

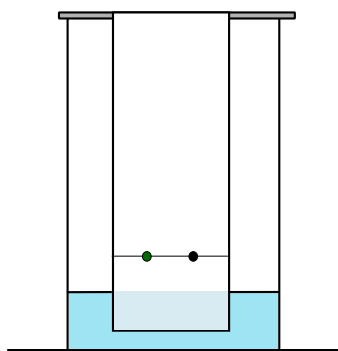
## CHROMATOGRAPHY

*General* Chromatography can be used to separate and analyse small amounts of mixtures. Methods involve a **stationary phase** and a **mobile phase**.

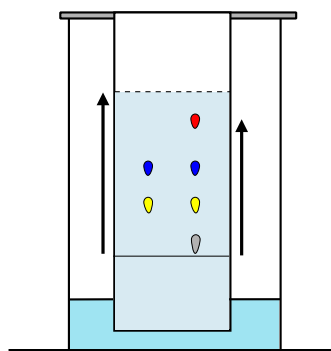
<i>type of chromatography</i>	<i>stationary phase</i>	<i>mobile phase</i>
<b>paper</b>	solid (filter paper)	liquid
<b>thin layer (tlc)</b>	solid (silica)	liquid
<b>column</b>	solid (silica)	liquid
<b>high pressure liquid (hplc)</b>	solid (silica)	liquid
<b>gas liquid (glc)</b>	solid or liquid	gas

### PAPER CHROMATOGRAPHY

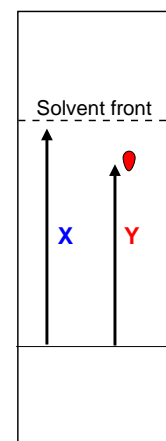
*How it works*



Place small a spot of the mixture to be analysed (and any possible component for comparison purposes) on the paper. Dip the paper in the solvent.



Allow the solvent to rise up the paper. Each component dissolves in the solvent. Those which are more soluble travel further up the paper.



Finished chromatogram

<b>Stationary phase</b>	chromatography paper
<b>Mobile phase</b>	suitable solvent (water, ethanol or other organic solvent)
<b>Separation</b>	As the solvent moves up the paper it dissolves the components and moves them up the paper. The more soluble a component is the further it moves.

*R<sub>f</sub> value* Under similar conditions, a component should always travel at the same speed. Its identity can be found by comparing the distance it moves relative to the solvent.

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

Comparison can be a problem if

- substances have similar  $R_f$  values
- there is nothing to compare it with

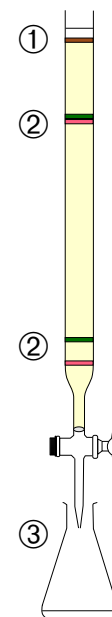
## THIN LAYER CHROMATOGRAPHY

<i>How it works</i>	<b>Stationary phase</b>	silica mounted on a glass plate
	<b>Mobile phase</b>	suitable organic solvent
	<b>Separation</b>	similar technique to paper chromatography
	<b>Limitations</b>	similar to paper chromatography

## COLUMN CHROMATOGRAPHY

<i>How it works</i>	<b>Stationary phase</b>	silica
	<b>Mobile phase</b>	suitable organic solvent
	<b>Separation</b>	components interact with the stationary phase to different extents

- Method*
- a chromatography column is filled with solvent and silica
  - drops of the mixture are placed on top of the silica - ①
  - the tap is opened to allow the solvent to flow out
  - additional solvent is added on top to replace that leaving
  - the components travel through at different rates and separate - ②
  - batches of solvent are collected at intervals - ③
  - the solvent in each batch is evaporated to obtain components



## HIGH PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

*How it works* A better form of column chromatography. Instead of draining down through the stationary phase, the solvent is forced through under high pressure.

- it is fast
- the path is short - *usually under 30cm*
- it gives better separation

<b>Stationary phase</b>	silica
<b>Mobile phase</b>	suitable solvent
<b>Separation</b>	similar to column chromatography

- Method*
- a sample is injected
  - solvent and sample are pushed through under pressure
  - different compounds have different retention times
  - output can be detected by compounds absorbing UV light
  - can be connected to a mass spectrometer

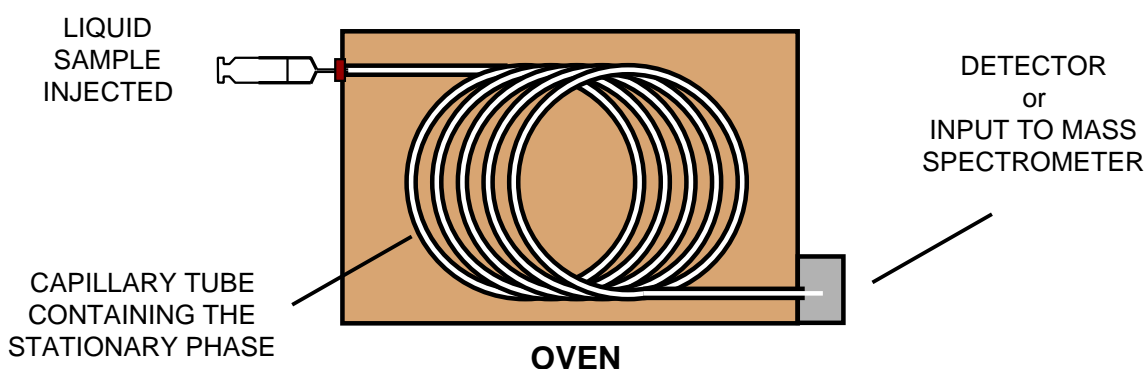
## GAS LIQUID CHROMATOGRAPHY

*How it works*

<b>Stationary phase</b>	liquid adsorbed on an inert solid / solid on a solid support
<b>Mobile phase</b>	gas

*Method*

- a very small amount of a sample is injected into the machine using a syringe
- the injector is contained in an oven
- the sample boils and is carried into a long thin column by an inert carrier gas
- the column contains a liquid stationary phase, adsorbed onto an inert solid
- the time a compound takes to travel through the tube will depend on how much time is spent moving with the gas rather than being attached to the liquid.



*Retention time*

**The time taken for a compound to travel through the column to the detector.**

It is measured from the time the sample is injected to the time its peak shows maximum height.

For a particular compound, the retention time depends on...

- |                                  |                           |                              |
|----------------------------------|---------------------------|------------------------------|
| • boiling point                  | <b>high boiling point</b> | <b>= long retention time</b> |
| • solubility in the liquid phase | <b>greater solubility</b> | <b>= long retention time</b> |

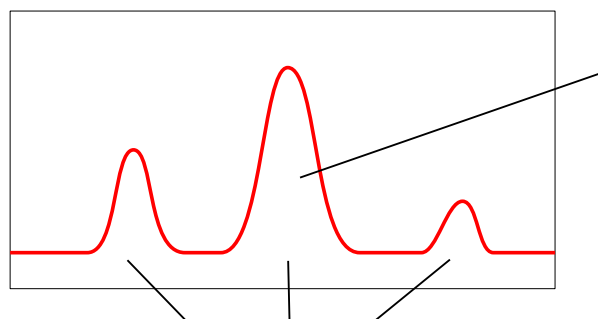
*Detection*

- there are several ways to detect components
- most involve destruction of the sample
- one method is an **FID - flame ionisation detector**

*The FID*

- as a component exits the capillary tube, it is burned in a hydrogen flame
- ions are produced in the flame
- a detector produces an electric current
- the greater the amount of a component, the larger the current produced
- the current can be represented by a chromatogram
- as the component is destroyed, an FID is not used in GCMS

- Interpretation**
- each compound in the mixture will produce a peak
  - the areas under the peaks are proportional to the amount of each compound
  - retention times can be used to identify compounds - *found out by putting known compounds through the system under similar conditions*



The area under a peak is proportional to the amount present.

Because each compound responds differently, the machine is calibrated beforehand to show the actual amount.

Each component has a different retention time.

## GAS CHROMATOGRAPHY - MASS SPECTROMETRY (GCMS)

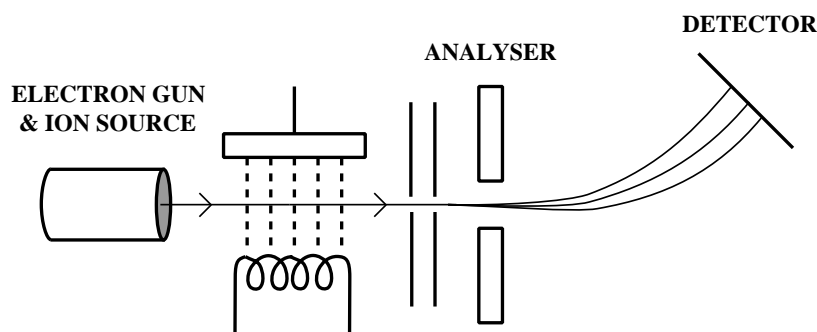
**Process** When a peak is detected, some of the component is sent to a mass spectrometer

A mass spectrometer has three main parts...

- Ioniser**
- the sample is bombarded with electrons and ionised
  - a positive molecular ion is formed
  - the molecular ion can break up into smaller ions
  - positive ions are accelerated towards the analyser

- Analyser**
- positive ions are separated according to their mass/charge ratio
  - the greater the mass/charge ratio, the smaller the deflection

- Detector**
- records the identity and abundance of each ion
  - the mass spectrum obtained is unique to a particular compound
  - the final peak is the molecular ion and gives the molecular mass



For more information, consult the notes on 'Mass Spectrometry'.