Application Of HPLC In Pharmaceutical Industry

ABSTRACT

High-performance liquid chromatography (HPLC) is a chromatographic process used in the fields of analytical chemistry, biochemistry and industrial separation of a mixture of compounds. The key objectives for using HPLC are the detection, quantification and purification of the mixture's specific components.HPLC plays an important and critical role in the pharmaceutical industry as it is used to test the products and detect the raw ingredient used to make them, i.e. qualitative and quantitative analyzes. In addition, the value of HPLC uses in these fields comes under the U.S.-stringent regulations. Health and Drug Agency (FDA). This requires all pharmaceutical companies to detect their product quality by using the HPLC before allowing them to sell it on the global market.The most important advantages gained from industrial and analytical uses of HPLC technique are that it helps in structural elucidation and quantitative determination of impurities and degradation products in bulk drug materials and pharmaceutical formulations. These benefits that gain from using HPLC for synthetic drugs and formulas are not only limited, but also include herbal medicine.

INTRODUCTION

Liquid chromatography is an analytical technique widely employed in the pharmaceutical sector. All manufactured products have to be of the highest quality in the pharmaceutical industry to ensure the lowest risk to patients. During the development process, researchers, manufacturers and developers use numerous technology equipment and analytical techniques, including liquid chromatography, to ensure that the goods pass certain standards. Liquid chromatography is an analytical procedure used to isolate a specified sample into its elements. The separation takes place when the sample comes into contact with both the mobile (liquid) and stationary (column) phases.Based on their polarities, the various sections of the sample are separated; they should have differing degrees of preference for the mobile process, resulting in column migration at specific velocities. The mixed components are placed atop the stationary phase column, which is usually a fine adsorbent solid such as silica. This must be evenly distributed to minimize the presence of air bubbles which may influence the test results. The column exit is stopped with glass, wool, or a porous plate. The mixture seperates into bands when the mobile phase passes through. Then, these can be collected and analyzed using other methods.The method works as the components in a mixture are drawn to the adsorbent surface of the stationary phase with different degrees based on the individual polarity and unique structural features; a component with a higher affinity for the stationary phase will migrate quieter down the column than a component that has more affinity for the mobile phase. High-performance liquid chromatography (HPLC) is the most common form of liquid chromatography in use today which pumps the sample mixture at high pressure via the column[1].

The method of extracting the chemical substances that are in the sample is liquid chromatography. Those chemical compounds can then identify what is and quantitate what it is. Due to its speed, column stability, and ability to separate a wide range of compounds, Reversed-phase HPLC (RP-HPLC) is one of the more popular methods. There are two variants of HPLC in the cycle, depending on the (stationary) step scheme. This approach distinguishes analytes

according to polarity. NPHPLC uses stationary phase polar and mobile phase non-polar.The stationary phase is indeed usually silica, with typical mobile phases being hexane, methylene chloride, chloroform, diethyl ether and mixtures of these. Therefore, polar samples are retained on the polar surface of the packing column longer than less polar materials. In the situation of Reverse Phase HPLC the stationary phase is in nature nonpolar (hydrophobic), while the mobile phase is a polar liquid, such as water, methanol, acetonitrile (or) mixtures. It relies on the basis of hydrophobic interactions hence the longer it is retained, the more nonpolar the material is. The most popular mode of chromatography is by far the reversed-phase HPLC.Nearly 90 percent of all low molecular weight sample analyzes are performed using RP HPLC. The following table offers a description of a large range of pharmaceutical drugs' specific analytical parameters such as substance, column form, mobile phase structure, flow rate, and form of detector. The main pharmaceutical applications with HPLC are shown in Table 1. They are listed by the order A to Z[2].

HPLC INDUSTRY APPLICATIONS

There is a wide variety of applications throughout the process of creating a new drug from drug discovery to the manufacture of formulated products that will be administered topatients. This Process to create a new drug can be divided into 3 main stages

- Drug discovery
- Drug development
- Drug manufacturing.

LC-MS is the best tool for compound identification and characterization. It may be used as a measurement tool during high throughput screening. Preparative HPLC is also used to isolate and purify hits and lead compounds as required. Eg: a combinatorial synthesis. The ability to prove purity of enantiomeric molecules is a standard in pharmaceutical assays, for which HPLC is suitable[3].

PHARMACEUTICAL APPLICATIONS

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

Food and Flavor analysis

- Rapid screening and analysis of components in non alcoholic drinks.
- Measurement of quality of soft drugs and water.
- Sugar analysis in fruit juices.
- Analysis of polycyclic compounds in vegetables.
- preservative analysis.
- Multiresidue analysis of lots of pesticides in food samples by LC triple quadrupole MS.

Environmental applications

- Detection of phenol compounds in drinking water.
- Identification of diphenhydramine in sedimented samples.
- Bio-monitoring of pollutant.
- Rapid separation and identification of carbonyl compounds by HPLC.
- LC/MS/MS solution for pharmaceuticals and personal care products in water, sediment, soil and biosolids by HPLC/MS/MS.
- Determination of 3-mercaptopropionic acid by HPLC6.

Forensics applications:

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

Clinical applications:

- Catecholamines such as epinephrine and dopamine are highly important for many biological functions. Analyzing their precursors and metabolites can provide diagnosis of diseases such as Parkinson’s disease, heart disease, and muscular dystrophy.
- Quantification of ions in human urine analysis ofantibiotics in blood plasma.
- Estimation of bilirubin &biliviridin in blood plasma incase of hepatic disorders.
- Detection of endogenous neuropeptides in extracellularfluids of the brain.

Pharmaceutical impurity profiling analysis

- Structure elucidation of impurities with LC/MS.
- Rapid condition scouting for method development.
- Using a fast LC method for higher sample throughput.

Pharmaceutical drug discovery analysis

Developing a fast, generic method for rapid resolutionLiquid chromatography with quadrupole MS detection.Fast, generic LC/MS method enables drug analysis in lessthan one minute[4].

RECENT APPLICATIONS

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

1. It is used in inorganic chemistry for separating anions& cations.
2. It is used in forensic science for the separation ofphenyl alkylamines (morphine and its metabolites)from blood plasma, and for the detection of poisonsor intoxicants such asalcohol, carbon monoxide,cholinesterase inhibitors, heavy metals, hypnotics,etc.

3. It is used in environmental studies for analyzing thepesticide content in drinking water
4. It is utilized in food analysis for separating watersoluble and fat-soluble vitamins from variety of foodproducts, fortified food and animal feed.
5. It is also used for determining antioxidants andpreservatives present in the food.
6. It is used in the cosmetic industry for the assay andquality control of various cosmetics like lipsticks,creams, ointments, etc.
7. It is used for separating various components of plantproducts with bear structuralresemblanceEg: Analysis of cinchona, digitalis, ergot extracts andlicorice.
8. It is used in the agrichemical industry for theseparation 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.
18. In production, development and product control it is used in nucleic acids research for numerous purposes like

- a. For studying the regulatory effects of cyclic nucleotides.
- b. For determining the composition of hydrolysates of nucleic acids
- c. For studying the diseased processes.
- d. For metabolite profiling of normal and diseased subjects
- e. For separation and purification of nucleic acids[5].

HOW IS LIQUID CHROMATOGRAPHY USED IN PHARMA?

HPLC is the form of liquid chromatography usually used in the pharmaceutical industry, as it can provide the accurate results needed. The results can be used to quantitatively and qualitatively analyze finished drug products and their ingredients during manufacturing process. This is achieved by separating, quantifying and identifying components in a mixture, and it can be used to reveal a drug's identity and monitor the progress of a disease therapy[6].

Though originally intended to be used as a complementary tool for gas chromatography, the pharmaceutical industry now uses HPLC as a chromatographic technique almost solely.

HPLC 's capacity to elucidate the composition and assess the concentrations of impurities in pharmaceutical formulations is one of the key advantages.3 HPLC is best suitable for

substances that are not readily volatilized, thermally unstable and have high molecular weight. It can therefore quantify a drug in its pure, dosage form.

Other forms of HPLC used in the pharmaceutical industry include reversed-phase enzyme reactor (IMER) HPLC, denaturing, and immobilized. However, one of HPLC 's drawbacks is that calibration tests must be preceded which can increase costs [7].

COMBINING ANALYTICAL TECHNIQUES

Because HPLC is simple, specific, rapid, accurate and accurate, it can be successfully and efficiently introduced in bulk and pharmaceutical dosage form for routine quality control analysis of drugs. It may also be used to further elucidate the components of mixtures in combination with other analytical methods.

HPLC-UV uses the UV as a detection form. The benefit of this is that it does not need the complicated treatment and procedures usually associated with the conventional chromatographic method5 which allows it less time consuming and economical [8].

However, if UV detection is used or is completely retained on the liquid chromatography column some parts may have weak UV chromophores. A diode array and fast scanning detector are useful for peak recognition and peak purity monitoring for detection of the components. Instead, fluorescence and electrochemical detectors are for certain substances significantly more sensitive to appropriate analytes and more reliable than UV detectors.

The most sensitive method for HPLC detection, according to Nikolin et al., is reductive electrochemical detection, which has produced excellent results on some classes of drugs in the inquiry [9].

Another technique with which HPLC can be integrated is mass spectrometry (HPLC / MS), and the chromatograph is connected to a mass spectrometer via an interface. This form of analysis can examine a wide range of components, including thermally labile components, display high polarity, or have high molecular mass. On the specialized interface the components eluted from the column are introduced into the mass spectrometer. The two most common interfaces used for HPLC/MS are the interfaces for electrospray ionization and chemical ionization of the atmospheric pressure [10].

CONCLUSION

Liquid chromatography is a useful analytical tool for determining the components of the formulation of a drug, allowing researchers to quantify the formulation and discover whether a product contains any impurities. The other techniques that can be combined with HPLC's further capabilities, making it an ideal pharmaceutical analytical technique for ensuring high drug quality. High-performance liquid chromatography is only the first technique for analyzing organic and inorganic compounds by Trace. Determination of trace compounds in pharmaceutical, biological, toxicological and environmental studies is very important since even a trace substance can be harmful or poisonous.HPLC is used in analytical chemistry, pharmaceutical and drug sciences, clinical sciences, food technology , and consumer products, combinatorial chemistry, polymer chemistry , environmental chemistry, and green chemistry for molecular

weight determination. HPLC 's role in the pharmaceutical industry is very vital , especially in preformulation, process development, during the development of formulations and drug discovery, and for verifying drug purity.All the research performed in medicinal drugs, processing of pure ingredients, trace inspection, environmental health where we have to test for contaminants and harmful chemicals contained in food and food items all of these activities are achieved through high-performance liquid chromatography or GC systematically and quickly on a daily basis.An significant function of chromatography is the QC of food quality but also drugs regulating the raw materials and regulating the finished goods maintaining people's health, we are so reliant on chemicals in the world today, minimal organic chemicals created by chemists with this mixed blessing, and I think mainly also pesticides that are very good for crops, very dangerous to humans if they are manufactured by chemists. HPLC is therefore the best separation technique for quantitative trace analysis of toxic chemicals, impurities, high pure products manufacturing, medicinal uses, and research purposes.