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A review on high performance liquid chromatography technique for pharmaceutical analysis

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ABSTRACT
HPLC is an analytical approach extensively used for identification, separation, spotting and quantification of various medicines and its affiliateddegradants. Applicable mobile phase, stationary phase, column, column size, temperature, wavelength and grade selection is importantfor the suitable comity and stability of pharmaceutical as well as contaminants and degradants. Most of the medicines as well as other compounds canbe assayed by HPLC method because of the several advantages like rapidity, specificity, accuracy, precision and ease of automation inthis method. This article containing different aspects of Chromatographic technique including characteristics, instrumentation, important parameters and various applications of HPLC in different fields.

I. INTRODUCTION

High- performance liquid chromatography or High pressureliquid chromatography(HPLC) is a specific form of columnchromatography generally used in biochemistry and analysis to separate, identify, and quantify the active compounds.HPLC is the system of choice for checking peak purity ofnew chemical substances, covering reaction changes insynthetic procedures or gauge up, appraising newPharmaceutical formulation and carrying out quality control/ assurance ofthe final medicine products.HPLC is now one of the most important tools inanalytical chemistry. It has the capability to separate, identify,and quantify the composites that are present in any samplethat can be dissolved in a liquid. HPLC is the most accurateanalytical approaches broadly used for the quantitative as wellas qualitative analysis of medicine product and used fordetermining medicine product stability.In HPLC, the essential equipment consists of an eluent,reservoir, a high- pressure pump, and an injector forintroducing the sample, a column containing the stationaryphase, a detector and annalist.The development of broadlyeffective micro particulate related phases has increased theversatility of the approach and has greatly enhanced theanalysis of multi component syntheses.

Principle[3-7]

HPLC principle is that solution of sample is injected into a column of passable material(stationary phase) and liquid phase(mobile phase) is pumped at high pressure through the column. The principle of separation followed is the adsorption of solute on stationary phase rested on its affinity towards stationary phase.HPLC is a special 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 ordersof magnitude relative to classical column chromatography and the use of much lower particles of the adsorbent or support becomes possible amplifying the column effectiveness mainly.

Types of HPLC

1. **Normal Phase chromatography**

In NP- HPLC the nature of stationary phase is polar and mobile phase isnon-polar. In a combination of elements to be separated, those analytes which are fairly more polar will be retained by the polar stationary phase longer than those which are fairly less polar. hence the least polar component will elute first. The attractive forces which are generally dipole- dipole and hydrogen bonding interaction.It is first choice for mixtures of isomers and for preparative scale HPLC and second choice for lipophilicsamples that cannot dissolve well in water-organic mixtures.[8-9]

2. **Reversed Phase Chromatography**

RP- HPLC has anon-polar stationary phase and polar or fairly polar mobile phase. RP- HPLC is grounded on the principle of hydrophobic interaction. In a combination of elements, those analytes which are moderately less polarwill be retained by thenon-polar stationary phase longer

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than those which are moderately more polar. thus the most polar element will elute first.11 Molecules that retain some degree of hydrophobic character can be separated by reversed phase chromatography with excellent recovery and resolution.[10]

3. **Size Exclusion Chromatography**

SEC, also called as gel permeation chromatography or gel filtration chromatography mostly separates molecules on the base of size. The column is filled with material having precisely controlled notch sizes, and the sample is simply screened or filtered. Larger particles are quickly washed through the column; lower particles pierce inside the penetrable of the packing particles and elute afterward . This technique is comprehensively used for the molecular weight determination of polysaccharides.[11]

4. **Ion Exchange Chromatography**

In Ion- exchange chromatography, retention is grounded on the attraction between solute ions and charged spots bound to the stationary phase. This methodology is used nearly solely with ionic or ionizable samples. The stronger the charge on the sample, the stronger it'll be attracted to the ionic shell and therefore, the longer it'll take to elute.[11]

5. **Bio-Affinity Chromatography**

Separation Grounded on specific reversible dealings of proteins with ligands. Ligands are covalently affixed to solid support on abio-affinity matrix, retains proteins with dealings to the column- bound ligands.[12]

Mode of separation[13]

There are two modes of separation in HPLC technique/method:

1. Isocratic

In this mode the separation includes continuous eluent the composition and maintain the equilibrium condition in the column and the velocity of compound moving through the column at constant speed. Here the peak capacity is less and the longer component is stayed on the column the wider its peak.

2. Gradient

In this type of mode, the separation contain different eluent composition, this help in increasing the separation power of the system mainly due to increase of the efficiency. Peak width changes depending on the rate of the eluent composition variation**.**

Instrumentation[14-15]

The required equipment consists of a high-pressure pump,and an injector for acquainting the sample, a column containing the stationary phase, a detector and recorder.

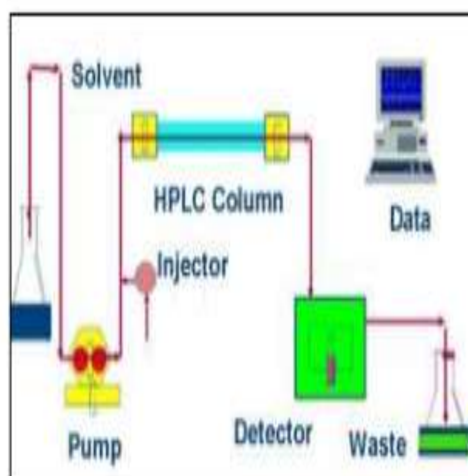

Figure:1 flow diagram of instrumentation of HPLC

Injection of the Sample

Septum injectors are available; using which sample solution is injected. An injector(sample injector or autosampler) is suitable to introduce(inject) the sample into the continuously flowing mobile phase trough that carries the sample into the HPLC column. A new advanced rotary cock and loop injector can be used to produce reproducible results. Typical sample volumes are 5- to 20- microliters(μl).

Pump

A high- pressure pump(solvent delivery system or solvent reservoir) is used to induce and measure a specified inflow rate of mobile phase, generally milliliters per minute. The pump suctions the mobile phase from solvent reservoir and forcesit to column and also passes to detector. The operating pressure depends on column confines, particle size, inflow rate and composition of mobile phase. Normal inflow rates in HPLC are in the 1 to 2ml/ min range. Typical pumps can reach pressures in the range of 6000- 9000 psi(400- to 600-bar).

Columns

It's where the effective separation takes place. The column contains the chromatographic packing material required to effect the separation.

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This packing material is called thestationary phase because it's held in place by the column tackle. It's stainless steel tube 5- 25 cm in length and 2-cm internal diameter. Material of packing is superficially passable or fully penetrable.

Detector

The detector can determine(discover) the individual particles that come out(elute) from the column. A detector serves to measure the volume of those particles so that the analyst can quantitatively analyze the sample elements. Thedetector provides an produce to a recorder or computer thatresult in the liquid chromatogram(i.e., the graph of thedetector response).There are several ways of detecting when a substancehas passed through the column. Generally, UV spectroscopyis attached, which discover the specific composites. Multipleorganic compounds absorb UV light of multi-colouredwavelengths. The volume of light absorbed will depend onthe volume of a particular compound that's passing throughthe ray at the time.

System Suitability Parameters of HPLC
System Resolution

System resolution = 2(t2 – t1)/ (W2 + W1) Where t2 and t1, are the retention times of the two components and W2 and W1 are the corresponding peak widths. The resolution factor should be greater than 2.00 for two HPLC peaks.

Determination of System Precision

After a standard solution is injected a number of times, the relative standard deviation of the peak responses is measured as either the peak height or peak area. When using an internal standard method, the response ratio is calculated.Maximum allowable system related standard deviations made at the 99% confidence level have been tabulated. For the USP monographs, five replicate chromatograms are used if the stated limit for relative standard deviation is 2% or less. Six replicate chromatograms are used if the stated relative standard deviation is more than 2.0%.

Asymmetry Factor or Tailing Factor

The increase in the peak asymmetry is responsible for a falloff in chromatographic resolution, detection limitsand precision. Measurement of peaks on solvent tails should be avoided. The peak asymmetry factor or tailing factor can be calculated by following formula: T = W0.05/ 2f, Where W0.05 is the width of the peak at 5 peak height.

Column Efficiency

Column efficiency is generally determined by calculating the number of theoretical plates for a column. It is mainly required for the assay of antibiotics and antibiotic containing drugs.
N = 5.545 (t / Wh/2) 2 Or N = 16(t / W)2
Where t is the retention time of the analyte and
Wh/2 is the peak width at half-height or
W is the width at the base of the peak. The height equivalent to one theoretical plate is calculated by h = L / n Where L is the length of the column.

Column Capacity (Capacity factor / Retention factor)

The column capacity factor is figured by K = (tr – tm)/tm,Where the retention time of the solute is tr and the retention time of solvent orun retained substance is tm. Retention volumes are occasionally preferred, because trvaries with inflow rate. The factor is also calculated by V = Vr – Vm)/ Vm, Where Vr is the retention volume of the solute and Vm is the elution volume of an unretained substance.

Application of HPLC
HPLC industry Applications

There's a wide variety of applications throughout the process of creating a new medicine from drug discovery to the manufacture of pharmaceutical formulation that will be administered to patients.

Pharmaceutical applications:[18]

1. Tablet dissolution study of the pharmaceutical dosage form.
2. To control drug stability, Shelf-life determination.
3. Identification of active ingredients.
4. Pharmaceutical quality control.
5. Tablet dissolution of pharmaceutical dosage forms.

Food and Flavour analysis[17]

1. Rapid screening and analysis of components in nonalcoholic drinks.
2. Measurement of quality of soft drugs and water.
3. Sugar analysis in fruit juices.
4. Analysis of polycyclic compounds in vegetables.

5. preservative analysis.
6. Multiresidue analysis of lots of pesticides in food samples by LC triple quadrupoleMS.

Forensics applications:[18]

1. Quantification of the drug biological samples.
2. Identification of anabolic steroids in serum, urine, sweat & hair.
3. Forensic analysis of textile dyes.
4. Determination of cocaine and other drugs of abuse in blood, urine, etc.
5. Determination of benzodiazepines in oral fluid using
LC/MS/MS

Pharmaceutical drug discovery analysis

Developing a quickly, generic method for fast resolution Liquid chromatography with quadrupole MS detection. Fast, general LC/ MS method enables medicine analysis in lower than one minute.

Recent applications[19]

Analytic method developmentand validation are key elements of any pharmaceutical development program.HPLC analysis method is developed to identify, quantity or purifying compounds of interest. HPLC helps a lot in stability studies of drug formulations. HPLC helps a lot in stability studies of atropine, antibiotics, &biotechnologybased drugs like insulin, streptokinase, etc.

1. It is used in inorganic chemistry for separating anions & cations.
2. It is used in forensic science for the separation of phenyl alkylamines (morphineand its metabolites) from blood plasma, and for the detection of poisons or intoxicants such as alcohol, carbon monoxide, cholinesterase inhibitors, heavy metals, hypnotics, etc.
3. It is used in environmental studies for analyzing the pesticide content in drinking water.
4. It is utilized in food analysis for separating watersoluble and fat-solublevitamins from variety of food products, fortified food and animal feed.
5. It is also used for determining antioxidants and preservatives present in the food.
6. It is used in the cosmetic industry for the assay and quality control of various cosmetics like lipsticks, creams, ointments, etc.
7. It is used for separating various components of plant products with bear structural resemblance.
8. It is used in the agrichemical industry for the separation of herbicides.
9. It is used in the separation and analysis of amino acids, carbohydrates, proteins, lipids and steroidal hormones.
10. It is used for separating coal and oil products from their crude sources.
11. It is used for separation and identification of Psychotropic drugs such as antidepressants, benzodiazepines, butyrophenones, neuroleptics, phenothiazines, etc.
12. It can be used for determining the stability of various pharmaceuticals. This is done by analyzing the degradation products of the drugs Eg: Stability studies of atropine
13. It can be used in bioassays of compounds like chloramphenicol,Cotrimoxazole,Penicillins, peptide hormones, and sulphonamides.
14. It is used for controlling microbiological processes used in the production of the number of antibiotics such as chloramphenicol, tetracyclines, and streptomycins.
15. It is used for monitoring the course of organic synthesis and also for isolating products in the reaction.
16. It gives an idea about the biopharmaceutical properties of a dosage form and the pharmacokinetics of the drugs. Thus, it is used in dosage form design.
17. It is utilized as an analytical method for numerous natural and synthetic drugs. It is used in different levels of pharmacy and pharmacology.

II. CONCLUSION:

High- performance liquid chromatography is just thepremier technique for Trace analysis of organic and inorganic composites. Determination of trace compositesis veritably important in medicinal, biological, toxicologyand environmental studies since indeed a trace substance canbe dangerous or toxic. HPLC is applied for molecularweight determination, in logical chemistry,pharmaceutical and medicine science, clinical sciences, foodtechnology, and consumer products, combinatorialchemistry, polymer chemistry, environmental chemistryand green chemistry. The role of HPLC in thepharmaceutical industry is veritably vital particularly inpreformulation, process development, during formulationdevelopment and drug discoveryand to corroborate medicine purity.All the work which has it being done in pharmaceuticalsubstances, Preparation of pure composites, trace analysis,food safety where we've to analyze for fungicides andpoisonous chemicals founds in food and food products all ofthese things are done routinely and

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daily quickly by high performance liquid chromatography.

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