Analytical Techniques

Chromatography

Chromatography is an analytical technique that separates components in a mixture between a mobile phase and a stationary phase.

The mobile phase may be a liquid or a gas.
The stationary phase may be a solid (as in thin-layer chromatography, TLC) or either a liquid or solid on a solid support (as in gas chromatography, GC)

Types of chromatography include:

- thin-layer chromatography (TLC) – a plate is coated with a solid and a solvent moves up the plate
- column chromatography (CC) – a column is packed with a solid and a solvent moves down the column
- gas chromatography (GC) – a column is packed with a solid or with a solid coated by a liquid, and a gas is passed through the column under pressure at high temperature.

In gas-liquid chromatography GC the **mobile** phase is a inert **gas** such as nitrogen, helium, argon.
The **stationary** phase is a **liquid** on an inert solid.

Separation by column chromatography depends on the balance between solubility in the moving phase and retention in the stationary phase.

If the stationary phase was polar and the moving phase was non- polar e.g. hexane. Then non-polar compounds would pass through the column more quickly than polar compounds as they would have a greater solubility in the non-polar moving phase. (Think about intermolecular forces)

A solid stationary phase separates by adsorption,
A liquid stationary phase separates by relative solubility

Retention times and Rf values are used to identify different substances.

Method: **Thin-layer chromatography**

a) **Wearing gloves**, draw a **pencil line** 1 cm above the bottom of a TLC plate and mark spots for each sample, equally spaced along line.
b) Use a capillary tube to add a **tiny drop** of each solution to a different spot and allow the plate to air dry.
c) Add solvent to a chamber or large beaker with a lid so that is no more than **1cm in depth**
d) Place the TLC plate into the chamber, **making sure that the level of the solvent is below the pencil line**. Replace the **lid to get a tight** seal.
e) When the level of the solvent **reaches about 1 cm from the top of the plate**, remove the plate and mark the solvent level with a pencil. Allow the plate to **dry in the fume cupboard.**
f) Place the plate under a **UV lamp or** if using amino acids spray with ninhydrin in order to see the spots. Draw around them lightly in pencil.
g) Calculate the Rf values of the observed spots.

Wear plastic gloves to prevent contamination from the hands to the plate

pencil line –will not dissolve in the solvent

tiny drop – too big a drop will cause different spots to merge

Depth of solvent– if the solvent is too deep it will dissolve the sample spots from the plate

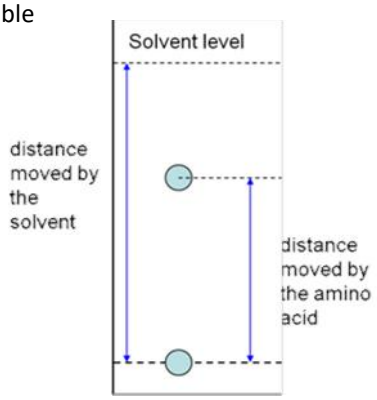
lid– to prevent evaporation of toxic solvent

Will get more accurate results if the solvent is allowed to rise to near the top of the plate but the Rf value can be calculated if the solvent front does not reach the top of the plate

dry in **a fume** cupboard as the solvent is toxic

UV lamp used if the spots are colourless and not visible

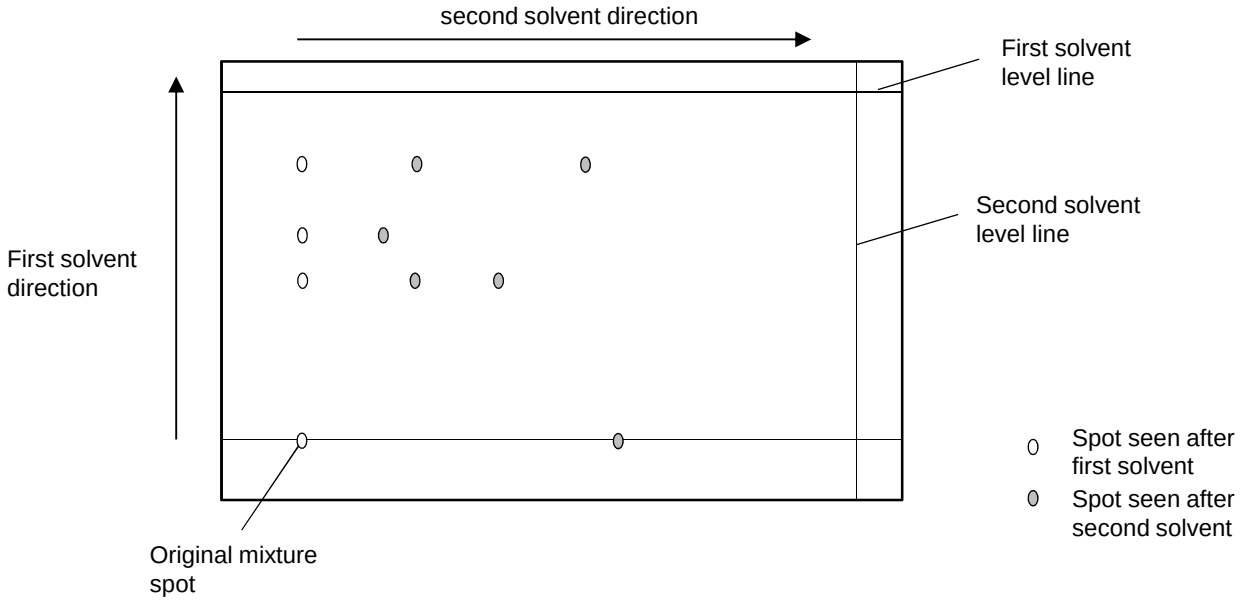
R_f value = distance moved by amino acid / distance moved by the solvent



Two directional chromatography

In order to separate a complex mixture that has components of different solubility in solvents, it may be necessary to do chromatography with two different solvents.

A spot of the mixture on a TLC plate is first separated with one solvent.
Then the TLC plate is rotated 90° and the plate is placed in a second solvent for a second separation to take place



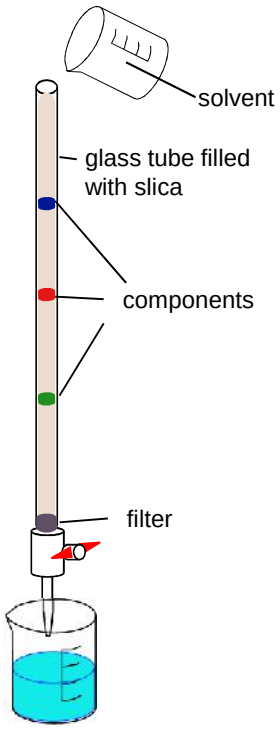
In total this mixture has 6 different components.

This process would be done if components in the mixture have the same Rf value or if some components are not soluble in the first solvent

Column chromatography (CC)

Simple column chromatography

- A glass tube is filled with the stationary phase usually silica or alumina in powder form to increase the surface area.
- A filter or plug is used to retain the solid in the tube. Solvent is added to cover all the powder.
- The mixture to be analysed is dissolved in a minimum of a solvent and added to the column.
- A solvent or mixture of solvents is then run through the column.
- The time for each component in the mixture to reach the end of the column is recorded (retention time)



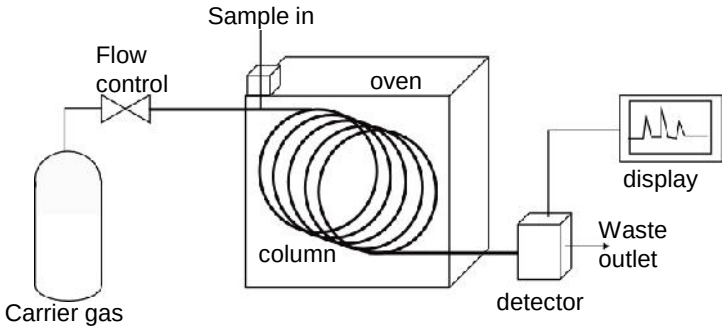
HPLC stands for high performance liquid chromatography and it type of column chromatography commonly used in industry.
HPLC: **stationary** phase is a **solid** silica
HPLC: **mobile** phase a **liquid**

Gas-Liquid Chromatography

Gas-liquid chromatography can be used to separate mixtures of volatile liquids.

In gas-liquid chromatography, the mobile phase is an inert gas such as helium and the stationary phase is a high boiling point liquid absorbed onto a solid.

The time taken for a particular compound to travel from the injection of the sample to where it leaves the column to the detector is known as its **retention time.** This can be used to identify a substance.



Some compounds have similar retention times so will not be distinguished.

Basic gas-liquid chromatography will tell us how many components there are in the mixture by the number of peaks. It will also tell us the abundance of each substance. The area under each peak will be proportional to the abundance of that component.

It is important to use an inert carrier gas such as helium or nitrogen. These will not react with the components being separated in the GC column.

The following factors can be changed to change the retention times of substances being separated: GC column temperature, column length , flow rate.
If the temperature or the flow rate is higher then substance will move more quickly through the column to give shorter retention times.

It is also possible for gas-liquid chromatography machine to be connected to a mass spectrometer, IR or NMR machine, enabling all the components in a mixture to be identified.

Most commonly a mass spectrometer is combined with GC to generate a mass spectra which can be analysed or compared with a spectral database by computer for positive identification of each component in the mixture.

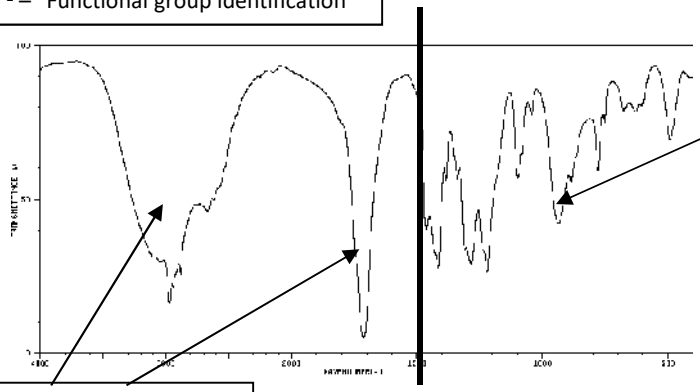
GC-MS is used in analysis, in forensics, environmental analysis, airport security and space probes.

Infrared spectroscopy

Certain bonds in a molecule absorb infra-red radiation at characteristic frequencies causing the covalent bonds to vibrate

Complicated spectra can be obtained than provide information about the types of bonds present in a molecule

ABOVE 1500 cm^{-1} – "Functional group identification"



BELOW 1500 cm^{-1} – "Fingerprinting"

Complicated and contains many signals – picking out functional group signals difficult.

This part of the spectrum is unique for every compound, and so can be used as a "fingerprint".

e.g. C=O 1640 – 1750 cm^{-1}

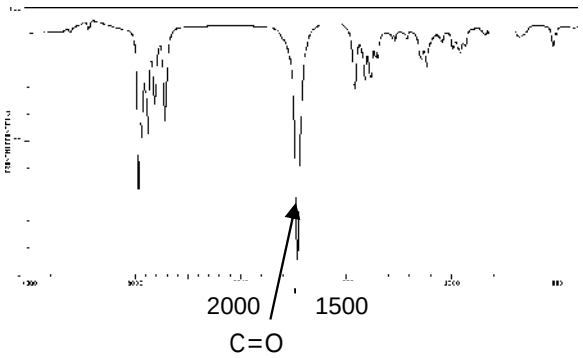
O-H (acid) 2500- 3300 cm^{-1}

A computer will compare the IR spectra against a database of known pure compounds to identify the compound

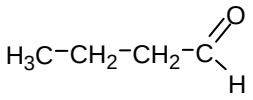
Use the IR absorption table provided in the data book to deduce presence or absence of particular bonds or functional groups

Bond	Wavenumber
C-O	1000-1300
C=O	1640-1750
C-H	2850 -3100
O-H Carboxylic acids	2500-3300 Very broad
N-H	3200-3500
O-H Acohols, phenols	3200- 3550 broad

use spectra to identify particular functional groups limited to data presented in wavenumber form e.g. an alcohol from an absorption peak of the O–H bond,



Spectra for butanal

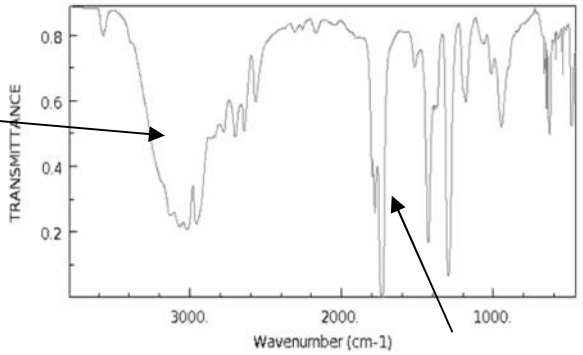


Absorption or trough in between 1640-1750 cm^{-1} range indicates presence of C=O bond

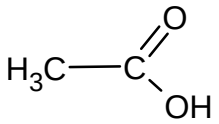
Always quote the wave number range from the data sheet

O-H absorptions tend to be broad

Absorption or trough in between 2500-3300 cm^{-1} range indicates presence of O-H bond in an acid



Spectra for ethanoic acid

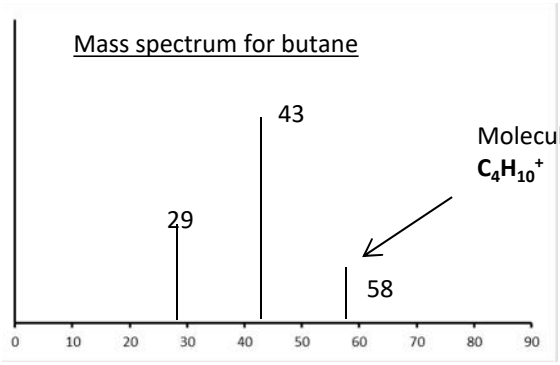


Mass spectrometry

Measuring the M_r of an organic molecule

If a molecule is put through a mass spectrometer it will often break up and give a series of peaks caused by the fragments. The peak with the largest m/z, however, will be due to the complete molecule and will be equal to the M_r of the molecule. This **M** peak is called the **molecular ion**

Spectra for C_4H_{10}



M+1 peak

A small peak will often occur in a mass spectrum next to the molecular ion peak. This is due to the presence of the C^{13} isotope in the molecule.
The relative height of this **M+1** peak compared to the **M** peak can be used to calculate the number of carbons in a molecule
Use the following equation to calculate the number (n) of carbons in a molecule

$$n = \frac{100 \times \text{height of } \mathbf{M+1} \text{ peak}}{1.1 \times \text{height of } \mathbf{M} \text{ peak}}$$

Learn this equation

Example
A hydrocarbon showed a ratio of heights of the M : M+1 peaks of 9.3 : 0.5.
Calculate the number of carbon atoms present in one molecule of this hydrocarbon

$$n = \frac{100 \times \text{height of } \mathbf{M+1} \text{ peak}}{1.1 \times \text{height of } \mathbf{M} \text{ peak}}$$

$$n = \frac{100 \times 0.5}{1.1 \times 9.3}$$

=4.89
Round to 5 carbons

M+2 peak

If a compound contains a chlorine or a bromine atom then a **M+2** peak will occur due to the two naturally occurring isotopes of chlorine or bromine.

Chlorine exists as Cl^{35} (75%) and Cl^{37} (25%)
Bromine exists as Br^{79} (50%) and Br^{81} (50%)

CH_3Cl will have a m/z value of **M** of 50 CH_3Cl^{35} and **M+2** of 52 CH_3Cl^{37}
The ratio of heights **M:M+2** will be 3:1

CH_3Br will have m/z value of **M** of 94 CH_3Br^{79} and **M+2** of 96CH_3Br^{81}
The ratio of heights **M:M+2** will be 1:1

If a compound contains two chlorine or bromine atoms then a **M+2** and a **M+4** peak will occur

$C_2H_4Cl_2$ will have a m/z value of **M** of 98 $C_2H_4Cl^{35}Cl^{35}$, a **M+2** of 100 $C_2H_4Cl^{35}Cl^{37}$ and a **M+4** of 102 $C_2H_4Cl^{35}Cl^{37}$
The ratio of heights **M:M+2: M+4** will be 9:6:1

Relative abundances
$Cl^{35}Cl^{35}$ = 0.75 x0.75 = 0.5625 =>**9**
$Cl^{35}Cl^{37}$ / $Cl^{37}Cl^{35}$ = 0.75 x0.25**x2**= 0.375 =>**6**
$Cl^{37}Cl^{37}$ = 0.25 x0.25 =0.0625 =>**1**
÷ smallest to get whole number ratio

$C_2H_4Br_2$ will have a m/z value of **M** of 186 $C_2H_4Br^{79}Br^{79}$, a **M+2** of 188 $C_2H_4Br^{79}Br^{81}$ and a **M+4** of 190 $C_2H_4Br^{81}Br^{81}$
The ratio of heights **M:M+2: M+4** will be 1:2:1

$C_2H_3Cl_3$ will have a m/z value of **M** of 132 $C_2H_4Cl^{35}Cl^{35}Cl^{35}$, a **M+2** of 134 $C_2H_4Cl^{35}Cl^{35}Cl^{37}$, a **M+4** of 136 $C_2H_4Cl^{35}Cl^{37}Cl^{37}$ and a **M+6** of 138 $C_2H_4Cl^{37}Cl^{37}Cl^{37}$
The ratio of heights **M:M+2:M+4:M+6** will be 27:27:9:1

Relative abundances
$Cl^{35}Cl^{35}Cl^{35}$ = 0.75 x0.75x0.75 = 0.4219 =>**27**
$Cl^{35}Cl^{35}Cl^{37}$ = 0.75 x0.75x0.25**x3**= 0.4219 =>**27**
$Cl^{35}Cl^{37}Cl^{37}$ = 0.75 x0.25x0.25**x3**= 0.1406 =>**9**
$Cl^{37}Cl^{37}Cl^{37}$ = 0.25 x0.25x0.25 =0.0156 =>**1**
÷ smallest to get whole number ratio

High Resolution Mass spectroscopy

High resolution mass spectrometry can be used to determine the molecular formula of a compound from the accurate mass of the molecular ion

High resolution mass spectroscopy can measure the mass to 5 d.p. This can help differentiate between compounds that appear to have similar Mr (to the nearest whole number)

For example, the following molecular formulas all have a rough M_r of 60, but a more precise M_r can give the molecular formula.

e.g. M_r = 60.02112 molecular formula = $C_2H_4O_2$

M_r = 60.05751 molecular formula = C_3H_8O

M_r = 60.03235 molecular formula = CH_4N_2O

Accurate masses of atoms:
H = 1.0078
C = 12.0000
O = 15.9949
N = 14.0031

Example A compound is found to have an accurate relative formula mass of 46.0417. It is thought to be either CH_3CH_2OH or $H_2NCH_2NH_2$. Calculate the M_r of each compound to 4 decimal places to work out which one it is.

$CH_3CH_2OH = (12.0000 \times 2) + (15.9949 \times 1) + (1.0078 \times 6) = 46.0417$

$H_2NCH_2NH_2. = (12.0000 \times 1) + (14.0031 \times 2) + (1.0078 \times 6) = 46.0530$

Fragmentation

When organic molecules are passed through a mass spectrometer, it detects both the whole molecule and fragments of the molecule.

Molecular ion formed: $M \rightarrow [M]^{+\cdot} + e^-$

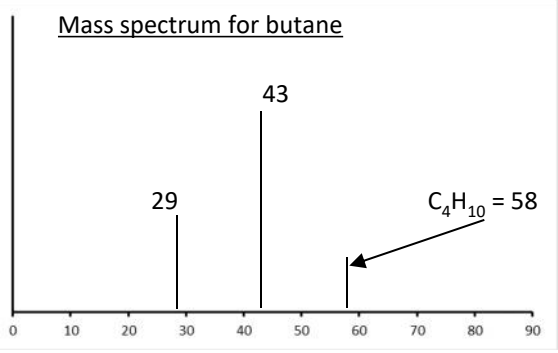
The molecule loses an electron and becomes both an ion and a free radical

Several peaks in the mass spectrum occur due to fragmentation.
The Molecular ion fragments due to covalent bonds breaking: $[M]^{+\cdot} \rightarrow X^+ + Y\cdot$

This process produces an ion and a free radical. The ion is responsible for the peak

Relatively stable ions such as carbocations R^+ such as $CH_3CH_2^+$ and acylium ions $[R\text{-}C{=}O]^+$ are common. The more stable the ion, the greater the peak intensity.

The peak with the highest mass/charge ratio will be normally due to the original molecule that hasn't fragmented (called the molecular ion) . As the charge of the ion is +1 the mass/ charge ratio is equal to *M*r.



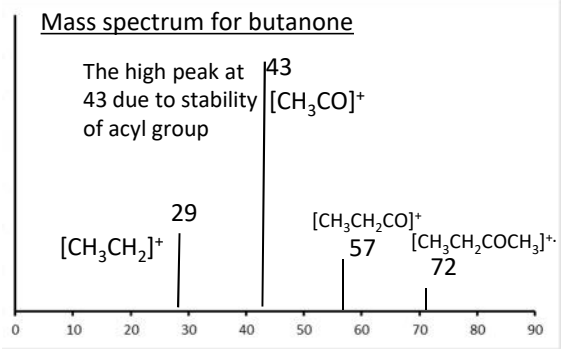
Equation for formation molecular ion

$C_4H_{10} \rightarrow [C_4H_{10}]^{+\cdot} + e^-$ m/z 58

Equations for formation of fragment ions from molecular ions

$[C_4H_{10}]^{+\cdot} \rightarrow [CH_3CH_2CH_2]^+ + \cdot CH_3$ m/z 43

$[C_4H_{10}]^{+\cdot} \rightarrow [CH_3CH_2]^+ + \cdot CH_2CH_3$ m/z 29



Equation for formation molecular ion

$CH_3CH_2COCH_3 \rightarrow [CH_3CH_2COCH_3]^{+\cdot} + e^-$ m/z 72

Equations for formation of fragment ions from molecular ions

$[CH_3CH_2COCH_3]^{+\cdot} \rightarrow [CH_3CH_2CO]^+ + \cdot CH_3$ m/z 57

$[CH_3CH_2COCH_3]^{+\cdot} \rightarrow [CH_3CO]^+ + \cdot CH_2CH_3$ m/z 43

$[CH_3CH_2COCH_3]^{+\cdot} \rightarrow [CH_3CH_2]^+ + \cdot COCH_3$ m/z 29

NMR spectroscopy

Different types of NMR

NMR spectroscopy involves interaction of materials with the low-energy radiowave region of the electromagnetic spectrum

NMR spectroscopy is the same technology as that used in 'magnetic resonance imaging' (MRI) to obtain diagnostic information about internal structures in body scanners

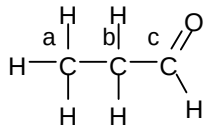
There are two main types of NMR
1. C^{13} NMR
2. H (proton) NMR

There is only around 1% C^{13} in organic molecules but modern NMR machines are sensitive enough to give a full spectra for C^{13}
The C^{13} spectra is a simpler spectrum than the H NMR

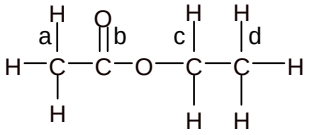
The radio waves used in proton nmr cause the hydrogen nucleus to **change its spin state.**

Equivalent Carbon atoms.

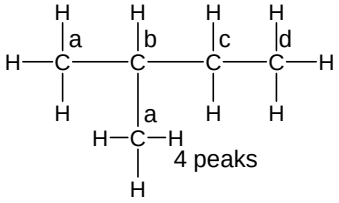
In a C^{13} NMR spectrum, there is one signal (peak) for each **set of equivalent C atoms.**



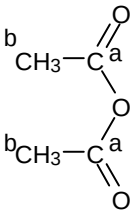
3 peaks



4 peaks

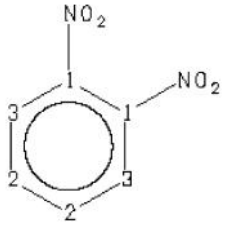


4 peaks



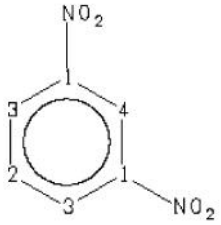
2 peaks

1,2 dinitrobenzene



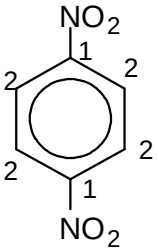
3 peaks

1,3 dinitrobenzene

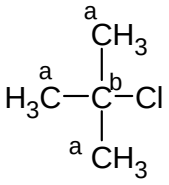


4 peaks

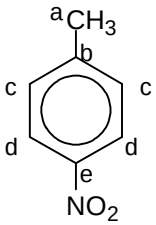
1,4 dinitrobenzene



2 peaks



2 peaks

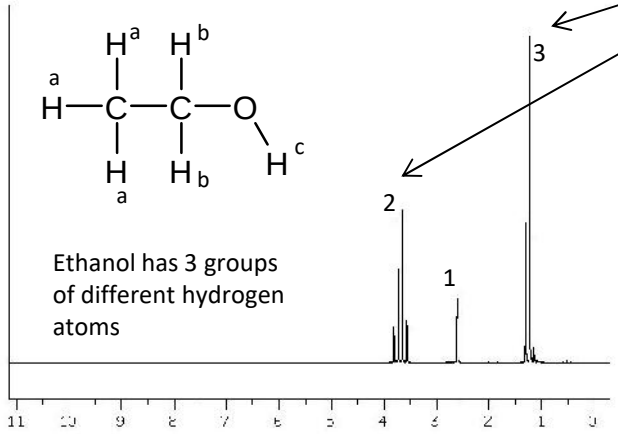


5 peaks

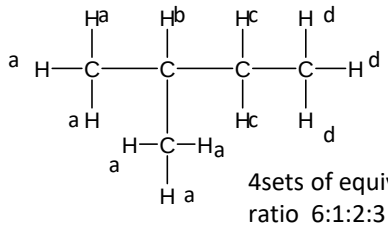
Equivalent Hydrogen atoms.

In an H NMR spectrum, there is one signal for each set of equivalent H atoms.

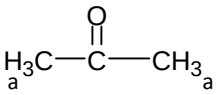
In addition the **intensity (integration value**) of each signal is proportional to the **number of equivalent H atoms** it represents.



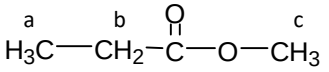
Ethanol has 3 groups of different hydrogen atoms



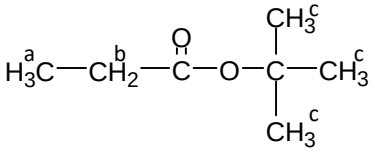
4sets of equivalent H's: ratio 6:1:2:3



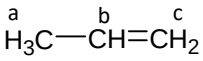
1 signal



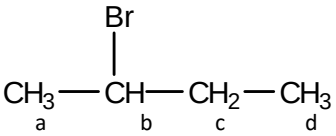
3 sets of equivalent H's: ratio 3:2:3



3 sets of equivalent H's: ratio 3:2:9



3 sets of equivalent H's: ratio 3:1:2



4 sets of equivalent H's: ratio 3:1:2:3

Solvents

Samples are dissolved in solvents without any ^{1}H atoms, e.g. CCl_4, $CDCl_3$.

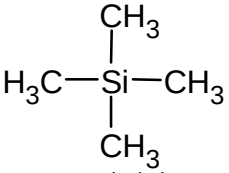
This means that in the H NMR the solvent will not give any peaks

The same solvent is used in C^{13} NMR and in this case there will be one peak due to the solvent that will appear on the spectrum. However, it is known where this peak is so it can be ignored.
In the exam it is likely this peak will not occur on the spectra.

Calibration and shift

A small amount of TMS (tetramethylsilane) is added to the sample to calibrate the spectrum

TMS is used because:
- its signal is away from all the others
- it only gives one signal
- it is non-toxic
- it is inert
- it has a low boiling point and so can be removed from sample easily

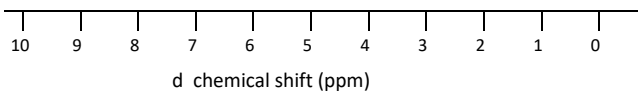


tetramethylsilane

The same calibration compound is used for both H and C NMR

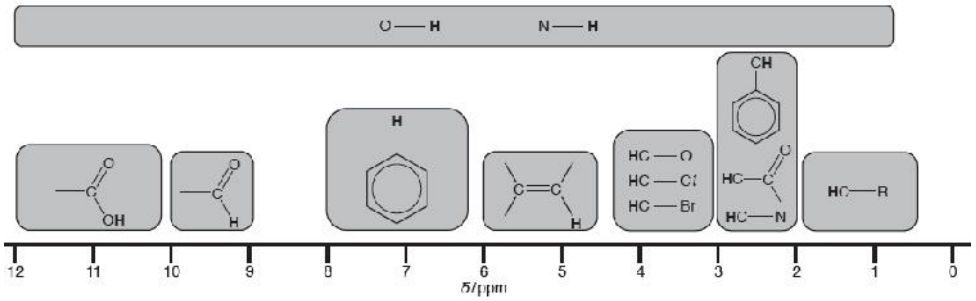
The spectra are recorded on a scale known as the chemical shift (δ), which is how much the field has shifted away from the field for TMS..

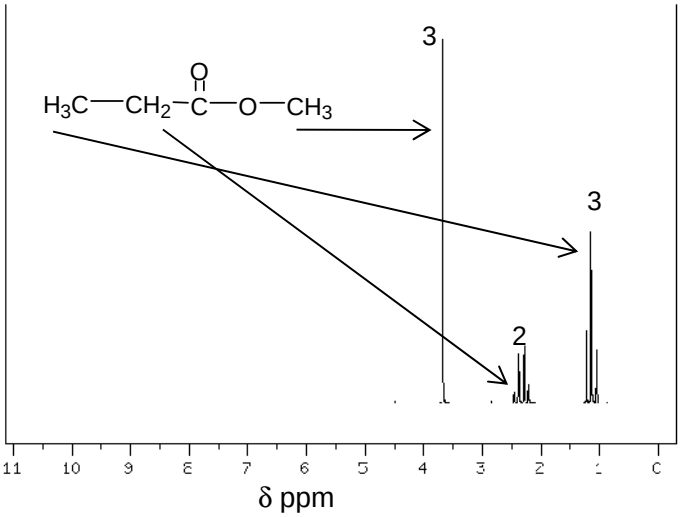
The δ is a measure in parts per million (ppm) is a relative scale of how far the frequency of the proton signal has shifted away from that for TMS.

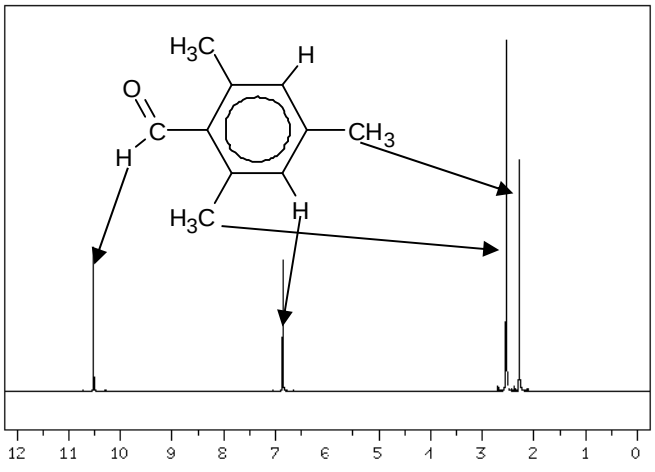


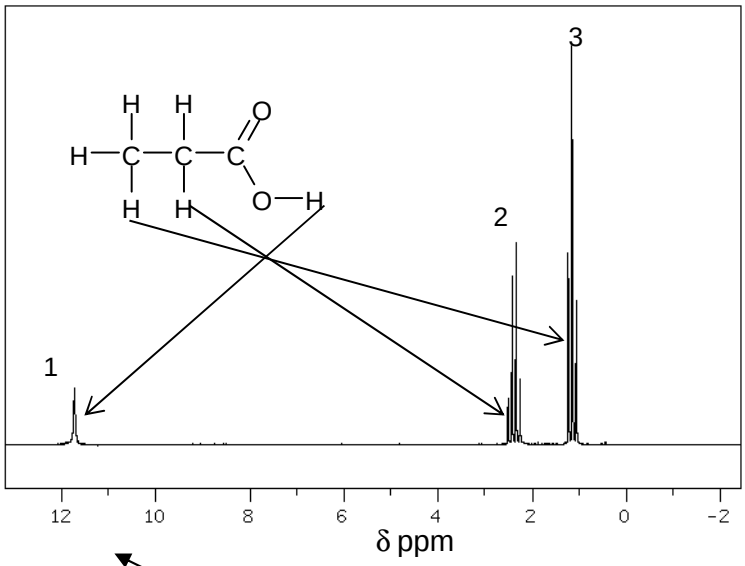
H NMR shift

The δ depends on what other atoms/groups are near the H – more electronegative groups gives a greater shift.







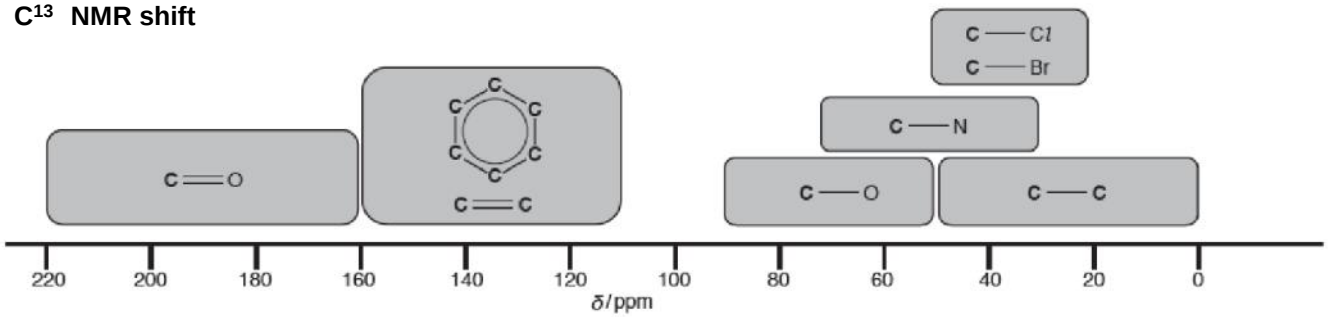


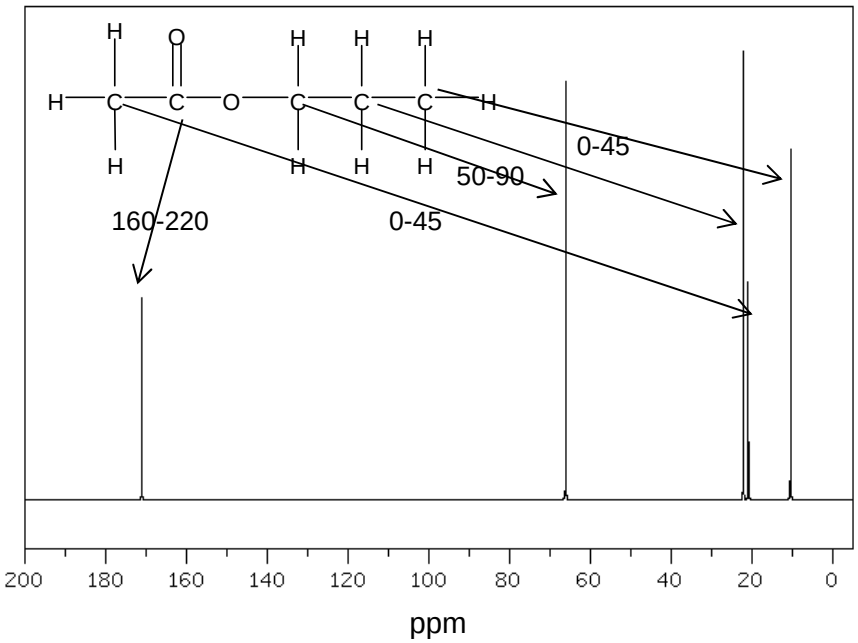
Proton exchange using D_2O

If D_2O is added to a sample then a process of proton exchange happens with the H in any O-H and N-H bonds. This has the effect of removing the peaks from the H-NMR spectra. This can help with the identification of O–H and N–H peaks on the spectra.

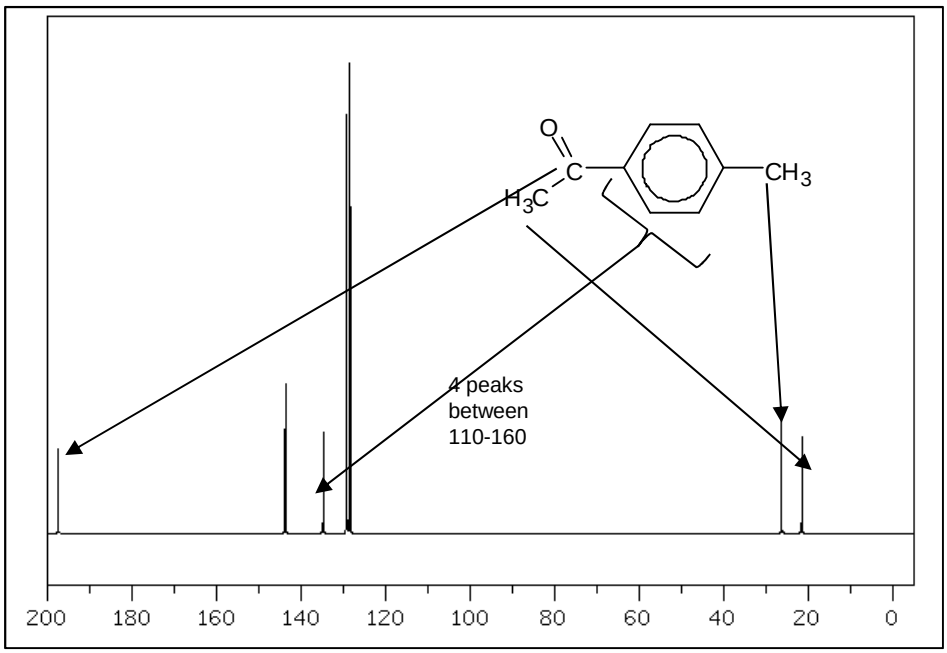
Addition of D_2O to the sample of Propanoic acid would make the peak at δ = 11.7 (ppm) in the above spectrum disappear

C^{13} NMR shift





It will not be possible to identify the exact carbon corresponding to each peak if several carbons are in the same range



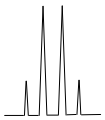
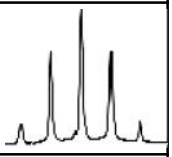
It is not possible to distinguish between similar shifts for each carbon in a benzene ring. In this example it should be possible to work out there are four different carbons in the benzene ring and these correspond to the four peaks between 120-145

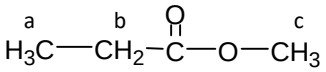
Spin-Spin coupling in H NMR

In high resolution H NMR each signal in the spectrum can be split into further lines due to inequivalent H's on neighbouring C atoms.

Nuclei in identical chemical environments do not show coupling amongst themselves!

Splitting of peak = number of inequivalent H's on neighbouring C atoms + 1

signal	singlet	doublet	triplet	quartet	quintet
appearance					
Split number of peaks	1	2	3	4	5
number of neighbouring inequivalent H atoms	0	1	2	3	4
relative size		1:1	1:2:1	1:3:3:1	1:4:6:4:1

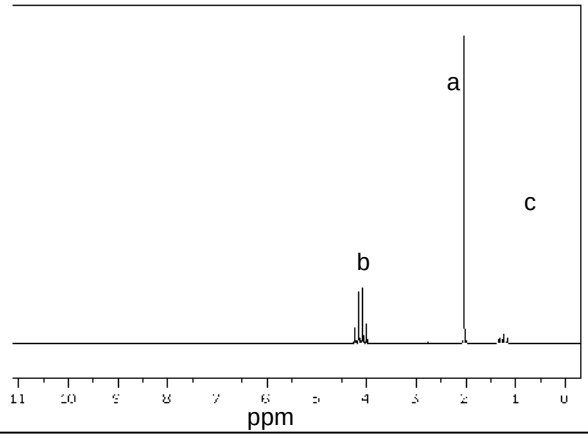


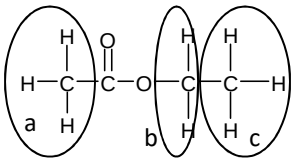
The peak due to group **a** will be a **triplet** as it is next to **b** (a carbon with 2 H's)

The peak due to group **b** will be a **quartet** as it is next to **a** (a carbon with 3H's)

The peak due to group **c** will be a **singlet** as it is next to a carbon with no H's)

For 6 split peaks use the term hextet or multiplet





The peak due to group **a** will be a **singlet** as it is next to a carbon with 0 H's
Shift 2.1-2.6
Integration trace 3

The peak due to group **b** will be a **quartet** as it is next to a carbon with 3 H's
Shift 3.7 -4.1
Integration trace 2

The peak due to group **c** will be a **triplet** as it is next to a carbon with 2 H's
Shift 0.7-1.2
Integration trace 3

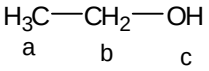
Hydrogens bonded to a nitrogen or oxygen usually do not couple with other protons and appear as singlets on the NMR spectra

The peak due to group **a** will be a **triplet** as it is next to a carbon with 2 H's
Shift 0.7-1.2
Integration trace 3

The peak due to group **b** will be a **quartet** as it is next to a carbon with 3 H's
Shift 3.7 -4.1
Integration trace 2

The peak due to group **c** will be a **singlet** as the Hydrogen is bonded to an oxygen and this does not split
Shift 0.5-5.0
Integration trace 1

Ethanol



You will not be asked to interpret splitting patterns for the protons attached to a benzene ring

Bringing it all together

1. Work out empirical formula

Elemental analysis C 66.63% H 11.18% O 22.19%

C	H	O
66.63/12	11.18/1	22.19/16
=5.5525	=11.18	=1.386875
=4	=8	=1

2. Using molecular ion peak m/z value from mass spectrum calculate Molecular formula

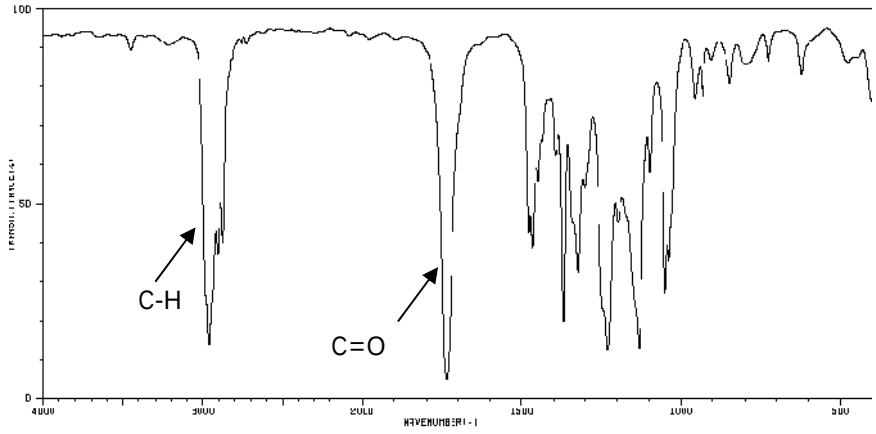
molecular ion peak m/z value= 144

Mr empirical formula C_4H_8O = 72

If Mr molecular formula 144 then compound is $C_8H_{16}O_2$

3. Use IR spectra to identify main bonds/functional group

$C_8H_{16}O_2$ could be an ester, carboxylic acid or combination of alcohol and carbonyl. Look for IR spectra for C=O and O-H bonds

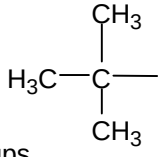


There is a C=O but no O-H absorptions, so must be an ester.

4. Use NMR spectra to give details of carbon chain

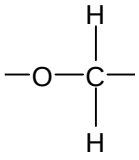
4 peaks – only 4 different environments.

singlet of area 9
At δ =0.9
Means 3 CH_3 groups



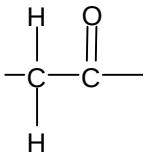
Peak at δ 4 shows H–C–O

Area 2 suggests CH_2
Quartet means next to a CH_3



Peak at δ 2.2 shows H–C=O

Area 2 suggests CH_2
Singlet means adjacent to C with no hydrogens



Peak at δ 1.2 shows R-CH_3
Area 3 means CH_3
Triplet means next to a CH_2

Put all together to give final structure