Edexcel International A Level (IAL) Chemistry

Your notes

Organic Analytical Techniques

Contents

Your notes

High Resolution Mass Spectrometry

- At AS level, you have used mass spectrometry to determine:
  - Relative atomic mass from isotopic abundance
  - Possible molecular formulae from molecular ion peaks
  - Organic structures from fragmentation patterns
- **High resolution mass spectrometry** can be used to deduce / confirm molecular formulae
- High resolution mass spectrometers provide data accurate to four, or sometimes more, decimal places
- This means that a compounds molecular formula can sometimes be deduced by using atomic masses accurate to four decimal places

Table of selected accurate relative atomic masses

Element	Hydrogen	Carbon	Nitrogen	Oxygen
Symbol	H	C	N	O
Accurate A_r	1.0078	12.0000	14.0031	15.9949

Worked Example

A compound is found to have an M_r value of 58.0417.

Determine if the compound is:

1. C_4H_{10}
2. $C_2H_6N_2$
3. C_3H_6O

Answer

Element	Hydrogen	Carbon	Nitrogen	Oxygen
Symbol	H	C	N	O
Accurate A_r	1.0078	12.0000	14.0031	15.9949

1. $C_4H_{10} = (4 \times 12.0000) + (10 \times 1.0078) = 58.0780$
2. $C_2H_6N_2 = (2 \times 12.0000) + (6 \times 1.0078) + (2 \times 14.0031) = 58.0530$

3. $C_3H_6O = (3 \times 12.0000) + (6 \times 1.0078) + (15.9949) = 58.0417$
- Therefore, the correct formula of the compound is C_3H_6O

Your notes

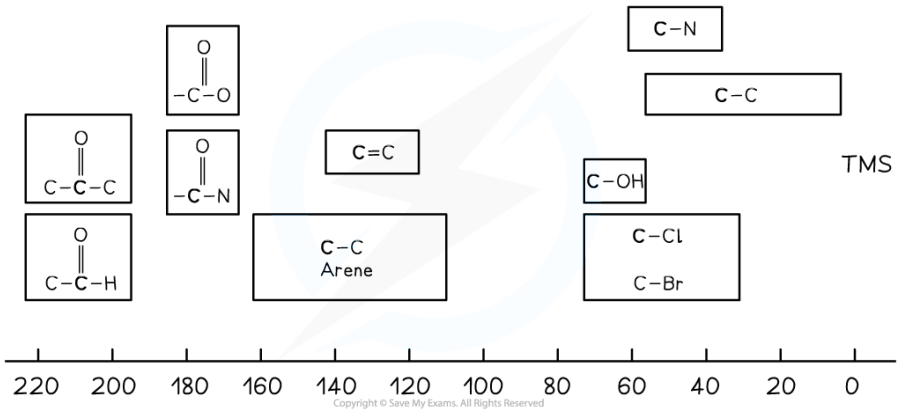
Examiner Tips and Tricks

- High resolution mass spectrometry can inform you about the molecular formula, it cannot tell you about the actual structure
  - In the worked example above, the final answer was C_3H_6O but you cannot say whether this is propanal or propanone
  - You would have to use the fragments on the mass spectrum for further detail that might help deduce more information about the structure
- Sometimes you may calculate the molecular mass of a compound and it won't match the value given in the question exactly
  - In this case, you should:
    - Firstly, double check your calculation(s)
    - Secondly, choose the compound that is the closest

Your notes

Carbon-13 NMR Spectroscopy

- Nuclear Magnetic Resonance (NMR) spectroscopy is used for analysing organic compounds
- Atoms with odd mass numbers usually show signals on NMR
  - For example, isotopes of atoms
  - Many of the carbon atoms on organic molecules are carbon-12
  - A small quantity of organic molecules will contain the isotope carbon-13 atoms
  - These will show signals on a ^{13}C NMR
- In ^{13}C NMR, the magnetic field strengths of carbon-13 atoms in organic compounds are measured and recorded on a spectrum
- Just as in ^{1}H NMR, all samples are measured against a reference compound – Tetramethylsilane (TMS)
- On a ^{13}C NMR spectrum, non-equivalent carbon atoms appear as peaks with different chemical shifts



Chemical shift values (relative to the TMS) for ^{13}C NMR analysis table

Environment of carbon	Groups	Chemical shift range δ / ppm
$-CH_2-CH_2-$	Alkyl	5–40
RCH_2Cl or Br	Halogenoalkanes	10–70
$RCOCH_2-$	Carbonyls	20–50
RCH_2NH_2	Amines	25–60
$-CH_2-O-$	Alcohols, ethers or esters	50–90
$-CH=CH-$	Alkenyl	90–150
$R-C\equiv N$	Nitriles	110–125
C_6H_6	Benzene	110–160
$R-COO-$	Ester or acids	160–185
$R-CO-$	Aldehyde or ketones	190–220

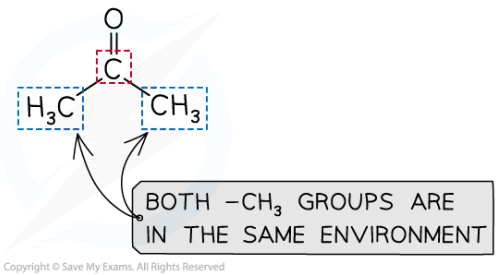
Your notes

Features of a ^{13}C NMR spectrum

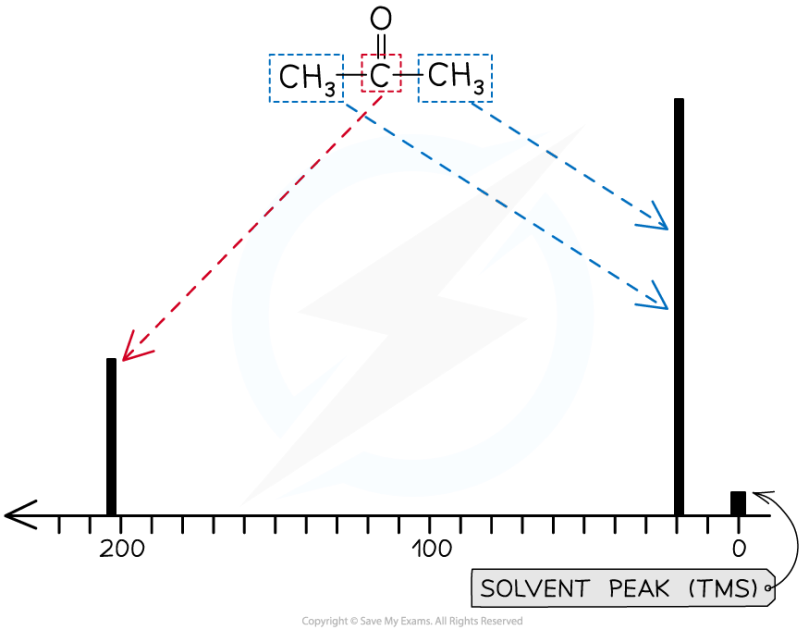
- ^{13}C NMR spectrum displays sharp single signals
  - There are no complicated spitting patterns like ^{1}H NMR spectra
- The height of each signal is **not proportional** to the number of carbon atoms present in a single molecular environment
- Carbon atoms in different chemical environments will give resonances at different chemical shifts in a ^{13}C spectrum
- As with ^{1}H NMR, tetramethylsilane is used as the standard reference point for ^{13}C at 0 ppm

Identifying ^{13}C molecular environments

- On an organic molecule, the carbon-13 environments can be identified in a similar way to the proton environments in ^{1}H NMR
- For example propanone
  - There are 2 molecular environments
  - 2 signals will be present on its ^{13}C NMR spectrum



There are 2 molecular environments in propanone



The ^{13}C NMR of propanone showing 2 signals for the 2 molecular environments

Examiner Tips and Tricks

Counting the number of ^{13}C resonances should be the first step in analysing a spectrum. For example, it is possible to differentiate the three isomers of dihydroxybenzene quickly be considering the symmetry of the molecules and therefore the number of resonances expected in their spectra.

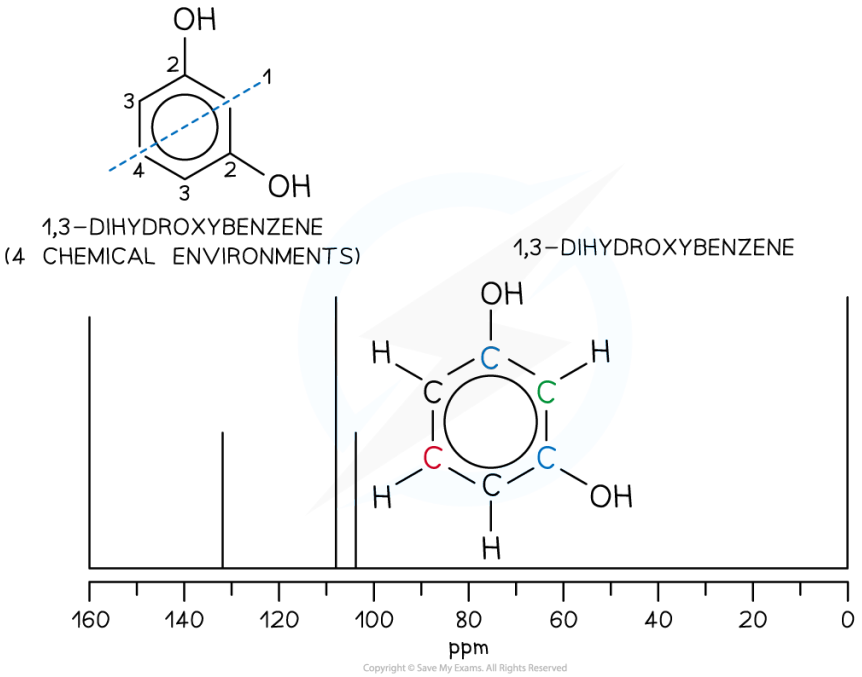
Worked Example

Your notes

How many chemical environments and therefore number of peaks / resonances would be in a ^{13}C spectra of 1,3-dihydroxybenzene?

Answer:

4 chemical environments and therefore four peaks / resonances on the spectra



Your notes

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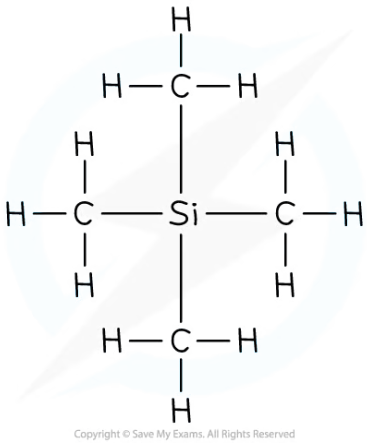
Low Resolution Proton NMR

- **Nuclear Magnetic Resonance (NMR)** spectroscopy is used for analysing organic compounds
- All samples are measured against a reference compound – **Tetramethylsilane (TMS)**



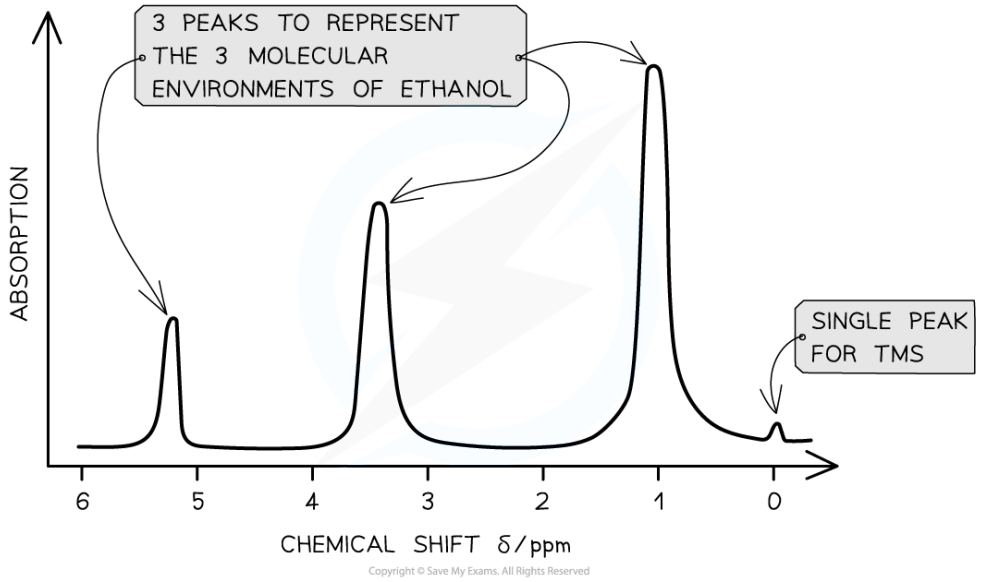
Tetramethylsilane is the common reference compound for NMR spectroscopy

- TMS shows a single sharp peak on NMR spectra, at a value of zero
- TMS is also used because it is:
  - Non toxic.
  - Does not react with the sample.
  - Easily separated from the sample molecule due to its low boiling point.
  - Produces one strong, sharp absorption peak on the spectrum.
- Sample peaks are then plotted as a 'shift' away from this reference peak
- This gives rise to 'chemical shift' values for protons on the sample compound
- Chemical shifts are measured in parts per million (ppm)

Features of a ^{1}H NMR spectrum

- NMR spectra shows the intensity of each peak against their chemical shift
- The area under each peak gives information about the number of protons in a particular environment
- The height of each peak shows the intensity / absorption from protons
- A single sharp peak is seen to the far right of the spectrum

- This is the reference peak from TMS
- Usually at chemical shift 0 ppm



A low resolution ^1H NMR for ethanol showing the key features of a spectrum

Molecular environments

- ^1H nuclei that have different neighboring atoms (said to have different **chemical environments**) absorb at slightly different field strengths
- The difference environments are said to cause a **chemical shift** of the absorption
  - Ethanol has the structural formula CH_3CH_2OH
  - There are 3 chemical environments: $-CH_3$, $-CH_2$ and -OH
- The hydrogen atoms in these environments will appear at 3 different chemical shifts
- Different types of protons are given their own range of chemical shifts

Worked Example

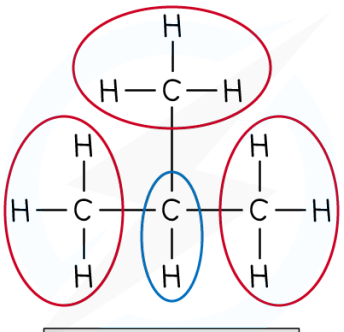
How many different ^1H chemical environments occur in 2–methylpropane?

Answer:

Two different ^1H chemical environments occur in 2–methylpropane

- The three methyl groups are in the same ^1H environment
  - The lone hydrogen is in its own ^1H environment

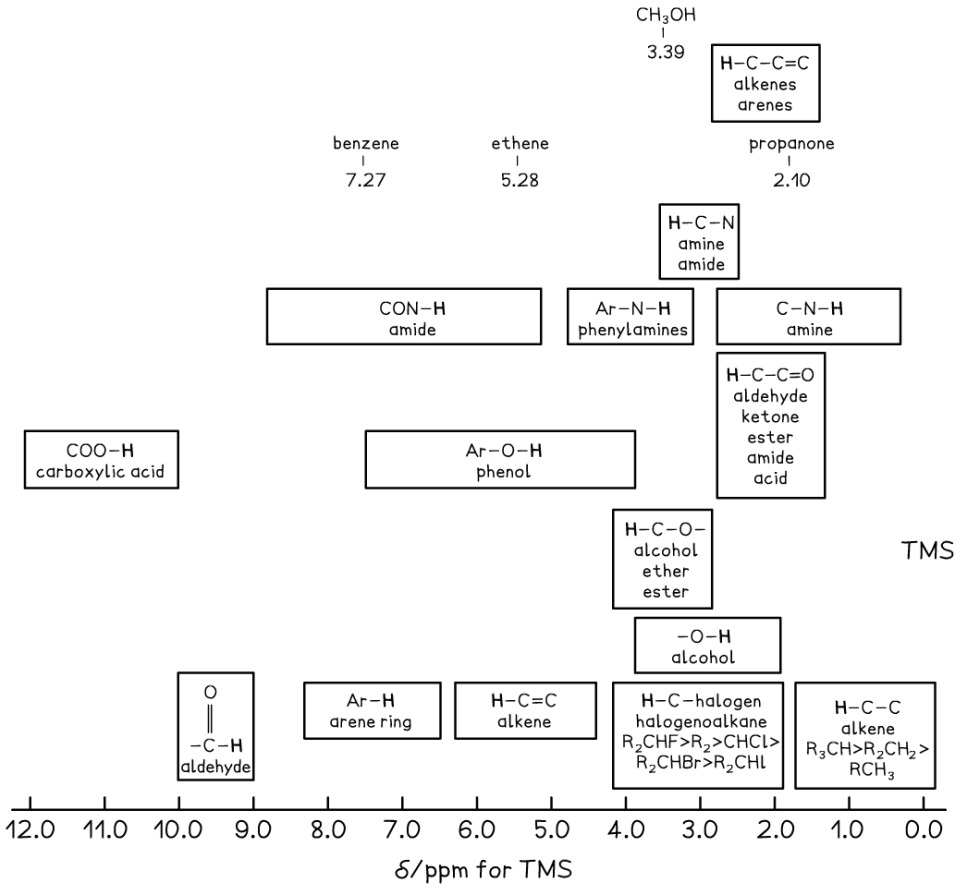
Your notes



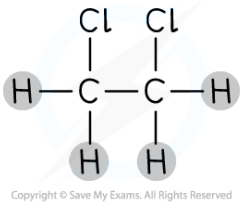
Your notes

Chemical Shift Values for ^1H Molecular Environments Table

Environment of proton	Chemical shift range δ/ppm
ROH	0.5–5.5
RCH_3	0.7–1.2
RNH_2	1.0–4.5
R_2CH_2	1.2–1.4
R_3CH	1.4–1.6
RCOCH–	2.1–2.6
ROCH–	3.1–3.9
RCH_2Cl or Br	3.1–4.2
RCOOCH–	3.7–4.1
RC=CH–	4.5–6.0
RCHO	9.0–10.0
RCOOH	10.0–12.0



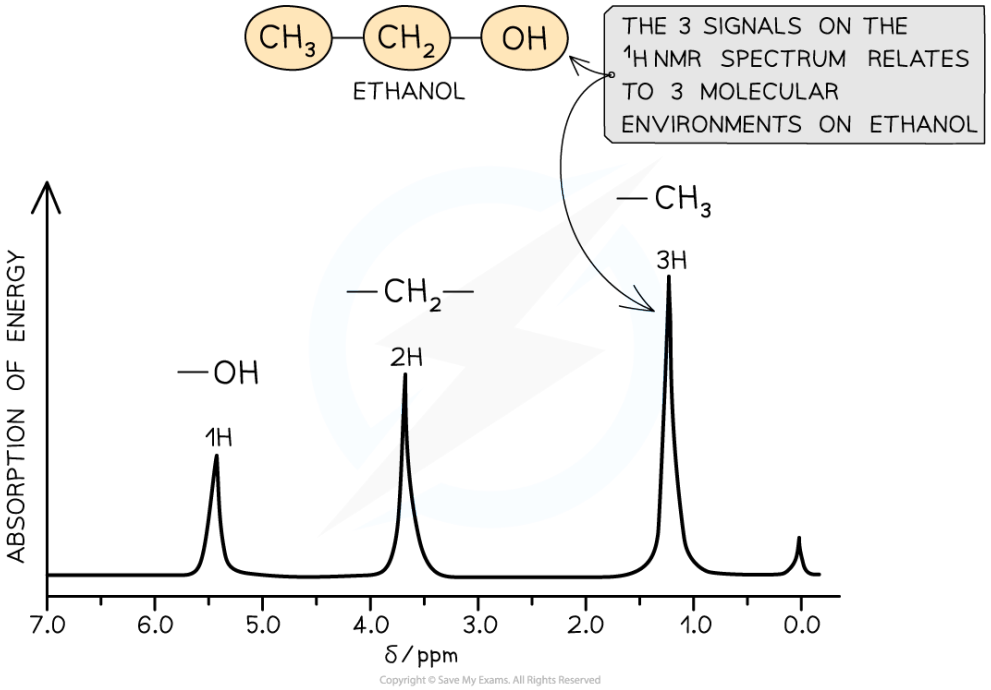
- Protons in the same chemical environment are chemically equivalent
  - 1,2-dichloroethane, Cl-CH_2-CH_2-Cl has one chemical environment as these four hydrogens are all exactly equivalent
- Each individual peak on a ^{1}H NMR spectrum relates to protons in the same environment
  - Therefore, 1,2-dichloroethane would produce one single peak on the NMR spectrum as the protons are in the same environment



Low resolution ^{1}H NMR

- Peaks on a low resolution NMR spectrum refers to molecular environments of an organic compound
  - Ethanol has the molecular formula CH_3CH_2OH

- This molecule as 3 separate environments: -CH_3, -CH_2, -OH
- So 3 peaks would be seen on its spectrum at 1.2 ppm (-CH_3), 3.7 ppm (-CH_2) and 5.4 ppm (-OH)
- The strengths of the absorptions are proportional to the number of equivalent ^1H atoms causing the absorption and are measured by the area underneath each absorption peak
- Hence, the areas of absorptions of -CH_3, -CH_2, -OH are in the ratio of 3:2:1 respectively



A low resolution NMR spectrum of ethanol showing 3 peaks for the 3 molecular environments

Your notes

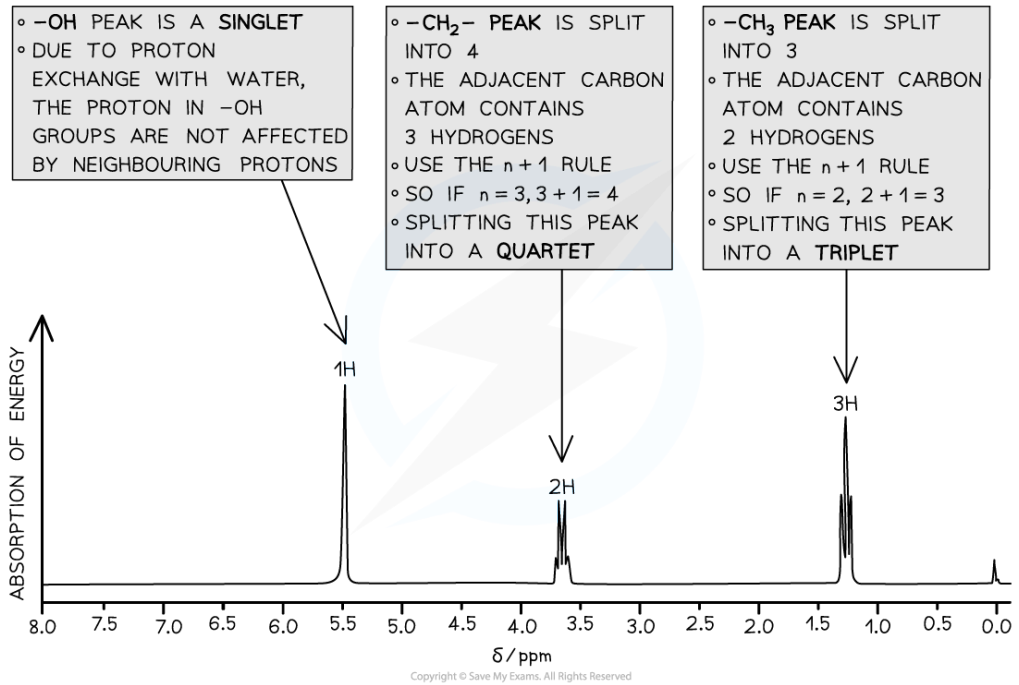
Your notes

High Resolution Proton NMR

- More structural details can be deduced using high resolution NMR
- The peaks observed on a high resolution NMR may sometimes have smaller peaks clustered together
- The splitting pattern of each peak is determined by the number of protons on neighbouring environments

The number of peaks a signal splits into = n + 1

(Where n = the number of protons on the adjacent carbon atom)

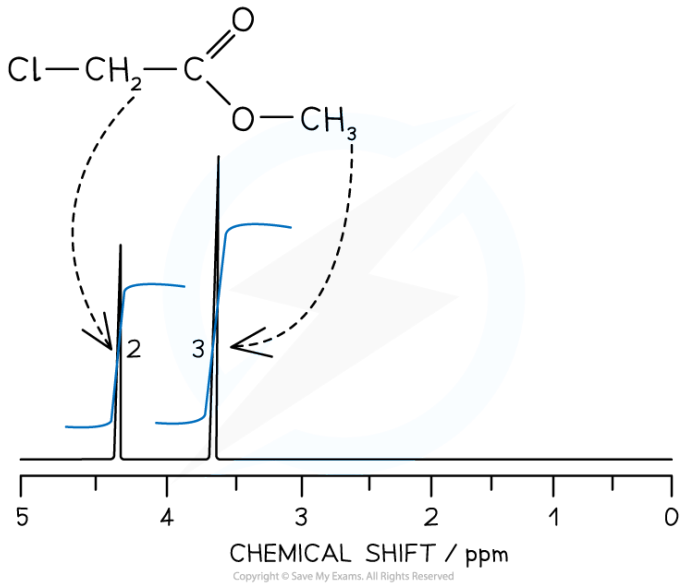


High resolution ^1H NMR spectrum of ethanol showing the splitting patterns of each of the 3 peaks. Using the n+1, it is possible to interpret the splitting pattern

- Each splitting pattern also gives information on relative intensities
  - A doublet has an intensity ratio of 1:1 – each peak is the same intensity as the other
  - In a triplet, the intensity ratio is 1:2:1 – the middle of the peak is twice the intensity of the 2 on either side
  - In a quartet, the intensity ratio is 1:3:3:1 – the middle peaks are three times the intensity of the 2 outer peaks

Integrated spectra

- In ^{1}H NMR, the relative areas under each peak give the ratio of the number of protons responsible for each peak
- The NMR spectrometer measures the area under each peak, as an integration spectra
  - This provides invaluable information for identifying an unknown compound
- The ^{1}H NMR of methyl chloroethanoate, $ClCH_2COOCH_3$, will show an integration spectra in the peak area ratio of 2:3
  - 2 for the protons in the CH_2
  - 3 for the protons in CH_3



Spin-Spin Splitting

- A ^{1}H NMR peak can show you the structure of the molecule but also the peaks can be split into sub-peaks or splitting patterns
- These are caused by a proton's spin interacting with the spin states of nearby protons that are in different environments
  - This can provide information about the number of protons bonded to adjacent carbon atoms
  - The splitting of a main peak into sub-peaks is called spin-spin splitting

The n+1 rule

- The number of sub-peaks is one greater than the number of adjacent protons causing the splitting
  - For a proton with *n* protons attached to an adjacent carbon atom, the number of sub-peaks in a splitting pattern = *n*+1
- When analysing spin-spin splitting, it shows you the number of hydrogen atoms on the immediately adjacent carbon atom

Your notes

- These are the splitting patterns that you need to be able to recognise from a ^1H spectra:

Your notes

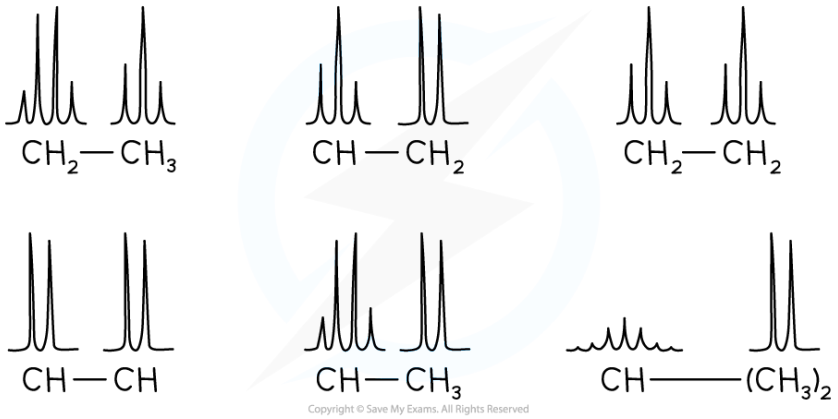
^1H NMR Peak Splitting Patterns Table

Number of adjacent protons (n)	Splitting pattern using the n+1 rule the peak will split into...	Relative intensities in splitting pattern	Shape
0	1, singlet	1	
1	2, doublet	1 : 1	
2	3, triplet	1 : 2 : 1	
3	4, quartet	1 : 3 : 3 : 1	

- Splitting patterns must occur in pairs, because each protons splits the signal of the other
- There are some common splitting pairs you will see in a spectrum however you don't need to learn these but can be worked out using the *n*+1 rule
  - You will quickly come to recognise the triplet / quartet combination for a CH_3CH_2 because it is so common

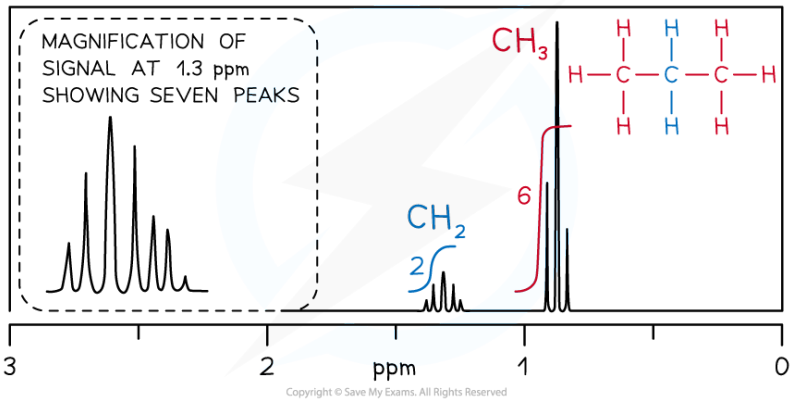
Common pair of splitting patterns

- A quartet and a triplet in the same spectrum usually indicate an ethyl group, CH_3CH_2-
- The signal from the CH_3 protons is split as a triplet by having two neighbours
- The signal from the CH_2 protons is split as a quartet by having three neighbours
- Here are some more common pairs of splitting patterns



Common pairs of splitting patterns

^{1}H NMR spectrum of propane



- The CH_2 signal in propane (blue) is observed as a heptet because it has six neighbouring equivalent H atoms (n+1 rule), three either side in two equivalent CH3 groups
- The CH3 groups (red) produce identical triplets by coupling with the CH_2 group

Worked Example

For the compound $(CH_3)_2CHOH$ predict the following:

i) the number of peaks

ii) the type of proton and chemical shift (using the Data sheet)

iii) the relative peak areas

iv) the split pattern

Answers:

Your notes

i) There are 3 different hydrogen environments - the methyl CH_3 hydrogens, the CH hydrogen and the OH hydrogen. This means that there are 3 peaks

ii) By looking the 3 different environments on the data sheet, we can assign chemical shifts:

- $(C\mathbf{H_3})_2CHOH$ at 0.7 - 1.2 ppm
- $(CH_3)_2C\mathbf{H}OH$ at 3.1 - 3.9 ppm
- $(CH_3)_2CHO\mathbf{H}$ at 0.5 - 5.5 ppm

iii) There are 6 hydrogens in the $(CH_3)_2$, 1 hydorgen in the CH and 1 hydrogen in the OH. This gives an overall ratio of 6 : 1 : 1

iv) The methyl groups in $(C\mathbf{H_3})_2CHOH$ have one neighbouring hydrogen in the CH, which means that its splitting pattern is a doublet (1 + 1 = 2)

The CH in $(CH_3)_2C\mathbf{H}OH$ has 6 neighbouring hydrogens in the 2 methyl groups, which means that it ssplitting pattern is a heptet (6 + 1 = 7) - this is more commonly just referred to as a multiplet

Your notes

Your notes

Thin-Layer Chromatography

Principles of Chromatography

- Chromatography is a technique that enables the separation of mixtures and includes:
  - thin-layer chromatography (TLC)
  - column chromatography (CC)
  - gas chromatography (GC) - sometimes called gas-liquid chromatography (GLC)
- All of these chromatography techniques makes use of the principle that components in a mixture when dissolved in a fluid (**mobile phase**), will flow through another material (**stationary phase**) at varying rates
- The rate of separation depends upon how the components in the mixture interact with the stationary phase (their **retention**) and how soluble they are in the mobile phase

Summary Table of Chromatography Component Phases

Chromatography technique	Stationary phase	Mobile Phase
Thin-layer (TLC)	Solid silica on a plastic or glass plate	Liquid solvent, e.g. water or organic
Column (CC)	Solid silica	Liquid solvent, e.g. water or organic
Gas – Liquid (GLC)	Microscopic liquid film on a solid support	Inert carrier gas, e.g. nitrogen

Thin-layer chromatography

- Thin-layer chromatography (TLC) is a technique used to analyse small samples via separation
  - For example, we could separate a dye out to determine the mixture of dyes in a forensic sample
- There are 2 phases involved in TLC:
  - **Stationary phase**
    - This phase is commonly thin metal sheet coated in alumina (Al_2O_3) or silica (SiO_2)
    - The solute molecules **adsorb** onto the surface
    - Depending on the strength of interactions with the stationary phase, the separated components will travel particular distances through the plate

- The more they interact with the stationary phase, the more they will 'stick' to it

- **Mobile phase**
  - Flows over the stationary phase
  - It is a polar or non-polar liquid (solvent) that carries components of the compound being investigated
  - Polar solvents - water or alcohol
  - Non-polar solvents - alkanes

- If the sample components are coloured, their spots are easily identifiable on the chromatogram
- If the sample components are not coloured, then we can **locate** the spots on the chromatogram and draw around them in pencil
  - To **locate** the spots we can use:
    - UV light
    - Ninhydrin (carcinogenic)
    - Iodine vapour

Conducting a TLC analysis

- **Step 1:**

  Prepare a beaker with a small quantity of solvent

- **Step 2:**

  On a TLC plate, draw a horizontal line at the bottom edge (in pencil)

  This is called the **baseline**

- **Step 3:**

  Place a spot of pure reference compound on the left of this line, then a spot of the sample to be analysed to the right of the baseline and allow to air dry

  The reference compounds will allow identification of the mixture of compounds in the sample

- **Step 4:**

  Place the TLC plate inside the beaker with solvent - making sure that the solvent does not cover the spot - and place a lid to cover the beaker

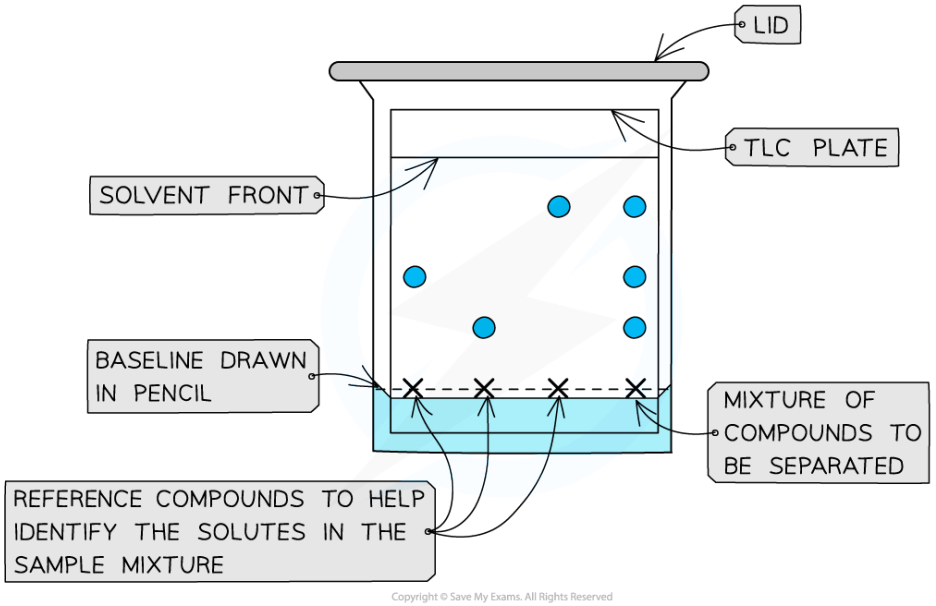
  The solvent will begin to travel up the plate, dissolving the compounds as it does

- **Step 5:**

  As solvent reaches the top, remove the plate and draw another pencil line where the solvent has reached, indicating the **solvent front**

Your notes

The sample's components will have separated and travelled up towards this solvent front



A dot of the sample is placed on the baseline and allowed to separate as the mobile phase flows through the stationary phase; The reference compound/s will also move with the solvent

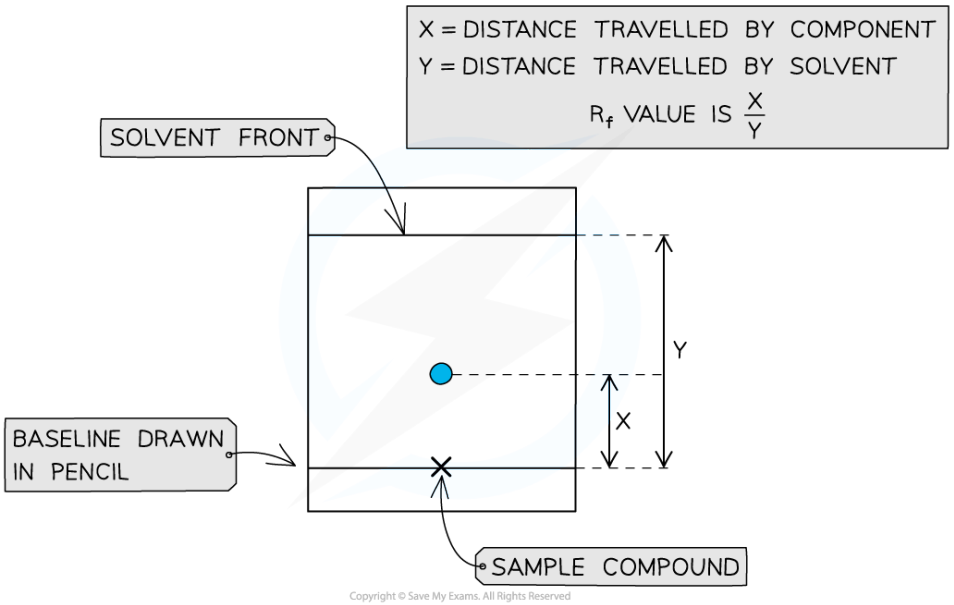
Retention factor, R_f, values

- A TLC plate can be used to calculate R_f values for compounds

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

- These values can be used alongside other analytical data to deduce composition of mixtures

Your notes



R_f values can be calculated by taking 2 measurements from the TLC plate

Examiner Tips and Tricks

The baseline on a TLC plate must be drawn in pencil

Any other medium would interact with the sample component and solvents used in the analysis process.

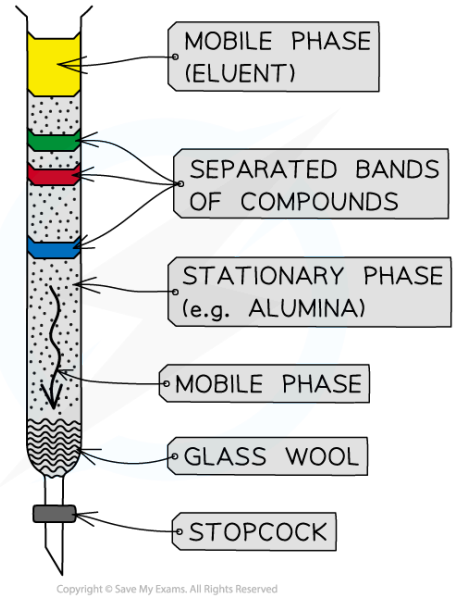
Your notes

Your notes

High-Performance Liquid & Gas Chromatography

Column Chromatography

- In column chromatography, the column is often a long vertical glass tube or in the laboratory a burette is suitable
- An inert solid (usually powdered silica gel or alumina) is the **stationary phase** which is placed in the column
- A liquid solvent phase, **mobile phase**, is added into the column until it is saturated with solvent
  - Care should be taken when setting up the column because cracks in the stationary phase can lead to issues during separation
- The sample mixture is dissolved in the solvent and introduced at the top of the column
  - A pipette is usually used to carefully add the dissolved sample to the top of the column
  - The aim here is to add the sample without disturbing the surface of the column so that the sample runs from one level through the column
- Once the sample has been added, more solvent (**eluent**) is added on top of the sample
- As the solvent runs through, fresh solvent is added to the top of the column so that it does not dry out
- The sample flows through the column via gravity
  - This process can be sped up by pushing the sample and mobile phase through the column
  - In school laboratories, this can be achieved by attaching a gas syringe to the top of the chromatography column
  - In industrial / research laboratories, this is achieved by attaching an air line to the top of the chromatography column
- The component with the greatest attraction / affinity to the stationary phase takes the longest time to flow through the column
- If the components are coloured, then they can be identified using the R_f value
- If the components are colourless, then other techniques such as fluorescence under UV light can be used to show their position in the column

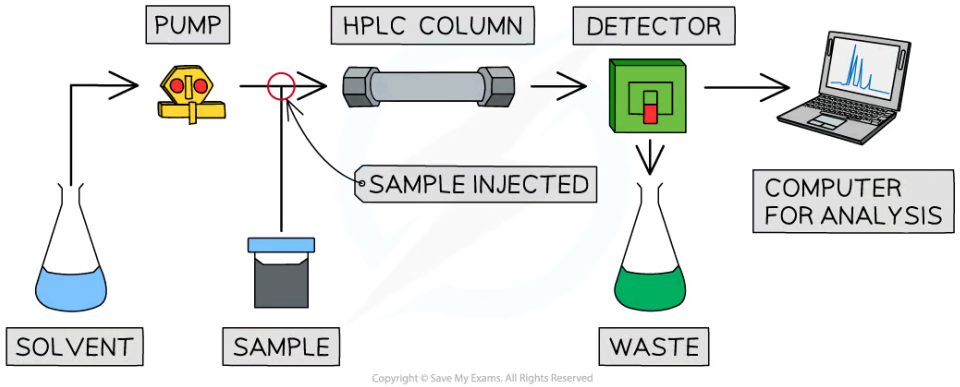


Column chromatography showing the separation of coloured compounds in a glass burette

High performance liquid chromatography, HPLC

- This is essentially the same as column chromatography
- The main differences are that:
  - The column doesn't work via gravity, the sample is pumped through by the solvent
  - The particles of the stationary phase are much smaller, leading to greater separation of compounds
  - There is a detector at the end of the column which measures **retention time**
    - Retention time is the time taken from the sample being injected to the sample being detected
  - HPLC is automated so the results are obtained quicker
  - The HPLC equipment typically includes a computer which allows for quicker analysis and comparison of results against known compounds in a database

Your notes



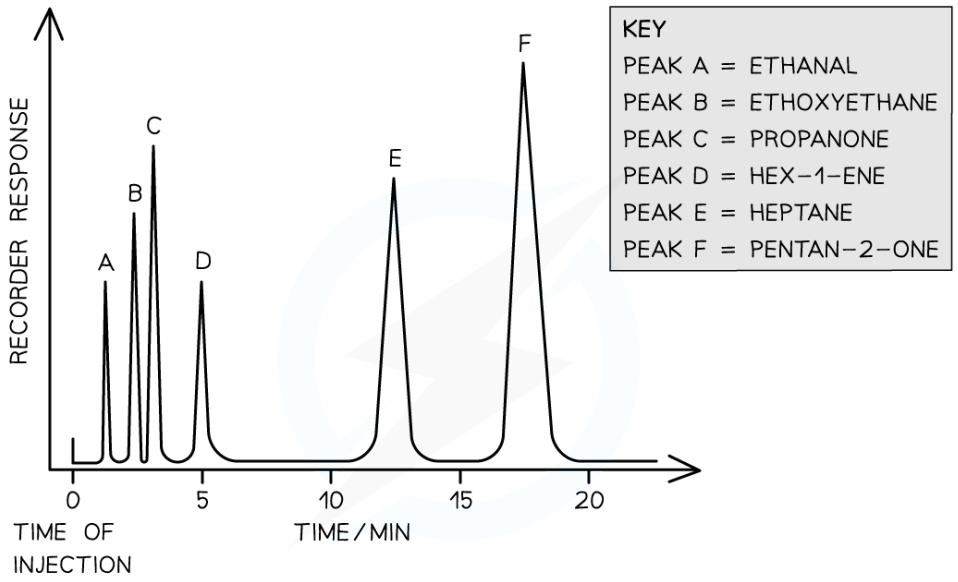
Your notes

Gas-Liquid Chromatography, GLC

- Gas-Liquid Chromatography (GLC) is used for analysing:
  - Gases
  - Volatile liquids
  - Solids in their vapour form
- The stationary phase:
  - This method uses a long coiled column for the stationary phase
  - Normally a non-volatile liquid is the stationary phase in GLC
- The Mobile phase
  - An **inert** carrier gas (e.g. helium, nitrogen) moves the sample molecules through the stationary phase
- The sample is injected into the column through a self-sealing disc and the vapour formed is carried through the stationary phase using the inert-gas mobile phase

Retention times

- Once sample molecules reach the detector, their **retention times** are recorded
  - This is the time taken for a component to travel through the column
  - It depends upon the attraction between the solute and the stationary and mobile phases as well as the nature of the solute
- The retention times are recorded on a chromatogram where each peak represents a volatile compound in the analysed sample
  - The relative sizes (i.e. areas) of the peaks are related to how much of each compound is present in the mixture
- Retention times are then compared with data book values to identify unknown molecules



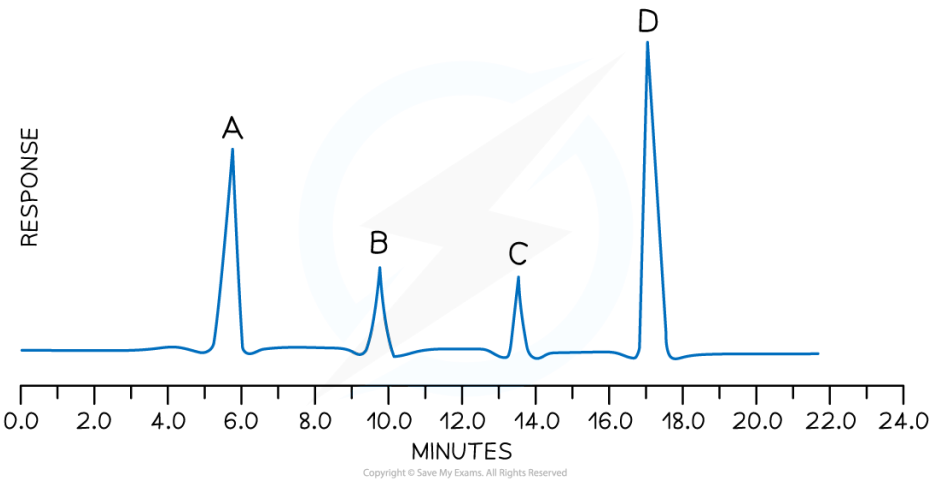
THE RETENTION TIME OF EACH PEAK IS DEPENDENT ON THE TIME TAKEN FOR THE SAMPLE MOLECULES TO TRAVEL THROUGH THE GLC COLUMN

Your notes

A gas chromatogram of a volatile sample compound has six peaks. Depending on each molecule's interaction with the stationary phase, each peak has its own retention time

Worked Example

Analysis of a compound by GLC shows the presence of four components, **A**, **B**, **C** and **D**.



i) Which compound is present in the greatest quantity?

ii) Which compounds were present in equal amounts?

iii) Which compound had the strongest interaction with the stationary phase?

Answers:

i) **D** (the larger the relative size of the peak, the greater the quantity of that substance present)

ii) **B** and **C** (the peak sizes are equal)

iii) **D** (the larger the retention time, the greater the interaction of that component with the stationary phase)

Your notes

Uses and limitations of HPLC and GLC

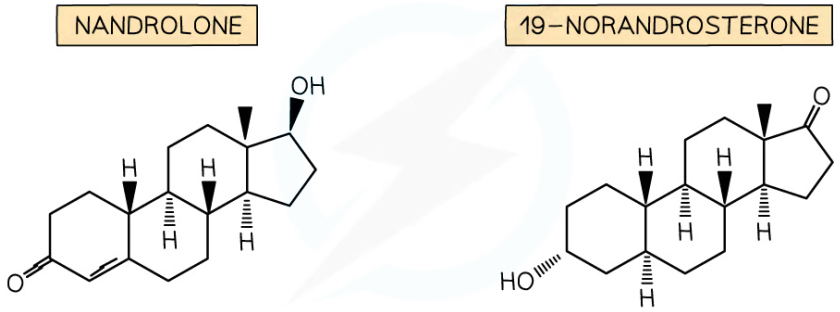
- HPLC and GLC are used to separate small amounts of components from a mixture
- They are often used to:
  - Provide **forensic evidence**
  - **Drug testing**, particularly in sports
  - Analysis of environmental pollution
  - Detecting explosives in baggage
- However, they are not very useful at identifying specific compounds, in legal terms - positive identification or beyond reasonable doubt
- This is because:
  - Different compounds may have the same retention time
  - It can be difficult to manage the conditions, e.g. temperature and pressure
  - Some unknown compounds may not have a reference for comparison in the databases
- For this reason, HPLC and GLC are often coupled with other analytical techniques, most commonly mass spectrometry
  - This results in HPLC-MS and GC-MS (GLC is sometimes abbreviated to GC)
  - This means that components can be separated from mixtures and then analysed all within one machine

Problems with drug testing

- GC-MS is the most common method of drug detection in sports due to the accepted reliability of the results
- Even then, there can be problems
- One publicised problem is around the use of anabolic steroids
  - Anabolic steroids can be used by athletes to improve muscle growth, increase production of red blood cells and strengthen bones by increasing their density

- They are also used to treat conditions such as osteoporosis, anaemia and some cancers

- One high profile anabolic steroid is nandrolone which is metabolised into a similar chemical called 19–norandrosterone
  - Competitors in the Olympic Games are routinely urine tested for the presence of 19–norandrosterone
  - A urine content above 2 nanograms per cm^3 (0.000000002 g per cm^3) is a positive test and can result in the athlete being disqualified and risking further sanctions
  - There is debate about nandrolone due to its genuine medical applications and the fact that it may be in some nutritional and dietary supplements



Your notes