HPLC

CHROMATOGRAPHY

Stationary phase may be solid (adsorption) or liquid (partition)

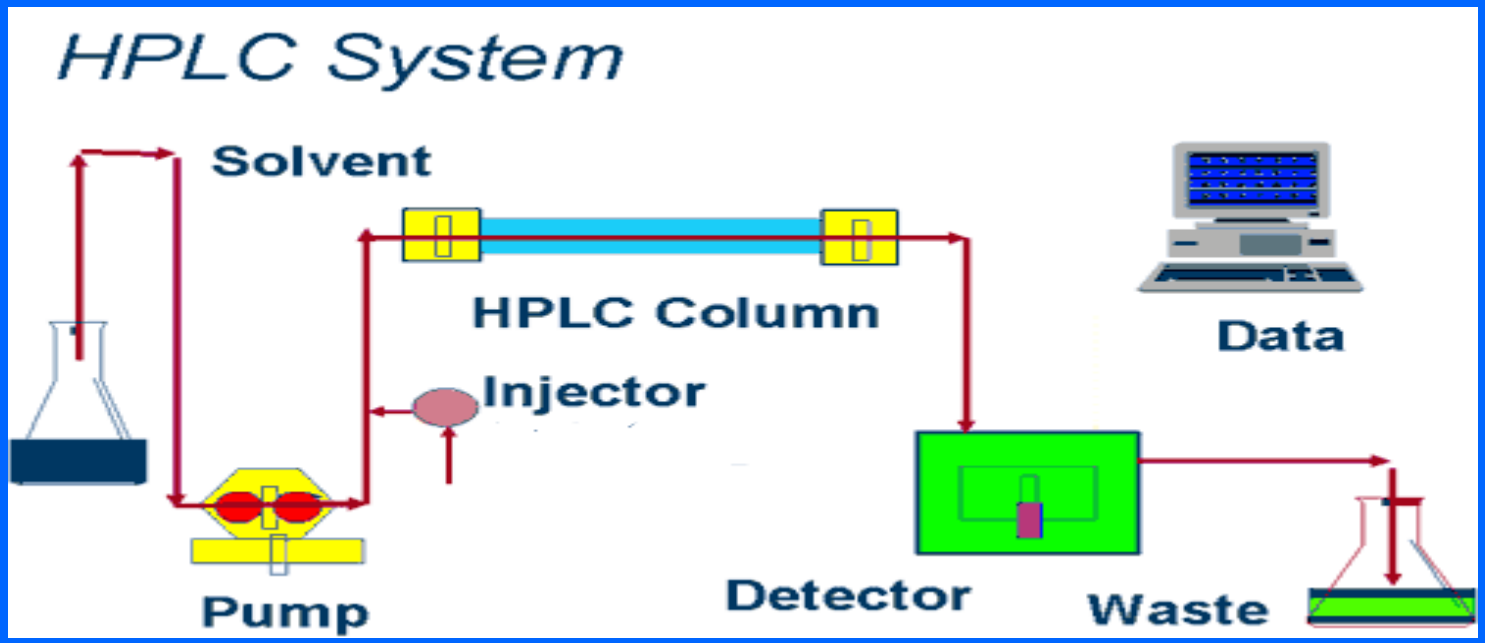
Mobile phase may be gas (GC) or liquid(LC)

High Performance Liquid Chromatography

HPLC principle

- it is a technique by which a mixture sample is separated into components for identification, quantification and purification of mixtures

Instrumentation



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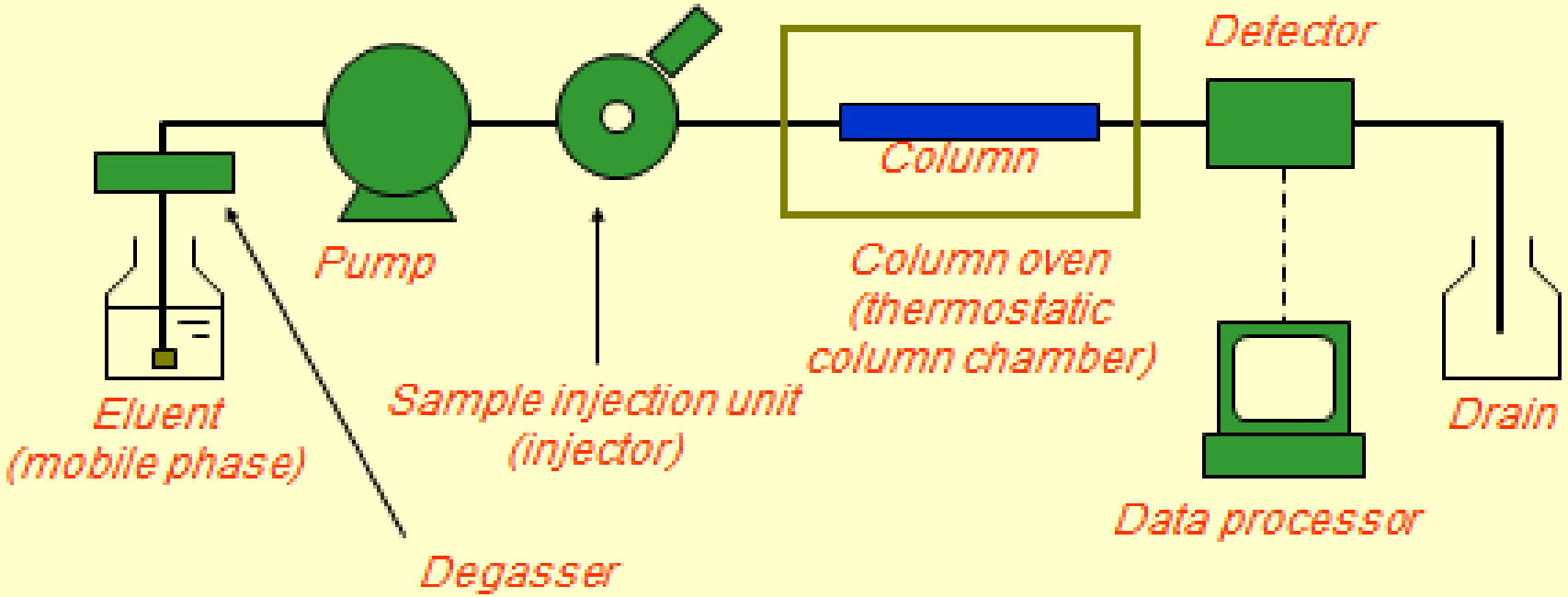
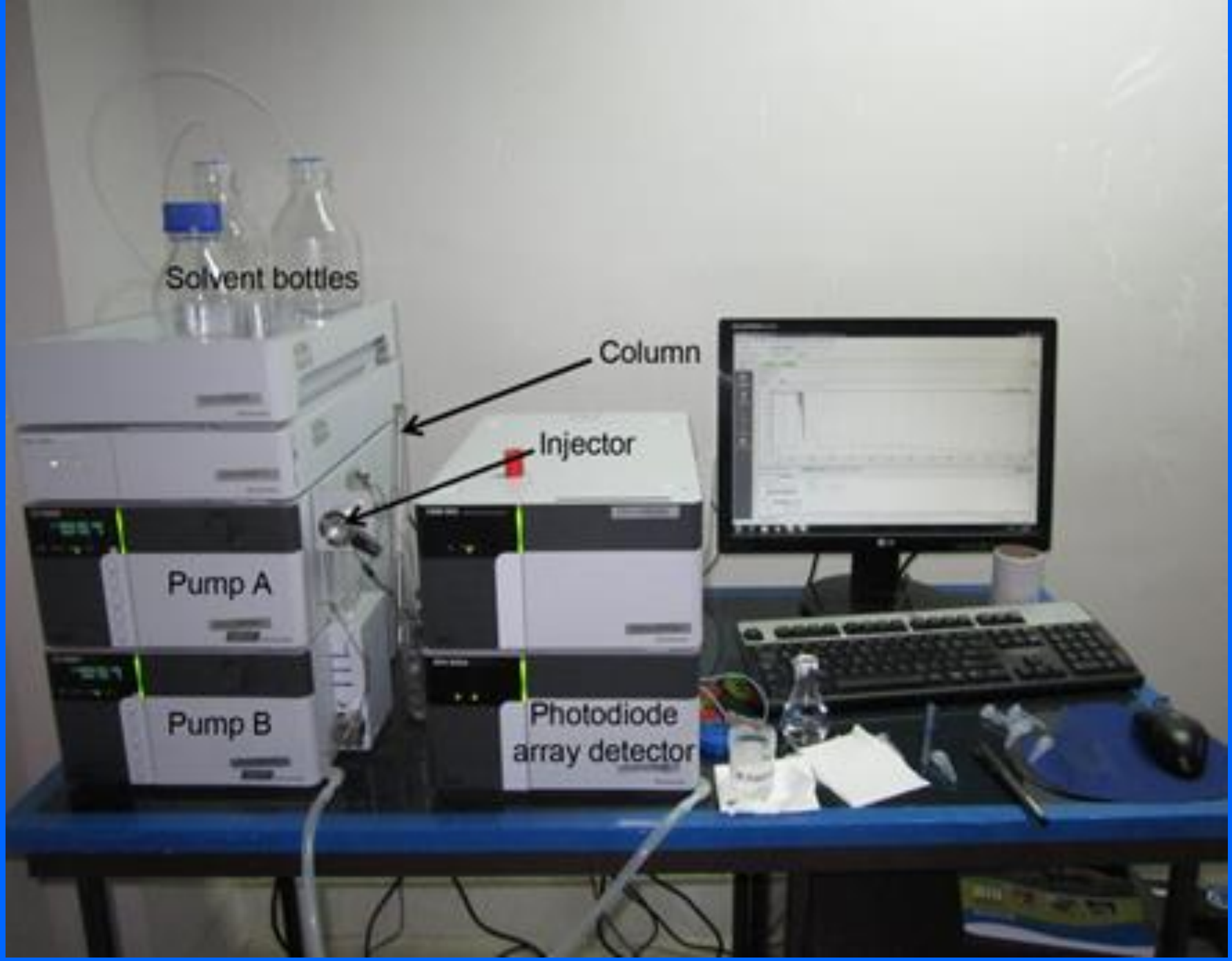
Flow Channel Diagram for High Performance Liquid Chromatograph




Solvent bottles
Column
Injector
Pump A
Pump B
Photodiode
array detector

- The heart of a HPLC system is the column.
- The column contains the particles that contains the stationary phase.
- The mobile phase is pumped through the column by a pump
- Solvents must be degassed to eliminate formation of bubbles .

HPLC column examples

1. Pump:

The role of the pumpis to force a liquid (mobile phase) through the liquid chromatograph at a specific flow rate

a pump can deliver a constant mobile phase composition (isocratic) which the m.ph composition remains unchanged during the analysis.

or (gradient) which the m.ph changed during the analysis..

Pump types:

- **Isocratic pump -** delivers constant mobile phase composition;
- solvent must be pre-mixed;
- lowest cost pump
- **Gradient pump -**delivers variable mobile phase composition;
- can be used to mix and deliver an isocratic mobile phase or a gradient mobile phase

2. Injector:

- The injector serves to introduce the liquid sample into the flow stream of the mobile phase.

May be auto-sampler or manual

There are a wide variety of stationary phases available for HPLC :

- **Normal Phase**.
  - Polar stationary phase and non-polar solvent.

  E.g. silica gel
- **Reverse Phase.**
  - Non-polar stationary phase and a polar solvent.

  E.g. silica gel -C18

- **ion exchange:**

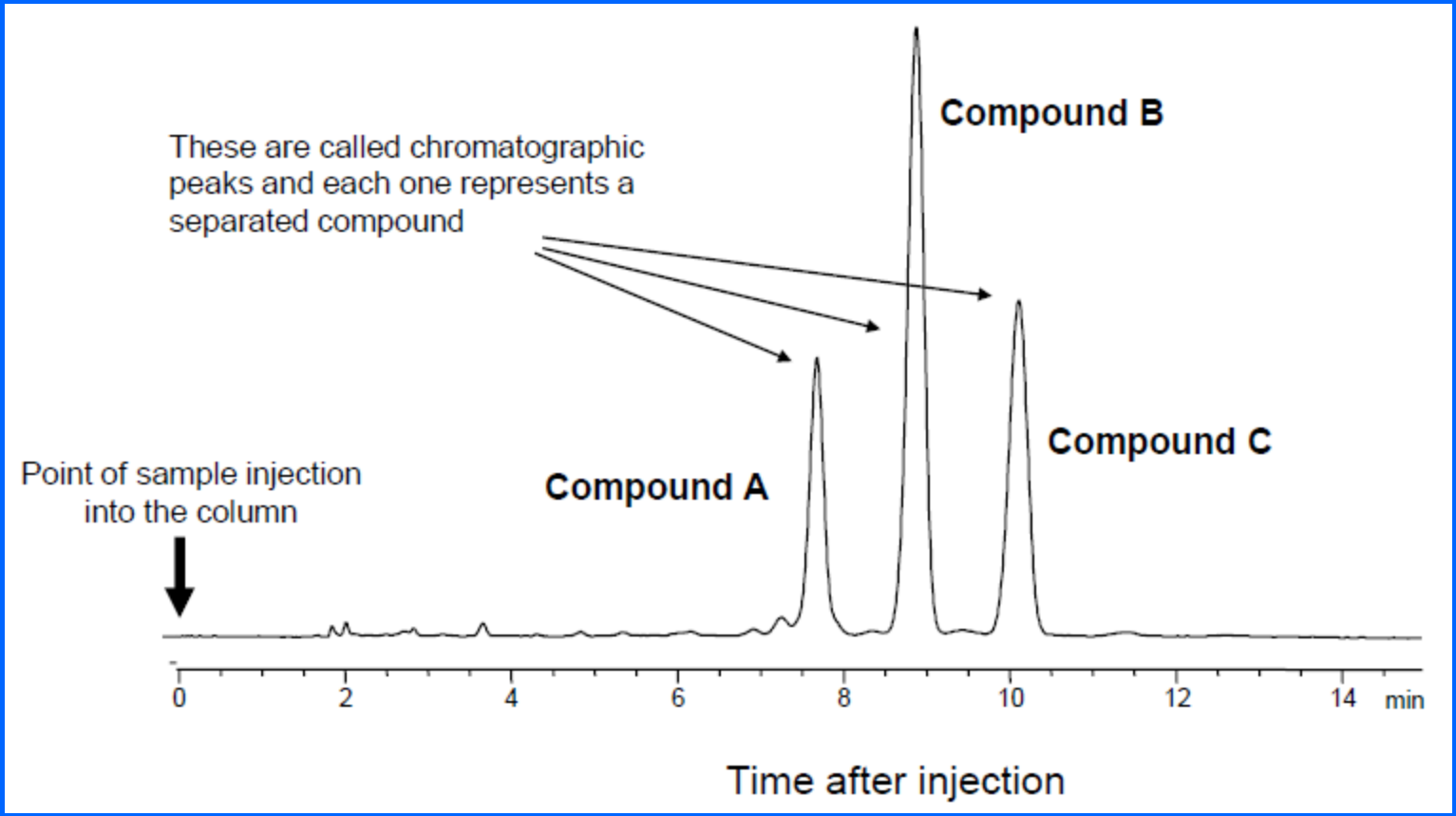
  stationary phase contains ionic groups and the mobile phase is an aqueous buffer

- **Size Exclusion**

there is no interaction between the sample compounds and the column .

Large molecules elute first. Smaller molecules elute later

Chromatogram

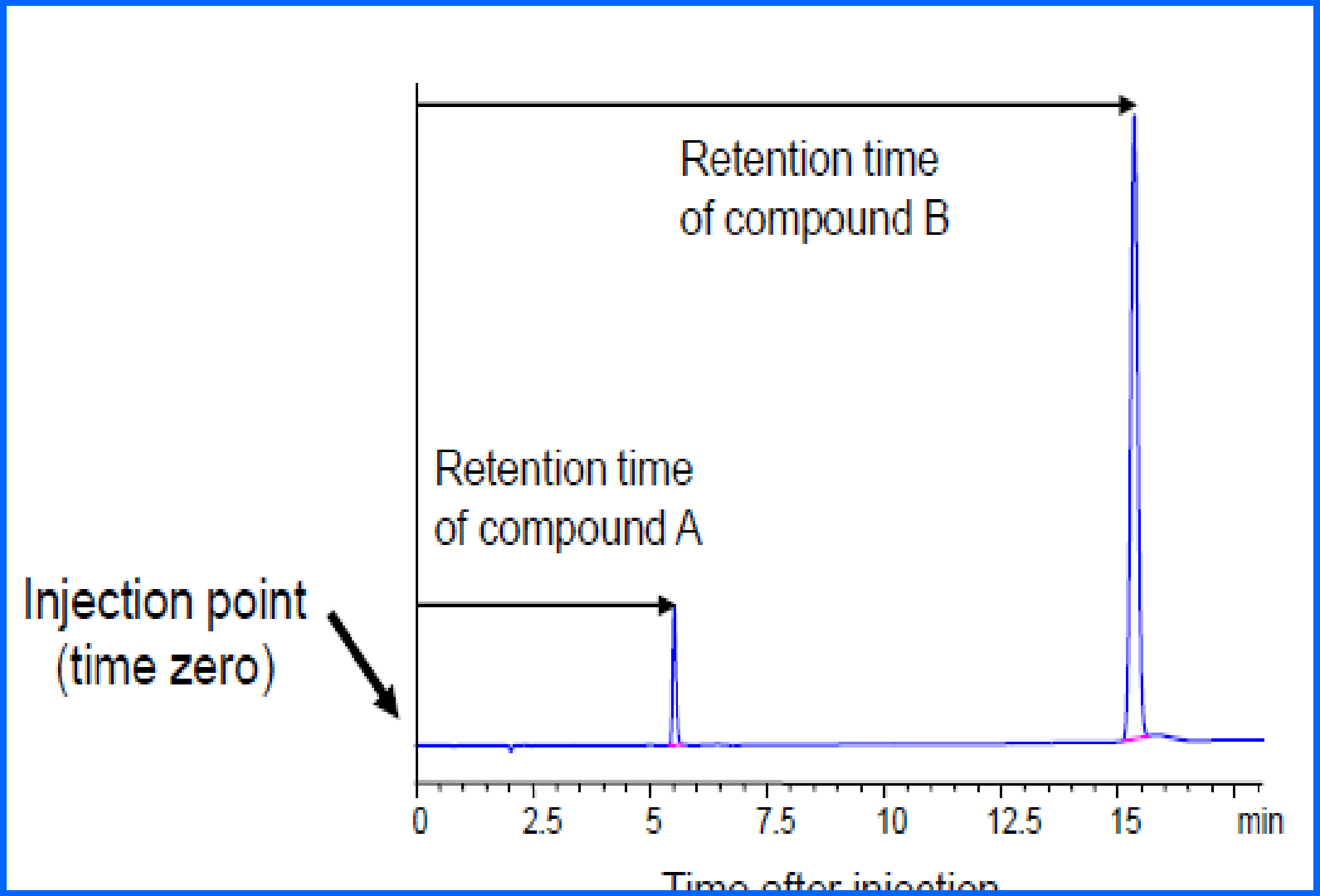


parameters of HPLC :

- 1- Qualitative analysis

the most common parameter for compound is retention time

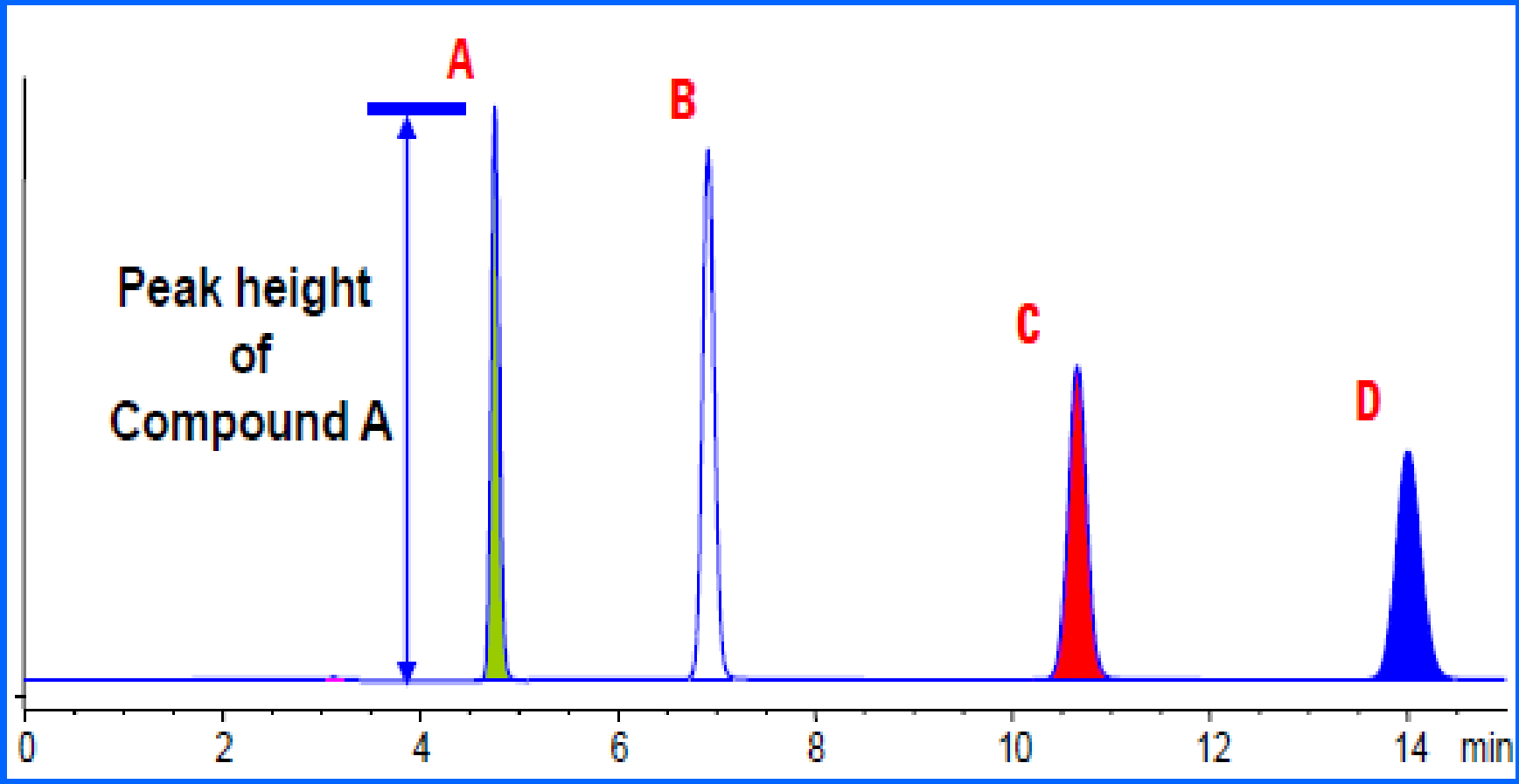
(the time it takes for that specific compound to elute from the column after injection)


Retention time
of compound B
Retention time
of compound A
Injection point
(time zero)
0
2.5
5
7.5
10
12.5
15
min
Time after injection

- **2- Quantitative Analysis**

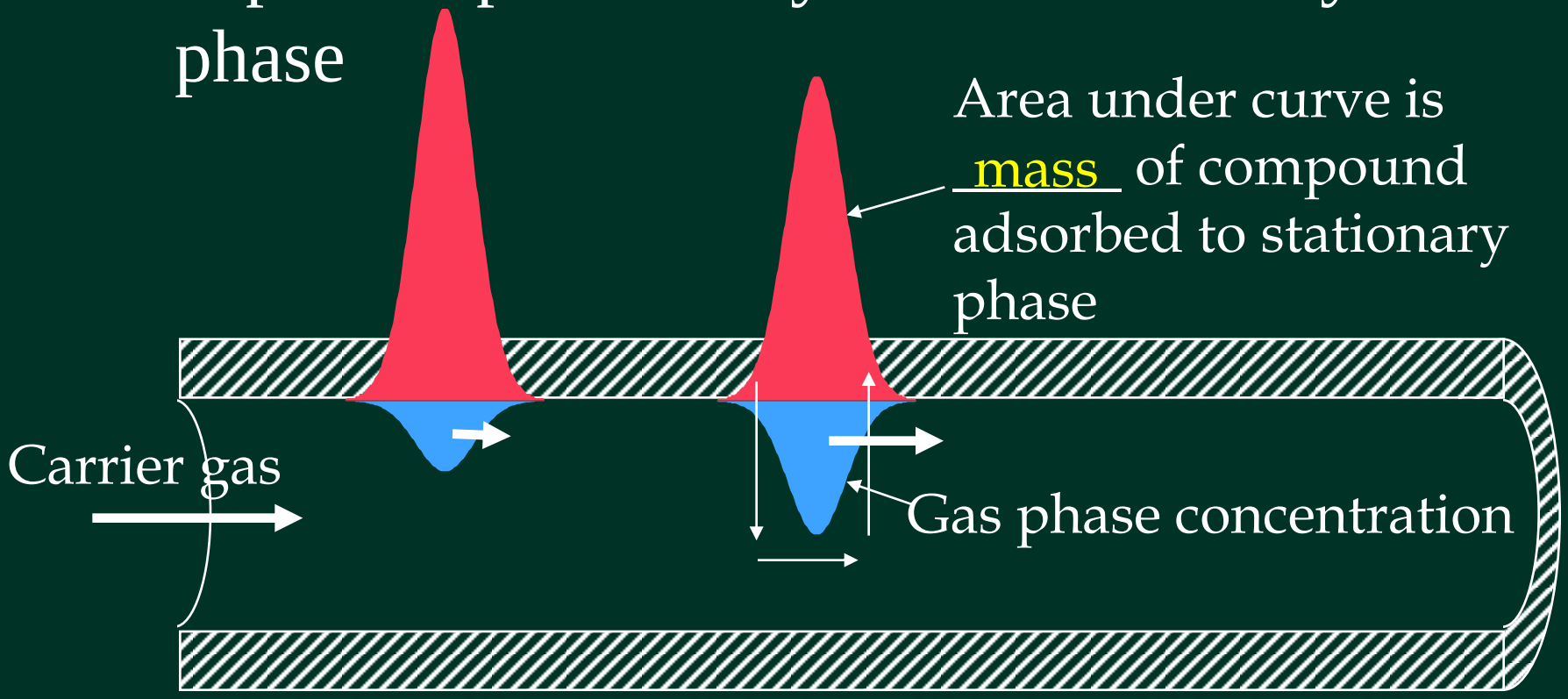
The measurement of the amount of compound in a sample (concentration)

1. determination of the peak height
2. determination of the peak area


A
B
C
D
Peak height
of
Compound A
0
2
4
6
8
10
12
14
min

Theory of Operation

- Velocity of a compound through the column depends upon affinity for the stationary phase



UV/Vis detector

A solute property detector.

Sample must exhibit absorption in UV/Vis range. Solvent must not absorb significantly at the measured wavelength.

Types " Filter photometer - single

- Variable wavelength
- Multiwavelength.

Refractive index detector

Bulk property detector - general purpose.

Based on refraction of light as it passes from one media to another. Presence of a solute changes the refractive index of the solvent.

Heat of absorption detector

A small amount of heat is released when a sample absorbs on a suitable surface.

This detector can measure this.

Electrochemical detectors

A number of properties have been evaluated

Detector types

! ! Dielectic constant

! ! Amperometric

! ! Conductometric

! ! Polarographic

! ! Potentiometric

Dielectric constant detector

Bulk property detector.

Measures changes in polarity of the liquid phase passing through the cell.

Conductometric detector

Measures conductivity of the solvent. Useful for solutions of ions.

Amperometric detectors

Most frequently applied type of electrochemical detector.

A known potential is applied across a set of electrodes - typically a glassy carbon type.

Ability to oxidize or reduce a species can be measured.

Typically limited to working with a specific class of materials per analysis

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Advantages of High Performance Liquid Chromatography

- High separation capacity, enabling the batch analysis of multiple components
- Superior quantitative capability and reproducibility
- Moderate analytical conditions
  - Unlike GC, the sample does not need to be vaporized.
- Generally high sensitivity
- Low sample consumption
- Easy preparative separation and purification of samples


Reveleris
X2

Components of a Gas Chromatograph

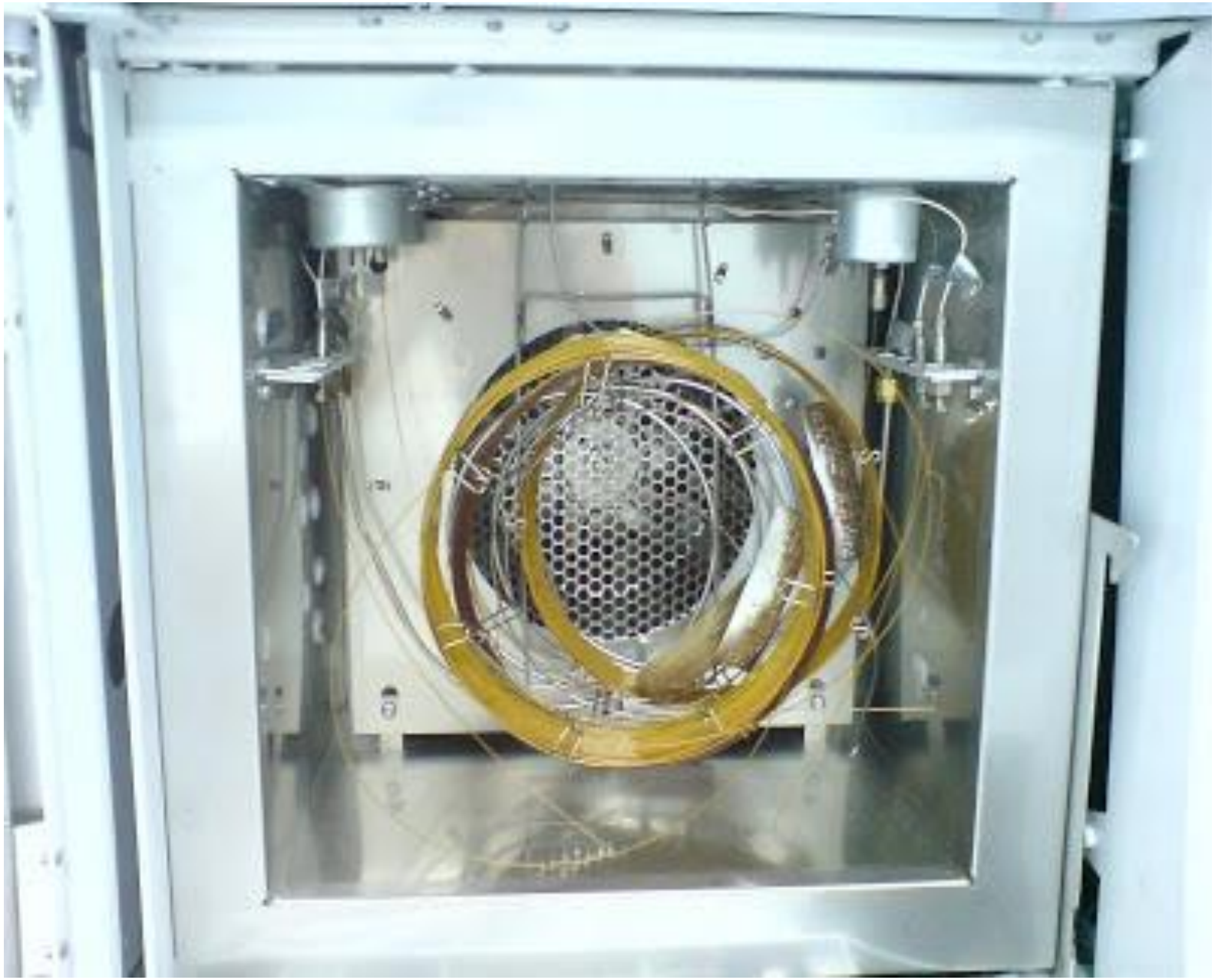
Gas Supply: (usually N_2 or He)

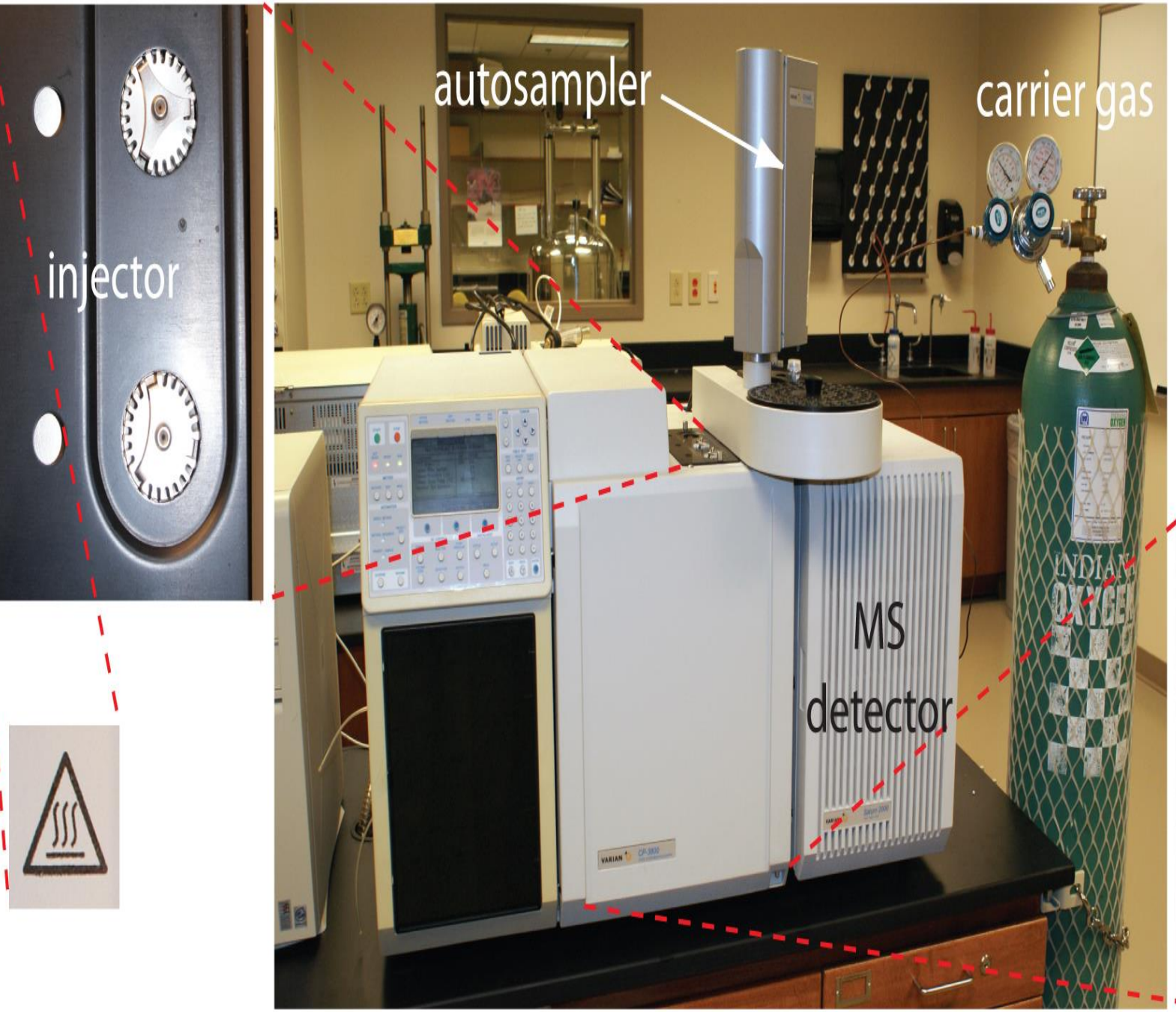
Sample Injector: (syringe / septum)

Column: 1/8" or 1/4" x 6-50' tubing packed with small uniform size, inert support coated with thin film of nonvolatile liquid

Detector: TC - thermal conductivity
FID - flame ionization detector


Glass
Columns
Custom Packed
Columns
Capillary
Columns




injector
autosampler
carrier gas
capillary
column
MS
detector
oven

GC Theory

- An inert gas such as helium is passed through the column as a carrier gas and is the moving phase. A sample is injected into a port which is much hotter than the column and is vaporized. The gaseous sample mixes with the helium gas and begins to travel with the carrier gas through the column. As the different compounds in the sample have varying solubility in the column liquid and as these compounds cool a bit, they are deposited on the column support. However, the column is still hot enough to vaporize the compounds and they will do so but at different rates since they have different boiling points. The process is repeated many, many times along the column. Eventually the components of the injected sample are separated and come off of the column at different times (called "retention times").
- There is a detector at the end of the column which signals the change in the nature of the gas flowing out of the column. Recall that helium is the carrier gas and will have a specific thermal conductivity, for example. Other compounds have their own thermal conductivities. The elution of a compound other than helium will cause a change in conductivity and that change is converted to an electrical signal. The detector, in turn, sends a signal to a strip chart recorder or to a computer. Detectors come in several varieties, for example, thermal detectors, flame-ionization and electron capture detectors.