1. High-Powered Liquid Chromatography (HPLC)

HPLC CHROMATOGRAPHY

- A scientific method used to separate components from their materials.
- Separation of analytes using polarity properties. Normal phase HPLC involves a polar stationary phase and a non-polar mobile phase. Polar analyte samples are retained on the polar surface of the stationary phase, which is selective against less polar materials.

STATIONARY PHASE

- An immiscible medium made from a solid or liquid, where the analyte passes through. E.g. silica.

MOBILE PHASE

- A medium made from a gas, liquid or supercritical fluid (any substance at a temperature and pressure above its critical point – distinct liquid and gas phases do not exist). E.g. hexane, chloroform, diethyl ether.
- It is selective, penetrates the stationary phase normally.
- Samples are transported or dissolved via the mobile phase.
- Normal phase (non-polar solvents).
- Reversed phase (mixtures of water, ethanol, methanol or acetonitrile). Reverse phase consists of neutral packing material, mobile and stationary phase.

COLUMN CHROMATOGRAPHY

- Involves a vertical cylinder column where components are separated and eluted through the other end of the column.
- The solvent or eluent (mobile phase) is inserted into the column and penetrates the stationary phase (column packing made from silica gel commonly).
- The solute travels through the column and its components get separated depending on how fast the flow rate is, the easier the particles are separately. This also depends on the interactions of the solute with the stationary phase. The longer the neutral solute interacts with the stationary phase, the longer it is eluted from the column.
- Changing the mobile phase can assist the solute to come out faster.

ADSORPTION CHROMATOGRAPHY

Stationary phase is solid and mobile phase is typically liquid or gas. The mobile phase carries the mixtures of substances in the sample through the stationary phase (adsorptive material). The separation process involves the adsorption of solutes onto the surface of the solid particles (stationary phase). E.g. normal phase chromatography

PARTITION CHROMATOGRAPHY

Thin film on a solid support makes up the stationary phase. The mobile phase is either a liquid or gas. The separation depends on the equilibration of the solute and the stationary and mobile phase. E.g. GC, reversed LC.

ION-EXCHANGE CHROMATOGRAPHY

Involves an ionically charged stationary phase and oppositely charged analyte sample ions. This method is ideal for ionic samples. The stronger the charge on the sample, the better interaction between the ionic surface and the analyte ionic sample. The mobile phase is of an aqueous buffer – pH and ionic strength are important parameters or independent variables that can be changed to manipulate the elution time.

SIZE-EXCLUSION CHROMATOGRAPHY

The column is of pore sized materials and the separation is based on molecular size. The larger molecules will elute first and rapidly, whereas the smaller molecules have longer interactions with the pore sized materials on the surface of the column. This produces a longer retention time of the smaller molecules.