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REVIEW article

The role of High-Performance Liquid Chromatography in the pharmaceutical analysis

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Abstract: High-Performance Liquid Chromatography (HPLC) is a frequently utilized analytical method that has numerous characteristics, including high selectivity, sensitivity, and a reduced time requirement. Upon using this fabulous technique, the limit of detection is reduced, and the accuracy, precision, reliability, and specificity can be expanded. This technique plays a crucial role in both qualitative and quantitative analysis throughout the various stages of drug production, from the initial discovery of the drug to its excretion from the body. The role of High-Performance Liquid Chromatography (HPLC) in pharmaceutical analysis during various stages of drug discovery, production, and clinical use, including drug pharmacokinetics, is reviewed and discussed in detail. This provides an overview of the benefits of High-Performance Liquid Chromatography from the chemical, pharmaceutical, and clinical perspectives.

Introduction

High-Performance Liquid Chromatography (HPLC) is a critical analytical method in pharmaceuticals for qualitative and quantitative drug analysis, covering over 90.0% of drugs listed in official pharmacopeias [1]. It is considered an essential analytical tool for assessing drugs in pharmaceutical products [2]. HPLC plays a crucial role in the discovery, development, and manufacture of pharmaceutical drugs, and is utilized in various studies related to humans and animals [3]. HPLC has replaced classical methods, such as filtration, offering speed, precision, and the ability to analyze various compounds simultaneously [4, 5]. Its main applications in pharmaceuticals include assessing drug purity, potency, and pharmacokinetics, as well as quality control throughout drug development and manufacturing processes [6]. HPLC operates by separating components based on their distribution between a stationary and mobile phase [7]. Types of HPLC vary by separation mode (normal and reversed phase), the principle of separation (ion exchange and size exclusion), elution technique (isocratic or gradient), and scale (analytical or preparative). Key system components include solvent reservoirs, degasser, pump, injector, column, detector, and data acquisition devices, each playing a role in the effective separation, detection, and analysis of compounds [5, 8-10]. This study provides a brief review that focuses on the significant role of HPLC in drug analysis and pharmaceutical applications over the last decade.

Role of HPLC in drug analysis: During the previous two decades, the most characteristic highlight of the advancement in the methodology of drug and biomedical analysis is that HPLC has turned out to be without a doubt the main insightful technique for the identification and quantification of medications [7, 11-13]. This

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method has become the main technique in the quality control of bulk medications and drug formulation, even at the pharmacopeial level [11]. In addition, to get the high affectability and selectivity of the system, the HPLC is combined with Mass Spectrometry (HPLC/MS) or with Liquid Chromatography/Mass Spectroscopy (LC/MS), which have become the overwhelming technique in bioassays, pharmacokinetics, and metabolic studies, such as in the design clarification of medication impurities and degradation items [11, 14]. The HPLC has been utilized to tackle no under half of the issues, leaving the other half to around 15.0% to other chromatographic, spectroscopic, and different methods, about 10.0% to Gas Chromatography (GC), 5.0% to Thin-Layer Chromatography (TLC), 10.0% to Ultraviolet (UV) Spectrophotometry and the rest to electro-analytical methods [11, 14]. To understand the role of the HPLC analytical technique in pharmaceutical analysis, it is necessary to think about its applications in the pharmaceutical field [12].

The main consistent application of HPLC in pharmaceutical analysis is the determination of active pharmaceutical ingredients (API); moreover, it assesses impurity and degradation of the drug substances and drug products. The HPLC also utilizes the improvement of the drug formulations [12, 15], as well as the extra employments of HPLC, including the determination of content uniformity of dosage forms, determination of antioxidant and microbial preservative content, and support of cleaning validations [15].

The second significant application of HPLC in pharmaceutical analysis is the determination of enantiomeric impurities in drugs administered as unadulterated enantiomers [16]. The more prominent change in the pharmacopeias in the previous years has been the expanding significance of purity tests. At the start, just a small number of monographs contained tests identified with impurities. Because of the advancement of TLC and HPLC, as of now, a staggering larger part of the monographs on bulk drugs and, to a great extent, those on definitions contain these tests. The impurity profile has become the foremost informative indicator of the standard of bulk drug materials. Simultaneously, the significance of testing bulk drugs has diminished extensively; there are conclusions that even this significance is questionable [14]. For instance, vitamin D in drug substances has been controlled by numerous methods (chromatographic and other alternative techniques) that are categorized into biological and chemical determination. A large portion of these methods incorporates complex stages, time-consuming, and failure to segregate between vitamin D forms. Notwithstanding its lack of selectivity, accuracy, and precision, it is due to ingredients within the formulation. However, in any case, the HPLC technique was broadly utilized for the examination of lipid-soluble vitamins from a sample. Somewhat recently, the HPLC was declared the most reasonable method for the determination of vitamin D in food, baby formula, and pharmaceutical preparations. It is a basic, quick, comparatively low price and direct HPLC strategy that is represented for the determination of vitamin D_2 and vitamin D_3 in their business pharmaceutical preparations without purification [17].

The third extensive application of HPLC in pharmaceutical analysis is the analysis of natural drugs. High separation effectiveness, speedy analysis, good sensitivity, and a wide choice of chromatographic columns and mobile phases have been created in the HPLC technique, which is common within the Chinese Pharmacopeia. The use of the HPLC was initially restricted by the detection technology, which needed the ultraviolet radiation absorption of the test compounds in the beginning phase. With the advancement of various kinds of HPLC detectors, the applicability has expanded extraordinarily to meet the various quality control functions of ancient Chinese Medicine [18]. The HPLC is utilized for isolating different parts of plant items with similar underlying structures, for example, analysis of cinchona, digitalis, ergot extracts, and licorice [4].

The fourth extremely useful application of HPLC in pharmaceutical analysis is the evaluation of drug stability. It is specific, quick, vigorous, and sensitive. The special properties of the HPLC incorporate different detection wavelengths that may be set for detection, adjustable flow rate, and the mobile phase elution profile. The HPLC could, at the same time, identify numerous analytes in pharmaceutical formulations. It is immensely

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utilized as a stability-indicating assay for bulk medicine and drug products, separating medication substances and degradation impurities at the same time. For example, it recently developed a stability-indicating assay utilizing the HPLC to separate fourteen impurities from the Excedrin pill, which comprised acetaminophen, aspirin, and caffeine by gradient elution. The separation was wonderful, and also the peaks were consummately resolved within the chromatogram. Many studies have announced that the HPLC showed promising sensitivity, reliability, linearity, accuracy, precision, repeatability, robustness, limit of detection, and limit of quantification, and it is very valuable to be utilized as a stability-indicating technique for different sorts of pharmaceutical ingredients and products. This can additionally be the explanation why HPLC is a popular method utilized in drug stability analysis [19]. For instance, a simple, speedy, and precise HPLC technique is developed for the simultaneous determination of atorvastatin, ezetimibe, and fenofibrate in their ternary mixture of business pharmaceutical preparations. The HPLC technique is successfully applied to the synchronous quantitative measurement analysis of the medicine in tablets [20]. The reason for HPLC analysis of any medicine is to verify the identity of a drug and supply quantitative results, and additionally to monitor the advancement of the treatment of an illness. It might likewise be utilized to further our comprehension of the normal and illness methods within the human body through biomedical and therapeutical analysis throughout the examination before the medicine registration [21].

The fifth advanced application of HPLC in pharmaceutical analysis is an examination of medications and their metabolites in body fluids. To undertake the analyses of medication and metabolites in body fluids the analyst is faced with many issues, The primary issue is because of the complex nature of the body liquid, the medications should be segregated by an extraction method, that ideally ought to give a comparatively spotless extract, and therefore the separation system should be capable of breakdown the medicine of interest from co-extractives. All referenced when we are utilizing the HPLC system, in this case, it requires great determination of detectors, a good stationary phase, eluents, and a satisfactory program [21].

The sixth progressive application of HPLC in pharmaceutical analysis is the separation and quantification of enantiomeric mixtures. The separation and quantification of enantiomeric mixtures are among the most difficult tasks in drug and biomedical analysis. The fundamental issues to be addressed are to decide the enantiomeric purity of medications being utilized in treatment as unadulterated enantiomers and the concurrent determination of the elements of race-mates in the biological samples. The current circumstance will be described by the spread of this method and, thus, the continuous development and commercialization of recent kinds of chiral HPLC columns [9, 11, 14]. Isomer separation is very necessary for drug substances since isomers can actually have different pharmacological and, in some cases, toxicological properties [22]. It can be mentioned that the enantiomers are kinds of isomers that have aroused a good interest once the forceful agent effects of the drug sedative-hypnotic (children born to mothers who had been taking sedative-hypnotic were born with truncated limbs) were attributed to at least one of the two enantiomers present within the drug substance. Enantiomers have indistinguishable physicochemical properties, and in this way, cannot be isolated by traditional chromatography. Therefore, in the liquid chromatography separation of enantiomers, it is important to utilize what is called the chiral selector. This might take the form of a chiral derivation agent, a chiral mobile phase additive, or a chiral stationary phase [23].

The seventh advantageous application of HPLC in pharmaceutical analysis is the study of the interactions of drugs. It is used for the study of the interactions between small molecules and large molecules, specifically to study drug-protein binding. Many specialists have utilized immobilized human serum albumin phase to study the interaction of drugs such as benzodiazepines, warfarin, ibuprofen, and others. The utilization of this stage as a model of interactions that occur *in vivo* could also be taken a step further by adding a retardant to the mobile phase, which has the potential to check how the interaction of one drug with human serum albumin is influenced by the presence of another [23].

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In the trendy pharmaceutical industry, HPLC is the major and integral analytical device applied toward making another medication from drug discovery to the manufacture of formulated products, which in turn will be administered to patients. This method to create a new drug will be divided into three main stages [4, 21].

(i) *Drug discovery:* The objective in the discovery phase of medication improvement is to find another, safe, and active chemical that will become a medication for the illness. Throughout the last decade, parallel synthesis of potential lead compounds utilizing combinatorial science has been done [7]. The enormous number of products made by combinatorial chemistry is then distinguished by quick LC-MS techniques and screened by *in vitro* bioassays and, additionally, pharmacological or chemical tests to permit the selection of a few chosen drug candidates. At the point when the lead compounds are chosen, the subsequent stages include pharmacological studies. Because of its high sensitivity and selectivity, HPLC coupled with tandem mass spectrometry (HPLC-MS/MS) has become an overwhelming technique in bioassays and pharmacokinetic and metabolic studies [4, 7].

(ii) *Drug development:* The advancement stage is the part during which some of the HPLC techniques that will be utilized throughout the ensuing manufacturing stages are created, approved, and then transferred [7].

(iii) *Drug producing:* This can be explained in the following points:

a. Drug identification: The identification test by the HPLC is aimed at verifying the identity of the active pharmaceutical ingredient within the samples of either drug substance or drug product. Two independent tests are required, for instance, one chromatographic and one spectroscopic. Thus, a chromatographic run with a diode-array or MS detector will give both parameters, the retention time in the chromatogram and the UV or MS spectrum of the eluting peak, matched against a known standard [7].

b. Assay and content uniformity: An assay of a sample containing either drug substance or drug product quantitatively by the HPLC estimates the actual quantity of the active ingredient compared to that expected within the medication substance test, whereas, in drug products, the actual quantity measured is verified against the label claim. The official assays are described in the pharmacopeias, such as the United States Pharmacopeia (USP). A content uniformity is analogous to the drug product assay; however, an individual solid dosage form is tested rather than a pool of the tablets/capsules as within the assay tests [7].

c. Dissolution: The dissolution test estimates the discharge of the drug substance from its dosage form into a dissolution bath below physiological conditions, and therefore, the permeability across the gastrointestinal tract. Owing to the important nature of the first two steps, *in vitro* dissolution could also be relevant to the prediction of *in vivo* performance [7, 23]. Since most drugs have absorption in the UV-VIS range, their dissolution has been traditionally measured by UV-VIS spectrum analysis and HPLC-UV. The benefit of the HPLC over ultraviolet radiation spectrum analysis is its separation abilities, providing higher specificity and sensitivity as well as its relevance in formulations with multiple APIs or extremely low doses [7]. The HPLC gives a thought regarding the biopharmaceutical properties of the dosage form and the pharmacokinetics of the medications. Accordingly, it is utilized in dosage form design. It is used as an insightful technique for various natural and manufactured medicines. In other general words, HPLC is utilized in various degrees of pharmacy and pharmacology [4].

d. Drug Impurities: Numerous potential impurities result from the API-producing method, as well as beginning materials, isomers, intermediates, reagents, solvents, catalysts, and reaction by-products; therefore, impurity control is crucial throughout the method of drug development and production. The HPLC has become a significant procedure during this process [7]. It gives us a structure clarification of impurities and a fast condition exploratory survey for technique development [4].

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e. Drug Stability: The investigation of the stability of drugs is important as a result of the need to stay away from the possibly poisonous degradation products [23]. Stability testing of drug substances or products is performed to make sure that their quality does not vary with time under the influence of a range of environmental factors like temperature, humidity, and light [7, 19, 23]. Additionally, if degradation happens, it will be important to identify and quantify the degradation products. A good clarification of this can be the liquid chromatography conditions developed for the determination of pilocarpine in ophthalmic solutions. The identical conditions are utilized for the determination of pilocarpine degradation products, i.e., isopilocarpine and pilocarpic acid [4, 7, 23]. The study of stability is not restricted to watching the degradation of the active. It is prudent to keep in mind that any degradation of formulation excipients could result in an amendment to the drug release characteristics of the formulation. An example is lactose, which is often utilized as an excipient with anomerisation in resolution between its α and β forms [23].

Almost altogether, the laboratories of the quality control of medications, the official ways for the assay of antibiotics are microbiological estimations. Presently, the HPLC technique will be utilized for the quantitative determination of antibiotics. That is a good advantage within the field of quality control of medicine [23]. It is used for controlling microbiological processes utilized in the production of many antibiotics such as chloramphenicol, tetracyclines, and streptomycin [4]. The HPLC is the technique of choice for checking the peak purity of the latest chemical entities, observing reaction changes in synthetic procedures or scale-ups, evaluating new formulations, and finishing up internal control/assurance of the final drug products [17]. The HPLC is employed in the separation and analysis of amino acids, carbohydrates, proteins, lipids, and steroidal hormones. It is used for the separation and identification of psychotropic drugs like antidepressants, benzodiazepines, butyrophenones, neuroleptics, phenothiazines, etc. Additionally, it is utilized in bioassays of compounds like chloramphenicol, cotrimoxazole, penicillins, peptide hormones, and sulphonamides [4]. Nowadays, within the Pharmacopoeias of the USA, Europe, Britain, and other countries, the high-performance liquid chromatography process is employed rather than chemical and many instrumental methods for the management of medicine [23].

Conclusion: The HPLC is a frequently utilized analytical method that has numerous positive characteristics, including high selectivity, sensitivity, and reliability. The HPLC may be utilized for the habitual analysis of the natural and artificial compounds in the pharmaceutical formulations and in the bulk drug preparations, in addition to the quality assurance of the associated extracts and marketplace samples. The role of the HPLC in the drug industry is essential, especially in pre-formulation, process improvement, formulation advancement, medication discovery, and checking drug purity. The important roles of the HPLC are controlling the raw materials and controlling the finished products, ensuring the safety of the people.

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