

## An Overview of HPTLC and HPLC: Theory, Practice, and New Advancements

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### Abstract

In order to guarantee that the production equipment is free of any undesirable contaminant that might affect the drug product's safety and effectiveness, the analytical approach must be sensitive, specific, rapid, and accurate. The use of HPLC and UPLC methods in pharmaceutical cleaning validation has been well-established. When it comes to qualitative and quantitative analysis, HPTLC is just as effective as any other contemporary analytical instrument. Today, high-performance thin-layer chromatography (HPTLC) is an integral part of analytical practice, working in tandem with HPLC rather than against it. The fundamental usage of reversed phases in high-performance liquid chromatography (HPLC) and unaltered silica gel in high-performance thin-layer chromatography (HPTLC) is one of the most glaringly orthogonal elements of the two methods. An improved and automated variant of thin-layer chromatography (TLC) that offers higher detection limits and greater separation efficiency is known as high performance thin-layer chromatography (HPTLC).

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### Introduction

Chromatography involves the interaction of a solute mixture with two physically separate components, the stationary phase and the mobile phase, in order to separate the solutes. A common solvent dissolves the mixture of solutes, which are then separated by a difference in solute distribution between the stationary and mobile phases. The solute-binding capability of the immobilized solid, gel, liquid, or solid-liquid combination makes it a potential stationary phase. Additionally, a gas or liquid known as the mobile phase moves past a stationary phase.

This short column prevents bigger particles and contaminants from entering the analytical column, extending its lifetime and protecting it. The analytical column's composition is comparable to this one. An HPLC may be equipped with a wide variety of detectors. They

include Ultra-Violet, Refractive Index, Fluorescent, Electrochemical, Mass Spectroscopy, and Light Scattering.

### History Mikhailswett

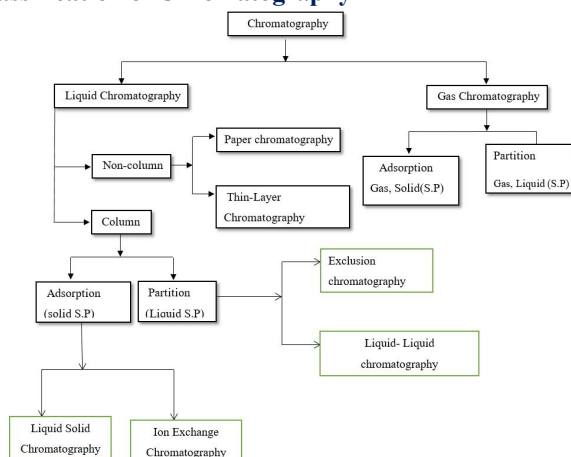
Russian, 1872-1919 Botanist In 1906 Tswett used to chromatography to separate plant pigments He called the new technique chromatography because the result of the analysis was 'written in color' along the length of the adsorbent column Chromameans "color" and graphein means to"write". Chromatography has 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

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 spend 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 ( $K_d$ ) describes the way an analyte distributes between the two immiscible phases.  $K_d = \frac{\text{Concentration in stationary phase}}{\text{Concentration in mobile phase}}$ . Thus the difference in  $K_d$  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.

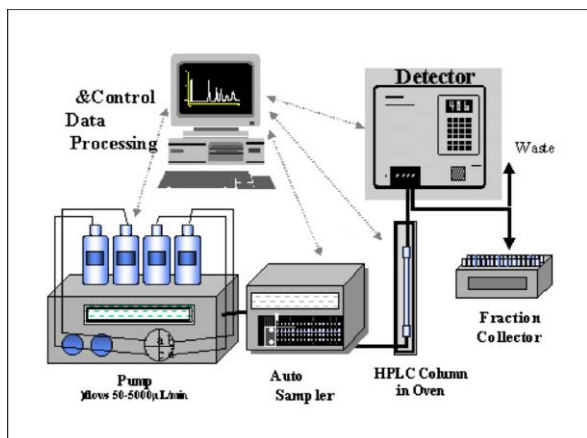
### HPLC System

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

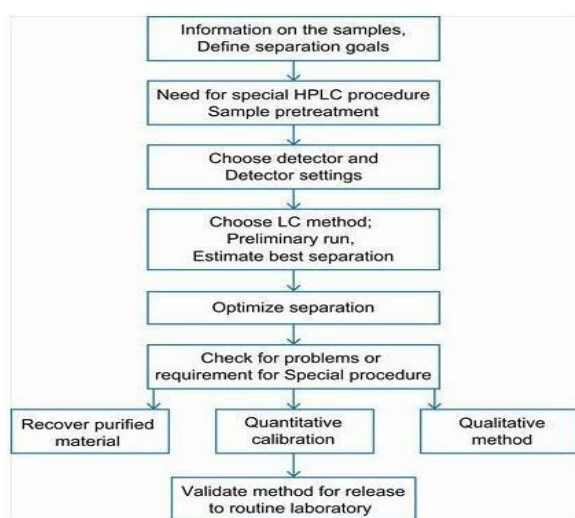
of a large syringe type reservoir, with a plunger that is activated by a motorized lead screw. The flow rate can be controlled by changing the voltage on the motor. Constant pressure pumps use pressure from a gas cylinder to drive the mobile phase through the column. In order to generate high liquid 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

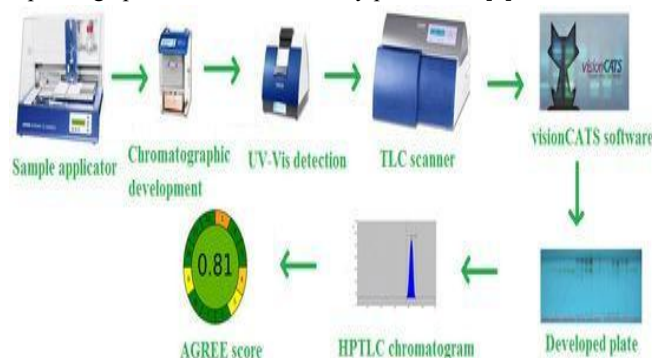


### High-Performance Thin-Layer Chromatography (HPTLC)

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 model<sup>9</sup>for 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, high-resolution 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.<sup>4</sup>

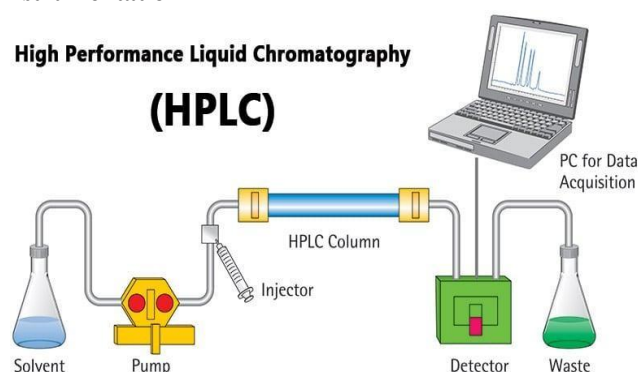
HPTLC an important alternative method to HPLC or gas chromatography. Specifically, HPTLC is one of the ideal TLC techniques for the analytical purposes because of its increased accuracy, reproducibility, and ability to document the results, compared with standard TLC. Because of this, HPTLC technologies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

#### High Performance Liquid Chromatography (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.<sup>6</sup>
- High-pressure generation is a “standard” requirement of pumps besides which, it should also 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.<sup>9</sup>

#### 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<sup>10</sup>

#### 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 heavily 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 drug formulations.<sup>12</sup>

#### Drug Discovery and Development

Drug discovery and development is a complex and meticulous process that involves identifying and designing potential drug compounds, conducting pre-clinical trials, and performing clinical trials to assess the safety and efficacy of the drug candidates [13].

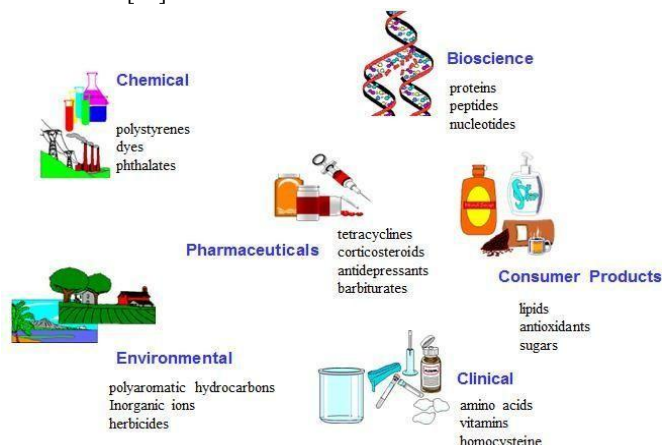
#### Environmental Monitoring and Analysis

Plays a crucial role in determining the presence and levels of various contaminants in the environment. High performance liquid chromatography has emerged as a powerful tool in this field, as it allows for the identification and qualification of a wide range of organic and inorganic compounds [14].

#### Forensic Science

Forensic science, a multidisciplinary field, plays a crucial role in solving criminal investigations and providing justice. By

utilizing various scientific techniques, such as HPLC, a forensic scientist can accurately analyze and identify substances found at crime scenes [15].



HPLC VERSUS HPTLC	
HPLC	HPTLC
A form of liquid chromatography to separate compounds dissolved in a solution	A most advanced form of planar chromatography
High-pressure liquid chromatography or high-performance liquid chromatography	High-performance thin-layer chromatography
A type of column chromatography	A type of planar chromatography
Consists of a pump-driven flow system through the stationary phase filled in a column	A type of planar chromatography in which the solvent moves through a stationary phase fixed on a plate
The stationary phase is filled into a column	The stationary phase is fixed on to a plate
Mainly a reverse phase chromatography	Normal phase chromatography
A closed system	An open system
Uses high pressure	Operates at atmospheric pressure
Takes 2-60 min per sample	Takes 1-30 min per sample
Does not allow parallel analysis	Allows parallel analysis
The results come through the machine	The results come through either machine or by eyes
Has a higher resolution	Has a moderate resolution

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#### 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

- 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.<sup>13</sup> 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

Last thoughts As a development from traditional TLC, HPTLC is a logical step forward. Time savings, cost effectiveness, and the ability to screen several samples at once are just a few of the important advantages offered by this advanced analytical method. In order to identify meloxicam and piroxicam independently or in combination, we created a fast, sensitive, and sustainable HPTLC technique. At very low concentrations, the approach could identify meloxicam and piroxicam: 0.04 µg per band for meloxicam and 0.05 µg per band for piroxicam. This approach has potential for both experimental and operational quality assurance purposes.

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