# 19. Spectroscopy and chromatography

The effect of different types of radiation on molecules
i **infrared in analysis** – infra red energy causes bonds to vibrate. This can be used to identify the types of bond in a molecule
ii **microwaves for heating-** certain molecules absorb the microwaves causing them to rotate
iii **radio waves in nmr** – can cause the hydrogen nucleus to **change its spin state.** This can give us information about the arrangements of hydrogens in a molecule.
iv **ultraviolet in initiation of reactions** – UV energy can break bonds such as the Cl-Cl bond or C-Cl bond

## NMR spectroscopy

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

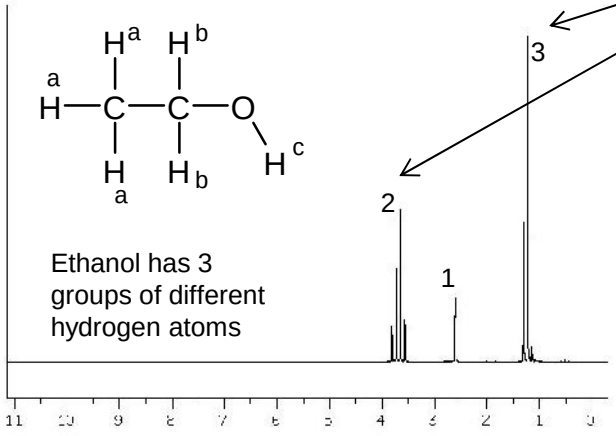
NMR spectroscopy is the same technology as that used in 'magnetic resonance imaging' (MRI) to obtain diagnostic information about internal structures in body scanners e.g. scanning for brain disorders

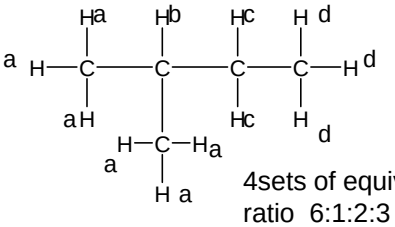
The radio waves used in proton nmr cause the hydrogen nucleus to **change its spin state.**

**Equivalent Hydrogen atoms.**

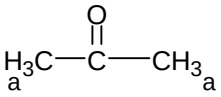
In an H NMR spectrum, there is one signal for each set of equivalent H atoms.

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

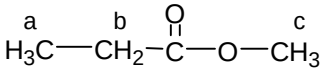


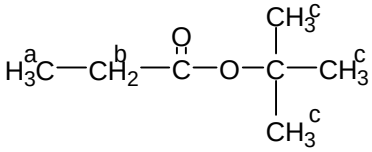
4sets of equivalent H's: ratio 6:1:2:3



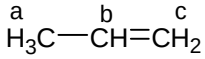
1 signal



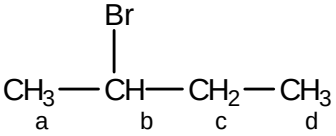
3 sets of equivalent H's: ratio 3:2:3



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



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



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

**Solvents**

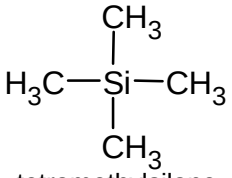
Samples are dissolved in solvents without any $^1H$ atoms, e.g. $CCl_4$, $CDCl_3$.

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

**Calibration and shift**

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

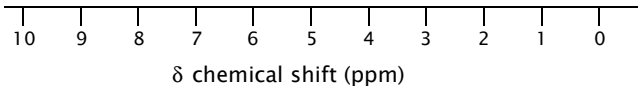
TMS is used because:
- its signal is away from all the others
- it only gives one signal
- it is non-toxic
- it is inert
- it has a low boiling point and so can be removed from sample easily



tetramethylsilane

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

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

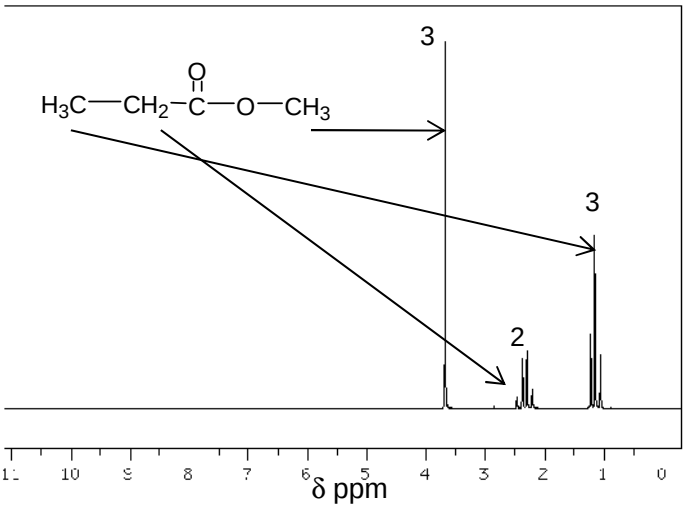


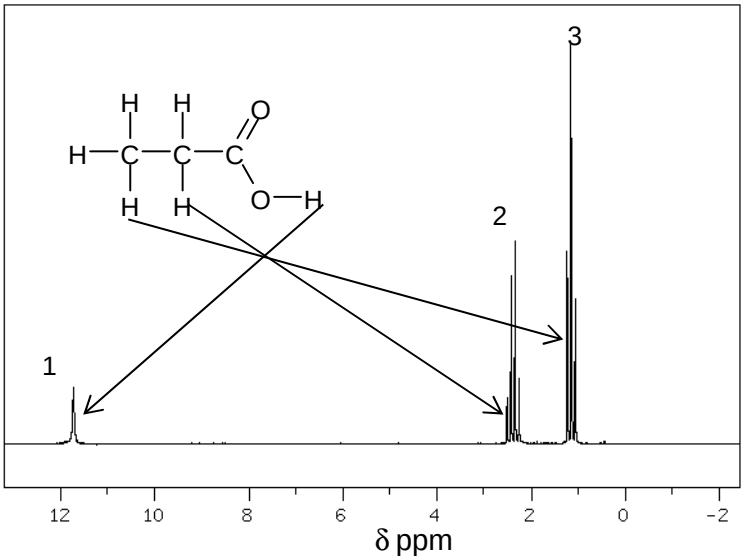

**H NMR shift**

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

$^1$H n.m.r. chemical shift data

| Type of proton | δ/ppm |
|---|---|
| ROH | 0.5–5.0 |
| $RCH_3$ | 0.7–1.2 |
| $RNH_2$ | 1.0–4.5 |
| $R_2CH_2$ | 1.2–1.4 |
| $R_3CH$ | 1.4–1.6 |
| R–C(=O)–C(H)– | 2.1–2.6 |
| R–O–C(H)– | 3.1–3.9 |
| $RCH_2Cl$ or Br | 3.1–4.2 |
| R–C(=O)–O–C(H)– | 3.7–4.1 |
| R(H)C=C(H) | 4.5–6.0 |
| R–C(=O)–H | 9.0–10.0 |
| R–C(=O)–O–H | 10.0–12.0 |



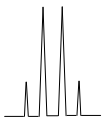
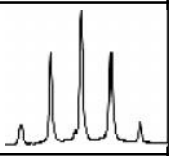



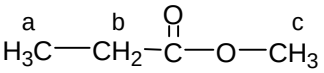
**Spin-Spin coupling in H NMR**

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

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

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

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

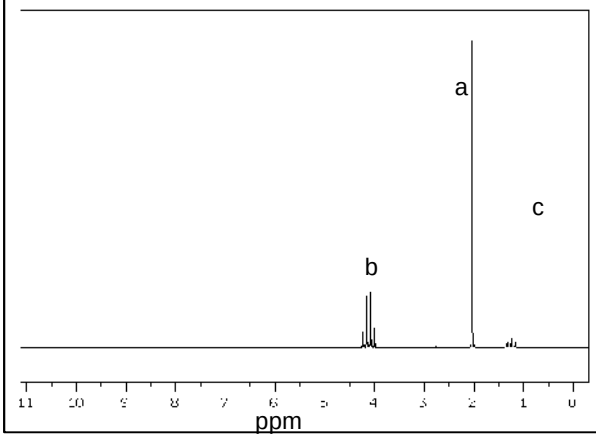


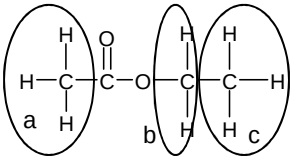

The peak due to group **a** will be a **triplet** as it is next to **b** (a carbon with 2 H's)

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

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

For 6 split peaks use the term hextet or multiplet




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

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

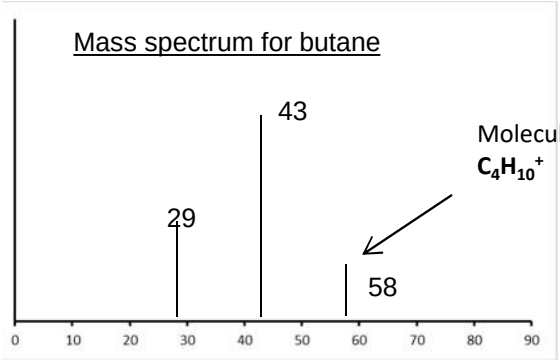
The peak due to group **c** will be a **triplet** as it is next to a carbon with 2 H's
Shift 0.7-1.2
Integration trace 3

## Mass spectrometry

### Measuring the $M_r$ of an organic molecule

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

**Spectra for $C_4H_{10}$**




### Fragmentation

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

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

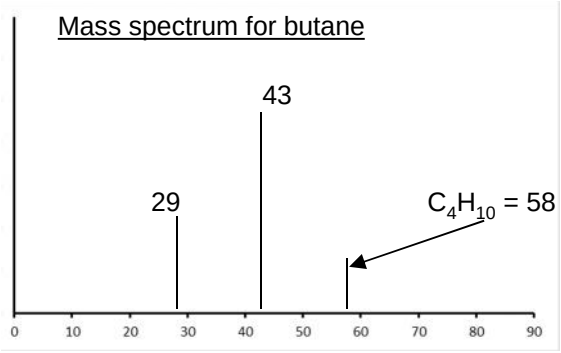
The molecule loses an electron and becomes both an ion and a free radical

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

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

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

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



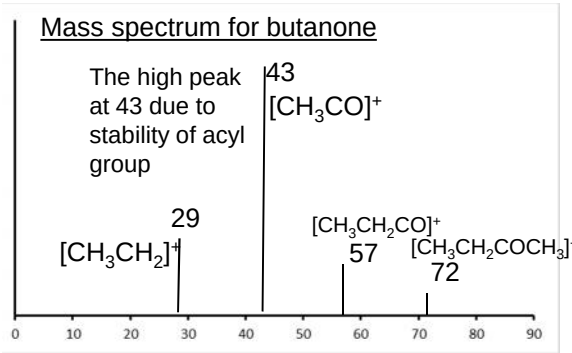

Equation for formation molecular ion

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

Equations for formation of fragment ions from molecular ions

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

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




Equation for formation molecular ion

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

Equations for formation of fragment ions from molecular ions

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

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

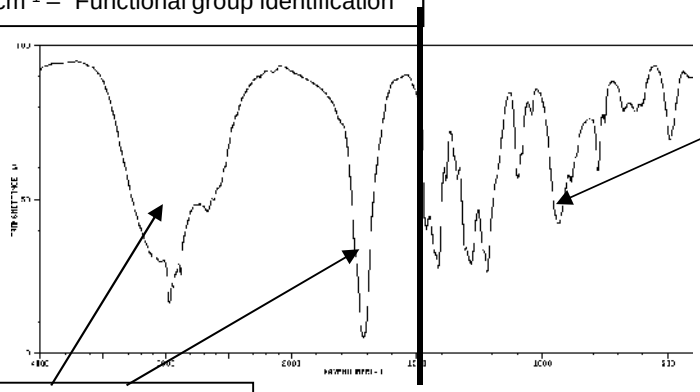
$[CH_3CH_2COCH_3]^{+\cdot} \rightarrow [CH_3CH_2]^+ + \cdot COCH_3$ m/z 29

**Infrared spectroscopy**

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

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

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



BELOW 1500 $cm^{-1}$ – "Fingerprinting"

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

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

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

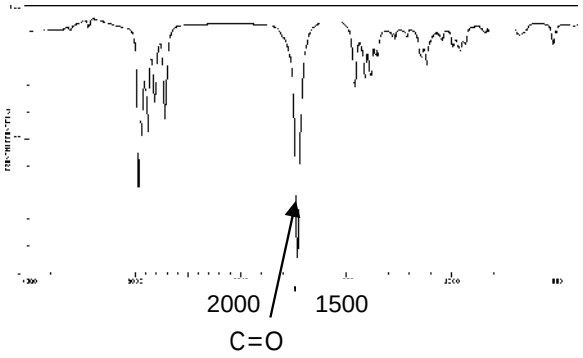
e.g. C=O 1640 – 1750 $cm^{-1}$

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

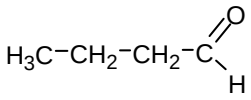
Use an IR absorption table provided in exam to deduce presence <u>or</u> absence of particular bonds or functional groups

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

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




Spectra for butanal

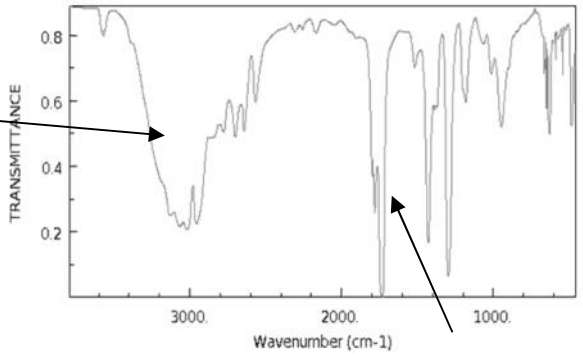



Absorption or trough in between 1640-1750 $cm^{-1}$ range indicates presence of C=O bond

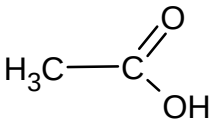
Always quote the wave number range from the data sheet

O-H absorptions tend to be broad

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




Spectra for ethanoic acid




Modern breathalysers measure ethanol in the breath by analysis using infrared spectroscopy

## Chromatography

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

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

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

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

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

HPLC stands for high performance liquid chromatography.
HPLC: **stationary** phase is a **solid** silica
HPLC: **mobile** phase a **liquid**

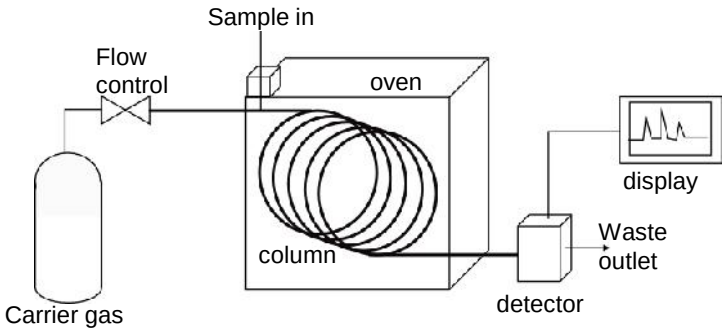
In gas-liquid chromatography GC the **mobile** phase is a inert **gas** such as nitrogen, helium, argon.
The **Stationary** phase is a **liquid** on an inert solid.

## Gas-Liquid Chromatography

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

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

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




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

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

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

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

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

**TLC Chromatography (thin-layer chromatography)**

A mixture can be separated by chromatography and identified from the amount they have moved. (Can be used with mixtures of amino acids)

Method: **Thin-layer chromatography**

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

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

**pencil** line –will not dissolve in the solvent

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

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

**lid**– to prevent evaporation of toxic solvent

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

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

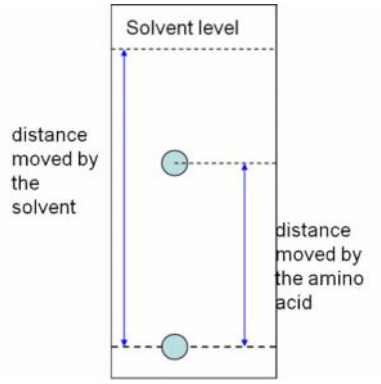
**UV lamp** used if the spots are colourless and not visible

If using amino acids then ninhydrin spray can be used instead of UV lamp to locate the spots

$R_f$ value = $\frac{\text{distance moved by amino acid}}{\text{distance moved by the solvent}}$

Each substance has its own $R_f$ value

Measure how far each spot travels relative to the solvent front and calculate the *R*f value.
Compare *R*f values to those for known substances.




Some substances won't separate because similar compounds have similar *R*f values. So some spots may contain more than one compound

## Bringing it all together

1. Work out empirical formula

Elemental analysis C 66.63% H 11.18% O 22.19%

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

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

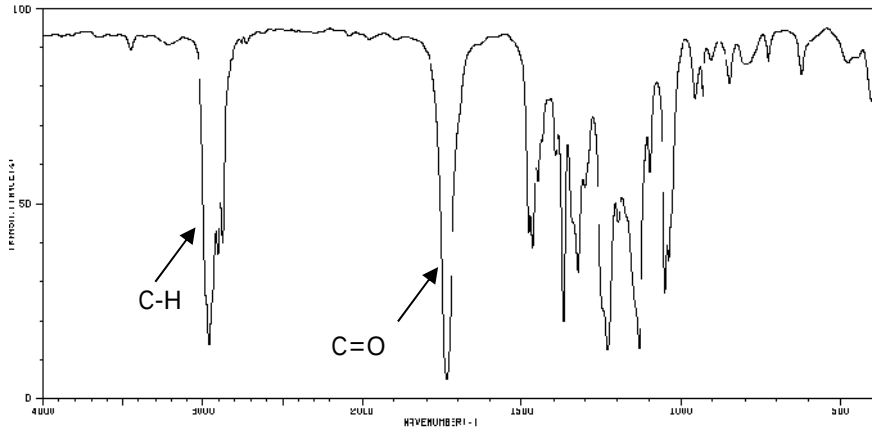
molecular ion peak m/z value= 144

Mr empirical formula $C_4H_8O$ = 72

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

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

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

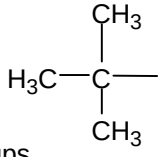



There is a C=O but no O-H absorptions, so must be an ester.

4. Use NMR spectra to give details of carbon chain

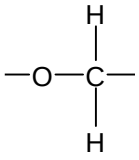
4 peaks – only 4 different environments.

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



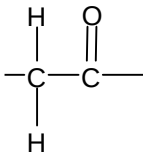
Peak at δ 4 shows H–C–O

Area 2 suggests $CH_2$
Quartet means next to a $CH_3$



Peak at δ 2.2 shows H–C=O

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



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




Put all together to give final structure