EUROPEAN ACADEMIC RESEARCH
Vol. VI, Issue 10/ January 2019

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Using high performance liquid chromatography (HPLC) in pharmaceutical industry: a short review

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Abstract

The analytical technique of High Performance Liquid Chromatography (HPLC) is used extensively throughout the pharmaceutical industry. It is used to provide information on the composition of drug related samples. The information obtained may be qualitative, indicating what compounds are present in the sample or quantitative, providing the actual amounts of compounds in the sample. HPLC is used at all the different stages in the creation of a new drug, and also is used routinely during drug manufacture. The aim of the analysis will depend on both the nature of the sample and the stage of development.

Key words: HPLC, pharmaceutical, mobile phase, detector

INTRODUCTION

High performance liquid chromatography (HPLC) play an important and critical role in the field of pharmaceutical industries and analysis, since it is used to test the products and to detect the raw ingredient used to make them i.e., qualitative and quantitative analysis. The most important benefits gain from the uses of HPLC technique in the industrial and analytical field that it is help in structure elucidation and quantitative determination of impurities and degradation products in bulk drug materials and pharmaceutical

formulations. These benefits which gain from the using of HPLC not only limited for the synthetic drugs and formulas but also include herbal medicine too (Shethi P.D. *et al*., 2010).

HPLC is an essential analytical tool in assessing drug product. HPLC methods should be able to separate, detect, and quantify the various drugs and drug related degradants that can form on storage or manufacturing, detect and quantify any drugs and drug-related impurities that may be introduced during synthesis (Bansal V. *et al*., 2010).

HPLC mainly utilizes a column that holds packing material (stationary phase), a pump that moves the mobile phase(s) through the column, and a detector that shows the retention times of the molecules. Retention time varies depending on the interactions between the stationary phase, the molecules being analyzed, and the solvent(s) used. The sample to be analyzed is introduced in small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase (Kaushal C and Srivastava B.A., 2010).

MATERIAL AND METHODS

The HPLC System

Instrumentation is required to enable the flow of the mobile phase through the stationary phase and also to convert the separated components into meaningful information. A typical configuration of a HPLC system is shown in Figure 1.

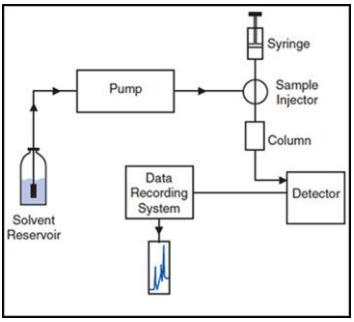

Figure 1: Configuration of a typical HPLC System

Solvents

The most common solvents used for HPLC are listed below in order of increasing polarity:

- n-hexane
- methylene chloride
- chloroform
- methyl-t-butyl ether
- tetrahydrofuran (THF)
- isopropanol (IPA)
- acetonitrile (MeCN or ACN)
- methanol (MeOH)
- water

A blend of two (or more) of these solvents is used as the mobile phase in a HPLC analysis. The proportions of the different solvents in the blend act to adjust the polarity of the mobile phase. This is combined with a suitable stationary phase to achieve the separation of a mixture. Ideally, the components in the mixture will be separated fully and will all elute within a practical time scale.

By convention, chromatographers usually refer to the strong solvent in a mobile phase as the 'B' solvent and the weak solvent as the 'A' solvent. Generally, solvent strength is related to polarity, with non-polar solvents being 'strong' solvents for reversed phase HPLC and polar solvents being 'strong' for normal phase HPLC Mobile phase reservoir (Snyder L.R. *et al.*, 2011) .

Mobile phase reservoir

The mobile phase is usually stored in glass containers, often these are plastic coated as a safety measure. Plastic containers are not used since additives in the plastic may leach into the mobile phase. The container needs to be of an appropriate size so that it contains enough mobile phase for the analysis being performed (e.g. 1, 2 and 5 liter flasks are often used). PTFE

tubing (or a similarly inert tubing material) connects the contents of the reservoir with the HPLC system. This tubing is typically of outer diameter (OD) 1/8 inch and of inner diameter (ID) 1/16 inch. The size of the tubing in a HPLC system is usually measured using the imperial system of inches (contrasting with the column which uses metric measurements (Lindholm J 2004).

At the end of the tubing which is in contact with the mobile phase there is usually a filter to remove any particulate matter, this also acts as a 'sinker' to hold the tubing at the bottom of the container. This is commonly glass, stainless steel or PEEK. A diagram of a typical mobile phase reservoir is shown in Figure 2.

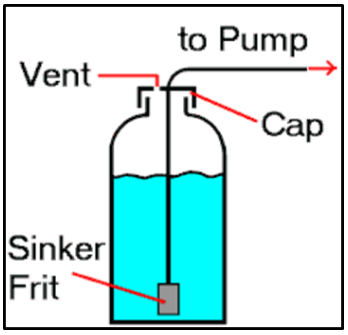

Figure 2: Typical mobile phase reservoir

Sample preparation

The drug substance being analyzed should be stable in solution (diluent). During initial method development, preparations of the solutions in amber flasks should be performed until it is determined that the active component is stable at room temperature and does not degrade under normal laboratory conditions. The sample solution should be filtered; the use of a 0.22 or 0.45 µm pore-size filter is generally recommended for removal of particulates. Filtration is a preventive maintenance tool for HPLC analysis.

Sample preparation is a critical step of method development that the analyst must investigate. The

effectiveness of the syringe filters is largely determined by their ability to remove contaminants/insoluble components without leaching undesirable artifacts (i.e., extractable) into the filtrate. If any additional peaks are observed in the filtered samples, then the diluents must be filtered to determine if a leachable component is coming from the syringe filter housing/filter (Mayer M.L., 1997).

Injection of the sample

Septum injectors are available; using which sample solution is injected. Sample can be injected when the mobile phase is flowing or it is stopped. A new advanced rotary valve and loop injector can be used to produce reproducible results.

The detector

There are several ways of detecting when a substance has passed through the column. Generally UV spectroscopy is attached, which detect the specific compounds. Many organic compounds absorb UV light of various wavelengths. The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time.

Interpreting the output from the detector

The output is recorded as a series of peaks, each one representing a compound in the mixture passing through the detector and absorbing UV light. The area under the peak is proportional to the amount of substance, which is passed through detector, and this area can be.

Applications of HPLC in pharmaceutical analysis

There are a wide variety of applications throughout the process of creating a new drug, from initial drug discovery to the manufacture of formulated products which will be administered to patients. The information that can be obtained by 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 (Donald D.H. and Mumtaz S, 2008).

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 in pharmaceutical industries

Other applications of HPLC are:

1. Tablet dissolution study of pharmaceutical dosages form.
2. Shelf-life determinations of pharmaceutical products.
3. Identification of active ingredients of dosage forms.

4. Pharmaceutical quality control.

CONCLUSION

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

REFERENCES

1. Bansal V; Malviya R ; Pal O.P. and Sharma P.K. (2010). High performance liquid chromatography: a short review. Journal of Global Pharma Technology, 2(5): 22-26.
2. Donald D.H. and Mumtaz S (2008). Development and validation of HPLC Stability-indicating Assays, In: Sens T. Carstensen, C.T.Rhodes, editors Drug Stability-Principle & Practice. 3rd Edition. New York: Marcel Dekker Inc., USA.
3. Kaushal C and Srivastava B.A. (2010). Process of Method Development: A Chromatographic Approach. J Chem Pharm Res, 2(2): 519-545.
4. Lindholm J (2004). Development and Validation of HPLC Method for Analytical and Preparative Purpose, Acta Universities Upsaliensis Uppsala, 13-14.
5. Mayer M.L. (1997). HPLC for Pharmaceutical scientist. Am. Lab., 29: 34- 37.
6. Shethi P.D. ; Rajat S ; Santosh V.G. ; Nitin D and Neeraj S (2010). Sethi's HPLC : Quantitative Analysis of Pharmaceutical Formulations, Vol. 6 : High Performance Liquid Chromatography. CBS Publishers & Distributors, UK.
7. Snyder L.R. ; Kirkland, J.J. and Glajch, J.L. (2011). Practical HPLC Method Development 2nd Ed., John Wiley & Sons Inc., USA.