Chromatography

- High performance liquid chromatography (HPLC)

Retention time depends on:
1. the nature of the solvent
2. the pressure used
3. the temperature inside the column

Substances are separated due to different retention times in the column.

Gas Chromatography

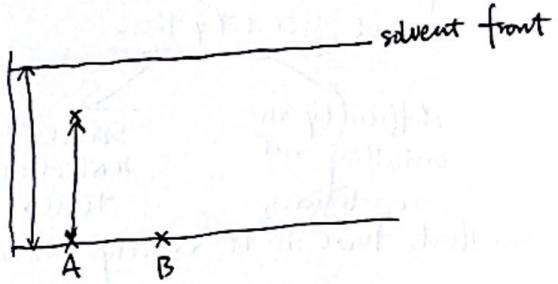
The stationary phase: solid or liquid coated on the inside of the tube.

The mobile phase: inert carrier gas (do not react) (N_2 or He usually)

Gases move in different speeds, depending on how strongly they are attracted to the stationary phase.

- The weaker attractions, the faster they move.
  The shorter the retention time.

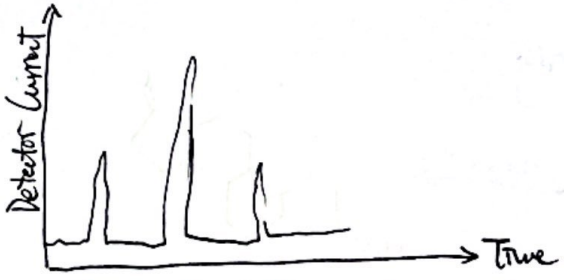
Chromatogram of Amino Acid



$$Rf = \frac{D \text{ amino acid}}{D \text{ solvent}}$$

- Amino acids have different Rf values because they have different solubility in both stationary phase and mobile phase.
- Ninhydrin is used to locate the amino acid spots. (toxic)

Chromatogram from GC.



Area (height) under peaks represents the concentration.

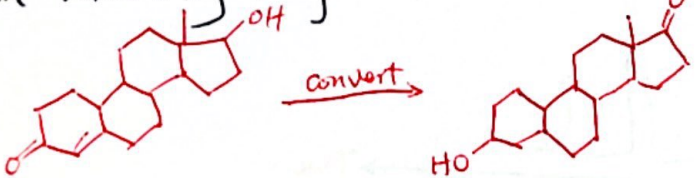
Limitation

- HPLC and GC can separate small quantities of substances but cannot identify them
  - difficulty in controlling all conditions
  - Same retention times
- Chromatography methods have to be exactly correct:
  1. forensic
  2. detecting drugs, pollutant, explosive items

GC – MS

1. Inject mixture
2. At a time, each component enters MS (due to different retention times)
3. m/z values and relative abundance are compared with known data.

Problem with drug testing



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