proton NMR, the chemical shifts are measured with reference to the TMS peak at 0 ppm
- Typical carbon 13 NMR shifts are shown:

Hybridisation of carbon atom	Environment of carbon atom	Example structures	Chemical / ppm shift range (δ)
sp^3	alkyl	CH_3 -, CH_2 -, $-\text{CH}$ -	0 – 50
sp^3	next to alkene/arene	$-\text{CH}_2-\text{C}=\text{C}$, $-\text{CH}_2-$ 	10 – 40
sp^3	next to carbonyl/carboxyl	$-\text{CH}_2-\text{COR}$, $-\text{CH}_2-\text{CO}_2\text{R}$	25 – 50
sp^3	next to nitrogen	$-\text{CH}_2-\text{NH}_2$, $-\text{CH}_2-\text{NR}_2$, $-\text{CH}_2-\text{NHCO}$	30 – 65
sp^3	next to chlorine	$-\text{CH}_2-\text{Cl}$	30 – 60
sp^3	next to oxygen	$-\text{CH}_2-\text{OH}$, $-\text{CH}_2-\text{O}-\text{CO}-$	50 – 70
sp^2	alkene or arene	$-\text{C}=\text{C}-$ 	110 – 160
sp^2	carboxyl	$\text{R}-\text{CO}_2\text{H}$, $\text{R}-\text{CO}_2\text{R}$	160 – 185
sp^2	carbonyl	$\text{R}-\text{CHO}$, $\text{R}-\text{CO}-\text{R}$	190 – 220
sp	alkyne	$-\text{C}\equiv\text{C}-$	65 – 85
sp	nitrile	$\text{R}-\text{C}\equiv\text{N}$	100 – 125

- Analysis of carbon 13 NMR spectra are similar to that of proton NMR
- however the signals produced in carbon 13 NMR appear as discrete vertical lines on a spectra
- The heights of the lines are not usually proportional to the number of equivalent ^{13}C atoms present

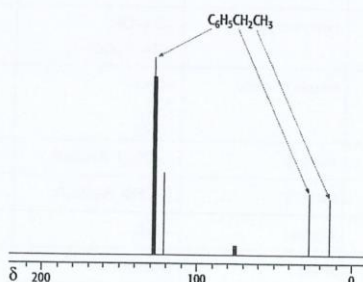
- The solvent used to prepare samples of ^{13}C NMR analysis is CDCl_3
- This accounts for the small signal near the 80 ppm that can be ignored when interpreting a spectrum, as it is caused by the atoms of ^{13}C in the solvent molecules

The ^{13}C NMR spectrum for propanone, $(\text{CH}_3)_2\text{CO}$.



Note that there are only two peaks: one for the carbon atom in the carbonyl group, $\text{C}=\text{O}$, and the other for the carbon atoms in the methyl groups, $-\text{CH}_3$. Although there are two $-\text{CH}_3$ groups in propanone, they are both equivalent and so appear as only a single peak (just as equivalent H atoms do in proton NMR).

The ^{13}C NMR spectrum of ethylbenzene, $\text{C}_6\text{H}_5\text{CH}_2\text{CH}_3$.



The carbon atoms in the benzene ring are almost equivalent but will be affected to slightly different extents by the presence of the ethyl group in the molecule. Hence the series of lines clustered near 125 ppm.

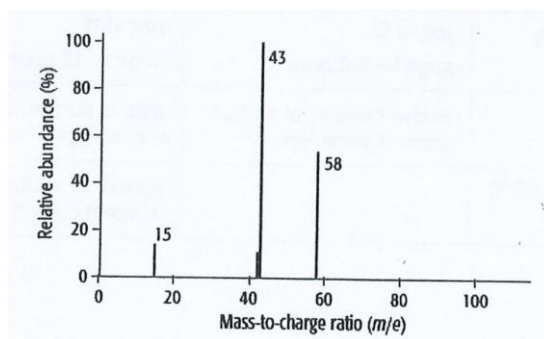
Comparison

Feature	Carbon-13 NMR	^1H NMR
number of peaks	number of types of C	number of types of H
chemical shift	type of C range 0–220 ppm	type of H range 0–12 ppm
peak area	relative number of carbon atoms of each type	relative number of protons of each type
spin-spin coupling		number of protons on adjacent carbon

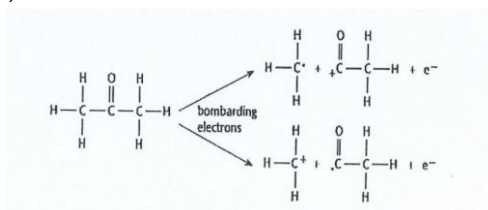
Mass Spectroscopy

- In a mass spectrometer the sample is first vaporised
- when vapour from the sample enters the machines
- it is bombarded by high-energy electrons
- this knocks electrons from the molecules and breaks covalent bonds
- fragmenting the molecules

e.g. Propanone



- The peak at the highest mass-to-charge ratio is caused by the molecular ion (M^+)
- this ion is formed by the sample molecules with one electron knocked out
- it gives us the relative molecular mass of the sample
- we can assume the ions detected carry a single positive charge
- so the reading on the horizontal axis gives us the mass
- in the case of propanone, the molecular ion has a relative mass of 58
- $(3 \times 12) + (1 \times 16) + (6 \times 1)$
- We also get peaks at 15 and 43 on the mass spectrum
- These peaks are due to fragments that are produced when propane molecules are broken apart by the electron bombardment



- The electron bombardment has caused the C-C single bonds in the propane molecules to break
- this has resulted in the fragments at m/e 15 and 43
- The breaking of single bonds of C-C C-O C-N are the most common fragmentations

High Resolution Mass Spectra

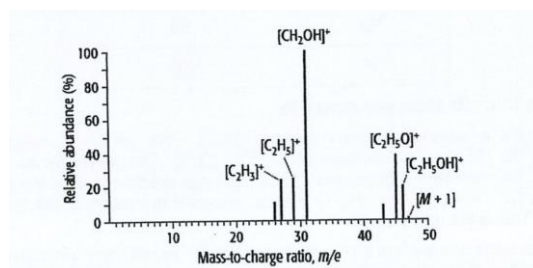
- This can distinguish between ions that appear to have the same mass on a low resolution spectra

Isotope	Relative isotopic mass
^1H	1.007 824 6
^{12}C	12.000 000 0 (by definition)
^{14}N	14.003 073 8
^{16}O	15.994 914 1

These accurate isotopic masses enable us to measure the mass of the molecular ion so accurately that it can only correspond to one possible molecular formula. For example, a molecular ion peak at 45 could be caused by C_2H_7N or CH_3NO . However, a high-resolution mass spectrum would show the $C_2H_7N^+$ peak at 45.057 846 and the CH_3NO^+ peak at 45.021 462. We can, therefore, be sure which molecule is being analysed.

Using the $[M + 1]$ peak

- There will always be a small peak just beyond the MIP at a mass of $[M+1]$
 - This is caused by molecules in which one of the carbon atoms is the ^{13}C
- e.g. ethanol



- In any organic compound there will be 1.10% carbon-13
- We can use this fact to work out the number of carbon atoms in a molecule, using the equation:

$$n = \frac{100}{1.1} \times \frac{\text{abundance of } [M+1]^+ \text{ ion}}{\text{abundance of } M^+ \text{ ion}}$$

Using the [M+2] and [M+4] peaks

- If the sample contains chlorine or bromine atoms, we also get peaks beyond the MIP because isotopes of Cl and Br.
- Chlorine has two isotopes
 - ^{13}C
 - ^{37}C
- Bromine has two isotopes
 - ^{79}Br
 - ^{81}Br

Isotope	Approximate %
^{35}Cl	75
^{37}Cl	25
^{79}Br	50
^{81}Br	50

One Cl or Br atom per molecule

Imagine a sample of chloromethane, CH_3Cl . We will have molecules of $\text{CH}_3^{35}\text{Cl}$ (75%) and molecules of $\text{CH}_3^{37}\text{Cl}$ (25%). The molecular ion will be $\text{CH}_3^{35}\text{Cl}^+$, and two units beyond that on the mass spectrum will be the peak for $\text{CH}_3^{37}\text{Cl}^+$. The peak for $\text{CH}_3^{37}\text{Cl}^+$ will be one-third the height of the molecular ion. This is the $[M+2]$ peak.

In the mass spectrum of bromomethane, CH_3Br , we will have two molecular ion peaks of approximately the same height – one for $\text{CH}_3^{79}\text{Br}^+$ and the other for $\text{CH}_3^{81}\text{Br}^+$ (the $[M+2]$ peak).

You should look out for the relative heights mentioned here when interpreting mass spectra.

- if the $[M+2]$ peak is one-third the height of the M peak, this suggests the presence of one chlorine atom per molecule
- if the $[M+2]$ peak is the same as the height of the M peak, this suggests the presence of one bromine atom per molecule.