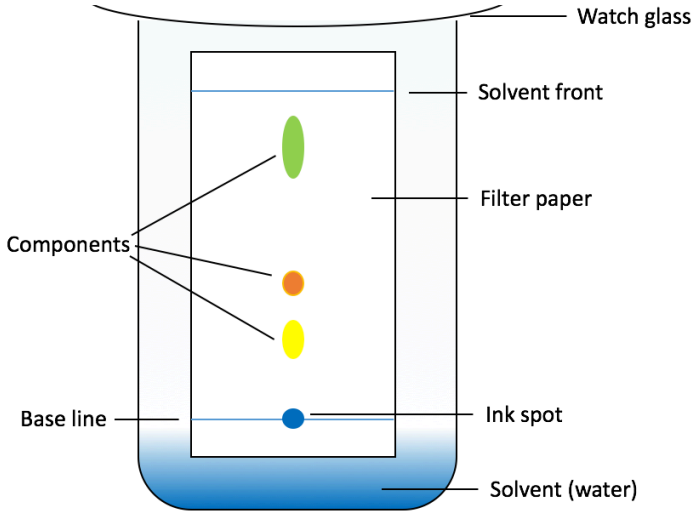
3.16 Chromatography

What is chromatography

- Chromatography is a separation technique
- There are 3 types of chromatography that you will study:

1) Thin Layer Chromatography - TLC
2) Column Chromatography - CC
3) Gas Chromatography – GC

Recap from GCSE – paper chromatography:



- The paper is placed in a beaker with the solvent (water) below the sample.
- The beaker is covered and the water allowed to rise near the top of the paper.
- The paper is removed, the solvent front marked.
- The data is analysed.
- Chromatography is used in analysis but is more widely used as a method of separation.

How does chromatography work?

- All chromatography works on the same principle:
- Components (molecules) have different affinities for a **stationary phase** and for a **mobile phase**

Phase:
Is the physical state – solid, liquid or gas

Stationary phase:
The molecules can't move – a solid, can be a liquid (viscous) on a solid support

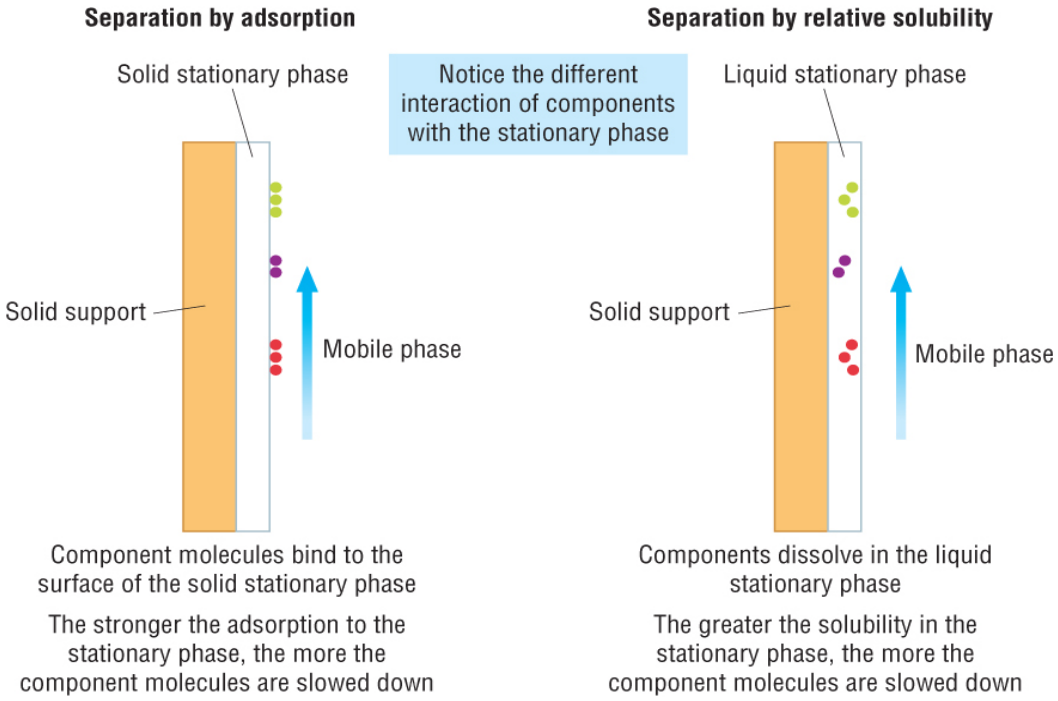
Mobile phase:
The molecules can move – always a liquid or gas

Phases and intermolecular forces of attraction:
Solid phase:
- Molecules interact with solid phases by – **ADSORPTION**

Liquid or gas phase:
- Molecules interact with the mobile phase by – **SOLUBILITY**

Stationary phase – slows down their movement - **RETENTION**
Mobile phase – speeds up their movement

OVERALL – It is the difference between the SOLUBILITY in the MOBILE PHASE and RETENTION in the STATIONARY PHASE that separate the components



1) Thin Layer Chromatography - TLC
- Solid stationary phase and liquid mobile phase
  - Retention by adsorption with stationary phase vs movement by solubility in the mobile phase

- Liquid stationary phase and liquid mobile phase
  - Retention by solubility with stationary phase vs movement by solubility in the mobile phase

2) Column Chromatography - CC
- Solid stationary phase and liquid mobile phase
  - Retention by adsorption with stationary phase vs movement by solubility in the mobile phase

3) Gas Chromatography - GC
- Solid stationary phase and gas mobile phase
  - Retention by adsorption with stationary phase vs movement by solubility in the mobile phase

- Liquid stationary phase and gas mobile phase
  - Retention by solubility with stationary phase vs movement by solubility in the mobile phase

1) Thin Layer Chromatography - TLC

Phases:

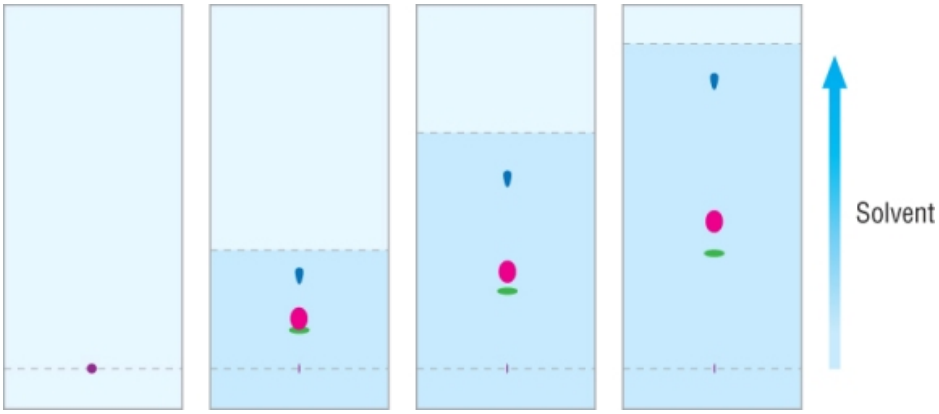
Stationary phase - Silica, SiO_2 or Aluminium oxide, Al_2O_3

Mobile phase – Solvent (liquid)

Producing the chromatogram:

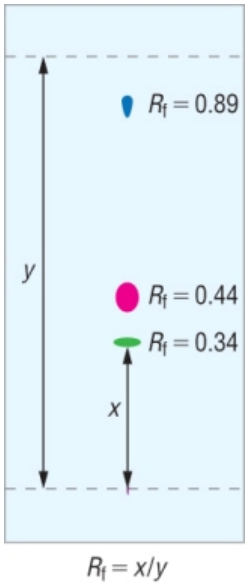
1) Dissolve sample.
2) Draw a pencil base line and spot sample using a capillary tube, allow to dry.
3) Place plate in a tank of solvent - solvent must be below the base line, seal the tank.
4) Separation is by adsorption - allow solvent to almost reach the top, draw a line here - solvent front.
5) Place in a fume cupboard to dry

The chromatogram:



Retention factor, R_f values:

- An R_f value shows how far the centre of a component has travelled compared with the solvent front:



$$R_f = \frac{\text{Distance moved by component}}{\text{Distance moved by solvent front}}$$

$$R_f \text{ Green} = \frac{1.65}{4.85} = 0.34$$

$$R_f \text{ Pink} = \frac{2.15}{4.85} = 0.44$$

$$R_f \text{ Blue} = \frac{4.30}{4.85} = 0.89$$

- R_f is a ratio of the distance of the component moved : solvent front.
- This means that the R_f values in this solvent will always be the same:

What do R_f values mean:

For substances that are very soluble in the liquid mobile phase and small retention due to weak adsorption with the stationary phase, R_f will be close to: **1**

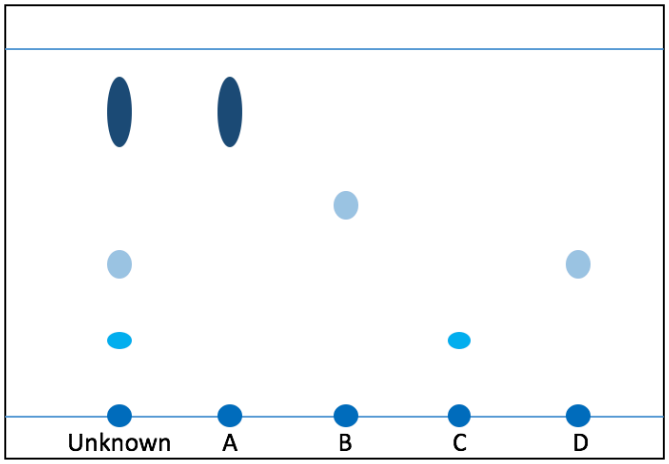
For substances that are not very soluble in the liquid mobile phase and large retention due to strong adsorption with the stationary phase, R_f will be close to: **0**

Limitations:

- Similar compounds often have too similar R_f values.
- Unknown compounds have no R_f value for comparison.
- Even a small temperature change can affect the R_f value
- It is hard to find a solvent that will have the correct amount of solubility - Goldilocks!!

Alternatively:

- Comparisons can also be made against pure components run against the mixture:



- It is clear to see that the unknown is made up of the known components A, C and D.

Colourless components:

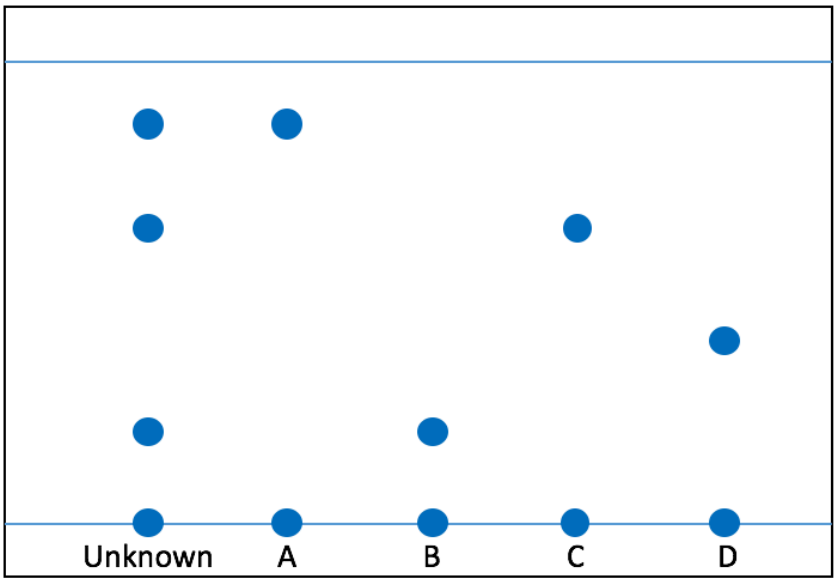
- Obviously, if the components are clear and colourless, you won't be able to see them on a dry chromatogram.
- Locating agents are added to reveal the location of the separated components / spots.
- This is done in one of 2 ways:

1) **Fluorescent dye:** Added to the stationary phase (can be sprayed on before drying). These show a dark spot under UV light.

2) **Locating agent:** Iodine vapour sticks to the chemicals on the plate. Seen as purple spots.

Question:

An unknown mixture contains 3 of4 compounds A – D. TLC was used to separate the mixture alongside the 4 known A – D:



a) State and explain which of the 4 compounds is present in the unknown mixture:

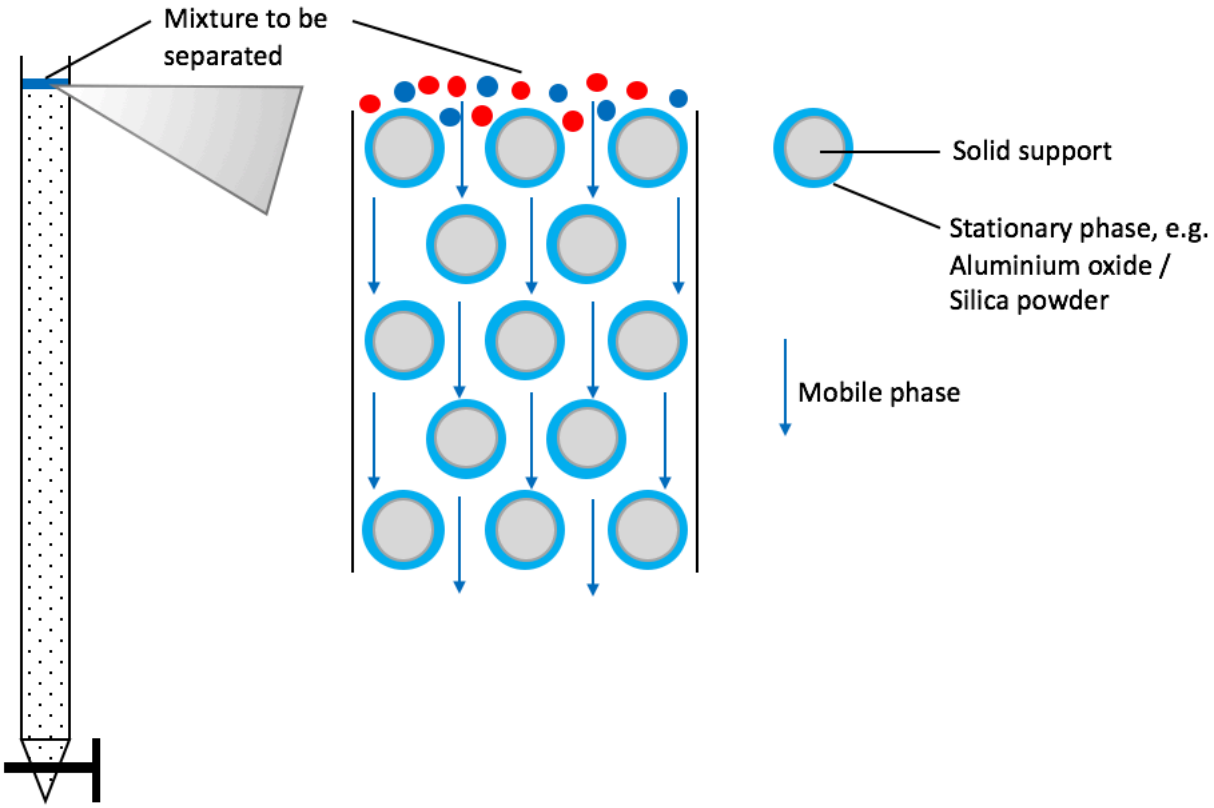
b) Calculate the R_f value for compound A and C

c) Which of the 4 known compounds, A – D is the least soluble in the solvent? Explain your answer:

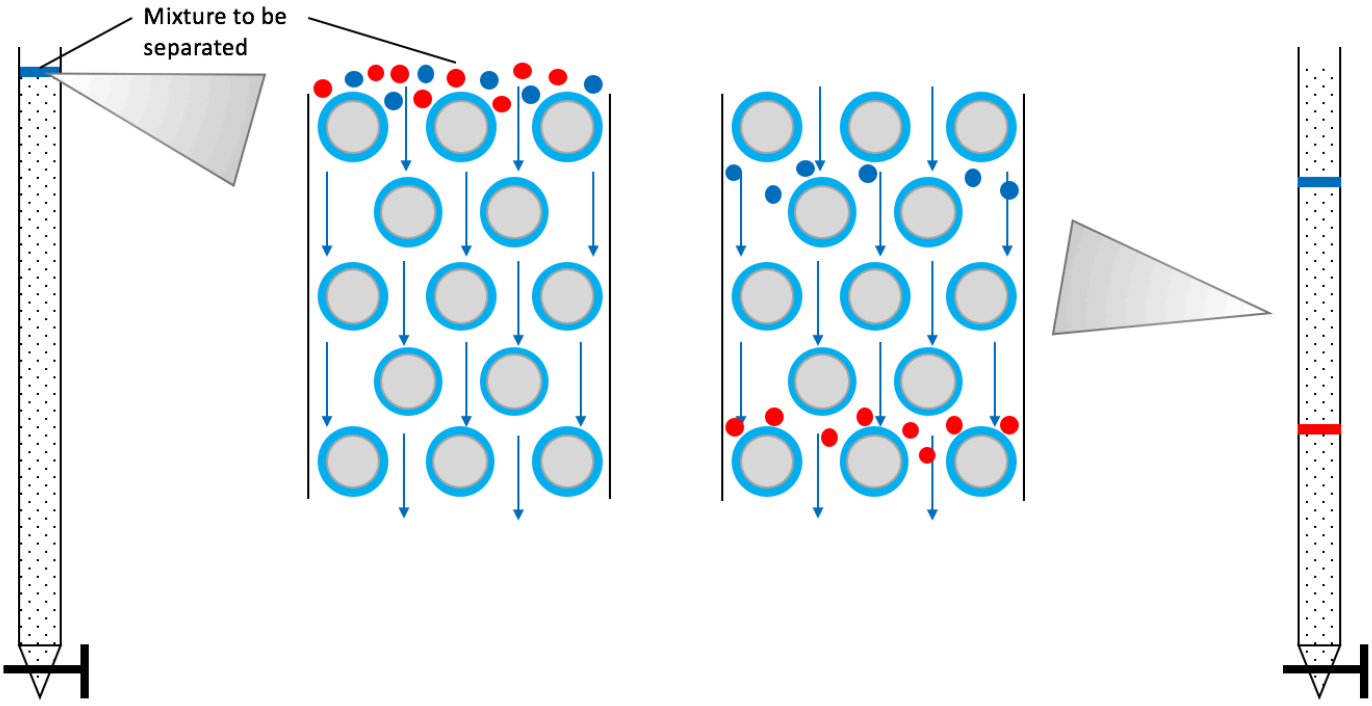
d) Explain why compound A has the largest R_f value. You must refer to both the stationary and mobile phase in your answer:

2) Column Chromatography - CC

- A column is set up as shown below.



- The mobile phase is added at the top.
- The relative retention and solubility's separate the components as they move down the column:



- Usually used to separate an organic compound from the reaction mixture.
- The pure liquid can be tapped of and identified by its **Retention time** or using **mass spectroscopy**.

Question:

An organic compound is made and needs separating from the reaction mixture and impurities. The pure organic product is more strongly adsorbed to the stationary phase than the impurities and reaction mixture. Will the pure product leave the column first or last? Explain your answer?

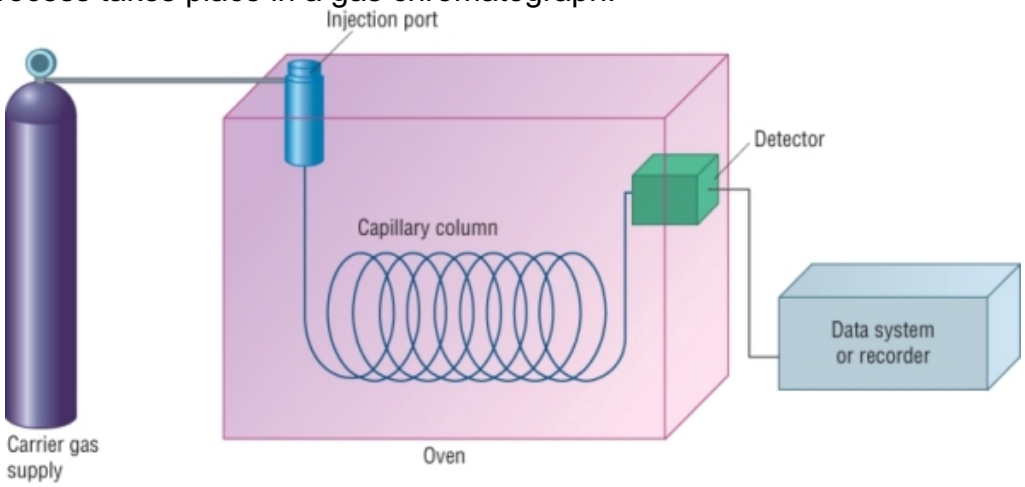
Two pure organic compounds A and B are to be separated using column chromatography. B leaves the column first.

a) Which compound has the largest retention time? Explain your answer.

b) Explain why compound B leaves the column first. You must refer to both the stationary and mobile phase in your answer:

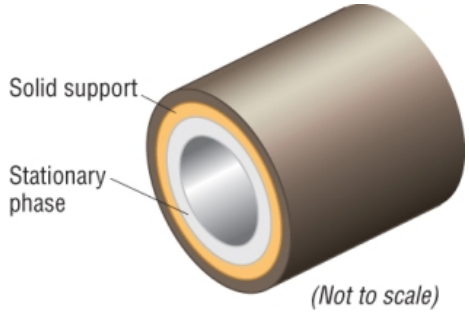
3) Gas Chromatography - GC

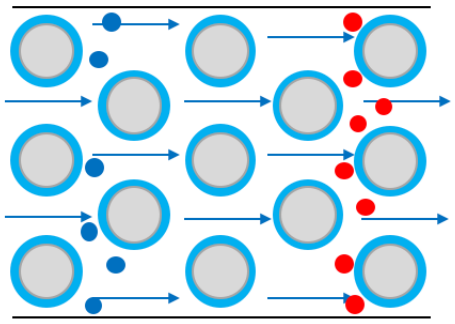
- Is used to separate volatile compounds (gases) in a mixture.
- The compounds, being volatile will have low boiling points (to evaporate easily)
- This process takes place in a gas chromatograph:



Producing the chromatograph:

- The mixture is injected into the chromatograph, it is vaporised and the mixture is carried through the column by the mobile inert carrier gas, eg Nitrogen
- As the mixture flows through the column, the components are slowed down by adsorption to the stationary phase lining the column:





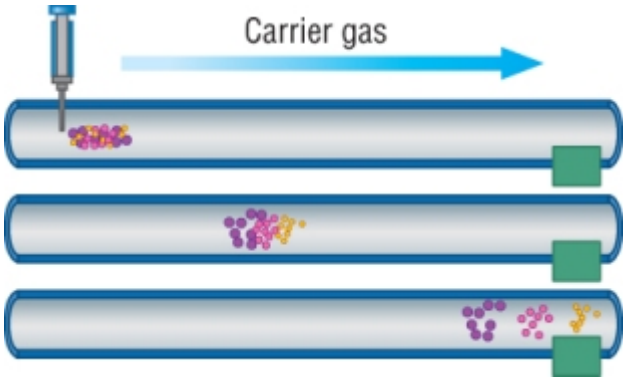
The stationary phase:

- The **stationary phase** is a liquid or solid lining of the capillary tube.
- A suitable **liquid** lining for the **stationary phase** is usually a long chain alkane (high boiling point) - **solubility**
- A suitable **solid** lining for the **stationary phase** is usually a silicone polymer - **adsorption**
- Depending on what is separated depends on whether you use a liquid or solid stationary phase.

The mobile phase:

- Is an inert carrier gas such as helium or nitrogen.

- The mixtures are separated by their relative adsorption / solubility to the stationary phase

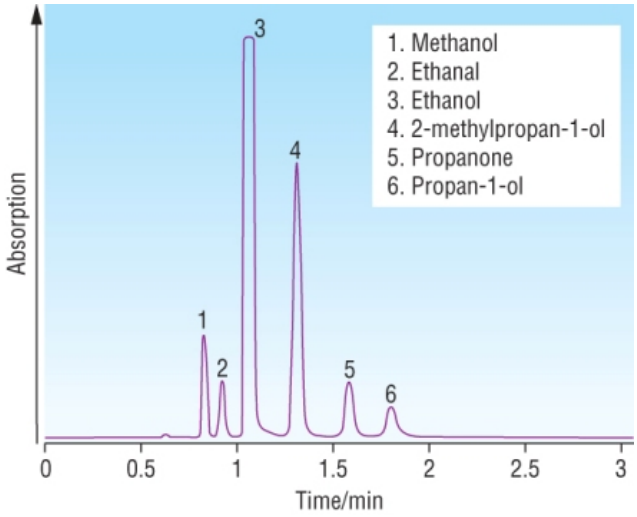


- Separation can be improved by using different flow rates and oven temperatures.
- Each component leaves the column at a different time and is detected as it leaves the column.
- The time taken for a component to leave the column is called the **retention time**:

Retention time and gas chromatography:

- Is the time taken for a component to pass from inlet to detector.
- As temperature is controlled, the retention times are more precise and can be used to identify a component.
- Known compounds will have known retention times at the same temperature, carrier gas and stationary phase.

A gas chromatogram:



Area under each peak

- The area under each peak (component) is equivalent to the amount of that component in the sample:
- The relative concentrations of each component can be estimated by comparing peak areas.
- It is used to find the alcohol level in blood.

Limitations of gas chromatography:

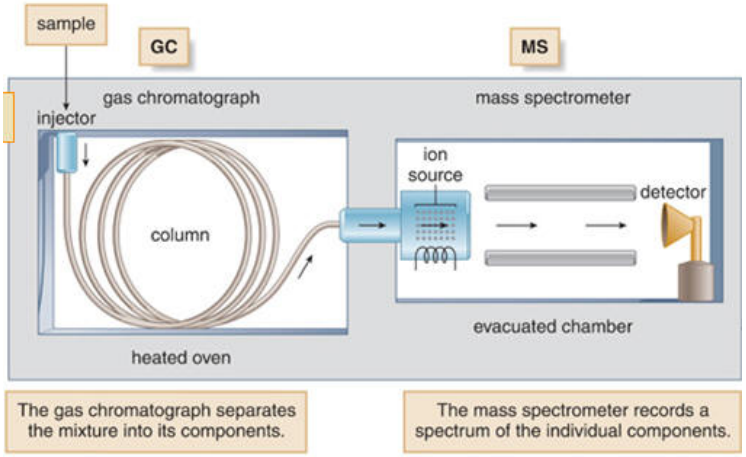
- Thousands of chemicals have similar retention times, peak shapes. This means that most compounds cannot be positively identified.
- Not all substances can be separated. Some substances can 'hide' under others. This can give a higher concentration of the other.
- Unknown compounds have no reference retention times. Analysts need to know what is expected.
- Due to the limitations, gas chromatography is usually used in conjunction with spectroscopy.

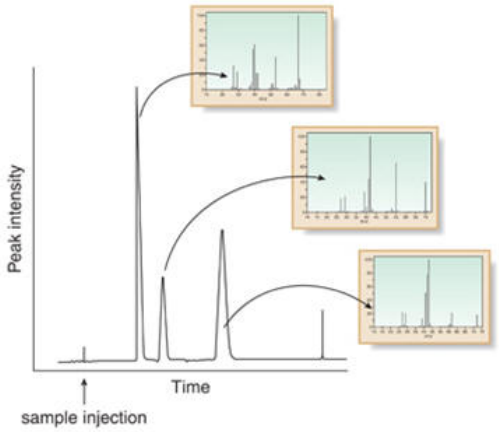
Gas Chromatography - Mass Spectroscopy - GC-MS

Combining gas chromatography with mass spectroscopy:

- This is 2 techniques combined to provide a powerful analysis tool.

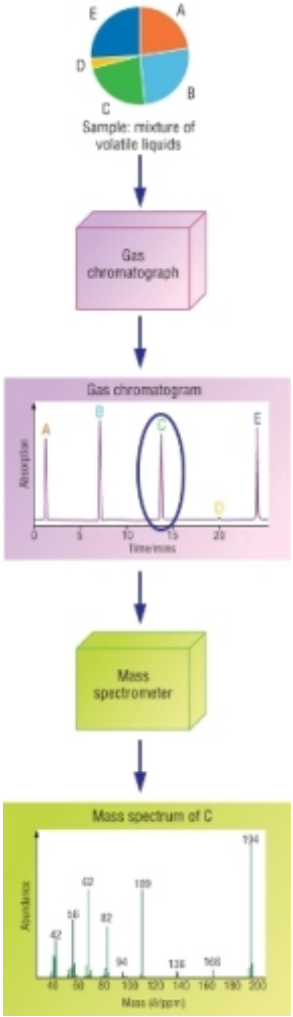
Gas Chromatography, GC	**Separates components**
Mass Spectroscopy, MS	**Gives detailed structural information**





- Each component is separated using GC then analysed using MS.
- Mass spectroscopy is much accurate than retention times as the mass spectra is unique to a compound – like a fingerprint.
- The mass spectra can be compared to a spectral database.

Summary:



Uses for GC-MS

1) Forensics - scenes of crime

2) Environmental analysis - air pollutants, waste water, pesticides in food.

3) Airport security - explosives in luggage / airport security

4) Space probes - planetary atmospheres