# Chromatographic Performance Parameters

The successful chromatographic separation of analytes in a mixture depends upon the

- **Selection of the most appropriate process** of chromatography
- **Optimization of the experimental conditions** associated with the separation.

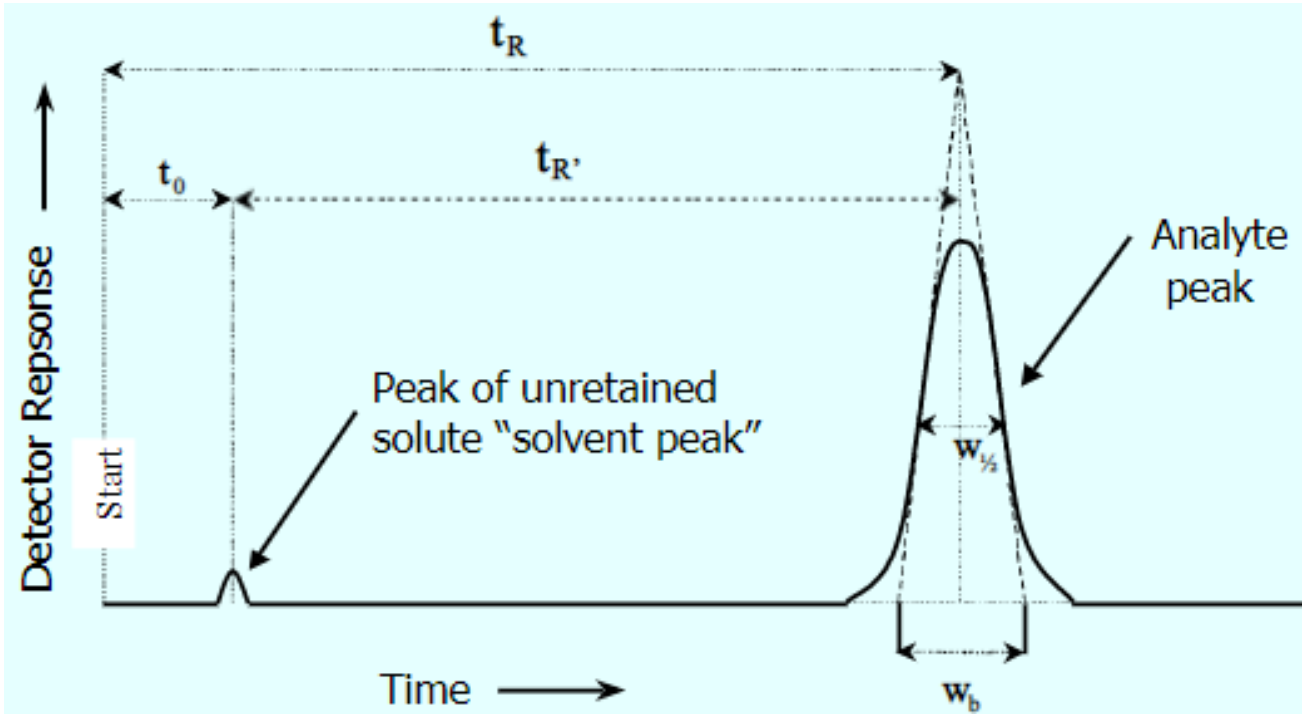
# CHROMATOGRAM

A chromatogram is a pictorial record of the detector response as a function of elution volume or retention time. It consists of a series of peaks or bands.



- Chromatogram tells us about:

1. **Qualitative data-** what is present? Through retention time.
2. **Quantitative data-** how much analyte is present?– Area under the peak



# CHROMATOGRAPHY CAN BE DIVIDED ON THE BASIS OF THE DEVELOPMENT AND ELUTION MODES:



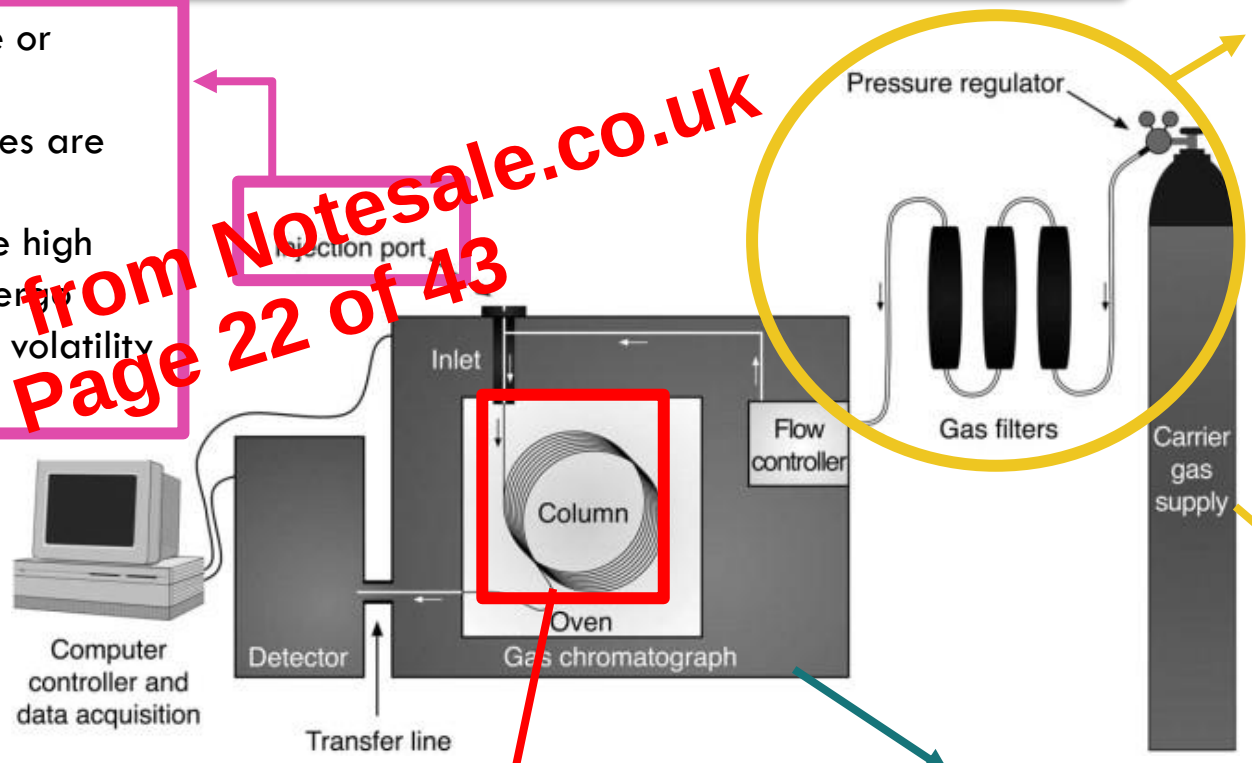
## Zonal development:

Separation on the **basis of their distribution coefficient** between the two phases.
Sample is dissolved in solvent which is **allowed to flow continuously** over stationary phase leading to Progressive separation and elution of sample.

## Displacement/ affinity development:

Analytes separated **on the basis of their affinity for the stationary phase**.
Analytes binds to stationary phase with a strength determined by their affinity which are **then selectively eluted by using a mobile phase that has higher affinity for stationary phase than them.**

Solvent used: Acetone, Hexane or methanol
Non polar or low polar analytes are directly inserted.
Polar analytes – needs to have high retention time or needs to undergo derivatisation to increase their volatility and distribution coefficient.

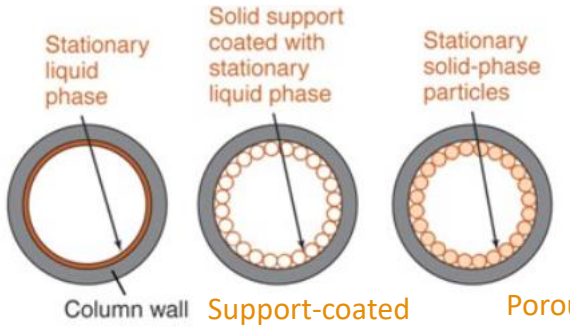



Removal of $O_2$, or hydrocarbons and needs to maintain a constant pressure and temperature

**Mobile phase:**
Inert gas used.
Nitrogen- conventional column
Helium or Argon in capillary columns.

Partition coefficient of analytes are sensitive to temperature.

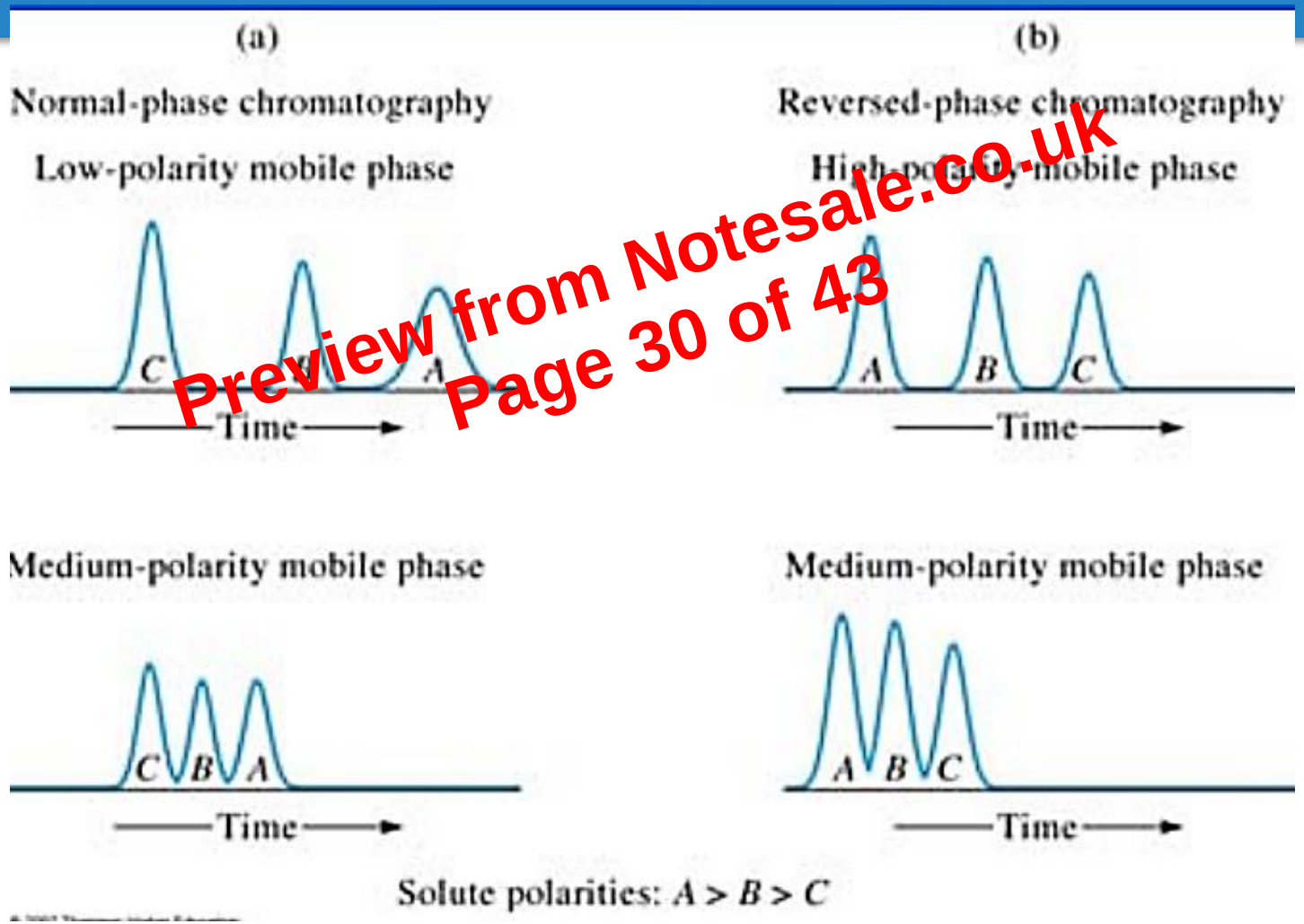
Can be conventional or capillary columns.
Capillary columns further divided into:



**Detectors:** Sensitivity of the detector system is sufficiently high and stable to respond to the low concentrations of each analyte in the eluate. Most commonly used detectors are:



| | |
|---|---|
| **Variable wavelength detectors** | based upon **ultraviolet–visible spectrophotometry** |
| **Scanning wavelength detectors:** | **record the complete absorption spectrum of each analyte, thus aiding identification.** -temporarily stop the eluent flow or by the use of diode array techniques that give 3D plot on VDU screen. |
| **Mass spectrometer detectors** | enable the analyte to be detected and its **structure determined** simultaneously. |
| **Refractive index detectors:** | rely on a change in the refractive index of the eluate as analytes emerge from the column. Are commonly used in the **analysis of carbohydrates.** |
| **Evaporative light-scattering detectors (ELSD):** | These rely on the **vaporization of the eluate, evaporation of the eluent** and the quantification of the analyte by **light scattering.** |
| **Fluorescence detector** | **UV detector** is used for identification. But only few analytes show fluorescence. |

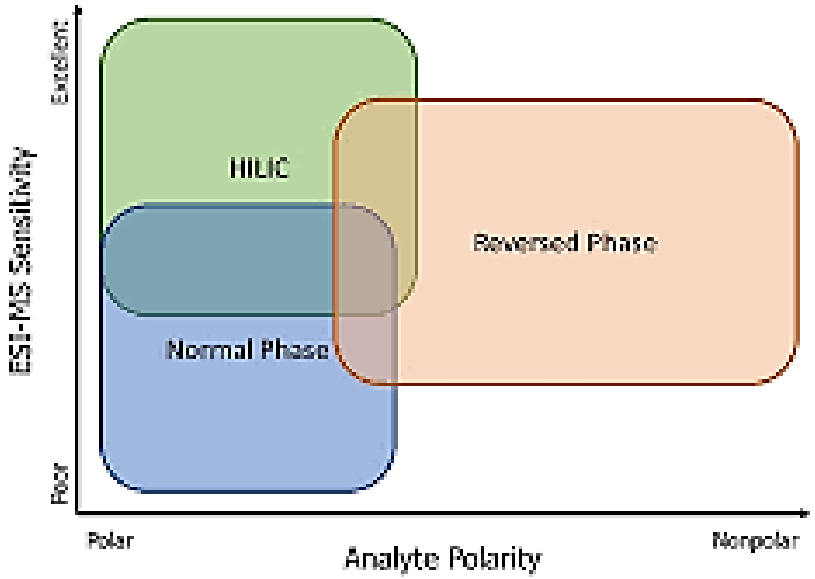



NORMAL PHASE

Polar stationary phase

REVERSE PHASE

Non Polar stationary phase



# WHAT TYPE OF CHROMATOGRAPHY DO I NEED?



**VOLATILE AND STABLE**

**Gas chromatography**

**NON VOLATILE**

**Liquid chromatography**

**Low Molecular weight**

**Surface techniques**

**Neutral**

**Adsorption chromatography**

**Partition chromatography**

**Ionic /charged**

**Ion exchange chromatography**

| +ve charge: | -ve charge: |
| --- | --- |
| Cation Exchange | Anion Exchange |

**High Molecular weight**

**Size exclusion chromatography**