



A REVIEW OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY PRINCIPLE, INSTRUMENTATION, APPLICATIONS

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ABSTRACT

In pharmaceutical industry various instrumental techniques are used for manufacturing , evaluation and identification of drugs and pharmaceutical ingredients . The chromatography is one of the most popular instrumental technique used in pharmaceutical industry for separation and purification of drug substance. The separation of substance is depends on the partition coefficient of mobile phase and stationary phase . The chromatography involves the various types like paper chromatography , gas chromatography , thin layer chromatography and high performance liquid chromatography . In this review the high performance liquid chromatography discussed through its basic information, principle , instrumentation , and applications . It involves detail information about principle , instrumentation and uses of high performance liquid chromatography .

Keywords: Chromatography, HPLC, separation, column, mobile phase, detector , solvent , stationary phase ,

1. INTRODUCTION [1 , 4]

The high performance liquid chromatography (HPLC) is also know as high pressure liquid chromatography . High performance liquid chromatography was developed between years 1960s and 1970s . Currently it is widely used as separation technique for purification and sample analysis in various pharmaceutical, environmental, food and polymer industries .

The high performance liquid chromatography is a type of column chromatography which includes a mobile phase and stationary phase for separation of analyt. In the high performance liquid chromatography the pump's , solvent , injectors , column , detectors and data collection devices are involved . The solvent flows from the column with high pressure are known as mobile phase. the sample mixture is filled in column with chromatographic packing material . This chromatographic packing material is known as stationary phase . During the separation process the continuous high pressure is maintained for proper flowing of mobile phase for separation.

2. PRINCIPLE [6, 7]

The high performance liquid chromatography have same mode of separation like column chromatography , but it differs from column chromatography in that the mobile phase is pumped in high pressure from packed column . The basic principle of high performance liquid chromatography is depend on the diffusion of sample between mobile phase and stationary phase . The principle advantage of HPLC compared with column chromatography are improved resolution of separated substance, increased accuracy , faster separation time and sensitivity .

3. INSTRUMENTATION [1, 4]

The basic components present in the instrumentation of High performance liquid chromatography are :

- Solvent reservoir
- Pumps
- Sample injection system
- Columns
- Detectors
- Data collection device

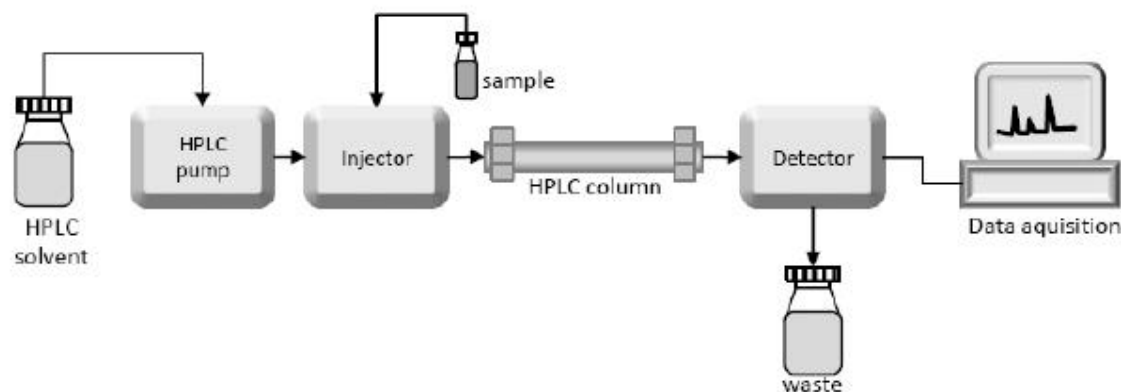


Fig .1. Instrumentation of High performance liquid chromatography

SOLVENT RESERVOIR:

In high performance liquid chromatography the mixture of solvent or single solvent is used as mobile phase . The mobile phase is store in solvent reservoir which is made up of glass . Mobile phase is made up of polar and non polar liquid Components . Generally glass and stainless steel reservoirs of 500 – 1000 ml capacity are used in High performance liquid chromatography.

PUMPS:

Pump is important component of High performance liquid chromatography . The mobile phase pumps from solvent reservoir to the column with the help of pump at high pressure . The pressure range of pump is 4000 – 6000 psi . Composition of mobile phase , particle size , flow rate of mobile phase and dimensions of column are the factors on which the proper operating process of pumps are depend . Ideal properties of pumps are solvent compatibility and constant pressure .

SAMPLE INJECTION SYSTEM:

Due to high sensitivity of High performance liquid chromatography it is very important to introduce accurate quantity of sample in the column . The fixed volume of sample solution is introduced in mobile phase present in column with the help of sample injection system . In High performance liquid chromatography , generally rotary sampling valve , slider valve and stop valve are used as sample injection system .

COLUMNS:

Column is the most important part or heart of the High performance liquid chromatography because the stationary phase is packed in column and mobile phase moves from this stationary phase of column . Columns are made up of stainless steel . Generally columns are 50 mm and 300 mm long and it's inward distance is up to 2 mm and 5 mm . Commonly the silica gel is used as packing material or stationary phase for column due to its microporous structure . There are two main types of High performance liquid chromatography columns are guard column and analytical column . The analytical column protected by guard column . Guard column increases the life of analytical column and protects from contaminants and particulate matters of solvents.

DETECTORS:

The HPLC detectors are present at the end of column for determination of analyte . As solutes are separated from their mixture then their presence in eluate should be detected. For the detection of compounds of mixture , various detectors are used in High performance liquid chromatography they are:

- Ultraviolet visible detector
- Refractive index detector
- Conductivity detector
- Fluorescence detector

ULTRAVIOLET VISIBLE DETECTOR:

Ultraviolet visible detector is most commonly used HPLC detector . The components which shows absorption spectrum in uv or visible region are identified by uv visible detector . The instrumentation of uv visible detector includes source of radiation like tungston lamp , wavelength selector or monochromator , sample containers or cuvette , detectors and recording devices .

As the eluate coming out from column, the uv visible radiation is passed through eluate. The signals are produced after absorption of uv visible radiation by organic compounds in eluate. The signal is then amplified and recorded to provide the chromatogram.

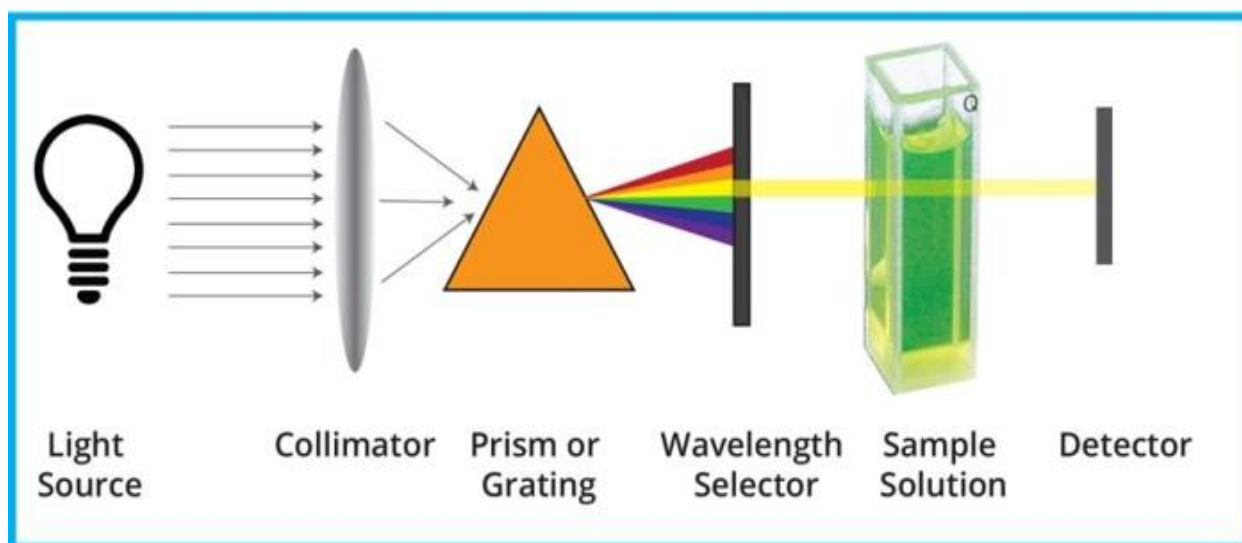


Figure .1. Instrumentation of UV visible detector

DATA COLLECTION DEVICE

When the detection of the components are completed then the detected signals are transformed into electrical signals. By using integrator the peak area, retention time and peak height are described. By using chromatographic software the collected data is converted into the presentable format.

4. APPLICATIONS [1, 3]

1) Pharmaceutical applications

- HPLC is used for determination of purity of drug substance.
- HPLC is used for quantitative and qualitative analysis.
- HPLC IS used for analysing purified compounds.
- HPLC is used For the determination of self life of pharmaceutical product .
- HPLC is used for complex molecules separation.

2) Environmental applications

- HPLC is used for the identification of contaminants in pesticides.
- HPLC is used for determination of quality of air and checking the drinking water.

3) HPLC is used in the treatment of various disease and disorder for clinical diagnosis.

4) HPLC is used in food industry for analysis of proteins, vitamins, amino acids and preservatives.

5) HPLC is used in forensic lab.

6) HPLC is used in biotechnology and DNA fingerprinting.

5. CONCLUSION [2, 3]

The HPLC is the highly used advanced technique which produces a very pure compounds. The HPLC is used in both laboratories, industries and clinical studies. High performance liquid chromatography shows high accuracy for identification and evaluation of biological and pharmaceutical samples. The HPLC process is carry out by production workers, chemist, students and biologist in large quantities.

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