Use Of High-Performance Liquid Chromatography in the Pharmaceutical Industry

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Abstract **- In the pharmaceutical industry, all manufactured products need to be of the highest quality to ensure the least risk to patients. To guarantee that goods pass certain standards, researchers, manufacturers and developers use various technical equipment and analytical techniques, including liquid chromatography, during the development process.**

Liquid chromatography is an analytical technique that is used to separate a certain sample into its individual components. The separation occurs when the sample interacts with the mobile (liquid) and stationary phases (column). The various parts of the sample are separated out based on their polarities; they will have varying levels of affinity for the mobile phase, resulting in migration through the column at different speeds.

The mixed components are placed at the top of the column of the stationary phase, which is generally a fine adsorbent solid such as silica. This must be distributed evenly to minimise the presence of air bubbles that could influence the results of the test. The exit of the column is stoppered with glass, wool or a porous plate. When the mobile phase passes through, the mixture separates into bands. These can then be collected and analysed via other methods.

The technique works as the components in a mixture are attracted to the adsorbent surface of the stationary phase with varying degrees depending on their individual polarity and their unique structural characteristics; a component with a higher affinity for the stationary phase will migrate down the column slower than a component which has more affinity for the mobile phase.

The most common form of liquid chromatography in use today is high-performance liquid chromatography (HPLC), which pumps the sample mixture through the column at high pressure.

INTRODUCTION

How Is Liquid Chromatography Used In Pharma?

HPLC is the form of liquid chromatography that is generally used in the pharmaceutical industry, as it can provide the precise results that are required. The results can be used to analyse finished drug products and their ingredients quantitatively and qualitatively during the manufacturing process. This is achieved through the separation, quantification and identification of components in a mixture and can be used to reveal the identity of a drug and monitor the progress of a therapy on a disease.

Although expected at first to be used as a complimentary method to gas chromatography, the pharmaceutical industry now almost exclusively uses HPLC as a chromatographic technique.

One of the main benefits of HPLC is its ability to elucidate the structure and determine the quantities of impurities in pharmaceutical formulations. HPLC is especially suitable for compounds that are not easily volatilised, thermally unstable and have high molecular weights. Therefore, it can quantify a drug in its pure and dosage form.

Other forms of HPLC that are used in the pharmaceutical industry include reversed phase, denaturing and immobilised enzyme reactor (IMER) HPLC.

However, one of the disadvantages of HPLC is that is must be preceded by calibration tests which can increase costs.

COMBINING ANALYTICAL TECHNIQUES

As HPLC is simple, specific, rapid, precise and accurate, it can be successfully and efficiently adopted

for routine quality control analysis of drugs in bulk and pharmaceutical dosage form. It can also be used in combination with other analytical methods to further elucidate the components of mixtures.

HPLC-UV uses UV as a form of detection. The advantage of this is that it does not require the elaborate treatment and procedures usually associated with the traditional chromatographic method, making it less time consuming and economical.

However, some components may have weak UV chromophores if UV detection is being used or be completely retained on the liquid chromatography column. For the detection of the components, a diode array and rapid scanning detector are useful for peak identification and for the monitoring of peak purity. Instead, fluorescence and electrochemical detectors are considerably more sensitive towards appropriate analytes and more selective than UV detectors for many compounds.

According to Nikolin et al., the most sensitive method for HPLC detection is reductive electrochemical detection, which has yielded excellent results in the investigation on some classes of drugs.

Another technique that HPLC can be combined with is mass spectrometry (HPLC/MS); the chromatograph is attached via an interface to a mass spectrometer. This form of analysis can examine a wide range of components, including those that are thermally labile, exhibit high polarity or have a high molecular mass. The components eluted from the column are introduced to the mass spectrometer on the specialised interface. The two most common interfaces used for HPLC/MS are electrospray ionisation and atmospheric pressure chemical ionisation interfaces.

CLINICAL DIAGNOSIS

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.

However, given how physiologically widespread these molecules are, their analysis and subsequent conclusions about patient health must be done carefully. HPLC has the ability to separate and compare molecules to a higher magnitude than other techniques, making it a great candidate for such diagnostic purposes.

Reversed-phase HPLC (RP-HPLC) is one of the more popular methods due to its speed, column stability, and capacity to separate a wide range of compounds.

Identification of molecules in HPLC is done by measuring retention time. Retention time is the time it takes a molecule to pass through a column lined with adsorbents which interact differently with different molecules. This is done under varying conditions. In 1976, the potential use for RP-HPLC in diagnostic settings was shown.

Researchers exploited hydrophobic properties to separate catecholamine metabolites and amines in the same run, thereby speeding up the process. This is partly due to an interaction with pH, as acidic catecholamine metabolites are retained for longer at low pH values, but vice versa for amines.

Several conditions and settings can be modified in HPLC protocols. HPLC can then be used not only to detect diseases as mentioned, but also to monitor the progression of diseases.

Pheochromocytoma is a potentially fatal tumor of the sympathetic nervous system. It is derived from tissue in the neural crest, which implies that it secretes catecholamines. It can cause hypertension, which can complicate diagnosis, because it may only differ from hypertension in the format of its metabolites.

This makes HPLC ideal for diagnosis, however, the origin of the sample to be analyzed can affect the results. Urinary samples will reflect metabolites from both the central nervous system and the periphery.

Using cerebrospinal fluid offers results more localized to the central nervous system and is therefore preferred.

PHARMACEUTICAL INDUSTRY

With the widespread production of pharmaceuticals, came the legislation to ensure proper production and purity of drugs distributed. HPLC is among the most commonly used methods to verify drug purity globally.

Its use in assessing drugs on an industrial scale started in the 1980s, though its use in some countries is prevalent but still less widespread.

This can potentially be due to cost. HPLC is capable of providing sufficient precision for the industry standard, but only when it is preceded by calibration tests. This can increase the costs, but this sacrifice leads to high accuracy and specificity.

This means HPLC can be more beneficial to ensure purity than other methods. Multiple crystallization method was previously used but had the drawback of potentially wasting expensive drugs. HPLC is much more efficient, and it minimizes losses to pharmaceutical manufactures.

Even at the start of HPLC usage in pharmaceutical industry, the method showed its usefulness. HPLC was used in the analysis of alkaloids, antibiotics, and steroids.

Steroids, in particular, were previously somewhat difficult to analyse owing to low dosages in medicines, and the impractical forms they often came in (creams and ointments).

Early discussion focused on the detector used, a debate which still continues and evolves, but given the multitude of methods currently available, the debate is much more complex than it once was and can vary depending on the type of HPLC being considered.

HPLC is not only used for analysis of the finished drug products. Since HPLC can separate compounds, it is also applied during manufacture.

Through this separation, HPLC can provide critical starting products for the manufacture of new drugs, or characterization of molecules with the potential to be manufactured into drugs.

These lead compounds can be derived from plants, animals, or fungi. HPLC can be used to separate enantiomers, the molecules which are mirror images of each other, using chiral stationary phases (CSPs).

The ability to prove purity of enantiomeric molecules is a standard in pharmaceutical assays, for which HPLC is suitable.

The most popularly used CSPs in pharmaceutical chemistry are polysaccharide benzoate and phenylcarbamate derivatives.

USE OF HPLC IN PHARMACEUTICAL APPLICATIONS

HPLC is one of the most useful analytical methods in the development and manufacture of pharmaceuticals. Its applications are not confined to just one area and it is instrumental in a number of critical steps necessary for robust pharmaceutical analysis.

Jade C. Byrd, a director in the Liquid Phase Separation Division (LPSD) at Agilent Technologies, notes that, "HPLC is a very versatile technique that can help (bio) pharmaceutical researchers and manufacturing facilities fully characterize potential drug or treatment candidates, and ensure the medicines are manufactured in a safe and consistent way." According to Byrd, typical research experiments might include understanding the chemical properties of small molecules or potential bio therapeutics, ranging from assessing the hydrophobicity of a particular molecule to the sugar structures on a monoclonal antibody that affect immune response.

One specific use case is ensuring the consistency of active pharmaceutical ingredients (API). HPLC can provide quantitative analysis of select molecules, so you can confirm the correct dosage of active ingredients. "For QA/QC testing, HPLC can be useful in ensuring critical quality attributes such as strength/concentration, content uniformity, the detection and quantification of impurities, and the quality and identity of raw materials," notes Byrd.

Impurities can pose a serious safety risk to patients, and their detection and identification is often facilitated by the use of HPLC. Standard HPLC techniques may be combined with highly efficient detection methods (such as UV detection) to provide a complete and accurate impurity profile.

In a similar vein, HPLC can be vital in evaluating the stability of pharmaceutical products. The composition of formulations can alter over time due to a variety of environmental factors, such as exposure to humidity, oxygen, heat, and light. HPLC can assist in the identification of degradation products as well as determine the extent of change over time.

Indeed, Byrd notes that HPLC can also be useful in determining shelf life; "for example, some biotherapeutics are sensitive to aggregation over time, or if not stored properly, and HPLC can be used to monitor this aggregation."

FACTORS TO CONSIDER WHEN USING HPLC

Although HPLC is extremely useful, the right methods and equipment must be chosen for each specific application. There are several major factors to consider here. In pharmaceuticals in particular, regulatory requirements will determine the right methods, equipment, and specifications to use. For example, the Food and Drug Administration (FDA) provides a guidance document, titled Validation of Chromatographic Methods, which covers various types of HPLC methods. Other regulatory bodies that

may dictate how products are tested in their respective regions include Health Canada and the UK's Medicines and Healthcare products Regulatory Agency.

Byrd explains that "There are many factors that affect the quality of the separation (instrument design, column and mobile phase chemistries, injection parameters, and column temperature)," and these variables need to be well understood.

HPLC is not a one-size-fits-all method and there are several options to choose from. Two common approaches are normal phase and reverse phase; in both, the separation is based on polarity. In normal phase HPLC, the adsorbent material is polar and the solvent non-polar (typically an organic liquid), resulting in fewer polar components of the sample being eluted first. Reverse phase involves the opposite setup, so the more polar components will exit the column quicker.

Other types of HPLC include ion-exchange (based on ionic charges) and chiral (for separating enantiomers). In the latter, the stationary phase material has pores that slow down smaller molecules. There's also ultra-high performance liquid chromatography (UHPLC), which uses a smaller column and smaller particles than HPLC, ultimately resulting in a more efficient process.

HPLC is incredibly versatile, especially when combined with different detectors, such as UV-Visible spectroscopy (UV-Vis), mass spectrometry (MS), and fluorescence. For example, UV-Vis can help determine the concentration of molecules following elution from the column. A highly specific detection method is MS, which can help measure a molecule's mass-to-charge-ratio and thus its molecular weight. Fluorescence can be particularly sensitive for the right kinds of analytes with parts per billion (ppb) detection limits.

There's no doubt that HPLC plays an important role in pharmaceutical analysis. Its accuracy and versatility make it a suitable tool for many stages of the development and production of biotherapeutics. While there are a number of factors to take into consideration when designing an HPLC analysis, the technique's innate flexibility, including its compatibility with multiple advancing technologies, makes it an excellent choice for a broad range of applications.

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 to patients.

This Process to create a new drug can be divided into 3 main stages

1. Drug discovery
2. Drug development
3. 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.

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 nonalcoholic 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

1. Detection of phenol compounds in drinking water.
2. Identification of diphenhydramine in sedimented samples.
3. Bio-monitoring of pollutant.
4. Rapid separation and identification of carbonyl compounds by HPLC.

5. LC/MS/MS solution for pharmaceuticals and personal care products in water, sediment, soil and biosolids by HPLC/MS/MS.
6. Determination of 3-mercaptopropionic acid by HPLC

FORENSICS APPLICATIONS

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.

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 of antibiotics in blood plasma.
- Estimation of bilirubin & biliviridin in blood plasma in case of hepatic disorders.
- Detection of endogenous neuropeptides in extracellular fluids of the brain.

PHARMACEUTICAL IMPURITY PROFILING ANALYSIS

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

PHARMACEUTICAL DRUG DISCOVERY ANALYSIS

Developing a fast, generic method for rapid resolution Liquid chromatography with quadrupole MS detection. Fast, generic LC/MS method enables drug analysis in less than one minute.

RECENT APPLICATIONS

Analytic method development and 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, & biotechnology-based 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 alkyl amines (morphine and 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 analysing the pesticide content in drinking water
4. It is utilized in food analysis for separating water-soluble and fat-soluble vitamins 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 Eg: Analysis of cinchona, digitalis, ergot extracts and liquorice.
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, tetracycline's, 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 hydro lysates 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.

CONCLUSION

Liquid chromatography is a useful analytical tool for establishing the components of a drug's formulation, enabling researchers to quantify the formulation and discover whether there are any impurities in a product. The other techniques that HPLC can be combined with further its capabilities, making it an ideal analytical technique for pharma to ensure the high quality of drugs.

REFERENCES

[1] Chemistry LibreTexts. 2020. Chromatography. [online] Available at: https://chem.libretexts. org/Bookshelves/Analytical_Chemistry… [Accessed 2 April 2020].
[2] Nikolin Be. High perfomance liquid chromatography in pharmaceutical analyses. – PubMed – NCBI [Internet]. Ncbi.nlm.nih.gov. 2020 [cited 2 April 2020]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/1562901
[3] [Internet]. Longdom.org. 2020 [cited 2 April 2020]. Available from: https://www.longdom. org/open-access/hplc-uses…
[4] Liquid Chromatography News, Research [Internet]. News-Medical.net. 2020 [cited 2 April 2020]. Available from: https://www.news-medical.net/?tag=/Liquid-Chromatography
[5] Chromatography Conferences 2020 | HPLC Conferences 2020 | Pharmaceutical Chemistry Conferences | Meetings | Events | 2020 | Europe USA Asia [Internet]. Chromatography. pharmaceuticalconferences.com. 2020 [cited 2 April 2020]. Available from: https://chroma tography.pharmaceuticalconferences.com/
[6] Thammana M. A review on high-performance liquid chromatography. Research & Reviews: Journal of Pharmaceutical Analysis, 5(2), 2016, 22.
[7] Malviya. R., et al., High-performance liquid chromatography: A short review, Journal of Global Pharma Technology, 2, 2010, 22 – 26.
[8] Heewon Lee, Pharmaceutical applications of liquid chromatography coupled with mass spectrometry (LC/MS), Journal of liquid chromatography & related technologies, 28, 2005, 7-8.