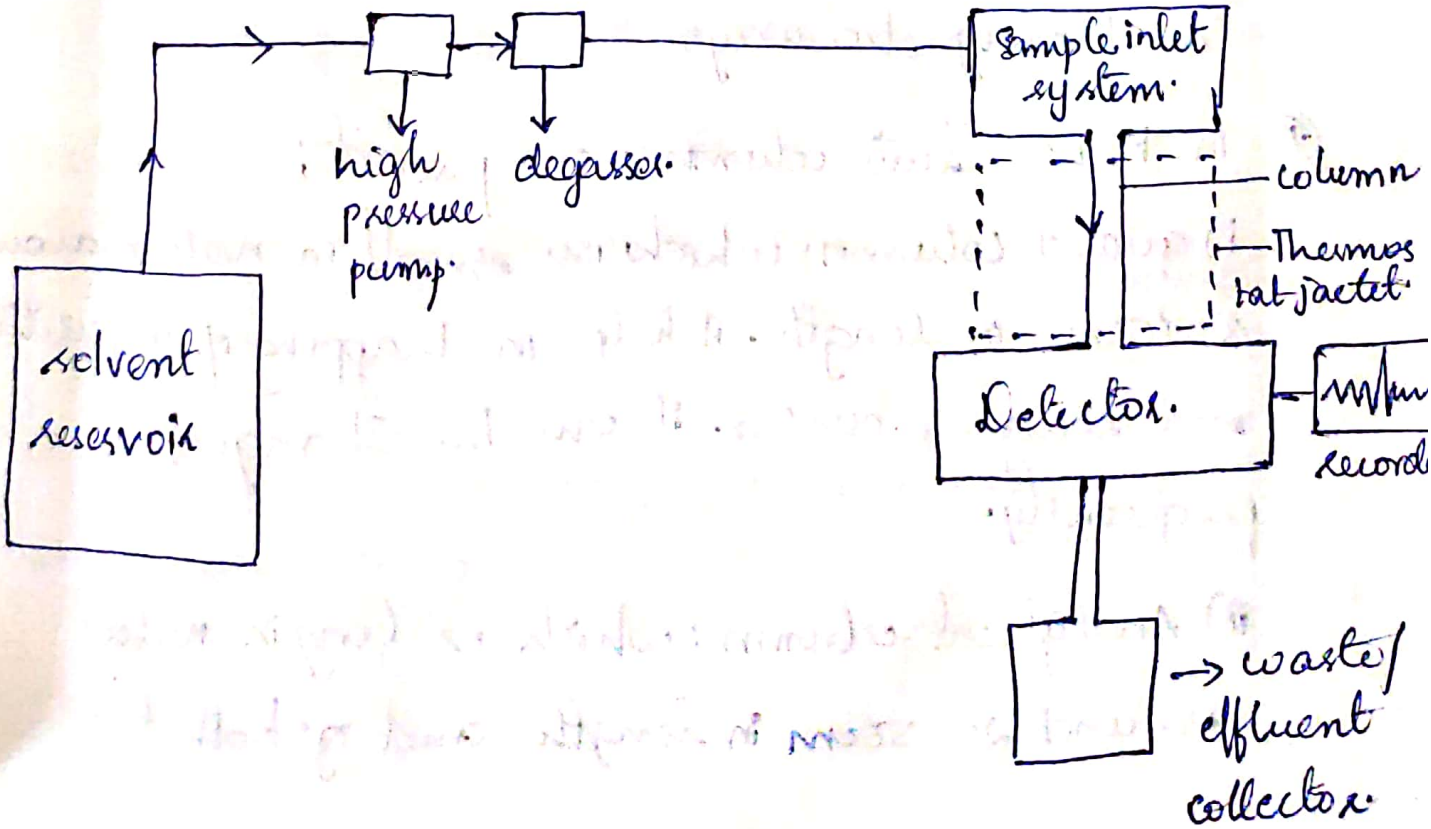
HPLC

HPLC: it stands for high performance liquid chromatography.

- Normal phase: stationary phase is polar and mobile phase is non polar.
- Reverse phase: stationary phase is nonpolar and mobile phase is polar.
- stationary and mobile phase both are in liquid state.
- It is used for thermally labile components.
- The principle of HPLC is partition.



① Columns are made up of stainless steel and can withstand pressure upto 8000 psi (pound force per square inch)

② HPLC column is straight 20 to 50 cm in length and 1-4 millimetre in diameter.

③ HPLC Column packing material (adsorbent / stationary phase) includes silica in the stationary phase.

④ High pressure pump in HPLC to control flow rate.

⑤ positive displacement pump which provides, constant pressure from reservoirs to sample inlet system.

⑥ degasses - to remove air bubbles or other ~~ess~~ material that will hinder the flow of solvent. it can be done through ultra sonic cavitation, vaccuming, and magnetic ways.

⑦ In HPLC two columns are present;

i) guard column: which is small in nature around 2-10cm in length. It helps in trapping of impurities and rough sources. it can be changed frequently.

ii) Analytical column: which is long in nature around 20-50cm in length and of both the..

columns having same packing material.

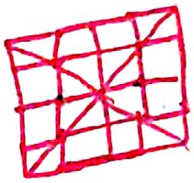
(8) Column thermostat for regulating the temperature.

(9) Solvent in HPLC has three functions:

(i) Introduction of sample.

(ii) development of Chromatogram.

(iii) purpose of Elution.



(10) detectors are of two types:

(i) bulk detector: to detect mobile phase properties

(ii) ie; refractive index, density, intensity and viscosity

(ii) solute property detectors: will detect UV Adsorbent IR Adsorbents, fluorescence .....

(11) Ideal Component for column:

i) sample and solvent should not react with the column.

ii) longer the column, better will be the resolution of Chromatogram.

iii) Adsorbent should be spherical and of uniform size.