ejpmr, 2022,9(11), 578-583

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A REVIEW ON COMPARISON BETWEEN NORMAL PHASE AND REVERE PHASE HPLC

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Article Received on 21/09/2022 **Article Revised on 11/10/2022** **Article Accepted on 01/11/2022**

ABSTRACT

This review mainly focuses on comparing between normal phase and reverse phase HPLC also its instrumentation and applications. The importance of RPHPLC in analytical method development and their strategies along with brief knowledge of critical chromatographic parameters needed for optimized an efficient method development has been mentioned. The key difference between reverse phase and normal phase HPLC is that the reverse phase HPLC uses a nonpolar stationary phase and a polar mobile phase whereas the normal phase HPLC uses a polar stationary phase and a less polar mobile phase.

KEYWORDS: Chromatography, RPHPLC, Instrumentation of HPLC, Applications.

INTRODUCTION[1,2]

Chromatography is a laboratory technique for the separation of a mixture into its components.

- The mixture is dissolved in a fluid solvent (gas or liquid) called the mobile phase, which carries it through a system (a column, a capillary tube, a plate, or a sheet) on which a material called the stationary phase is fixed.
- Because the different constituents of the mixture tend to have different affinities for the stationary phase and are retained for different lengths of time depending on their interactions with its surface sites, the constituents travel at different apparent velocities in the mobile fluid, causing them to separate.
- The separation is based on the differential partitioning between the mobile and the stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation.

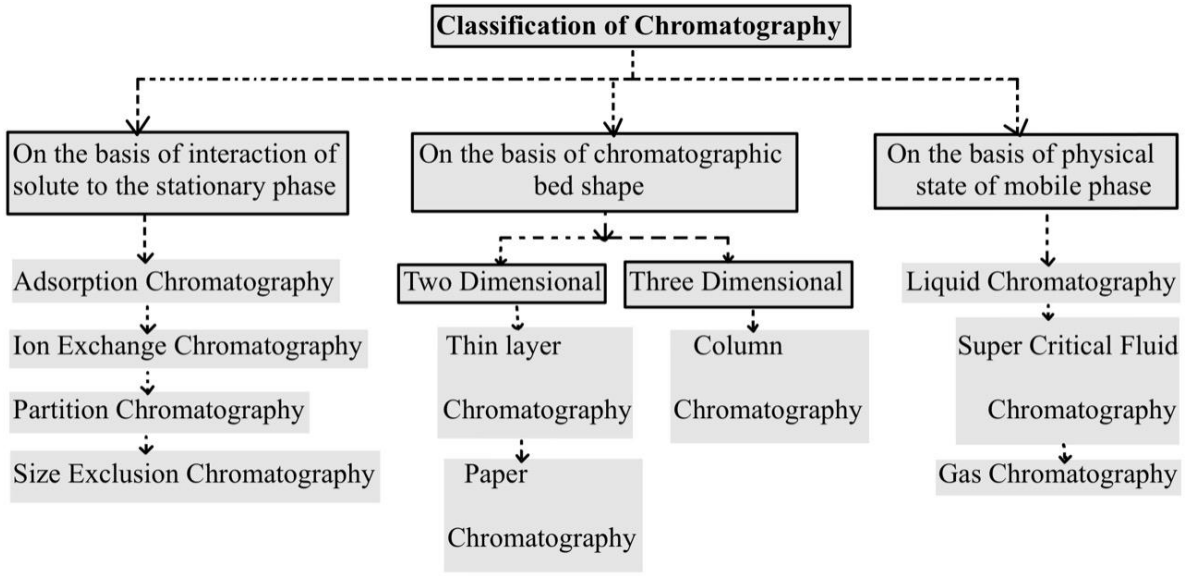

Fig. 1: Classification of chromatography.

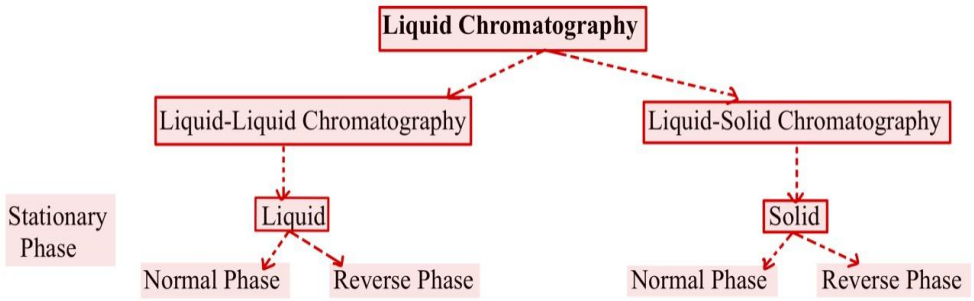

Fig. 2: Classification of liquid chromatography.

Normal Phase Chromatography

The components in a mixture will elute at different rates depending on each one's polarity relative to the next.

- When the column to be used for the separation is more polar than the mobile phase, the experiment is said to be a normal phase method.
- In normal phase chromatography, the stationary phase is polar, and so the more polar solutes being separated will adhere more to the stationary adsorbent phase.
- When the solvent or gradient of solvents is passed through the column, the less polar components will be eluted faster than the more polar ones.
- The components can then be collected separately, assuming adequate separation was achieved, in order of increasing polarity.
- This method of chromatography is not unique to liquid-solid column chromatography and is often used when performing High Performance Liquid Chromatography (HPLC).

Reverse Phase Chromatography[4]

In reverse phase chromatography, the polarities of the mobile and stationary phases are opposite to what they were when performing normal phase chromatography.

- Instead of choosing a non-polar mobile phase solvent, a polar solvent will be chosen. Or, if the experiment requires a solvent polarity gradient, the gradient must be carried out with the most polar solvent first and the least polar solvent last (reverse order of normal phase chromatography).
- Common polar solvents mixtures of solvents include water, methanol, and acetonitrile. It is slightly more difficult and expensive to obtain a column where the stationary phase is non polar, as all solid adsorbents are polar by nature.
- The non polar stationary phase can be prepared by coating silanized silica gel with a non polar liquid. Silanizing the silica gel reduces the silica gel's ability to absorb polar molecules.
- Common non polar liquid phases include silicone and various hydrocarbons. An alternative to this type of column is used in HPLC, in which a bonded liquid phase is used as the stationary phase.
- The less polar liquid is chemically bonded to the polar silica gel in the column. So using reverse phase, the most polar compounds in the sample solution will be eluted first, with the components following having decreasing polarities.

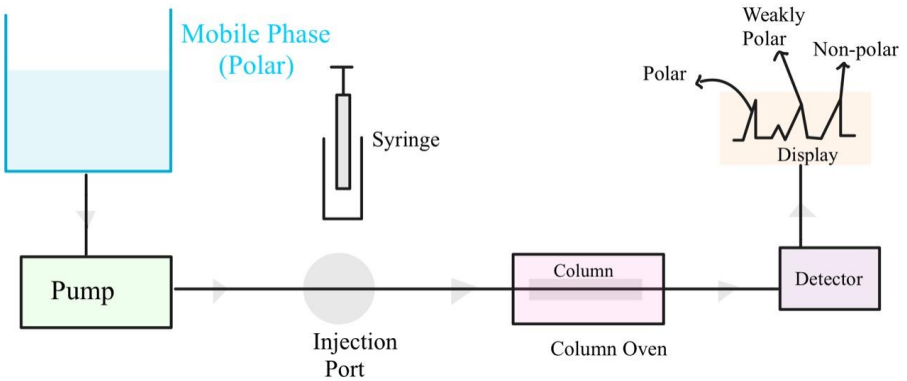

Fig. 3: Instrumentation of Reverse phase HPLC.

Advantages[5]

- It is an economical method compared to other chromatographic techniques.
- RP-HPLC allows water to be used in the composition of the mobile phase with other solvents.
- • Another advantage of using reversed-phase chromatography is that it provides accurate results with small amounts of sample.
- RP-chromatography also has the advantage of being able to use pH selectivity to improve the separation.
- The hydrophobic stationary phase in reverse-phase columns works well for the retention of most organic molecules.
- In RP-chromatography we can use pH selectivity to get better separations.

- About 75 percent of all HPLC methods use reversed-phase chromatography.

Disadvantages

- Water-insoluble compounds and amines can be more difficult to analyze.
- In RP-HPLC need to create pressure.
- It requires technical capability and skill to handle the system
- The silica of the reversed-phase column can be dissolution at pH > ~7.5.
- The eluted sample from the column cannot be recovered.
- Additional techniques are needed to confirm the identity of the analytes.

DIFFERENCE BETWEEN NORMAL PHASE AND REVERSE PHASE HPLC

Table 1: Normal Phase Vs Reverse Phase HPLC.

	Normal Phase (NP-HPLC)	Reverse Phase (RP-HPLC)
Definiton	HPLC is with hydrophilic stationary phase	HPLC is with hydrophobic stationary phase
Stationary Phase	Polar (silica gel)	Non- Polar (C_{18}, C_{12}, C_8)
Mobile Phase	Non- Polar (organic solvent)	Polar (aqueous / organic)
Components is mobile phase	Hexane, Dichloromethane, Isopropanol, Methanol	Water, Methanol, Acetonitrile, Tetrahydrofuran
Sample Movement	Non- Polar fastest	Polar fastest
Separation based on	Different polarities (functionality)	Different hydrocarbon content
Stabilization	Stabilizes slowly and is prone to fluctuations in retention time	Stabilizes quickly and stationary phase has long service life
Usage	NP-HPLC is not that much in use	RP-HPLC has usage is around 70%

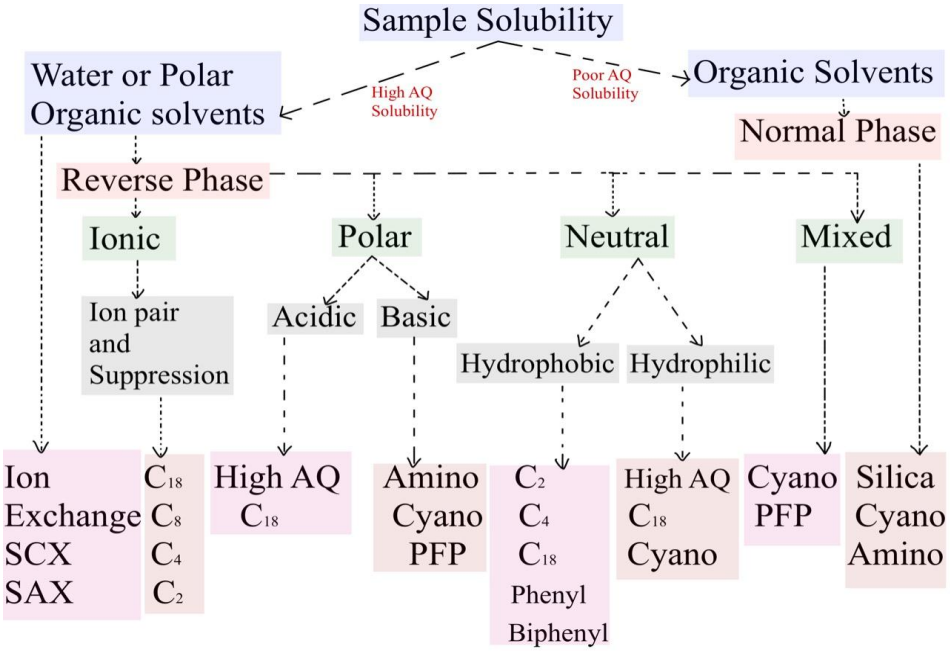

Fig. 5: Basics of HPLC.

Instrumentation of High-Performance Liquid Chromatography (HPLC)[10]

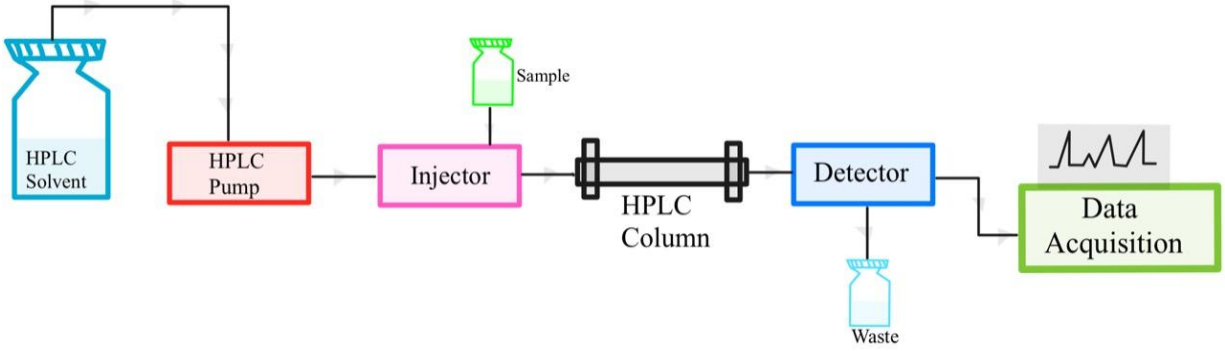

Fig 6: Flow diagram of HPLC.

The Pump

- The development of HPLC led to the development of the pump system.
- The pump is positioned in the most upper stream of the liquid chromatography system and generates a flow of eluent from the solvent reservoir into the system.
- High-pressure generation is a "standard" requirement of pumps besides which, it should also to be able to provide a consistent pressure at any condition and a controllable and reproducible flow rate.
- Most pumps used in current LC systems generate the flow by back-and-forth motion of a motor-driven piston (reciprocating pumps). Because of this piston motion, it produces "pulses".

Injector

- An injector is placed next to the pump.
- The simplest method is to use a syringe, and the sample is introduced to the flow of eluent.
- The most widely used injection method is based on sampling loops.
- The use of the auto sampler (auto-injector) system is also widely used that allows repeated injections in a set scheduled-timing.

Column

- The separation is performed inside the column.
- The recent columns are often prepared in stainless steel housing, instead of glass columns.
- The packing material generally used is silica or polymer gels compared to calcium carbonate. The eluent used for LC vary from acidic to basic solvents.
- Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.

Detector[12]

- Separation of analytes is performed inside the column, whereas a detector is used to observe the obtained separation.
- The composition of the eluent is consistent when no analyte is present. While the presence of analyte changes the composition of the eluent. What detector does is to measure these differences.
- There are different types of detectors available. They are as follows-

a) Photodiode Array (PDA) Detectors

- Responds to chromophoric analytes in the range 190 – 800nm
- Multi-wavelength monitoring
- Complete spectral profiles of chromatographic peaks
- Good for the majority of organic analytes
- Gradient elution compatible

b) Fluorescence Detectors

- Responds only to analytes which fluoresce naturally or can be made to fluorescence through derivatization
- Extremely sensitive
- Gradient elution compatible

c) Refractive Index Detectors

- Considered a universal detector for all analytes
- Comparatively insensitive
- Not compatible with gradient elution

d) Evaporative Light Scattering Detectors

- Considered a near-universal detector, responding to both chromophoric and non-chromophoric analytes
- Good sensitivity
- Gradient elution compatible

e) Mass Spectrometric Detectors

- The most discriminating detector
- Delivers mass information for analyte ID and structure elucidation
- Highly sensitive
- Gradient elution compatible

Degasser

The eluent used for LC analysis may contain gases such as oxygen that are non-visible to our eyes.

- When gas is present in the eluent, this is detected as noise and causes an unstable baseline.
- Degasser uses special polymer membrane tubing to remove gases.
- The numerous very small pores on the surface of the polymer tube allow the air to go through

Column Heater

The LC separation is often largely influenced by the column temperature.

- In order to obtain repeatable results, it is important to keep consistent temperature conditions.
- Also for some analysis, such as sugar and organic acid, better resolutions can be obtained at elevated temperatures (50 to 80°C).
- Thus columns are generally kept inside the column oven (column heater).

Recorder

- The change in eluent detected by a detector is in the form of an electronic signal, and thus it is still not visible to our eyes.
- In older days, the pen (paper)-chart recorder was popularly used. Nowadays, a computer based data processor (integrator) is more common.
- There are various types of data processors; from a simple system consisting of the in-built printer and word processor while those with software that are specifically designed for an LC system which not only data acquisition but features like peak-fitting, baseline correction, automatic concentration calculation, molecular weight determination, etc.

Fig 7: Instrumentation of HPLC.

Applications of High-Performance Liquid Chromatography (HPLC)[15]

The HPLC has developed into a universally applicable method so that it finds its use in almost all areas of chemistry, biochemistry, and pharmacy.

- Analysis of drugs
- Analysis of synthetic polymers
- Analysis of pollutants in environmental analytics
- Determination of drugs in biological matrices
- Isolate ion of valuable products
- Product purity and quality control of industrial products and fine chemicals
- Separation and purification of biopolymers such as enzymes or nucleic acids
- Water purification
- Pre-concentration of trace components
- Ligand-exchange chromatography
- Ion-exchange chromatography of proteins
- High-pH anion-exchange chromatography of carbohydrates and oligosaccharides.

Advantage of HPLC:

- HPLC offers a rapid, automated and highly precise method to recognize certain chemical components in a sample.
- High-performance liquid chromatography offers a fast and precise quantitative analysis.
- A gradient solvent system can be applied in certain methods.
- It is highly reproducible.
- HPLC can be upgraded to mass spectroscopy (MS).
- The HPLC is very rapid, efficient, and delivers high resolution as compared to other chromatographic techniques, such as TLC, column chromatography, and paper chromatography.
- Manages all areas of analysis to increase productivity

Disadvantage of HPLC

- HPLC can be an expensive method, it required a large number of expensive organics, needs a power supply, and regular maintenance is required.
- It can be complicated to troubleshoot problems or develop new methods.
- The lack of a universal detector for HPLC, however, the UV-Vis detector only detects chromophoric compounds.
- The separation in High-performance liquid chromatography has less efficiency than GC.
- It is more difficult for the beginner.
- HPLC pump process reliability relies on of cleanliness of the sample, mobile phase, and proper operation of the system.

CONCLUSION

Analytical methods development plays important roles in the discovery, development and manufacture of pharmaceuticals. RP-HPLC is probably the most universal, most sensitive analytical procedure and is unique in that it easily copes with multi component mixtures. Reverse phase and normal phase HPLC techniques are two liquid chromatographic techniques. The key difference between reverse phase and normal phase HPLC is that the reverse phase HPLC uses a nonpolar stationary phase and a polar mobile phase whereas the normal phase HPLC uses a polar stationary phase and a less polar mobile phase.

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