Chromatography

* Can be BOTH qualitative and quantitative
* Encompasses a variety of techniques, each with a specialized purpose
* Not all methods will do both qualitative and quantitative analysis
* Not all techniques will allow the recovery of sample material
* A term which describes a range of techniques which are used to separate, identify and quantify the components of a mixture.
* All chromatographic techniques have two ‘phases’, a **stationary** phase and a **mobile** phase.
* The mobile phase moves over or through the stationary phase, carrying with it the various components of the mixture.
* The components of the mixture are separated in the stationary phase, where the different components ‘**adsorb’** (stick) to the stationary phase, and then **‘desorb’** back into the mobile phase.
* The stronger the component adsorbs to the stationery phase, the longer it takes for that component to move pass through stationery phase.
* Components undergo continuous **adsorption** and **desorption**.

The rate of movement of each compound is determined by two things:  
- How strongly it adsorbs to the stationery phase  
- How easily it dissolves in the mobile phase

* The choice of the correct stationery phase and mobile phase is vital in order to achieve the correct **resolution.**
* **Resolution** describes how well the components in a mixture have been separated.  
  The better the resolution is, the more helpful it is in determining what the components of the mixture are.

Read the following pages from you text books, and answer the questions below for each of these techniques (paper chromatography, thin layer chromatography, HPLC, GC):

1. Identify both the mobile and stationary phases used in this technique.
2. Is this technique useful for qualitative or quantitative analysis, or both?
3. Is it cheap and easy or more expensive and difficult?
4. Can the sample be retrieved at the end of the test?
5. What chemical compounds is this technique suitable to analyse?
6. What is the sensitivity of this technique (how little concentrations can it detect)?
7. What is the readout (i.e. is it digital, or do you have to interpret it)
8. For GC and HPLC, learn how standards are made, and then how they are used to find the concentration of something in an unknown solution.
9. Rachael has done all this already, so ask her for help.

**TLC**

* Uses Fused silica or Alumia as the stationary phase, and some kind of solvent as the mobile phase.
* The mobile phase is chosen so as to give the max reolution (seperation between spots)
* Draw the noral set up on the board
* It is qualitative but only is if the sample these is something to use as a reference
* **Origin** is where the spots are located to start with, **solvent front** is where the folvent moves to**,**
* The Rf can be calculated by dividing the distance moved by the centre of the spot by the total distance moved.
* The spots will either need to be stained or run under UV light normally to be seen.
* Disadvantages: Uses a lot of the sample, cant be recovered, is not quantitive, and is only qualitative when there is a reference to compare with.

**Colum Chromatography**

* A long glass tube (like a big burette) is filled with silica (with cotton wool in the end.
* This is the stationry phase.
* The mobile phase is chosen to give the maxium resolution, and is often a very polar solvent such as diethyl ether.
* ALL of the sample is placed into the collum (dissolved in the solvent) and the mobile phase carries it through, but with the components of the sample moving at different rates as they; have different solubilities in the solvent, and adsorb more or less strongly to the stationry phase.
* The solvent drips out the bottom, into test tubes (this is called the eluent), and each test tube is tested with TLC to see whether it contains any of the sample. This can then be Rotovaped to get the sample back
* Advantages: All of the sample is recovered, and separated, disadvantages, takes time and a lot of solvent to do.

**HPLC**

* Uses a similar method to what we just described, by instead uses a very thin, and long column, and also uses much smaller particles as a stationery phase. This allows for more frequent adsorption and desorption
* Imagine how must resistance this would make!! Therefore we use a super high pressure solvent so it pushes though the stationery phase.
* The detector works like this: Most organic compounds absorb UV light, so a beam of UV is passed through the eluent, and when a reduced signal is picked up the detector records this..
* Draw a chromatogram on the board
* Explain both qualitative and quantitative
* Retention times are chartacteristic under those certain condirtions
* This is a very sensitive technique (talk about running standards) and will be both quant and qual
* Unfortunatle the sample is lost int the process.

**GC**

* Can detect 10-12 of a gram of a compound. Very sensitive
* Uses a calibration curve
* Limited to compounds which can be readily vapourised (MW < 300)
* Draw a diagram on the board
* Mobile phase is a carrier gas like nitrogen (something which wont burn in the process)
* Collum is 2-3m in total, and 4mm thick
* Porus solid coated in liquid hydrocarbon as the stationery phase.
* **The least soluable are swept out first**
* **The detector is a flame ionisation detector**
* This works by hot flame to ionise the ocmpounds, the ions are then attracted to electrodes causing a current to flow. The amount of current flowing is relative to the amount of compound.
* Do a few problems