HPLC- High Performance Liquid Chromatography
OR
High Pressure Liquid Chromatography

- High performance liquid chromatography is now one of the most powerful tools in analytical chemistry.
- It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in a liquid.
- Today, compounds in trace concentrations as low as parts per trillion (ppt) may easily be identified.

PRINCIPLE

High-performanceliquid chromatography (HPLC) is a form of column chromatography that pumps a sample mixture in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase).

COMPONENTS

1.Column
2.Stationary phase
3.Mobile phase
4.Injector
5.Detector
6.Recorder

1.COLUMN

- It is modified form of column chromatography, It is made up of stainless steel which can withstand hogh pressure up to 50MP
- length of the column is 5 to 25cm,internal diameter of the column is 4.5mm and flow rate of mobile phase to the column is 1.3ml/min

2.STATIONARY PHASE;

- Stationary phase is packed inner walls of the column
- Stationary phase is made up of adsorbent materials like silica or alumina that has very small particle size and possess larger surface area, the particle size is kept uniform to obtain better performance.

3.MOBILE PHASE

- The Mixture of different solvents can be used as mobile phase,The solvent used depends upon the the sample which have to be separated.The mobile phase is usually kept in the solvent reservoir, it is attached with **High Pressure Pump which pumps the mobile phase to the column with high pressure (up to 40 MP)**

4.INJECTOR

Injector which is placed just before the column which allows the sample in to the column

5.DETECTOR;

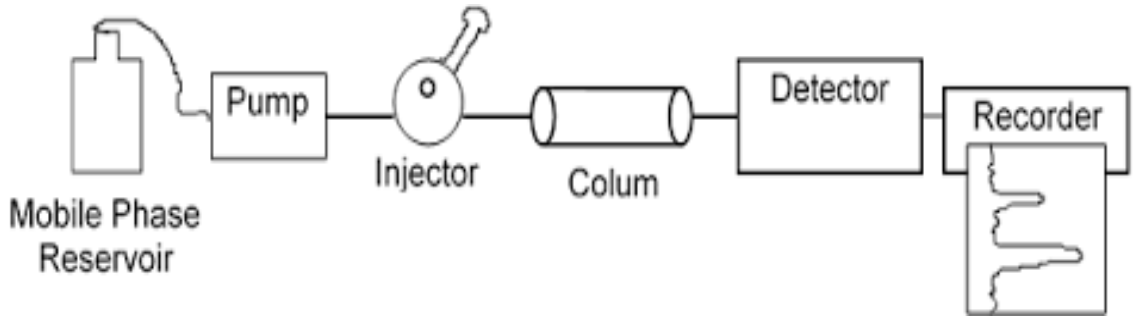
Detector detects components present in sample which is attached at the end of the column.Commonly used detectors are UV Detector /IR Detector/Electrochemical detector.

6.RECORDER

Detector is connected with Recorder (Computer) which collects the informations.

WORKING

BLOCK DIAGRAM



High pressure pump pumps Solvent (Mobile phase) in to the column and the Sample molecules are injected into the column ,the sample molecules are separated in the column and is detected by the detector and the peak is obtained on the computer ,this peak is plotted with respect to the retention time.

APPLICATIONS

HPLC can be applied to just about any sample, such as pharmaceuticals, food, nutraceuticals, cosmetics, environmental, forensic samples, and industrial chemicals**.**