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Preparative High Performance Liquid Chromatography- Instrumentation and its Applications- A Review

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ABSTRACT

Pharmaceutical analysis involves both quantitative and qualitative analysis of drug or drug substance which done by using various types of analytical techniques like chromatographic techniques, spectroscopic techniques, electrochemical, radioscopic etc., Preparative High Performance Liquid Chromatography [PHPLC] is type of chromatographic technique which used to isolation and purification of organic compounds or any other products. It's more important and versatile chromatographic technique in quality of drugs. This is an attempt to review instrumentation of PHPLC in this article.

Keywords*: Preparative high performance liquid chromatography , Instrumentation, Fraction Collector, Application*

1. Introduction

Pharmaceutical analysis may be defined as the application of analytical procedures used to determine the purity, safety and quality of drugs and chemicals Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination of structure of chemical compounds [1] . Preparative HPLC is used for the isolation and purification of valuable products in the chemical and pharmaceutical industry as well as in biotechnology and biochemistry. [2] Depending on the working area the amount of compound Preparative HPLC is a liquid chromatography (LC) for separating and purifying the target compound from the mixed solution after the synthesis reaction and/ or extract from the natural product and recovering it with high purity. By obtaining the target compound with high purity, it is possible to conduct structural elucidation analysis, various functional evaluation / analysis or the next step analysis more reliably. The technique is also sometimes used for purification of the final product on an industrial scale basis. Appropriate scaling up from the required amount and degree of purity of the target compound, it is possible to select a system configuration that is superior in efficiency and cost performance. In the preparative HPLC system, the key objective is to separate desired compound from the coexisting components in the crude material. The same Liquid Chromatograph principles apply in the Preparative workflow. Therefore, as with ordinary HPLC systems, its basic instrument setup includes a liquid feed pump, a sample injection unit, a separation column, and a detector. Additionally, as a characteristic feature of the preparative system, a mechanism for fractionating the target substance has been added after elution. In the case of a very simple preparative HPLC system, manual injection / manual fractionation is adopted by using a manual injector for the sample injection part and a manual flow path switching valve for the fractionating part. It is also possible to configure a fully automated system by setting an autosampler or fraction collector. [3] This articles briefly explains about the appropriate knowledge and instrumentation of preparative HPLC and its applications. The major difference between preparative HPLC and high performance liquid chromatography as shown in table 1.

Table 1: difference between HPLC AND PHPLC

Components	*Analytical HPLC*	*Preparative HPLC*
Flow rate	*Upto 10ml/min*	*Upto 100ml/min*
Sample requirements	*Low concentration*	*High concentration*
Injection volume	*Low*	*High*
Fraction collector	*Absent*	*Present*
Column	*1-5mm*	*1-10cm*
Sample size	*Less than 2 mg*	*Upto 500mg*
Purpose	*Main goal is to quantification of compound*	*Main goal is to isolation or purification of compound*

2. Instrumentation of (PHPLC)

The instrumentation of preparative high performance liquid chromatography resembles same as high performance liquid chromatography in addition to HPLC additionally it consists of fraction collector.

The major parts of PHPLC are as shown in figure 1

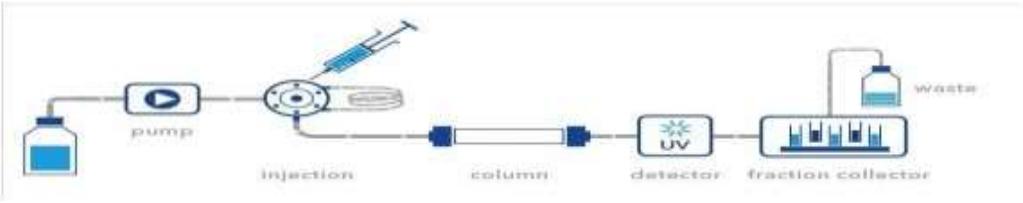

Fig 1 instrumentation Of preparative high performance chromatography Reservoir

- ✓ *Pump*
- ✓ *PHPLC column*
- ✓ *Sample injector*
- ✓ *Detector*
- ✓ *Fraction collector*

2.1 Solvent Reservoir

Depend upon the type of samples it's varies, usually made up of glass or stainless steel, that requires larger amount. Large capacity (Upto several gallons) reservoirs made up of glass or stainless steel are used in prep. HPLC. [4]

2.2 Pump

When compared to Analytical HPLC PHPLC requires a more flow rate(10 to 100ml/min) and more internal diameter of column. In prep. HPLC, analytical pumps are modified with the respect to high pumping rates and large volume. That achieved by that is required to work at flow rate of 10-100ml/min is a larger piston head with higher volume liquid filled chamber. [4]

2.3 PHPLC Column

***Specification of the Column**: C8, 50x1200mm, 7µm, 100Å*

- ***The first one**, C8 is a chain length of carbon in the stationary phase i.e. resin.*
- ***The second one** is the column dimension in terms of length and internal diameter.*
- ***Third**, the particle size of the resin.*
- ***Fourth**, the pore size of the resin.*

Particle size for silica-based carbon chain resins generally ranges between 3 to 15µm. Also, there are different carbon chain lengths ranging from C4 to C18. These resins come with their own mechanical stability with different maximum packing pressure they can sustain. Following are the parameters looked into while selecting the resin:

- *Particle Size and Distribution*
- *Pore Size and Distribution*
- *Specific Surface Area*
- *Pore Volume*
- *Chemical Purity and Stability*
- *Packing Density*
- *Coverage etc.*

For a Chromatographic technique's the column occupies the chief position for analysis of compound, in that way the heart of PHPLC is column compartment, it should withstand high pressure in order to obtain high flow rate. It have a usually more internal diameter and more silica particle size when compared to Analytical column. For preparation of column dry packing technique or slurry technique is used. Preparative column as shown in figure 2 [5]

Figure 2: columns in PHPLC

2.4 Sample Injection

In PHPLC, it should inject the sample up to range of 100ml with high pressure and high flow rate. Process of sample injector as shown in figure3

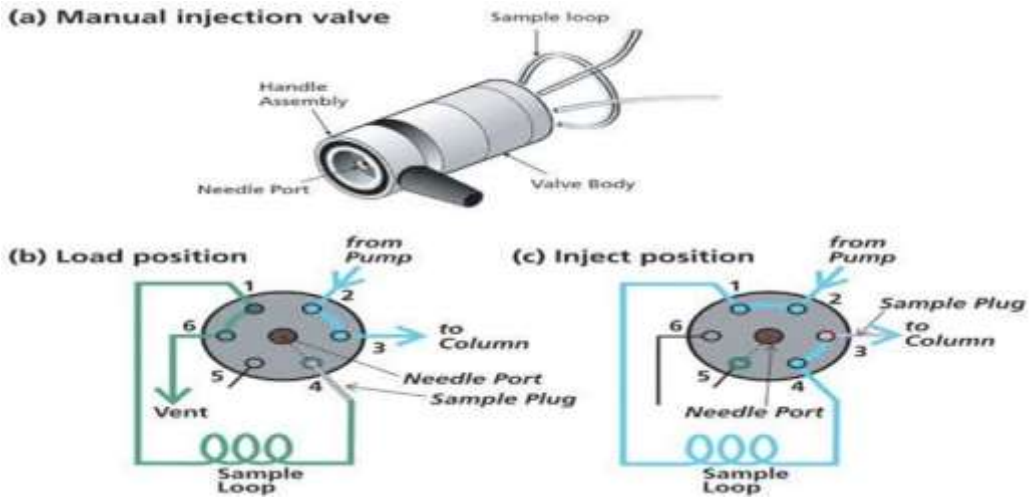

Figure 3,: sample injection in PHPLC

2.5 Detector

Detectors which are used for Analytical HPLC are used for PHPLC. Samples after mixed with mobile phase it reach to detector then to fraction collector.

2.6 Fraction Collector

Fraction collection is the process of capturing the eluting fluid in vessels They are different types of fraction collector,

a. ***Manual fraction Collector***

Manual fraction collection means that triggers the switching of the diverter valve manually, based on the signal plot. It offers the highest flexibility because of only the desired parts of the purification are collected. The drawback in this method is the lack of automation.

b. Peak-Based Fraction Collector

Peak-based fraction collection is an automated way of fraction collector based on a detector signal

c. Mass-Based Fraction Collector

In peak-based fraction collector we collected all compounds based on their peak elucidated. While in Mass based fraction collector we selectively collected the compound with desired mass.

3.Applications

3.1 Purification in medicinal or high-throughput chemistry

Mostly all the compounds are synthesized by high throughput chemistry. All newly synthesized compounds must be purified . Prep. HPLC is widely used for this purpose.

3.2 Purification of compounds in natural product compound

PHPLC is used to isolate and purify individual component from mixture of crude extract.

3.3 Purification of by-products, formed during impurity analysis

In drug discovery and development process it is not only important to isolate the compound of interest in pure form but also with traces of impurities. These isolated impurities are collected in PHPLC

3.4 Recovery collection

It is necessary to collect all fractions of a sample at specific location during the prep. HPLC analysis . In prep. HPLC, diverter valve is used to isolate fraction . This recovery collection is very valuable for reanalysis.

3.5 Automated fraction re-analysis [6]

4.Conclusions

Preparative High Performance Liquid Chromatography is expensive technique when compared to other purification technique thus it's used for rare or expensive product. In industrial scale PHPLC work's continuously to isolate the large number of products. In PHPLC we can reuse the sample with help of fraction collector and also it's majorly employed in purification of impurities, organic compound and crude extract.

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Declarations

a. ***Ethics approval and consent to participate***

Not applicable

b. ***Consent for publication***

Not applicable

c. ***Availability of data and materials***

Not applicable

d. Competing interests

Not applicable

e. Funding

Not applicable

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List of Abbreviation

- *HPLC – High Performance Liquid Chromatography*
- *PHPLC – Preparative High Performance Liquid Chromatography*
- *GC – Gas Chromatography*
- *TLC -Thin Layer Chromatography*
- *HPTLC- High Performance Thin Layer Chromatography*