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A Review on High Performance Liquid Chromatography

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ABSTRACT: The chromatography technique is the subject of this review. The estimate of pharmaceutical and biological materials typically uses the key qualitative and quantitative technique known as high performance liquid chromatography (HPLC). In addition to several other human and animal investigations, the development and validation of HPLC techniques play crucial roles in the discovery, development, and production of innovative drugs. The majority of pharmaceuticals and other chemicals may be examined using the HPLC method because of its many benefits, including speed, specificity, accuracy, precision, and ease of automation. The component can be distributed throughout the mixture using the High-Performance Liquid Chromatography (HPLC) Method. Chromatography is employed across the board in pharmaceutical and research labs.This article was prepared with an aim to review different aspects of HPLC, such as principle, types, instrumentation, application, Advantages, Metod development and Validation parameters.

KEY WORDS: HPLC, Chromatography, Instrumentation, Stationary Phase, Mobile Phase, Application.

I. INTRODUCTION:

- To make drugs serve their purpose various chemical and instrumental methods were developed at regular intervals which are involved in the estimation of drugs. These pharmaceuticals may develop impurities at various stages of their development, transportation and storage which makes the pharmaceutical risky to be administered thus they must be detected and quantitated. For this analytical instrumentation and methods play an important role.High-performance liquid chromatography a specific form of column chromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds. HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used.[1]
- The retention time is the time it takes for a certain analyte to elute.Any miscible combination of water or organic liquids is a common solvent. Gradient elution has been used to change the mobile phase composition during the analysis. The gradient separates analyte mixtures based on the analyte's affinity for the current mobile phase. The nature of the stationary phase and the analyte influence the choice of solvents, additives, and gradients. [2]

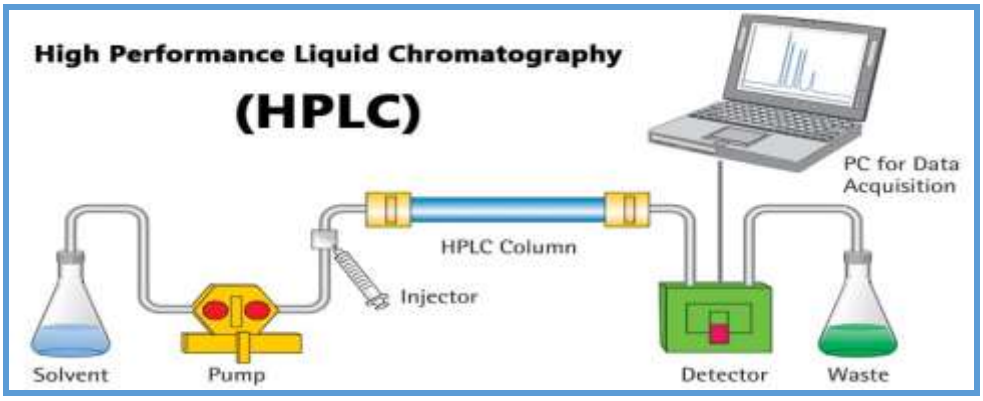

Fig. 1: FLOW DIAGRAM OF HPLC

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II. PRINCIPLE OF HPLC: [3]

- HPLC principle is that solution of sample is injected into a column of porous material (stationary phase) and liquid phase (mobile phase) is pumped at higher pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase based on its affinity towards stationary phase.
- HPLC is a branch of column chromatography in which the mobile phase is forced through the column at high speed. As a result the analysis time is reduced by 1-2 orders of magnitude relative to classical column chromatography and the use of much smaller particles of the adsorbent or support becomes possible increasing the column efficiency substantially.

III. TYPES OF HPLC:[4]

- Depending on the substrate used i.e. stationary section used, the HPLC is split into following type

a) Normal phase HPLC: Section HPLC- in this approach the separation is based totally on polarity. The stationary phase is polar, broadly speaking Silica is used and the non-polar segment used is hexane, chloroform and diethyl ether

b) Reverse phase HPLC- It's reverse to normal phase HPLC. The mobile part is polar and therefore the stationary part is non polar or hydrophobic. The additional is that the non-polar nature the additional it'll be maintained.

c) Size-exclusion HPLC- Size-exclusion HPLC- The column is incorporating with precisely controlled substrate molecules. On the distinction in molecular sizes the separation of constituents can occur.

d) Ion-exchange HPLC- The stationary part has ironically charged surface opposite to the sample charge. The mobile part used is binary compound buffer which can management pH scale and ionic strength.

IV. MODE OF SEPARATION:[5]

- There are two modes of separation in HPLC, based on eluent composition these are:

I) Isocratic: Isocratic mode of separation involves the constant eluent composition; that means the equilibrium conditions in the column and the actual velocity of compounds moving through the column are constant.

II) Gradient: Gradient mode of separation involves the varying eluent composition. This technique significantly increases the separation power of a system mainly due to increasing the apparent efficiency (decrease of the peak width). The width of the peak depends on the rate of the composition of the eluent.

V. INSTRUMENTATION:[6,7]

(a) Solvent reservoir:

- The contents of mobile phase are present in glass container. In HPLC the mobile phase or solvent is a mixture of polar and non-polar liquid components. Depending on the composition of sample, the polar and non-polar solvents will be varied.

(b) Pump:

- A high-pressure pump (solvent delivery system or solvent manager) is used to generate and meter a specified flow rate of mobile phase, typically milli litters per minute. The pump suctions the mobile phase from solvent reservoir and forces it to column and then passes to detector. Typical pumps can reach pressures in the range of 6000-9000 psi. Pump pressure depends on column dimension, particle size, flow rate and composition of mobile phase.

(c) Sample injector:

- The injector serves to introduce the liquid sample into the flow stream of the mobile phase. Typical sample volumes are 5- to 20-microliters (µL). The injector must also be able to withstand the high pressures of the liquid system. An auto sampler is the automatic version for when the user has many samples to analyse or when manual injections are not Practical.

(d) Column:

- Considered the "heart of the chromatograph" the column's stationary phase separates the sample components of interest using various physical and chemical parameters. The pump must push hard to move the mobile phase through the column and this resistance causes a high pressure within the chromatograph. Provides separation through high pressure created by the small particles.
- The types of columns are: **A guard column** is introduced before the analytical column to increase the life of the analytical column by removing not only particulate matter and contaminants from the solvents but also sample components that bind irreversibly to the stationary phase. **Analytical columns** is the heart of High-performance liquid

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chromatography. Liquid-chromatographic columns range in length from 10 to 30 cm. normally, the columns are straight, with added length, where needed, being gained by coupling two or more columns together. The inside diameter of liquid columns is often 4 to 10 mm; the most common particle size of packing is 5 or 10 m. The most common column currently in use is one that is 25 cm in length, 4.6 mm inside diameter, and packed with 5 mm particles. Columns of this type contain 40,000 to 60,000 plates/meter.

(e) Detector:

- The detector can see (detect) the individual molecules that come out (elute) from the column. A detector serves to measure the amount of those molecules so that the chemist can quantitatively analyse the sample components. The detector provides an output to a recorder or computer that results in the liquid chromatogram (i.e., the graph of the detector response).

(f) Data collection devices or Integrated:

- Signals from the detector might be gathered on graph recorders or electronic integrators that fluctuate in many-sided quality and in their capacity to process, store and reprocess chromatographic information. This chromatogram can be analysed manually or by specialized software used in the procedures that aim to purify a certain compound from a mixture. i.e. the output of this system is data only.

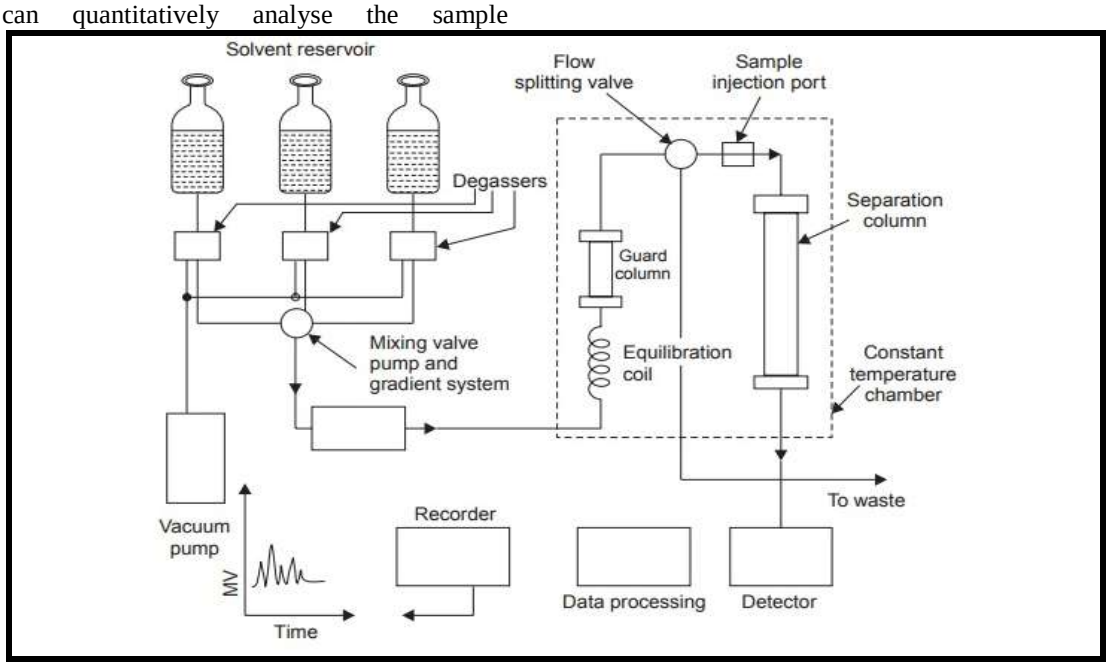

Fig. 2: INSTRUMENTATION OF HPLC

VI. METHOD DEVELOPMENT:[8]

- When there are no authoritative methods are available, new methods are being developed for analysis of novel products. To analyze the existing either pharmacopoeial or non-pharmacopoeial products novel methods are developed to reduce the cost besides time for better precision and ruggedness. These methods are optimized and validated through trial runs. Alternate methods are proposed and put into practice to replace the existing procedure in the comparative laboratory data with all available merits and demerits.

A. Purpose of analytical method development

- Drug analysis reveals the identification characterization & determination of the drugs in mixtures like dosage forms & biological fluids.
- The reasons for the development of novel methods of drug analysis are:

a) When there is no official drug or drug combination available in the pharmacopoeias.

b) When there is no decorous analytical process for the existing drug in the literature due to patent regulations.

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c) When there are no analytical methods for the formulation of the drug due to the interference caused by the formulation excipients.
d) Analytical methods for the quantitation of the analyte in biological fluids are found to be unavailable.
e) The existing analytical procedures may need costly reagents and solvents. It may also involve burdensome extraction and separation procedures.

B. Steps for the development of the method

- Development procedure follows with the proper documentation. All data relating to these studies must be recorded either in laboratory notebook or in an electronic database.

C. Choosing a method

- Duly utilizing the information available from the literature, methodology is evolved since the methods are changed wherever required. Occasionally it is imperative to get additional instrumentation to develop, modify or reproduce and validate existing procedures for analytes and samples.
- If there are no past suitable methods available to analyze the analyte to be examined.

D.Instrumental setup and initial studies

- Installation, operational and performance qualification of instrumentation with reference to laboratory standard operating procedures is verified by setting up appropriate instrumentation.

E. Optimization

- While performing optimization, one parameter is changed at a time and a set of conditions are isolated, before utilizing trial and error approach. The said work need to be accomplished basing on a systematic methodical plan duly observing all steps and documented with regard to dead ends.
- In HPLC analysis method development define and follow the following steps to achieve the goal.[9]

a) Describe the sample,
b) Establish goals,
c) Consider sample preparation
d) Choose detector
e) Solubility of Analytes
f) Choice of Column

- **Sample Pretreatment Options:**[14]

• Sample Collection: Obtain representative sample using statistically valid processes.
• Sample Storage and Preservation: Use appropriate inert, tightly sealed containers; be especially careful with volatile, unstable, or reactive materials; biological samples may require freezing.
• Preliminary Sample Processing: Sample must be in a form for more efficient sample pretreatment (e.g., drying, sieving, grinding, etc.); finer dispersed samples are easier to dissolve or extract.
• Weighing or Volumetric Dilution: Take necessary precautions for reactive, unstable, or biological materials; for dilution, use calibrated volumetric glassware.
• Alternative Sample Processing Methods: Solvent replacement, desalting, evaporation, freeze drying, etc.
• Removal of Particulates: Filtration, solid-phase extraction, centrifugation

VII. METHOD VALIDATION:

- The act of demonstrating that a method's presentation characteristics meet the requirements for the anticipated scientific application through research facility inspections is the approval of an insightful procedure. Each new or modified approach must first receive approval in order to verify that it will provide results that are predictable and trustworthy when applied by various administrators using the same hardware in the same or a different research institution. The method being discussed and its anticipated goals will determine exactly what kind of approval programme is needed. Method approval research can be used to assess the calibre, dependability, and consistency of scientific findings; it is an essential component of any competent perceptive practise.The technique approval methodology requires the utilization of hardware that is inside determination, works well, and is adequately adjusted. Approval and revalidation of scientific methodology are required.[10]

- Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for its intended use. The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedure.All analytical methods that are intended to be used for analyzing any clinical samples will need to

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be validated. The validation of analytical methods is done as per ICH guidelines.[11]

- ❖ Method validation results can be used to assess the quality, reliability, and consistency of analytical results; it is an essential component of any good analytical practice. The use of equipment that is within specification, working properly, and properly calibrated is critical to the method validation process. Validation or revalidation of analytical methods is required. • Before they are put into routine use; • When the conditions for which the method has been validated change; • When the method is changed. [12]
- ➢ The analytical methods which need to be validated are classified as per ICH are classified as following:[13]
- Identification tests: To ensure identity of an analyte.
- Quantitative test for impurities: to accurately and quantitatively reflect the purity of a sample
- Limit test for impurities: to reflect purity characteristics of the sample
- Assay of drug substance and drug products: to measure accurately and quantitatively the analyte present in the sample. These methods also include analysis for content uniformity and measurement of analyte from dissolution samples.
- ❁ **Typical parameters to validate are include;**
- ❁ General recommendation for analytical method validation. i.e. for pharmaceutical methods, can be found in the FDA guidelines. [15]

1. System Suitability
2. Accuracy
3. Precision

Repeatability
Intermediate precision

4. Linearity
5. Detection limit
6. Quantitation limit
7. Selectivity
8. Stability
9. Range
10. Robustness

- ❁ **System suitability:**
- ❖ The system suitability test represents an integral part of the method and is used to ensure the adequate performance of the chosen chromatographic system.

Efficiency, capacity factor, resolution factor, and symmetry factor are the parameters that are normally used in assessing the column performance. Factors that can affect chromatographic behavior include mobile phase composition, temperature, ionic strength, apparent pH, flow rate and column length and stationary phase characteristics such as porosity, particle size and type, and specific surface area. [16]

VIII. APPLICATIONS OF HPLC: [17]

- ➢ The HPLC has several applications in the fields of pharmacy, forensic, environment and clinical. It also helps in the separation and purification of compound.
- **Pharmaceutical Applications:** The pharmaceutical applications include controlling of drug stability, dissolution studies and quality control.
- **Environmental Applications**: Structure elucidation and Monitoring of unknown pollutants and detecting components of drinking water.
- **Forensic Applications:** Analysis of textile dyes, quantification of drugs and steroids in biological samples.
- **Food and Flavour Applications:** Sugar analysis in fruit juices, detecting polycyclic compounds in vegetables, analysis of preservatives.
- **Clinical Applications:** Detecting endogeneous neuropeptides, analysis of biological samples like blood and urine.

IX. CONCLUSION:

- ➢ After a thorough review, it can be said that HPLC is a multilateral, reliable chromatographic method for approximating medicinal products. In terms of estimating active molecules quantitatively and qualitatively, it has several applications in numerous domains. It is applicable to both clinical and laboratory science. HPLC can be used to increase accuracy, precision, and specificity.

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