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High Performance Liquid Chromatography (HPLC)

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Abstract

The chromatography term is derived from the Greek words namely chroma (colour) and graphein (to write). The chromatography is very popular technique and it is mostly used analytically. High performance liquid chromatography (HPLC) is an important qualitative and quantitative technique, generally used for the estimation of pharmaceutical and biological samples. It is the most versatile, safest, dependable and fastest chromatographic technique for the quality control of drug components. This article was prepared with an aim to review different aspects of HPLC, such as principle, types, instrumentation and application. A brief description of the instrumentation and the method development is provided. The principles of HPLC including different separation modes and detection methods for the quantitative analysis are summarized. This review mainly focuses on the HPLCtechnique its principle, types, instrumentation and applications.

Keywords:-*chromatography, technique, pharmaceutical, HPLC, instrumentation and application.*

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I. INTRODUCTION:-

High Performance Liquid Chromatography which is also known as High Pressure Liquid Chromatography. It is a popular analytical technique used for the separation, identification and quantification of each constituent of mixture. HPLC is an advanced technique of column liquid chromatography.[1-2] Liquid chromatography was initially discovered as an analytical technique in the early twentieth century and was first used as a method of separating colored compounds. This is where the name chromatography chroma means color, graphy means writing, was derived. A Russian botanist named Mikhail S. Tswett used a rudimentary form of chromatographic separation to purify mixtures of plant pigments into the pure constituents. He separated the pigments based on their interaction with a stationary phase, which is essential to any chroma-tographic separation. The stationary phase he used was powdered chalk and aluminia, the mobile phase in his separation was the solvent[3]

HPLC is recognized from traditional ("low weight") liquid chromatography because operational pressures are fundamentally higher (50 bar to 350 bar), while normal liquid chromatography regularly depends on the power of gravity to pass the portable stage through the segment. Because of the small sample amount isolated in scientific HPLC, column section measurements are 2.1 mm to 4.6 mm distance across, and 30 mm to 250 mm length. Additionally, HPLC segments are made with smaller sorbent particles (2 μm to 50 μm in normal molecule size). This gives HPLC high determining or resolving power (the capacity to recognize components) while isolating mixtures, which makes it a prominent chromatographic meth

HPLC has advantages like

- Simultaneous Analysis
- High Resolution
- High Sensitivity
- Good repeatability
- Small sample size
- Moderate analysis condition.
- Easy to fractionate the sample and purify. HPLC has numerous advantages like[14].

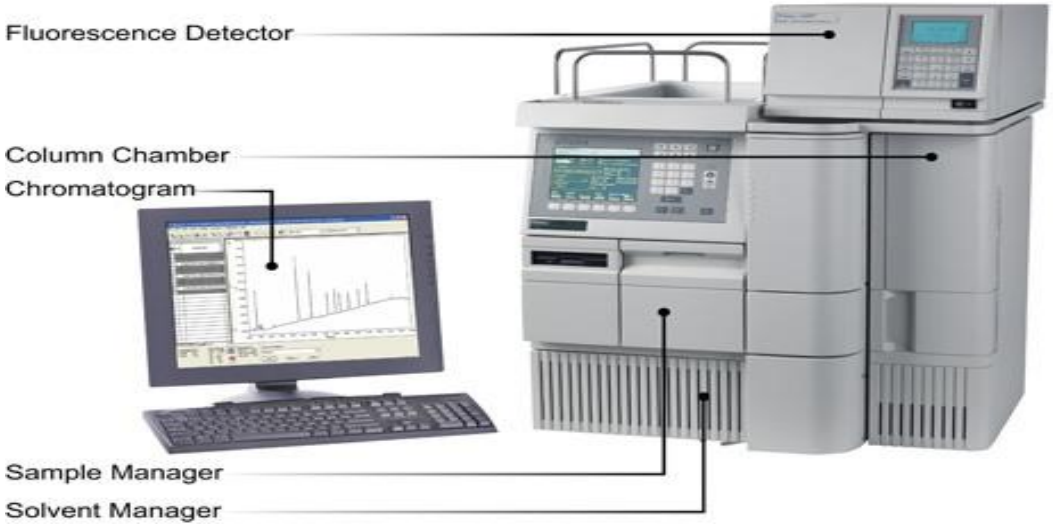

Fig:- HPLC

❖ **Different types of HPLC:-**

There are following variants of HPLC, depending upon the phase system (stationary) in the process:

1. **Normal phase HPLC:-**This method separates analytes on the basis of polarity. NP-HPLC uses polar stationary phase and non-polar mobile phase. Therefore, the stationary phase is usually silica and typical mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these.

Polar samples are thus retained on the polar surface of the column packing longer than less polar materials [15].

2. **Reversed phase chromatography :-** Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. RPC oper-ates on the principle of hydrophobic interactions, which result from repulsive forces between a polar eluent, the relatively non-polar ana-lyte, and the non-polar stationary phase. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent [16].

3. **Ion exchange chromatography:-**In Ion-exchange chromatography, retention is based on the attraction between solute ions and charged sites bound to the stationary phase. Ions of the same charge are excluded. This form of chromatography is widely used in purifying water, Ligand-exchange chromatography, Ion-exchange chromatography of proteins, High-pH anion-exchange chromatography of carbohydrates and oligosaccharides, etc [17].

4. **Size exclusion chromatography** Size exclusion chromatography (SEC), also called as gel permeation chromatography or gel filtration chromatography mainly separates particles on the basis of size. It is also useful for determining the tertiary structure and quaternary structure of proteins and amino acids. This technique is widely used for the molecular weight determination of polysaccharides [18].

5. **Bio-affinity chromatography:-**Separation based on specific reversible interaction of proteins with ligands. Ligands are covalently attached to solid support on a bio-affinity matrix, retains proteins with interaction to the column-bound ligands.

Proteins bound to a bio affinity column can be eluted in two ways:

- Bio specific elution: inclusion of free ligand in elution buffer which competeswith column bound ligand.
- A specific elution: change in pH, salt, etc. which weakens interaction protein with column-bound substrate.

Because of specificity of the interaction, bioaffinity chromatography can result in very high purification in a single step (10 - 1000-fold) [19].

II. INSTRUMETATION:-

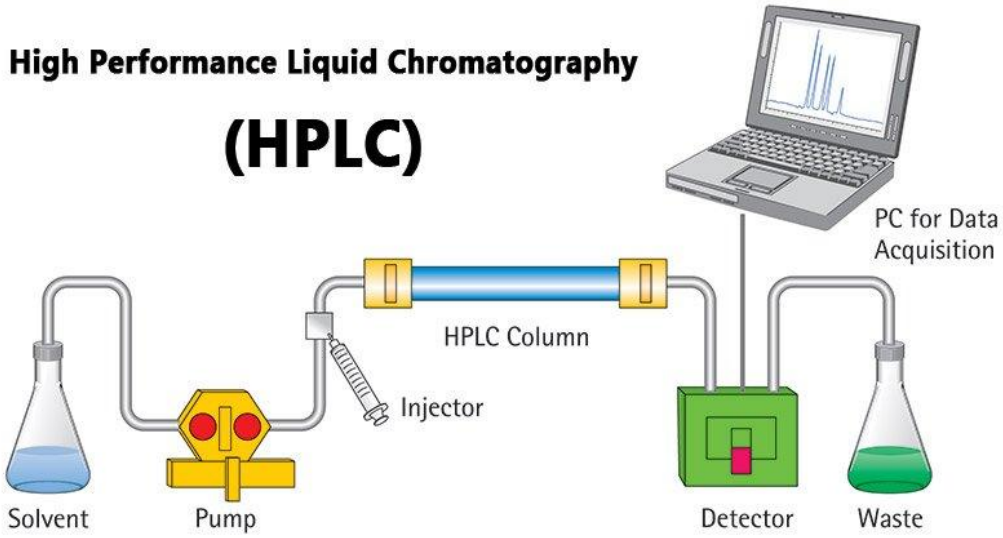

Fig. Instrumentation of HPLC

1. Mobile phase/solvent reservoir

- The reservoir that holds the mobile phase is often no more than a glass bottle. Often, the reagent bottle that holds our HPLC solvent can be used as a reservoir. Solvent is delivered from the reservoir to the pump by means of Teflon tubing -- called the "inlet line" to the pump. Some HPLC systems like the Agilent 1100 shown at the right have special compartments to hold one or more mobile phase reservoirs. The reservoirs in these systems may have additional features that allow the mobile phase to be degassed and isolated from contact with air.

2. **Solvent delivery system**

- The solvent delivery system is described like a deliver system of continuous pulse free flow of mobile phase to the HPLC regardless of the system back pressure[20].

3. **Pump**:

- The role of the pump is to force a liquid (called the mobile phase) through the liquid chromatograph at a specific flow rate, expressed in millilitres per min (mL/min).
- Normal flow rates in HPLC are in the 1- to 2-mL/min range.
- Typical pumps can reach pressures in the range of 6000-9000 psi (400- to 600-bar).
- During the chromatographic experiment, a pump can deliver a constant mobile phase composition (isocratic) or an increasing mobile phase composition (gradient).

4. **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 autosampler is the automatic version for when the user has many samples to analyse or when manual injection is not practical.

5. **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 small particles inside the column are what cause the high backpressure at normal flow rates.
- The pump must push hard to move the mobile phase through the column and this resistance causes a high pressure within the chromatograph.

6. **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).

7. Computer:

- Frequently called the data system, the computer not only controls all the modules of the HPLC instrument but it takes the signal from the detector and uses it to determine the time of elution (retention time) of the sample components (qualitative analysis) and the amount of sample (quantitative analysis)[21].

❖ **PRINCIPLE OF HPLC**

- The separation principle of HPLC is based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column).
- Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase.
- The specific intermolecular interactions between the molecules of a sample and the packing material define their time "on-column".
- Hence, different constituents of a sample are eluted at different times. Thereby, the separation of the sample ingredients is achieved.
- A detection unit (e.g. UV detector) recognizes the analytes after leaving the column. The signals are converted and recorded by a data management system (computer software) and then shown in a chromatogram.
- After passing the detector unit, the mobile phase can be subjected to additional detector units, a fraction collection unit or to the waste.
- In general, a HPLC system contains the following modules: a solvent reservoir, a pump, an injection valve, a column, a detector unit and a data processing unit.
- The solvent (eluent) is delivered by the pump at high pressure and constant speed through the system.
- To keep the drift and noise of the detector signal as low as possible, a constant and pulseless flow from the pump is crucial. The analyte (sample) is provided to the eluent by the injection valve.[22]

III. APPLICATION

- The information that can be obtained using HPLC includes identification, quantification, and resolution of a compound. Preparative HPLC refers to the process of isolation and purification of compounds. This differs from analytical HPLC, where the focus is to obtain information about the sample compound.
- **Chemical Separations** It is based on the fact that certain compounds have different migration rates given a particular column and mobile phase, the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.
- **Purification:** Purification is defined as the process of separating or extracting the target compound from a mixture of compounds or contaminants. Each compound showed a characteristic peak under certain chromatographic conditions. The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any other undesired compound.
- **Identification** Generally assay of compounds are carried using HPLC. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed.

- **Other applications of HPLC:** Other applications of HPLC includes

▪ **Pharmaceutical applications**

• Tablet dissolution study of pharmaceutical dosages form.
• Shelf-life determinations of pharmaceutical products
• Identification of active ingredients of dosage forms
• Pharmaceutical quality control

▪ **Environmental applications**

• Detection of phenolic compounds in Drinking Water
• Identification of diphenhydramine in sedimented samples
• Bio-monitoring of pollutant

- **Forensics**

• Quantification of the drug in biological samples.
• Identification of anabolic steroids in serum, urine, sweat, and hair
• Forensic analysis of textile dyes.
• Determination of cocaine and metabolites in blood

- **Clinical**

• Quantification of ions in human urine Analysis of antibiotics in blood plasma.
• Estimation of bilirubin and biliverdin in blood plasma in case of hepatic disorders.
• Detection of endogenous neuropeptides in extracellular fluids of brain.

- **Food and Flavour**

• Ensuring the quality of soft drink and drinking water.
• Analysis of beer.
• Sugar analysis in fruit juices.
• Analysis of polycyclic compounds in vegetables.
• Trace analysis of military high explosives in agricultural crops.[23-38]

IV. USES OF HPLC:-

- This technique is used for chemistry and biochemistry research analysing complex mixtures, purifying chemical compounds, developing process for synthesizing chemical compounds, isolating natural products, or predicting physical properties.
- It is also used in quality control to ensure the purity of raw materials, to control and improve process yields, to quantify assays of final products, or to evaluate products stability and monitor degradation.
- In addition, it is used for analysing air and water pollutants.
- Federal and state regulatory agencies use HPLC to survey food and drug products[39].

V. CONCLUSION:-

It can be concluded from the exhaustive review that HPLC is a multilateral**,** consistent chromatographic technique for the approximation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules.

REFERENCE:-

[1]. Rogatsky E. Modern high performance liquid chromatography and HPLC 2016 International Symposium. JChromatogr Sep Tech. 2016;7:e135.
[2]. Mulubwa M, et al. Development and validation of high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method for determination of tenofovir in small volumes of human plasma. J Chromatogr Sep Tech. 2015;6:300.
[3]. Joseph C. Touchstone (2006), History of Chromatography, Journal of liquid chromatography, volume 8 (16), 1647-1665. https://doi.org/10.1080/10826079308021679.
[4]. Akan JC, et al. Determination of organochlorine, organophosphorus and pyrethroid pesticide residues in water and sediment samples by high performance liquid chromatography (HPLC) with UV/visible detector. J Anal Bioanal Tech. 2014;5:226
[5]. Parbhunath OL, et al. Optimization and validation of a reverse-phase high performance liquid chromatography assay with ultra-violet detection for measuring total l-ascorbic acid in food and beverage products. J Anal Bioanal Tech. 2014;5:201
[6]. Szterk A, et al. Comparison of various detection systems coupled to high performance liquid chromatography for determination of tocopherols in meat. The influence and comparison of the most popular sample preparation method. J Anal Bioanal Tech. 2013;S2:005.
[7]. Lories IB, et al. High performance liquid chromatography, TLC densitometry, first-derivative and first-derivative ratio spectrophotometry for de-termination of rivaroxaban and its alkaline degradates in bulk powder and its tablets. J Chromatograph SeparatTechniq. 2013;4:202.
[8]. Chierentin L and Nunes Salgado HR. Development and validation of a simple, rapid and stability-indicating high performance liquid chromatography method for quantification of norfloxacin in a pharmaceutical product. J Chromat Separation Techniq. 2013;4:171.
[9]. Srinivasarao K, et al. Validated method development for estimation of formoterol fumarate and mometasone furoate in metered dose inhalation form by high performance liquid chromatography. J Anal Bioanal Tech. 2012;3:153.
[10]. Sun H, et al. A Rapid and effective method for simultaneous determination of residual sulfonamides and sarafloxacin in pork and chicken muscle by high performance liquid chromatography with accelerated solvent extraction–solid phase extraction cleanup. J Chromat Separation Techniq. 2012;3:154.
[11]. Virkar PS, et al. Development and validation of a high performance liquid chromatography method for determination of telmisartan in rabbit plasma and its application to a pharmacokinetic study. J Anal Bioanal Tech. 2012;3:133.
[12]. Gugulothu DB, et al. A versatile high performance liquid chromatography method for simultaneous determination of three curcuminoids in pharmaceutical dosage forms. Pharmaceut Anal Acta. 2012;3:156.
[13]. Devika GS, et al. Simultaneous determination of eprosartan mesylate and hydrochlorthiazide in pharmaceutical dosage form by reverse phase high performance liquid chromatography. Pharm Anal Acta. 2011;2:122.
[14]. Sonia K, Nappinnai M. Development and validation of HPLC and UV-visible spectrophotometric method for the pharmaceutical dosage form and biological fluid –review. European Journal of Biomedical and Pharmaceutical sciences. 2016; 3(3): 382-391.

[15]. Hamilton J.G., Comai K. (1988), Separation of neutral lipid, free fatty acid and phospholipid classes by normal phase HPLC. Lipids, (23). 1150–1153. https://doi.org/10.1007/BF02535282.

[16]. Horvath C., Melander W. (1977), Liquid Chromatography with Hydrocarbonaceous Bonded Phases; Theory and Practice of Reversed Phase Chro-matography, Journal of Chromatographic Science, 9 (15), 393–404, https://doi.org/10.1093/chromsci/15.9.393.

[17]. Gallant S.R., Vunnum S., Cramer S.M. (1996), Optimization of preparative ion-exchange chromatography of proteins: linear gradient separations, Journal of Chromatography A, 2 (23), 295-314. https://doi.org/10.1016/0021-9673(95)00909-4.

[18]. Oeyen E., Mol K. V., Baggerman G., Willems H., Boonen K., Rolfo C., Pauwels P. Jacobs A., Schildermans K., Cho W., Mertens. I. (2018), Ultra-filtration and size exclusion chromatography combined with asymmetrical-flow field-flow fractionation for the isolation and characterisation of ex-tracellular vesicles from urine. Journal of Extracellular Vesicles, 1-7. https://doi.org/10.1080/20013078.2018.1490143.

[19]. Malviya R, Bansal V, Pal O.P. and Sharma P.K. (2010), High Performance Liquid Chromatography: A Short Review, Journal of Global Pharma Technology 2(5), 22-26.

[20]. Wiese S., Teutenberg T. and Schmidt T. C. (2011), A general strategy for performing temperature-programming in high performance liquid chro-matography—Prediction of segmented temperature gradients, Journal of Chromatography A, 10. (39), 6898-6906. https://doi.org/10.1016/j.chroma.2011.08.022

[21]. https://polymer.ustc.edu.cn/_upload/article/files/23/46/8007190f4e29a2673dc0b5c292ba/P020110906263097048536.pdf

[22]. https://www.knauer.net/Application/application_notes/VSP0019_HPLC%20Basics%20-%20principles%20and%20parameters_final%20-web-.pdf

[23]. Bergh J. J., Breytenbach, J. C. Stability-indicating High-performance Liquid- chromatographic Analysis of Trimethoprim in Pharmaceuticals. J. Chromatogr. 1987; 387: 528-531.

[24]. Stubbs C., Kanfer, I. Stability-indi- cating High-performance Liquid-chromato- graphic Assay of Erythromycin Estolate in Pharmaceutical Dosage Forms. Int. J. Pharm. 1990; 3(2): 113-119.

[25]. MacNeil L., Rice J. J., Muhammad N. Lauback R. G. Stability-indicating Liquid-chromatographic Determination of Cefapirin, Desacetylcefapirin and Cefapirin Lactone in Sodium Cefapirin Bulk and Injectable Formulations. J. Chromatogr. 1986; 361: 285-290.

[26]. Bounine J. P., Tardif B., Beltran P. Mazzo D. J. High-performance Liquid-chromatographic Stability-indicating Determination of Zopiclone in Tablets. J. Chromatogr. 1994; 677(1): 87-93.

[27]. Lauback R. G., Rice J. J., Bleiberg B., Muhammad N., Hanna, S. A. 1984. Specific High-performance Liquid-chromato- graphic Determination of Ampicillin in Bulks, Injectables, Capsules and Oral Suspensions by Reversed-phase Ion-pair Chromatography. J. Liq. Chromatogr. 1984; 7(6): 1243-1265.

[28]. Wiklund A E., Dag B., Brita S. Toxicity evaluation by using intact sediments and sediment extracts. Marine Pollution Bulletin (2005); 50(6): 660-667.

[29]. Kwok Y. C., Hsieh D. P. H., Wong P. K. Toxicity identification evaluation (TIE) of pore water of contaminated marine sediments collected from Hong Kong waters. Marine Pollution Bulletin. 2005; 51(8-12): 1085-1091.

[30]. Hongxia Yu., Jing C., Cui Y., Shang H., Ding Z., Jin H. Application of toxicity identification evaluation procedures on wastewaters and sludge from a municipal sewage treatment works with industrial inputs. Ecotoxicology and Environmental Safety. 2004; 57(3): 426-430.

[31]. Ayerton J. Assay of ceftazidime in biological fluids using high-pressure liquid chromatography. J. Antimicrob. Chemother. 1981; 8: 227-231.

[32]. Bowden R.E., Madsen P.O. High- pressure liquid chromatographic assay of sulbactam in plasma, urine andtissue. Antimicrob. Agents Chemother. 1986; 30: 31-233.

[33]. Haginaka J., Yasuda H., Uno T., Nkagawa T. Alkaline degradation and determination by high-performance by high-performance liquid chromatography. Chem. Pharm. Bull. 1984; 32: 2752-2758

[34]. Fredj G., PailletAussel M. F., Brouard A., Barreteau H., Divine C., Micaud M. Determination of sulbactam in biological fluids by high-performance liquid chromatography. J. Chromatogr. 1986; 383: 218-222.

[35]. Rodenas V., Garcia M.S., Sanchez-Pedreno C., Albero M.I. Flow-injection spectrophotometric determination of frusemide or sulphathiazole in pharmaceuticals. J. Pharm. Biomed. Anal. 1997; 15: 1687-1693.

[36]. Shah A.J., Adlard M.W., Stride J.D. A sensitive assay for clavulanic acid and sulbactam in biological fluids by high-performance liquid chromatography and precolumn derivatization. J. Pharm. Biomed. Anal. 1990; 5: 437-443.

[37]. Abidi S.L. High-performance liquid chromatography of phosphatidic acids and related polar lipids. J.Chromatogr. 1991; 587: 193-203.

[38]. Christie W.W., Gill S., Nordbäck J., Itabashi Y., Sanda S., Slabas A.R. New procedures for rapid screening of leaf lipid components from *Arabidopsis*. Phytochemical Anal. 1998; 9: 53-57.

[39]. https://www.google.com/url?sa=i&url=https%3A%2F%2Fslideplayer.com%2Fslide%2F9274088%2F&psig=AOvVaw0BJZNvOr-F9BFjT1KUjLbH&ust=1628150153621000&source=images&cd=vfe&ved=0CAsQjRxqFwoTCNjqtZDylvICFQAAAAAdAAAAABAD.