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A REVIEW ON COMPARISION OF HPLC AND HPTLC

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Abstract

The analytical method should be sensitive, specific, fast and accurate to establish the assurance that the equipments used in manufacturing are free of the unwanted impurity, presence of which may alter the safety and efficacy of the drug product. HPLC, UPLC techniques have established their role in pharmaceutical cleaning validation. Among the modern Analytical tools HPTLC is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. HPTLC is playing an important role in today analytical world, not in competition to HPLC but as a complementary method. One of the most obvious orthogonal features of the two techniques is the primary use of reversed phases in HPLC versus unmodified silica gel in HPTLC, resulting in partition chromatography and adsorption chromatography respectively. High Performance Thin layer Chromatography (HPTLC) technique is a sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits.

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Introduction

Chromatography is a separation process where a mixture of solute is allowed to interact with two physically distinct entities, that is, the stationary phase and the mobile phase. The mixture of solutes is dissolved in a common solvent and is separated from one another by a differential distribution of solutes between the stationary and the mobile phase. The stationary phase can be the solid/gel/liquid/solid-liquid mixture that is immobilised and it may have the ability to bind some type of solutes on to it. And the mobile phase can be liquid or gas which passes over the stationary phase.

This short column helps protect the analytical column and increase its lifespan by removing larger particles and impurities before they can enter the column. The composition is similar to that of the analytical column. There are many different detectors that can be attached to an HPLC. They include Ultra-Violet, Refractive Index, Fluorescent, Electrochemical, Mass Spectroscopy, and Light Scattering.

History

Mikhailtswett

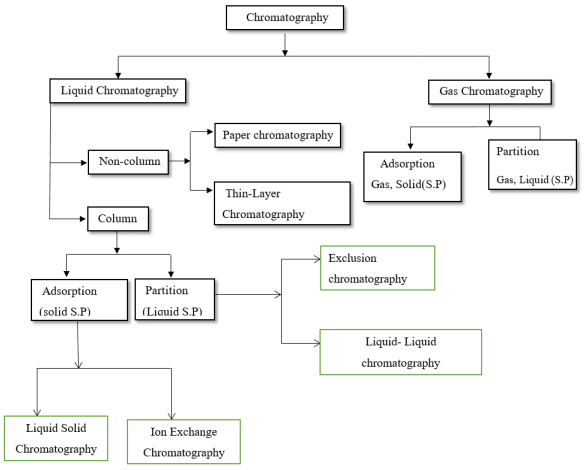
Russian, 1872-1919 Botanist In 1906 Tswett used to chromatography to separate plant pigments He called the new technique chromatography because the result of the analysis was 'written in color' along the length of the adsorbent column Chromameans “color” and graphein means to“write .Chromatography has application in every branch of the physical and biological sciences 12 Nobel prizes were awarded between 1937 and 1972 alone for work in which chromatography played a vital role [1].

General Principle of Chromatography

All forms of chromatography work on the same principle. They have a stationary phase (a solid or liquid supported on a solid) and a mobile phase (a liquid or a gas). During the process, the sample containing many molecular components comes into contact with the stationary phase and the components distribute themselves between the stationary and the mobile phase. If some components in the sample are bound to the stationary phase then they will spend more time in stationary

phase and hence their movement in the chromatographic system will be retarded. On the other hand, the molecules that show weak affinity/interaction with the stationary phase spends more time with the mobile phase and are rapidly removed and eluted from the system. Thus the rate of migration of the solute depends on the rate of interaction of it with the stationary and mobile phase. Distribution or Partition coefficient (Kd) describes the way an analyte distributes between the two immiscible phases. Kd = Thus the difference in Kd value of the components results in their separation. And the general process of moving a solute mixture through a chromatographic system is called development [2].

Classification of Chromatography



A Comparison Study of HPLC and HPTLC Principle instrumentation and Applications

Liquid chromatography is similar to gas chromatography but uses a liquid mobile phase. The stationary phase is usually an inert solid or a liquid held on the inert solid. Mobile phase travels through the column forcibly with the aid of the high pressure pump. Solutes of the sample separated on column and eluted with mobile phase [1-5]. The technique is applicable to thermally fragile samples, e.g. amino acids, proteins, nucleic acids, hydrocarbons, antibiotics, steroids, drugs, inorganic and may organic substances.

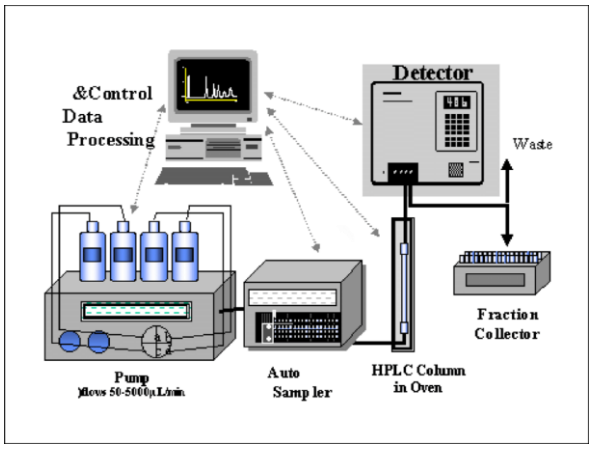
HPLC System

The HPLC consists [1-8] of pumps, an injector, column, mobile phase reservoir, oven, and detector. The injector introduces the sample into the HPLC system. This is either done by hand with a syringe, or automated with an auto sampler. Figure 1 & 2 shows a simplified schematic representation and analytical steps of HPLC system. There are several different types of pumps available for use with HPLC. They include reciprocating pumps, which are the most common, syringe type pumps, and constant pressure pumps. The reciprocating pumps use a motor driven piston to pump mobile phase into the column. On the backstroke, mobile phase is pulled in, and on the forward stroke, it is driven out to the column.

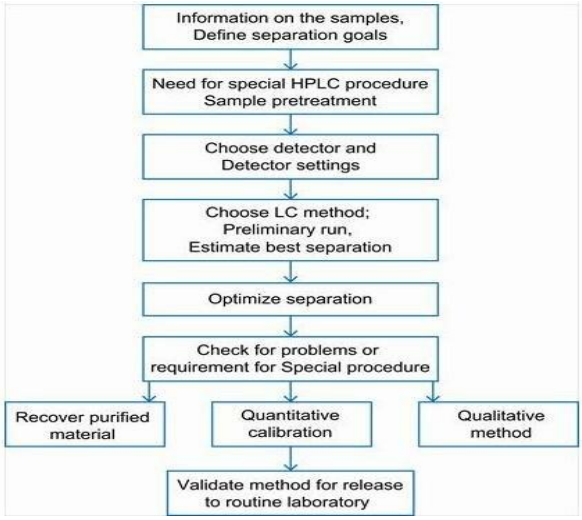
These have the advantage of being able to achieve a wide range of flow rates. Dual and triple head pumps consist of identical units, which are 120 or 180 degrees out of phase. Syringe type pumps, or displacement pumps, have a very small capacity, and are therefore most suited to small bore columns. They consist of a large syringe type reservoir, with a plunger that is activated by a motorized lead screw. The flow rate can be controlled by changing the voltage on the motor. Constant pressure pumps use pressure from a gas cylinder to drive the mobile phase through the column. In order to generate high liquid pressures, a low-pressure gas source is needed. The solvent chamber has a low capacity, but a valve arrangement allows for rapid refill, and provides continuous mobile phase flow rate. HPLC columns are usually made of stainless steel tubing. There are two types of columns that are distinguished by the relative polarities of the mobile and stationary phases. Guard columns are often used in front of the column. This short column helps protect the analytical column and increase its lifespan by removing larger particles and impurities before they can enter the column [3]. The composition is similar to that of the analytical column. There are many different detectors that can be attached to an HPLC. They include Ultra-Violet, Refractive Index, Fluorescent, Electrochemical, Mass Spectroscopy, and Light Scattering.

Liquid chromatographic separation modes Normalphase chromatography (Adsorption Chromatography): The principle of adsorption chromatography [1-8] is known from classical column and TLC. A relatively polar material (water-soluble, hydrophilic) with a high specific surface area is used as the stationary phase, silica being the most popular, but alumina and magnesium oxide are also often used. The mobile phase is relatively non-polar (fat soluble, lipophilic) as heptanes or tetra hydro furan. The different extents to which the various types of molecules in the mixture are adsorbed on the stationary phase provide the separation effect. Polar compounds are eluted later than non-polar compounds. RP-Chromatography RP- chromatography [1-8] is the term used to describe the state in which the stationary phase is less polar than the mobile phase. Chemically bonded octa decyl silane (ODS), an n-alkane with 18 carbon atoms, is the most frequently used stationary phase.

C8 and shorter alkyl chains and also cyclohexyl and phenyl groups provide other alternatives. Phenyl groups are more polar than alkyl groups. The reverse of the above applies [1-11]: (a) The stationary phase is very non-polar. (b) The mobile phase is relatively polar. (c) A polar solvent such as water elutes more slowly than a less polar solvent such as acetonitrile. So, non-polar compounds are eluted later than polar compounds. State-of-the-art HPLC equipment can automate HPLC separations, using automatic samplers, injectors, microprocessor-controlled analytical conditions and ChemStations for data evaluation. Important requirements for automation are: • Excellent precision of the liquid chromatography system, • Data evaluation with report printouts, the possibility to store chromatograms and results, • The possibility to detect leaks and other errors for safety reasons, and • implemented OQ/PV tools in the HPLC system.



Optimization Steps Involved In HPLC

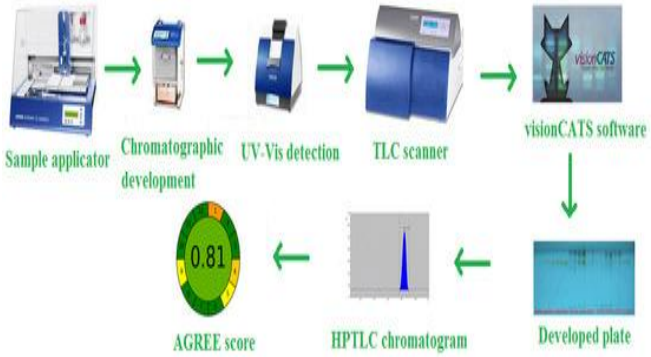


High-Performance Thin-Layer Chromatography (HPTLC)

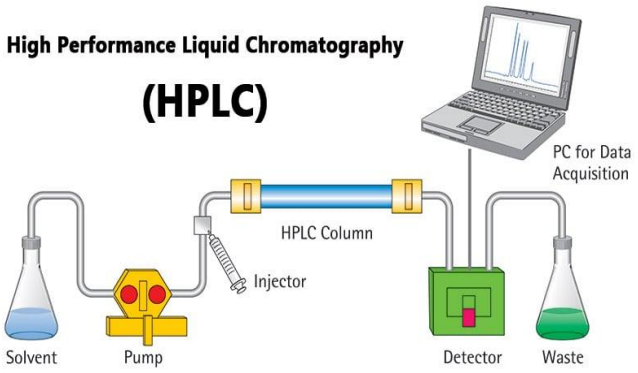
Is a form of thin-layer chromatography (TLC) that provides superior separation power using optimized coating material, automated procedures for mobile phase feeding, layer preconditioning, précised sample application, chromatogram development scanning, and photo documentation. Figure 3 & 4 shows a simplified schematic representations and analytical steps of HPTLC system. It promotes for higher separation efficiencies, shorter analysis time, lower amounts of mobile phase, and efficient data acquisition and processing. HPTLC has strong potentials as a surrogate chromatographic model9for estimating partitioning properties in support of combinatorial chemistry, environmental fate, and health effect studies. The method can be used to validate the simultaneous estimation of two or more drug combinations in a dosage form. One of the available chromatographic techniques is HPTLC, which is used for the identification of constituents, identification and determination of impurities, and quantitative determination of active substances. The use of modern apparatus such as video scanners, densitometers, and new chromatographic chambers, and more effective elution techniques, highresolution sorbents with selected particle size or chemically modified surface, the possibility of combining with other instrumental methods, and development of computer programs for method optimization.[4]

HPTLC an important alternative method to HPLC or gas chromatography. Specifically, HPTLC is one of the ideal TLC techniques for the analytical purposes because of its increased accuracy, reproducibility, and ability to document the results, compared with standard TLC. Because of this, HPTLC technologies are also the most appropriate TLC technique for conformity with GMPs

HPLC is a modern technique has chemical standardize technique a much more reliable and reproducible method for the standardization of both single and compound herbal formulation. High-pressure liquid chromatography, is a separation technique based on a stationary phase and a liquid mobile phase. Separations are achieved by partition, adsorption or ion exchange process, depending upon the size of stationary phase used [5].



Instrumentation



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.[6]
- 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” [5].

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 [7].

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 [8].
- The fluent used for LC varies from acidic to basic solvents.
- Most column housing is made of stainless steel since stainless is tolerant towards a large variety of solvents.[9]

Detector

- 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.
- This difference is monitored as a form of an electronic signal. There are different types of detectors available[10]

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 [11].

Applications of HPLC

Pharmaceutical Industry

The pharmaceutical industry hevily relies on HPLC chromatography for drug analysis and quality control.this technique allows for the separation identification and qualification of various chemical compounds present in a drug formulations.[12]

Drug Discovery and Development

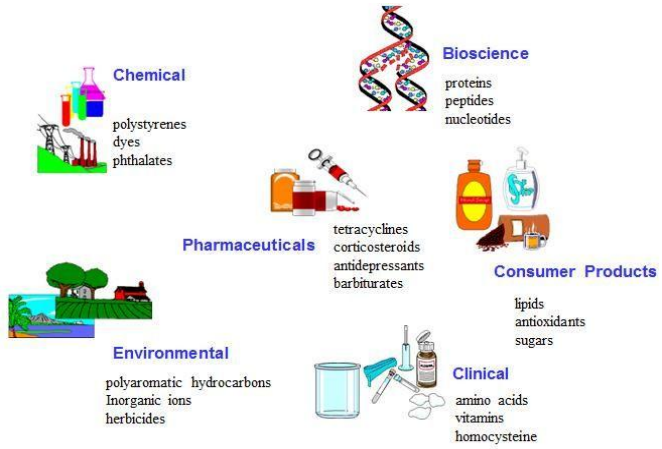
Drug discovery and development is a complex and meticulous process that involve identifying and designing potential drug compounds,conducting pre cleanical trails,and performing clinical trails to asses the safety and efficacy of the drug candidates [13].

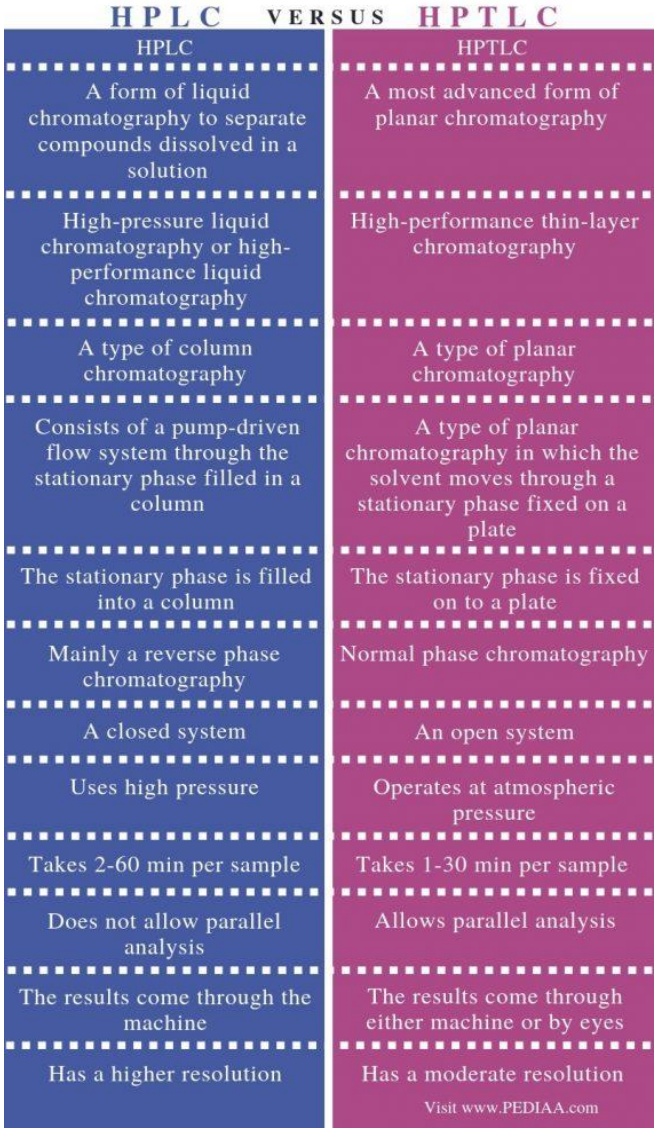
Environmental Monitoring and Analysis

Plays a crucuial role in determining the presence and levels of varios containments in the environment,high performance liquid chromatography as emerged as a powerful tool in this field, as it allows for the identification and qualification of wide range orgnic and inorganic and compounds [14].

Forensic Science

Forensic science,a multidisiplanory field,plays crucial role in solving criminal investigations and providing justice. By utilizing various scientific techniques, such as HPLC forensic scientist can accurately analyse and identify substance found at crime scenes [15].





Advantages of HPLC HPTLC

- Higher resolution and speed of analysis
- HPLC columns can be reused without repacking or generation.
- Greater reproducibility due to close control of the parameters affecting the efficiency of separation [16].

- Easy automation of instrument operation and data analysis
- Adaptability to large scale preparative procedures [17].

Disadvantages of HPLC HPTLC

- It can be costly complex to operate and does not work for all sample
- Need a skill to run the instruments
- Solvents consuming [18].

Current Developments in Chromatography

HPLC

There is a growing need for chemical analyses to be performed in the field, at the point of need. Technical advances in miniaturization and liquid chromatography are enabling the translation of these techniques out of the laboratory and into the field. Portable platforms capable of real-time reverse transcription polymerase chain reaction (RRT-PCR) and amplification assays for various diseases have revolutionized surveillance in sectors ranging from aquatic ecology to industrial quality control The conventional size of HPLC columns was 4.6 by 250 mm. Modern columns tend to be shorter now, with a length of 50 to 100 mm [19].

HPTLC

High-performance thin-layer chromatography (HPTLC) is a sophisticated instrumental technique based on the full capabilities of thin layer chromatography. It has several advantages such as automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, etc.[13] A comparative review on High-Performance Liquid Chromatography (HPLC) and HPTLC has been conducted to understand the improvements in HPTLC. The review states that the improvements in HPTLC are intended to up surge the resolution of compounds to be separated and to permit quantitative analysis of the compound [20].

Conclusion

Conclusions HPTLC represents a natural evolution of classical TLC. It is a sophisticated analytical technique that provides essential benefits, such as simultaneous screening of many samples, cost effectiveness, and time savings. We developed a rapid, sensitive, and sustainable HPTLC method that could be used for the identification of meloxicam and piroxicam simultaneously and separately. The method was able to detect meloxicam and piroxicam in the following very low concentrations: 0.04 µg per band for meloxicam and 0.05 µg per band for piroxicam. This method could be used in research, or for routine quality control.

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Conflict of Interest

No Conflict of interest declared by the authors.

Informed Consent & Ethical Statement

Not Applicable

Author Contribution

All authors are contributed equally.

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