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A Review on High Performance Liquid Chromatography (HPLC)

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Abstract: High Performance Liquid Chromatography (HPLC) used tosplit, recognize and compute the matter. In practice partition, detection and quantification may constitute the whole analysis with an additional method. Partition isolates analytes. Qualitative and quantitative identifies the analytes while determines the quantity of concentration.
Keywords Chromatography, HPLC, Mobile Phase, Stationary Phase, ICH.

I. GENERAL INTRODUCTION [1-5]

In 1906 the Russian botanist M.S. Tswett reported separation of different colored constituents of extra of green leaves into a series of colored bands by allowing a solvent to percolate through column bed of powered calcium carbonate. He termed this technique as 'Chromatography' from the Greek words meaning 'color(chroma)' and 'writing(graphy)'. As a matter of above fact, chromatography owes its origin to the efforts of him. Tswett's this technique was virtually unnoticed in the literature until the early 1940s, when the well known paper of Martin and Synge was published. They reported the discovery of liquid liquid partition chromatography, both on columns and on paper. They also provided a theoretical frame work for the basic chromatographic process and they received the Nobel prize in chemistry in 1952 for their work. The next major step that led to progress in this field was the development of gas - liquid chromatography by James and Martin. The success of modern chromatography is greatly due to the excellent extensive treatment of chromatographic theory by Giddings in 1965 through his book entitled Dynamics of chromatography. Afterwards, a number of well-known scientists whose contributions are too numerous to be recounted here and their work has led to the development of modern liquid chromatography, which is often called high-pressure or high performance liquid chromatography. This technique is also called as HPLC.HPLC plays a significant role in the Pharmaceutical product growth. This is a expert branch of analytical chemistry. It involves sorting out, identifying and decisive the virtual amounts of components in atest sample. Qualitative analysis reveals that the chemical identity of the samples, establishes the relative amount of one or more of these species or analytes in numerical terms and it is required before a quantitative analysis can be undertaken. A separation step is usually a necessary part of both a qualitative and quantitative analysis. The results of typical quantitative analysis can computed from two measurements. One is the mass or volume of sample to be analyzed and second is measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis. Instrument plays a vital role in the quantitative analysis of Pharmaceuticals.

II. INSTRUMENTAL ANALYSIS [6-11]

Instrumental methods are part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied sciences. Analytical Instrumentation plays an important role in the production and evaluation of new product and in the protection of consumers and environment. Instrumentation provides lower detection limits requires assuring safe foods, drugs, air and water. Instrumental methods are widely used by analytical chemists to save time to avoid chemical separation and obtain increased accuracy. Most Instrumental techniques fit to one of the four principle areas mentioned below.

III. SOME TECHNIQUES OF SPECTROPHOTOMETRY

U V - Visible Spectrophotometry
Fluorescene and Phosphorescene Spectrophotometry
Atomic absorption &Emission Spectrophotometry
Infrared Spectrophotometry
Raman Spectrophotometry
X-Ray Spectrophotometry

Nuclear Magnetic Resonance Spectrophotometry
Electron Spin Resonance Spetrophotometry
Mass Spectrophotometry

IV. SOME TECHNIQUES OF ELECTRO CHEMICAL

A. Potentiometry
B. Voltametry
C. Electrogravimetry
D. Conductometry
E. Amperometry

V. SOME TECHNIQUES OF CHROMATOGRAPHY

Thin Layer Chromatography
Gas Chromatography
High Performance Liquid Chromatography
High Performance Thin Layer Chromatography
GC-MS (Gas Chromatography- Mass Spectroscopy)
LC-MS (Liquid Chromatography- Mass Spectroscopy)

VI. SOME TECHNIQUES OF MISCELLANEOUS

A. Thermal analysis

Kinetic techniques Chromatographic techniques are predominantly used in the pharmaceutical industry for a large variety of samples. HPLC is one of the chromatographic techniques is widely used for the checking the purity of new drugs, monitoring changes or scale ups of synthetic procedures, evaluating new formulations and scrutinizing quality control/assurance of final drug products.

VII. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY [12-20]

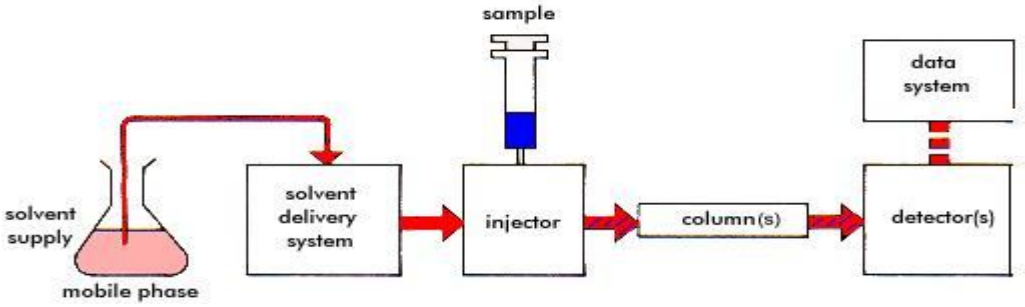
Chromatography is the technique in which the components of a mixture are separated based upon the rates at which they are distributed through two phases, one of which does not move(stationary phase) and the other that moves(mobile phase). When mobile phase is liquid, this technique is known as 'Liquid chromatography'. Liquid Chromatography (LC) is a method of chromatographic separation based on the difference in the distribution of species between two non miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column. It is mainly based on the mechanism of adsorption, mass distribution, ion exchange, size exclusion or stereo chemical interaction.

Early liquid chromatography is carried out in long glass columns with wide diameter. Now a days with the help of advent of latest technology, the particle diameters were reduced as small as to below 10 μm with replacement of glass columns to steel ones. The flow rate of the mobile phase was improved by applying high pressure to the column using pumps and hence the performance was improved. This development led to be mostly called as 'high performance liquid chromatography' or 'high pressure liquid chromatography' (HPLC). HPLC is most widely used analytical separation technique that offers major improvements over the old chromatography. The technique is more popular because it is non destructive and may be applied to thermally liable compounds (unlike GC). HPLC is ideally suitable for the separation of macromolecules and ionic species of biomedical interest, liable natural products and diverse less stable and/ or high molecular weight compounds. The majority of difficult separations are often readily attained by HPLC because both phases used in HPLC participate in the chromatographic process (as opposed to only one in GC) to increase more selective interactions with the sample molecule.

Short, small-bore columns containing densely packed particles of stationary phase provide for the rapid exchange of compounds between the mobile and stationary phases. A large variety of unique column packing's (stationary phase) provides a wide range of selectivity to separation through HPLC. HPLC also offers wide choice of detection methods as numbers of unique detectors are available. HPLC can easily be extended to trace determinations of compounds which do not usually provide adequate detector response with the use of post column derivation methods that improve selectivity and detection limits. Facility to arrange gradient flow of mobile phase is often use during method development and it is also provide the possibility to achieve difficult separation in reduced run-time. HPLC contains automatic instrument and calculation which is carried out by integrator itself that offers saving of manual labour. In addition to receiving and reporting detector outpour, computers are used to control chromatographic settings and

operations. All these advantages make HPLC more efficient over the all remaining chromatographic techniques in case of separation, speed, sensitivity, easy sample recovery, automation, integration and handling and maintained. The wide applicability of HPLC makes it as a most important separation tool in scientific the field of analysis.

A. Instrumentation and Designing of HPLC [21-25]



Schematic instrumentation of HPLC equipment

A liquid chromatography consists of a reservoir containing the mobile phase, a pump to force the mobile phase through the system at high pressure, an injector to introduce the sample into the mobile phase, a chromatographic column to attain retention, a detector to detect analyte response and a data collection device such as a computer, integrator or recorder. Further, in some cases, degasser with vacuum pump and pre-column facility can implement in the modern HPLC.

B. HPLC modes

Various modes of HPLC utilized to separate compounds are classified as follows:

C. Based Upon the Nature of Stationary and Mobile phase

There are different types of Chromatography based on the type of stationary and mobile phase used. They are

1) Gas - Solid Chromatography
2) Gas - Liquid Chromatography
3) Solid - Liquid Chromatography[Column Chromatography, Thin layer Chromatography, HPLC [High Performance Liquid Chromatography]
4) Liquid - Liquid Chromatography [Paper partition Chromatography, Column Chromatography]

Polar - Polar - Interaction or Affinity is more
Non Polar - Non Polar - Interaction or Affinity is more
Polar - Non Polar - Interaction or Affinity is less.

D. Based on the modes of Chromatography [26-30]

There are two types. They are based upon the polarity of the stationary phase and mobile phase used.

E. Normal Phase Chromatography

In this the stationary phase is Polar and mobile phase is Non-polar. This is not widely used in pharmacy.

F. Reverse phase Chromatography

In this, stationary phase is Non - polar and mobile phase is polar. This is most widely used pharmaceutical analysis.

Comparison of Normal phase and Reverse phase

Name of the Phase	Normal Phase	Reverse phase
Stationary phase	Polar	Non - Polar
Mobile phase	Non - Polar	Polar
Compound eluted first and retained	Non - Polar	Polar

less		
Compound eluted last and retained more	Polar	Non - Polar
Examples of stationary phase	Silica Gel	ODS(C_{18}), C_8, C_4 -bonded phases

G. *Functional Group*

The functional group present in stationary phase depends on the type of chromatography separation. In normal phase mode it contains the silanol groups(hydroxy group). In reverse phase mode it contains the following groups:

C_{18} - OctaDecylSilane(ODS) COLUMN
C_8 - Octyl column
C_4 - Bonded phases
CN - Nitrile column
NH_2 - Amino column

VIII. TYPES OF ANALYSIS

A. Qualitative Analysis

Which is used to identify the compound, detect the presence of impurities, to find out the no of components, etc., this is done by using retention time values.

B. Quantitative Analysis

Which is done to determine the quantity of the individual or several components in a mixture. This is done by comparing the peak area of the standard and sample.

IX. CONCLUSION

HPLC is used as a analytical technique. It is the versatile, universal and unique equipment. It is mainly deals with the drug analysis. It is very useful for researchers regarding method development and validation under ICH guidelines.

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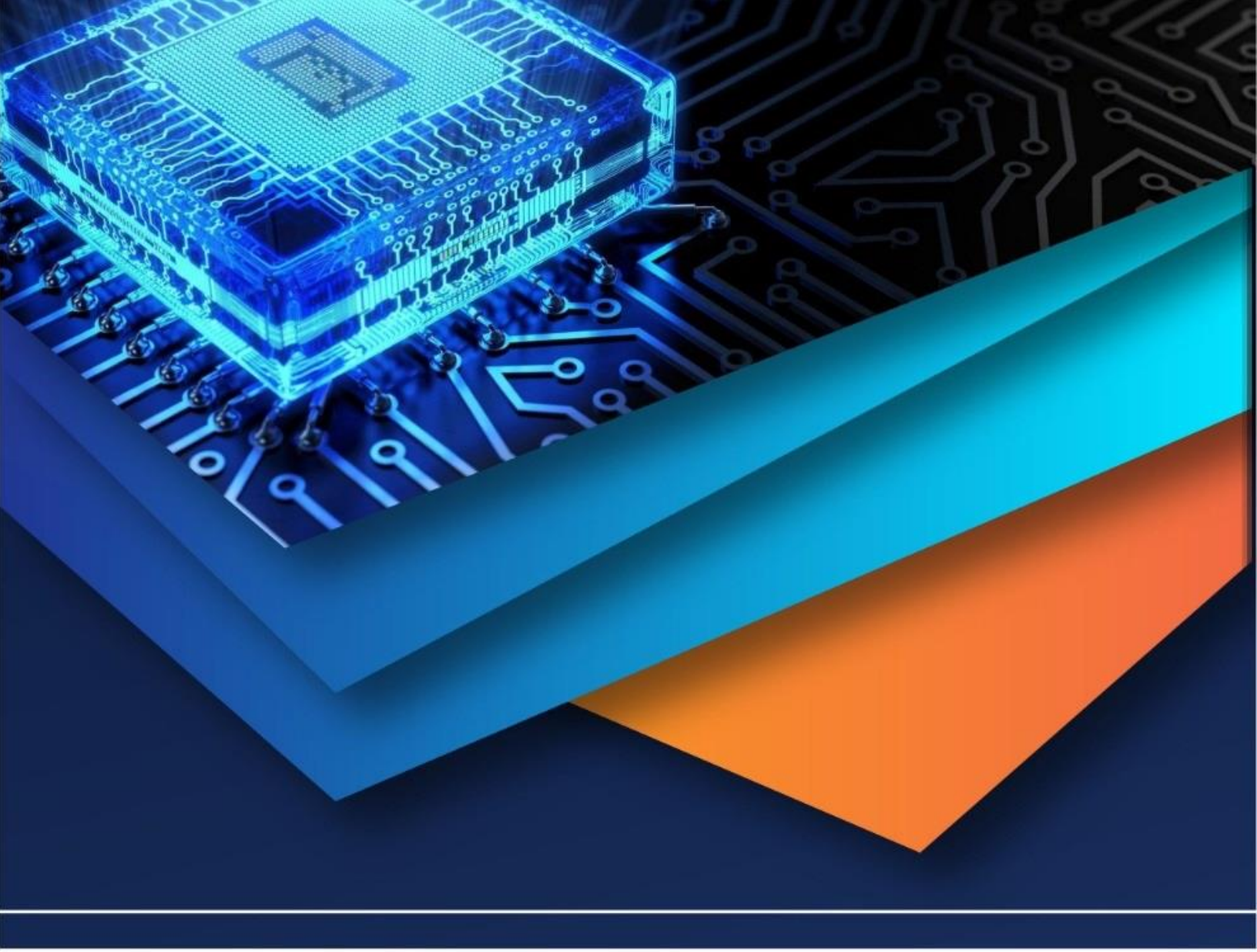

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