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A comparative review on High-Performance Liquid Chromatography (HPLC), Ultra Performance Liquid Chromatography (UPLC) & High-Performance Thin Layer Chromatography (HPTLC) with current updates

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ABSTRACT

Any chosen analytical method should be subtle, precise, fast and exact to begin the guarantee that the material used in the manufacturing is free of unsolicited impurity, the existence of which may vary the safety and effectiveness of the drug product. The techniques of HPLC and UPLC have established their part in pharmaceutical cleaning validation. High-Performance Liquid Chromatography (HPLC) is the main pharmaceutical and biomedical analysis approach utilized today because it generates highly efficient separations, and in most circumstances, it provides high detection sensitivity. Applying the HPLC method has several advantages compared to other methods, among others, specificity, rapidity, accuracy, precision, and the ease of automation. Due to the aforementioned, most drugs in a multi-component dosage form can be analyzed. Ultra-Performance Liquid Chromatography (UPLC) is a modern-day technique that gives a new track for liquid chromatography. UPLC provides the user with speed of application, resolution and sensitivity. The quantification and separation in UPLC are done under very high pressure (up to 100M Pa). High-Performance Thin Layer Chromatography (HPTLC) has improved and innovative separation efficacy and detection limits. It is a cultured and automated form of Thin Layer Chromatography (TLC) and is based on the use of an optimized silica gel 60 with a significantly smaller particle size than which is used for TLC. The previously stated analytical methods are employed for purity control of chemicals, steroids, pesticides, and water analysis, water-soluble food dyes, vitamins, pesticides in vegetables, fruits, and other foodstuffs. The current updates in the techniques allow us to understand the increased utilization of these methods in the current eras.

INTRODUCTION

Chromatography is a lab technique for separating mixtures. Chromatography is perhaps the most influential analytical technique accessible to modern chemists. Its authority ascends from its capacity to determine quantitatively many individual components present in mixtures by the application of a single analytical procedure. An accurate analytical methodology should be used for stability testing of

drug substances that will quantitate the active pharmaceutical ingredients (API) without interfering with degraded products, process impurities, and other potential impurities [1].

High-Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is also known as High-Pressure Liquid Chromatography. HPLC is a popular analytical technique and it is applied for identification, separation and quantification of each constituent of a mixture. The stability-indicating strategies

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of HPLC are employed in separating varied drug-related impurities which form during manufacturing or synthesis of drug products [2].

Ultra Performance Liquid chromatography

Ultra Performance Liquid chromatography UPLC contributes to the improvement of 3 areas: Speed, chromatographic resolution, and sensitivity analysis. UPLC methods use fine particles i.e., less than 2.5 μm which decreases the column length. Moreover, solvent consumption is also reduced and it saves time. UPLC works on chromatographic principles using columns packed with smaller particles and higher flow rates to run partitions. Smaller particles can be used for extending speed and peak capacity to new limits.

For this separation mechanism, the principle applied is the Van Deemter equation:

$$H = A + B/v + Cv$$

where,

H – reduced plate height, an immeasurable measure of the band broadening where:

A – Eddy mixing

B – Axial diffusion

C – Solute's mass transfer

v – linear velocity.

The above equation describes the relationship between linear velocity (flow rate) and plate height (Column efficiency). With the help of this equation, we can understand that with smaller particles, efficiency increases but this will lead to a quick increase in backpressure as most HPLC systems can operate to 400 bars only. To hasten the examination without loss of efficiency and maintaining an acceptable loss of loads, we use short columns filled with particles of 2 μm [3].

High-Performance Thin Layer Chromatography (HPTLC)

High-Performance Thin Layer Chromatography (HPTLC) is the most powerful and progressive form of Thin Layer Chromatography (TLC) and involves chromatographic layers of very unlike functionality and the use of modern equipment for all phases of the method. HPTLC embraces a complete standardized method based on the use of effective methods for qualitative and quantitative analysis built upon scientific facts. HPTLC allows more accurate quantitative measurements and it also meets all quality requirements for today's analytical laboratories. The improvements in HPTLC are intended to up surge the resolution of compounds to be separated and to permit quantitative analysis of the compound. Some developments, like the use of higher quality thin layer chromatography plates with finer particle sizes in the stationary phase, allows better resolution. Furthermore, the separation is enhanced by repeated plate development, using multiple development devices. At the moment, beyond better resolution, HPTLC a Lower Limit of Detection (LODs) than the previous mentioned approaches [4].

PRINCIPLE

High-Performance Liquid Chromatography (HPLC)

The separation of HPLC is based on the spreading of the analyte between a mobile phase and an immobile phase, it depends on the chemical structure of the analyte, and the molecules are retarded while passing the stationary phase. The specific intermolecular interactions between the molecules of a sample and the packing material express their time on-column. The different constituents of a sample are eluted at different times and separation of sample ingredients is promoted. The detection unit recognizes the analytes after leaving the column and signals are transformed and chronicled by a data management system.

Ultra Performance Liquid Chromatography (UPLC)

UPLC is Ultra Performance Liquid Chromatography. UPLC became the trendy standard HPLC platform due to its accumulative sampling throughput chromatographic potency, sensitivity and decreased run time. UPLC handles pressure up to 15,000 psi, and is the newest technology in liquid chromatography-based analysis. Basically, it is an upgraded form of high-performance liquid chromatography using high pressures, and offering outstanding peak resolution and sensitivity [5].

High-Performance Thin Layer Chromatography (HPTLC)

High-performance thin-layer chromatography is able to separate complex organic compounds. HPTLC utilizes a similar approach and applies similar physical principles as that of TLC (Adoption Chromatography) i.e. the principle of separation is adsorption. The critical usage of 'very fine particulate sorbents' in HPTLC enables a distinct display of the movement of the ensuing 'mobile phase' after relatively short traversed distances. In order to circumvent this marked 'limitation', a 'forced-flow technique' has been devised by making use of a 'pressurized chamber'. The mobile phase is incorporated with the help of a 'sophisticated pump' at an almost constant velocity via a strategically located 'slit' in a plastic sheet which eventually covers the stationary phase bearing the 'analyte samples' [6].

CLASSIFICATION

Classification of HPLC

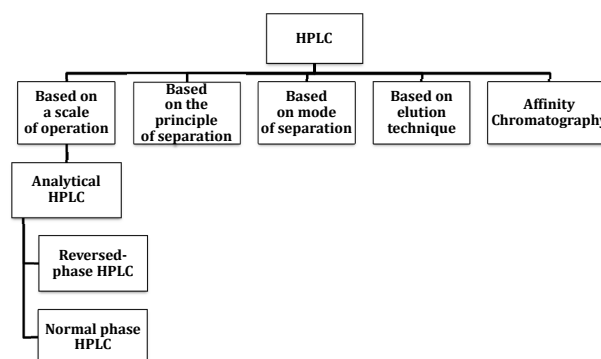


Figure 1. Classification of HPLC [7]

Classification of HPTLC

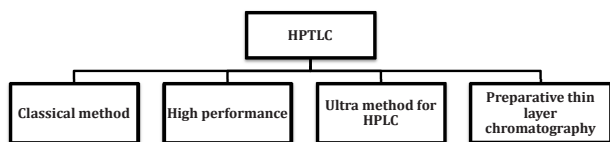


Figure 2. Classification of HPTLC [8]

INSTRUMENTATION

The representation of an HPLC includes:

Table 1. Instrumentation of HPLC, HPTLC, UPLC

Sr. no.	HPLC	UPLC	HPTLC
1	Detector – Generates signal proportional to sample component emerging from the column	Detector – These are UV/visible detectors	Plate coater – It should be coated and conveyed underneath the whooper filled with absorbent
2	Sampler – Brings sample mixture into the mobile phase stream which carries it into the column	Sample injection – To protect the column from extreme fluctuations	Drying racks – It consists of an individual aluminum plate dimension of 20×20 cm which can be stacked quickly
3	Column – Responsible for the separation of sample components	Column – Responsible for the separation of sample components	Plate cutter – Used to cut plates in thickness of 3 mm
4	Pump – Deliver the desired flow and composition of the mobile phase through the column	Column manager and cooler heater – Enables the laboratory to use temperature as a method parameter	Plate heater – Designed for heating TLC plate to a given temperature
5	Injector – Allows the introduction of precise sample volume into the column	Software – for controlling and diagnosing and monitoring via graphical system console	Development chamber – Should be sealed tight enough that the atmosphere inside the container is saturated with developing liquids
6	Degasser – Removes gases from the liquid which forms bubbles	Accessories – Recording technology, available on all acuity UPLC columns - records column history. Flex cart system platform improves usability, accessibility and convenience	Scanning densitometer – Designed to handle objects up to 200*200 mm - is connected to a computer which controls the action process and generates the report
7	Mixing unit – Used to mix solvents and pass through the column	Connection insight service (if provided by manufacturer) – Uses intelligent device management technology to provide diagnostic information for the UPLC system	Derivatization devices – Automatic developing chamber, horizontal developing chamber

WORKING

Working of HPLC

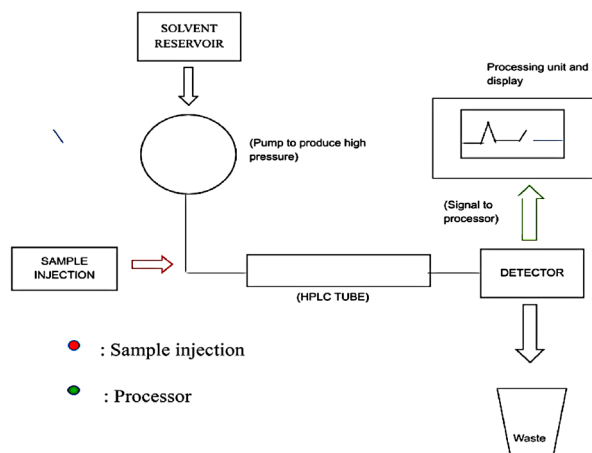


Figure 3. Working of HPLC

Working of UPLC

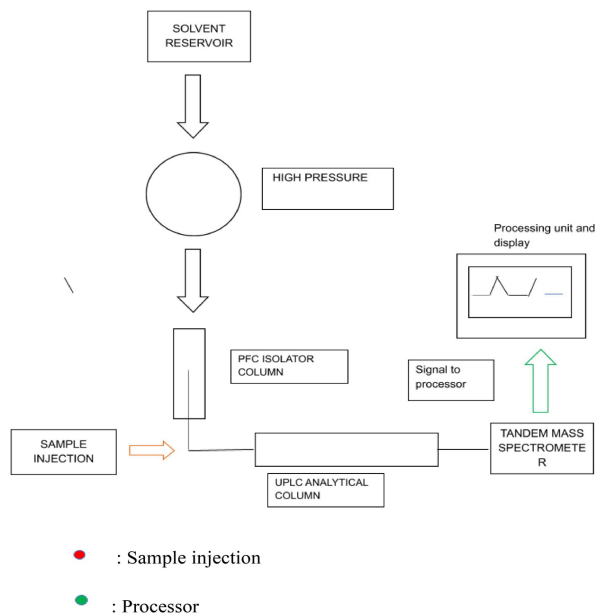


Figure 4. Working of UPLC

Working of HPTLC

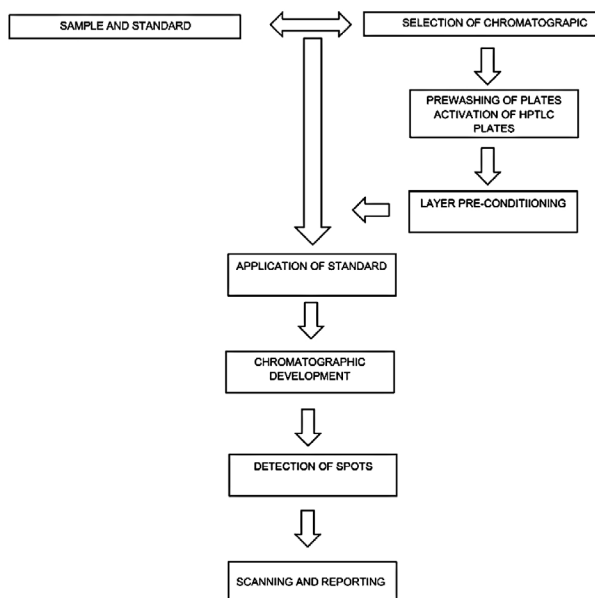


Figure 5. Working of HPTLC

Advantages, disadvantages and applications

Table 2. Advantages, disadvantages, application of HPLC, HPTLC, UPLC [9,10].

Sr. no.		Advantages	Disadvantages	Applications
6.1	HPLC	<ul style="list-style-type: none"> HPLC offers a fast, automated and extremely precise technique for recognizing certain chemical components in a sample. High-performance liquid chromatography offers a quick and precise quantitative analysis. A gradient solvent system can be applied in certain methods 	<ul style="list-style-type: none"> HPLC can be an expensive method, it requires 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. HPLC pump process reliability depends on cleanliness of the sample, mobile phase, and application of correct procedures 	<ul style="list-style-type: none"> Water Purification Ligand – exchange chromatography Ion- exchange chromatography High – ph anion exchange chromatography & oligosaccharides
6.2	UPLC	<ul style="list-style-type: none"> It requires less runtime and increases sensitivity. It supplies selectivity, sensitivity, and dynamic range of liquid chromatography analysis. Multiple residue methods are applied 	<ul style="list-style-type: none"> The main disadvantage of UPLC analysis is that the life of columns is less. Due to increase in pressure, the life column is affected and requires more maintenance. Utilising a stationary phase of particle size 2 µm better analysis is achieved without negative effects 	<ul style="list-style-type: none"> UPLC gives deeper explanation into the meaning of analysis For drug development and manufacturing processes Rapid analysis helps in studying pharmacokinetic absorption, distribution, metabolism, excretion (ADME)
6.3	HPTLC	<ul style="list-style-type: none"> HPTLC is a modern adaptation of TLC with better and advanced separation efficiency and detection limits. Samples in minute quantities like in nano-gram range. Samples rarely require clean-up 	<ul style="list-style-type: none"> Costly & requires large quantities of expensive organics Bulky instrumentation, large space requirement, many fold expensive, requires stringent conditions of operation (dust-free environment and temperature controlled conditions), and technically skilled people with the knowledge to run the system. Plate length is limited and, hence, separation takes place only up to a certain length 	<ul style="list-style-type: none"> Pharmaceutical applications include: Quality control Stability test Cosmetics Forensics Investigation of poisoning Detection of documents forgery Herbal medicines and botanical dietary supplements

Comparison between HPLC, UPLC and HPTLC

Table 3. Comparison between HPLC, UPLC, HPTLC [11]

Sr no.	Parameters	HPLC	UPLC	HPTLC
7.1	Definition	It is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture	UPLC is a modern technique that gives a new direction for liquid chromatography. UPLC refers to ultra-performance liquid chromatography, which is enhanced mainly in three areas: "speed, resolution and sensitivity	High-performance thin-layer chromatography is an improved form of thin-layer chromatography. A number of modifications can be made to the basic method of thin-layer chromatography to automate the different steps, to increase the resolution achieved, and to allow more accurate quantitative measurements
7.2	Type of Chromatography	Column chromatography	Column chromatography	Planar chromatography
7.3	Stationary phase	Filled into a column	Filled into a column	Fixed onto a plate
7.4	Chromatography phase	Reversed phase	Reversed phase	Normal phase
7.5	Particle size	Particle size of stationary phase is between 3 to 5 microns	Particle size of stationary phase is less than 2 microns	Particle size of stationary phase is less than 3 to 5 microns
7.6	Pressure	High pressure	High pressure (Higher than HPLC)	Atmospheric pressure
7.7	Resolution	Less resolving power	High resolving power	Moderate resolving power

Current developments in chromatography

HPLC

- Methods based on some form of HPLC separation continue to outnumber all other assays, as they have for over two decades. The vast majority of these are simple, isocratic, reversed-phase procedures that employ UV detection.
- In some cases, more novel approaches, often based on novel separation mode or the use of more selective detection systems, such as diode array and fluorescence detectors or mass spectrometers, are implemented.
- Coupled LC-MS has grown in popularity as electrospray ionisation and atmospheric pressure ionisation interfaces have become more reliable at higher throughput volumes [12].

UPLC


- utilizes a novel type of column temperature-control module;
- employs instrument dead volume and associated extra-column band broadening;
- applies gradient delay volume (also called 'dwell volume');
- provides an enhanced detector data-acquisition rate;
- noted for increased system-pressure drop beyond 1400 bar;
- mobile-phase temperature is elevated (>60°C);
- particle size reduced (<1.5 µm) or particle-size distribution modified;
- makes use of sub-2 µm superficially porous particles (SPPs) [13].

HPTLC

- In a significant development, the United States and Europe have opted to implement “HPTLC fingerprint” as an identification test for “materials of botanical origin.”
- The US Pharmacopeia has also published a standard operating procedure (SOP) for performing HPTLC, which has resulted in the application of standardized techniques for ascertaining the chemical composition of complex mixtures such as herbal extracts and formulations.
- HPTLC produces more data per “Chromatogram” than other methods. UV absorbance, fluorescence, visible colours, images in short and long wave UV, and visible light can all be included in the data. Using some of the 1100 known in-situ derivatization reagents, more information can be obtained. HPTLC plates are widely employed for bio-autography studies and effect directed analysis, and have been hyphenated to MS, NMR, and IR.
- Because of its “fingerprint” feature, the use of which has become mandatory, HPTLC will be widely applied in the herbal and food industries in the near future.

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