



A COMPARATIVE STUDY OF HPLC AND HPTLC: PRINCIPLES, INSTRUMENTATIONS AND APPLICATIONS

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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, and is often an excellent alternative to GC and HPLC. Applications of HPTLC include phytochemical and biomedical analysis, herbal drug quantification, active ingredient quantification, fingerprinting of formulations, and check for adulterants in the formulations. HPTLC is also useful in detecting chemicals of forensic concern.

Key Words: HPTLC, HPLC, Principles, instrumentations, applications, Quality control.

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INTRODUCTION

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 [4-7].

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. 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 Normal-phase 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]:

- The stationary phase is very non-polar.
- The mobile phase is relatively polar.
- 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 Chem-Stations 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 reason, and
- implemented OQ/PV tools in the HPLC system.

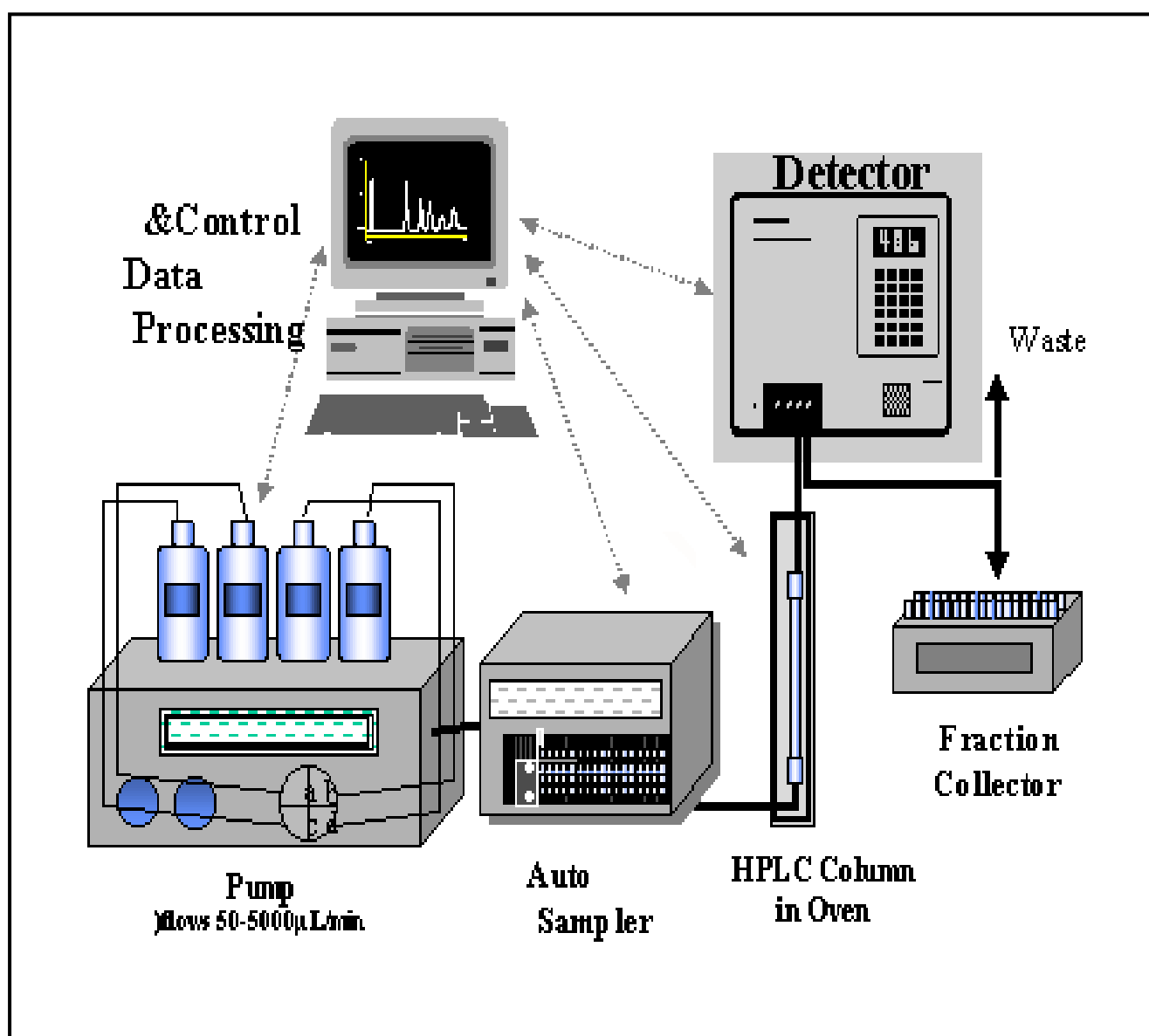


Figure 1: Simplified schematic representation of an HPLC system.

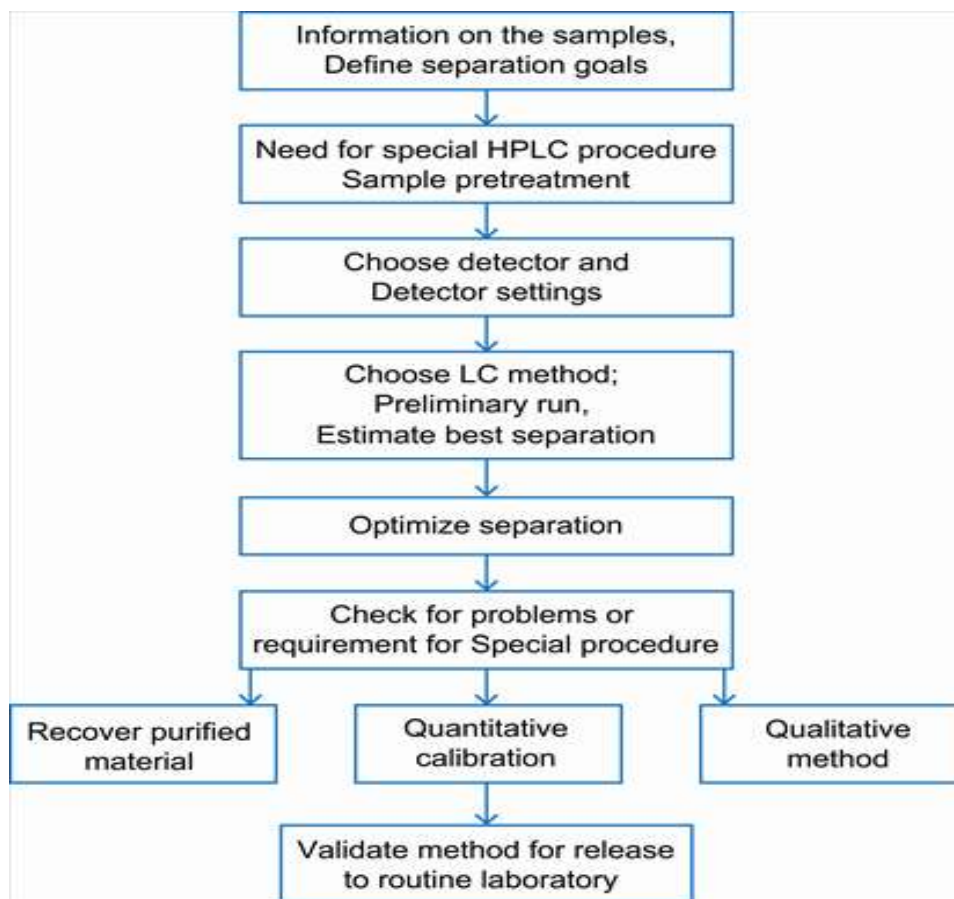


Figure 2: Optimizations steps involved in HPLC

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) [9-13] 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, precised 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 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 all make the

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.

High Performance Thin layer Chromatography (HPTLC) [13-19] is a sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits. It is also known as High Pressure Thin layer Chromatography/Planar chromatography or Flat-bed chromatography. It is a powerful analytical method equally suitable for qualitative and quantitative analytical tasks. Separation may result due to adsorption or partition or by both phenomenon's depending upon the nature of adsorbents used on plates and solvents system used for development.

Different aspects on HPTLC fundamentals: principle, theory, understanding; instrumentation: implementation, optimization, validation, automation and qualitative and quantitative analysis; applications: phyto-chemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis and potential for hyphenation (HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser) have been reported. Currently, a lot of HPTLC presence in the Chinese Pharmacopoeia and into the American Herbal Pharmacopoeia is promising.

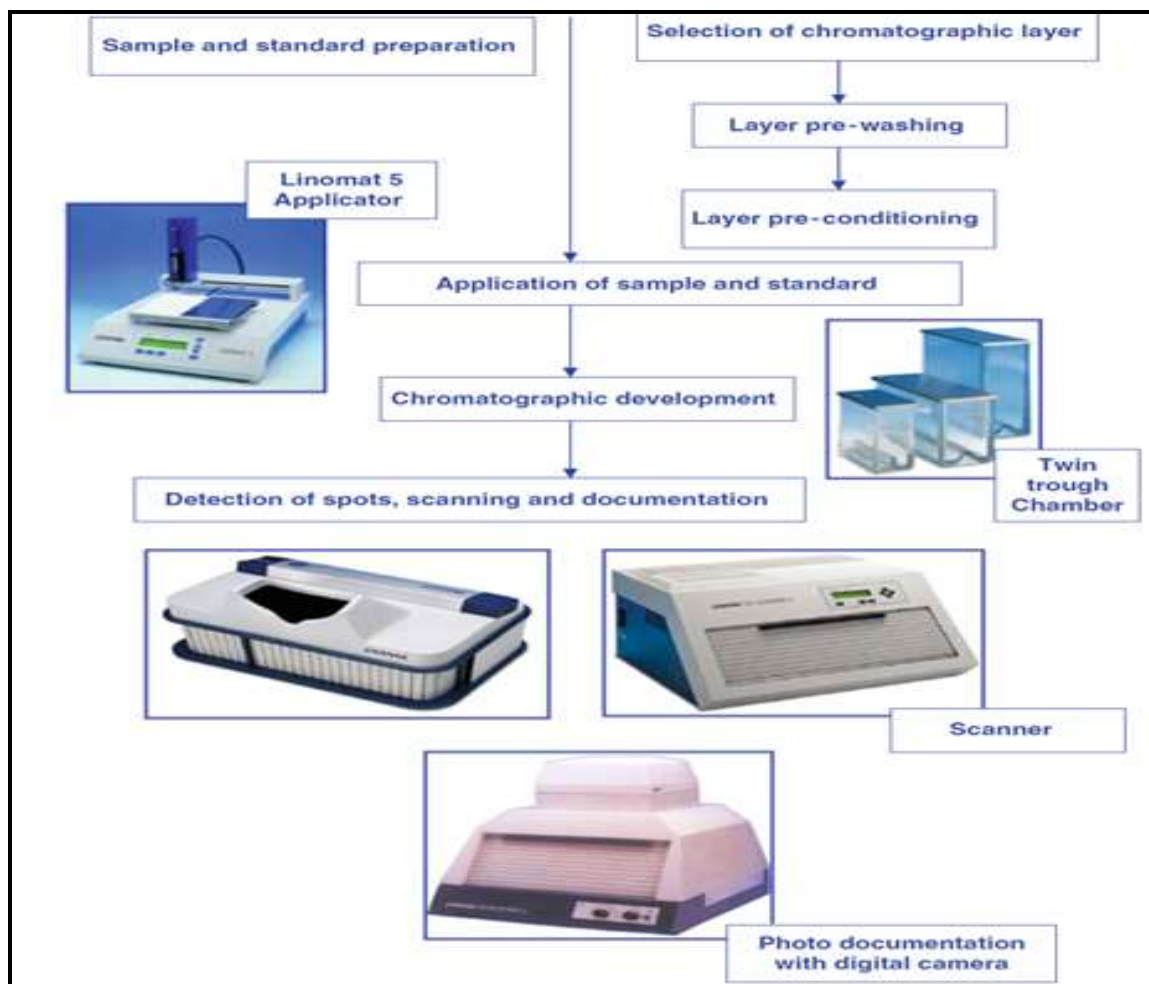


Figure 3: Simplified schematic steps of an HPTLC system.

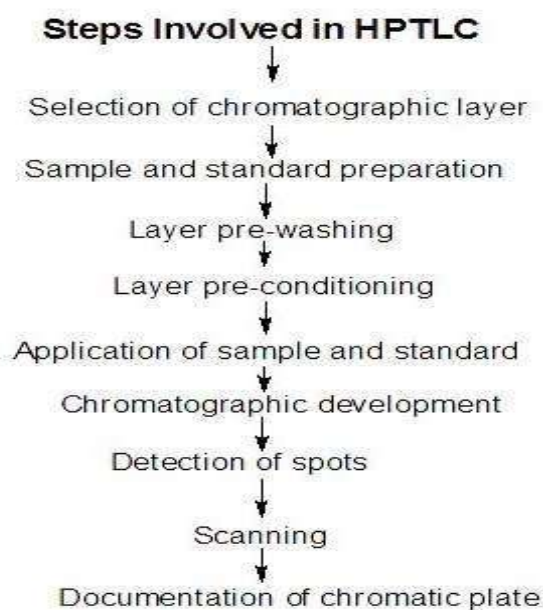


Figure 4: Steps involved in HPTLC

Advantages [18-22] of HPTLC include-

- i) Samples in minute quantities like in nano-gram range,
- ii) Minimum handling and human errors due to automation,
- iii) Better accuracy and sensitivity.

Disadvantages [23-25] includes-

- ii) Bulky instrumentation, large space requirement, many folds expensive, requires stringent condition of operation like dust free environment and temperature controlled conditions, and technically skilled person with the knowledge to run the system.

COMPARISON BETWEEN HPLC AND HPTLC
Figure 5 & tables 1, 2 & 3 shows a comparison, limitations

and Complimentary Aspects of HPLC & HPTLC system [1-8, 13-33] respectively.

Table 1: The comparison between HPLC and HPTLC on the basis of various parameters

Feature	HPLC	HPTLC
Stationary phase	Liquid	Solid
Mobile phase	Liquid	Liquid
Conditioning phase	None	Gas
Samples should be	Non-volatile	Non-volatile
Results	By machine	By machine + eyes
Analysis	On-line	Off-line
Resolution	Very high	Moderate to high
Chromatography System	Closed	Open
Separating medium	Tubular column	Planar layer (plate)
Analysis in parallel	No.	Yes
No. of samples	Only 1 at a time	Up to 100 samples
High temp. / pressure	High pressure	None
Time per sample	2-60 min	1-3 min
Data obtained from chromatography	Limited to very high (Detector dependent)	High to very high (Detector dependent)
Post chromatography derivatization	Limited possibilities. Cumbersome.	Simple, Possible for every sample. Gives additional info.
Fraction collection / micro-preparative chromatography	Requires preparative Scale chromatograph & fraction collector	Simple, No special requirements
Sensitivity	High to ultra-high	Moderate to ultrahigh
Fluorescence data	Possible, optional	Possible, built-in
Abs. spectra for identification	Yes (PDA)	Yes
Detectors	UV, fluorimetric and Electrochemical, Light scatter & MS detection	UV-Vis, bioluminescence, MS
Chromatogram image documentation	No	Yes, At 254 & 366 nm & visible
Sample clean-up	Through Column reusable	Not so important Layer disposable
Chromatographic fingerprint	Yes, but limited	Yes, Comprehensive
Cost per analysis	Very high	Low
Equipment maintenance	Very high	Low
Analyst's skills required	High to very high	Low (TLC) to high (HPTLC)

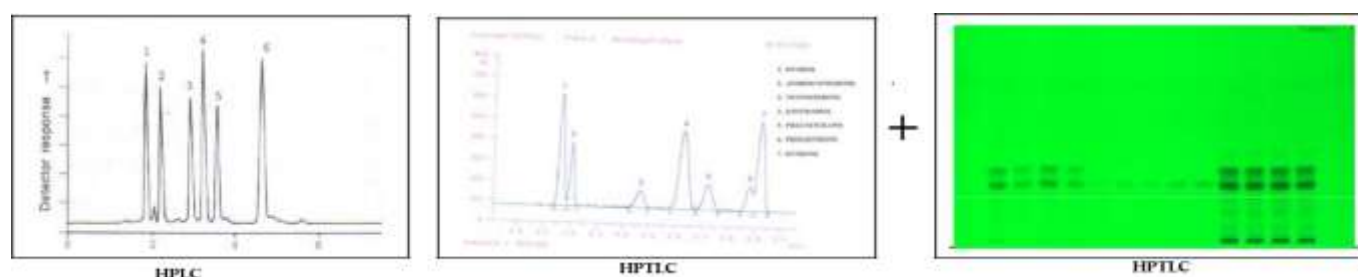


Figure 5: The comparison between HPLC and HPTLC graphs

Table 2: Limitations of HPTLC & HPLC

HPLC Limitations	HPTLC Limitations
High Pressure required	Separation medium length
Sample clean – up essential	Column ~ about 8 cm
High running cost	Narrow Linearity Range
High maintenance	Open System
Quite slow	

Table 3: Complimentary Aspects of HPTLC & HPLC

HPTLC	HPLC
Straight phase Chromatography (np)	Reverse phase Chromatography (rp)
Separation by adsorption	Separation by partition
Strongly retarded	Strongly retarded
Fractions seen as “sharp Peaks”	Fractions seen as “broad Peaks”
Irreversibly retained	Irreversibly retained
Fractions detected	Fractions not detected
Isomer separation easy	Isomer separation
Low cost, high speed	Difficult, High cost, low speed
Ideal for screening	Confirmation of Screened sample results

The development of high-performance liquid chromatography (HPLC) [1-10] has proven a milestone in the drug discovery and allowed faster separations of fragile biological macromolecules. Liquid chromatography LC/MS is more suitable for biomedical applications than GC/MS because almost all bio-molecules are heat sensitive. Furthermore, a combination of various mass spectrometers has been used even for proteins directly. Improving the sensitivity of nuclear magnetic resonance spectrometry (NMR) has permitted a direct connection with LC. Fast LC analysis using a column switching technique was introduced for aromatic amino acid metabolites and guanidino compounds. After the lead compounds are selected, the next steps involve pharmacological, metabolic and pharmacokinetic studies for lead optimization. For these studies high sensitivity and selectivity is required. HPLC coupled with tandem mass spectrometry i.e. HPLC-MS/MS. As well LC coupled with nuclear magnetic resonance methods LC-NMR, LC coupled with Infrared methods LC-IR methods has proved its application in the drug discovery. Assessment of identify of the active ingredient in the test sample or drug formulation is utmost important. It can be affected using LC-PDA or LC-MS. It is mandatory to characterize the impurity profile to establish the therapeutic safety of the drug candidate in pharmaceutical research. Many of the governing regulations like ICH, USFDA has emphasized on identification of impurities in Active Pharmaceutical ingredients. It will not be exaggerate if one says that impurity profiling is impossible without chromatographic

methods. Chromatographic methods along with spectroscopic detection technique in identification of the impurities has brought milestone in the drug discovery and development of pharmaceutical industry. Various hyphenated techniques LC-MS, LC-NMR, LC-NMR-MS, GC-MS, and LC-MS/MS are conducted for these purposes depending upon the chemical and physical status of the sample. Obviously assay, content uniformity and dissolution testing are important from the standpoint of consumers' safety and therapeutic effect of dosage form. One has to follow the pharmacopoeial norms for the methods to be used. IP, BP, USP and many more pharmacopoeias has described the chromatographic methods and conditions while performing the tests [1-10]. The stability testing is conducted to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of temperature, humidity, and light, and to establish a re-test period for the drug substance or a shelf life for the drug product and recommended storage conditions. The stability program also includes the study of product-related factors that influence its quality, for example, interaction of API with excipients, container closure systems and packaging materials [4-12]. In addition one need to ensure there is no risk associated with cross-contamination of active ingredients. The analytical method involved in here should be sensitive, specific, fast and accurate to establish the assurance that the equipments used in manufacturing are free of the above 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. IPQC testing are integral part during manufacturing process, which give assurance that any process in manufacturing is running as per the laid standards and will produce the products with predetermined specifications. HPLC, GC, UPLC studies are commonly utilized to check drug release, dissolution testing, content uniformity etc. Identification of leachable is utmost important in pharmaceutical manufacturing. Chromatographic techniques have justified its role in detection of leachable.

APPLICATIONS OF HPLC & HPTLC

APPLICATIONS HPLC

The information that can be obtained using HPLC [1-8] includes identification, quantification, and resolution of a compound. Preparative HPLC refers to the process of isolation and purification of compounds. This differs from analytical HPLC, where the focus is to obtain information about the sample compound.

Chemical Separations

It is based on the fact that certain compounds have different migration rates given a particular column and mobile phase, the extent or degree of separation is mostly determined by the choice of stationary phase and mobile phase.

Purification

Purification is defined as the process of separating or extracting the target compound from a mixture of compounds or contaminants. Each compound showed a characteristic peak under certain chromatographic conditions. The migration of the compounds and contaminants through the column need to differ enough so that the pure desired compound can be collected or extracted without incurring any other undesired compound.

Identification

Generally assay of compounds are carried using HPLC. The parameters of this assay should be such that a clean peak of the known sample is observed from the chromatograph. The identifying peak should have a reasonable retention time and should be well separated from extraneous peaks at the detection levels which the assay will be performed.

Other applications of HPLC

Other applications of HPLC includes

Pharmaceutical applications

- Tablet dissolution study of pharmaceutical dosages form.
- Shelf-life determinations of pharmaceutical products
- Identification of active ingredients of dosage forms
- Pharmaceutical quality control

Environmental applications

- Detection of phenolic compounds in Drinking Water
- Identification of diphenhydramine in sedimented samples
- Bio-monitoring of pollutant

Forensics

- Quantification of the drug in biological samples.
- Identification of anabolic steroids in serum, urine, sweat, and hair
- Forensic analysis of textile dyes.

- Determination of cocaine and metabolites in blood

Clinical

- Quantification of ions in human urine Analysis of antibiotics in blood plasma.
- Estimation of bilirubin and bilivirdin in blood plasma in case of hepatic disorders.
- Detection of endogenous neuro-peptides in extracellular fluids of brain.

Food and Flavor

- Ensuring the quality of soft drink and drinking water.
- Analysis of beer.
- Sugar analysis in fruit juices.
- Analysis of polycyclic compounds in vegetables.
- Trace analysis of military high explosives in agricultural crops.

APPLICATION OF HPTLC

Isolation and identification [6-14] of the crude extracts is very challenging, one can confidently and effectively satisfy the 100% identity rule of the cGMP to isolate and identify crude raw materials and their powdered or liquid extracts as well as identify a majority of such ingredients in finished products with diverse matrixes. HPTLC Technique can be used for Fingerprint analysis and the determination of identity and quality of Herbal drugs effectively. Applications of HPTLC for phyto-chemical analysis, biomedical analysis, herbal drug quantification, finger print analysis are in the routine practice. HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser mode are powerful analytical tools in the field of analysis. It is noteworthy that utilization of instrumental HPTLC toward the analysis of drug formulations, Bulk drugs, natural products, clinical samples food stuffs, environmental, and other relevant samples will increase in the future.

HPTLC method deals with qualitative and quantitative analytical applications such as herbal and dietary supplements, nutraceuticals, and various types of medicines. It is used [12-35] in quality control, purity check; clinical applications: metabolism studies, drug screening etc.; forensic : poisoning investigations, assaying radiochemical impurities of radiopharmaceuticals, detection and identification of pharmaceutical raw materials, drugs and their metabolites in biological media.

HPTLC in quality control of pharmaceuticals

HPTLC has been used for routine quality control of topiramate, dutasteride, nabumetone in pharmaceutical formulations. Validated sensitive and highly selective stability indicating methods were reported for simultaneous quantitative determination of sulphiride and mebeverine hydrochloride in presence of their reported impurities and hydrolytic degradates whether in pure forms or in pharmaceutical formulation. Stability-indicating HPTLC method for the analysis of ropinirole HCl was developed and validated for precision, accuracy, ruggedness, robustness, specificity, recovery, limit of detection (LOD) and limit of quantitation (LOQ). A significant difference of R_f when drug was subjected to acidic, alkaline, oxidative, dry heat, wet heat and photo degradation stress is observed. In herbal medicinal products, HPTLC is also an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability. HPTLC has been reported for development of a quality assurance program.

HPTLC as biomarker in pharmacognostical research

HPTLC analysis of many plants used in Indian Systems of Medicine has been performed for various pharmacological activities like CNS, hepato-protective etc. The HPTLC may be used as a rapid method by which to control the quality of raw plant materials and formulations based on the *Lawsonia inermis* plant. *Michelia champaca* L. (Magnoliaceae) popularly known as champa is a reservoir of numerous bio-markers. HPTLC method has been used for detection, and quantification of quercetin in *Michelia champaca* (leaves and stem bark) and the estimated values indicate that the leaves are the richest source of the quercetin. HPTLC method for the estimation of curcumin in marketed turmeric powder can be used routinely with good reliability and reproducibility.

HPTLC in the forensic sciences

In forensics, HPTLC has an important role in the analysis of drugs in complex matrixes like blood and urine. Researchers reported the standardization and utility of cannabinoid extraction, screening and detection by HPTLC in the urine samples of cannabis abusers and this method was found to be high throughput, sensitive, reproducible and cost-effective compared to commercial kits.

Hyphenations in HPTLC with MS, FTIR and bioassays

It comprises the different approaches to combine mainly spectrometers with chromatographic systems to get further information about the sample.

HPTLC and mass spectrometry (MS)

HPTLC/MS is looked into as better, viable and simple alternative to HPLC. HPTLC and its future in combinatorial approach adds new dimension for its potential for hyphenation, HPTLC-MS coupling, laser desorption-HPTLC chromatography coupled with chemical ionization mass spectrometry etc. The recent frontier of HPTLC is the coupling with MS spectrometry. The direct coupling of TLC/HPTLC with mass spectrometry (MS) is of particular interest because of the later are high sensitivity, rapid analysis, and ability to aid structural characterization. The conventional ionization method requires heating of the analyte and was suitable only for the analyses of volatile and thermally stable compounds.

HPTLC and FTIR:

In recent years, much effort has been devoted to the coupling of high-performance thin-layer chromatography (HPTLC) with spectrometric methods because of the robustness and simplicity of HPTLC and the need for detection techniques that provide identification and determination of sample constituents. IR is one of the spectroscopic methods that have been coupled with HPTLC. IR spectroscopy has a high potential for the elucidation of molecular structures, and the characteristic absorption bands can be used for compound-specific detection. HPTLC-FTIR coupled method has been widely used in the modern laboratories for the qualitative and quantitative analysis. The potential of this method has been demonstrated by its application in different fields of analysis such as drug analysis, forensic analysis, food analysis, environmental analysis, biological analysis, etc. The hyphenated HPTLC-FTIR technique will be develo-

ped in the future with the aim of taking full advantage of this method.

HPTLC-Bioluminescence:

This technique is used for the separation of the active constituents and screening for their antimicrobial activities.

CONCLUSION

It can be concluded from the entire review that HPLC is a versatile, reproducible chromatographic technique for the estimation of drug products. It has wide applications in different fields in term of quantitative and qualitative estimation of active molecules. HPTLC is an ideal tool for identification of herbal materials. In addition, it is used for semi-quantitative comparison to provide quantitative results. HPTLC use for screening pharmaceutical compounds for the antimicrobial activities is emerging. The uses in validation of new incoming products and its introduction into the regulatory systems are of much importance towards the future of HPTLC.

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