Spectroscopy in a Suitcase

Students' resource

Introduction to mass spectrometry, infrared spectroscopy, nuclear magnetic resonance spectroscopy and ultraviolet–visible spectroscopy. Also includes worksheets for spectroscopy activities.

Introduction to spectroscopy

What is spectroscopy?

One of the frustrations of being a chemist is the fact that no matter how hard you stare at your test tube or round-bottomed flask you can't actually see the individual molecules you have made! Even though your product looks the right colour and seems to give sensible results when you carry out chemical tests, can you be really sure of its precise structure?

Fortunately, help is at hand. Although you might not be able to 'see' molecules, they do respond when light energy hits them, and if you can observe that response, then maybe you can get some information about that molecule. This is where **spectroscopy** comes in.

Spectroscopy is the study of the way **light** (electromagnetic radiation) and matter interact.
There are a number of different types of spectroscopic technique and the basic principle shared by all is to shine a beam of a particular electromagnetic radiation onto a sample and observe how it responds to such a stimulus: This allows scientists to obtain information about the structure of the molecules.

What is light?

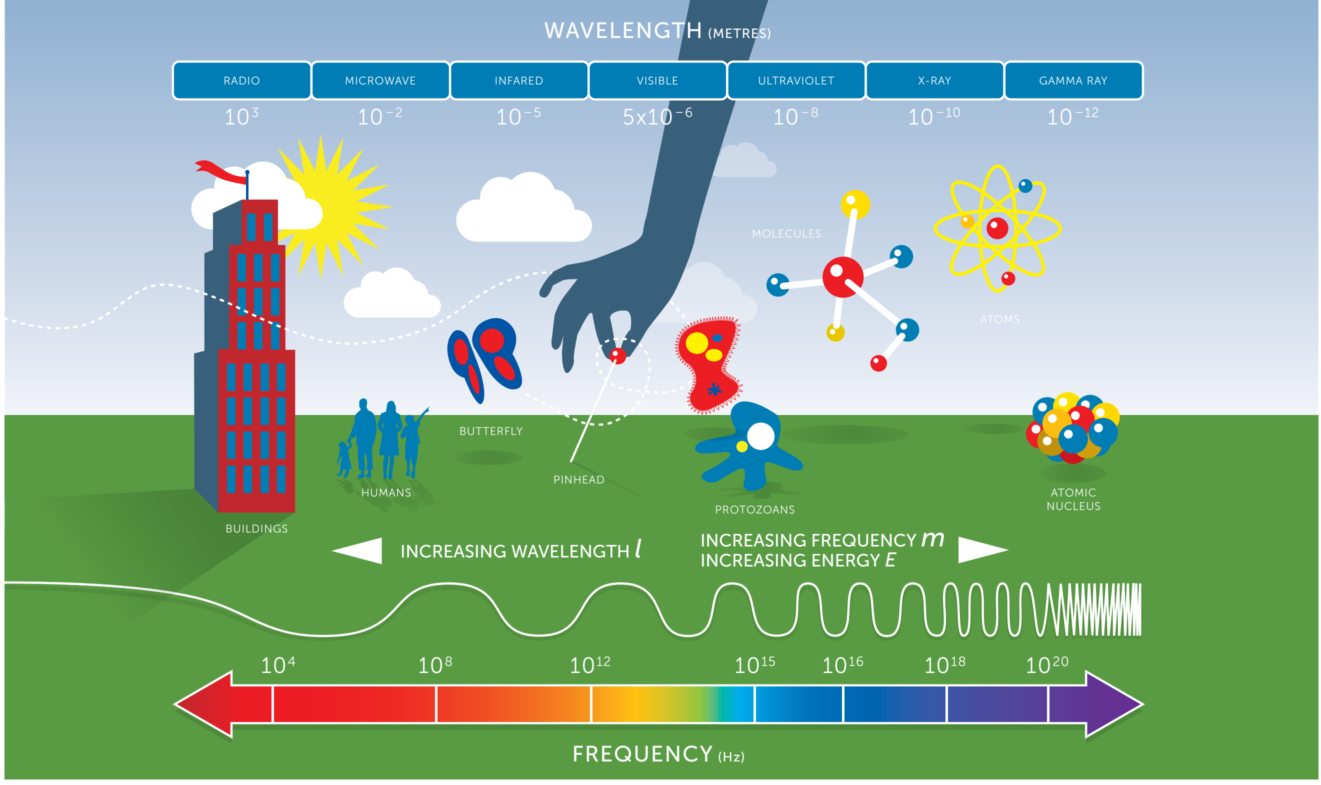
Light carries **energy** in the form of tiny particles known as photons. Each photon has a discrete amount of energy, called a quantum. **Light** has wave properties with **characteristic wavelengths** and **frequency** (see the diagram below).

The energy of the photons is related to the frequency (ν) and wavelength (λ) of the light through the two equations:

$E = h\nu$ and $\nu = c/\lambda$

(where h is Planck's constant and c is the speed of light). Therefore, high-energy radiation (light) will have high frequencies and short wavelengths.

The range of wavelengths and frequencies in light is known as the **electromagnetic spectrum**. This spectrum is divided into various regions extending from very short-wavelength, high-energy radiation (including gamma rays and X-rays) to very long-wavelength, low-energy radiation (including microwaves and broadcast radio waves). The visible region (white light) only makes up a small part of the electromagnetic spectrum, considered to be 380–770 nm. [Note that a nanometre is 10^{-9} metres.]



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When matter absorbs electromagnetic radiation the change which occurs depends on the type of radiation, and therefore the amount of energy, being absorbed. Absorption of energy causes an electron or molecule to go from an initial energy state (ground state) to a high-energy state (excited state) which could take the form of the increased **rotation, vibration or electronic excitation.** By studying this change in energy state scientists are able to learn more about the physical and chemical properties of the molecules.

- **Radio waves** can cause nuclei in some atoms to change magnetic orientation and this forms the basis of a technique called **nuclear magnetic resonance (NMR)** spectroscopy.
- Molecular rotations are excited by **microwaves.**
- Electrons are promoted to higher orbitals by **ultraviolet** or **visible** light.
- Vibrations of bonds are excited by **infrared** radiation.

The energy states are said to be quantised because a photon of precise energy and frequency (or wavelength) must be absorbed to excite an electron or molecule from the ground state to a particular excited state.

Because molecules have a unique set of energy states that depend on their structure, **IR, UV-visible** and **NMR** spectroscopy will provide valuable information about the structure of the molecule.

To 'see' a molecule we need to use light having a wavelength smaller than the molecule itself (approximately 10^{-10} m). Such radiation is found in the X-ray region of the electromagnetic spectrum and is used in the field of **X-ray crystallography.** This technique yields very detailed three-dimensional pictures of molecular structures – the only drawback being that it requires high-quality crystals of the compound being studied. Although other spectroscopic techniques do not yield a three-dimensional picture of a molecule they do provide information about its characteristic features and are therefore used routinely in structural analysis.

Mass spectrometry is another useful technique used by chemists to help them determine the structure of molecules. Although sometimes referred to as mass spectro***scopy*** it is, by definition, not a spectroscopic technique as it does not make use of electromagnetic radiation. Instead the molecules are ionised using high-energy electrons and these molecular ions subsequently undergo fragmentation. The resulting mass spectrum contains the mass of the molecule and its fragments, which allows chemists to piece together its structure.

In all spectroscopic techniques only very small quantities (milligrams or less) of sample are required, but, in mass spectrometry the sample is destroyed in the fragmentation process, whereas the sample can be recovered after using IR, UV-visible and NMR spectroscopy.

TECHNIQUE	RADIATION		WHAT CAN IT SEE?
Nuclear magnetic resonance (NMR) spectroscopy	Radio waves (10^3 m)	Nuclei flipping magnetic spin 10^3 m	How neighbouring atoms of certain nuclei (e.g. 1H, 13C, 19F, 31P) in a molecule are connected together, as well as how many atoms of these types are present in different locations in the molecule.
Infrared spectroscopy	Infrared (10^{-5} m)	10^{-5} m NOTE Molecule vibrations	The functional groups that are present in a molecule.
Ultraviolet–visible spectroscopy	Ultraviolet (10^{-8} m)	10^{-8} m NOTE Electrons promoted to higher energy state	Conjugated systems (i.e. alternating single and double bonds) in organic molecules as well as the metal–ligand interactions in transition metal complexes.
X-ray crystallography	X-rays (10^{-10} m)	10^{-10} m X-ray	How all the atoms in a molecule are connected in a three-dimensional arrangement.
Mass spectrometry	Non-spectroscopic technique	Molecules fragment	The mass to charge ratio of the molecular ion (i.e. the molecular weight) and the fragmentation pattern, which may be related to the structure of the molecular ion.

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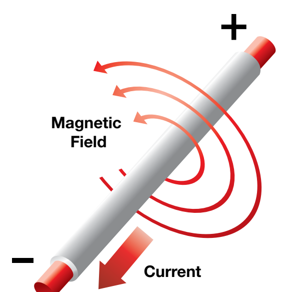
Introduction to mass spectrometry (MS)

Mass spectrometry (MS)

This is a very powerful analytical tool that can provide information on both molecular mass and molecular structure.

Background information

Any wire carrying an electric current – a flow of negative electrons – has a magnetic field surrounding it, known as an electromagnetic field. If a current-carrying wire is placed in an external magnetic field it would 'jump' as it is deflected when the two magnetic fields interact.



An electromagnetic field can also be generated by a flow of positively charged ions, such as those generated by a mass spectrometer.

Mass spectrometry

This technique is about 1000 times more sensitive than other analytical techniques such as IR or NMR spectroscopy.

VALUE	SYMBOL	NAME
10^{3} g	kg	kilogram
10^{-3} g	mg	milligram
10^{-6} g	μg	microgram
10^{-9} g	ng	nanogram

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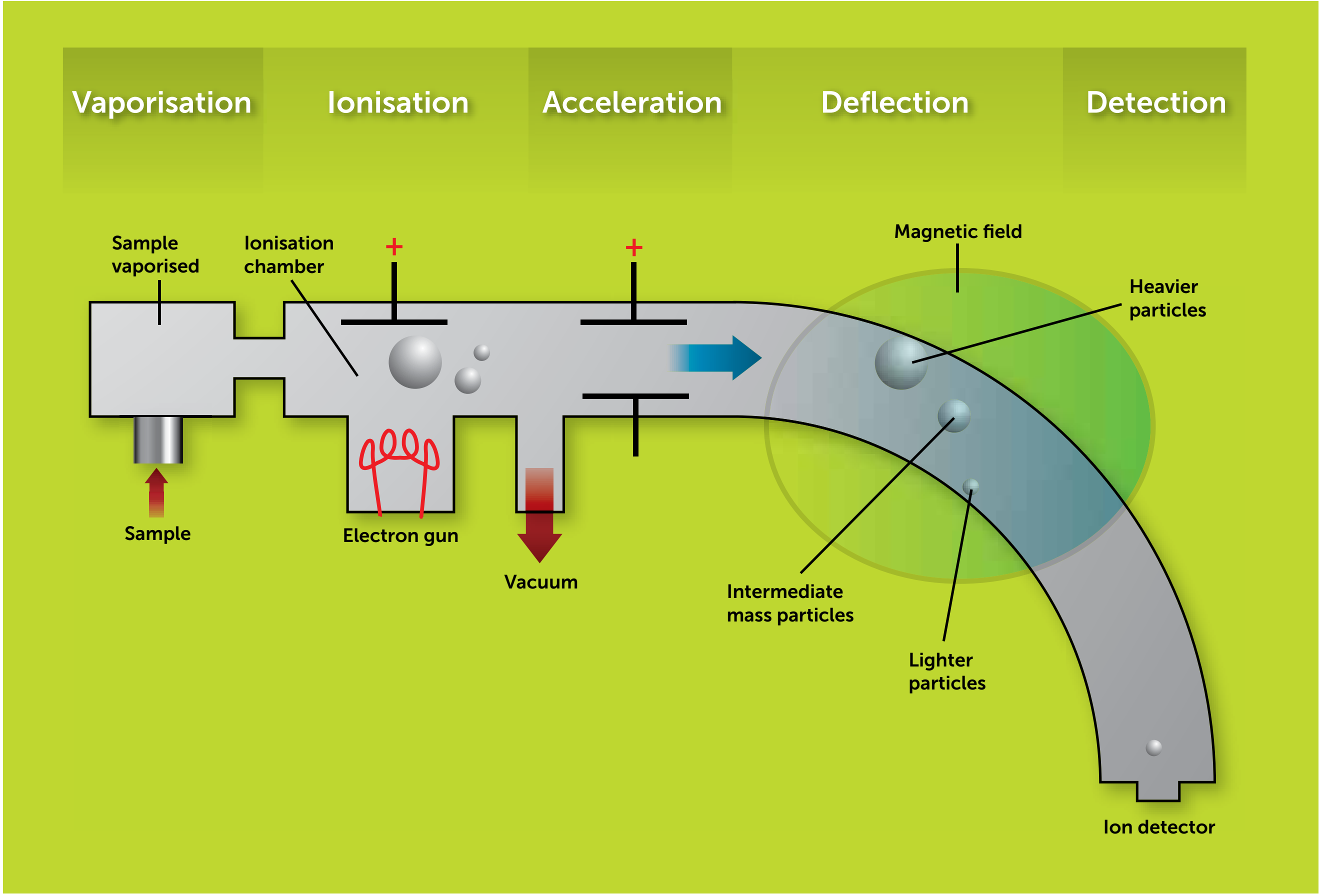
How it works

In a mass spectrometer a stream of positively charged ions is produced along with an associated magnetic field and their deflection in a controlled external magnetic field is studied in detail.

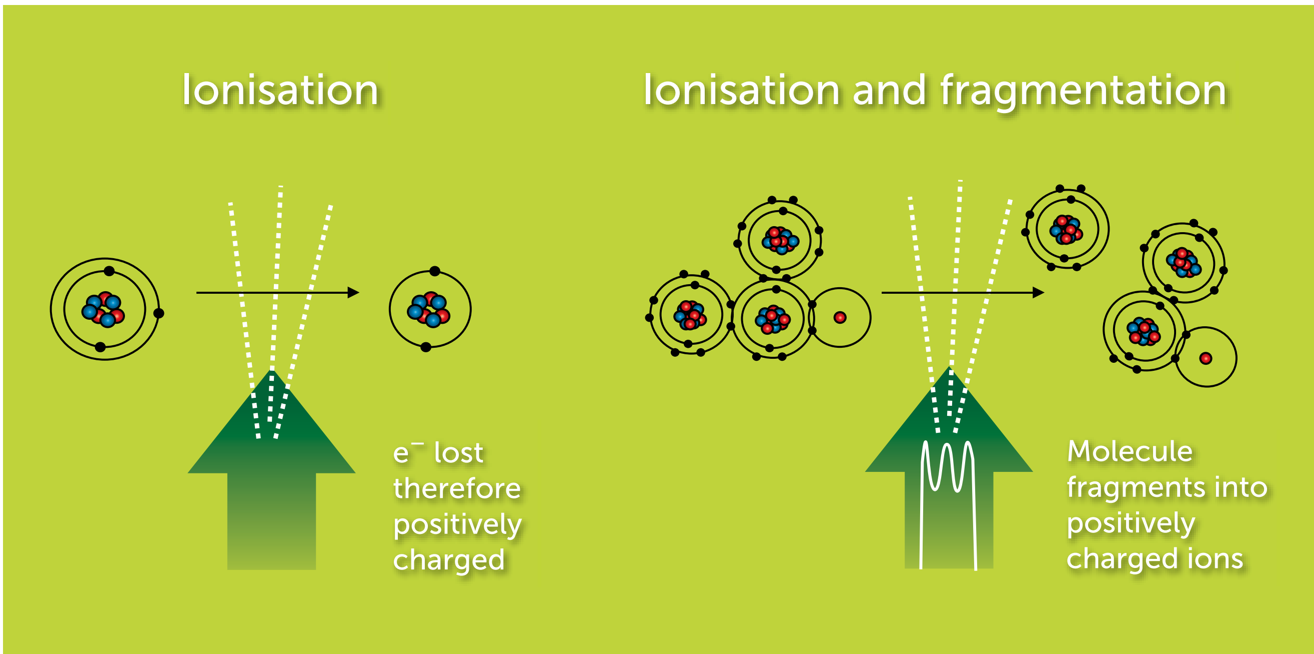
It is important that the atoms or the molecules of the substance being investigated are free to move so if the sample is not a gas it must first be **VAPORISED.**



Next, the sample must be **IONISED**. This is achieved by bombarding the sample with **high-energy electrons** from an electron gun. These knock off an electron to produce a **positive** ion.

e.g. consider a helium atom $He(g) + e^- = He^+(g) + 2e^-$

Sometimes doubly charged ions may also be produced but this only occurs in smaller amounts because more energy would be required.



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The high energy electron bombardment may also cause molecules to be broken into many different fragments.

e.g. methane molecules CH_4 can be fragmented to produce CH_3^+ CH_2^+ CH^+ and C^+

Fragmentation is dealt with in more detail in a later section.

NOTE: Because the positive ion formed has an unpaired electron it is sometimes shown with a dot indicating that it is a free radical, e.g. CH_3^{+}.

The positive ions are then **ACCELERATED** by an electric field and focused into a fine beam by passing through a series of slits with increasing negative potential. It is important that the ions can move freely through the apparatus without colliding with air molecules so the system has all the air removed to create a vacuum.

The beam of fast-moving positive ions is **DEFLECTED** by a strong external magnetic field. The magnitude of deflection depends upon two factors:

- The mass (*m*) of the ion – the lighter it is the more it will be deflected.
- The charge (*z*) on the ion – ions with 2^+ charges are deflected more than 1^+.

These two factors are combined into the **mass to charge ratio (*m/z*).** When *m/z* is small the deflection is large.

Finally ions which make it right through the machine are **DETECTED** electronically. As the positive ions arrive at the detector they pick up electrons to become neutral. This movement of electrons is detected, amplified and recorded. The external magnetic field involved in deflection can be adjusted so that ions with different *m/z* ratios can be detected. A printout of intensity vs *m/z* ratio is produced.

A simple mnemonic may help you remember these stages

VICTOR	**Vaporisation**
IS	**Ionisation**
A	**Acceleration**
DAFT	**Deflection**
DUCK	**Detection**

Interpreting the printouts

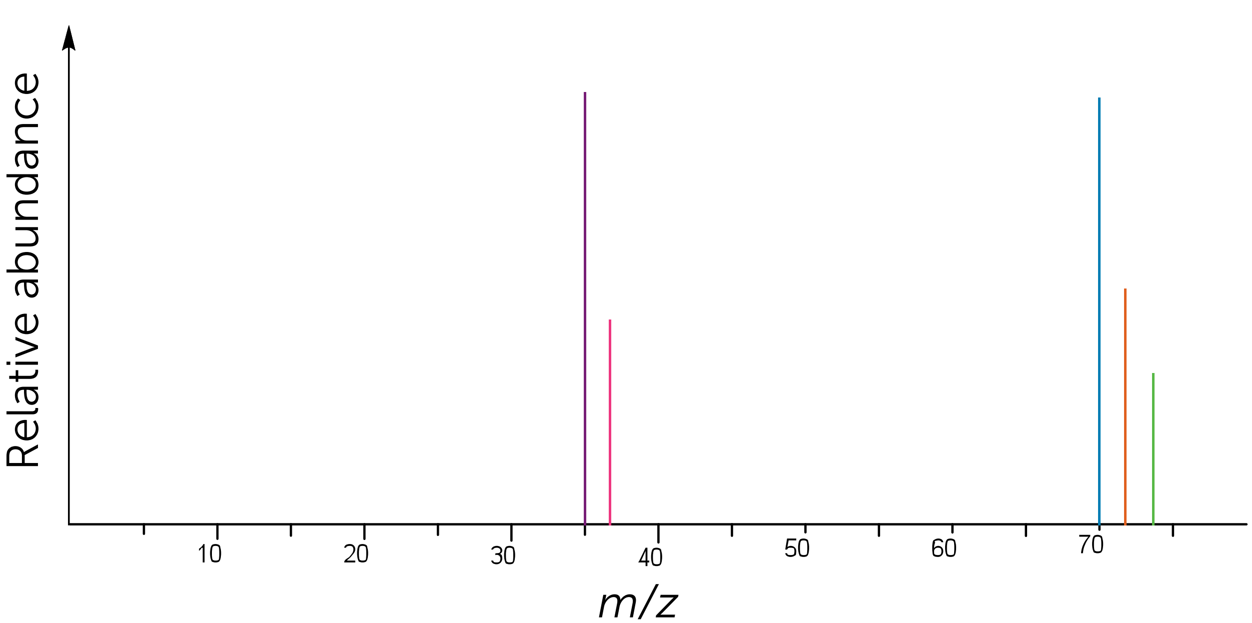
The mass spectrum of chlorine Cl_2

ISOTOPE	OBSERVED MASS
^{35}Cl	35 *m/z*
^{37}Cl	37 *m/z*
^{35}Cl-^{35}Cl	70 *m/z*
^{35}Cl-^{37}Cl	72 *m/z*
^{37}Cl-^{37}Cl	74 *m/z*

The multitude of peaks is seen because chlorine has two common isotopes ^{35}Cl and ^{37}Cl.

The peak at ***m/z*=35 represents the [^{35}Cl]$^+$ ion** and that at ***m/z*=37 the [^{37}Cl]$^+$ ion**. The ratio of the peak heights is 3:1 indicating the relative abundance of these isotopes; accounting for the relative atomic mass of 35.5 a.m.u.

The cluster of peaks at the higher mass result from the diatomic molecules, i.e. Cl_2 where ***m/z*=70 represents the [^{35}Cl-^{35}Cl]$^+$ ion**. That at ***m/z*=72 represents the [^{37}Cl-^{35}Cl]$^+$ ion** and that at ***m/z*=74 the [^{37}Cl-^{37}Cl]$^+$ ion**.



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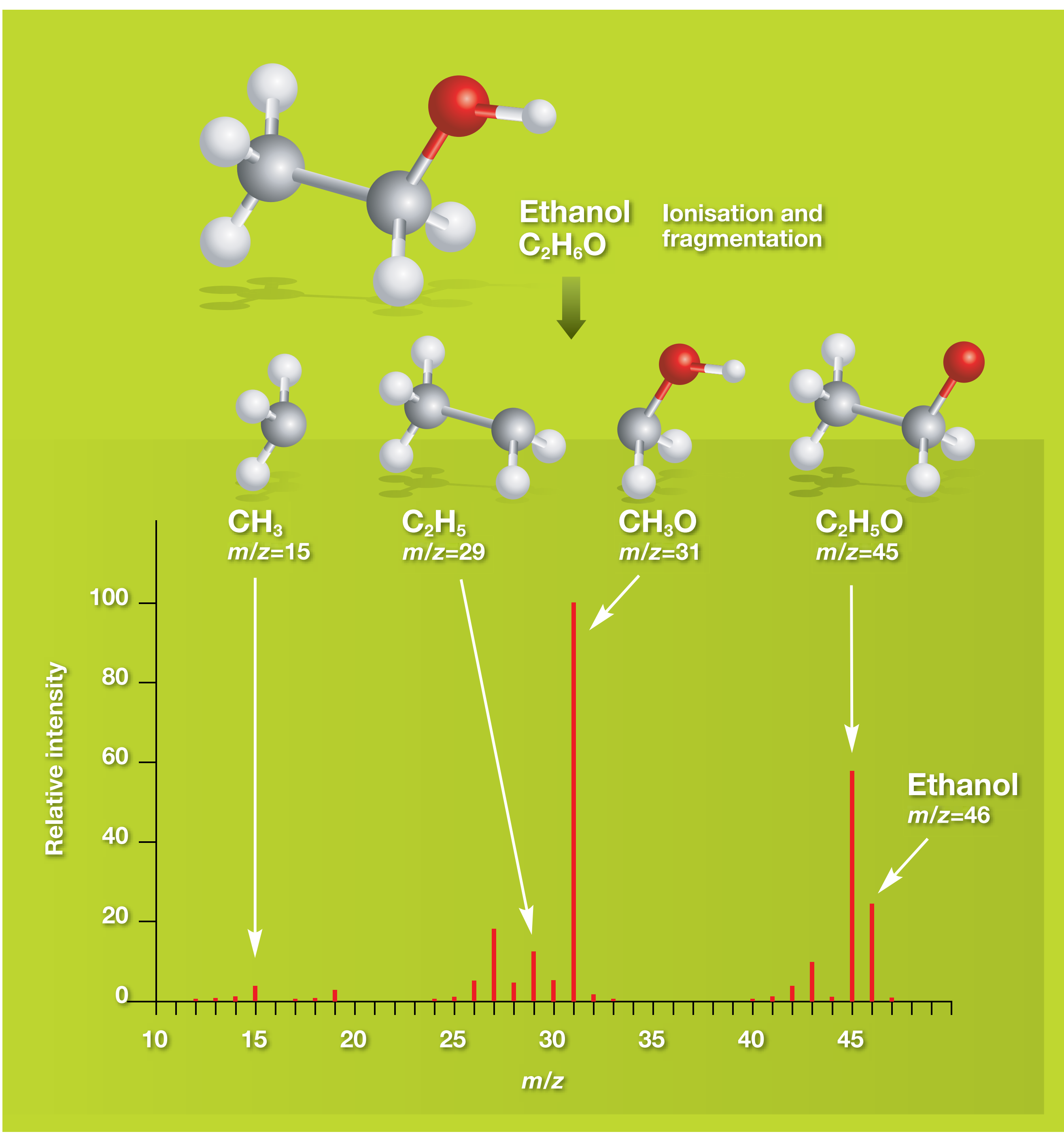
As the molecule gets bigger the possibility of fragmentation increases and the mass spectra become more complex. Final decisions about structure are made after combining evidence from mass spectrometry with other analytical tools such as infrared, ultraviolet–visible and nuclear magnetic resonance spectroscopy.



Many more mass spectra are available at www.le.ac.uk/spectraschool/

Modern applications of MS

LC–MS (liquid chromatography–mass spectrometry)

This process allows complex mixtures to be separated by liquid chromatography using small capillary columns. The most up to date are less than 100 μm across, allowing very small quantities of sample to be used. This is very important as MS destroys the sample. As the separated substances leave the column they are automatically fed into a mass spectrometer so that identification of each component of the mixture can be made.

This technique has many applications including:

- Proteomics – the study of proteins including digestion products.
- Pharmaceutics – drug development, identification of drugs and drug metabolites – remember the Olympics and the drug testing of competitors.
- Environmental – detection and analysis of herbicides and pesticides and their residues in foodstuffs.

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GC–MS (gas chromatography–mass spectrometry)

This technique is growing in popularity due to the compact nature of the equipment, the speed of use (less than 90 seconds for the best equipment) and its relatively low cost. Again it combines a chromatography step to separate out the components in a mixture, this time using an inert gas as the mobile phase.

Some of its many applications include:

- Airport security – for drug and explosive detection.
- Fire forensics – using the debris from fires to try to explain the causes.
- Astrochemistry – probes containing GC–MS have been sent to Mars, Venus and Titan to analyse the atmosphere and planet surfaces. The Rosetta space mission uses this technology to analyse comet constituents.

High-resolution MS

High-resolution MS can distinguish compounds with the same nominal mass but different actual mass caused by the different elemental composition. For example C_2H_6, CH_2O and NO all have a nominal mass of 30, however their exact masses are 30.04695039, 30.01056487 and 29.99798882, respectively. These subtle differences can be distinguished by this high-resolution technique.

It is becoming increasingly important as a technique for analysing the interactions between drugs and body tissues at the scale of DNA.

Common fragmentations

When a molecule is split during fragmentation the pieces formed tend to be the more stable types and the height of the detected peak provides an indication of how stable the fragment is. Some typical examples are provided in the table.

COMMONLY LOST FRAGMENTS		COMMON STABLE IONS	
m - 15	$\cdot CH_3$	*m/z* = 43	$H_3C\dot{C}^{+}\equiv O$
m - 17	$\cdot OH$	*m/z* = 77	phenyl cation radical ($C_6H_5^{+\cdot}$)
m - 26	$\cdot C\equiv N$	*m/z* = 91	benzyl cation radical ($C_6H_5\dot{C}H_2^{+}$)
m - 28	$H_2C=CH_2$	*m/z* = 91	tropylium cation radical
m - 29	$\cdot CH_2CH_3$	*m/z* = m - 1	$RC(=O^{+\cdot})H \rightarrow R-\overset{+}{C}\equiv O$
m - 29	$\cdot CHO$		
m - 31	$\cdot OCH_3$		
m - 35	$\cdot Cl$		
m - 43	$H_3C\dot{C}=O$		
m - 45	$\cdot OCH_2CH_3$		
m - 91	$C_6H_5\dot{C}H_2$		

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Introduction to infrared (IR) spectroscopy

Infrared (IR) spectroscopy

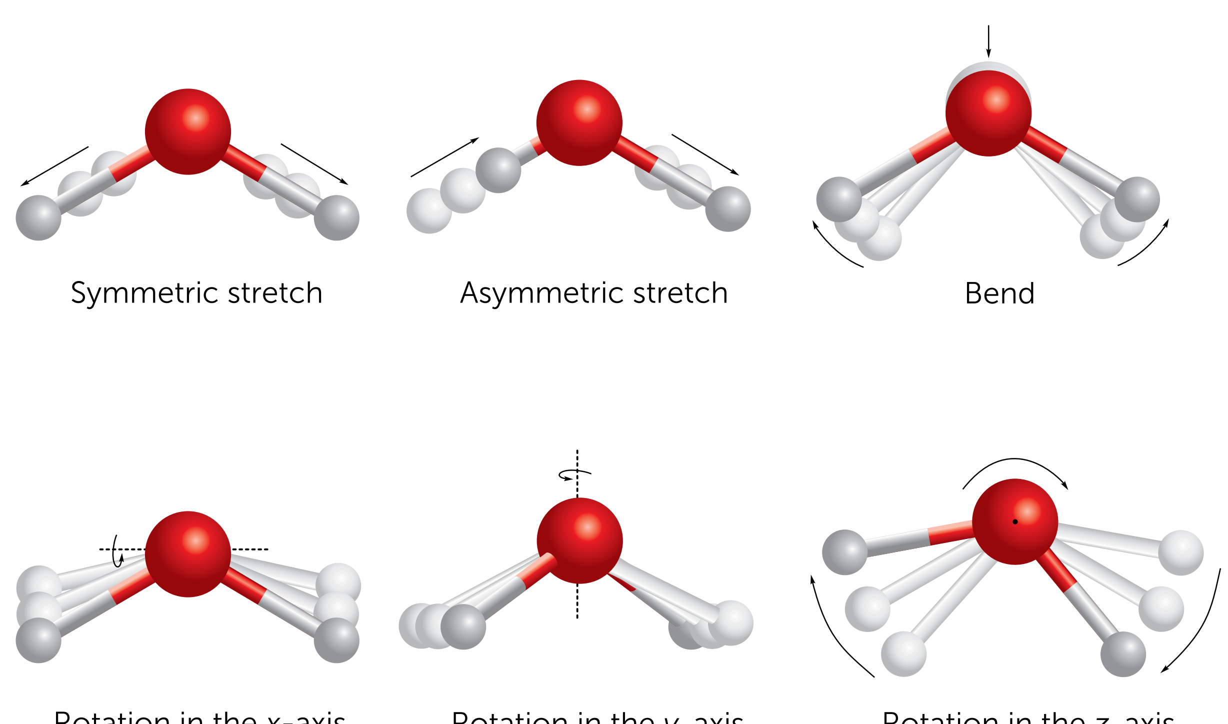
One of the first scientists to observe IR radiation was William Herschel in the early 19th century. He noticed that when he attempted to record the temperature of each colour in visible light, the area just beyond red light gave a marked increase in temperature compared to the visible colours. However it was only in the early 20th century that chemists started to take an interest in how IR radiation interacted with matter and the first commercial IR spectrometers were manufactured in the USA during the 1940s.

Interaction with matter

The bonds within molecules all vibrate at temperatures above absolute zero. There are several types of vibrations that cause absorptions in the IR region. Probably the most simple to visualise are bending and stretching, examples of which are illustrated below using a molecule of water.

Molecules will absorb IR energy at a frequency corresponding to the frequency of the bond's natural vibration. This absorption of energy results in an increase in the amplitude of the vibrations known as **resonance.**

An IR spectrometer detects how the absorption of a sample varies with wavenumber, cm^{-1}, which is the reciprocal of the wavelength in cm (1/wavelength). The wavenumber is proportional to the energy or frequency of the vibration of the bonds in the molecule.



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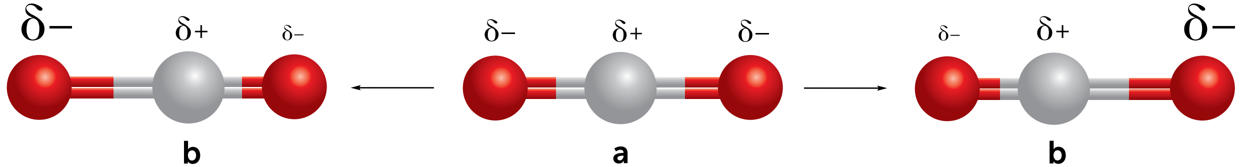
Carbon dioxide and IR

Carbon dioxide is probably the molecule most people associate with the absorption of IR radiation, as this is a key feature of the greenhouse effect. If carbon dioxide was perfectly still it would not have a permanent dipole moment as its charge would be spread evenly across both sides of the molecule.

However the molecule is always vibrating and when it undergoes an asymmetric stretch, an uneven distribution of charge results. This gives the molecule a temporary dipole moment, enabling it to absorb IR radiation.

Hence even some molecules without a permanent uneven distribution of charge can absorb IR radiation.



(a) No permanent dipole moment when the molecule is stationary due to equal and opposite dipoles.
(b) As molecules undergo asymmetric stretch the permanent dipoles become uneven and temporary dipole moments are created.

Factors that affect vibrations

Type of vibration

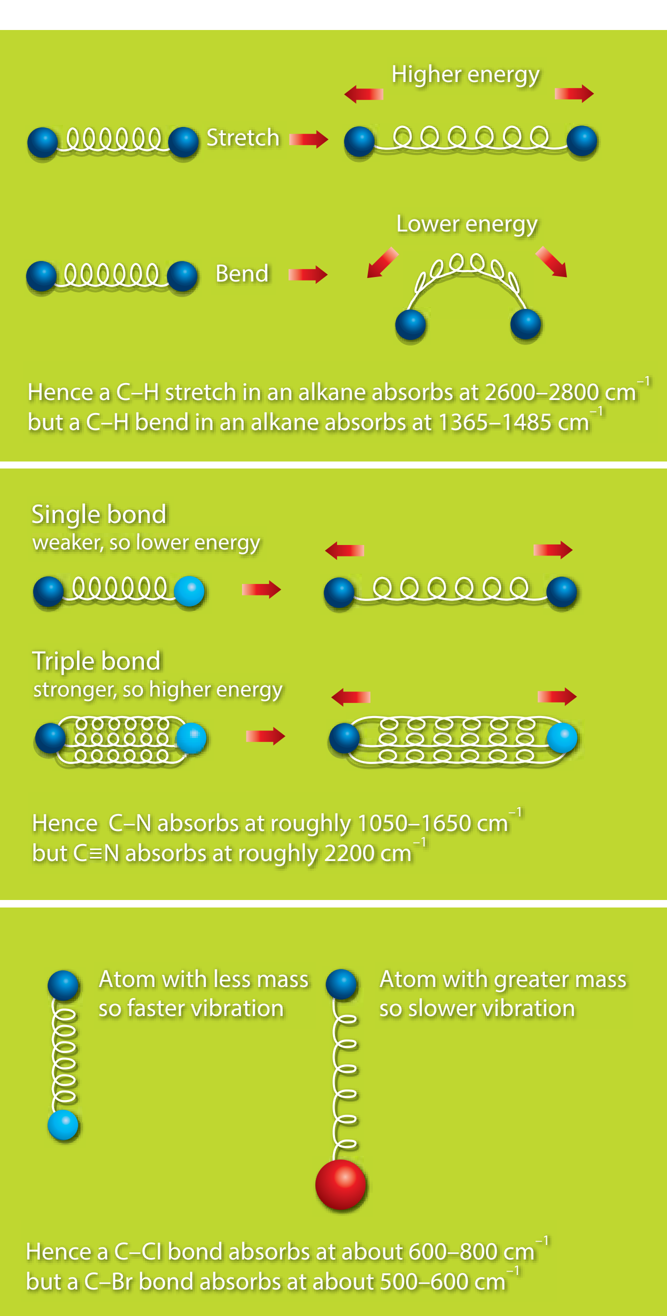
The energy absorbed when particular bonds vibrate depends on several factors. To get your head around this it is helpful to use an analogy; you can think of a bond as a spring between two atoms. Imagine trying to bend or stretch the spring. Generally it is easier to bend than stretch, so bending vibrations are of lower energy than stretching vibrations for the same bond. Therefore, absorptions due to bending tend to occur at lower wavenumbers than stretches.

Strength of bonds

You can think of a strong bond as a stiff spring. This will need more energy to make the 'spring' bond vibrate, so stronger bonds absorb at higher wavenumbers.

Mass of atoms

Finally the atoms in the bond can be thought of as masses at the end of the spring. Heavy masses on a spring vibrate more slowly than lighter ones. Using this analogy we can imagine that heavier atoms vibrate at a lower frequency than lighter ones. Therefore you would expect a C–Br bond to absorb at a lower frequency than a C–Cl bond as bromine is heavier than chlorine.



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IR spectrometers

IR spectrometers work in a variety of ways but all of them pass IR radiation across the full IR range through a sample. The sample can be a thin film of liquid between two plates, a solution held in special cells, a mull (or paste) between two plates or a solid mixed with potassium bromide and compressed into a disc. The cells or plates are made of sodium or potassium halides, as these do not absorb IR radiation.

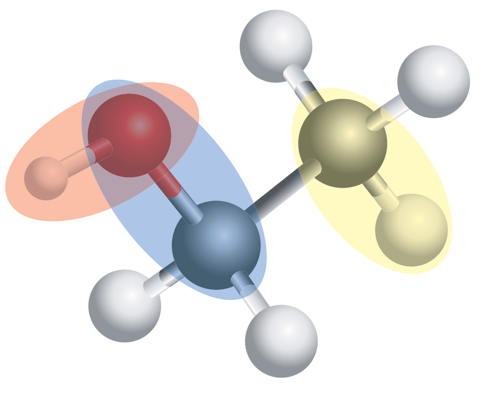
However the IR spectrometer 'in the suitcase' can use a technique called **attenuated total reflectance (ATR)**, which allows IR spectra to be run on solid and liquid samples without any additional preparation.

Interpreting a spectrum

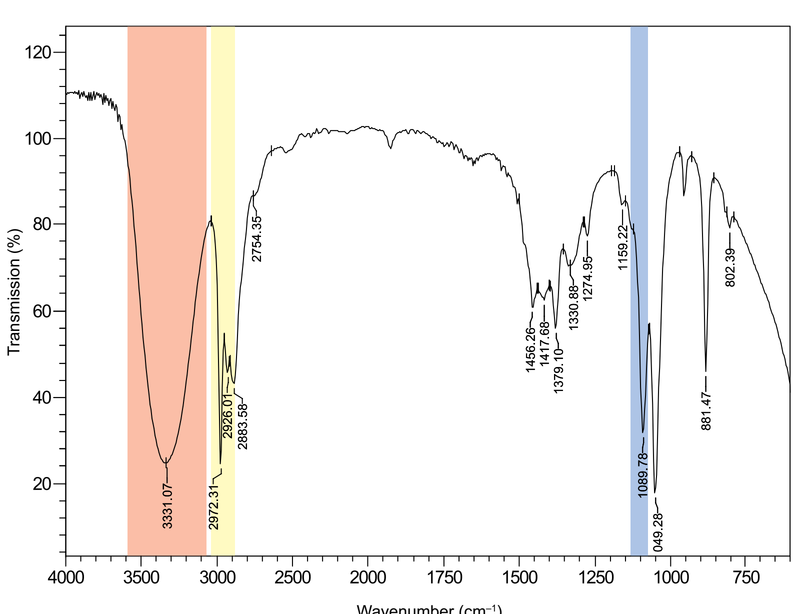
As molecules often contain a number of bonds, with many possible vibrations, an IR spectrum can have many absorptions. This can be helpful as it results in each molecule's spectrum being unique. If the spectrum of a molecule has already been recorded on a database, any spectrum produced can be compared to that database to help identify the molecule. The spectrum can also be used to highlight specific bonds and hence functional groups within a molecule, to help determine its structure.

Look at this spectrum of ethanol, CH_3CH_2OH, to see which bonds are responsible for particular absorptions.

Although IR is not able to provide enough information to find the exact structure of a 'new' molecule, in conjunction with other spectroscopic tools, such as NMR and MS, IR can help provide valuable information to help piece together the overall structure.



Ethanol
CH_2CH_3OH



Uses of IR spectroscopy

Students and research chemists regularly use IR in structure determination and IR continues to have a wide range of applications in both research chemistry and wider society. For instance, IR is being used to help identify the structure of complex molecules in space, to analyse works of art and in Formula 1 motor racing!

Many police forces across the world now routinely use IR, almost certainly without realising it. This is because many 'breathalysers' used to collect evidence to determine levels of alcohol in breath are IR spectrometers that look specifically for absorptions at around 1060 cm^{-1}, which corresponds to the vibration of the C–O bond in ethanol!

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IR ABSORPTION DATA

BOND	WAVENUMBER RANGE/cm^{-1}	FUNCTIONAL GROUP				
O–H	3400–2500 (broad)	Alcohols R—OH	Carboxylic acids R–C(=O)–OH	Phenols C_6H_5OH		
N–H	3600–3100	Amides R–C(=O)–N	Amines C—N			
C–H	3150–2850 (sharp)	(All organic compounds)				
C≡N	2260–2200	Nitriles C≡N				
C=O	1750–1630 (sharp)	Aldehydes R–C(=O)–H	Ketones C–C(=O)–C	Esters R–C(=O)–O–C	Carboxylic acids R–C(=O)–OH	Amides R–C(=O)–N
C=C	1680–1610	Alkenes C=C				
C–O	1300–1060	Alcohols R—OH	Carboxylic acids R–C(=O)–OH	Esters R_1–C(=O)–O–R_2	Ethers C—O—C	

'R' indicates where a number of different groups could be attached to the molecule.

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Introduction to nuclear magnetic resonance (NMR) spectroscopy

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy provides chemists with information on the environment in which the nuclei of atoms are found in molecules and compounds. We can therefore use NMR spectroscopy to determine the structure of a molecule.

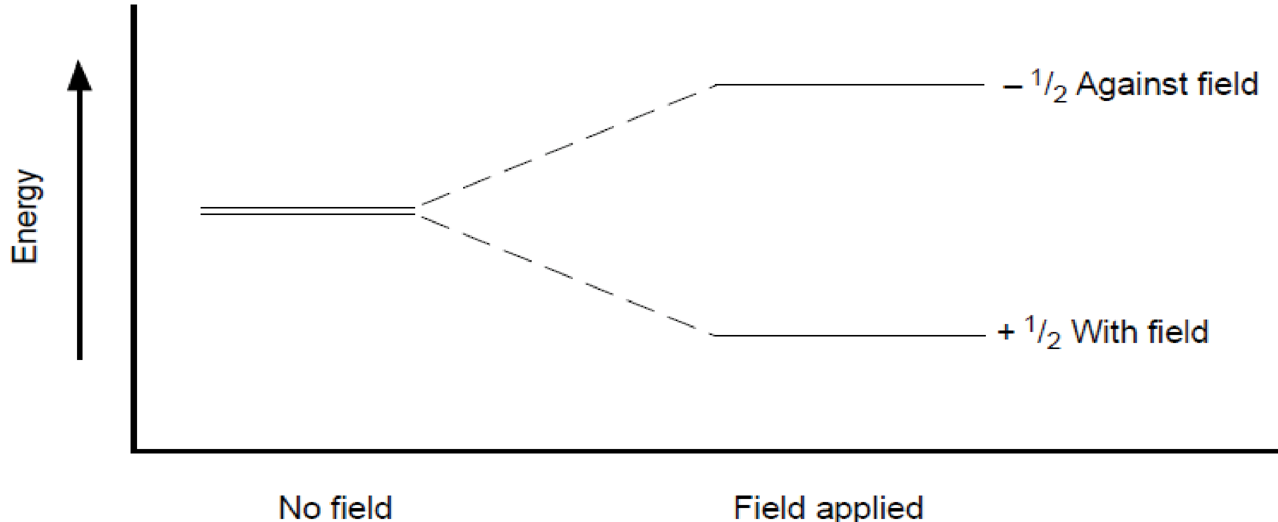
The theory behind the technique is rather more complex than for MS and IR spectroscopy, but the interpretation of the spectra is no more difficult, once a little familiarity has been gained.

Background

When some atoms are placed in a strong magnetic field, their nuclei behave like tiny bar magnets aligning themselves with the field. Electrons behave like this too, and for this reason both electrons and nuclei are said to possess 'spin', i.e. any spinning electric charge has an associated magnetic field.

Just as electrons with opposite spin pair up with each other in bonding orbitals, a similar thing happens with protons and neutrons in the nucleus. If a nucleus has an even number of protons and neutrons (e.g. ^{12}C), their magnetic fields cancel each other out and there is no overall magnetic field; however, if the number of protons and neutrons is odd (e.g. ^{13}C and ^{1}H), the nucleus has a magnetic field. If the substance is placed in an external magnetic field, the nuclear magnet lines up with the field, in the same way as a compass needle lines up with a magnetic field. The nuclear magnet can have two alignments, of low energy and high energy.

To make the nucleus change to the higher energy alignment, energy must be supplied. The energy absorbed corresponds to radio frequencies. The precise frequency of energy depends on the environment of the nucleus, that is, on the other nuclei and electrons in its neighbourhood.



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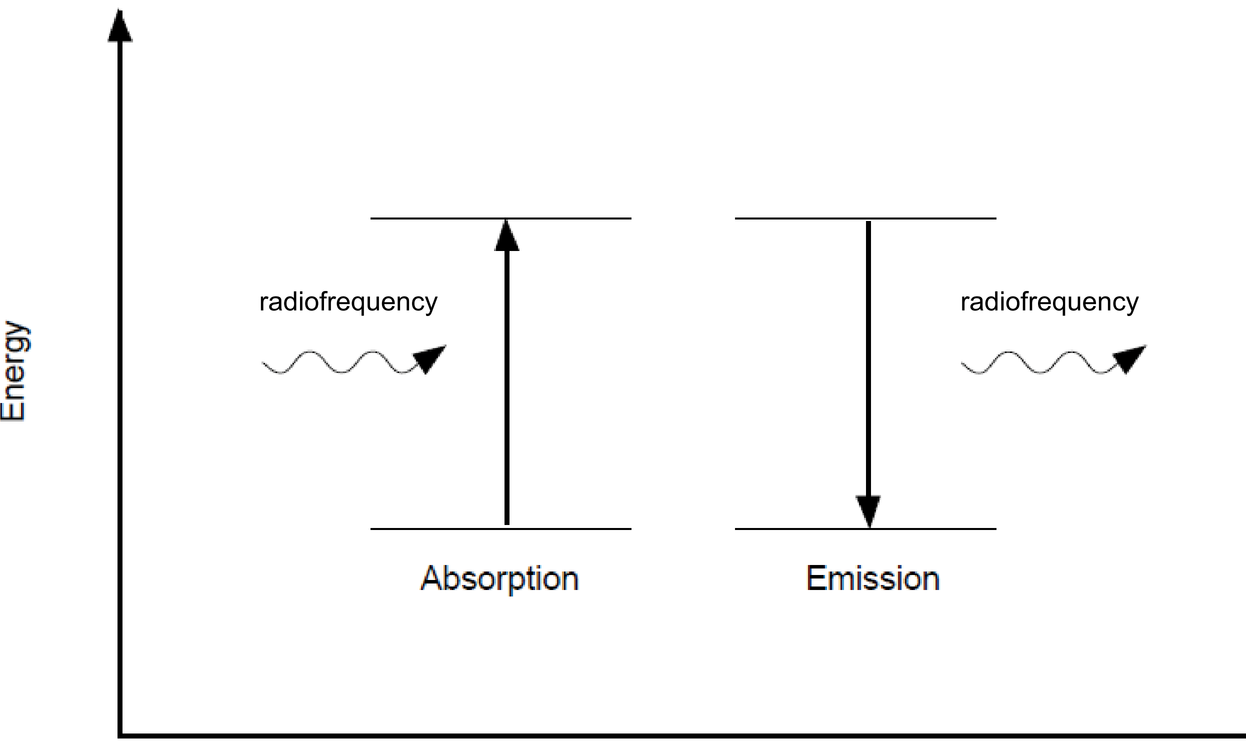
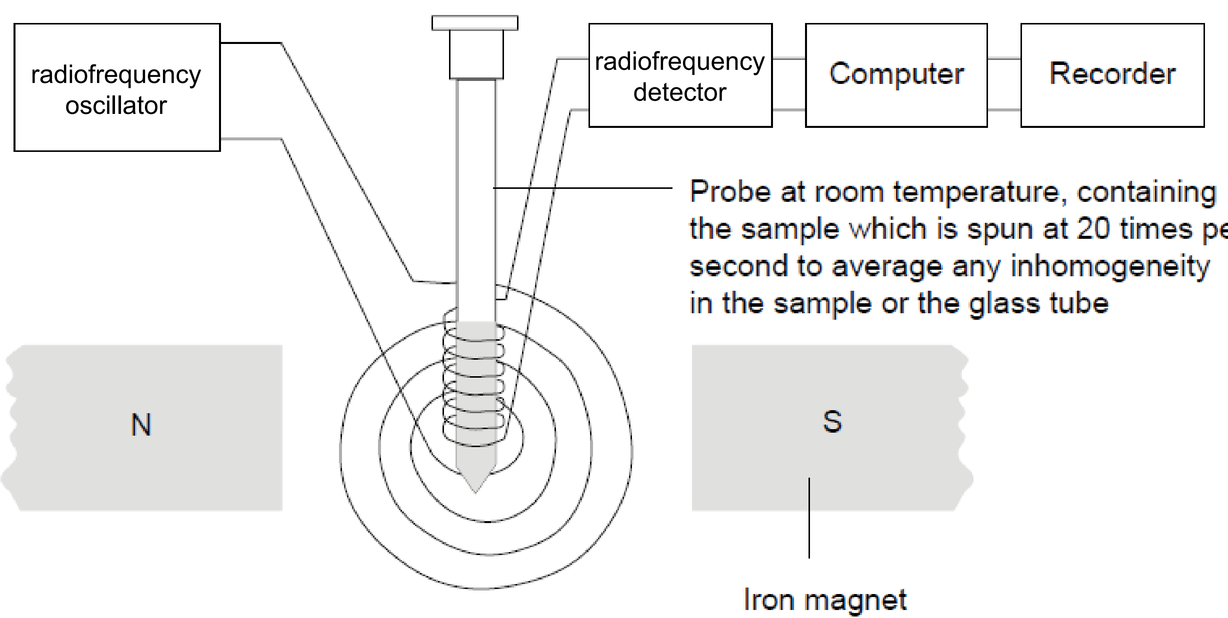


So, by placing the sample being examined in a strong magnetic field and measuring the frequencies of radiation it absorbs, information can be obtained about the environments of nuclei in the molecule.

There are two variables that can be altered when recording an NMR spectrum:

1. The magnetic field can be kept constant and the range of radiofrequencies scanned, or
2. The radiofrequency can be kept constant and the magnetic field scanned.



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^{1}H NMR spectroscopy

NMR spectroscopy is particularly useful for identifying the positions of hydrogen atoms (^{1}H) in molecules, which provides us with information about a molecule's structure. Analysing NMR spectra can be a bit like trying to piece together a puzzle made up of:

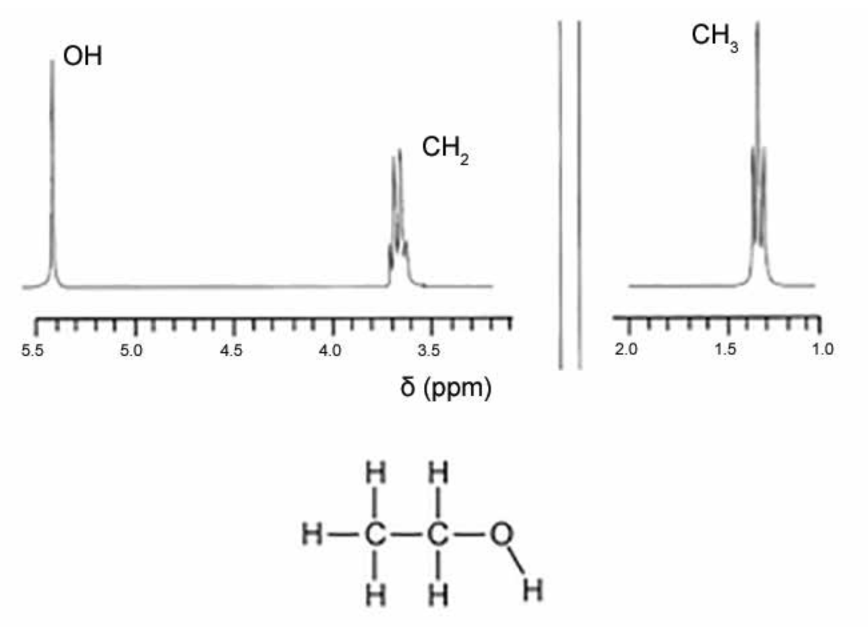
- **Hydrogen environments** – nuclei (e.g. hydrogen atoms) in different environments absorb different amounts of energy (radio waves) as they experience different magnetic fields inside the spectrometer; this this gives rise to different 'peaks' on an NMR spectrum, for each environment.
- **Chemical shift** – the hydrogen atoms in a particular type of environment have similar positions in an NMR spectrum. Normally, this position is measured as a chemical shift from a fixed reference point. The reference point normally used is the absorption of a substance called tetramethylsilane (TMS), which has the formula $(CH_3)_4Si$.

  TMS is a useful standard as it is non-toxic and inert and its boiling point is fairly low so it can be boiled off if the sample is required for anything else. TMS also gives a signal that resonates well away from almost all other organic hydrogen resonances because the protons are so well shielded. There are 12 protons in the same environment and they all resonate at the same frequency so a single intense peak is produced.
- **Peak integral** – area under each peak, which is proportional to the number of that type of hydrogen atom in the molecule, i.e. the number of hydrogens in each environment.
- **Spin–spin coupling** – in a molecule, the nucleus of an atom can induce a very weak magnetic moment in the electrons of the chemical bonds attached to it. This affects the magnetic field at a neighbouring atom's nucleus. The interaction is known as coupling and this causes the peaks to be split into a number of smaller ones. Protons can usually interact with other protons that are up to three bonds away, but protons in the same chemical environment do not show coupling with each other.
- **Splitting pattern** – as a result of spin–spin coupling, the peaks split into one more than the number of hydrogens on the neighbouring carbon atoms. This is known as the **'n + 1 rule'**. You can work out the number of neighbouring hydrogens by looking at how the peak splits.

Looking at the proton NMR spectrum of ethanol below, we can easily identify the hydrogen atoms by examining the pieces of the puzzle. There are three major peaks:

- The peak to the left represents the single H in the OH group (integration of 1).
- The middle peak represents the H's in the CH_2 group (integration of 2).
- The peak to the right represents the H's in the CH_3 group (integration of 3).

When the spectrum is recorded as a high-resolution spectrum, more detail is apparent and the peaks appear as singlets, doublets, triplets, quartets etc. due to interaction with neighbouring protons. In the spectrum of ethanol, the CH_3 group affects the CH_2 group and vice versa. The peak representing the CH_2 is therefore split into four as it is next to the CH_3 group (3+1) and the peak representing the CH_2 group is split into three as it is next to the CH_3 group (2+1). This spin–spin coupling provides essential information for a skilled NMR technician to interpret a spectrum.



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NMR spectrum of iodoethane CH_3CH_2I

NMR spectrum of iodoethane CH_3CH_2I

The CH_3 protons produce a peak at δ 1.8 but, instead of a single peak, a triplet is produced. This is because the CH_3 protons couple with the adjacent two CH_2 protons.

The CH_2 protons produce a peak at δ 3.2 but, instead of a single peak, a quartet is produced. This is because the CH_2 protons couple with the adjacent three CH_3 protons.

Applications of NMR spectroscopy

NMR is one of the most powerful methods for analysis of chemical samples, biological compounds, etc:

- Provides structural information on a range of different elements in molecules including hydrogen, carbon, fluorine, silicon, phosphorus and many others.
- Can be used to optimise reaction dynamics, measure reaction kinetics, monitor reaction progress and control product purity.
- Can be used to determine shapes and structures of large complex molecules, such as how proteins fold, twist and coil.
- Can be used to evaluate the proportions of solid and liquid components in fatty foodstuffs such as margarines and low-fat spreads.

NMR is also used a lot in pharmaceutical sciences and medicine, for example:

- Diagnosis of tissue abnormalities
- pH control in diabetics
- Body scanning by magnetic resonance imaging (MRI): strong magnetic fields are used to excite protons in tissues containing water molecules. Excited protons emit a radio frequency signal, which is measured and used to form an image of the body. The contrast between different tissues is determined by the rate at which excited atoms return to the equilibrium state.

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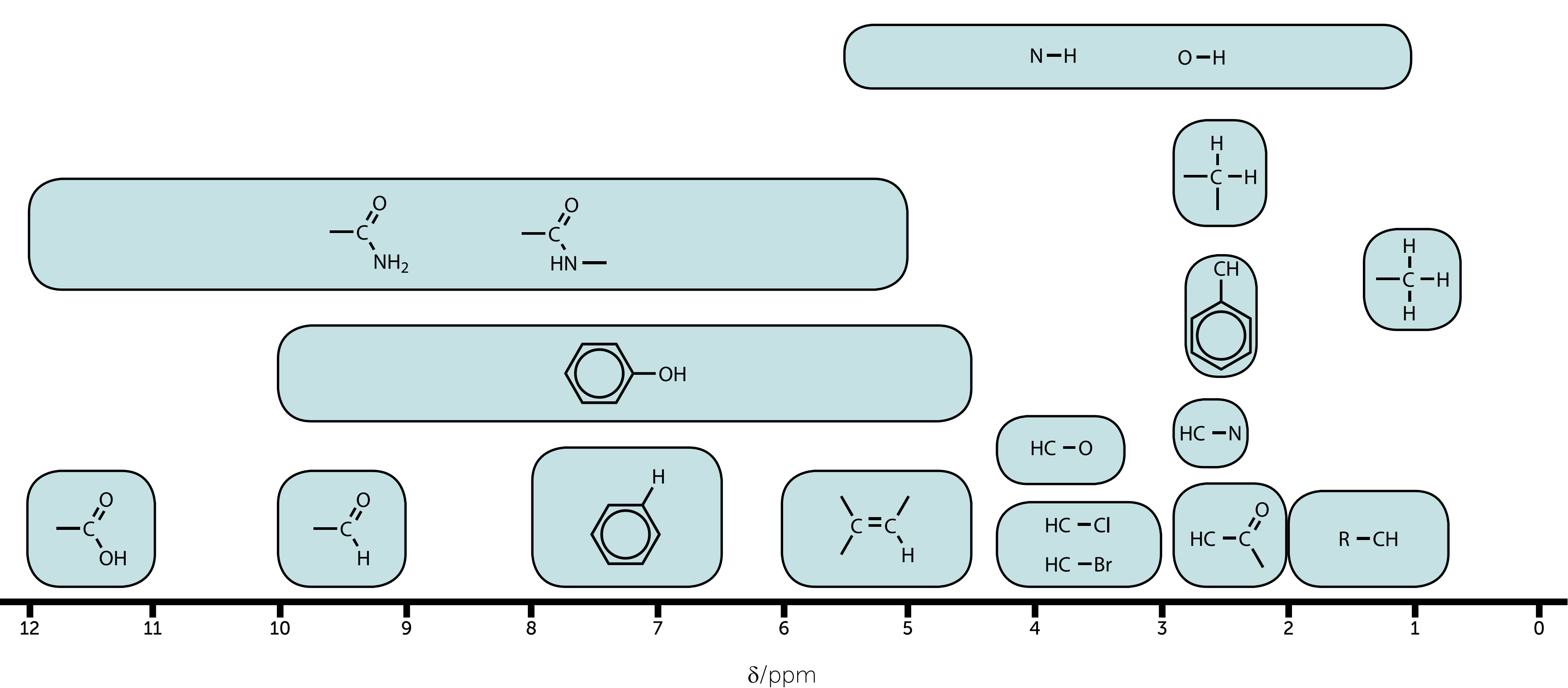
^{1}H NMR chemical shifts relative to TMS



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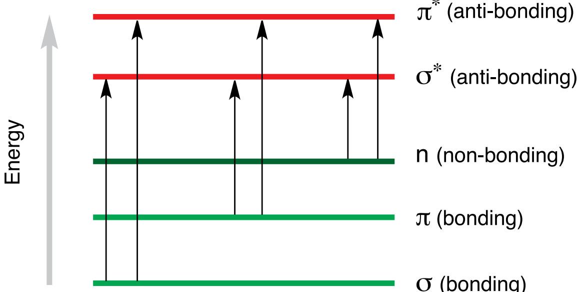
Introduction to ultraviolet–visible (UV-VIS) spectroscopy

Absorption of ultraviolet and visible radiation

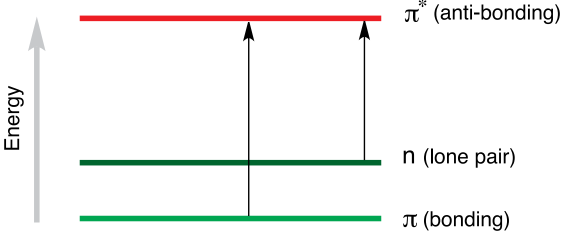
Absorption of ultraviolet (UV) and visible radiation is associated with **excitation of electrons**, in both atoms and molecules, from lower to higher energy levels. Because the energy levels of matter are quantised, only light with the precise amount of energy can cause transitions only light with a precise amount of energy can cause transition from one energy level to another. Light with a different energy level would not be absorbed and would not change the level. The possible electronic transitions that light might cause are:



In each possible case, an electron is excited from a full (low energy, ground state) orbital into an empty (higher energy, excited state) anti-bonding orbital. Each wavelength of light has a particular energy associated with it. If that particular amount of energy is just right for making one of these electronic transitions, then that wavelength will be absorbed.

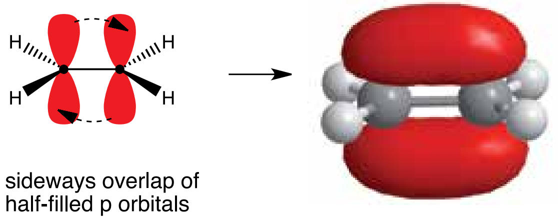
The larger the gap between the energy levels, the greater the energy required to promote the electron to the higher energy level, resulting in light of higher frequency, and therefore shorter wavelength, being absorbed.

All molecules will undergo electronic excitation following absorption of light, but for most molecules very high-energy radiation (in the vacuum ultraviolet, <200 nm) is required. Consequently, absorption of light in the UV–visible region will only result in the following transitions:



Therefore in order to absorb light in the region from 200 to 800 nm (where spectra are measured), the molecule
must contain either π bonds or atoms with non-bonding orbitals. A non-bonding orbital is a lone pair on, say, oxygen, nitrogen or a halogen.

π bonds are formed by sideways overlap of the half-filled p orbitals on the two carbon atoms of a double bond. The two red shapes shown in the diagram below for ethene are part of the same π bonding orbital.
Both of the electrons are found in the resulting π bonding orbital in the ground state.



sideways overlap of half-filled p orbitals

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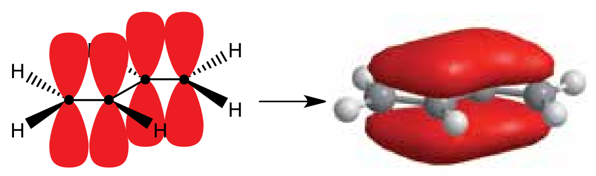
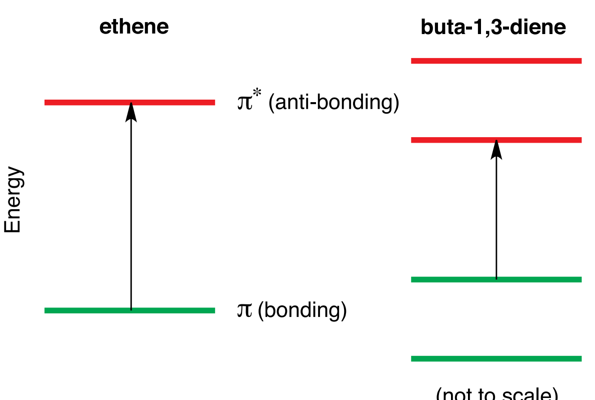
Molecules that contain **conjugated systems**, i.e. **alternating single and double bonds**, will have their electrons delocalised due to overlap of the p orbitals in the double bonds. This is illustrated below for buta-1,3-diene. Benzene is a well-known example of a conjugated



system. The Kekulé structure of benzene consists of alternating single and double bonds and these give rise to the delocalised V system above and below the plane of the carbon–carbon single bonds.

As the amount of delocalisation in the molecule increases the energy gap between the π bonding orbitals and π anti-bonding orbitals gets smaller and therefore light of lower energy, and longer wavelength, is absorbed.



Although buta-1,3-diene absorbs light of a longer wavelength than ethene it is still absorbing in the UV region and hence both compounds are colourless. However, if the delocalisation is extended further the wavelength absorbed will eventually be long enough to be in the visible region of the spectrum, resulting in a highly coloured compound. A good example of this is the orange plant pigment, beta-carotene, which has 11 carbon–carbon double bonds conjugated together.

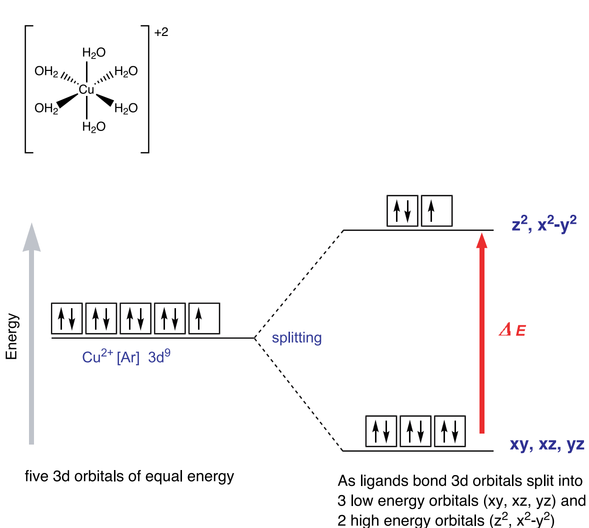
Beta-carotene absorbs throughout the UV region but particularly strongly in the visible region between 400 and 500 nm with a peak at 470 nm.

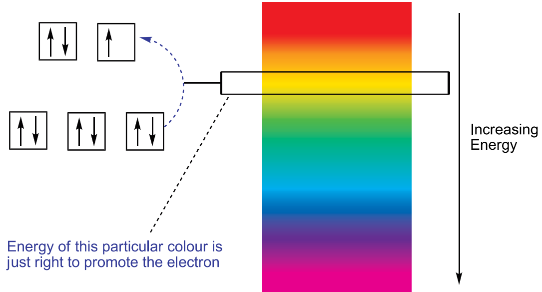
Groups in a molecule which consist of alternating single and double bonds (conjugation) and absorb visible light are known as **chromophores**.

Transition metal complexes are also highly coloured, which is due to the splitting of the d orbitals when the ligands approach and bond to the central metal ion. Some of the d orbitals gain energy and some lose energy. The amount of splitting depends on the central metal ion and ligands.

The difference in energy between the new levels affects how much energy will be absorbed when an electron is promoted to a higher level. The amount of energy will govern the colour of light which will be absorbed.

For example, in the octahedral copper complex, $[Cu(H_2O)_6]^{2+}$, yellow light has sufficient energy to promote the d electron in the lower energy level to the higher one.





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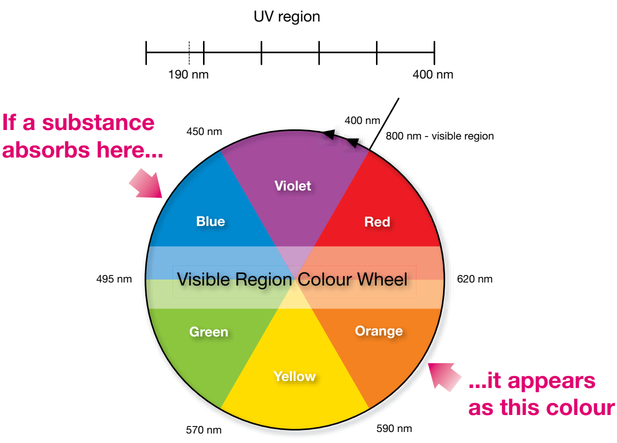
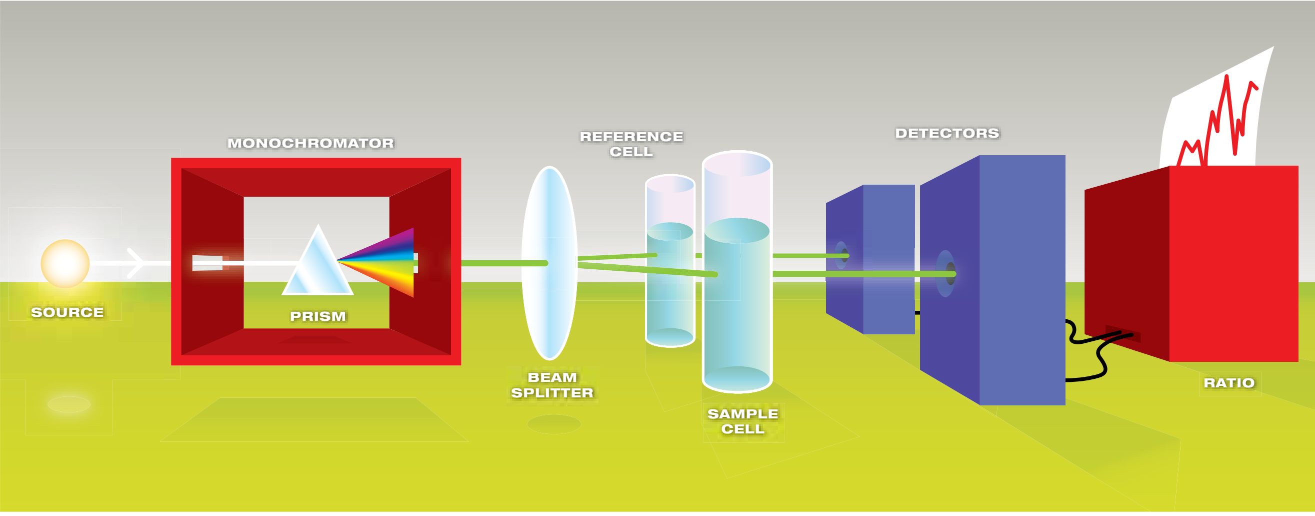


Red	620–750 nm
Orange	590–620 nm
Yellow	570–590 nm
Green	496–570 nm
Blue	450–495 nm
Violet	380–450 nm

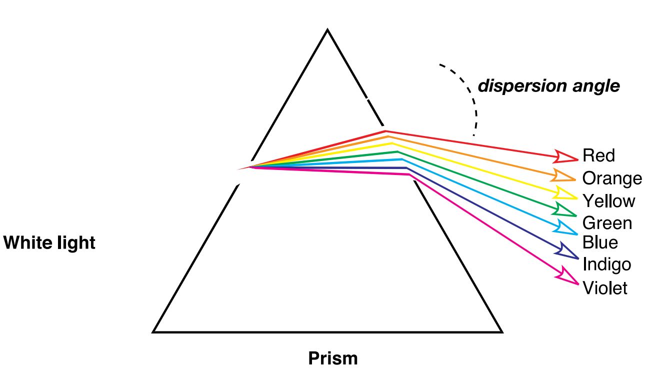
It is possible to predict which wavelengths are likely to be absorbed by a coloured substance. When white light passes through or is reflected by a coloured substance, a characteristic portion of the mixed wavelengths is absorbed. The remaining light will then assume the complementary colour to the wavelength(s) absorbed. This relationship is demonstrated by the colour wheel shown on the right. Complementary colours are diametrically opposite each other.

UV-VIS spectrometer



UV-VIS spectrometers can be used to measure the absorbance of UV or visible light by a sample, either at a single wavelength or by performing a scan over a range in the spectrum. The UV region ranges from 190 to 400 nm and the visible region from 400 to 800 nm. The technique can be used both quantitatively and qualitatively. A schematic diagram of a UV-VIS spectrometer is shown above.

The light source (a combination of tungsten/halogen and deuterium lamps) provides the visible and near ultraviolet radiation covering 200–800 nm. The output from the light source is focused onto the diffraction grating which splits the incoming light into its component colours of different wavelengths, like a prism (shown below) but more efficiently.



For liquids the sample is held in an optically flat, transparent container called a **cell** or **cuvette**. The reference cell or cuvette contains the solvent in which the sample is dissolved and this is commonly referred to as the **blank**.

For each wavelength the intensity of light passing through both a reference cell **(I_0)** and the sample cell **(I)** is measured. If I is less than I_0, then the sample has absorbed some of the light.

The absorbance **(A)** of the sample is related to I_0 and I_0 according to the following equation:

$$A = \log_{10} \frac{I_0}{I}$$

The detector converts the incoming light into a current, and the higher the current the greater the intensity. The chart recorder usually plots the absorbance against wavelength (nm) in the UV and visible section of the electromagnetic spectrum. *(Note: absorbance does not have any units.)*

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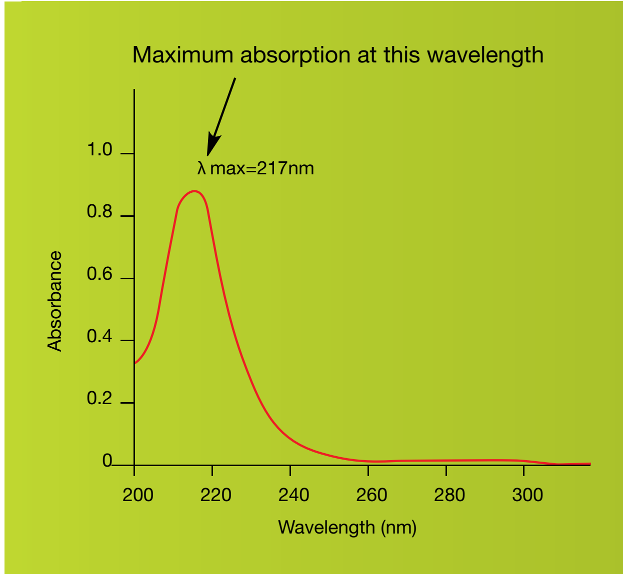
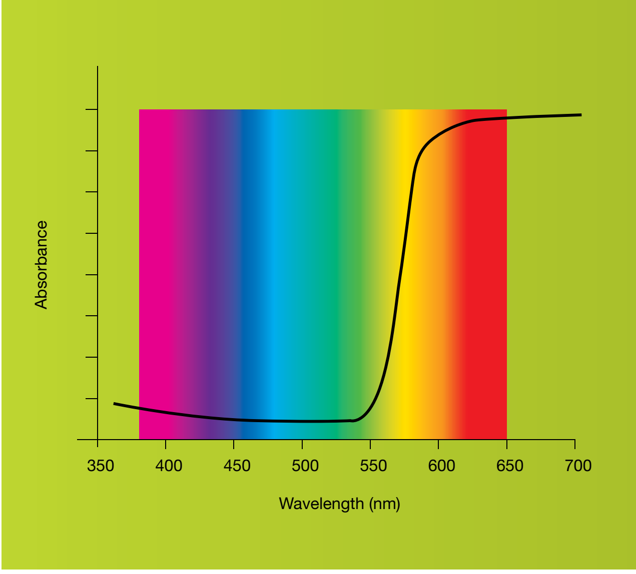
UV-VIS spectrum

The diagram below shows a simple UV-VIS absorption spectrum for buta-1,3-diene. Absorbance (on the vertical axis) is just a measure of the amount of light absorbed. One can readily see what wavelengths of light are absorbed (peaks), and what wavelengths of light are transmitted (troughs). The higher the value, the more of a particular wavelength is being absorbed.



The absorption peak at a value of 217 nm is in the UV region, and so there would be no visible sign of any light being absorbed, making buta-1,3-diene colourless. The wavelength that corresponds to the highest absorption is usually referred to as 'lambda-max' (λ_{max})

The spectrum for the blue copper complex shows that the complementary yellow light is absorbed.



The Beer–Lambert Law

According to the Beer–Lambert Law the **absorbance** is **proportional** to the **concentration** of the substance in solution and as a result UV-VIS spectroscopy can also be used to measure the concentration of a sample.

The Beer–Lambert Law can be expressed in the form of the following equation:

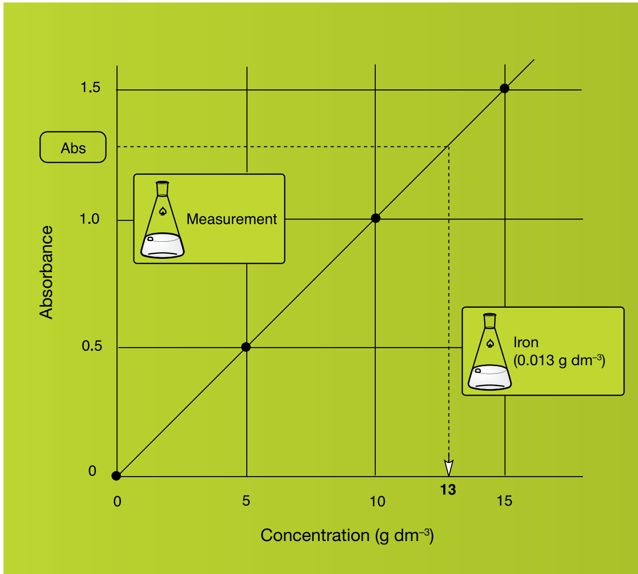
$$A = \varepsilon c l$$

Where

- A = absorbance
- l = optical path length, i.e. dimension of the cell or cuvette (cm)
- c = concentration of solution (mol dm^{-3})
- ε = molar extinction, which is constant for a particular substance at a particular wavelength (dm^3 mol^{-1} cm^{-1})

If the absorbance of a series of sample solutions of known concentrations are measured and plotted against their corresponding concentrations, the plot of **absorbance versus concentration** should be linear if the Beer–Lambert Law is obeyed. This graph is known as a **calibration graph**.

A calibration graph can be used to determine the concentration of an unknown sample solution by measuring its absorbance, as illustrated below.



Because the absorbance for dilute solutions is directly proportional to concentration, another very useful application for UV-VIS spectroscopy is studying reaction kinetics. The rate of change in concentration of reactants or products can be determined by measuring the increase or decrease of absorbance of coloured solutions with time. Plotting absorbance against time one can determine the orders with respect to the reactants and hence the rate equation, from which a mechanism for the reaction can be proposed.

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Modern applications of UV-VIS spectroscopy

UV-VIS spectroscopy is a technique that readily allows one to determine the concentrations of substances and therefore enables scientists to study the rates of reactions, and determine rate equations for reactions, from which a mechanism can be proposed. As such UV-VIS spectroscopy is used extensively in teaching, research and analytical laboratories for the quantitative analysis of all molecules that absorb UV and visible electromagnetic radiation.

Other applications include:

- In clinical chemistry UV-VIS spectroscopy is used extensively in the study of enzyme kinetics. Enzymes cannot be studied directly but their activity can be studied by analysing the speed of the reactions they catalyse. Reagents or labels can also be attached to molecules to permit indirect detection and measurement of enzyme activity. The widest use in the field of clinical diagnostics is as an indicator of tissue damage.

  When cells are damaged by disease, enzymes leak into the bloodstream and the amount present indicates the severity of the tissue damage. The relative proportions of different enzymes can be used to diagnose disease, say of the liver, pancreas or other organs, which otherwise exhibit similar symptoms.

- UV-VIS spectroscopy is used for dissolution testing of tablets and products in the pharmaceutical industry. Dissolution is a characterisation test commonly used by the pharmaceutical industry to guide formulation design and control product quality. It is also the only test that measures the rate of *in-vitro* drug release as a function of time, which can reflect either reproducibility of the product manufacturing process or, in limited cases, *in vivo* drug release.

- In the biochemical and genetic fields UV-VIS spectroscopy is used in the quantification of DNA and protein/enzyme activity as well as the thermal denaturation of DNA.

- In the dye, ink and paint industries UV-VIS spectroscopy is used in quality control in the development and production of dyeing reagents, inks and paints, and the analysis of intermediate dyeing reagents.

- In environmental and agricultural fields the quantification of organic materials and heavy metals in fresh water can be carried out using UV-VIS spectroscopy.

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Spectroscopy in a Suitcase exercises

CLASSROOM WORKSHOPS

Compound identification: Exercise 1 MS and IR analysis (optional inclusion of NMR)

Compound identification: Exercise 2 IR and NMR analysis

Food dye analysis: Exercise 3 UV-VIS analysis

Body in a lab: Exercise 1 Compound identification MS and IR analysis

Body in a lab: Exercise 2 Aspirin overdose UV-VIS analysis

Body in a lab: Exercise 3 Murder Mystery “Who Dunnit?” MS analysis

Olympic drug scandal: IR analysis investigation

N.B. Exercise 1 and Body in a lab Exercise 1 use the same samples and spectra. One focuses on compound identification and the other frames the investigation within a forensics setting.

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Compound identification: Exercise 1

MS and IR analysis

(optional inclusion of NMR)

Background

IR spectroscopy is a technique used by chemists to identify unknown substances based on the absorption of IR radiation by chemical bonds. IR spectroscopy has many benefits in the field of science and technology and portable spectrometers are often used in forensic analysis at a crime scene to provide rapid, sensitive and non-destructive analysis of blood samples or unknown substances. Computer databases have records of known IR absorbance graphs so that almost any substance or material can be identified with a high degree of confidence.

Objective

To try to establish the functional groups present in the chemical samples. The MS, IR and NMR spectra are provided for all samples. You may wish to use some or all of these in your investigation.

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METHOD

You are provided with unknown samples A – H

1. Analyse each sample using the ATR IR spectrometer. **(Note: Care must be taken with this expensive and fragile equipment; use only when supervised by a demonstrator.)**

Interpretation of spectra

To interpret the spectra obtained from a sample it is necessary to refer to correlation charts and tables of IR data.

2. Using the IR absorption data provided interpret your spectra and identify the functional groups present in each sample. Record your results in the table provided.

Identification of an unknown compound

While IR spectroscopy is a very useful tool for identifying the functional groups in an unknown compound, it does not provide sufficient evidence to confirm the exact structure. Chemists make use of a variety of techniques in order to piece together the structure of a molecule.

3. Use your interpreted IR spectra and the mass spectra provided to suggest the structure of all unknown compounds.
4. Suggest what other instrumental technique or techniques would be required to confirm the identity of the chemicals. (Your demonstrator may be able to provide you with additional data for confirmation of analysis.)

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STUDENT WORKSHEET

SAMPLE	IMPORTANT PEAK VALUES (cm^{-1})	FUNCTIONAL GROUP AND RANGE (cm^{-1})	MASS SPEC ANALYSIS (Mass of compound and fragments identified)	PREDICTED CHEMICAL STRUCTURE AND NAME
A				
B				
C				
D				
E				
F				
G				
H				

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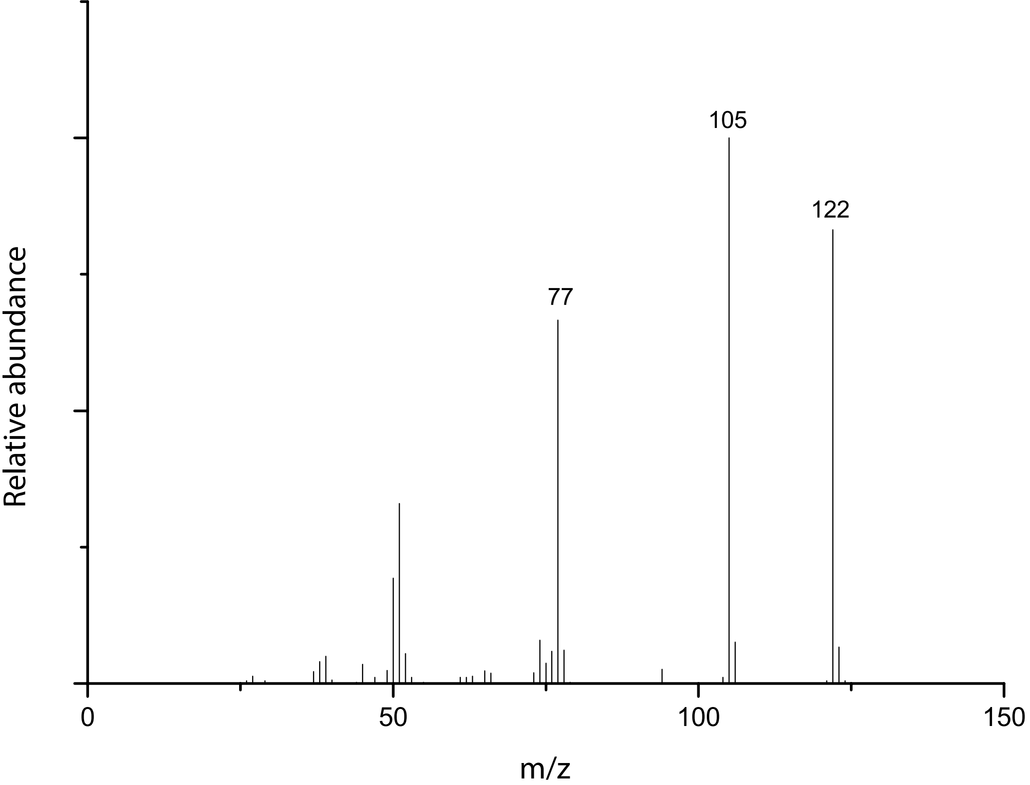
MASS SPECTRA FOR INTERPRETATION

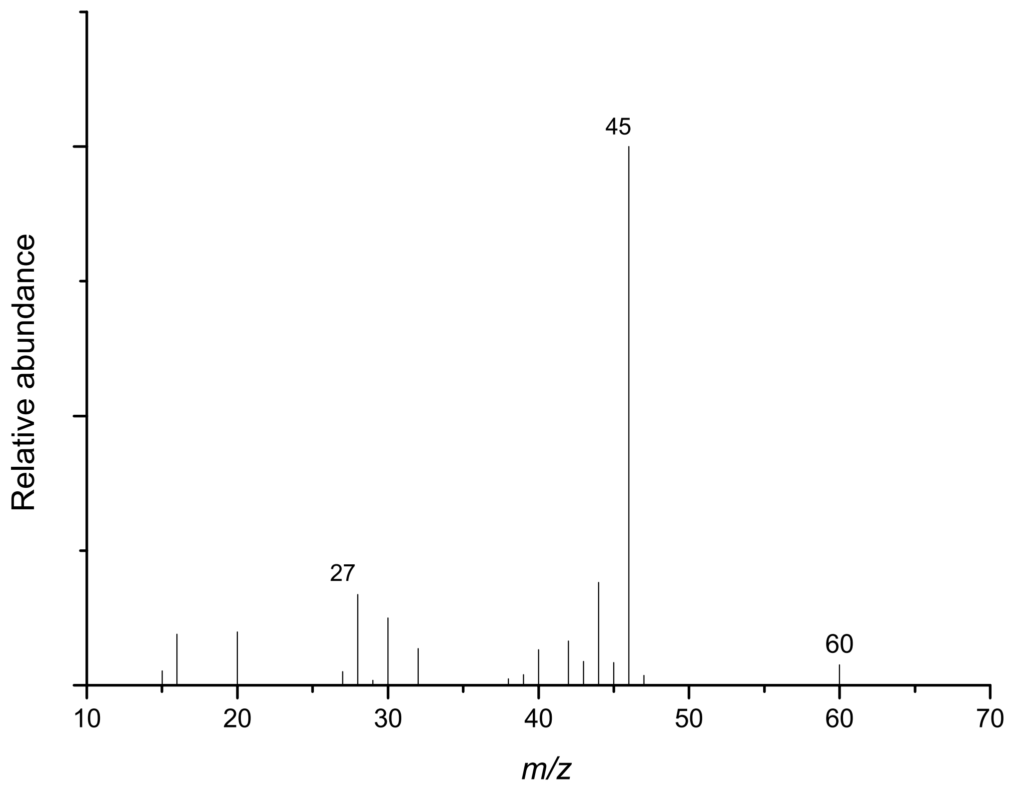
Sample A – MS

Empirical formula $C_7H_6O_2$



Sample B – MS

Empirical formula C_3H_8O



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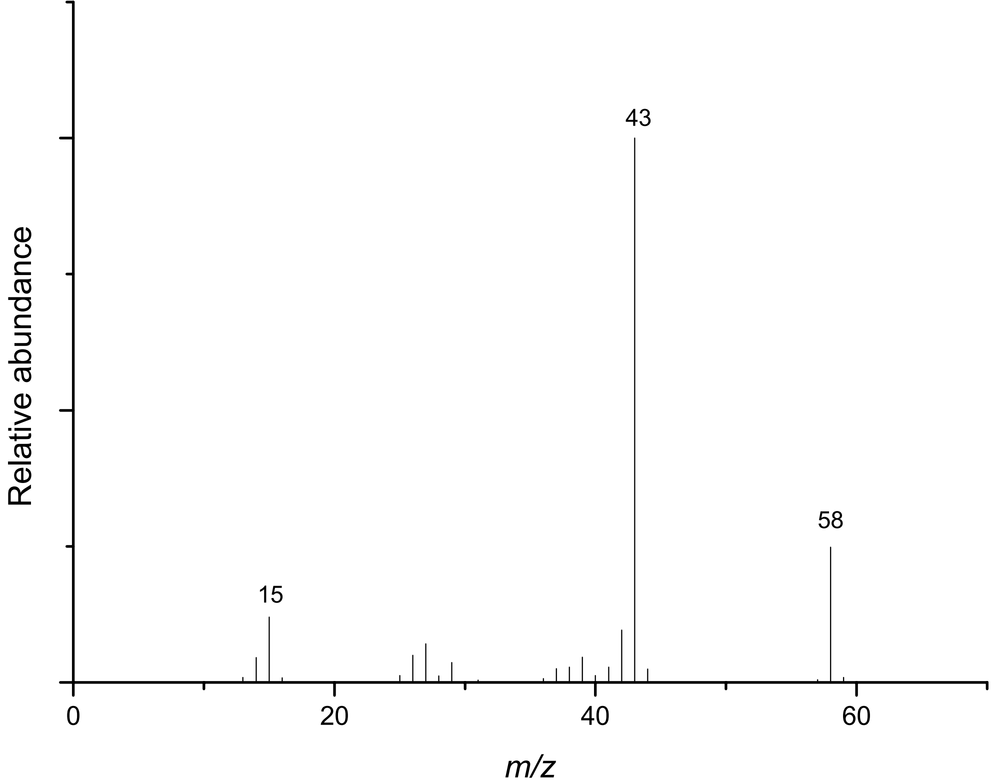
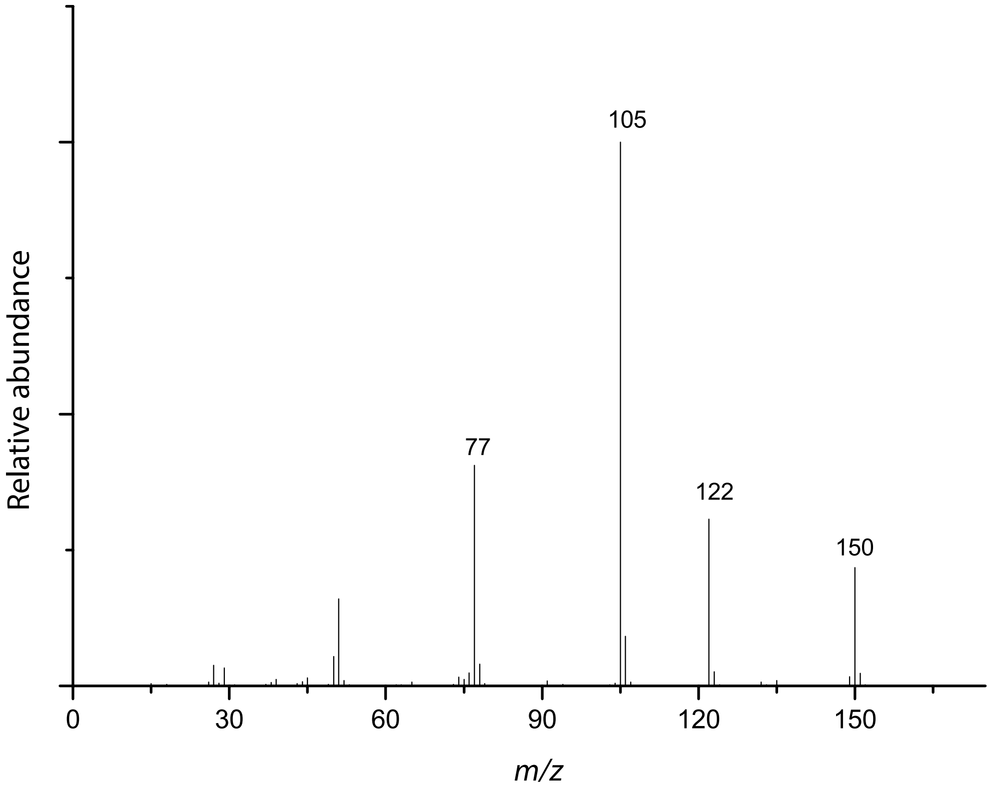
Sample C – MS

Empirical formula C_3H_6O



Sample D – MS

Empirical formula $C_9H_{10}O_2$



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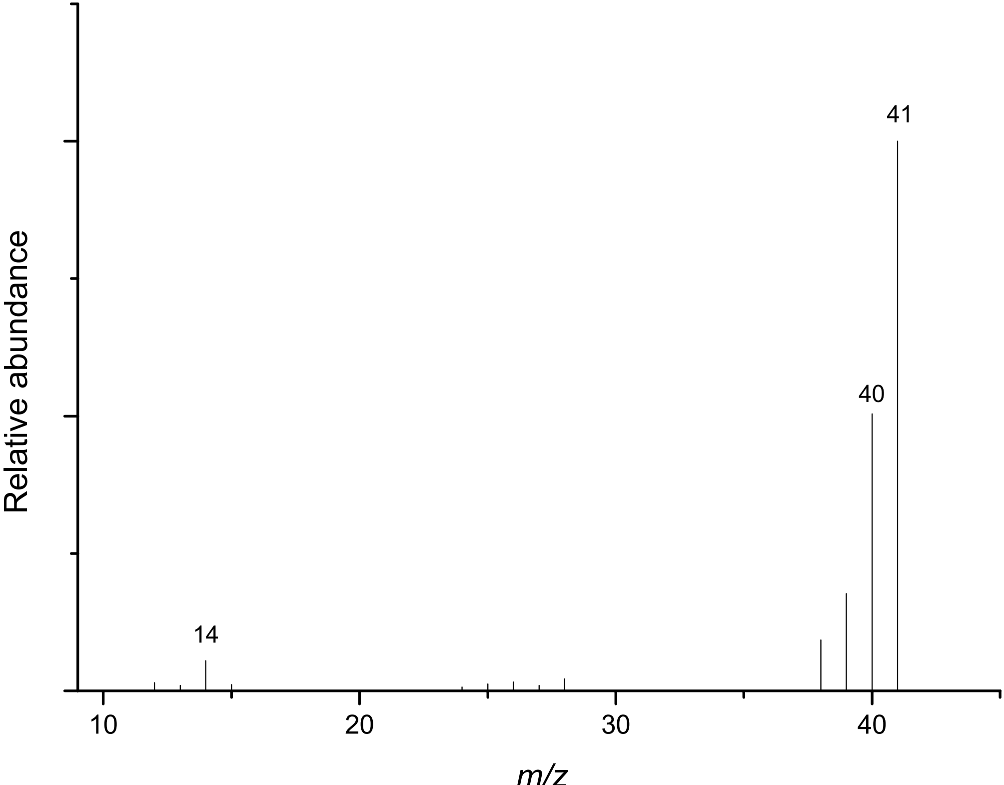
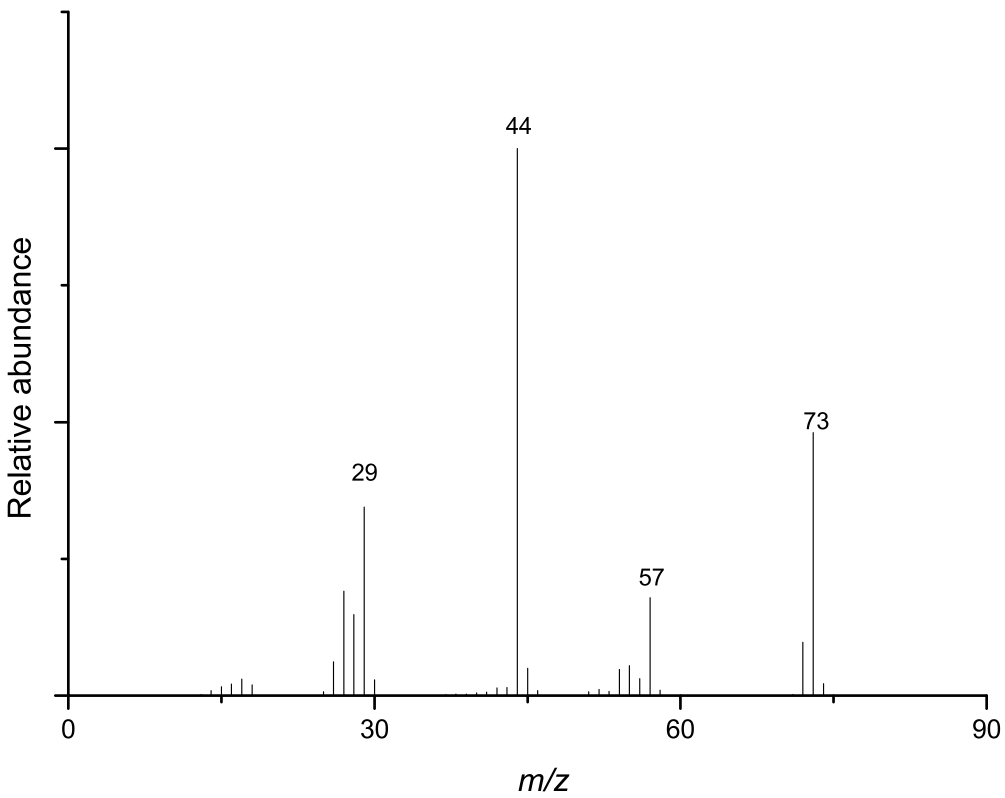
Sample E – MS

Empirical formula C_2H_3N



Sample F – MS

Empirical formula C_3H_7NO



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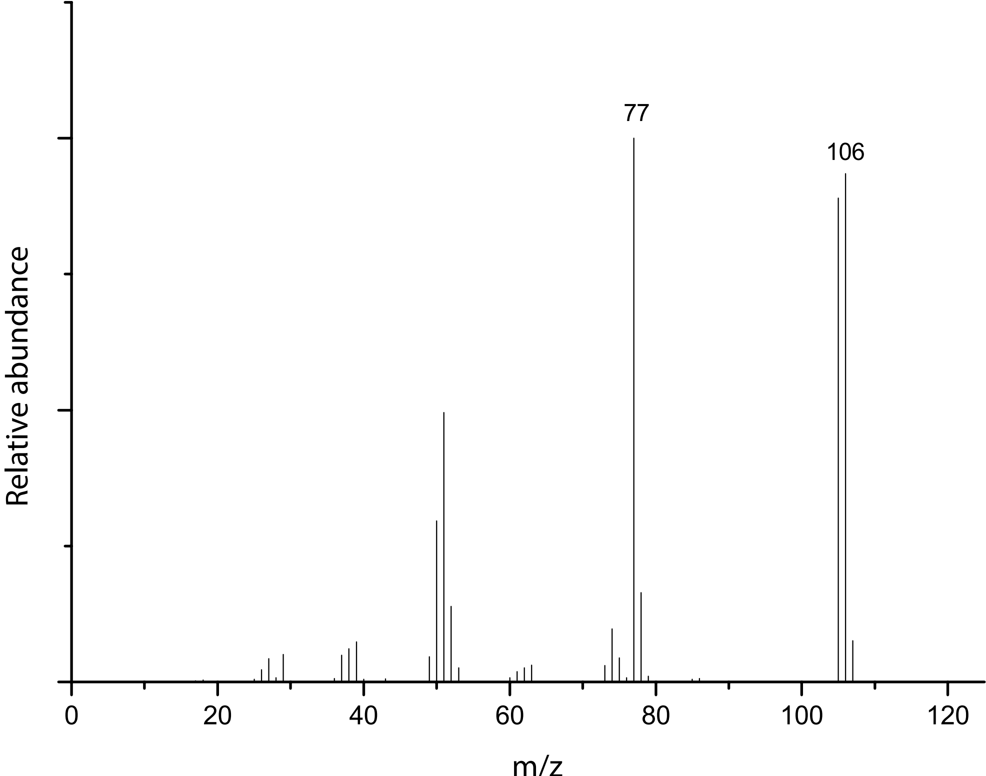
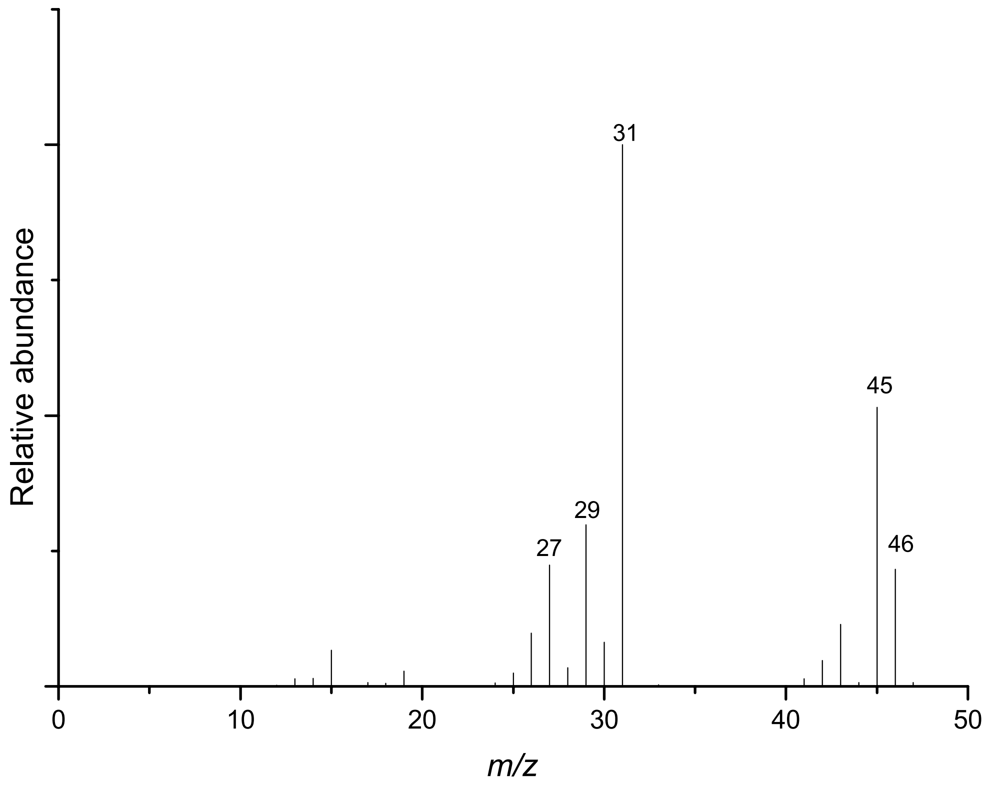
Sample G – MS

Empirical formula C_7H_6O



Sample H – MS

Empirical formula C_2H_6O



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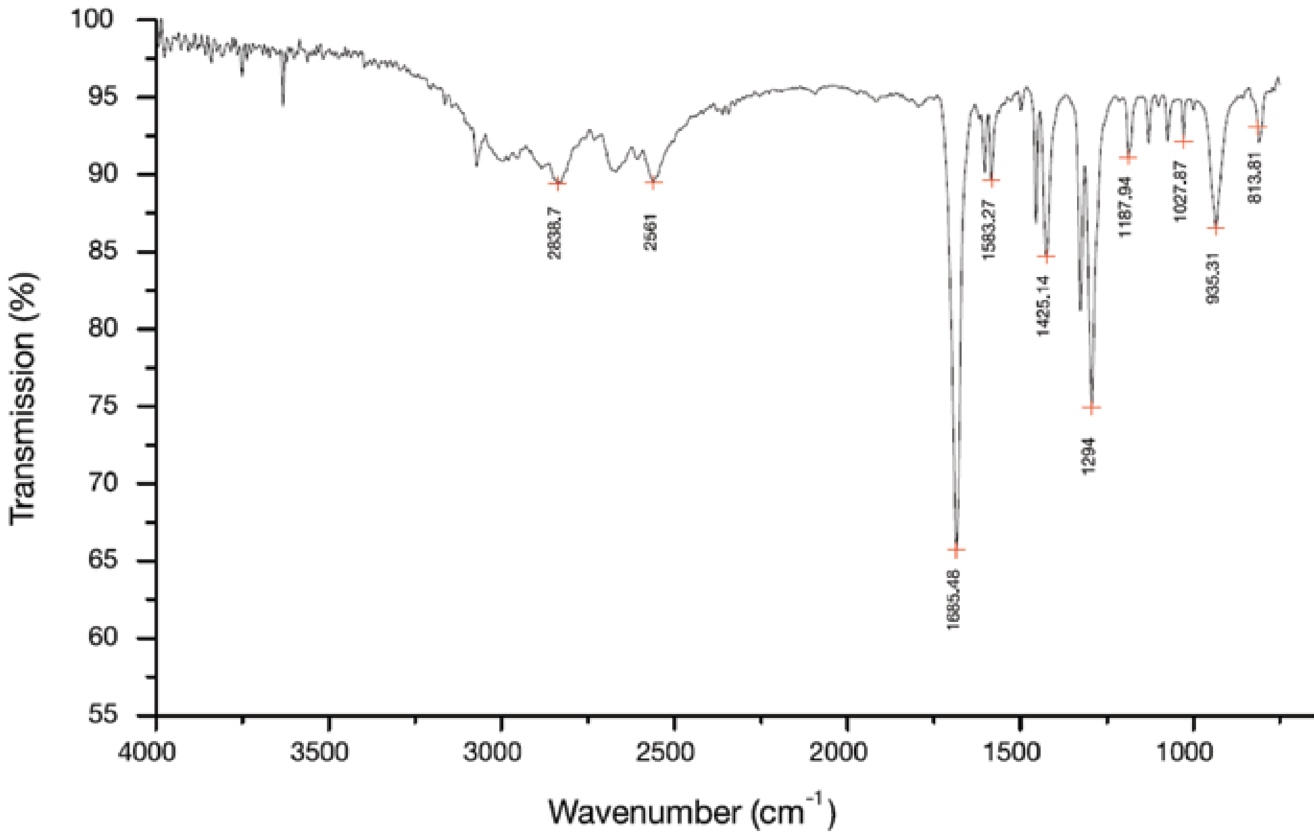
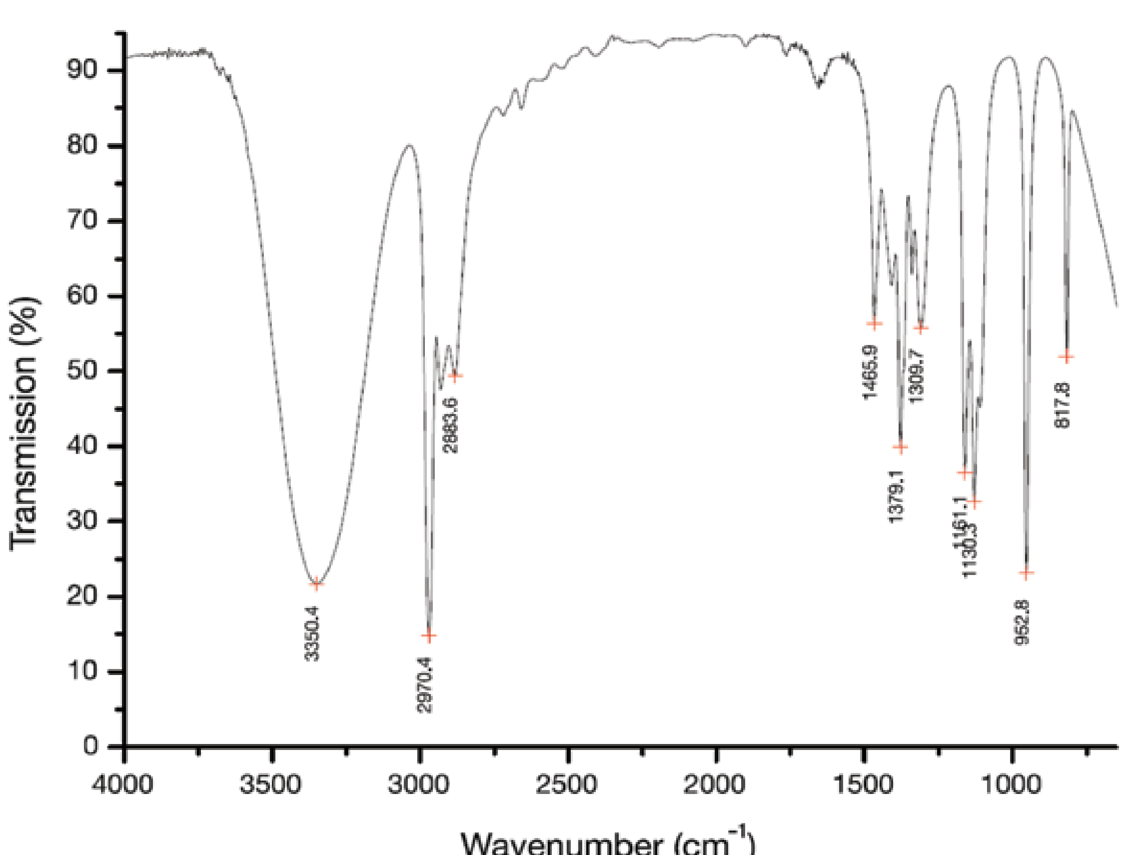
IR SPECTRA FOR INTERPRETATION

Sample A – IR



Sample B – IR



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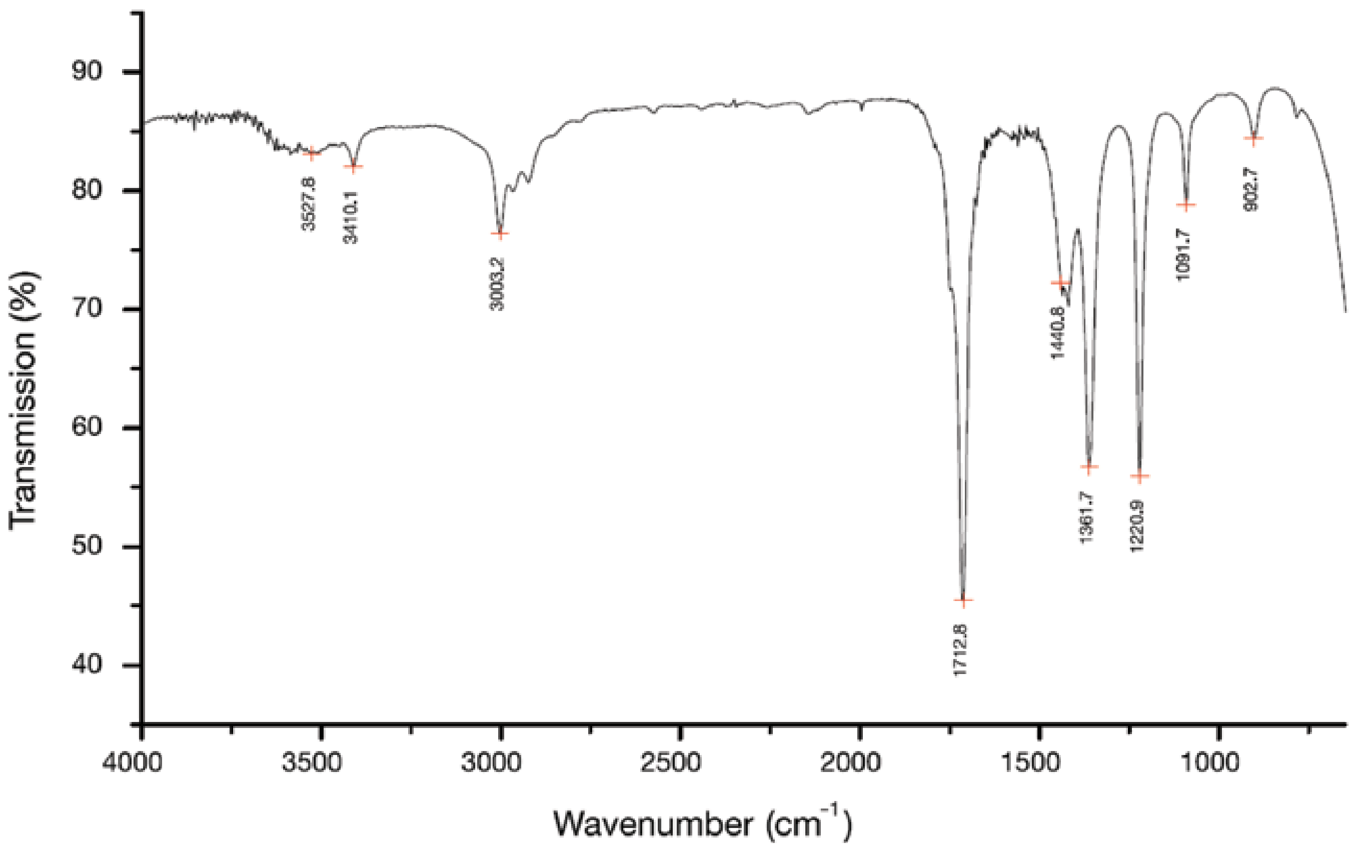
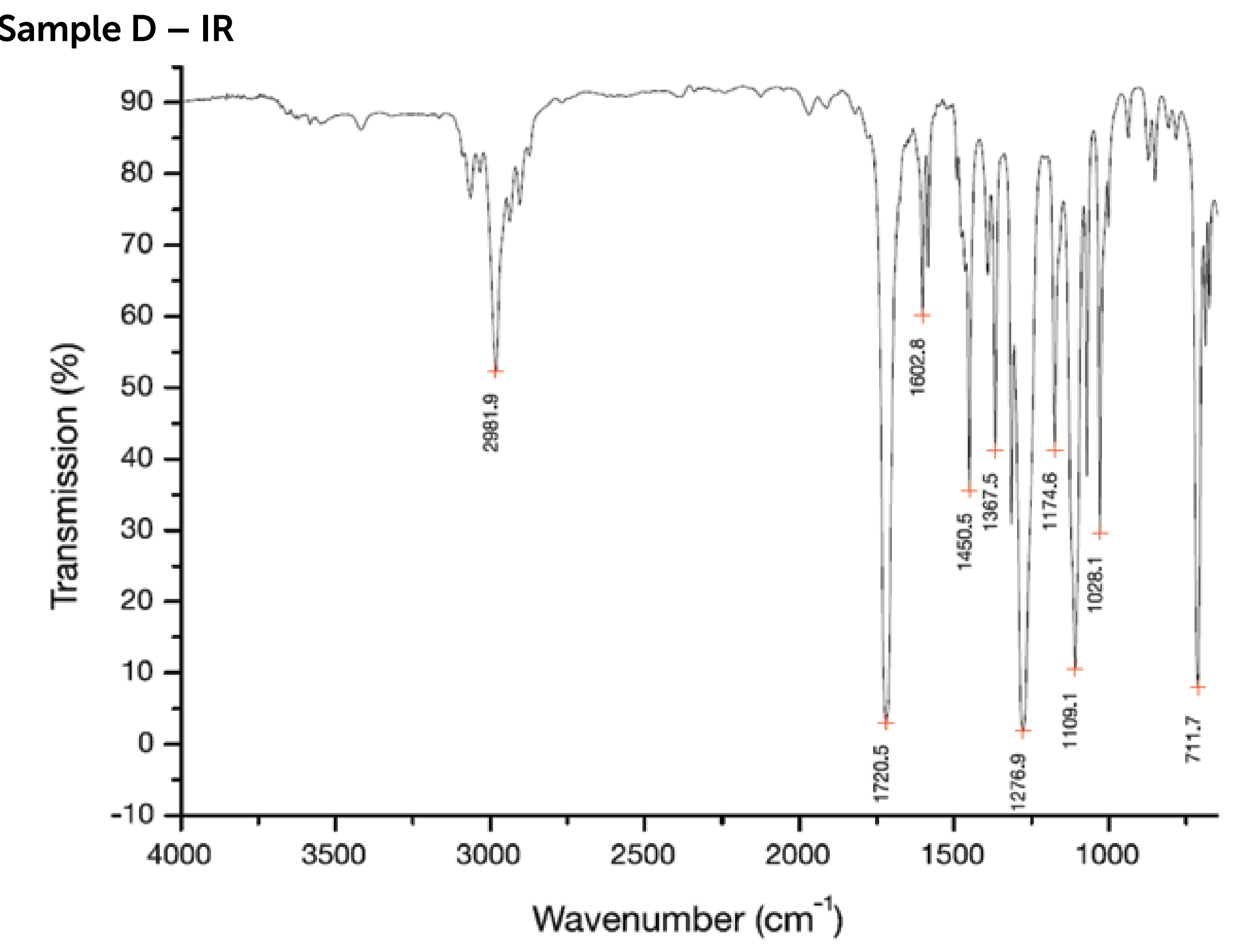
Sample C – IR



Sample D – IR



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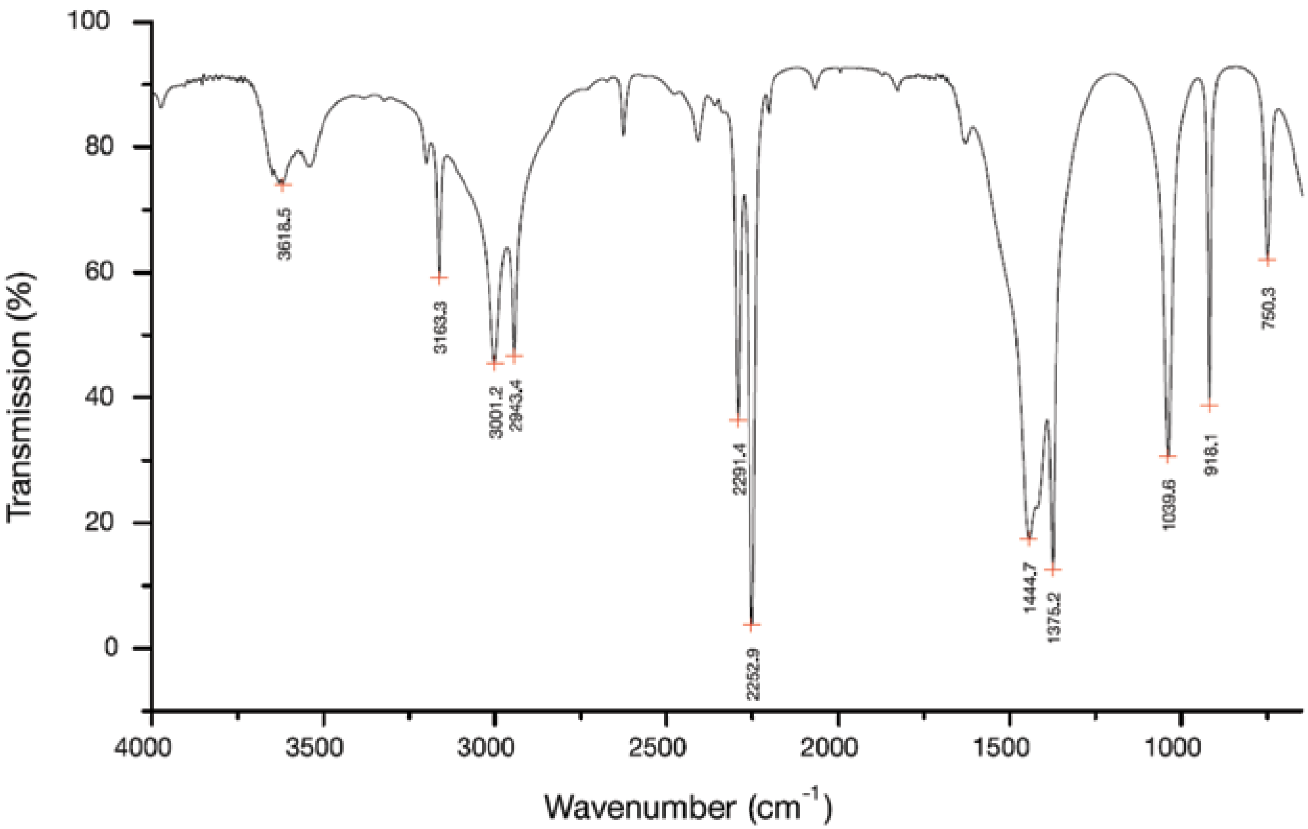
Sample E – IR



Sample F – IR

Transmission (%)
100
90
80
70
60
50
4000
3500
3000
2500
2000
1500
1000
Wavenumber (cm^{-1})
3359.39
3185.83
2979.48
1656.55
1419.35
1295.93
1141.65
821.53

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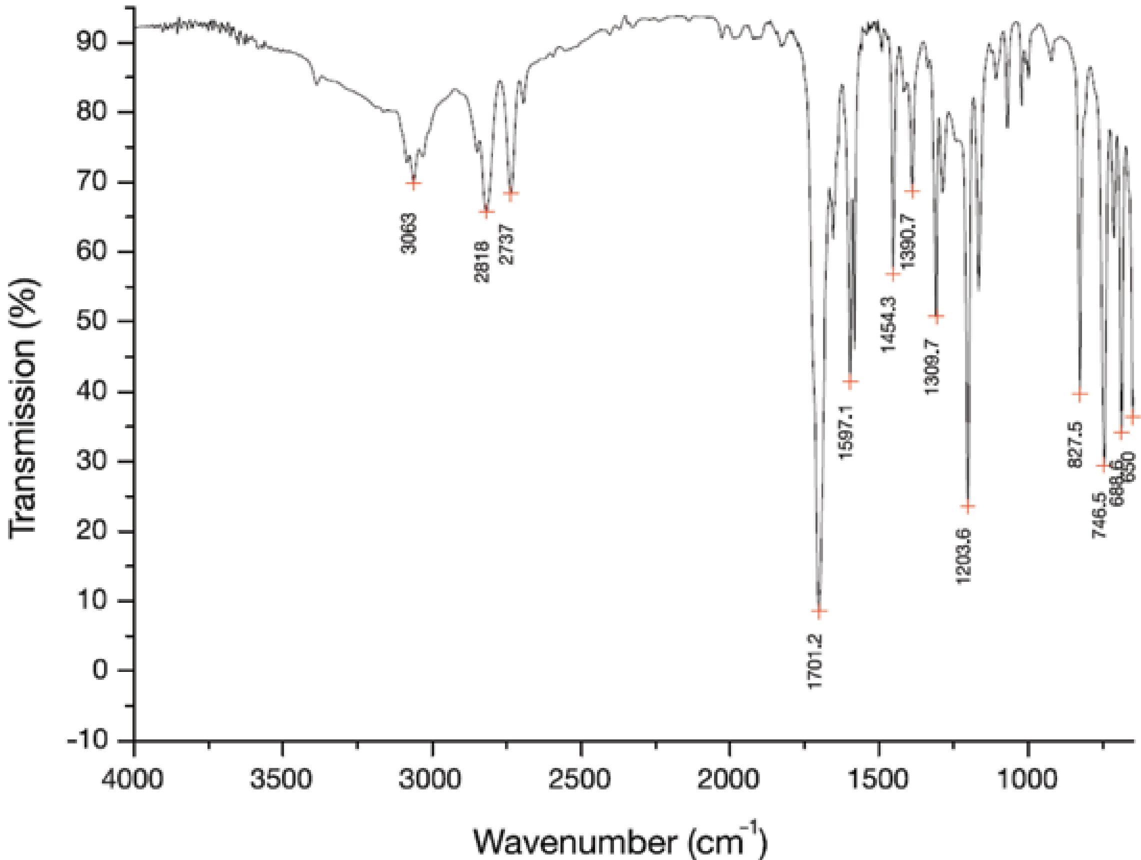
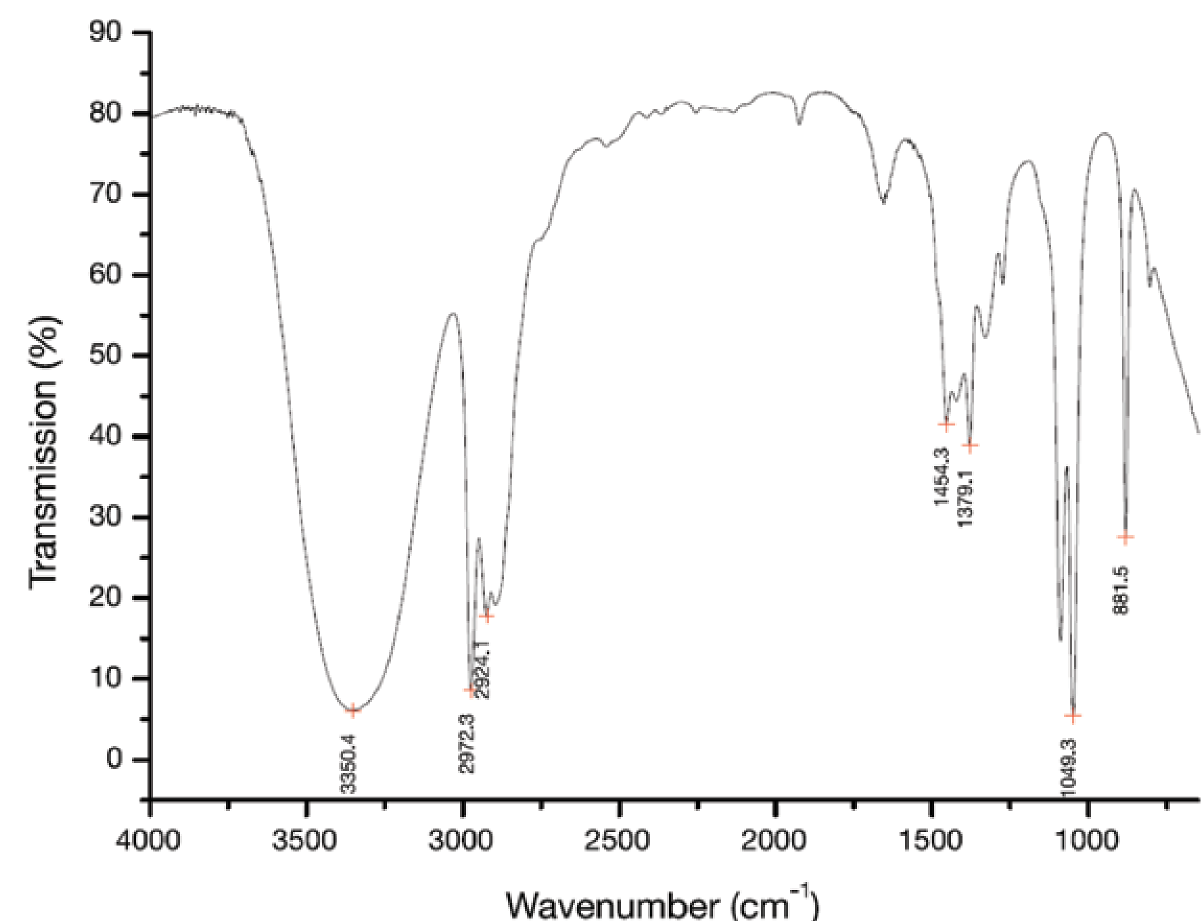
Sample G – IR



Sample H – IR



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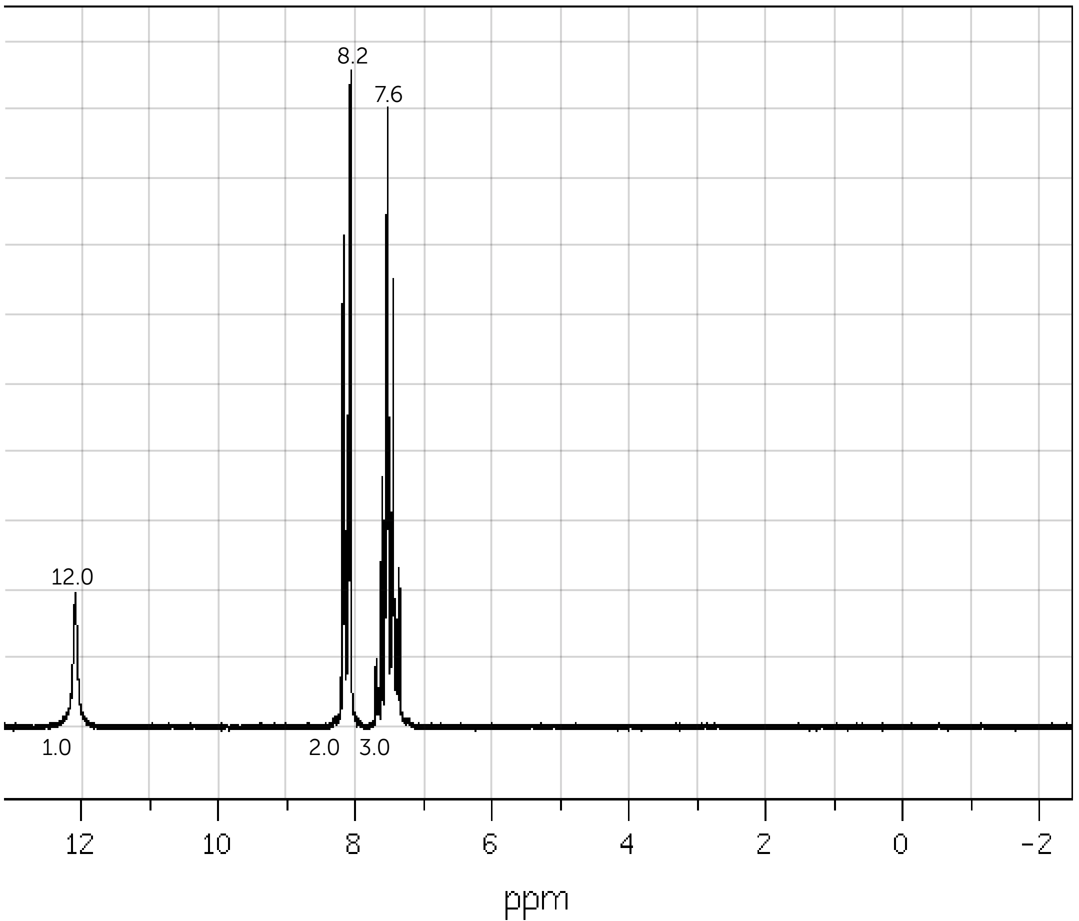
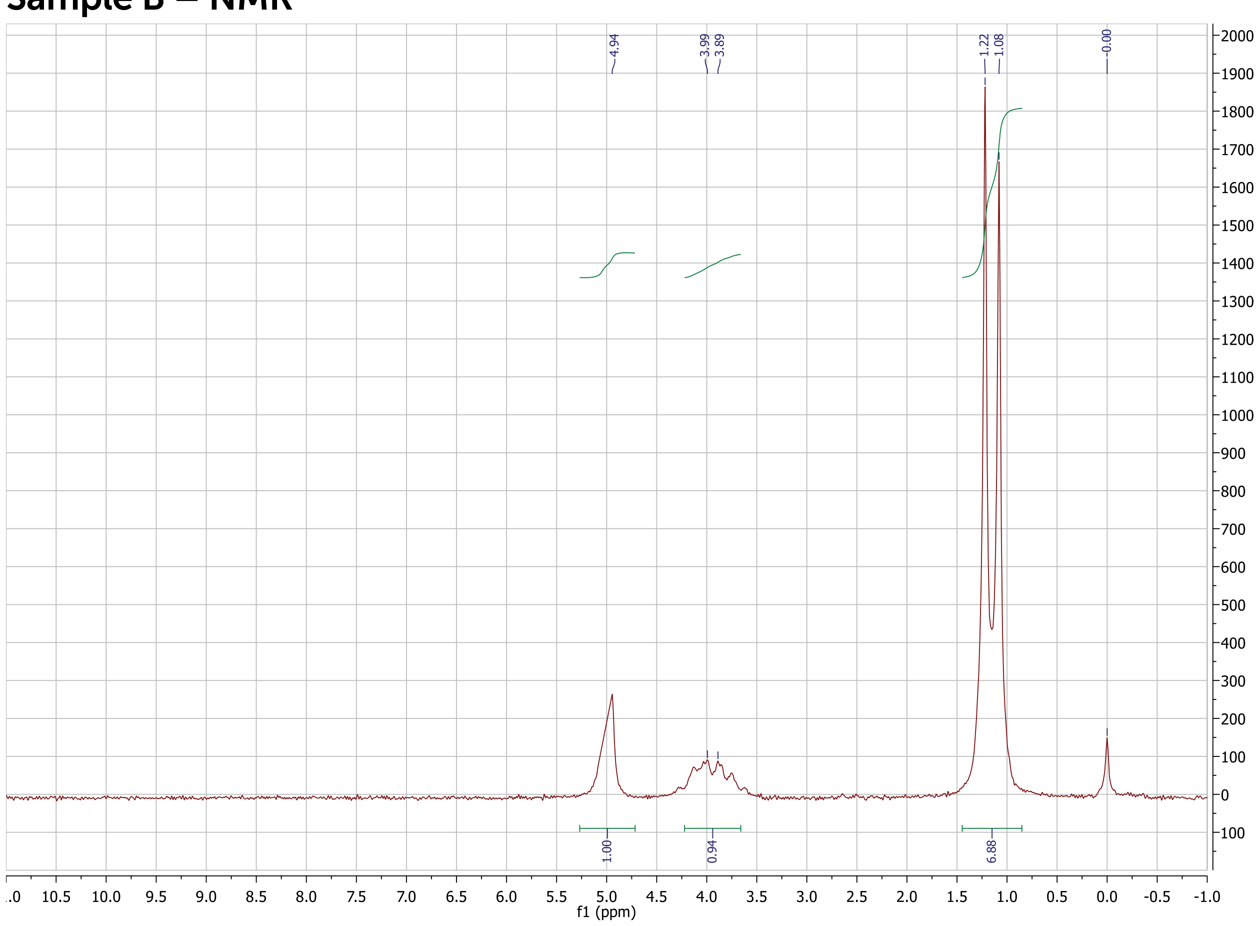
NMR SPECTRA FOR INTERPRETATION

Sample A – NMR



Sample B – NMR



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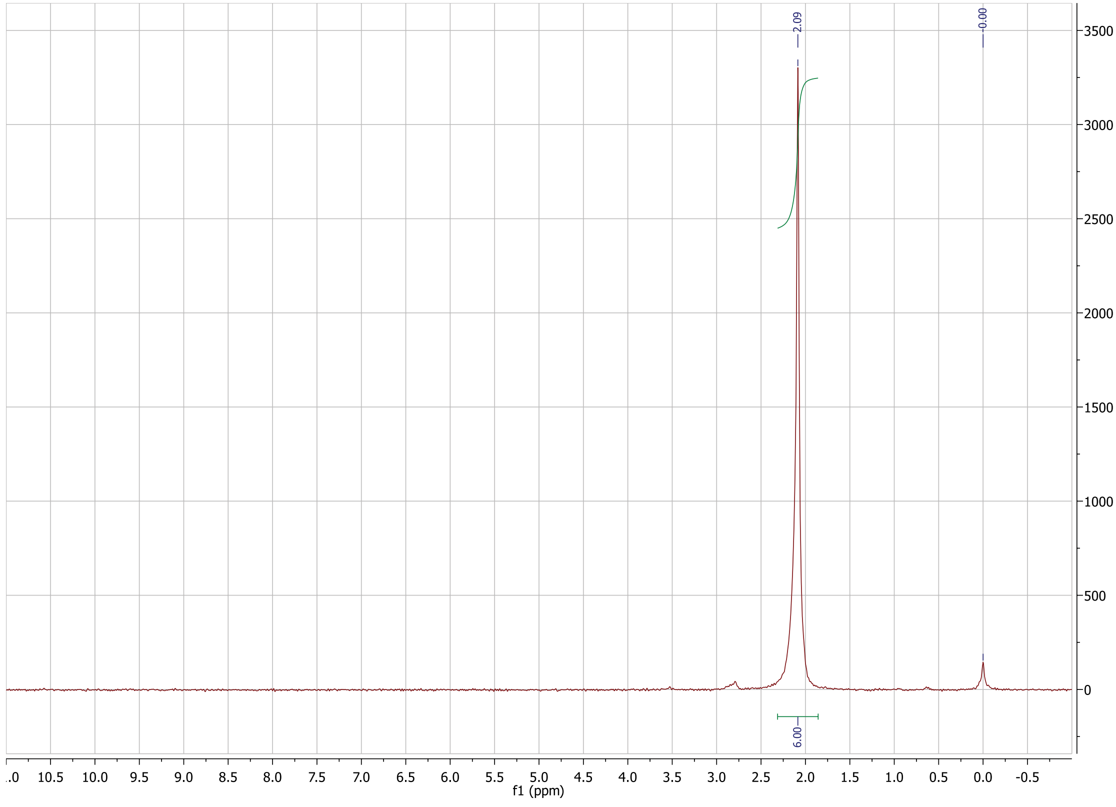
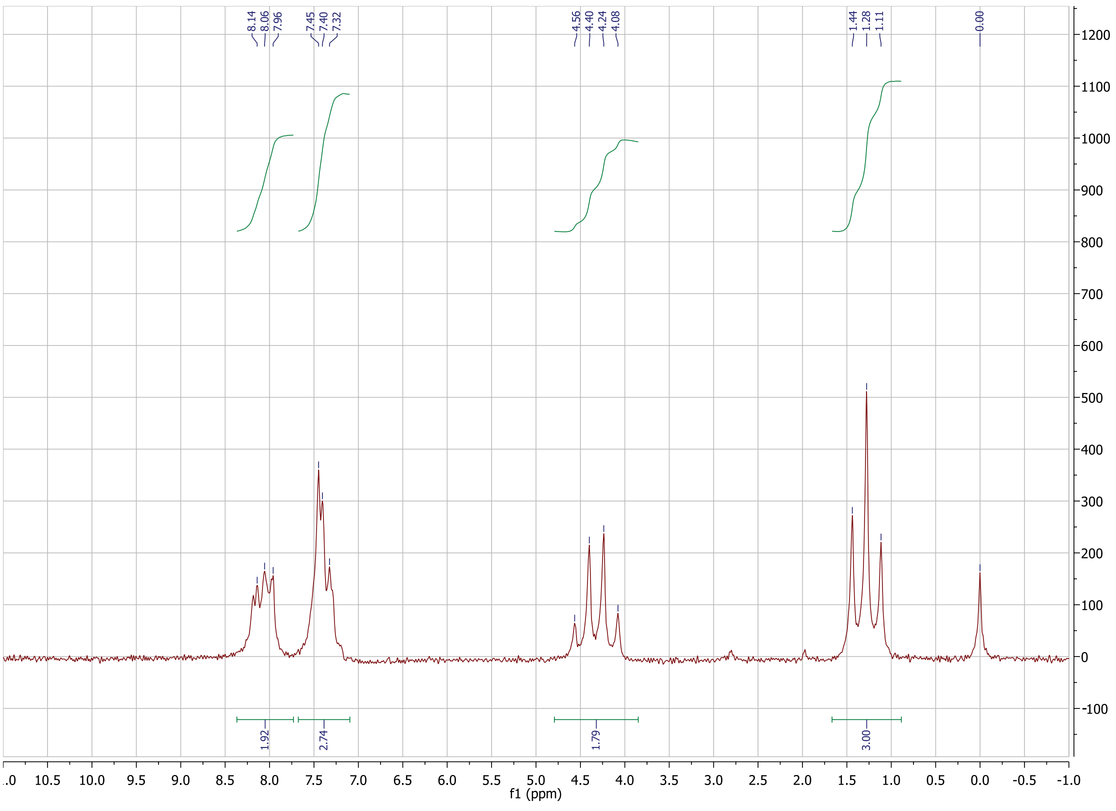
Sample C – NMR



Sample D – NMR



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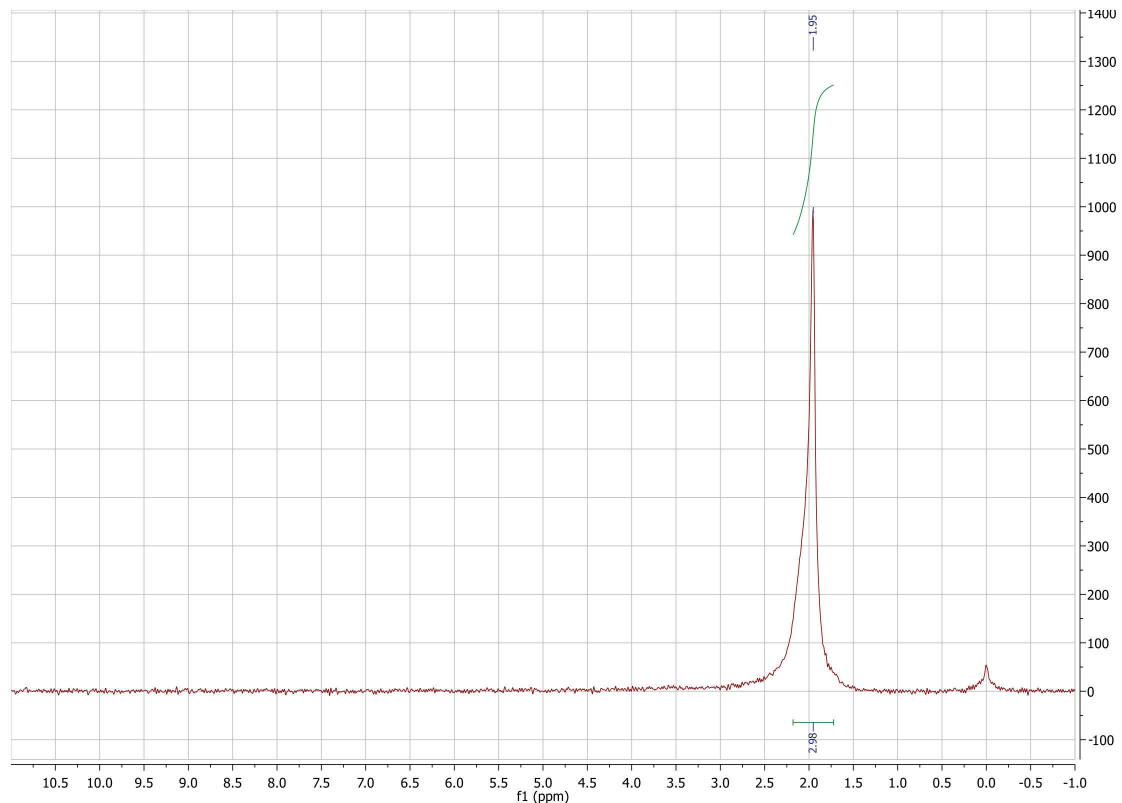
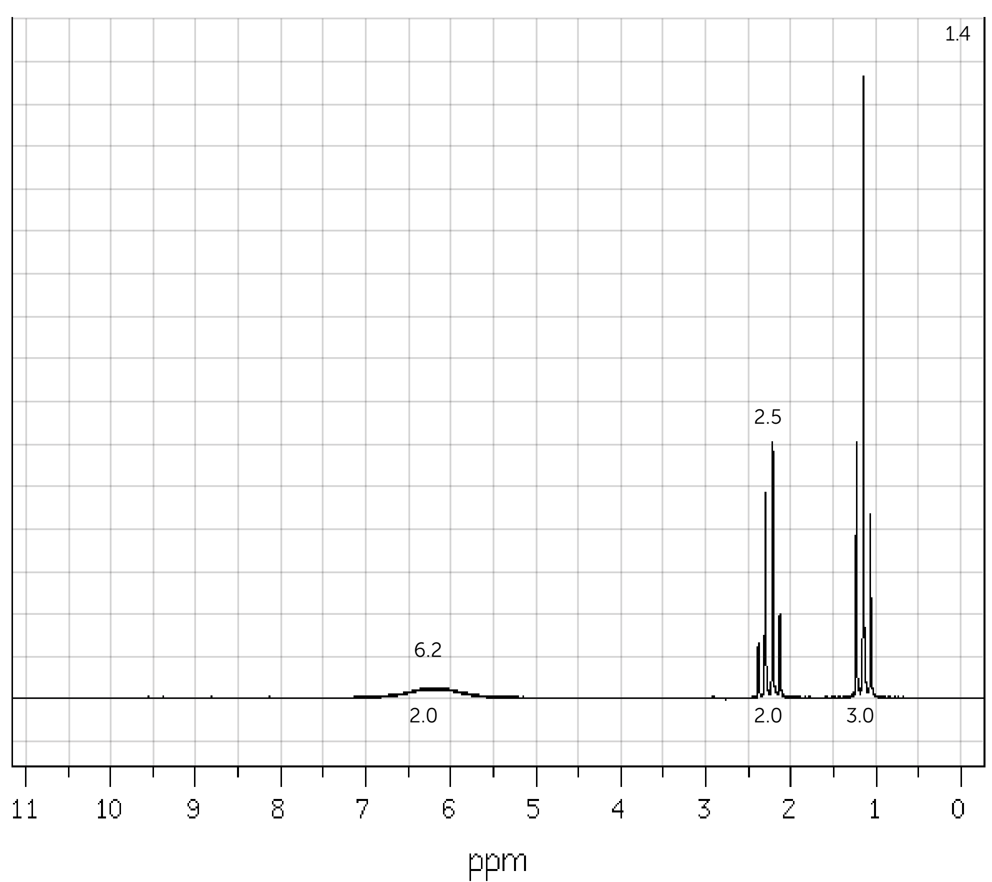
Sample E – NMR



Sample F – NMR



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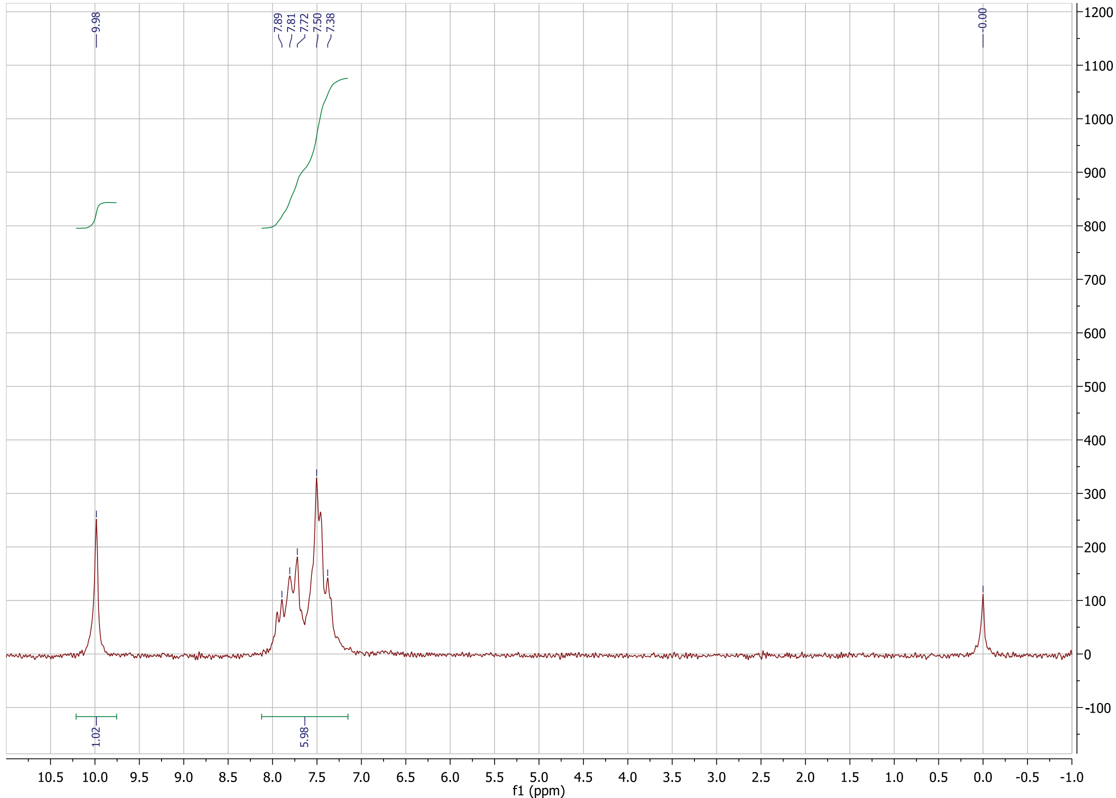
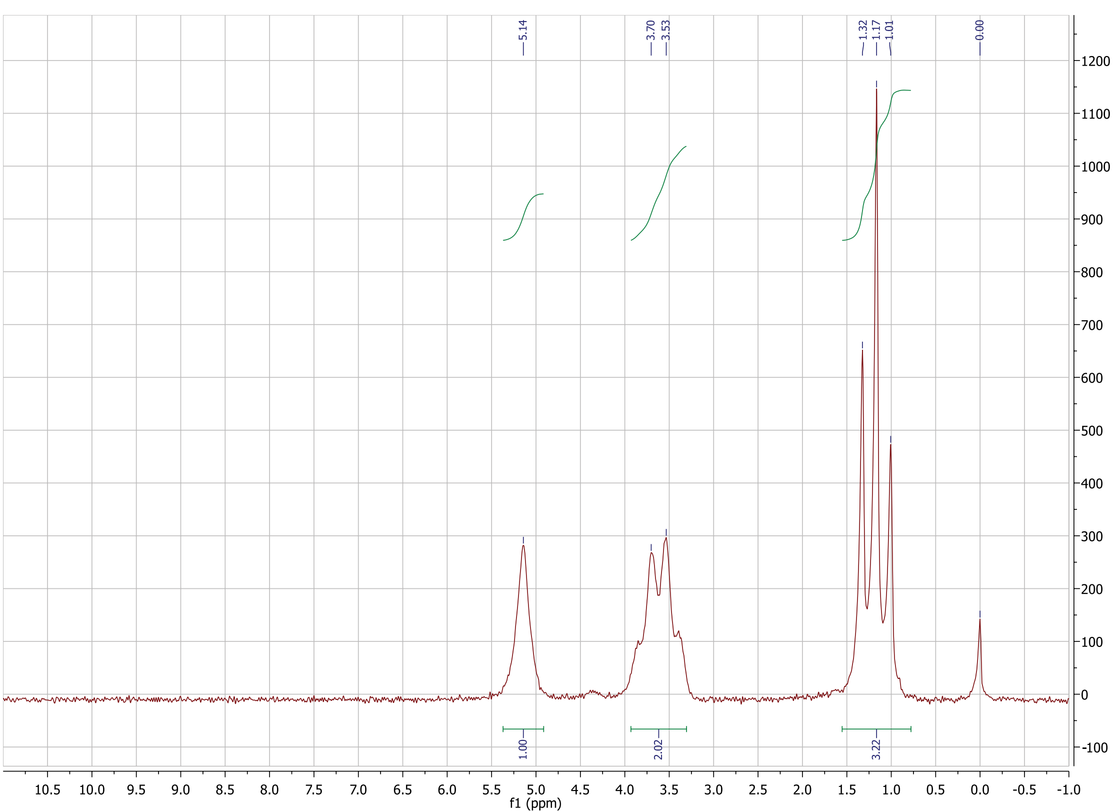
Sample G – NMR



Sample H – NMR



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Compound identification: Exercise 2
IR and NMR analysis

INTRODUCTION

NMR spectroscopy (NMR) provides us with useful information about a molecule's structure. Analysing NMR can be a bit like trying to piece together a puzzle made up of:

- Hydrogen environments – nuclei in different environments absorb different amounts of energy (radio waves) as they experience different magnetic fields inside the spectrometer and this gives rise to different 'peaks' for each environment.
- Chemical shift – the differences in absorption are measured relative to a standard chemical (e.g. tetramethylsilane) and this gives rise to a 'chemical shift' for all other chemical environments.
- Peak integral – area under each peak, which tells us the number of hydrogen nuclei in each environment.
- Splitting pattern – the peaks representing a particular hydrogen environment can be split by interactions with hydrogens on neighbouring carbon atoms. The peaks split into one more than the number of hydrogens on the neighbouring carbon atoms – this is known as the 'n + 1 rule'. You can work out the number of neighbouring hydrogens by looking at how the peak splits.

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METHOD

You will now use the following techniques to identify unknown organic compounds A to I:

- IR spectroscopy – provides information about the functional groups present.
- NMR spectroscopy – provides information about the structure.
- Elemental composition – can be used to calculate the molecular formula.

1. Use the elemental compositions and molecular masses on the next page to work out the molecular formula of each unknown and record this on your worksheet.
2. Analyse an unknown sample using the ATR IR spectrometer and identify the functional group(s) present using the correlation chart provided.

   (Note: Use only when supervised by a demonstrator.)
3. In groups, analyse a sample using the NMR spectrometer.

   (Note: Use only when supervised by a demonstrator.)

   - Count the number of peaks to find the number of hydrogen environments.
   - Compare integrals to determine the number of protons in each environment.
   - Determine the splitting pattern for each peak (remember the 'n+1 rule').
   - Look up the chemical shifts using the data tables provided if time allows.
4. Use your interpreted IR and NMR spectra and the copies of spectra provided to determine the structure of all unknown compounds and record your assignments on your worksheet.

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STUDENT WORKSHEET

Molecular mass and elemental composition data:

Sample A M_r: 60 C: 59.96% H: 13.42% O: 26.62%	**Sample B** M_r: 58 C: 62.04% H: 10.41% O: 27.55%	**Sample C** M_r: 86 C: 69.72% H: 11.70% O: 18.58%
Sample D M_r: 72 C: 66.63% H: 11.18% O: 22.19%	**Sample E** M_r: 74 C: 48.64% H: 8.16% O: 43.20%	**Sample F** M_r: 86 C: 69.72% H: 11.70% O: 18.58%
Sample G M_r: 88 C: 54.53% H: 9.15% O: 36.32%	**Sample H** M_r: 60 C: 59.96% H: 13.42% O: 26.62%	**Sample I** M_r: 88 C: 54.53% H: 9.15% O: 36.32%

Calculating molecular formulae from elemental composition and molecular mass: **Sample A**
M_r: 60 g mol^{-1}
C: 59.96%
H: 13.42%
O: 26.62%

1) Assume 100 g of the compound is present and change the percentages to grams:
   C = 59.96 g
   H = 13.42 g
   O = 26.62 g

2) Convert the masses to moles:
   C = 59.96 g / 12 g/mol = **5.0 mol**
   H = 13.42 g / 1 g/mol = **13.4 mol**
   O = 26.62 g / 16 g/mol = **1.66 mol**

3) Divide by the lowest number to find the smallest whole number ratio:
   C = 5.0 / 1.66 = **3**
   H = 13.4 / 1.66 = **8**
   O = 1.66 / 1.66 = **1**

4) Write the empirical formula:
   C_3H_8O

5) Determine the molecular formula:
   Effective formula weight = (3 x 12) + (8 x 1) + 16 = 60 g/mol
   60 / 60 = 1
   Molecular formula = C_3H_8O

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STUDENT WORKSHEET

Unknown	Molecular formula	Functional groups and wavenumber	NMR assignments	Name (inc. common name)	Structure
Sample A					
Sample B					
Sample C					
Sample D (paper copy only)					
Sample E (paper copy only)					
Sample F					
Sample G					
Sample H					
Sample I					

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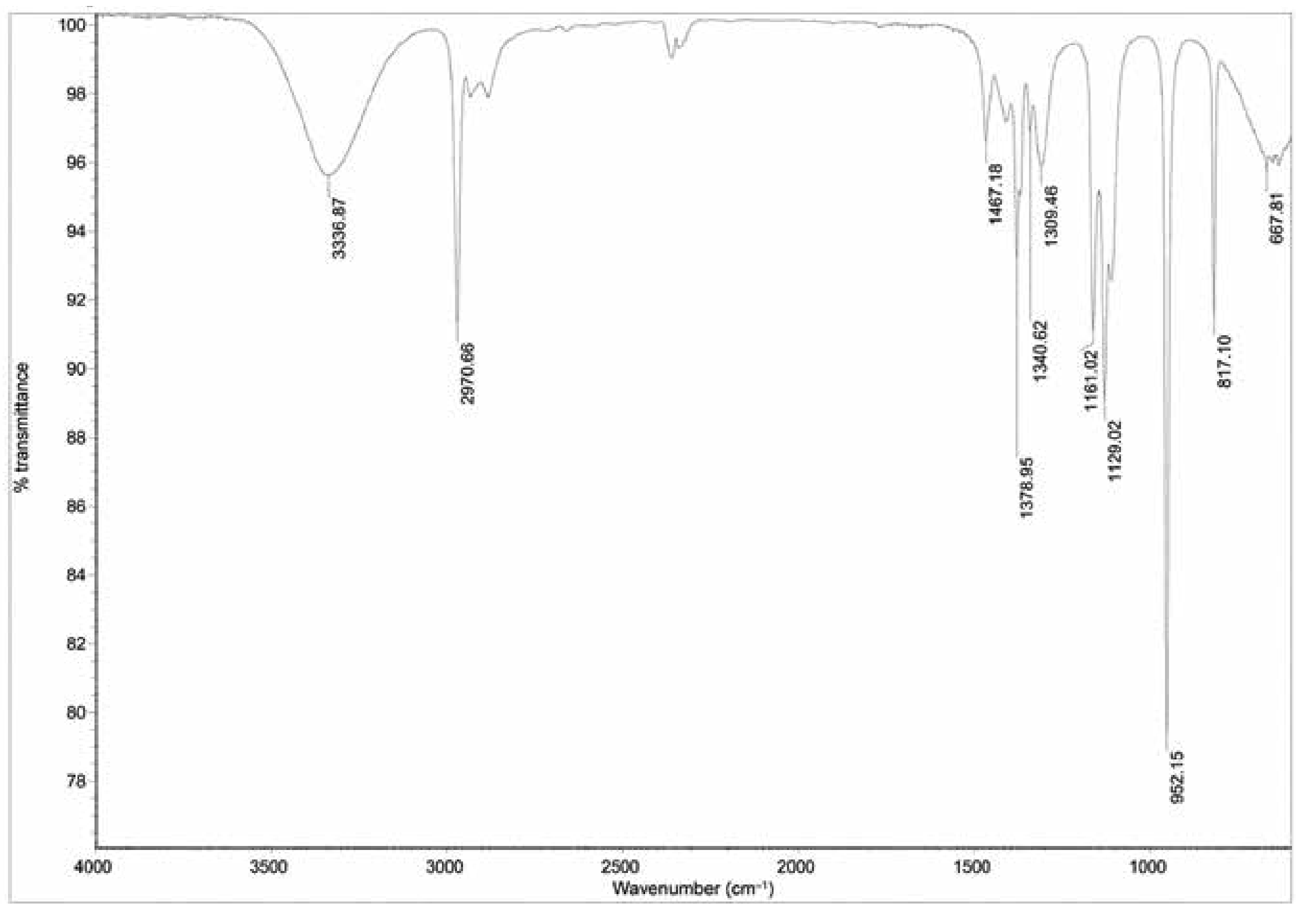
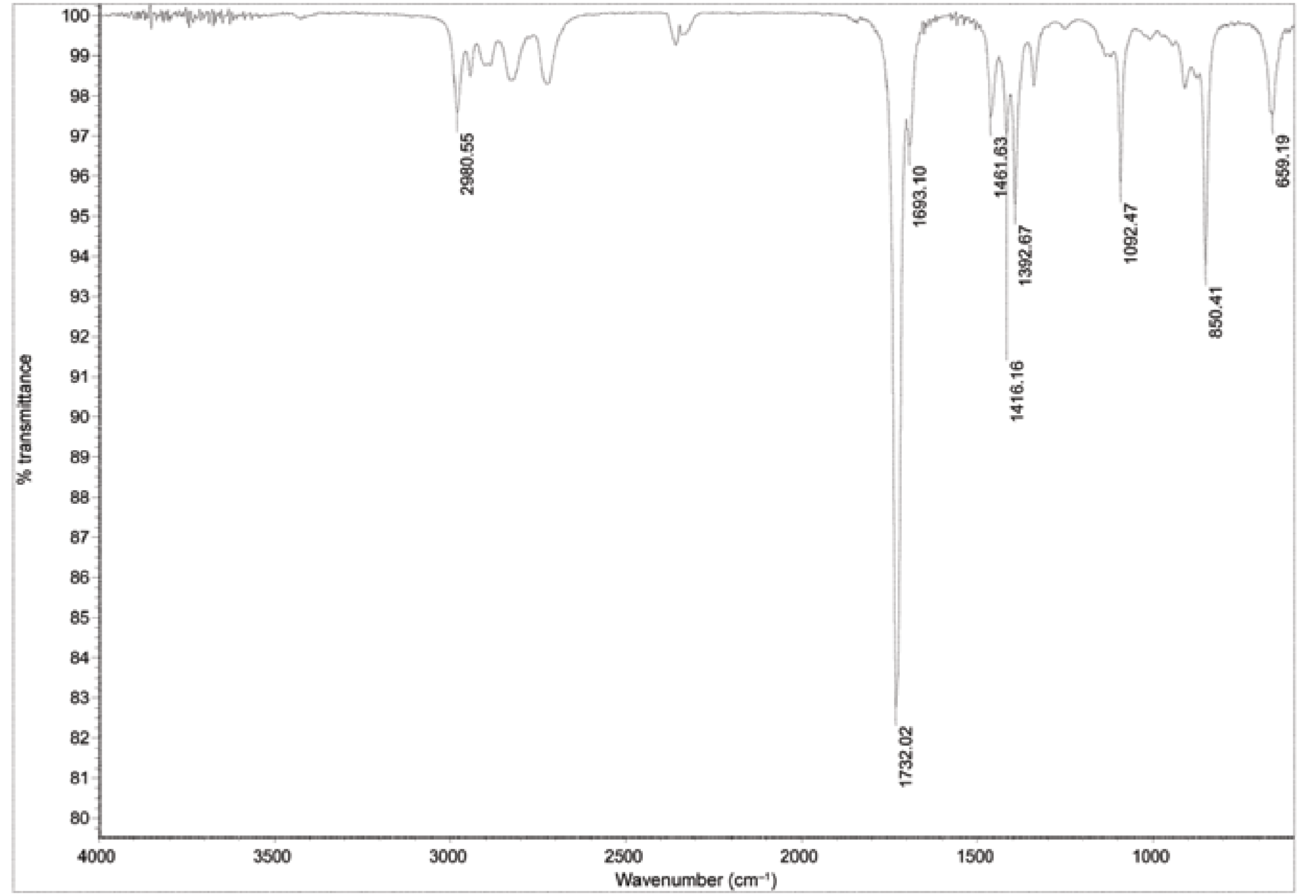
IR SPECTRA FOR INTERPRETATION

Sample A – IR



Sample B – IR



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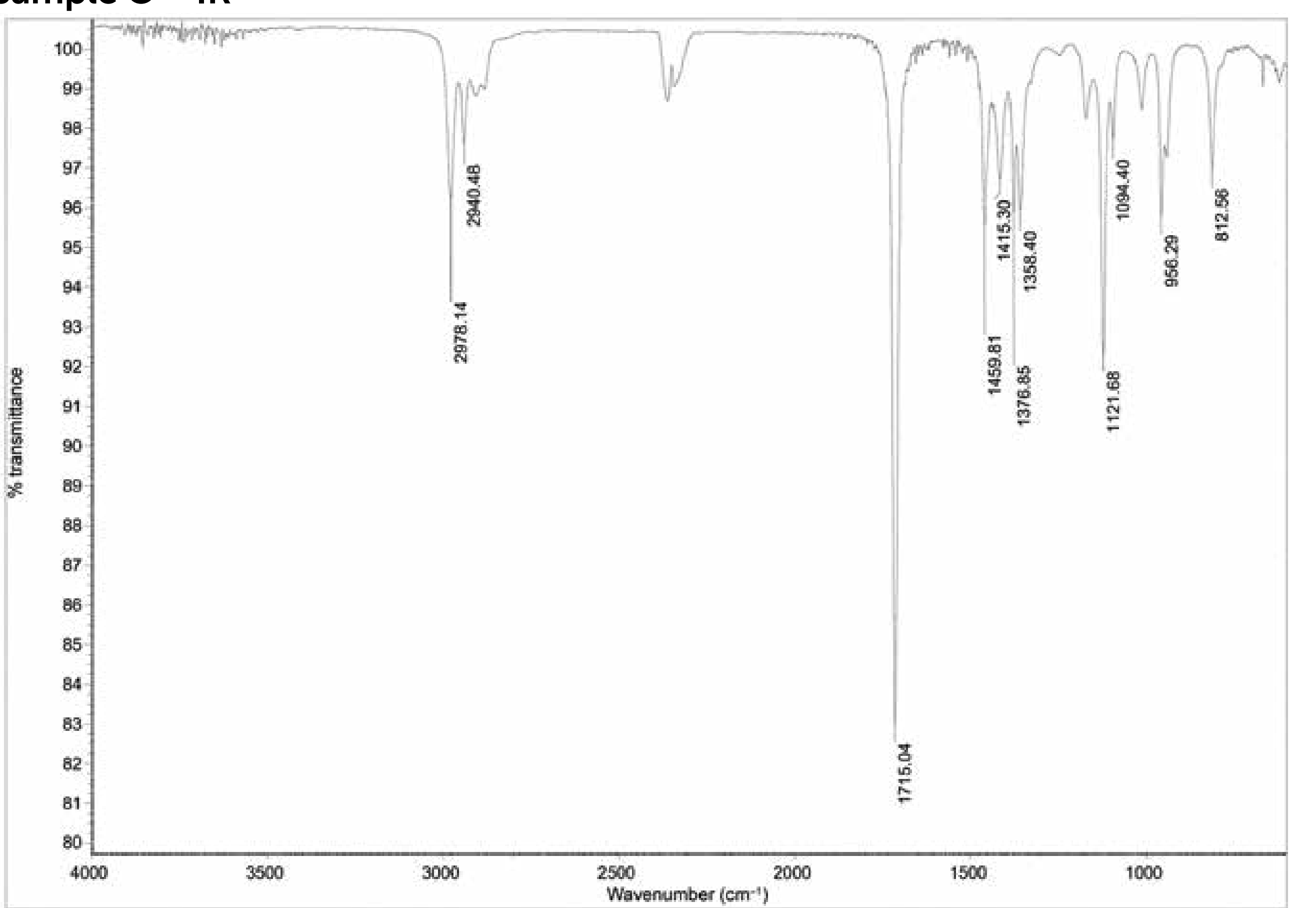
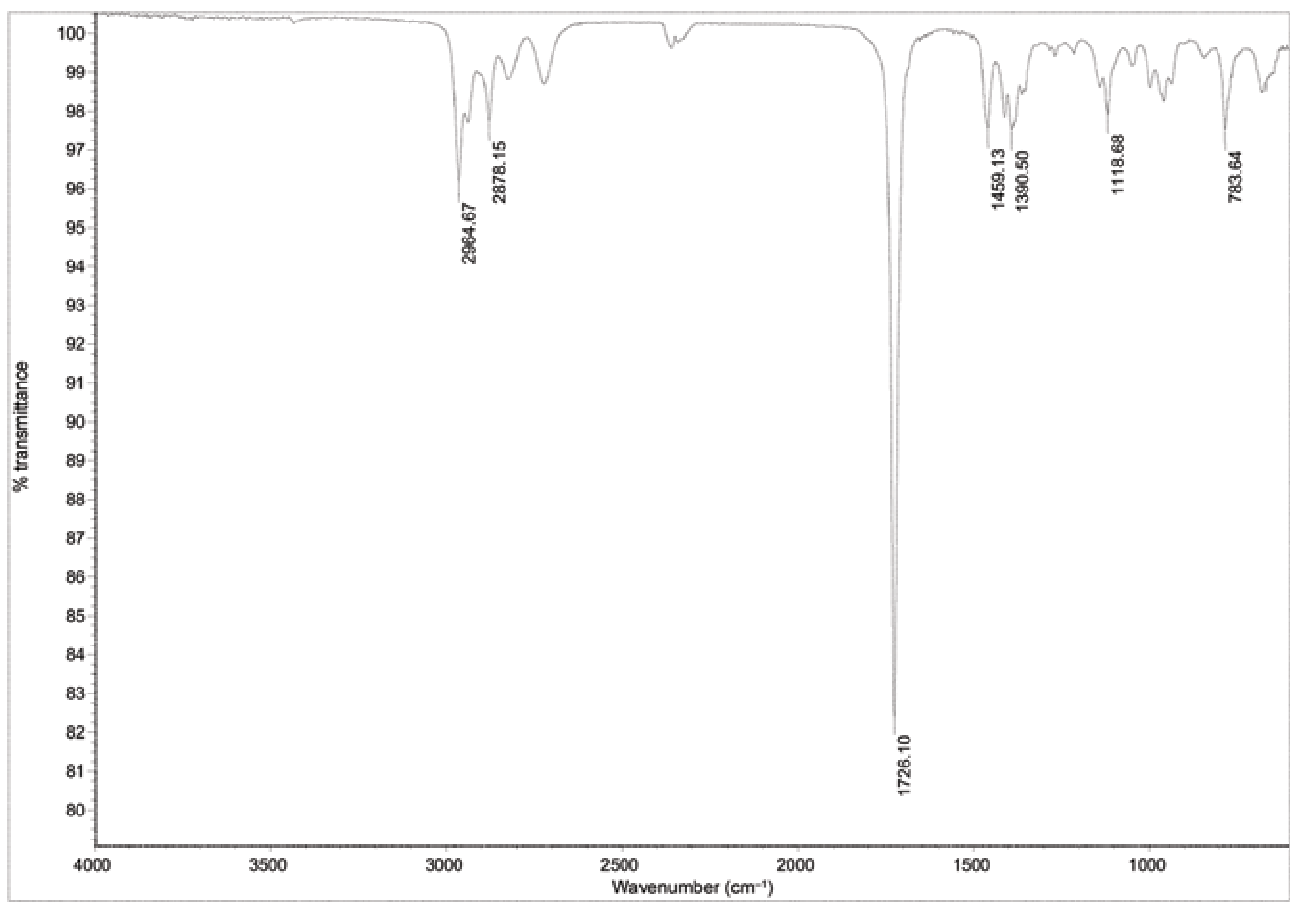
Sample C – IR



Sample D – IR



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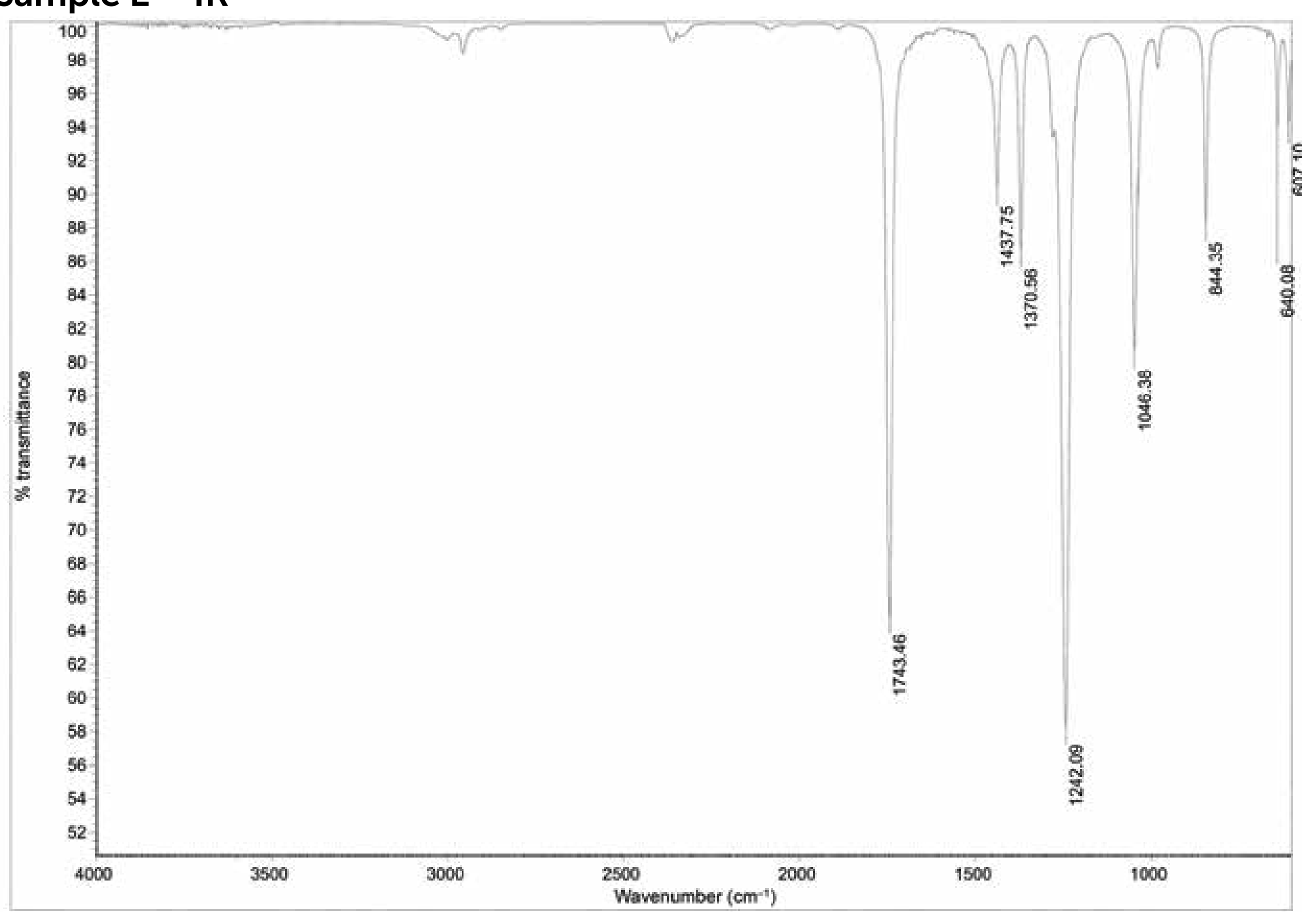
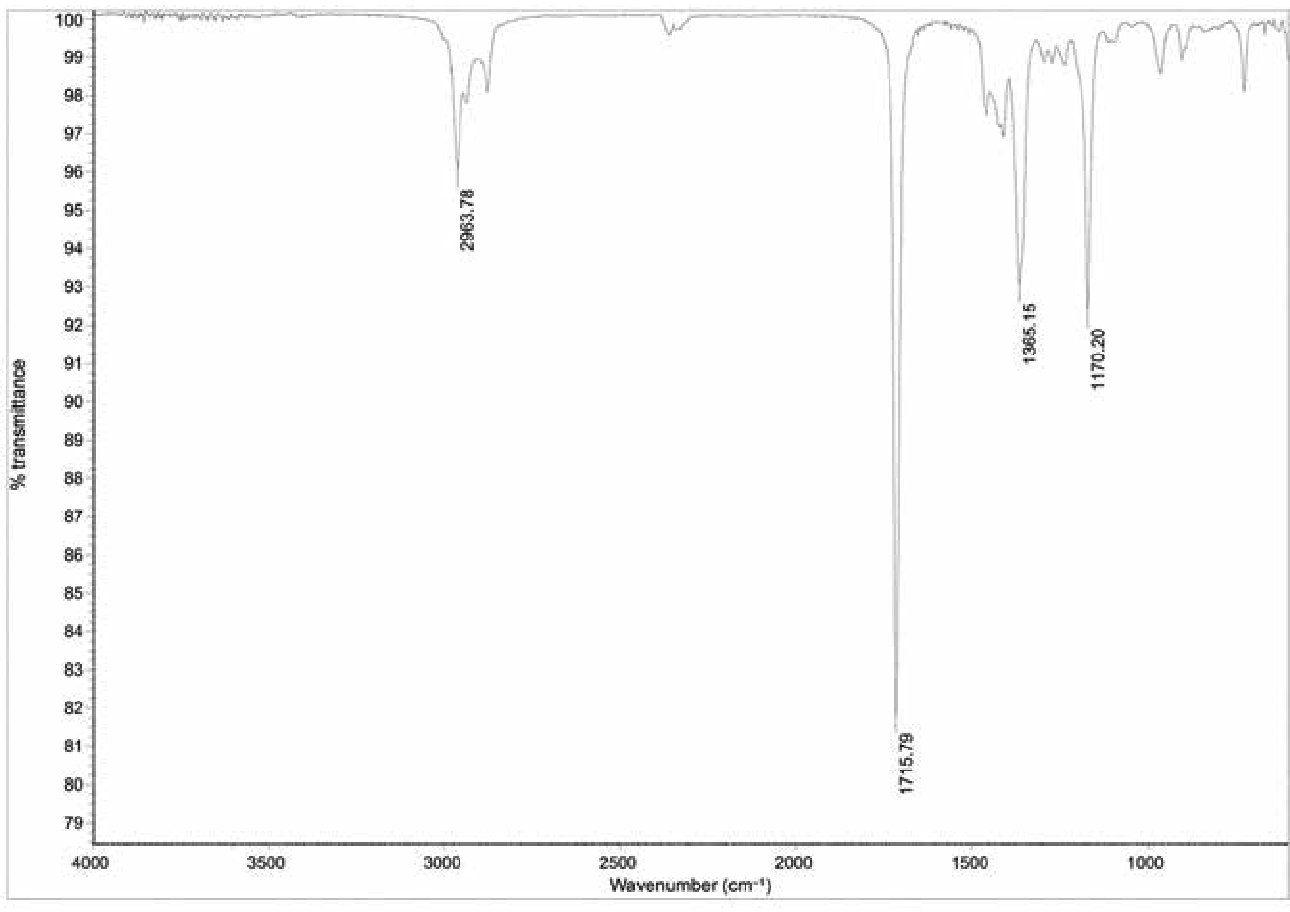
Sample E – IR



Sample F – IR



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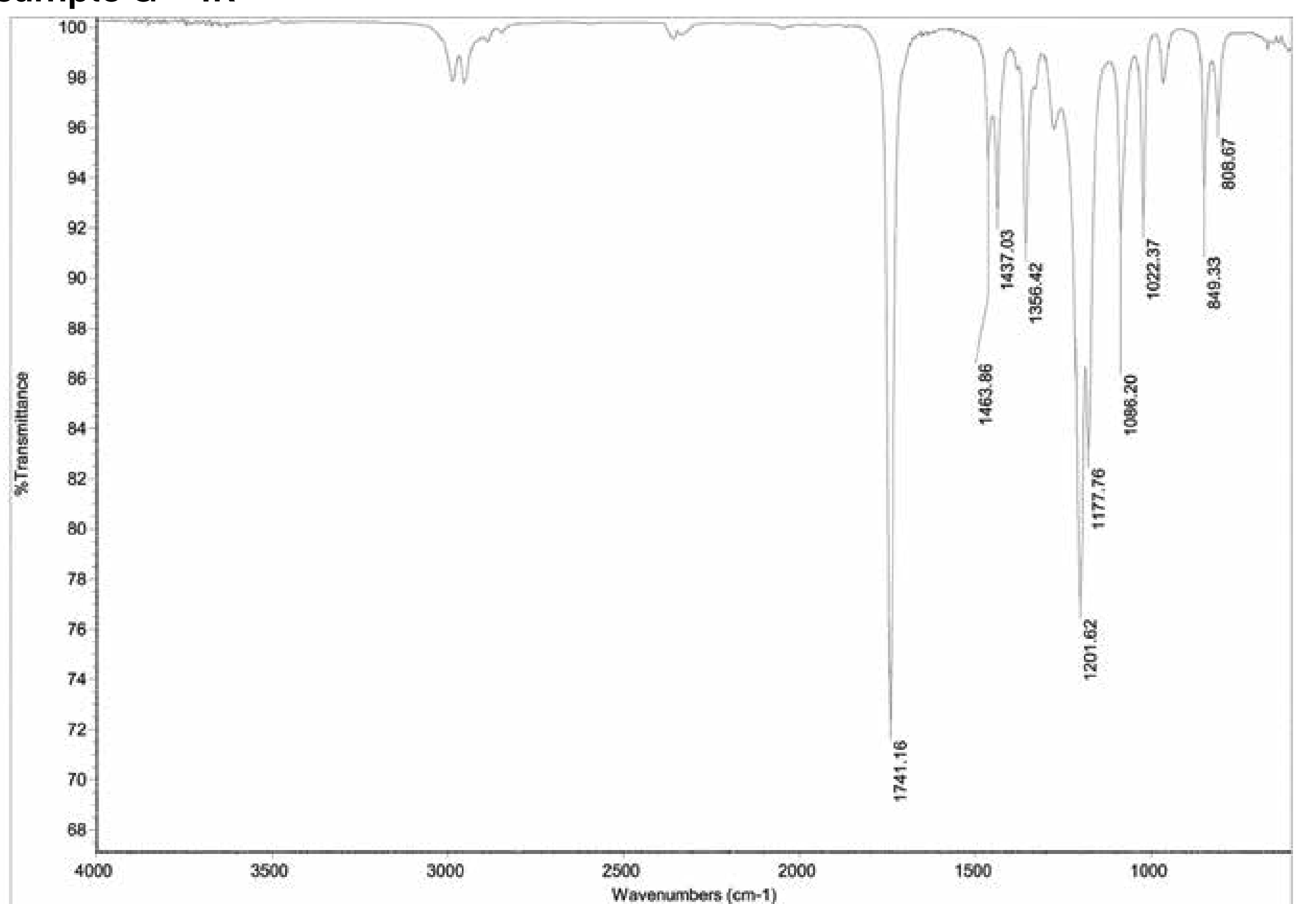
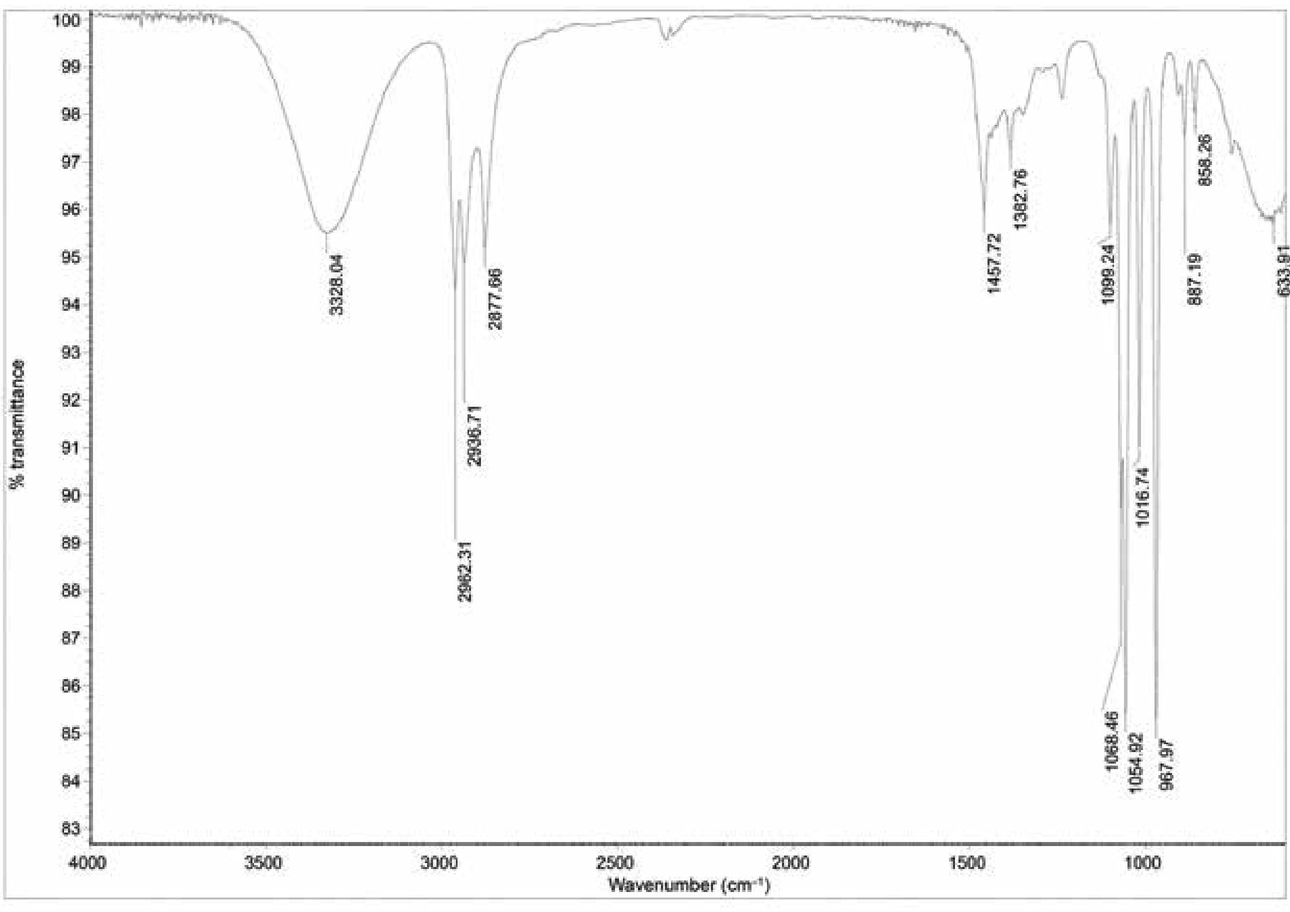
Sample G – IR



Sample H – IR



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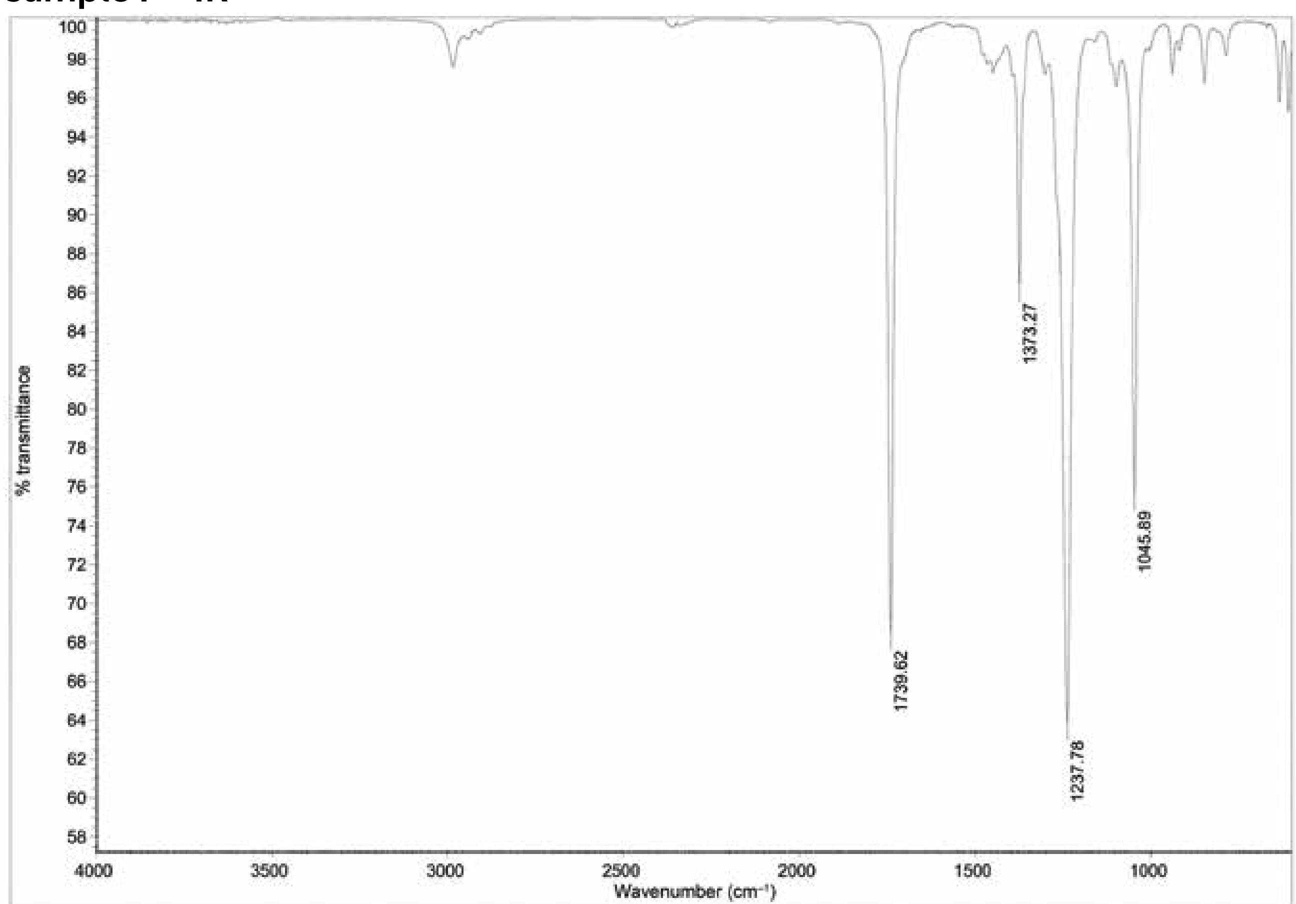
Sample I – IR



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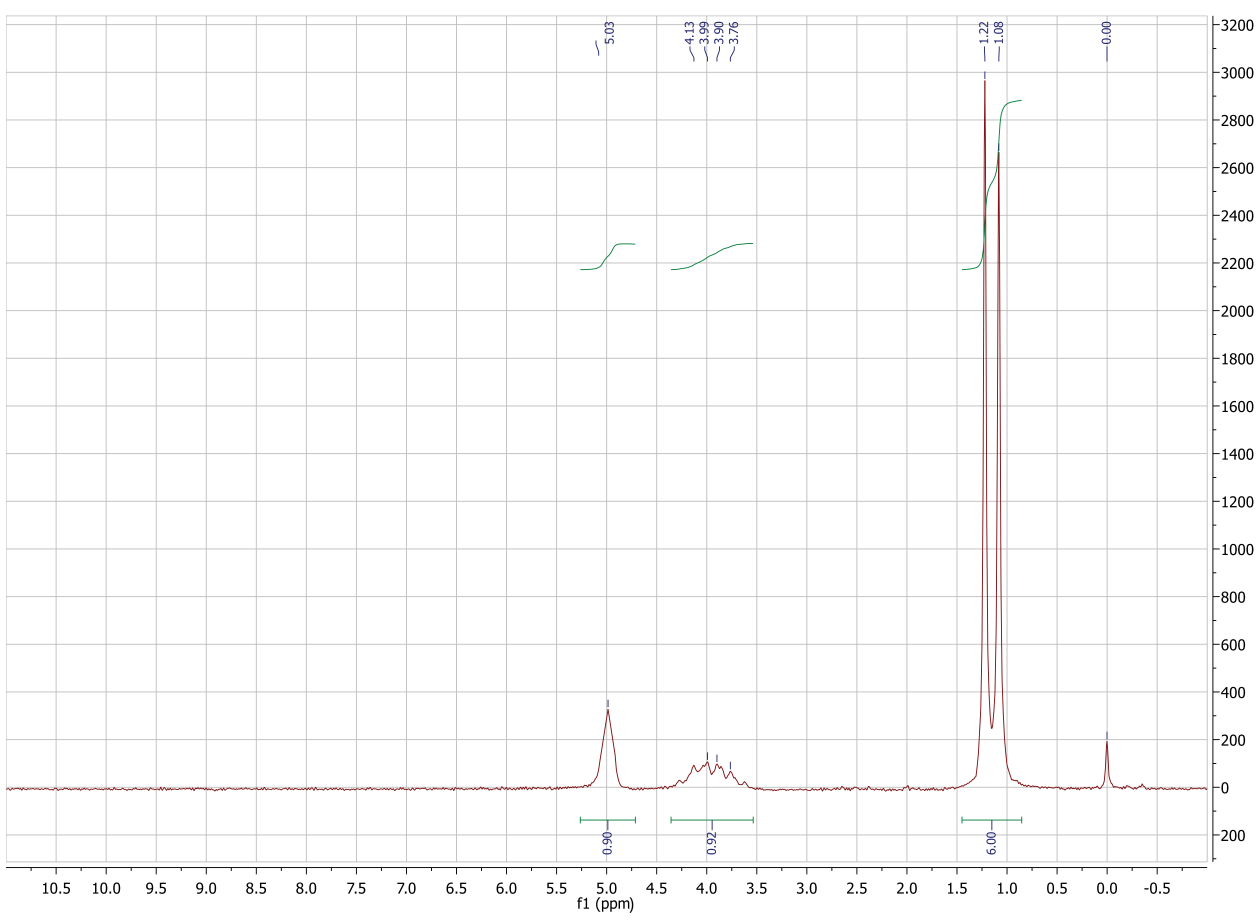
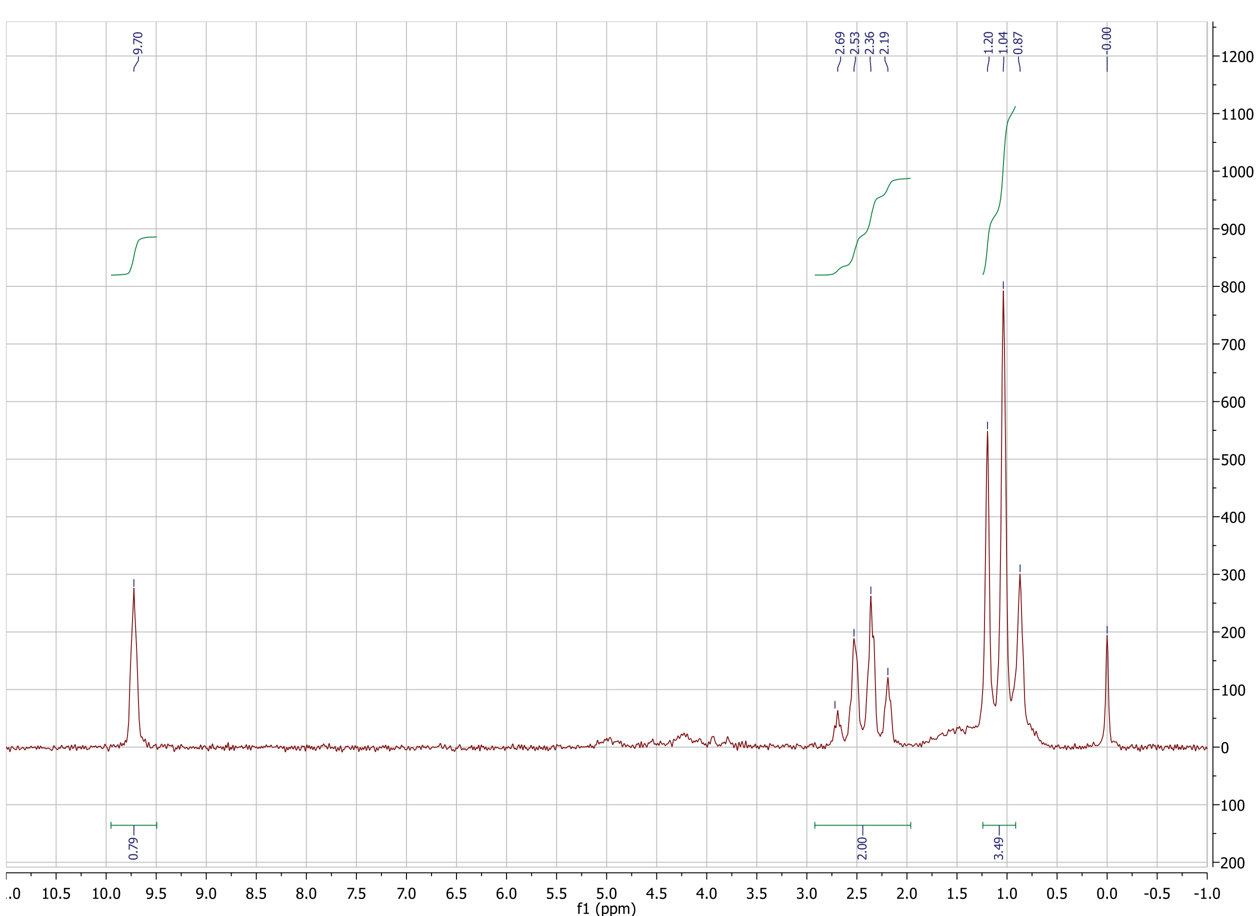
NMR SPECTRA FOR INTERPRETATION

Sample A – NMR



Sample B – NMR



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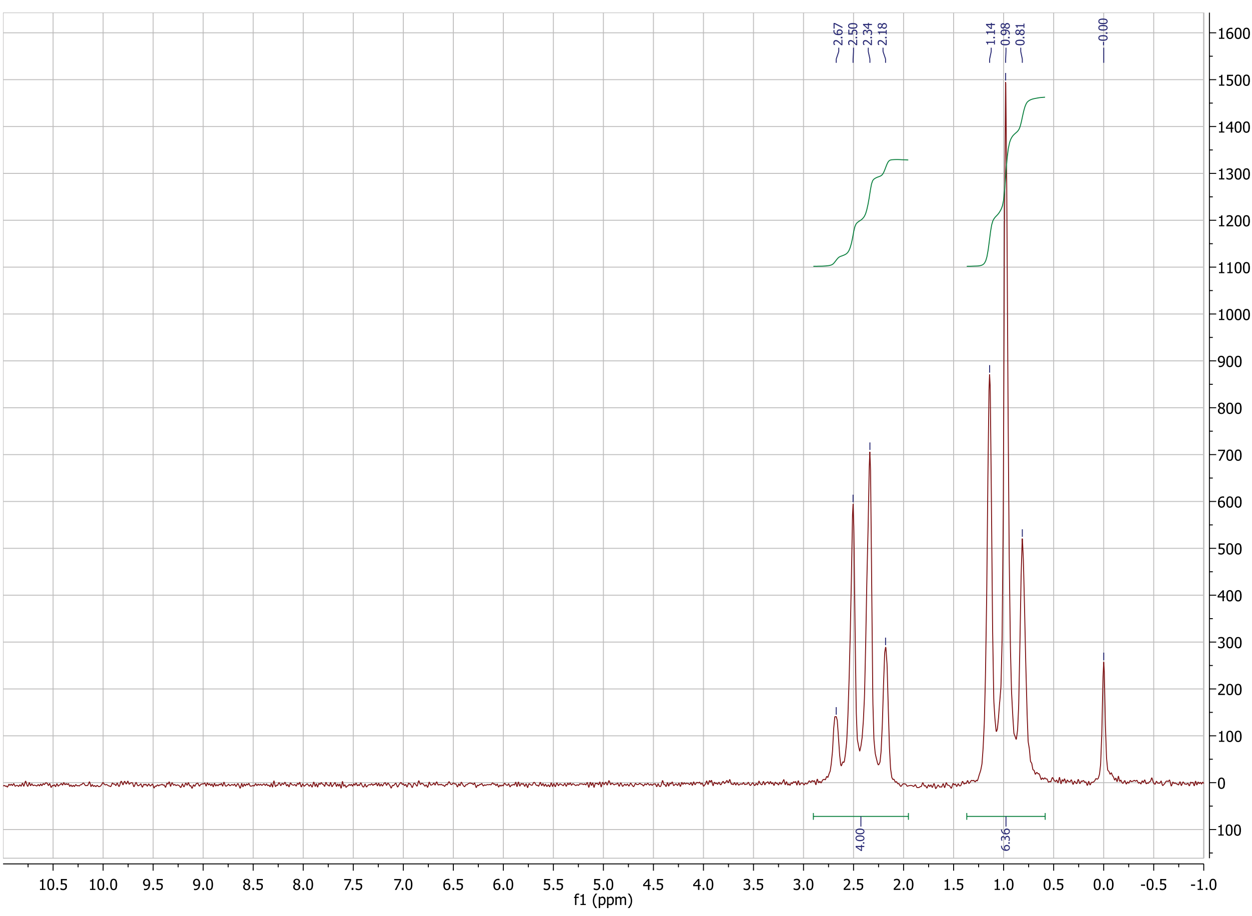
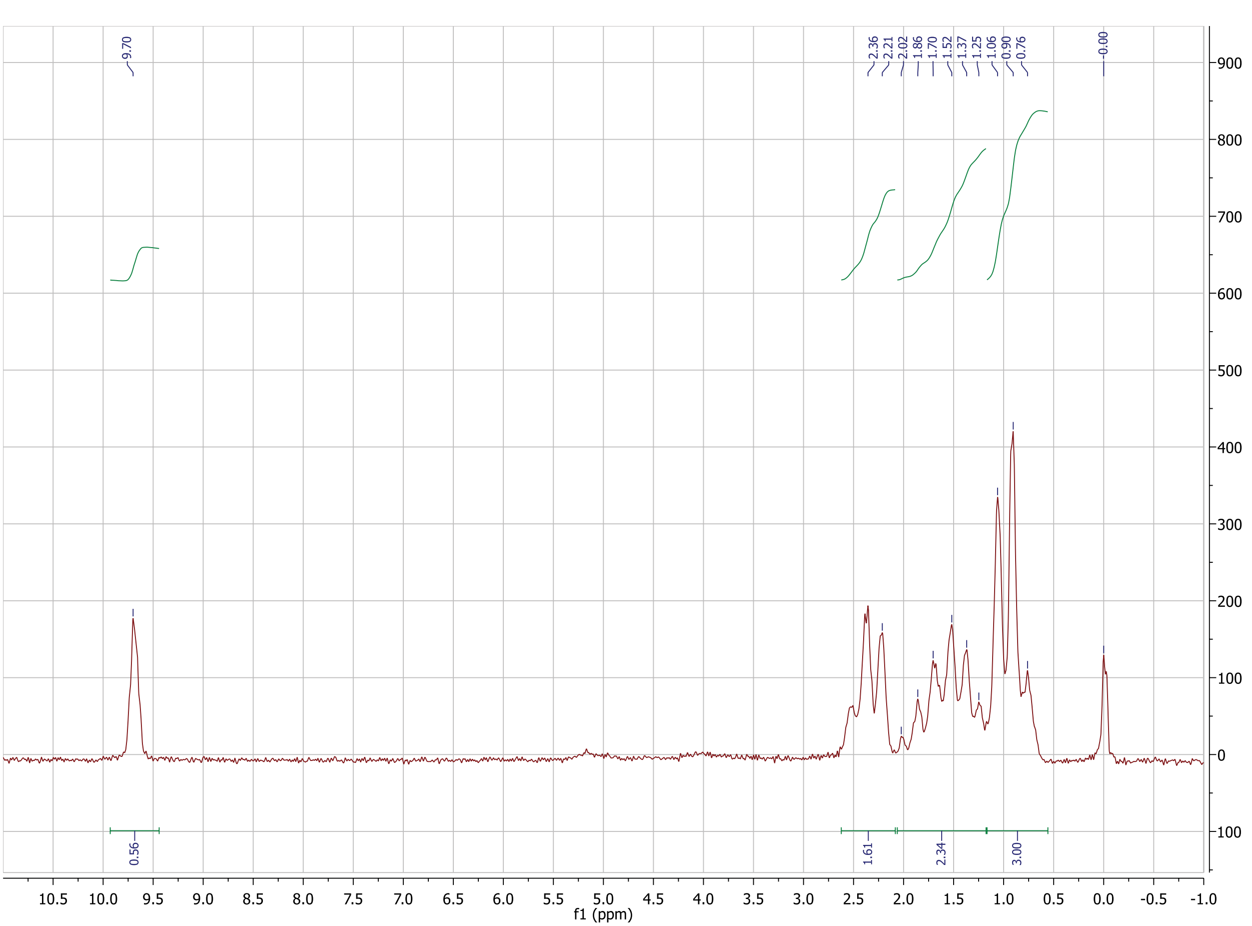
Sample C – NMR



Sample D – NMR



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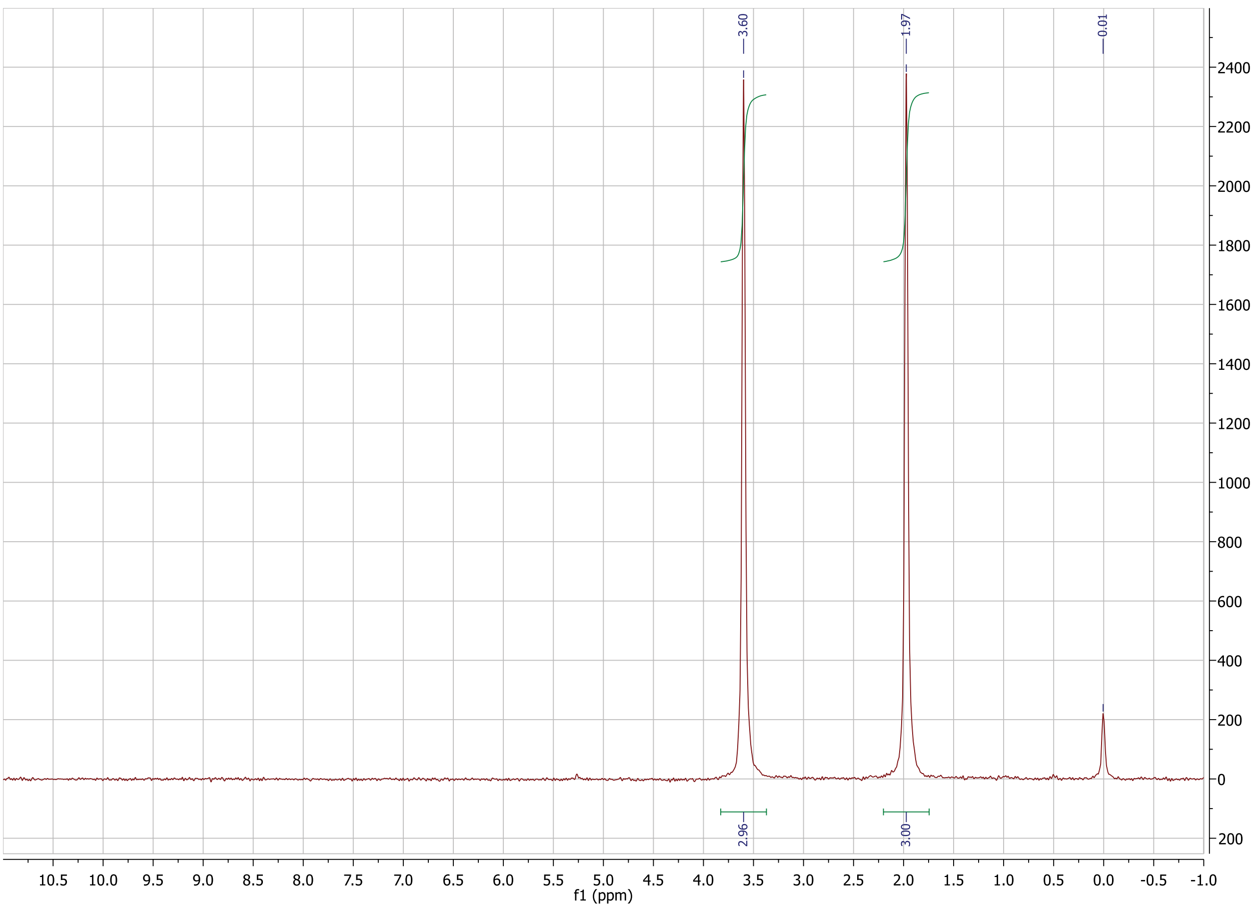
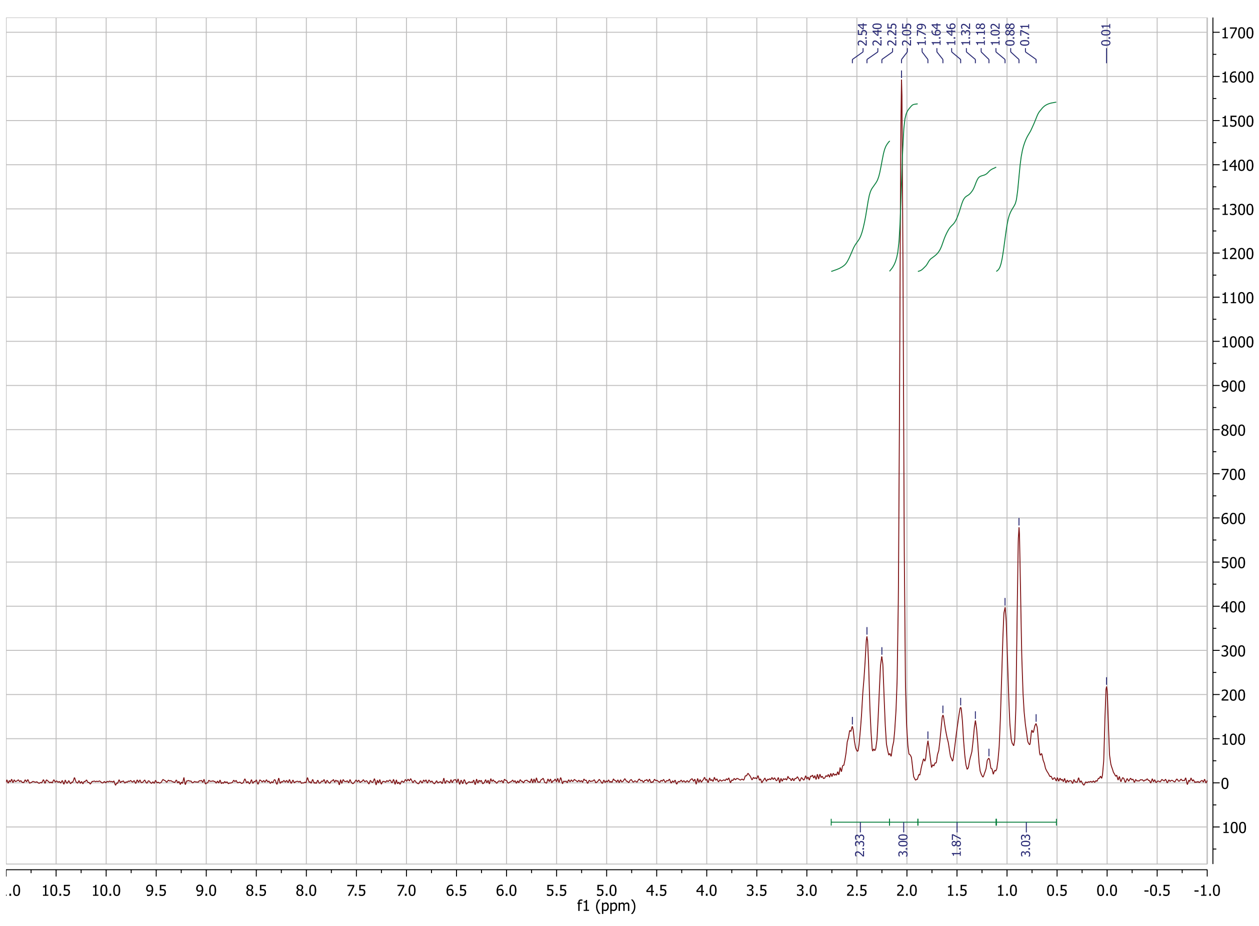
Sample E – NMR



Sample F – NMR



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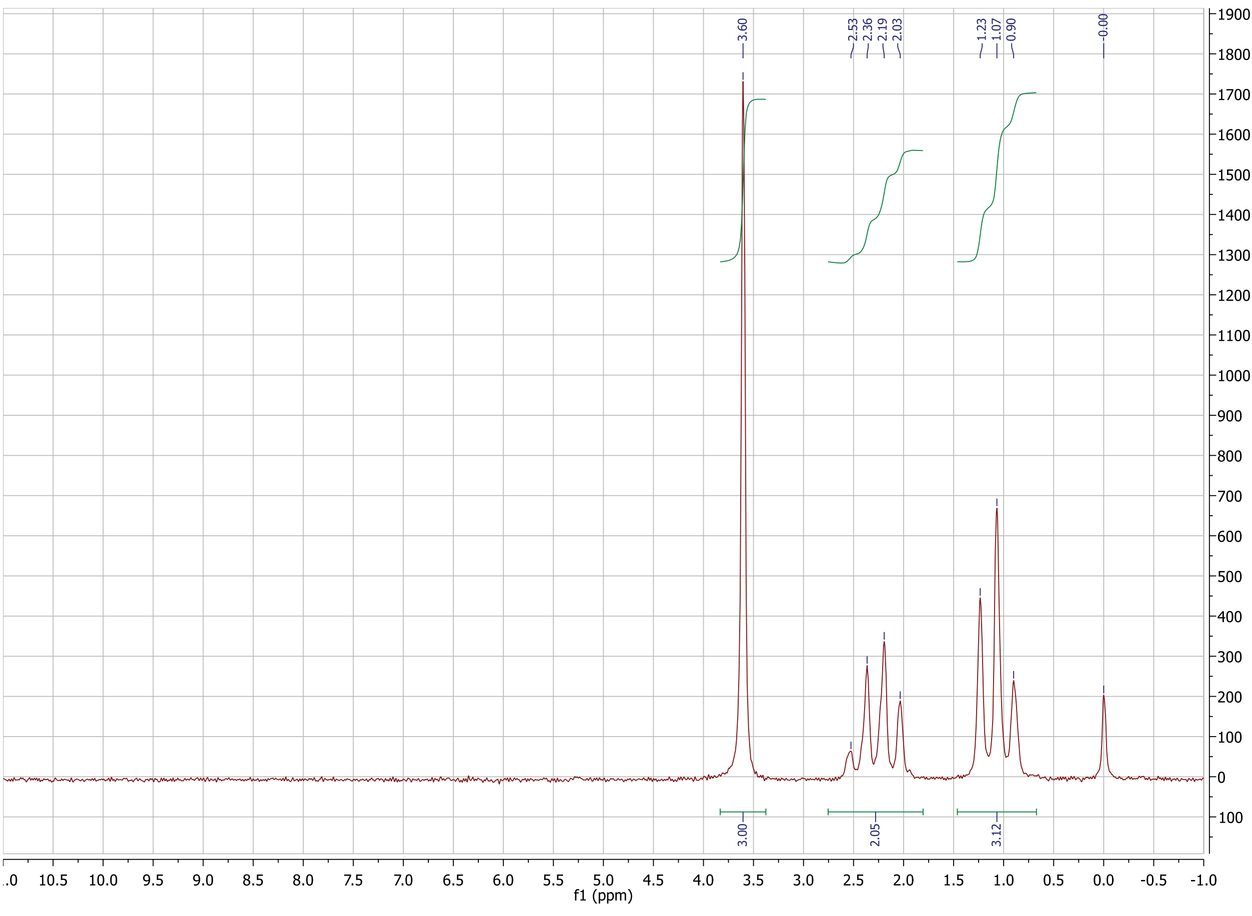
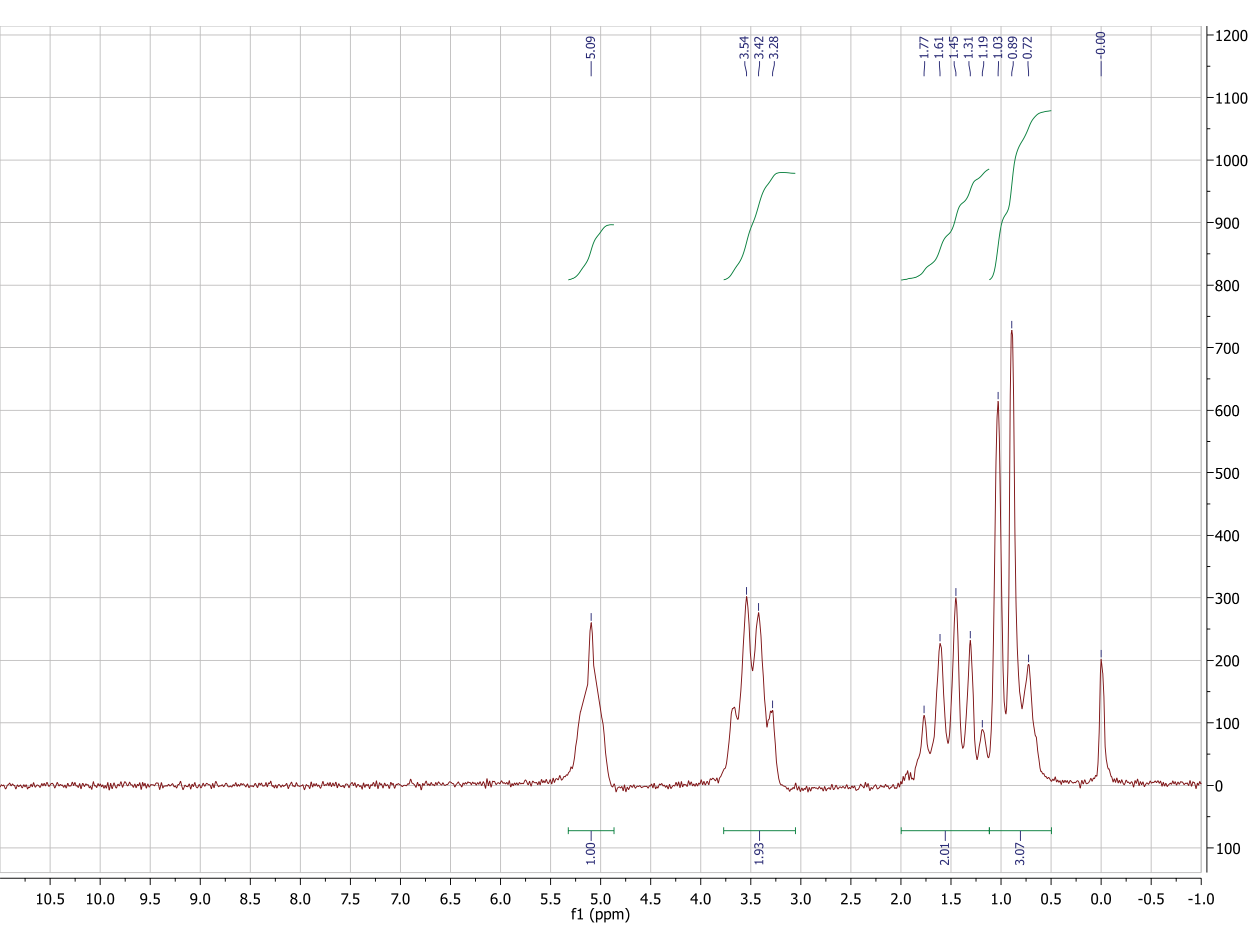
Sample G – NMR



Sample H – NMR



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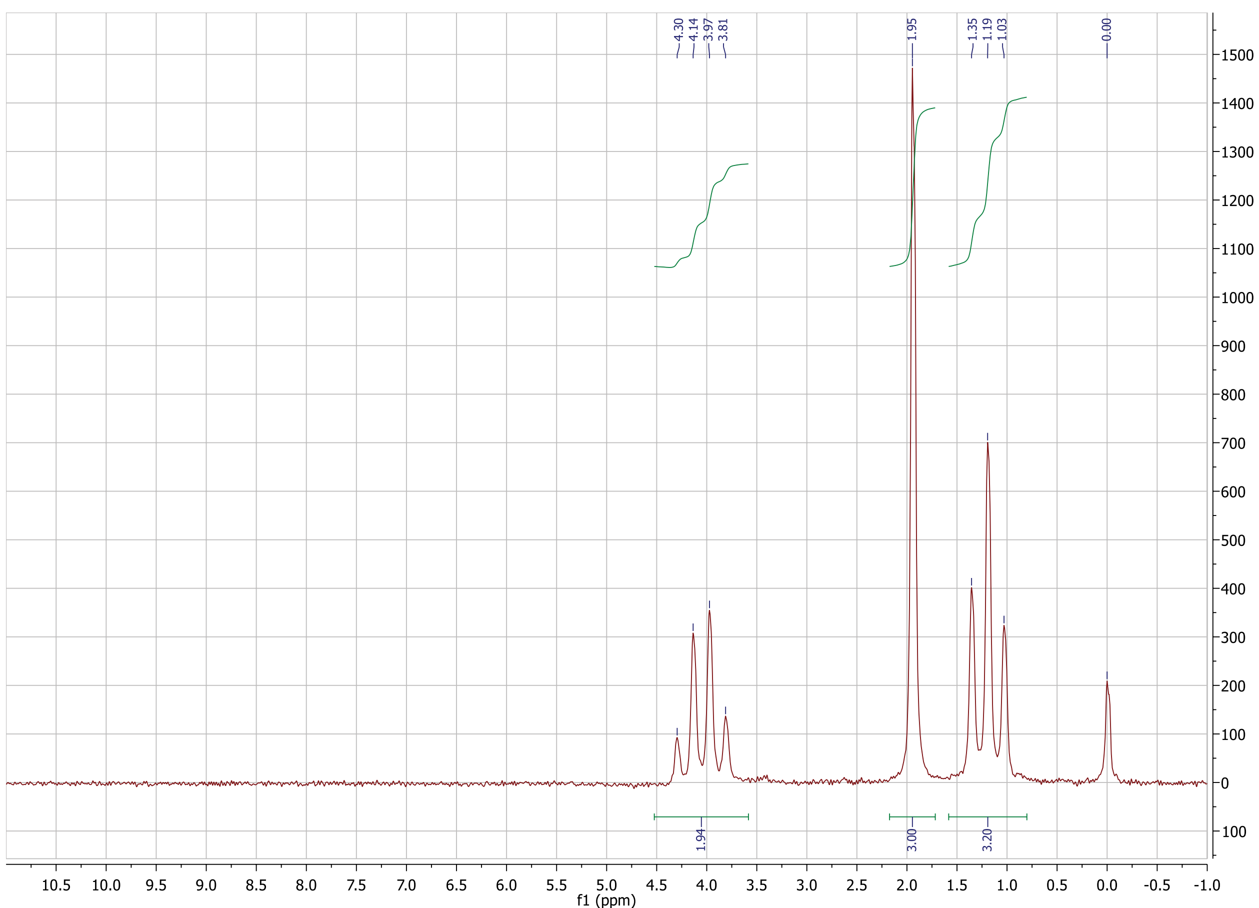
Sample I – NMR



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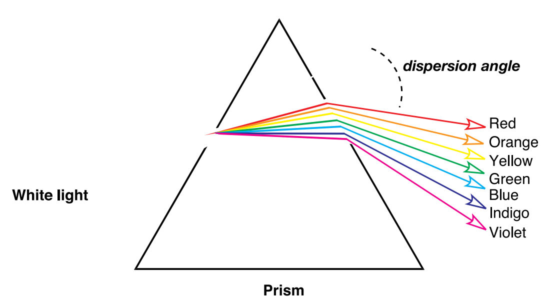
Food dye analysis: Exercise 3
UV-VIS analysis

INTRODUCTION

The electromagnetic spectrum ranges from radio waves with wavelengths the size of buildings down to gamma rays, the size of atomic nuclei. White light forms a small part of this spectrum and is composed of a range of different wavelengths that can be dispersed using a prism into its component colours. The colour an object, or a solution, appears will depend on which light is transmitted or reflected in the visible spectrum and which light is absorbed. Using a UV-VIS spectrometer and a range of food dyes you will test how the absorbance wavelength value relates to the colour of the solution.



UV-VIS spectrometer

UV-VIS spectrometers can be used to measure the absorbance of UV or visible light by a sample. The spectrum produced is a plot of absorbance versus wavelength (nm) in the UV and visible section of the electromagnetic spectrum. Instruments can be used to measure at a single wavelength or to perform a scan over a range in the spectrum. The UV region ranges from 190 to 400 nm and the visible region from 400 to 800 nm. The technique can be used both quantitatively and qualitatively.

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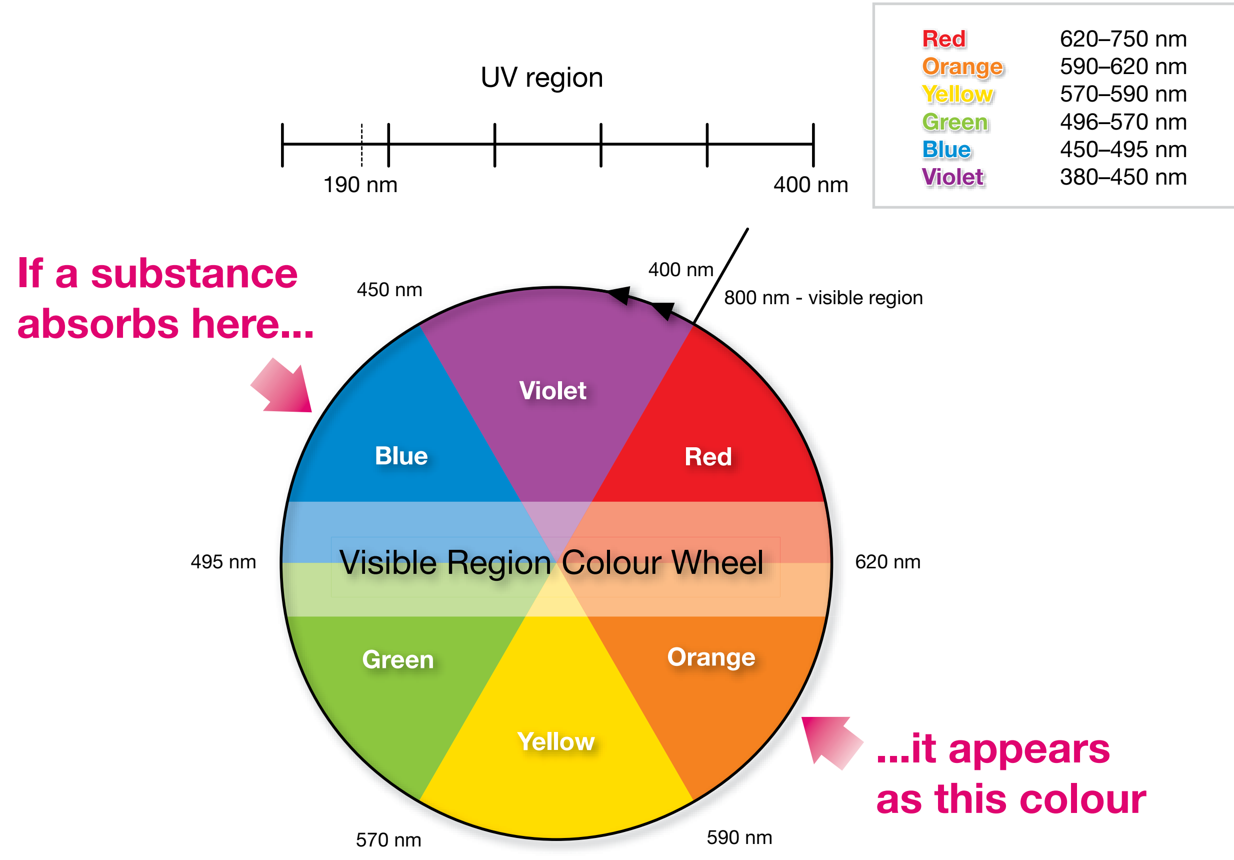
METHOD

1. Prepare a dilute sample for each colour to be tested using a cuvette and distilled water (approximately 1 drop food colouring to 100 cm^3 distilled water).
2. For each colour sample fill a plastic cuvette and stopper with a lid.
3. Prepare a blank sample cuvette containing distilled water only and stopper with a lid.
4. Use the colour wheel to predict absorbance values for each solution and record your predictions in the table provided.
5. Set up the spectrometer to scan the visible region from 350 nm to 800 nm and run each sample. Print out the spectrum and note the wavelength for each of the absorbance peaks. Compare these with your predictions.

Red	620–750 nm
Orange	590–620 nm
Yellow	570–590 nm
Green	496–570 nm
Blue	450–495 nm
Violet	380–450 nm



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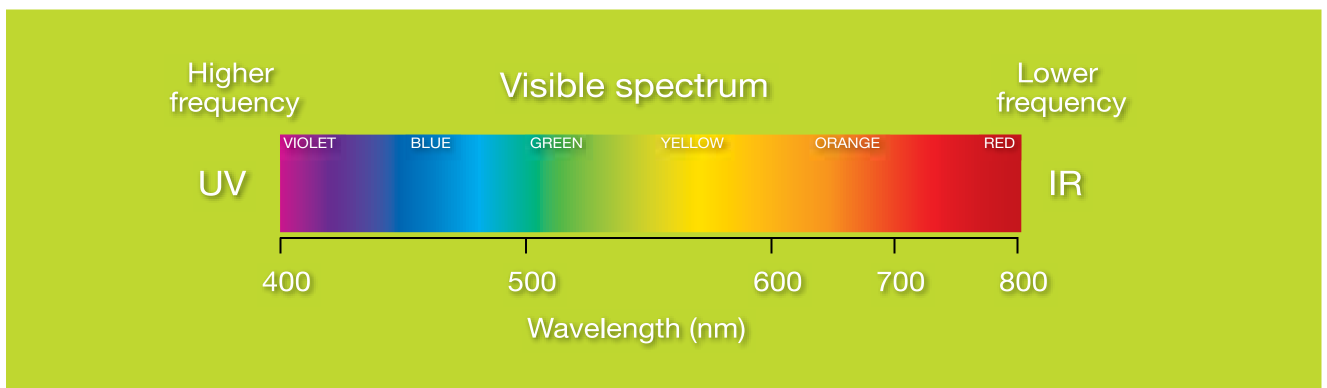
STUDENT WORKSHEET

COLOUR	PREDICTED ABSORBANCE VALUE (nm)	ACTUAL ABSORBANCE VALUE (nm)	NOTES
Red			
Yellow			
Green			
Blue			
Pink			
Black			



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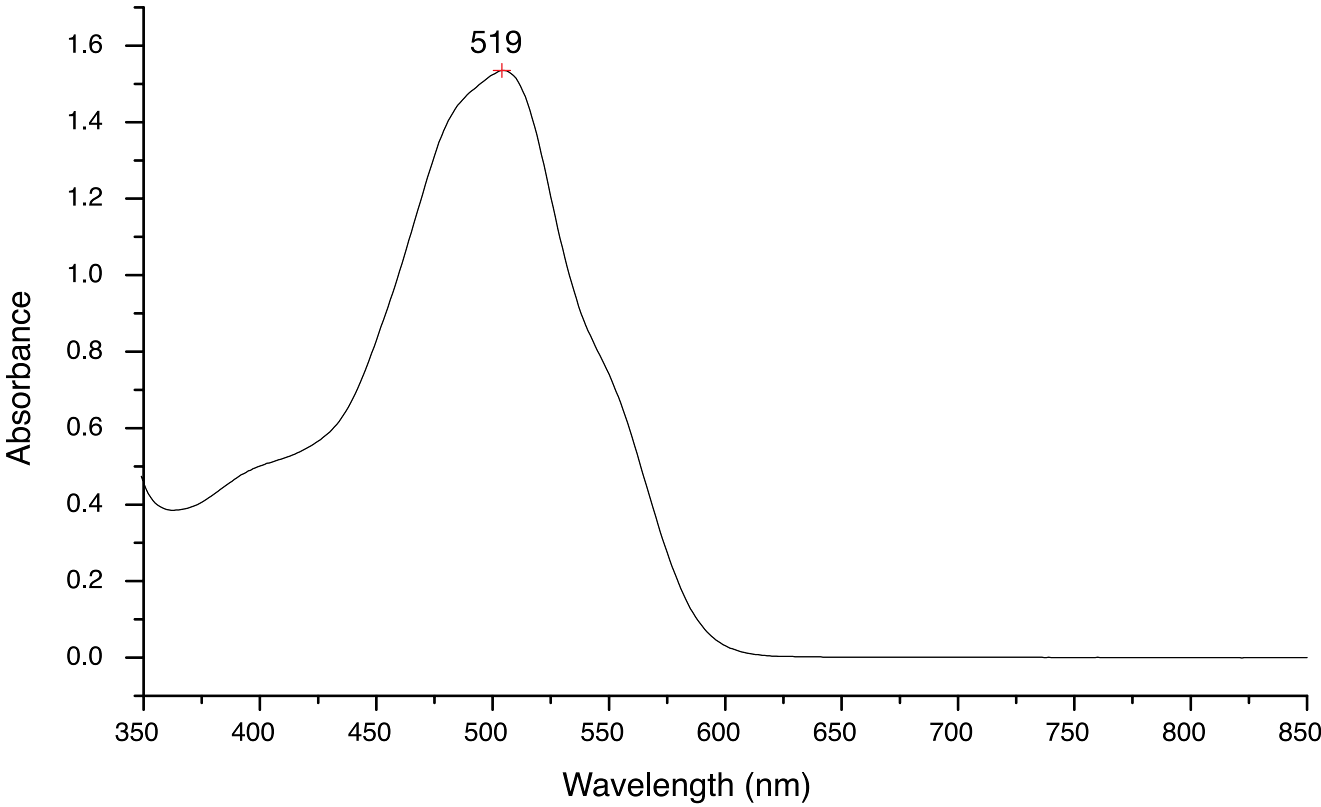
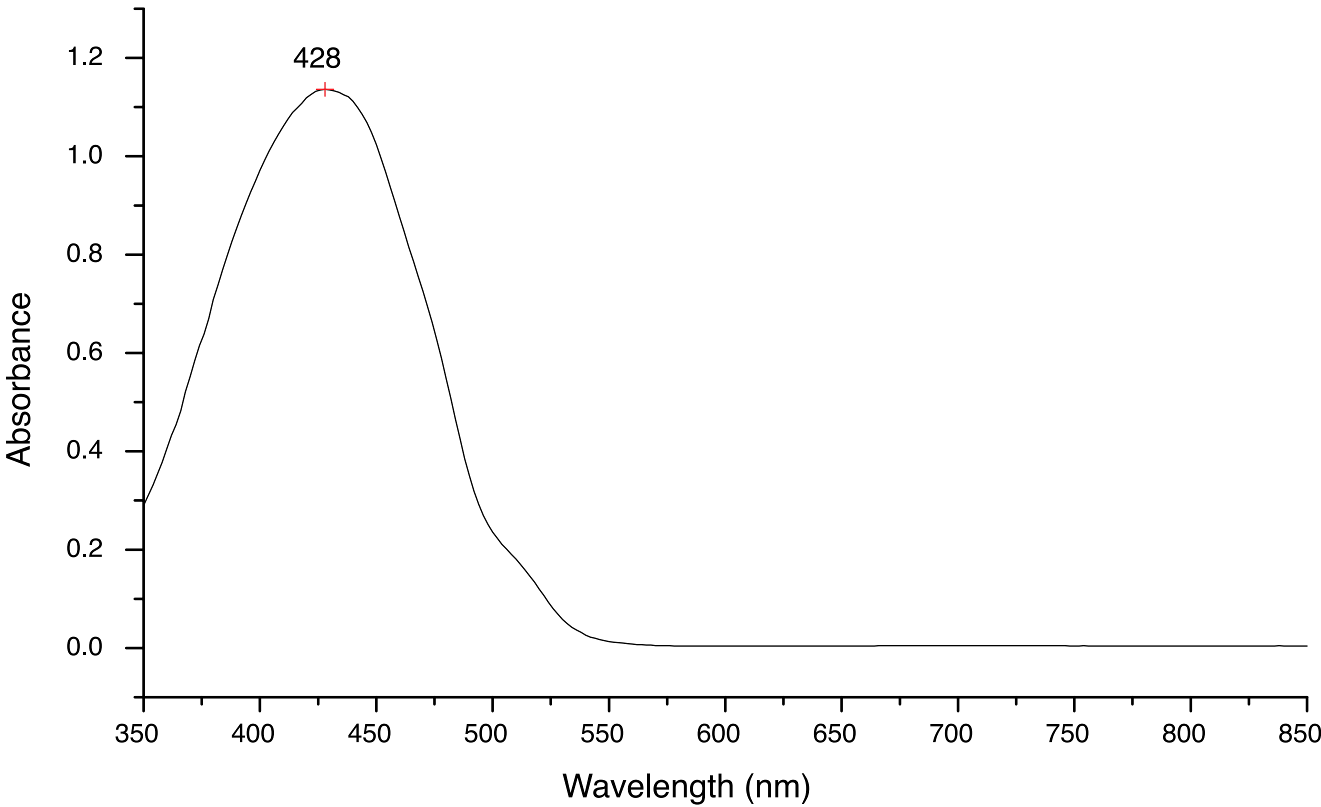
MODEL SPECTRA

Red: 519 and 528 nm



Yellow: 428 nm



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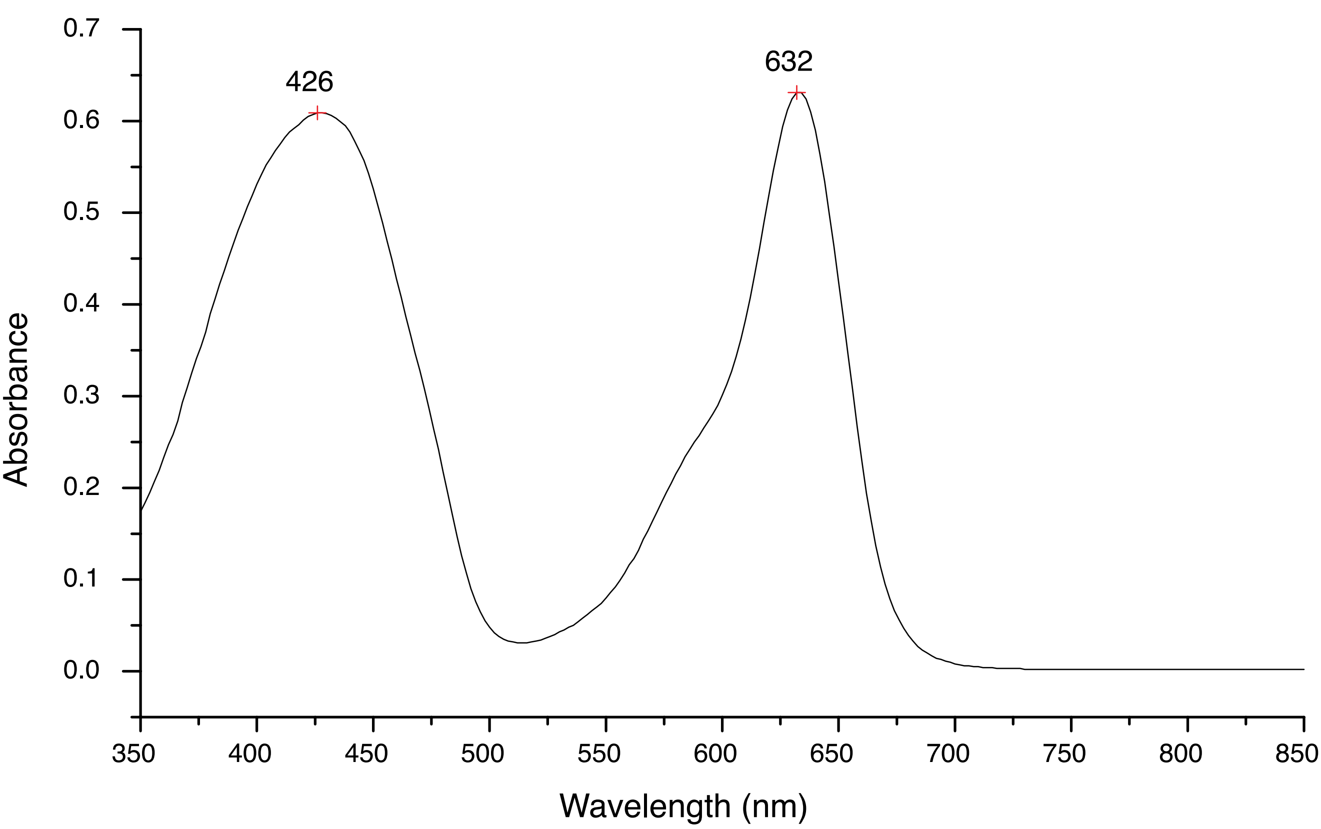
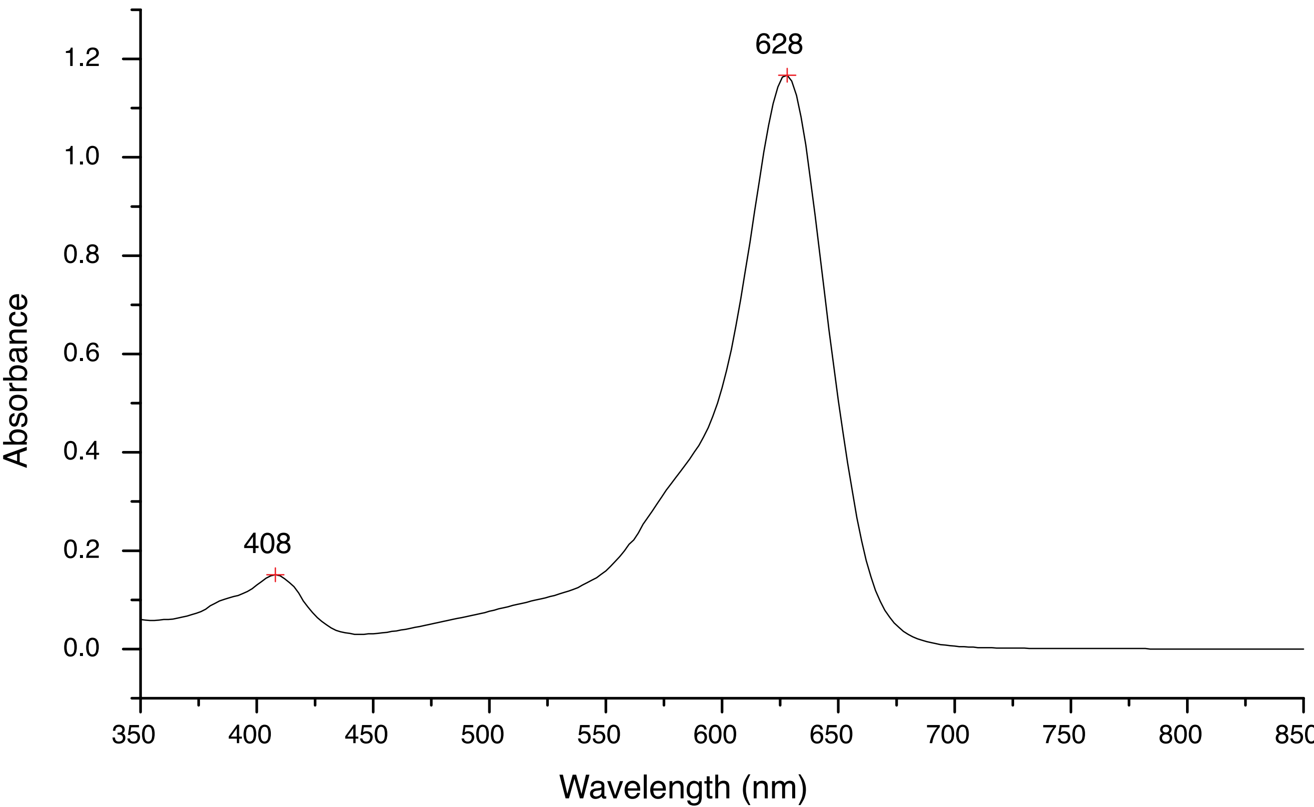
Green: 635 nm



Blue: 628 nm



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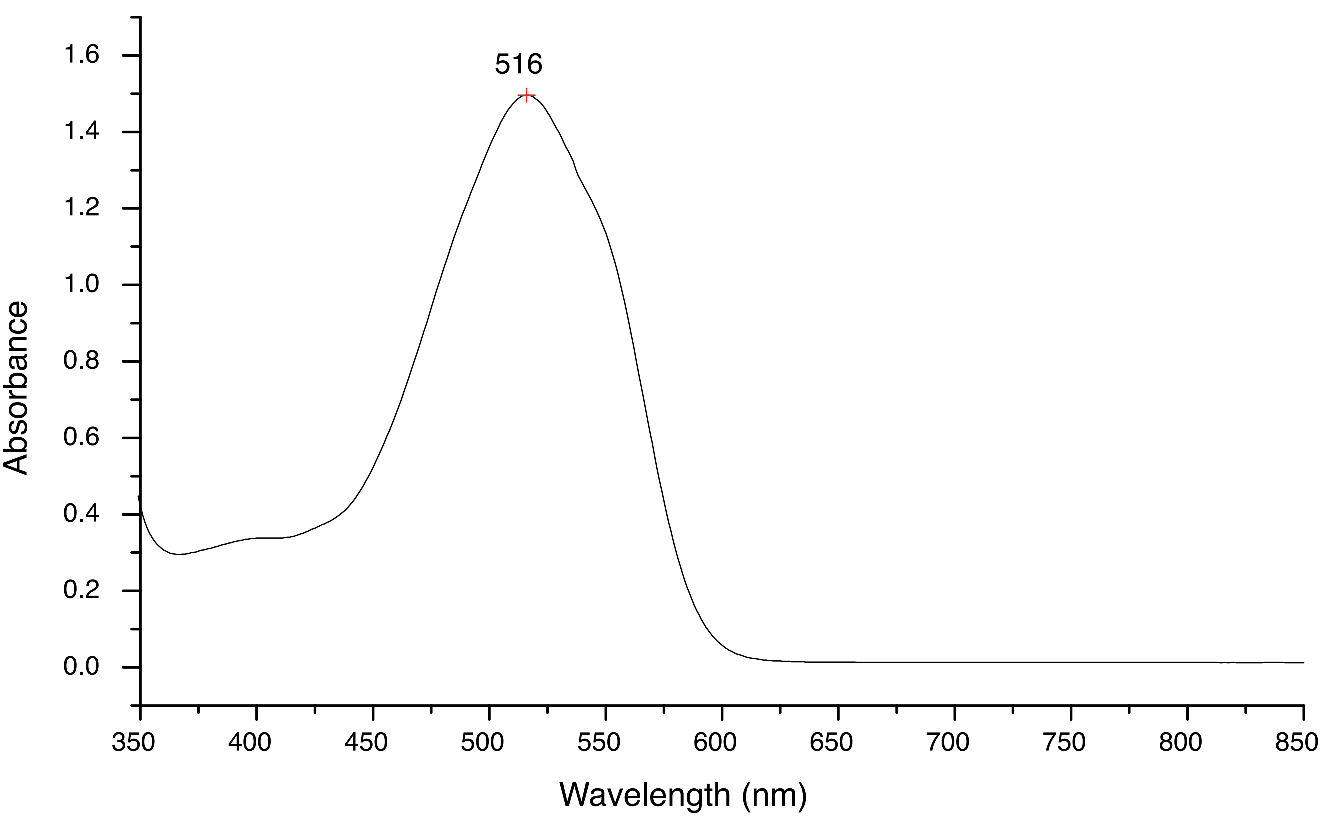
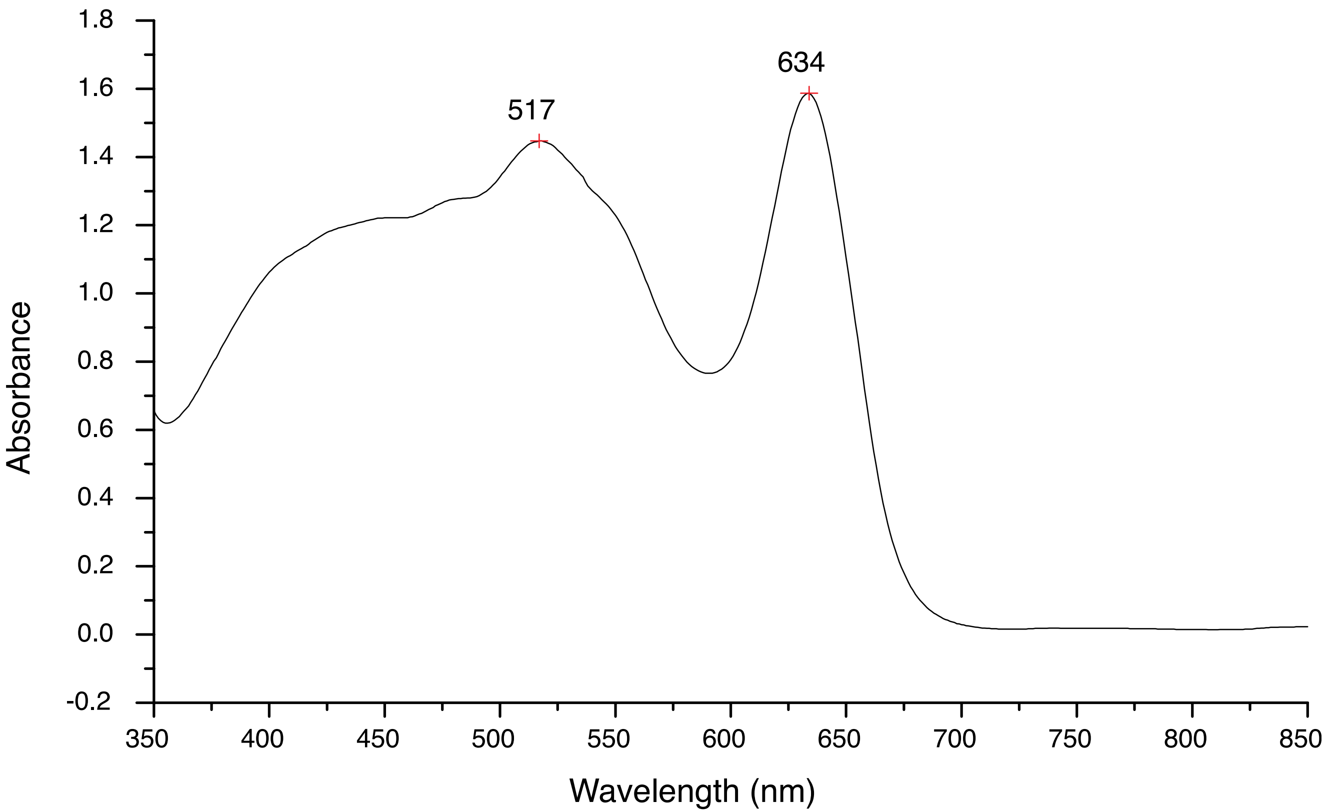
Pink: 510 nm



Black: 519 and 635 nm



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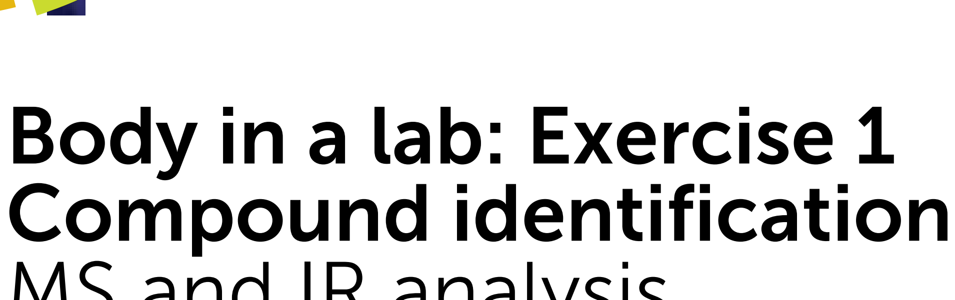

Body in a lab: Exercise 1
Compound identification
MS and IR analysis

INTRODUCTION

Background

A body has been found in the lab!

The victim, Mr Blue, was known to have a heart condition, but on the bench at the scene where the victim had been working a large bottle of concentrated acid had been upturned and spilt. All around this acid were different chemical bottles which had also had been knocked over and may have mixed with the acid. A medicine bottle was also present with unknown tablets inside (Sample X – the tablets have been ground ready for analysis).

Objective

Try to establish cause of death by using IR analysis to discover the functional groups present in the chemical samples collected. Decide whether any of these are likely to be toxic or may have formed a lethal toxic gas on contact with the spilt acid. Establish the identity of the medicine found by library comparison of the spectra and suggest possible implications.

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METHOD

You are provided with unknown samples A – H and medicine sample X

1. Analyse each sample using the ATR IR spectrometer.

   (Note: Care must be taken with this expensive and fragile equipment, use only when supervised by a demonstrator.)

Interpretation of spectra

To interpret the spectra obtained from a sample it is necessary to refer to correlation charts and tables of IR data.

2. Using the correlation chart provided interpret your spectra and identify the functional groups present in each sample. Record your results in the table provided.

Identification of unknown compound

While IR spectroscopy is a very useful tool for identifying the functional groups in an unknown compound, it does not provide sufficient evidence to confirm the exact structure. Chemists make use of a variety of techniques in order to piece together the structure of a molecule.

3. Use your interpreted IR spectra and the mass spectra provided to suggest the structure of all unknown compounds.
4. Identify any chemicals that you think may be toxic or where the functional group may possibly release a toxic gas on contact with an acid.
   ANSWER: The acid could have reacted with acetonitrile to release hydrogen cyanide gas.

6. Suggest what other instrumental technique or techniques would be required to confirm the identity of the chemicals. (Your demonstrator may be able to provide you with additional data for confirmation of analysis.)

6. Identify sample X by using the library spectra.

The MS and IR spectra for samples A to H can be found in 'Exercise 1: Compound identification', earlier in this booklet.

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STUDENT WORKSHEET

SAMPLE	IMPORTANT PEAK VALUES (cm^{-1})	FUNCTIONAL GROUP AND RANGE (cm^{-1})	MASS SPEC ANALYSIS (Mass of compound and fragments identified)	PREDICTED CHEMICAL STRUCTURE AND NAME
A				
B				
C				
D				
E				
F				
G				
H				
X				

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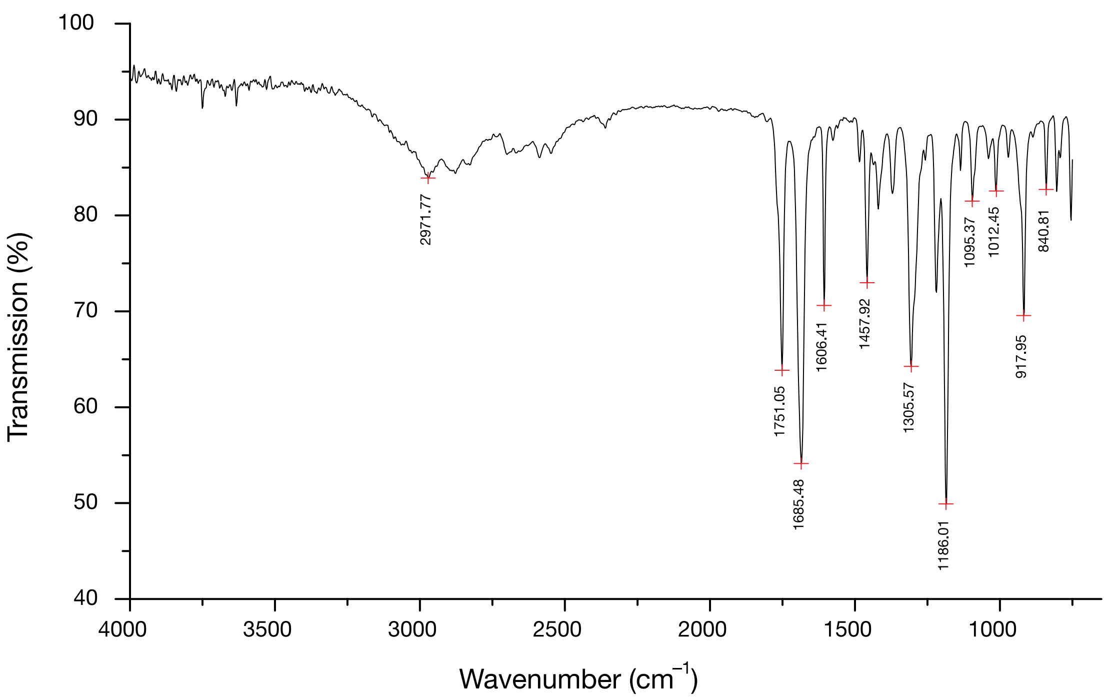
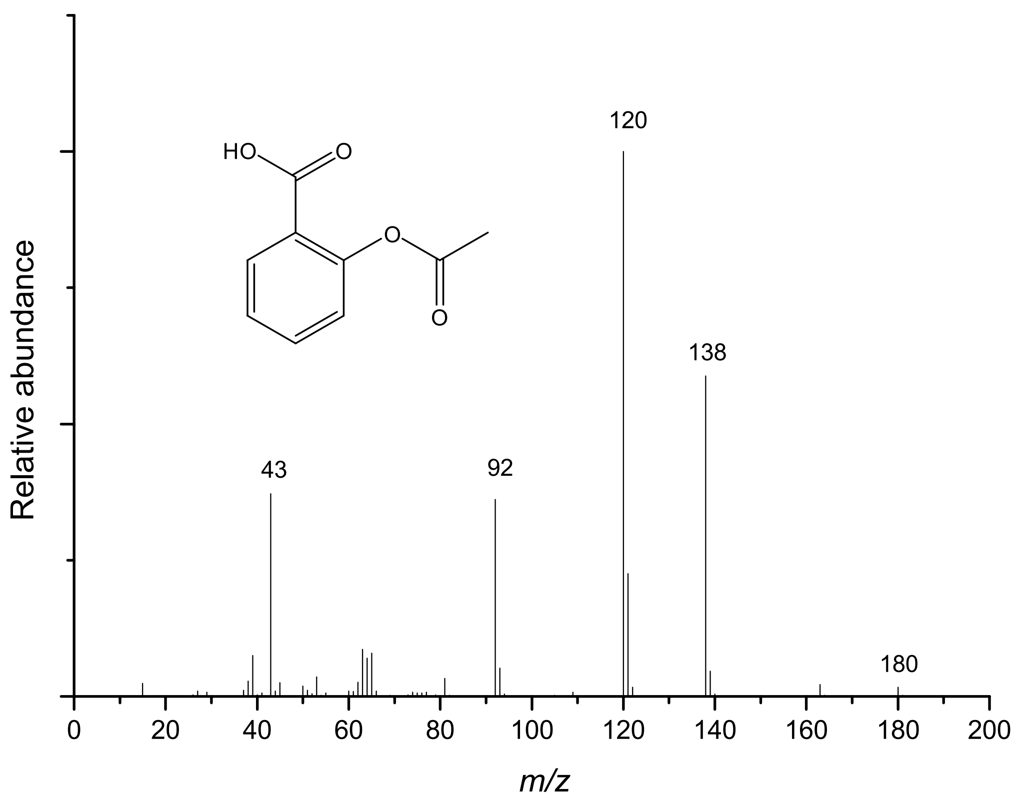
Sample X

IR spectrum



Mass spectrum

Empirical formula $C_9H_8O_4$



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Body in a lab: Exercise 2
Aspirin overdose UV-VIS analysis

INTRODUCTION

A body has been found in the Lab! The deceased, Mr Blue, was known to be taking aspirin and a sample of his blood plasma has been sent for analysis. Use UV spectroscopy to determine the concentration of aspirin in the body and ascertain if the amount present was enough to be the cause of death.

Analysis of salicylate in blood plasma by UV-VIS spectroscopy

Aspirin or acetyl salicylic acid is a widely available drug with many useful properties. It was one of the first drugs to be commonly available and it is still widely used with approximately 35,000 tonnes produced and sold each year, equating to approximately 100 billion aspirin tablets.

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Aspirin is prepared by the acetylation of salicylic acid using acetic anhydride. Its many properties as a drug include its uses as an analgesic to reduce pain, anti-inflammatory to reduce inflammation, antipyretic to reduce temperature, and platelet aggregation inhibitor to thin the blood and stop clotting.

Therapeutic levels taken after a heart attack are typically <0.3 mg dm^{-3} and for post by-pass operations 0.075 mg dm^{-3}.

The levels of salicylate present in blood plasma can be analysed using UV-VIS spectroscopy to indicate if the subject has taken a therapeutic dose or an overdose (see following table).

Therapeutic	<0.3 mg dm^{-3}
Moderate overdose	0.5–0.75 mg dm^{-3}
Severe overdose	>0.75 mg dm^{-3}

Most adult deaths occur when the measured plasma level is greater than 0.7 mg dm^{-3}. (Note: the maximum salicylate plasma levels usually occur approximately 4–6 hrs after ingestion.)

METHOD

This method involves measuring the absorbance of the red-violet complex of ferric and salicylate ions at about 530 nm using a UV-VIS spectrometer.

A 5% iron(III) chloride solution has been prepared for you (5 g iron(III) chloride in 100 cm^3 of deionised water).

1. Preparation of salicylate calibration standards

A stock solution of 2 mg dm^{-3} salicylate in 250 cm^3 of deionised water has been prepared for you by dissolving 580 mg sodium salicylate in a 250 cm^3 volumetric flask.

Make up standard calibration solutions (if this has not already been done for you)

In a 100 cm^3 standard volumetric flask dilute appropriate volumes of the stock solution to give 0.1, 0.2, 0.3, 0.4 and 0.5 mg dm^{-3} salicylate calibration standards using the dilutions given below.

2. Prepare a blank

In a test tube prepare a blank solution by taking **1 cm^3** of deionised water and adding **4 cm^3 of 5% iron(III) chloride** solution.

3. Prepare standards and unknown plasma sample for UV-VIS analysis'

Prepare each of the standards and the unknown plasma sample by pipetting 1 cm^3 into a separate test tubes and adding **4 cm^3 of 5% iron(III) chloride** solution to each (making sure each is carefully mixed).

4. Record the absorbance

Transfer the calibration solutions, blank and unknown sample to separate cuvettes to record the absorbance. For each sample record the absorbance in the visible region between 400 and 600 nm. A peak should be observed at about 530 nm (see your demonstrator for instructions on using the UV-VIS spectrometer).

CONCENTRATION	DILUTION
0.1 mg dm^{-3}	5 cm^3 stock salicylate solution in 100 cm^3 deionised water
0.2 mg dm^{-3}	10 cm^3 stock salicylate solution in 100 cm^3 deionised water
0.3 mg dm^{-3}	15 cm^3 stock salicylate solution in 100 cm^3 deionised water
0.4 mg dm^{-3}	20 cm^3 stock salicylate solution in 100 cm^3 deionised water
0.5 mg dm^{-3}	25 cm^3 stock salicylate solution in 100 cm^3 deionised water

ANALYSIS OF RESULTS

1. Using the Beer–Lambert Law plot the **absorbance versus concentration** calibration graph for the standards and using this find the unknown concentration of the salicylate present in the plasma.
2. Use this result to decide whether the subject had taken a therapeutic or life-threatening dose.

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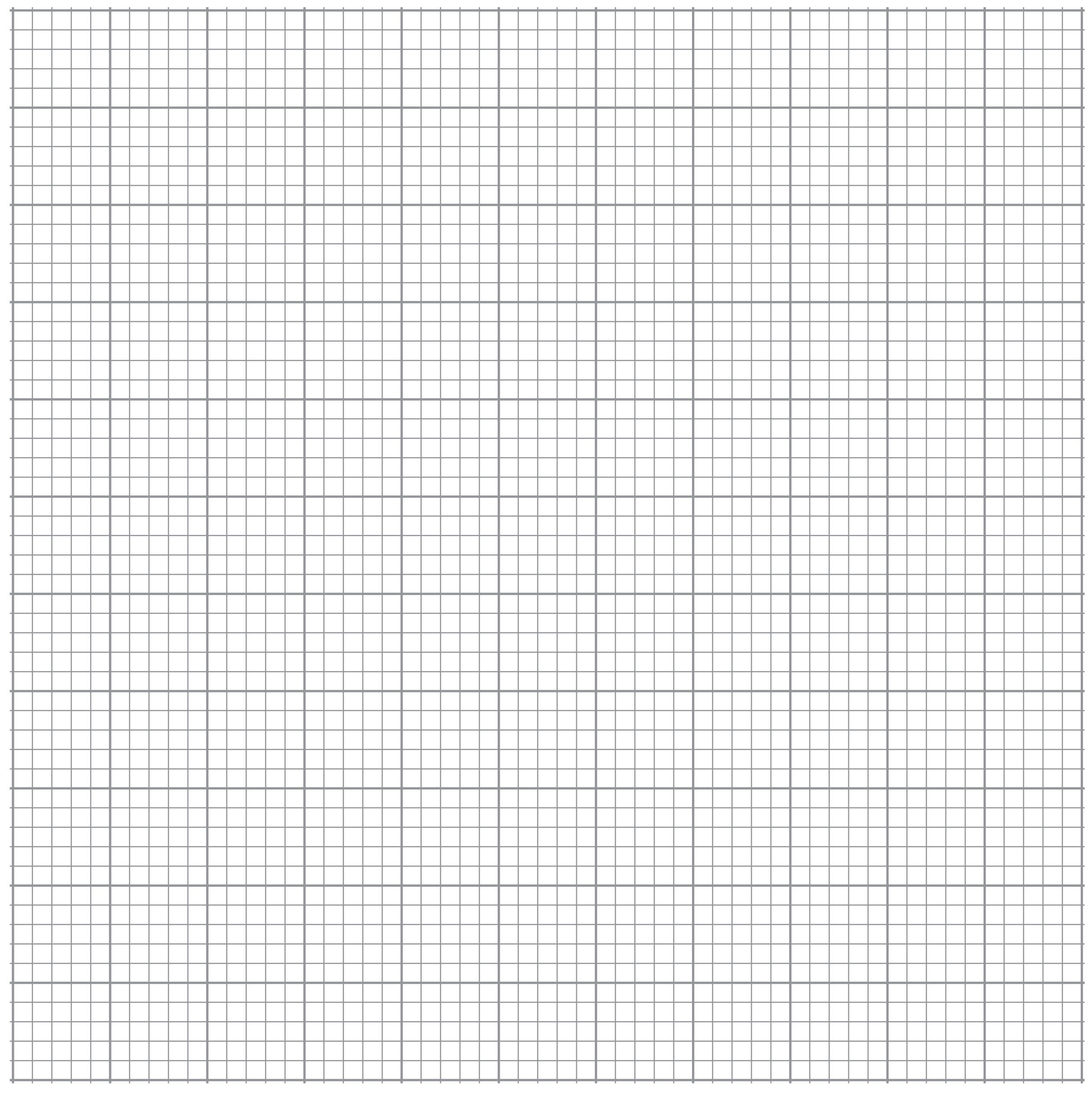
STUDENT WORKSHEET

SAMPLE	ABSORBANCE	CHOSEN PEAK WAVELENGTH (nm)
Blank deionised water		
0.1 mg dm^{-3} calibration solution		
0.2 mg dm^{-3} calibration solution		
0.3 mg dm^{-3} calibration solution		
0.4 mg dm^{-3} calibration solution		
0.5 mg dm^{-3} calibration solution		
Plasma sample		



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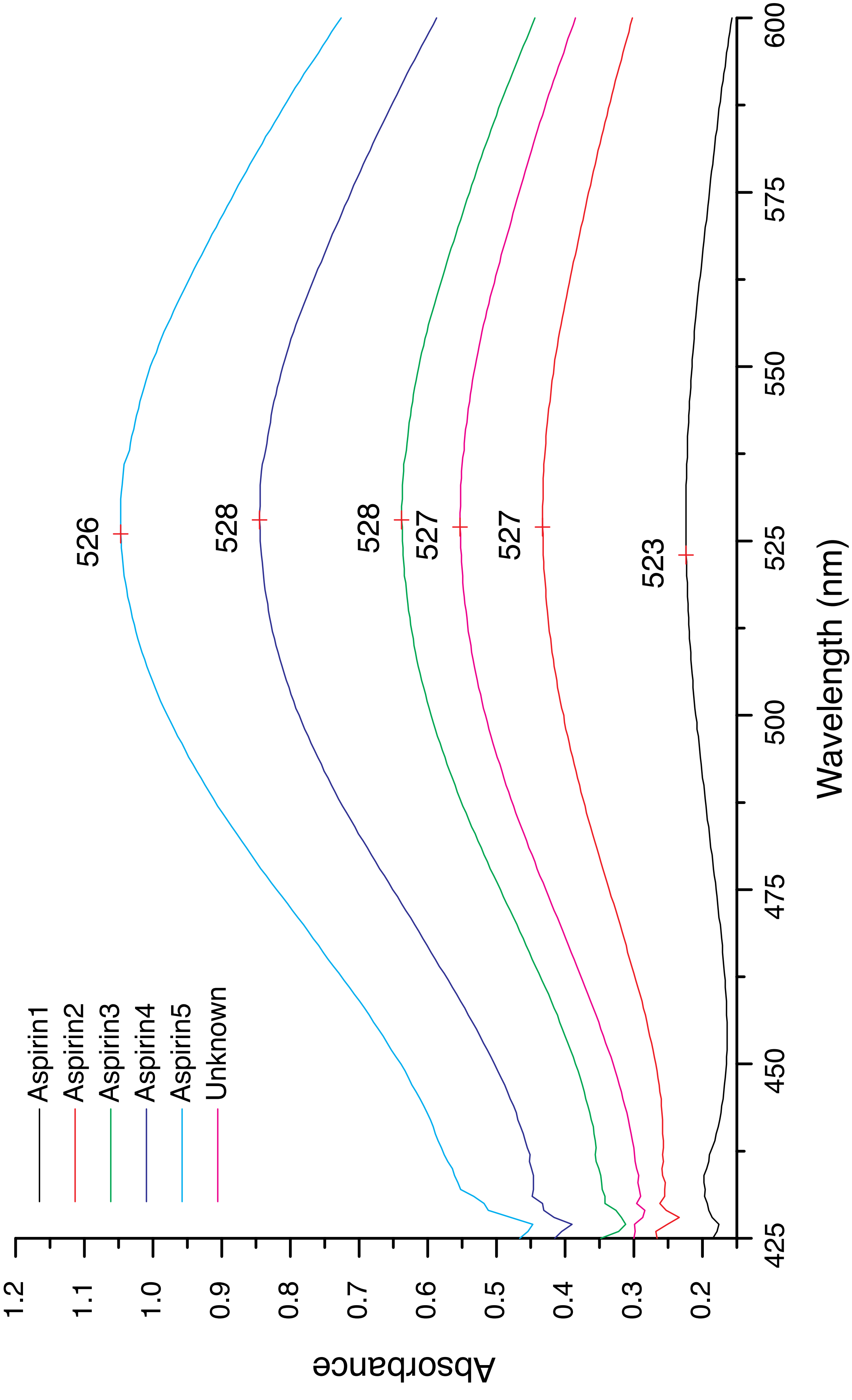
MODEL SPECTRA



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Body in a Lab: Exercise 3 Murder Mystery "Who Dunnit?"
MS analysis

INTRODUCTION

Background information

Story so far...

A body has been found in a lab and an investigation is being conducted to determine the cause of death.

IR spectroscopy

IR has been used to determine whether Mr Blue's death was caused by any of the various chemicals found around the body. This stage of the investigation concluded that the death was not accidental and identified a bottle of aspirin near the body.

UV spectroscopy

UV spectroscopy was used to analyse the blood plasma sample from Mr Blue to ascertain whether the quantity of aspirin present was a lethal dose. The result proved that Mr Blue possessed only therapeutic levels of medication in his blood, consistent with the dose prescribed by the doctor.

At this stage the death is looking increasingly suspicious and investigation is focusing on the people who had contact with Mr Blue on the lead-up to his death.

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EVIDENCE

Extract from Scene of Crime Report

Mr Blue, a university researcher, was found dead surrounded by chemical bottles that had been knocked over and spilt. On the bench concentrated acid and various organic chemicals were found. There was also a bottle of unlabelled tablets next to the body. Fingerprints from four other people were found at the scene: Mr Green, the laboratory technician, Mrs Blue (estranged wife) and Mr Maroon, both fellow researchers, and Miss Scarlet, a PhD student. A letter was found in Mr Blue's pocket addressed to the university Human Resources Department, which claimed that Mr Green had a suspected drug addiction and had been caught ordering chemicals for drug manufacturing and that he should be dismissed immediately.

Extract from the doctor's report

From the doctor's report it could be seen that the victim, Mr Blue, had previously had a mild heart attack and was taking aspirin daily as medication.

Witness/suspect statements

Mr Green – laboratory technician

Mr Green has worked for one year as a technician in the research laboratories. Mr Green said he saw the victim arguing with Mr Maroon about research results. He said the victim had claimed Mr Maroon had stolen the results from him and published it as his own work. Mr Maroon stood to gain significant funding and high-profile publicity from this research work. If it got out that the results were stolen from a colleague, his career and reputation would be ruined. Mr Green said that he had also seen Mr Blue return from lunch a little earlier with Miss Scarlet. He seemed to have been drinking and Miss Scarlet made him a drink.

Miss Scarlet – PhD Student

Miss Scarlet stated that she had been in a relationship with the victim for the last 6 months, and she claimed that they intended to get engaged in the New Year. Miss Scarlet was a keen horse rider, spending her spare time helping at a professional stables where she was good friends with Mrs Silver, the vet.

Mrs Blue – researcher and estranged wife

Mrs Blue, who had separated the previous year from Mr Blue, stated that the victim had no intention of getting married to Miss Scarlet. She said that Miss Scarlet had misunderstood Mr Blue's intentions, and that he was in fact planning to move jobs to another university without Miss Scarlet. She also claimed that Mr Blue had even talked about getting back together with her. Recently Mrs Blue had been on a trip to India for a conference on natural Indian medicines.

Mr Maroon – professor

Mr Maroon has worked at the university for 12 years as a high-profile research professor but of late had not made much progress in his field. Mr Maroon needed to secure further funding and was under pressure to produce some results. Mr Maroon also stated that Mr Green had financial problems and had approached him for a loan. Mr Maroon had recently called in the exterminators for a rat problem in one of the basement laboratories.

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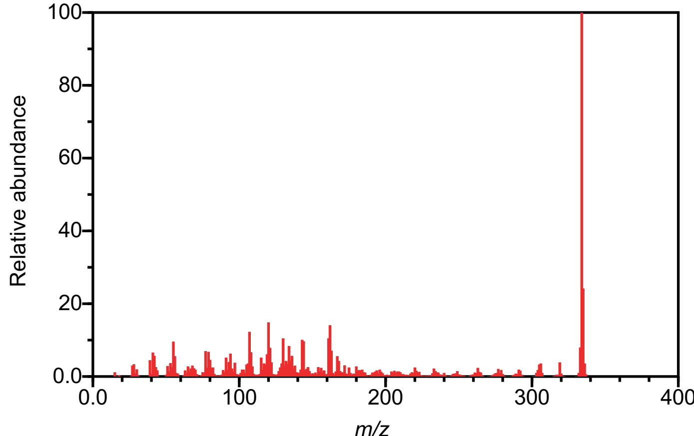
Forensic laboratory report

Sample:
Post mortem urine sample from Mr Blue

Analysis requested:
GC-MS analysis for common drugs or poisons

Results: Mr Blue urine sample



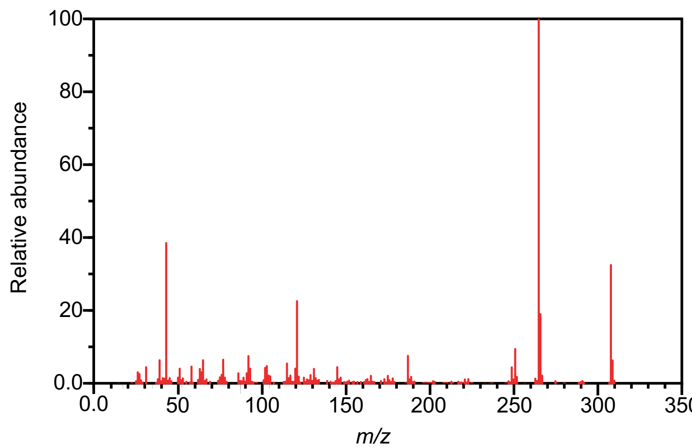
Ref NIST Chemistry webbook

Forensic laboratory report

Sample:
Rat poison sample from laboratory

Analysis requested:
GC-Mass spectrometry analysis

Results: Rat poison sample



Ref NIST Chemistry webbook

METHOD

Who did it?

Objective

Use the small database of common poisons and forensic report provided to identify the chemical found in the urine sample of the victim. From this evidence identify what further information would be necessary to establish that this poison was the cause of death, then use the witness statements to identify the most likely suspect.

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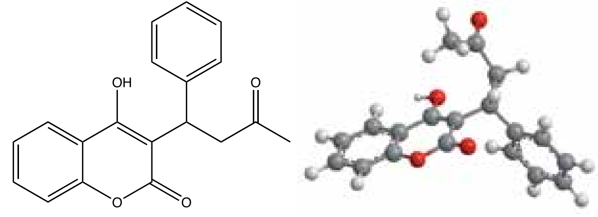
MASS SPECTROMETRY BACKGROUND INFORMATION

Common drugs and poisons

Warfarin $C_{19}H_{16}O_4$ RMM: 308.33



- Is a widely prescribed anticoagulant drug used to prevent thrombosis and blood clots.
- Is commonly used as a pesticide for rats and mice.
- Overdose can cause death by internal bleeding such as gastrointestinal haemorrhage.

Relative abundance

265

43

308

121

251

0 50 100 150 200 250 300 350

m/z

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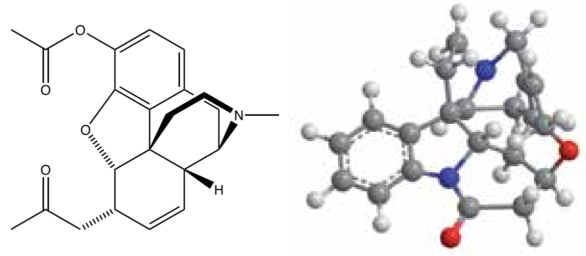
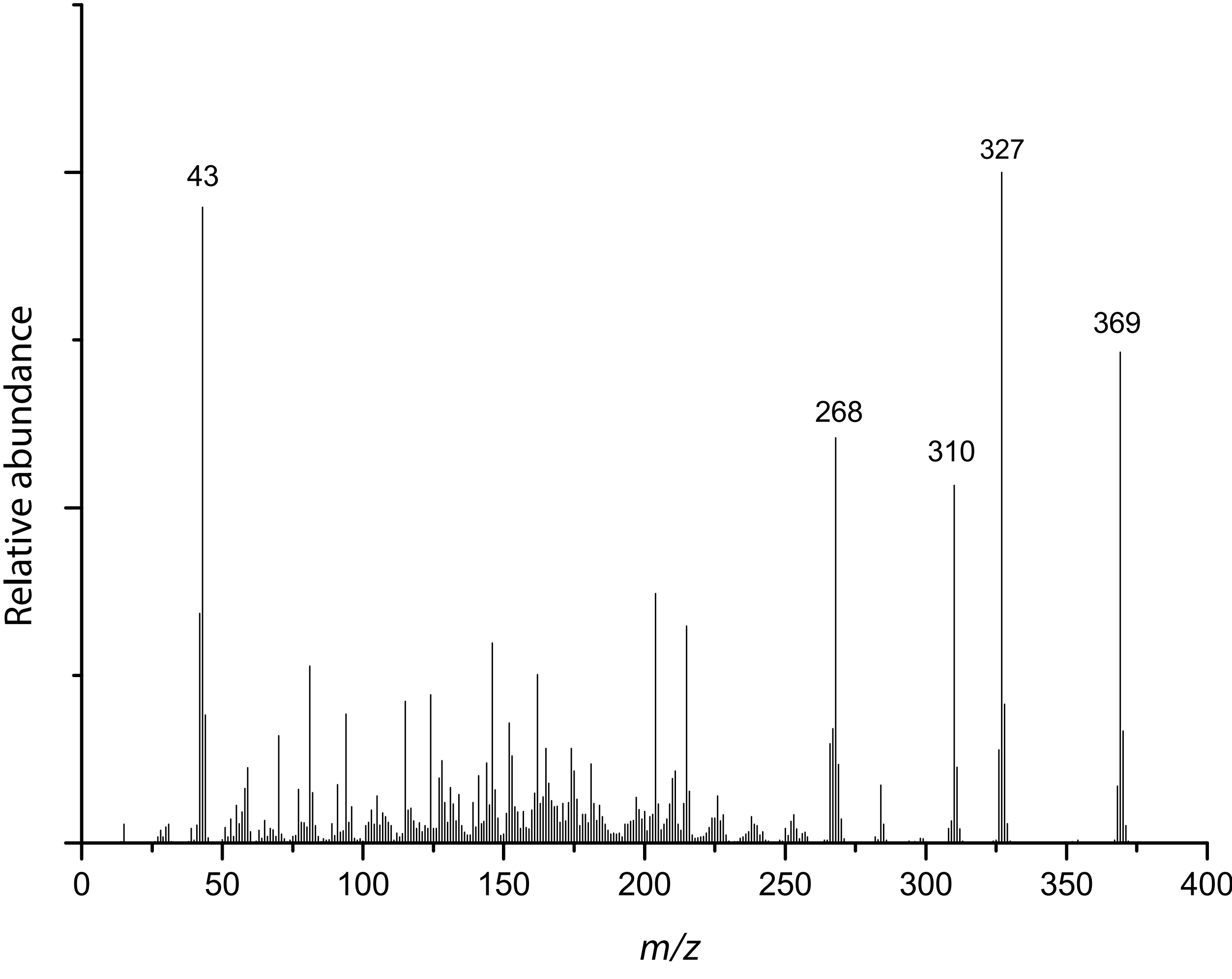
Heroin $C_{21}H_{23}NO_5$ RMM: 369.41



- Is used as a painkiller and illegal, highly addictive recreational drug.
- Large doses of heroin can cause fatal respiratory depression, and the drug has been used for suicide or as a murder weapon.



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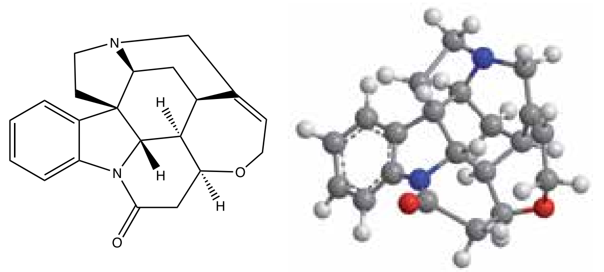
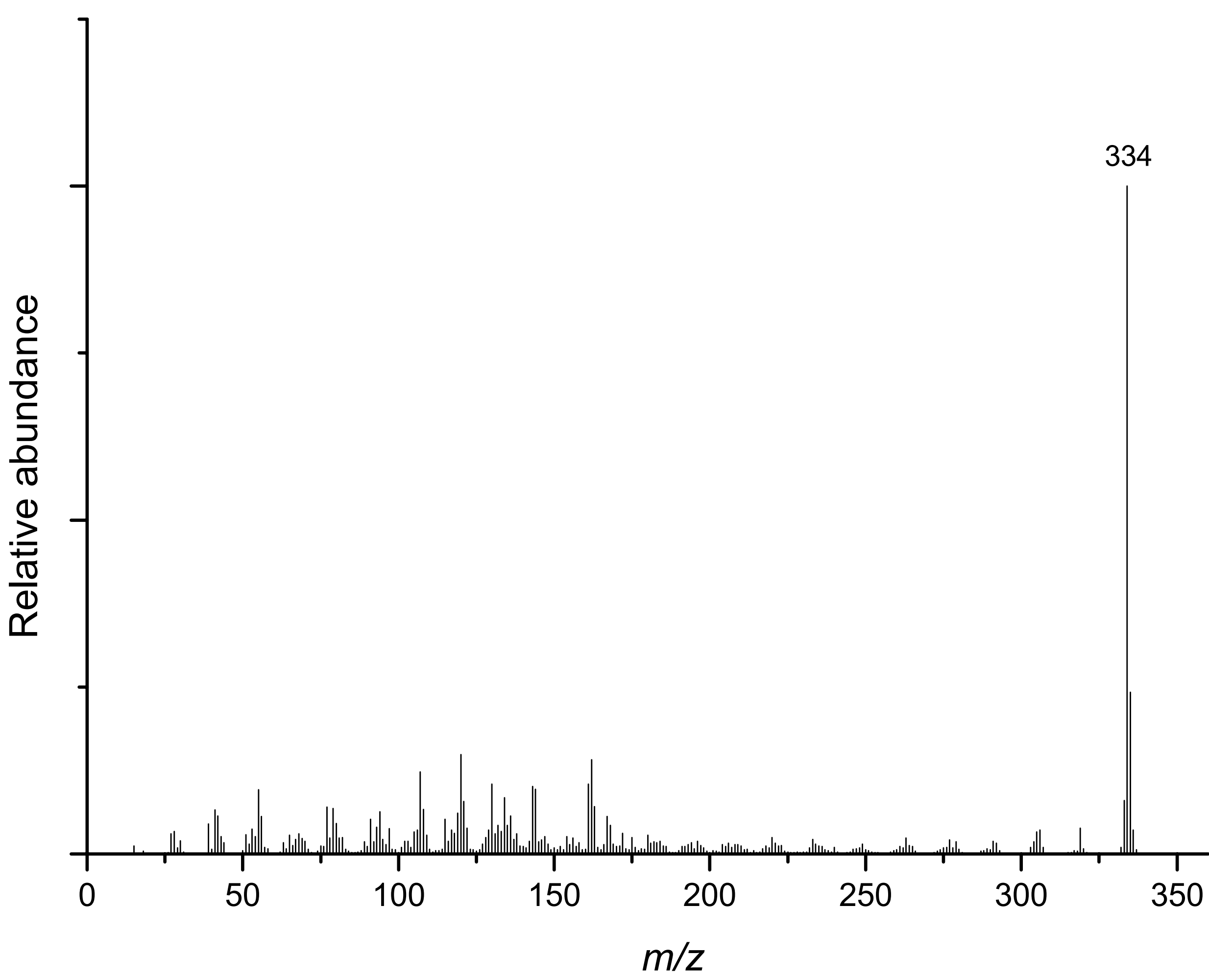
Strychnine $C_{21}H_{22}N_2O_2$ RMM: 334.41



- Is used as a pesticide for killing small mammals, birds and rodents.
- Strychnine causes muscular convulsions and eventually death through asphyxia or exhaustion.
- The use of strychnine as a medicine was abandoned once safer alternatives became available.
- The most common source is from the seeds of the *Strychos nux-vomica* tree found in Asia.



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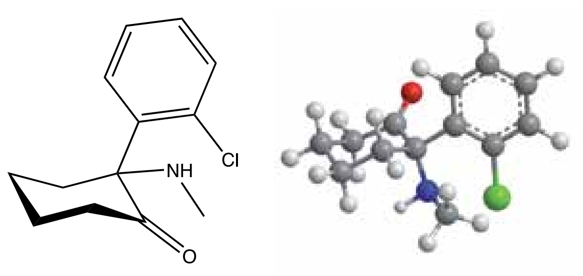
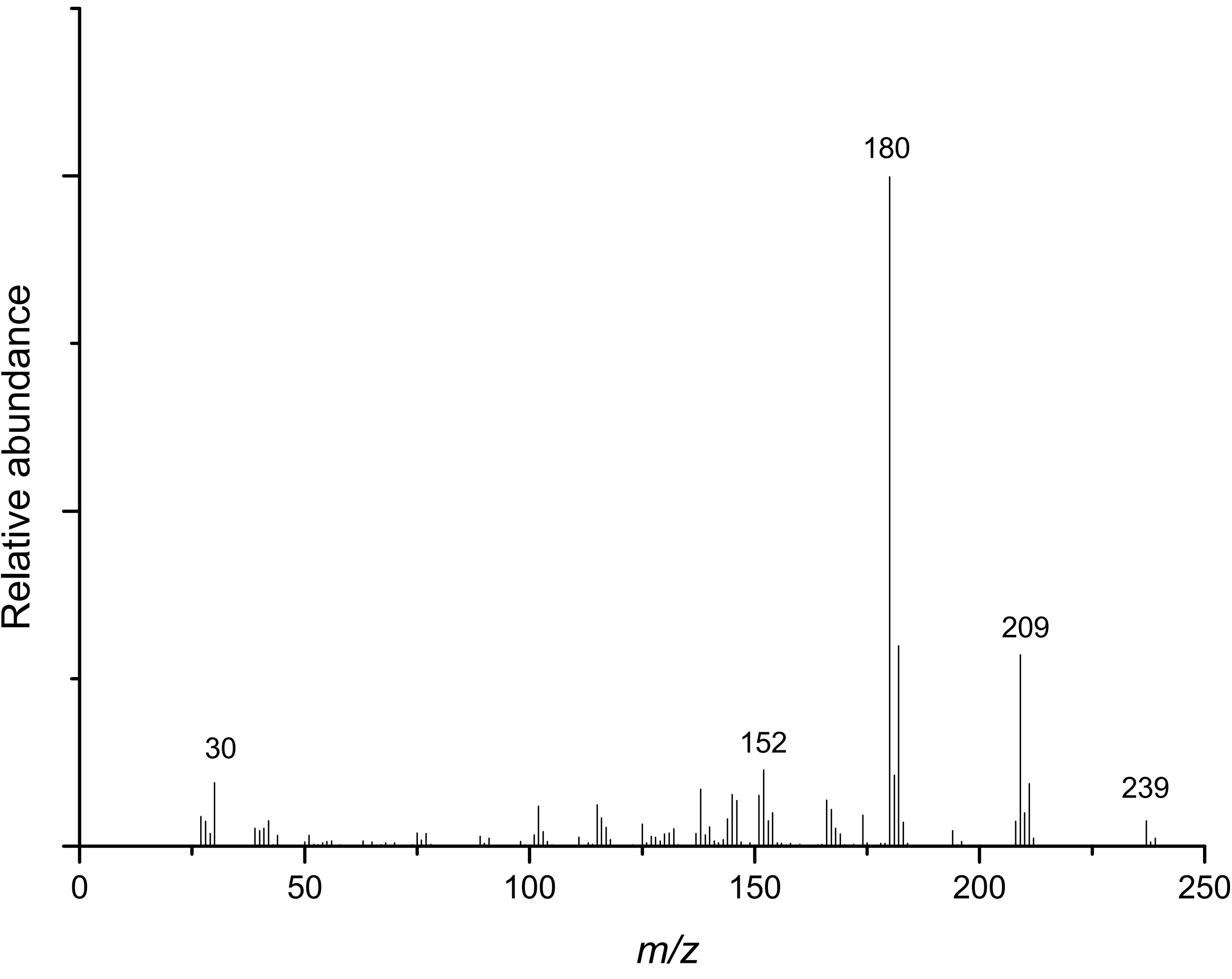
Ketamine $C_{13}H_{16}ClNO$ RMM: 237.73



- Is a drug used in medicine as an anaesthetic or analgesic.
- It is also widely used in veterinary medicine as an anaesthetic.
- It is a chiral compound and exists as two enantiomers, the (S) enantiomer being more active.
- Deaths have been attributed to ketamine due to choking, vomiting and overheating.



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Olympic drug scandal:
IR analysis investigation

History:

The Olympics are one of the greatest sports competitions in the world. Athletes fight for Gold medals, using skill, power and speed. Emotion levels are high, the competition is fierce and people will do anything to win. Sometimes people go too far and this can lead to the use of performance-enhancing drugs in many sports competitions. One of the most notorious cases of drug doping in athletics came from the 1976 Olympics, when the East German team won almost every competition. When Germany reunited, the athletes sued the government for forcing them to take anabolic steroids and stimulants.[1] During the London 2012 Olympics, the British Olympic Association was calling for tougher sanctions on athletes found using performance-enhancing drugs.[2,3] First, we need to be sure they are taking illegal substances, before anyone's career is ruined!

The illicit drugs found in sport range far and wide: anabolic steroids, which increase muscle mass; stimulants, such as amphetamines; and more recently, proteins used to increase blood cell production, thereby increasing the amount of oxygen supplied to the muscles.[4,5] And there are several drugs that are debated and are monitored from year to year.[6] There are several ways drug tests are performed: blood plasma samples, urine samples and hair. Spectroscopy is often used to determine what drugs are found in these samples or in unknown substances found in an athlete's possession.

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Scenario

The Olympics are on. Five competitors have been found with unlabelled pills in their lockers. You have been hired by the Olympics Drug Testing Association to determine what is in each pill and whether it is illegal in the competition. The pills have been ground, ready for analysis.

Your goal is to establish the functional groups found in each pill. Using a list of known drug molecules, you can narrow down your choices and use a library of spectra to further identify the compounds in the pills. Then you can decide if any of the pills has the possibility of being illegal in sport.

METHOD

1. Five unknown pills, one from each competitor, will be provided and an IR spectrum should be obtained for each sample.
   - Use the ATR attachment to run the samples.
   - Make sure you are supervised by a demonstrator, because the machine is expensive and fragile.
2. As a group, using the functional group reference table provided, determine which functional groups are present in each of the drug molecules provided on the laminated sheets.
3. Interpret your spectra and determine whether any important functional groups appear and if the IR spectra of the pills could match any of the compounds on the drug worksheet.
4. Using the IR library provided, determine whether any of the spectra of the drug molecules match any of the unknown compounds.
   - If any compounds match any of the drugs on the worksheet, do you think they might be illegal in sport?

Questions

1. If any compounds match any of the drugs on the worksheet, do you think they might be illegal in sport?
   - *Drugs that are illegal in sport on the worksheet are: ephedrine (amphetamine), cocaine (increased awareness, etc), heroin (painkiller), morphine (painkiller), and testosterone (increased muscle mass).*
   - *Drugs that are monitored and have been illegal in the past are: caffeine (increased awareness and painkilling effects) and pseudoephedrine (similar to ephedrine without the effects, but too similar in structure for early tests).*
   - *The other drugs are all over-the-counter common drugs and are not illegal in any quantity in sport.*
2. What other methods or instrumental techniques could be used to determine the identity of the pills? Would you trust just the IR results? Why or why not?
   - UV-VIS, NMR, MS, etc.
   - You should not trust merely one test, ever!
3. Testosterone/epitestosterone and ephedrine/pseudoephedrine are optical isomers. How might you be able to distinguish between them?
   - There are special tests such as circular dichroisms, based on the difference in polarity of optical isomers.
   - Technically the fingerprint region of the optical isomers is different, but only quality spectra and a good matching program would show this.

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STUDENT WORKSHEET

SAMPLE	FUNCTIONAL GROUP	IMPORTANT PEAK VALUES (cm^{-1})	NAME OF CHEMICAL (using additional information)
Competitor 1			
Competitor 2			
Competitor 3			
Competitor 4			
Competitor 5			

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DRUG MOLECULES STUDENT WORKSHEET

OH

HN

ephedrine

O

O

N

O

O

cocaine

O

O

H

N

HO

codeine

OH

HN

pseudoephedrine

O

O

O

OH

aspirin

OH

O

N
H

paracetamol

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DRUG MOLECULES STUDENT WORKSHEET

testosterone

caffeine

ibuprofen

morphine

heroin

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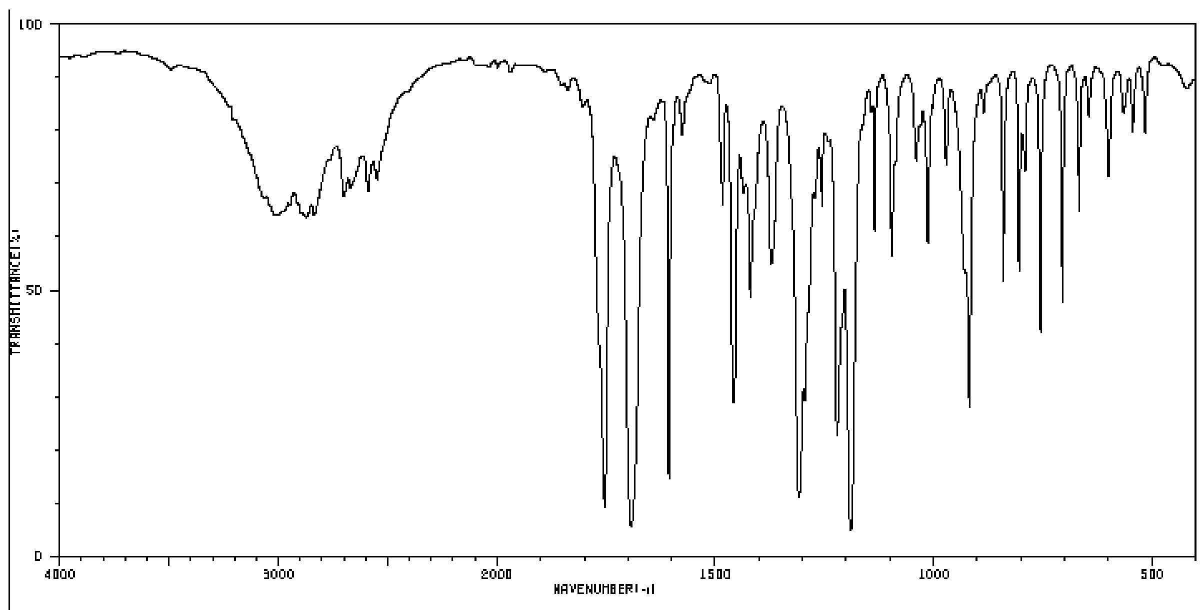
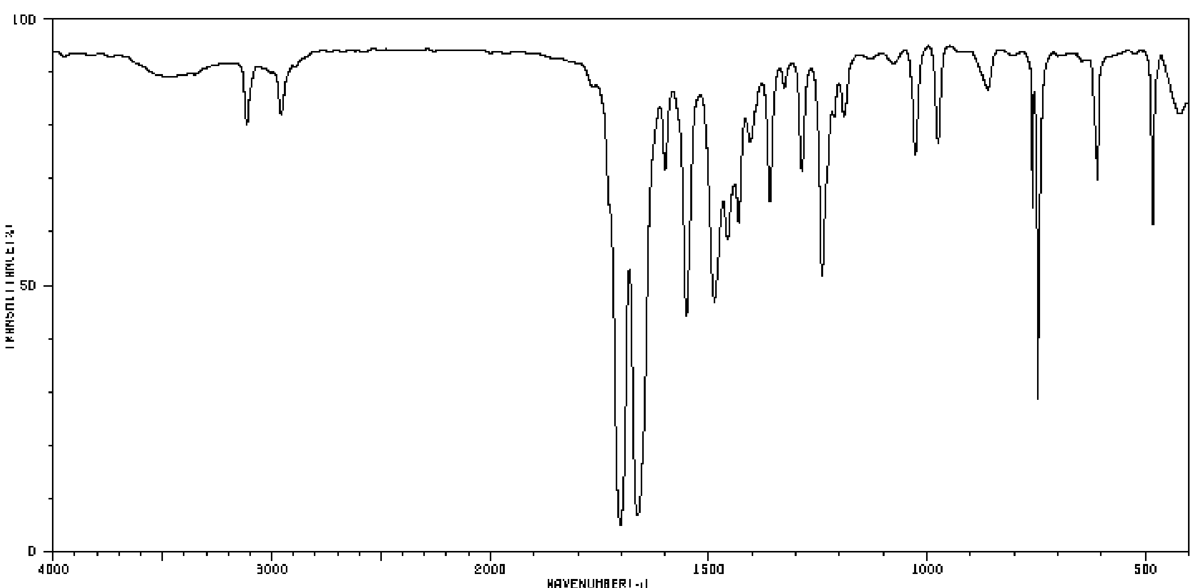
INFRARED SPECTRAL LIBRARY

Aspirin[7]



Caffeine[7]



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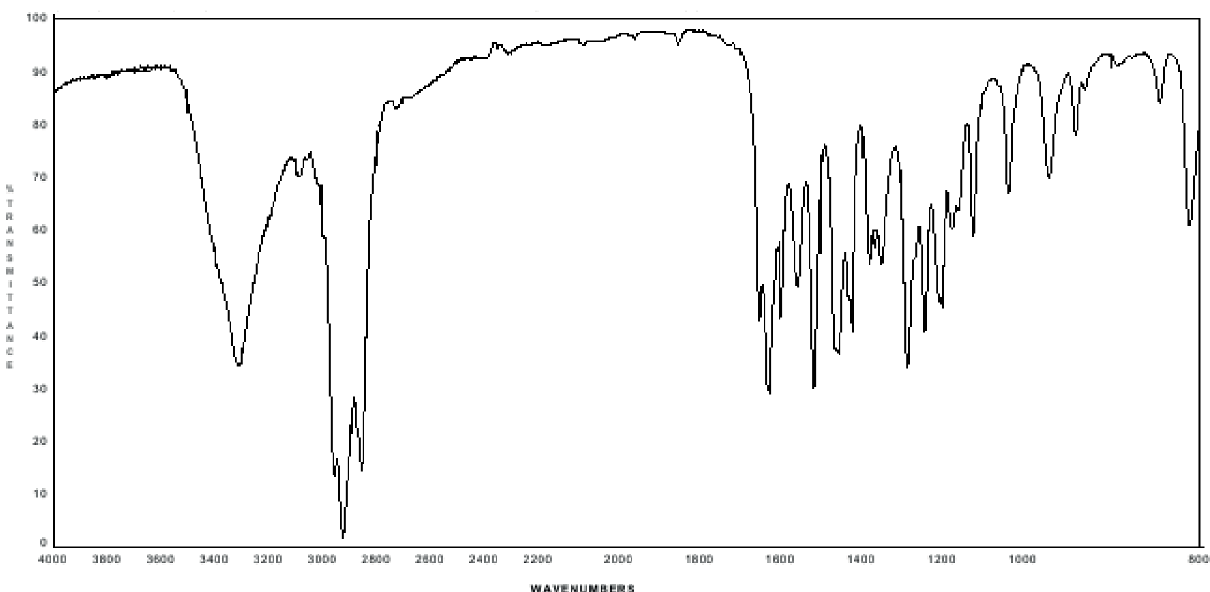
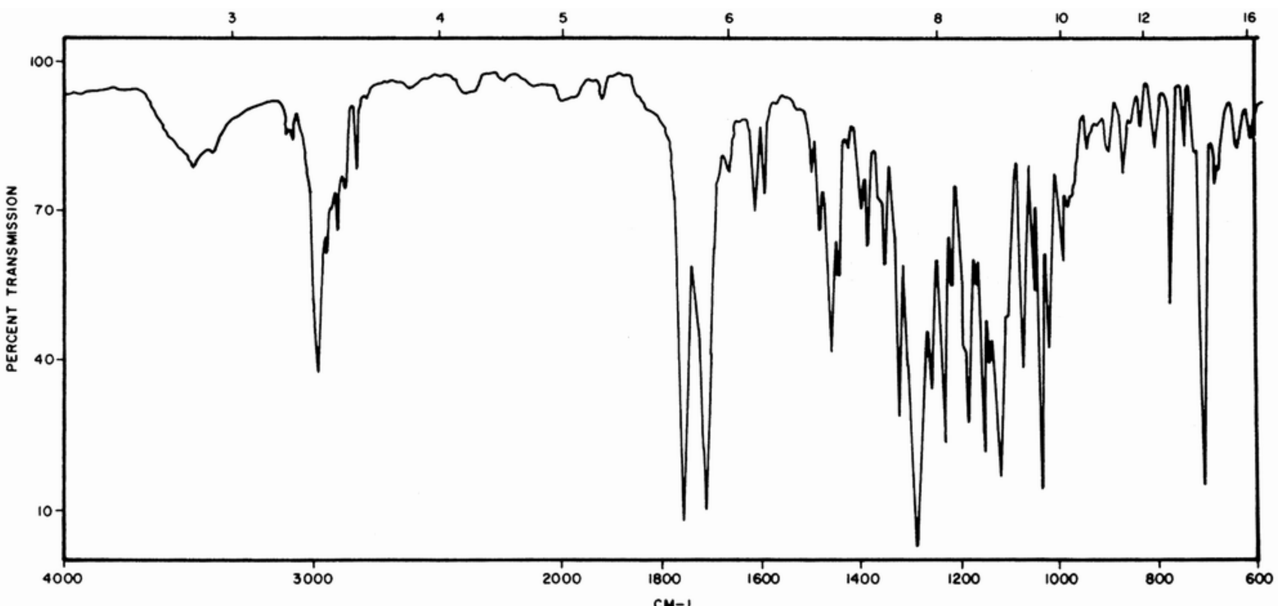
Capsaicin[8]



Cocaine[9]



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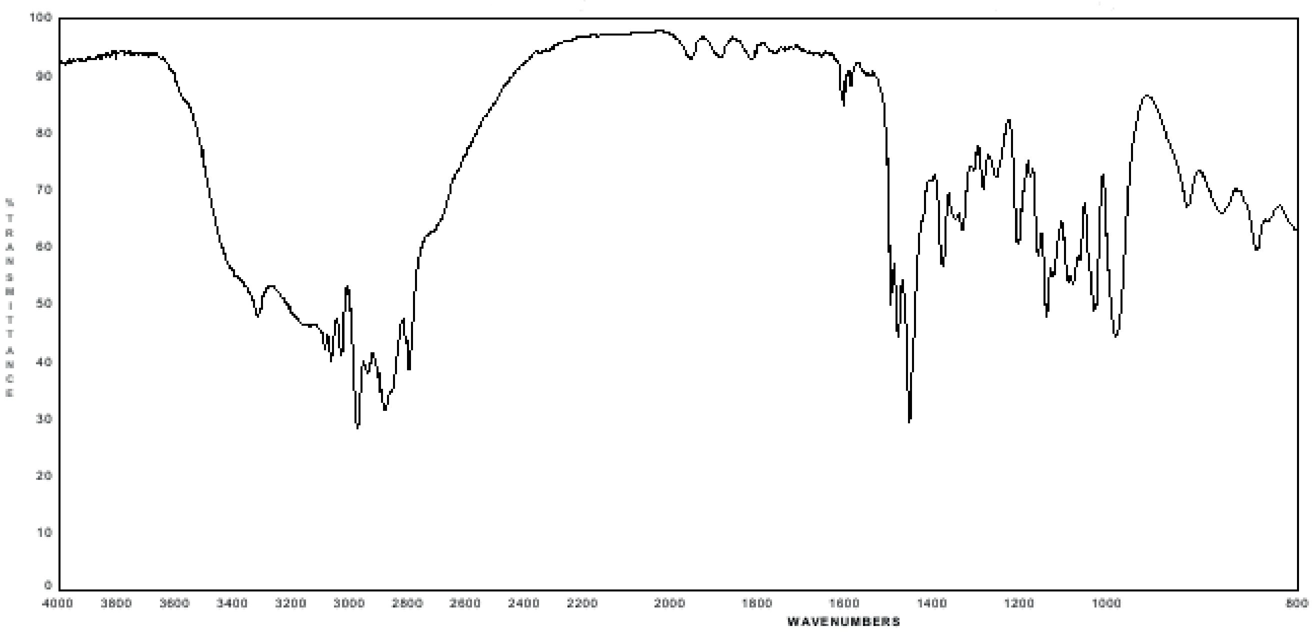
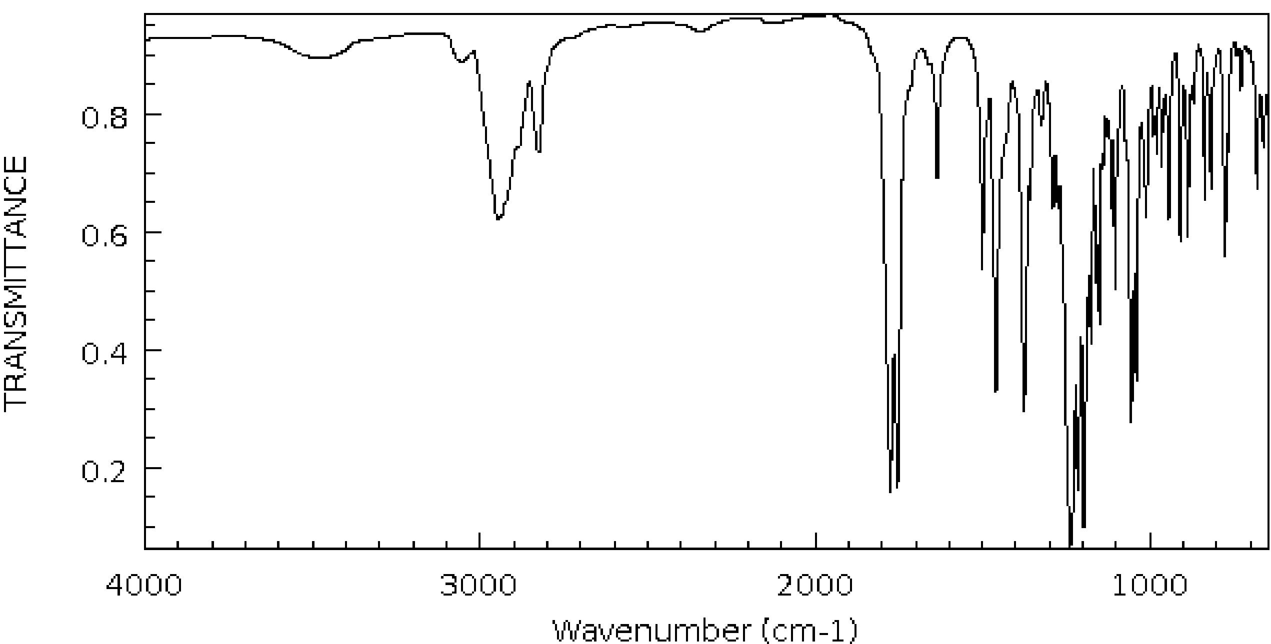
Ephedrine/Pseudoephedrine[8]



Heroin[7]



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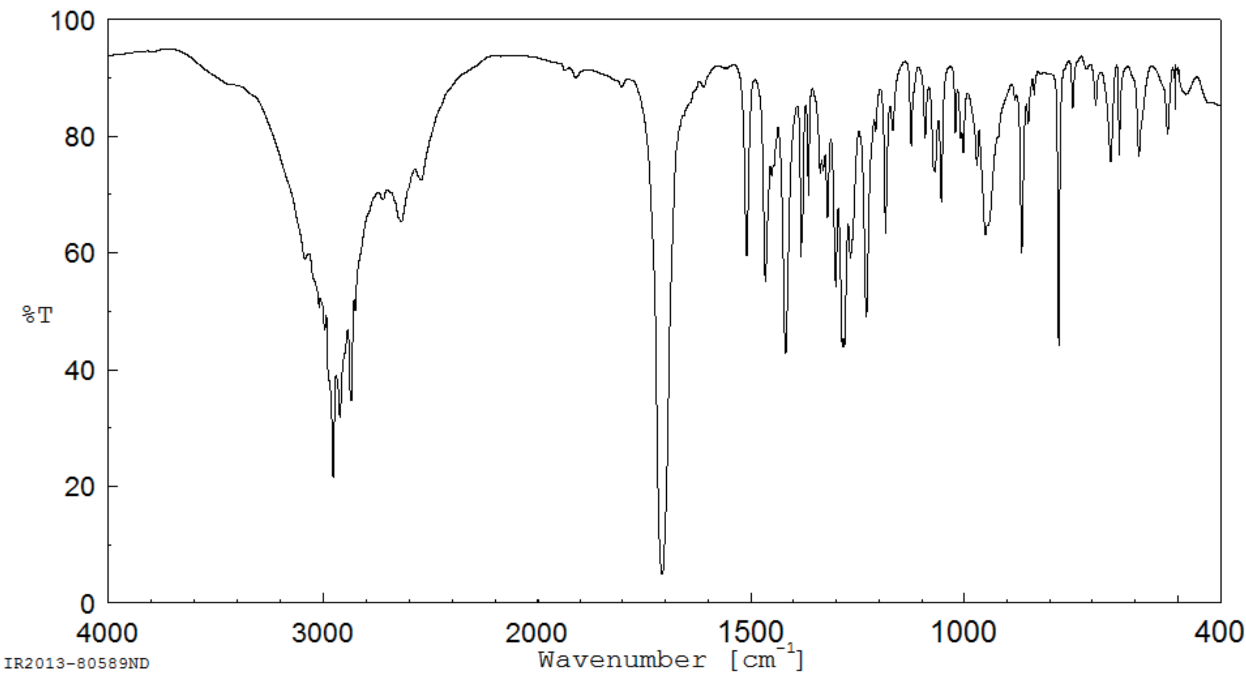
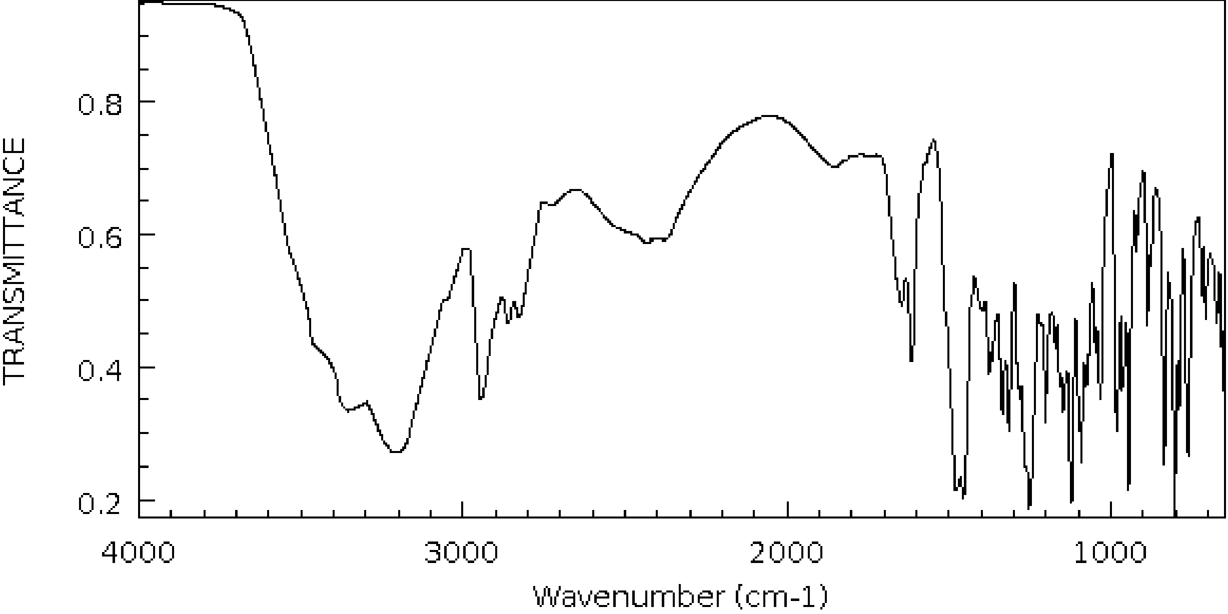
Ibuprofen[7]



Morphine[10]



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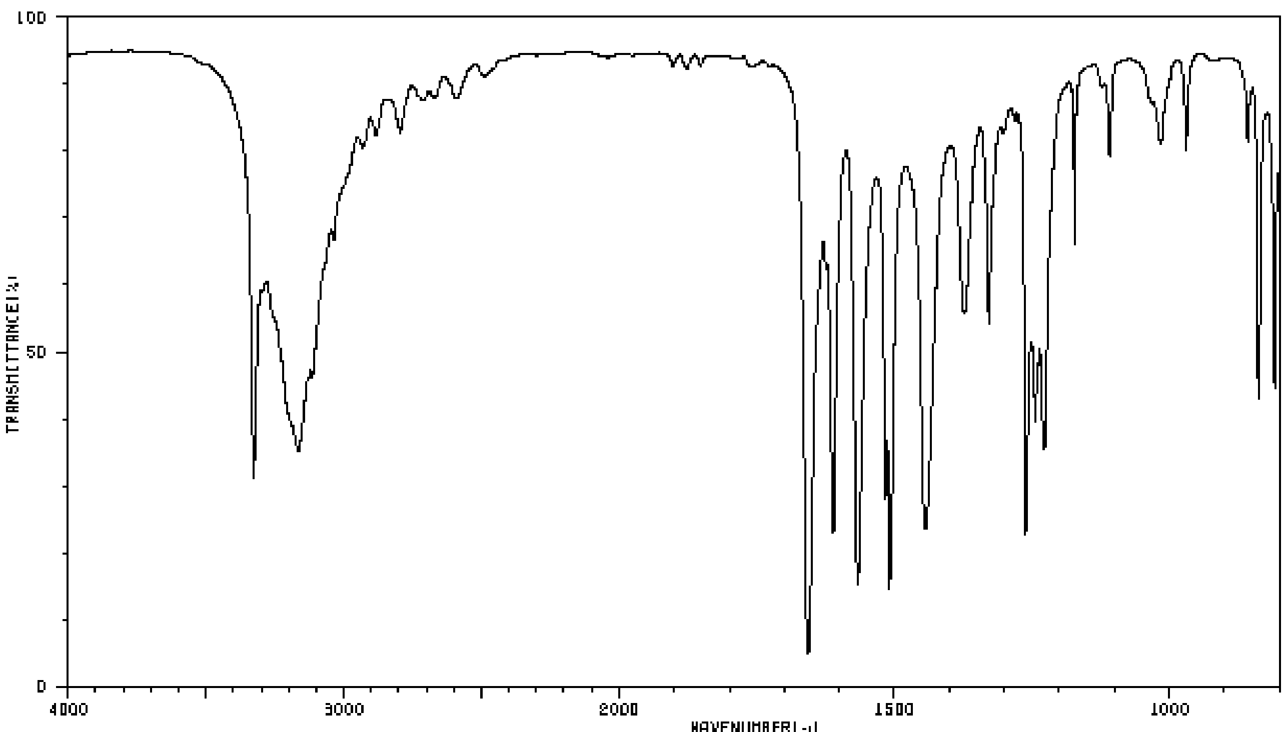
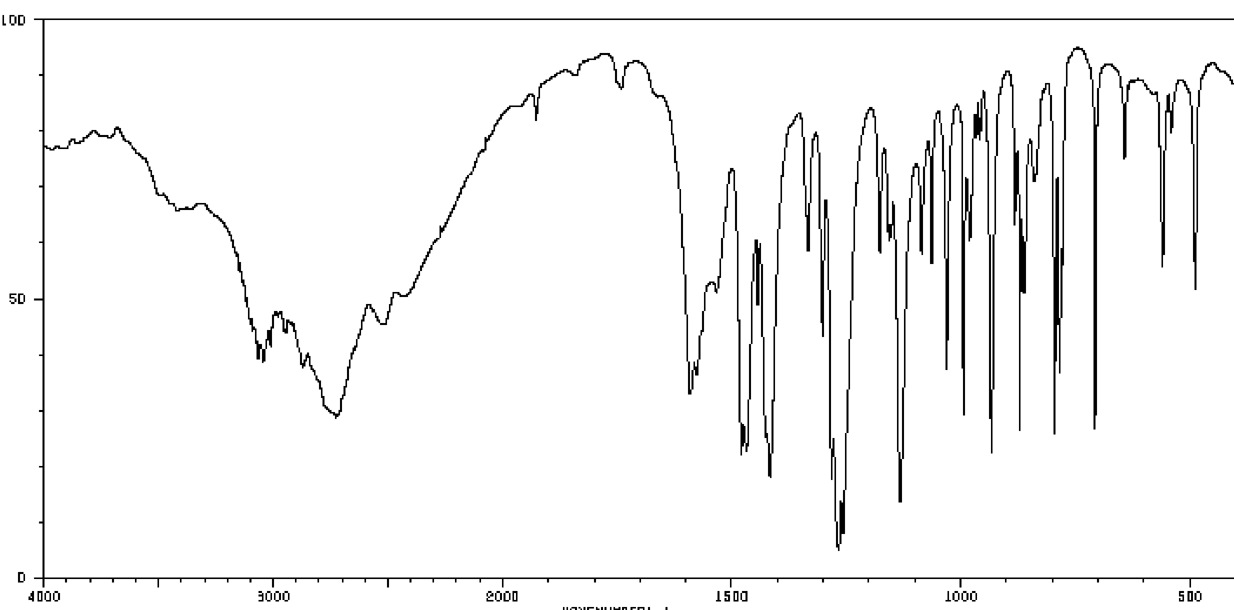
Paracetamol[7]



Phenylephrine[7]



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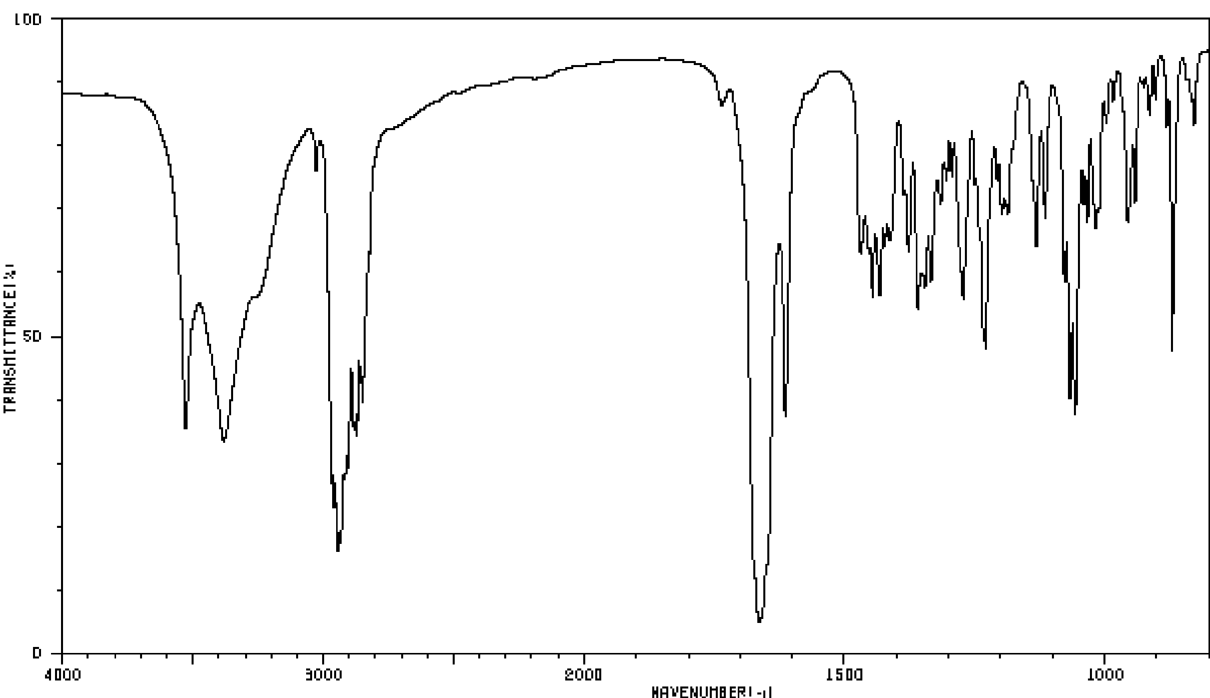
Testosterone/epitestosterone[7]



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[3]MacMichael, S. "WADA president to urge re-banning of caffeine". Road.cc, 11 Aug 2010, **road.cc/content/news/21341-wada-president-urge-re-banning-caffeine** Accessed 12 September 2016.

[4]BBC Sport, "Olympic Horses Fail Drug Tests". BBC Sport, 21 Aug 2008.

[5]World Anti-Doping Agency. "2012 Monitoring Programme", **www.wada-ama.org/en/resources/science-medicine/monitoring-program** Accessed 12 September 2016.

[6]World Anti-Doping Agency. "2012 Prohibited List", **www.wada-ama.org/Documents/World_Anti-doping_Program/WADP-Prohibited-list/2012/WADA_Prohibited_List_2012_EN.pdf** Accessed 30 April 2012.

[7]Spectra taken from the Spectral Database for Organic Compounds, SDBS. National Institute of Advanced Industrial Science and Technology (AIST), Japan.

[8]Spectra taken from the Sigma-Aldrich online catalogue, **www.sigmaaldrich.com/united-kingdom.html** Accessed 30 April, 2012

[9]John F. Casale, "A Practical Total Synthesis of Cocaine's Enantiomers". *Forensic Science International*, 33 (1987) 275-298.

[10]Spectra taken from the National Institute of Standards and Technology, Chemistry WebBook, **webbook.nist.gov/chemistry** Accessed 12 September 2016)

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