International Journal of Scientific Research in Engineering and Management (IJSREM)
Volume: 08 Issue: 11 | Nov - 2024 SJIF Rating: 8.448 ISSN: 2582-3930

A Review on High Pressure Liquid Chromatography

Sonali mirkut*1 Tushar jadhav 2 Vitthal kuchake3.

2 assistant professor (Pharmacology) department, Ojas College of pharmacy Jalna .

1*,2,3 Ojas College of pharmacy Jalna 431203.

❖ ABSTRACT:-

High performance liquid chromatography (HPLC) has a dominant position among the Modern techniques in both the qualitative and quantitative drugs analysis. After adequate Sample preparation it is also commonly used for the determination of drugs and metabolites in Biological material.This study is focused on the development and validation of a chromatographic Method suitable for the analysis of 2',4'-dihydroxyacetophenon isonicotinoyl hydrazone (DHAP-INH) – a novel biocompatible iron chelator, and its application for stability Evaluation of this drug candidate in rabbit plasma in vitro. DHAP-INH is among newly Synthesized salicylaldehyde isonicotinoyl hydrazone (SIH) analogues, which were prepared to Improve compound stability in plasma and thus make pharmacokinetic profile of Aroylhydrazones more favorable.

❖ Introduction: -

Is a technique in analytical chemistry used to separate, identify, purify specific components in the mixture.

It is characterized by the use of high pressure to push a mobile phase through a column of stationary phase.
Which allowing separation of complex mixtures with high resolution
HPLC instruments consist of pump, mobile phase, column and injector detector.

❖ Applications: -

- Water purification.
- Detection of impurities in pharmaceutical industries.
- Pre-concentration of trace components.
- Ligand-exchange chromatography.
- Ion-exchange chromatography of proteins.
- High-pH anion-exchange chromatography of carbohydrates and oligosaccharides.

❖ **Methodology : -**

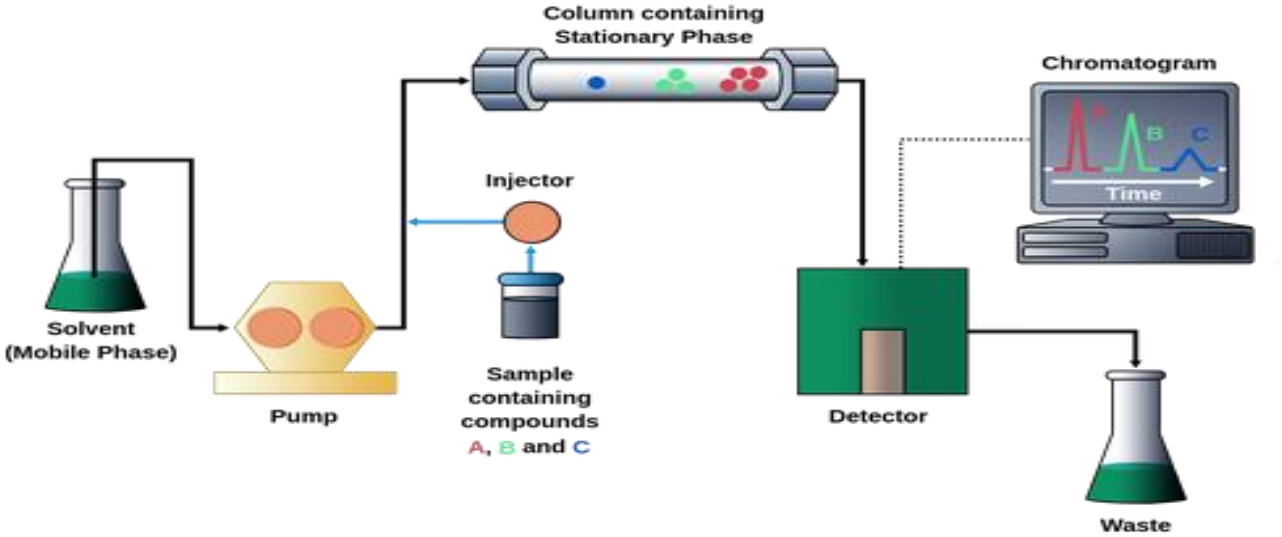

FIG1: - HIGH PRESSURE LIQUID CHROMATOGRAPHY

1. Pump: -

-Pump is used to force a liquid or eluent (m-P) through a specific flow
-It has the capacity to resist the high pressure at about up to 10,000 psi.
-Normal flow rate in HPLC is in 0.1-10ml/min

❖ **Types of pumps in HPLC:**

A. Constant Pressure Pumps
B. Syringe Type Pumps
C. Reciprocating Piston Pumps

A. Constant Pressure Pumps: -

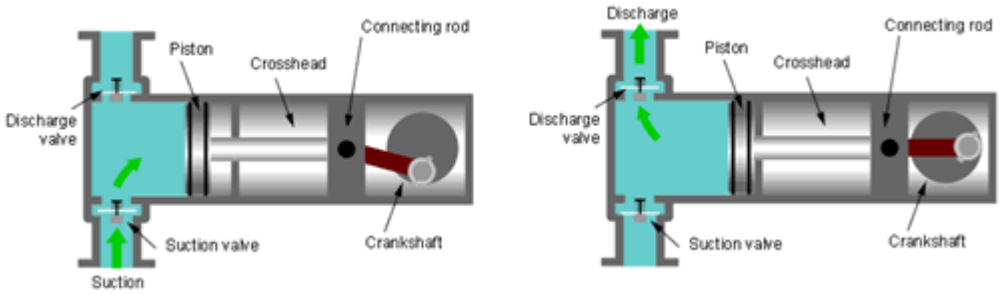

FIG 2. Constant pressure pump

-

-Constant pump is one the simplest pump, which uses gas to deliver mobile phase through the system.
-The key feature of a constant pressure Pump is that it maintain pressure a stable regardless of fluctuating flow rate.
-This system provides continuous pumping with high flow rates.

B. Syringe Type Pumps:-

FIG 3.Syringe type pump

- The rate of solvent delivery is controlled through voltage of motor.
- It consist of large stainless-steel Hypodermic syringe (cylindrical Jlike) Chamber that holds m. P
- Which is accelerated by a Piston
- Pump used to deliver a highly contained constant flow of liquid into HPLC System.
- Pressure capability up to 78,000 PSI

C. Reciprocating Piston Pump: -

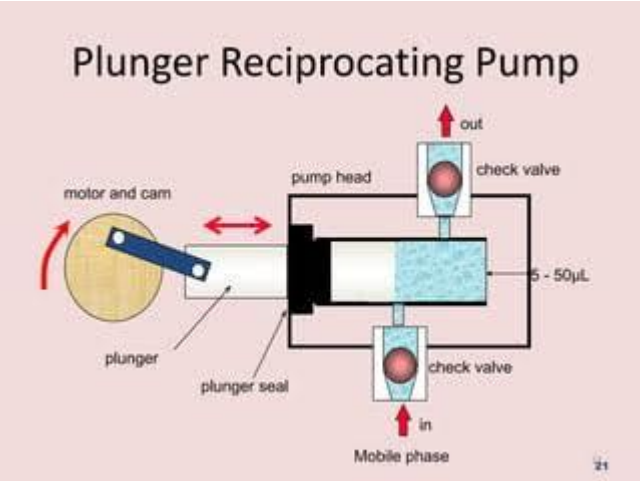

FIG 4.Reciprocating piston pump

- Currently used in 90% commercially available HPLC systems.
- Used to generate and maintain constant flow of the mop through the System under high pressure
- It consist of small chamber in which solvent is the pumped by back motion of motor doper piston
- High output pressure upto 10,000 psi

❖ Functions Of Pumps In HPLC: -

- Provides a precise flow of mobile phase of a standard composition to the column.
- Pumps provide a pressurized ability to the further column of the phases.

❖ Applications Of Pumps: -

- Packing of HPLC columns at pressure up to 1000 bar.
- Delivery of super critical fluids such as Co2.
- Metering against constant pressure.
- Provides precise flow of the mobile phases of specialised composition to the column.

2.Solvents:-

- The weak portion of the mobile phase for normal phase HPLC must be non- polar [lipophilic Solvent].
-The non-polar will not attract molecules to the analyte thus boosting the time of analyte in the stationary Phase.

❖ **MP for Normal phase HPLC must be non-polar (Lipophilic solvent)**

- Hexane
- Heptane
- Benzene
- Ethyl acetate
- **Hexane: -**

- HPLC grade is a colourless liquid used in chemical labs as a non polar mobile phase solvent in HPLC.
- They have low UV absorbance.

➢ **Heptane: -**

- They contain mobile phase consists of a non-polar solvent.
- They are ideal for transport and storage.

➢ **Benzene: -**

- It is used as an industrial solvent.
- Carcinogen
- Has non-polar solvent.

➢ **Ethyl acetate: -**

- Commonly used solvents in various chromatography technique.
- It is highly pure form is used as a solvent for spectrophotometric applications.
- Most suited for HPLC instrument.

❖ **MP for Reversed phase HPLC must be Polar :-**

➢ Water
➢ Methanol
➢ Acetonitrile
➢ Isopropanol

➢ **Methanol** – commonly used organic. Solvent with relatively high polarity
➢ **Acetonitrile**- relatively high polarity and low absorbance.
➢ **Isopropanol** - can improve sample solubility and enhance the separation of Polar solutes.

❖ **Stationary Phase :-**s.p for Normal phase HPLC must be polar

Ex- silica, Alumina etc.

- .S. P for Reversed phase must be Non-polar.
- E.g. (si-CH2-CH3)

❖ **Applications Of Solvents:-**

- Used for mobile phase.
- Water, methanol, and formic acid are staple reagents in HPLC .
- Helps mobile phase for the further chromatography.

3.Injectors: -

- It is a device that introduces a sample into the HPLC System.
- The injector is used to introduce the required accurately sample volume.
- Most widely used injection methods is based on Sampling 1000.

❖ **Types Of Injectors :-**

1. Manual Injectors
2. Automatic injectors

1. **Manual Injectors:-**

FIG 5. Manual Injectors

- User manually loads Sample into the injector using a syringe.
- And then inject sample into the flowing mobile phase.
- Which transports the sample into the beginning (head) of the column is ar high pressure.

2. **Automatic injectors:-**

- User loads voids filled with Sample solution, into the auto sampler tray.
- Measure the appropriate Sample Volume ,injects the sample ,flushes the injector to be ready for next sample.

❖ **Applications of injectors:-**

- HPLC injectors allow the introduction of precise sample volume into the column.
- A sample solution is introduced into the sample loop using a 22 – gauge blunt tip syringe in the load position.

4.Columns :-

- HPLC column most commonly used are made from stainless Steel tubing
- Typical dimensions being a 10-30 cm long and 4 to 5 mm internal diameter
- Each end with stainless steel frits with a mesh of 2um or less.

➢ **Types of columns based on scale of preparation :-**

1. Pre- Column (Guard column)
2. Analytical Column

A . Pre- Column (Guard column)

FIG 6.Pre - column

- It is short column present between the injector and analytical Column
- Packing composition of guard Column and analytical column same

B. Analytical Columns :-

FIG 7 Analytical Column

- Actual separation is carried out by analytical column
- The column is filled with small particle 5-10 micron and the solid support can be silica gel.

➢ **Types of column based on mode of Operation** :-

1. **Normal phase column**
2. **Reversed phase column**
3. **Size exclusion column**
4. **Ion exchange column**

1. Normal Phase Column:-

- In this column packing materiel must be more polar than the mobile Phase with respect to the sample .

FIG.8 Normal phase column

2. Reversed Phase Column:-

- In this Column, packing material must be non polar and solvent is polar .

FIG 9. Reversed Phase Column

3. Size Exclusion:-

- The column is Racked with material having controlled pore size
- Sample filtered according to molecular size
- Large molecules rapidly washed through column
- Smaller molecules penetrate inside the pores and elute later

4. Ion Exchange Phase Column:-

- This technique is used only for ionic or ionisable sample
- Mobile phase is buffer
- Column packing contain ionic group

❖ **Applications:-**

- To separate many types of analytes.
- Commonly used for separation of carbohydrates, amino acids, and proteins.
- Ion exchange and ligand exchange chromatography may be combined in a column.

5 . Detectors :-

- Detector is a device used in high performance liquid chromatography (HPLC) to detect components of the mixture being. Eluted off the chromatography column
- The detector converts effluents into electrical signal.

❖ **Types :-**

1. **U.V visible Detector**
2. **Photo diode array**
3. **Fluorescence Detector**
4. **Electro-chemical Detector**
5. **Refractive index detector (RID)**
6. **Mass spectrometry**

1. U.V Visible Detector :-

- Detector's used to monitor the Separation of compounds.
- It works by measuring the absorbance of (U.V) or visible light by the analytes as they pass through detector.
- U.V (190-400nm) or visible (400-7007m) wavelengths absorb.

2. Fluorescence Detector:-

- It is used to detect and quantify Component that exhibit fluorescence.

3. Electro- chemical Detector :-

- E.C.D is used to detect compound that under go oxidation or reduction

4. Refractive Index Detector :-

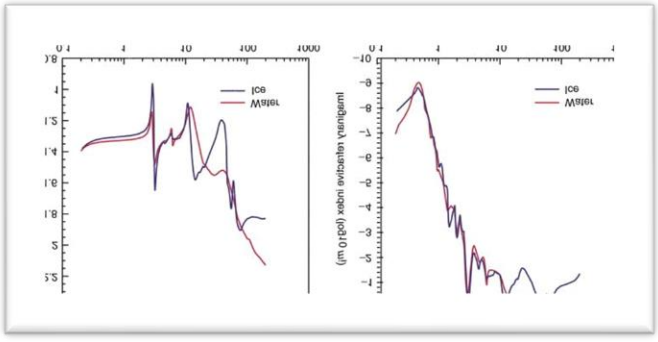

FIG 10.Refractive Index

-Measures the change in the refractive index of the mobile phase (eluent)
-As it passes through the detector which helps in identifying and Quantifying that compounds in a sample do not absorb U.V light

5. Photo Diode Array :-

- It detects absorbance of compounds at multiple wavelength simultaneously

- Useful for complex mixtures.

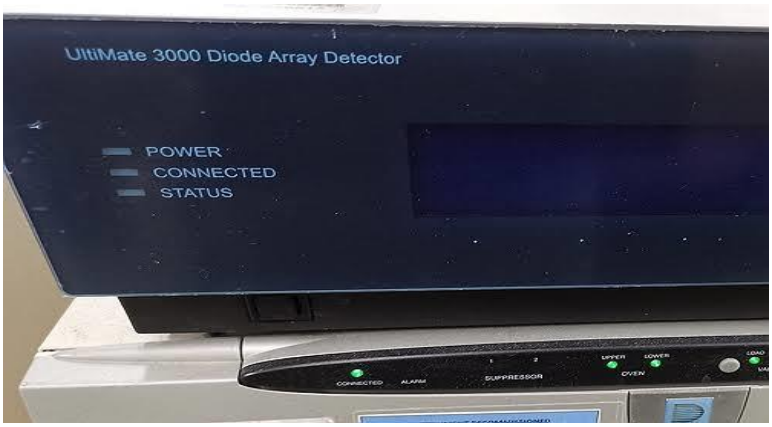

FIG 11 Photo Diode Array Detector

- **6.Mass Spectrometry:-**

- mass spectrometry used to distinguish between compounds with very similar chemical structures.

❖ **Advantages Of HPLC :-**

- HPLC has High resolution and speed of analysis.
- High surface area .
- It has High pressure gradient.
- It has wide range of stationary phase .
- Precise flow rate control .
- Sensitive detection methods .
- Low sample methods requirements.
- Accurate peak identification

❖ **Disadvantages Of HPLC:-**

- HPLC has High cost .
- High quality components are needed.
- The solvents and columns are used in HPLC are expensive.
- Regular maintenance and calibration is needed with add extra cost .
- Sophisticated software is required for data analysis.
- Research and development cost .
- Requires a large number of expensive organics ,need a power supply and regular maintenance is required.

❖ **Result**:-The results of a High Performance Liquid Chromatography (HPLC) analysis are displayed in chromatogram, which is a graph that shows detectors response over time .
The chromatogram's axes and other features can be used to interpret the results.

❖ **Discussion**:-High Performance Liquid Chromatography (HPLC) is a process of separating components in a liquid mixture.
A liquid sample is injected into a stream of solvent (mobile phase) flowing through a column packed with a separation medium (stationary phase).

❖ **Conclusion**:- High-performance liquid chromatography is important analytical method commonly used to separate and quantify components of liquid samples.In this technique, a solution (first phase) is pumped through a column that contains a packing of small porous particles with a second phase bound to the surface.

❖ REFERENCE:-

Ahmad, Z.; Coleman, N.; Shepherd, N. High Pressure Liquid Chromatography (HPLC). Standard Operating Procedure (SOP). Version: SMB019.3. The University of Sydney. Molecular Bioscience.

a. https://sydney.edu.au/science/molecular_bioscience/ohs/documents/sop/SOP%20SMB_019.3_High%20pressure%20liquid%20chromatography%20(HPLC)%20ZA%20NC%20NS%200714.pdf

2. High Performance Liquid Chromatography. Purdue University. Radiological & Environmental Management Division of Environmental Health and Public Safety.

a. https://www.purdue.edu/ehps/rem/waste/hplc.html

3. 8.4.5 Liquid Chromatography Waste. University of Minnesota Department of Environmental Health & Safety (DEHS).

a. https://dehs.umn.edu/845-liquid-chromatography-waste

4. BAE. HPLC Standard Operating Procedure. Aminex 87-H and 87-P analytical columns. University of Kentucky, Biosystems and Agricultural Engineering Dept. ‘

5. Instrumental method of analysis book of Nirali publication by Dr. K. R. Mahadik and Dr. L. Sathiyarayanan

6. ANA Notes of Pharmaceutical analysis Volume 1 of ANA GPAT by Dr, Mahesh S. Kale Dr. Machindra D. Bochare

7. https://en.wikipedia.org/wiki/High-performance_liquid_chromatography#:~:text=High%2Dperformance%20liquid%20chromatography%20(HPLC,quantify%20specific%20components%20in%20mixtures.

8. Rao BV, Sowjanyal GN, Ajitha A, Uma V, Rao M. A review on stability indicating HPLC method development. World Journal of Pharmacy and Pharmaceutical Sciences 2015;4(8)405-423.

9. Rasaiah JC. Molecular Theory of Solutions By Arieh Ben-Naim (The Hebrew University