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CHROMATOGRAPHY

Type	Stationary Phase	Mobile Phase
Column	Powder (SiO_2 or Al_2O_3)	solvent
Paper	Absorbent paper	solvent
TLC	Powder (SiO_2 or Al_2O_3) on glass/plastic sheet	solvent
Gas	Powder packed in tube or liquid coated on tube lining	Inert gas (e.g. He, Ar, N_2)

In ALL chromatography

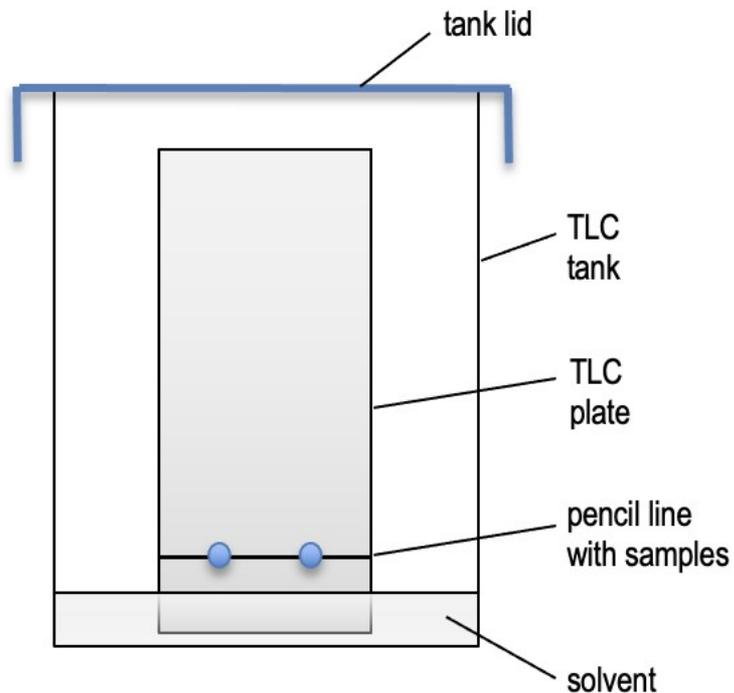
Substances separated according to relative affinity (attraction) to stationary and mobile phase.

If stronger affinity for mobile phase, then move quickly

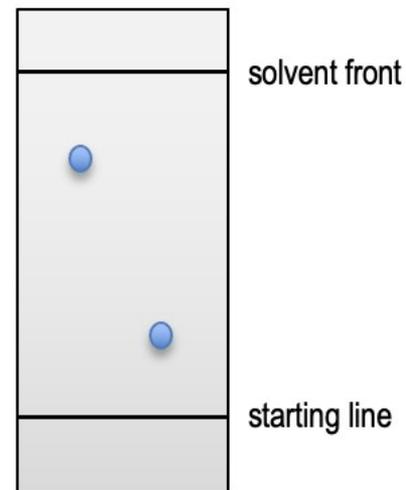
If stronger affinity for stationary phase, the move slowly

TLC – Thin layer chromatography

SET-UP



AFTERWARDS



TLC – Thin layer chromatography



TLC – Thin layer chromatography

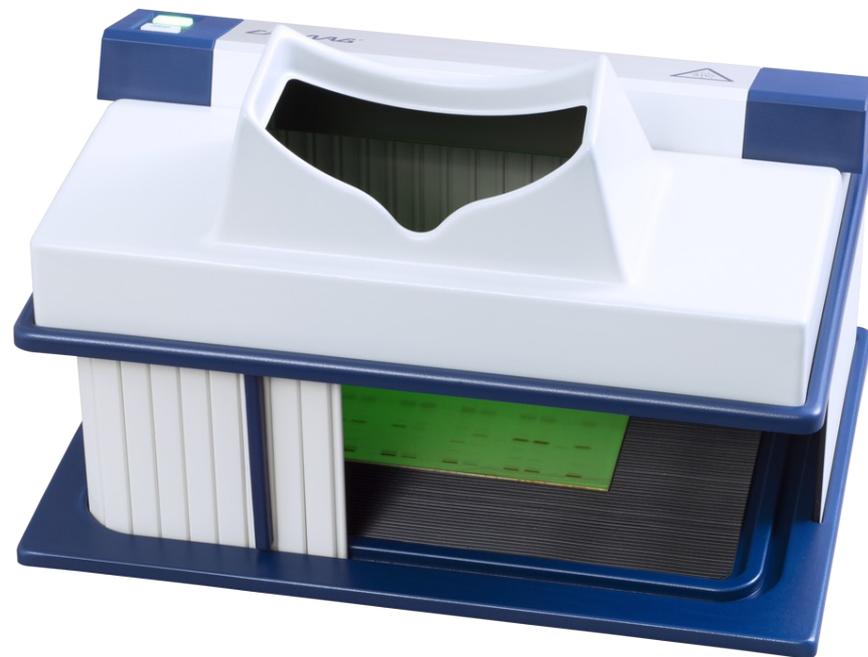
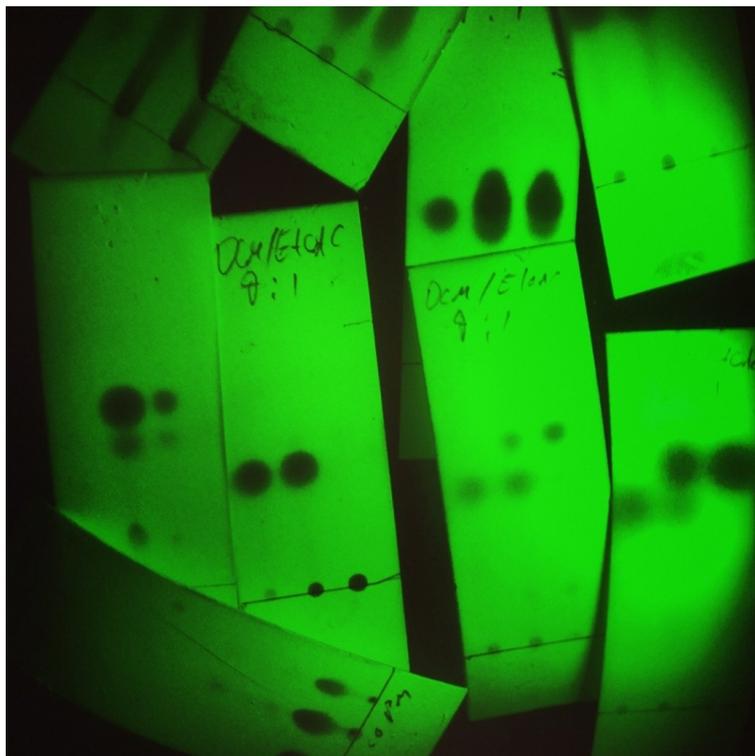
- TLC plates are plastic (or glass) sheets coated in SiO_2 (or Al_2O_3) powder.
- The samples are placed onto the powder as small dots on a pencil line.
- The plate is stood in a solvent (sometimes called eluent) in a tank / jar. The samples should be above the height of the solvent.
- The tank / jar should have a lid
 - to prevent solvent evaporating from the tank / jar
 - to maintain a constant atmosphere that is saturated with solvent vapour
 - to prevent solvent evaporating off the surface of the plate as it rises up
- The plate should not be moved during the experiment.
- The solvent soaks up the plate – the plate is removed once the solvent nears the top and the level that the solvent reaches marked with a pencil.

TLC – Thin layer chromatography

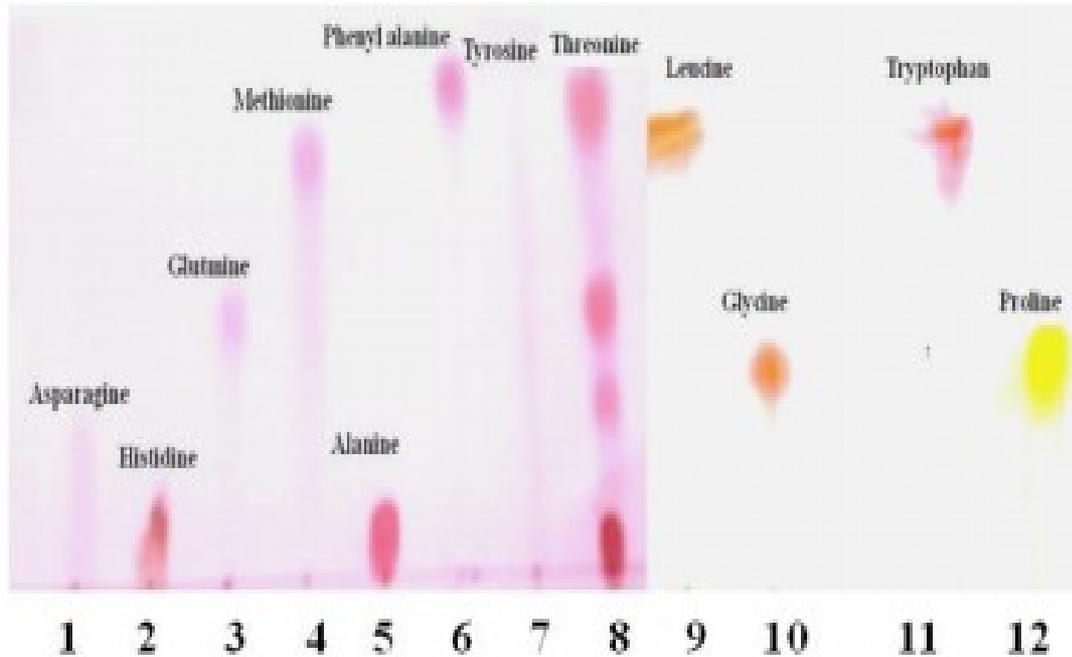
- The spots on the plate are usually colourless and cannot be seen with the naked eye.
- There are two common ways to make the spots visible:
 - view the plate under a UV light and mark the positions of the spots with a pencil (for all samples)
 - stain the spots with ninhydrin (specifically for amino acids)

TLC – Thin layer chromatography

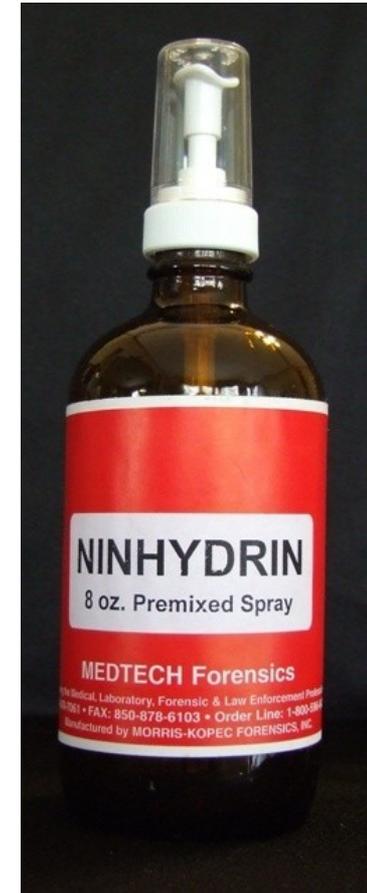
**For colourless samples:
View results under UV light**



TLC – Thin layer chromatography



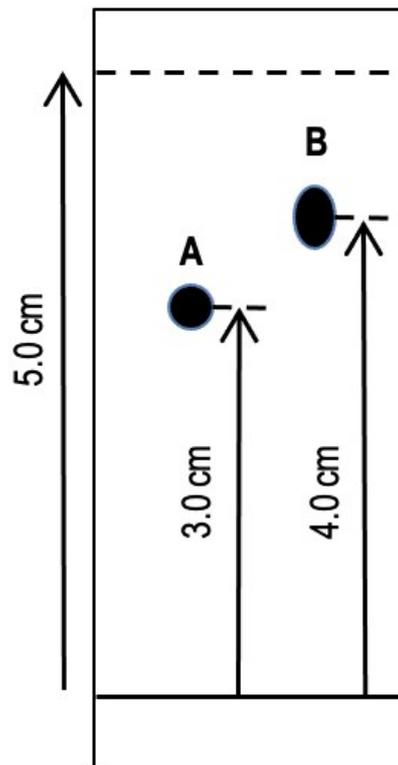
**Amino acids
can be stained using ninhydrin**



TLC – Thin layer chromatography

$$R_f = \frac{\text{distance moved by spot}}{\text{distance moved by solvent}}$$

- we always measure the distance travelled from the start line (so that we are measuring the distances travelled by solvent and sample in the same time period).
- we measure to the centre of the spots



for substance **A**

$$R_f = \frac{3.0}{5.0} = 0.60$$

for substance **B**

$$R_f = \frac{4.0}{5.0} = 0.80$$

TLC – Thin layer chromatography

- How fast/far each compound moves depends on the relative attraction (affinity) of each compound to the mobile and the stationary phases.
- This is a rough guide and there are many deviations from this to very specific interactions between compounds, the solvent(s) and the silica.

	Solvent is more polar than SiO ₂	Solvent is less polar than SiO ₂
Which substances move fastest (greatest R _f values)	Polar molecules (more attracted to the solvent)	Non-polar molecules (more attracted to the solvent)
Which substances move slower (lowest R _f values)	Non-polar molecules (more attracted to the SiO ₂)	Non-polar molecules (more attracted to the SiO ₂)

TLC – Thin layer chromatography

**Rough guide to
relative polarity**

most polar



water

alcohols

ketones (e.g. propanone)

esters (e.g. ethyl ethanoate)

SiO₂ on plate

halogenoalkanes (e.g. dichloromethane)

alkanes (e.g. hexane)

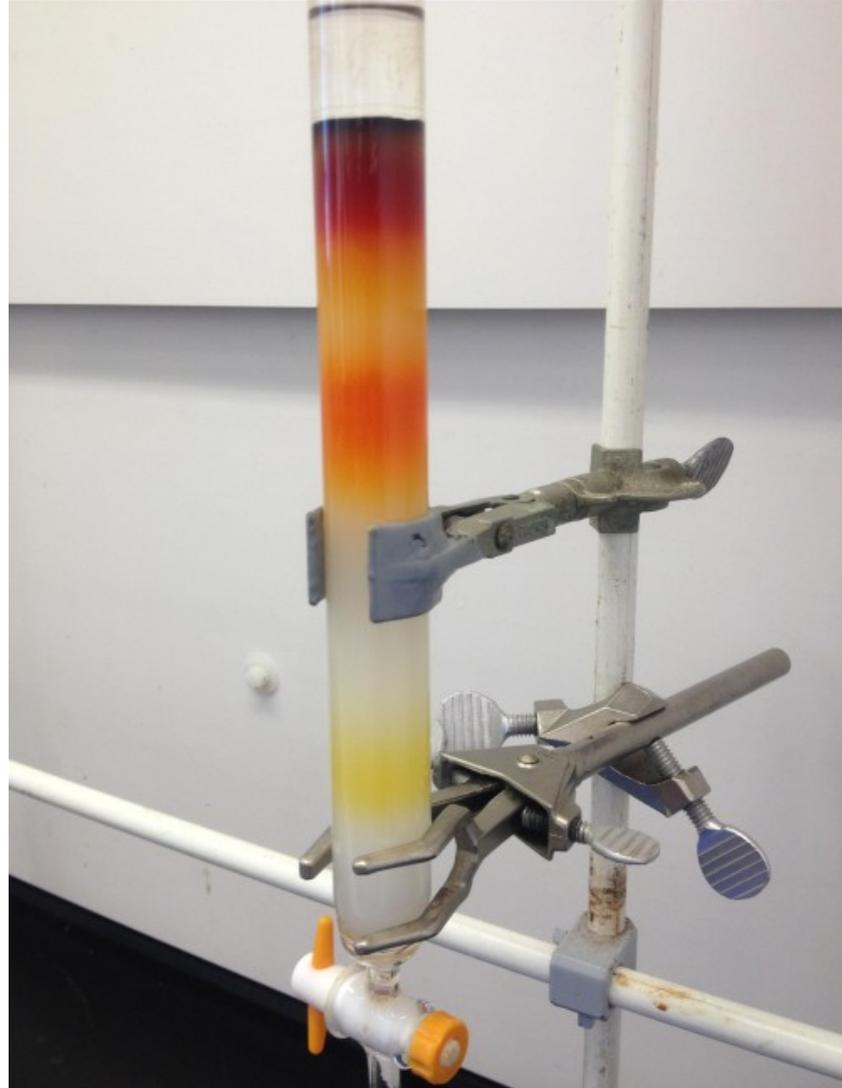
least polar

Column chromatography

“Large scale TLC”

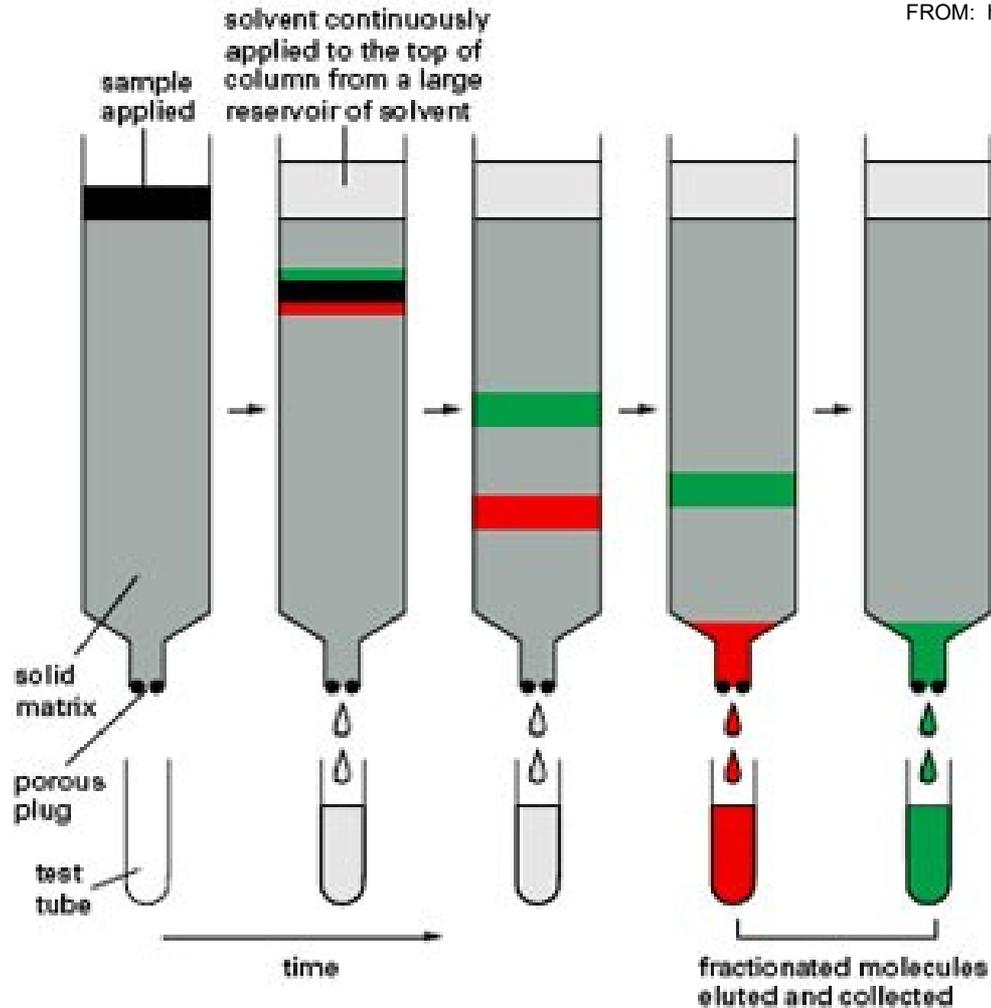
**Stationary:
Powder (SiO_2 or Al_2O_3)**

**Mobile:
Solvent**

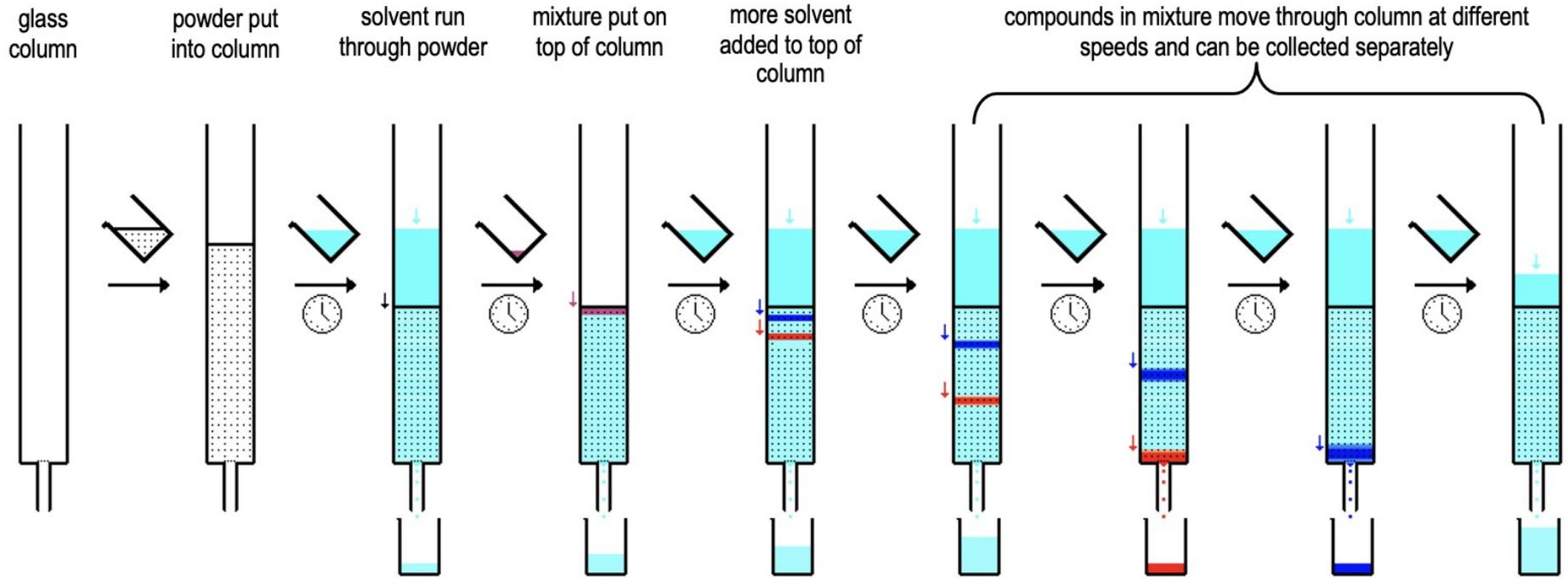


Column chromatography

FROM: http://www.mun.ca/biology/scarr/4241_RCMchromatogrpahy.jpg

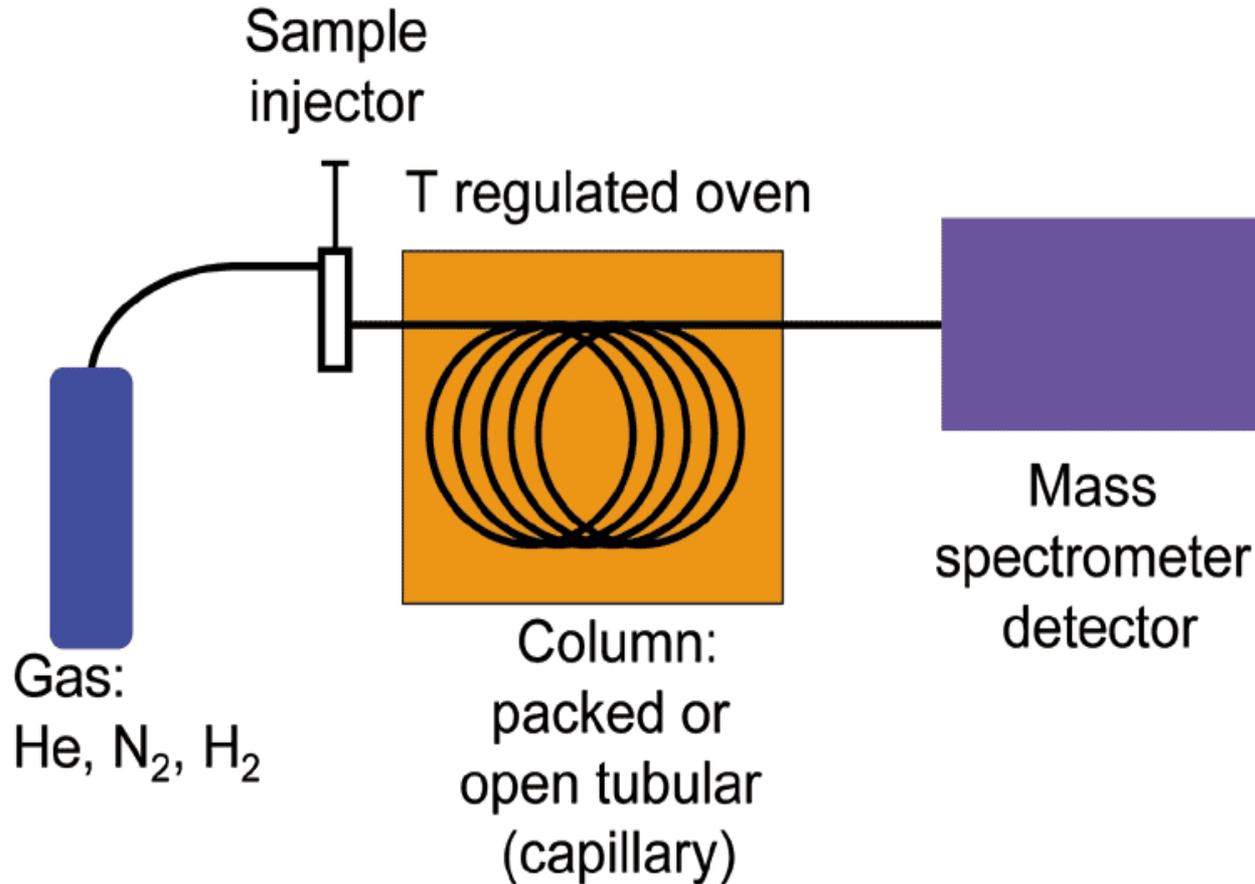


Column chromatography



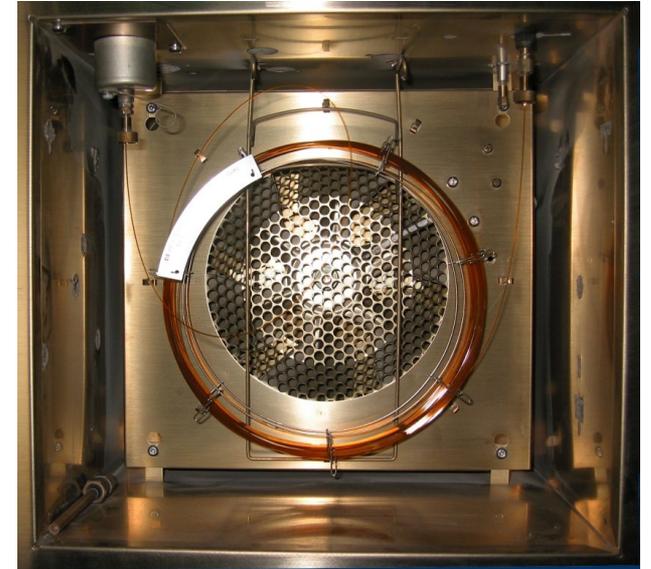
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Gas / Gas-liquid chromatography



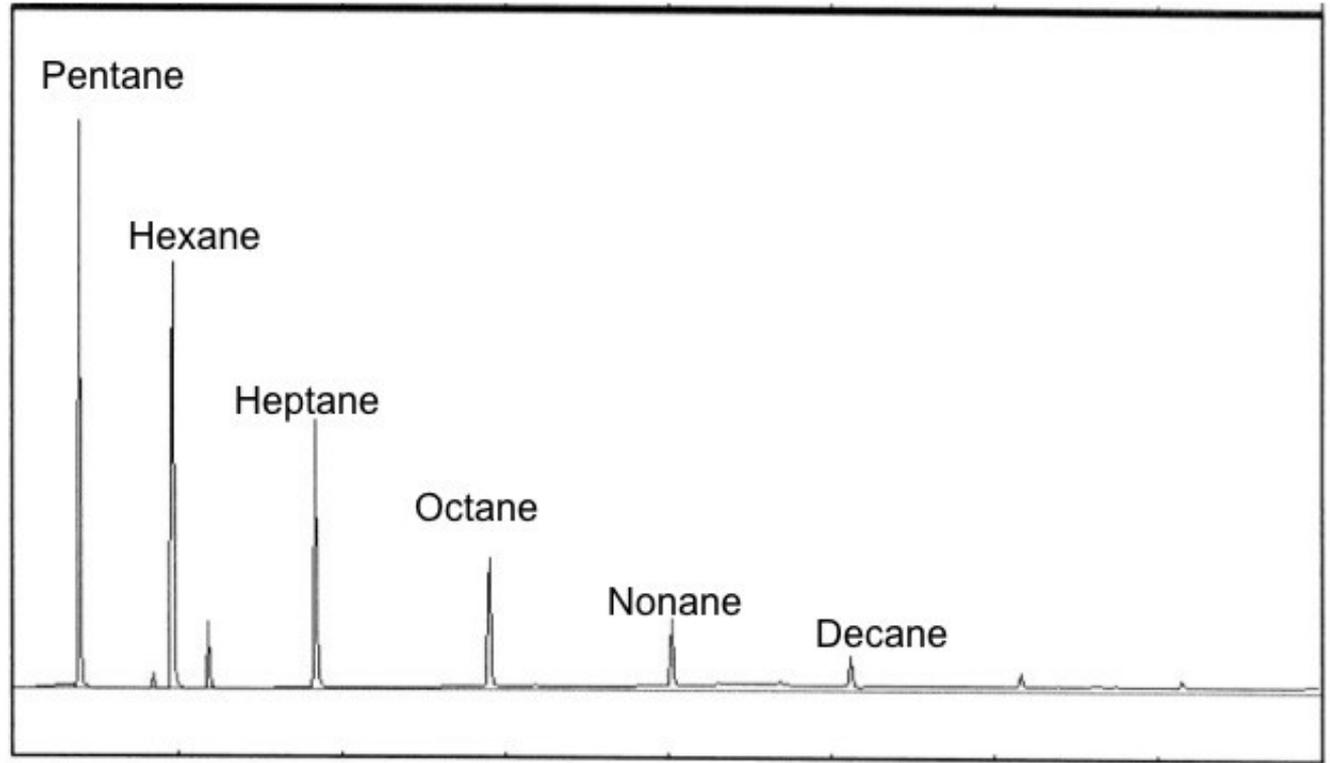
Gas / Gas-liquid chromatography

- Long coiled tube packed with powder / coated with liquid
- Inert gas passes through tube
- **Retention time** = time to travel through tube
- Samples often analysed by mass spectrometer as they leave tube



Gas / Gas-liquid chromatography

Gas chromatogram (e.g. of a fraction of crude oil)



Retention time (min)