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A Review on High Performance Thin Layer Chromatography

Miss. Payal Badhe[1], Dr. Vijaya Barge[2]

[1]Bachelor of Pharmacy, Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune, Maharashtra, India.

[2]Vice principal, pharmaceutical quality assurance department, PDEA's Shankarrao Ursal College of Pharmaceutical Science's and Research Centre, Kharadi, Pune, Maharashtra, India

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ABSTRACT

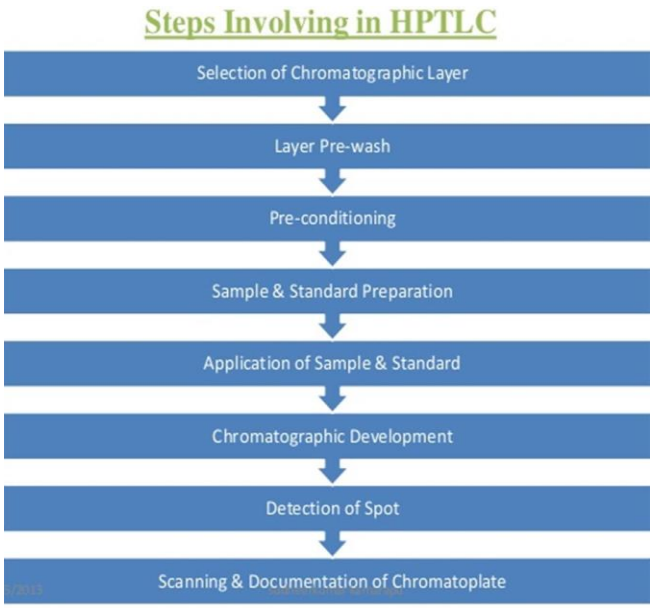
A common excellent replacement for GC and HPLC is High-Performance Thin Layer Chromatography (HPTLC), an improved and automated technique of thin-layer chromatography (TLC) that provides better separation performance and detection limits. Applications of HPTLC include the study of biological materials and phytochemicals, the measurement of herbal medications and active components, formulation fingerprinting, and the identification of adulterants in formulations. Using HPTLC, chemicals of forensic importance can be located. It is more sensitive and feasible to run many samples in a little period of time by using a small volume of solvent. It is one of the more intricate instrumental procedures, utilizing every feature available in thin-layer chromatography.

Keywords : High-performance thin-layer chromatography (HPTLC), Thin-layer chromatography (TLC), Application, High pressure liquid chromatography (HPLC)

I. INTRODUCTION

A more complex and automated version of thin-layer chromatography (TLC) with improved and enhanced detection limits and separation efficiency is called high performance thin-layer chromatography, or HPTLC. It is sometimes referred to as flat-bed chromatography, planar chromatography, or high pressure thin layer chromatography. It is an effective analytical technique that works well for both quantitative and qualitative analytical problems [1,2]. Separation can occur as a result of partition, adsorption, or both, depending on the type of adsorbents utilized on the plates and the solvent system used in the development. Applications: phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis, and potential for hyphenation (HPTLC-MS, HPTLCFTIR, and HPTLC-Scanning Diode Laser) have been discussed. Various aspects of HPTLC

fundamentals, including principle, theory, and understanding, have also been covered.[3]



Principle :

The physical principles of HPTLC and TLC (adsorption chromatography) are the same; that is, adsorption serves as the primary unit of separation.The solvent from the mobile phase flows through due to capillary action. The components migrate in accordance with their affinities with the adsorbent. Moving more slowly is the component that is more drawn to the stationary phase. Components with a weaker affinity for the stationary phase move at a faster rate swiftly. This leads to the components being separated using a chromatographic plate.(4)

Table 1 : Difference between TLC and HPTLC[37-40]

S. No.	Feature	TLC	HPTLC
1	Technique	Manual	Instrumental
2	Plates	Lab Made/ precoated	Pre-coated
3	Plate height	30 µm	12 µm
4	Layer of sorbent	250 µm	100 µm
5	Stationary phase	Silica gel, alumina &kiesulguhr	Wide choice of stationary phase like silica gel for normal phase and C8, C18 for reversed phase modes
6	Separations	10-15 cm	3-5 cm
7	Analysis time	20-200 min	1-3 min
8	Mean particle size	10-12 µm	5-6 µm
9	Efficiency	Less	High due to smaller particle size
10	Sample holder	Capillary/ pipette	Syringe
11	Sample spotting	Manual spotting	Auto sampler
12	Size of sample	Uncontrolled/ solvent dependent	Controlled solvent Independent
13	Shape of sample	Circular (2-4 nm dia)	Rectangular (6mm L X 1mmW)
1	Sample tracks per plate	≤10	≤36 (72)

4			
15	Vol. range	1 to 10 µL	0.1 to 500 µL
16	Development chamber	More amount	A new type that requires less amount of mobile phase
17	Wavelength range	254 or 366 nm, visible	190 or 800 nm, monochromatic
18	The detection limit (Absorption)	1-5 pg	100-500 pg
19	The detection limit (Fluorescence)	50-100 pg	5-10 pg
20	PC connectivity, method storage, Validation	No	Yes
21	Quantitative analysis	No	Yes
22	Scanning	No	The use of UV/ Visible/ fluorescence scanner scans the entire chromatogram qualitative and quantitatively and the scanner is an advanced type of densitometer
23	Analysis Judgment	By analyst	By machine

Advantages :

HPTLC has become a valuable tool for accurate identification and is being used as a substitute for classical TLC in recent times.

It is a software-controlled instrument. When determining impurities, HPTLC relies more on a basic yet highly effective silica gel hydrophilic phase that satisfies most pharmacopoeia criteria. [5,6]

There are several advantages of using HPTLC for the analysis of compounds as compared to other techniques, like HPLC, spectrophotometry, titrimetry, etc. Some of the advantages of HPTLC are [7-9]:

1.The separation procedure is simple to follow, particularly when dealing with colorful compounds.

2.Ability to test crude samples with several components.

3.On a single plate, multiple samples can be split parallel to one another, providing a high output, time savings, and quick, inexpensive analysis.

4.Since the mobile phases are completely evaporated before the detection step, there is a large range of solvents available for HPTLC development.

5. It is simple to separate two dimensions execute. During chromatography, stability bought should be examined through two-dimensional

6.Separated spots can be found using sensitive and specific color reagents (Kedde/Dragendroff reagent).

7.Because HPTLC may be combined with other methods of assessment, it can be utilized for various

modes of evaluation and identify substances with distinct qualities in light absorption or different hues.

8.Contact detection makes it possible to track radiolabelled chemicals and evaluate the amount of microbiological activity in specific areas.

9.The HPTLC approach has the potential to reduce the danger of exposure to harmful organic waste water and greatly lessen disposal issues, hence lowering environmental pollution.

Common Methodology for HPTLC Analysis :

Method development in thin-layer (planar) chromatography is one of the most significant steps for a qualitative and quantitative analysis. During establishing a new analytical procedure, always starts with wide literature survey [10] i.e. primary information about the physicochemical characteristics of sample and nature of the sample (structure, polarity, volatility, stability and solubility).

It involves considerable trial and error procedures. General steps involved in HPTLC method developments are as follow:

Basic Steps:

1. Selection of the stationary phase
2. Mobile phase selection and optimization
3. Sample Preparation and Application
4. Chromatogram Development (separation)
5. Detection

Quantitation:

HPTLC method validation for pharmaceutical analysis [11]:

1. Specificity
2. Linearity
3. Range
4. Accuracy
5. Precision
6. Detection Limit
7. Quantitation Limit
8. Robustness

Basic Steps:

Selection of the stationary phase-

During method development, stationary phase selection should be based on the type of compounds to be separated. HPTLC uses smaller plates (10*10 or 10*20 cm) with significantly decreased development distance (typically 6 cm) and analysis time (7–20 min).

HPTLC plates provide improved resolution, higher detection sensitivity, and improved in situ quantification and are used for industrial pharmaceutical densitometric quantitative analysis[12].

Mobile phase selection and optimization-

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte. [13,14]. The Table 2 gives the details of mobile phase generally used in detection of some chemical compounds.

Table 2 : Generally Used Mobile phase in detection of some Chemical compounds [41]

S. No.	Chemical Compounds	Mobile Phase
1	Polar Compounds Anthraglycosides, Arbutin, Alkaloids, Cardiac Glycosides,	Ethyl Acetate: Methanol: Water [100:13.5:10]

	Bitter Principles, Flavonoids, Saponin	
2	Lipophilic Compounds Essential oils, Terpenes, Coumarin, Napthoquinons, Velpotriate	Toluene: Ethyl Acetate [93:7]
3	Alkaloids	Toluene: Ethyl Acetate: Diethyl Amine [70:20:10]
4	Flavonoids	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water [100:11:11:26]
5	Saponin	Chloroform: Glacial Acetic Acid: Methanol: Water [64:32:12:8]
6	Coumarin	Diethyl Ether: Toluene [1:1] Saturated with 10% Acetic Acid
7	Bitter Drug	Ethyl Acetate: Methanol: Water [77:15:8]
8	Cardiac Glycosides	Ethyl Acetate: Methanol: Water [100:13.5:10] OR [81:11:8]
9	Essential Oil	Toluene: Ethyl Acetate [93:7]
10	Lignans	Chloroform: Methanol: Water [70:30:4] Chloroform: Methanol [90:10] Toluene: Ethyl Acetate [70:30]
11	Pigments	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water [100:11:11:26]
12	Pungent Testing	Toluene: Ethyl Acetate [70:30]
13	Terpenes	Chloroform: Methanol: Water [65:25:4]
14	Triterpenes	Ethyl Acetate: Toluene: Formic Acid [50:50:15] Toluene: Chloroform: Ethanol [40:40:10]

Sample preparation & application:

A excellent solvent system does not add any solvent front; instead, it moves every component of the combination off the baseline. It is necessary to resolve the interest peaks between Rf 0.15 and 0.85. Eluent strength, a characteristic that is connected to the polarity of the constituents of the mobile phase, determines the elution power of the mobile phase.[15] The more nonpolar the compound, the faster it will elute (or the shorter time it will remain on the stationary phase) and the more polar the compound the slower it will elute (or more time on the

stationary phase). The order of elution can be predicted with the help of the following chart.

To create a test solution that can be applied directly to an HPTLC plate, a pharmaceutical preparation with a suitably high analyte concentration is simply dissolved in an appropriate solvent that will completely dissolve the analyte and leave the excipients undissolved [16]. It is a known fact that the most important stage in obtaining adequate resolution for HPTLC quantification is sample application [17].

Sample application technique depends on factors such as the type of sample
matrix, workload and time constraints.

Table 3 : Common Mobile Phases listed by Increasing Polarity[42]

S. No.	Solvent
1	N- Pentane
2	Hexane
3	Cyclohexane
4	Carbon tetrachloride
5	Toluene
6	Chloroform
7	Methylene Chloride
8	Tetrahydrofuran
9	Acetone
10	Ethyl Acetate
11	Aniline
12	Acetonitrile
13	Ethanol
14	Methanol
15	Acetic Acid

Chromatogram Development (separation)-

Although chromatogram development is the most crucial step in the HTLC procedure, important parameters are generally overlooked.28 HPTLC plates are developed in twin-trough chambers, or horizontal-development chambers. In general, saturated twin-trough chambers fitted with filter paper offer the best reproducibility. Twin-through chamber avoids solvent vapor preloading and humidity [18]. Detection- Detection of separated compounds on the sorbent layers is enhanced by quenching of fluorescence due to UV light (ranged normally at 200-400 nm). This process is commonly called Fluorescence quenching. Visualization at UV 254 nm F254 should be described as phosphorescence quenching. In this instance the fluorescence remains for a short period after the source of excitation is removed. It is very short lived, but longer than 10 seconds. F254 fluorescent indicator is excited with UV wavelength at 254 nm and emits green fluorescence [19]. Compounds that absorb radiation at 254 nm reduce this emission on the layer, and a dark violet spot on a green background is observed where the compound zones are located [20]. This quenching is caused by all compounds with conjugated double bonds. Anthraglycosides, coumarins, flavonoids, propylphenols in essential oils, some alkaloid type such as indole, isoquinoline and quinoline alkaloids etc. should be detected under 254 nm [21]. Visualization at UV 366 nm F 366 should be described as Fluorescence quenching. In this instance the fluorescence does not remains after the source of excitation is removed. This quenching is shown by all anthraglycosides, coumarins, flavonoids, Phenolcarboxylic acids, some alkaloid types (Rauwolfia, Ipecacuanha alkaloids). [24] Visualization at white light Zone containing separated compounds can be detected by viewing their natural color in daylight (White light) [22].

Derivatisation Derivatization can be defined as a procedural technique that primarily modifies an analytes functionality in order to enable chromatographic separations. Derivatization can be performed either by immersing the plates or by spraying the plates with a suitable reagent [23-26]. For better reproducibility, immersion is the preferred derivatization technique. HPTLC vs. HPLC

High-performance thin-layer chromatography (HPTLC) is still increasingly finding its way in pharmaceutical analysis in some parts of the world. With the advancements in the stationary phases and

the introduction of densitometers as detection equipment, the technique achieves for given applications a precision and trueness comparable to high-performance liquid chromatography (HPLC). Basic differences between HPLC and HPTLC are given in following Table:

Table no 4 : A Comparison evaluation of HPLC and HPTLC [43-46]

S. No.	Criteria	HPTLC System	HPLC System
1	Validation	Relatively simple	Relatively Simple
2	Documentation	Meets all the Requirements	Meets all the requirements
3	Photo documentation	Possible	Not possible
4	Sample preparation	Very simple and fast; dissolve; centrifuge and supernatant for Application	Expensive, time-consuming, complex, extraction, and filtration is essential before chromatography
5	Number of CUT (content uniformity test) systems handled at a time	Up to 5 tests of 17 samples each	Maximum of 1 sample and 1 test at a time
6	Chromatography time of each CUT (content uniformity test)	45-60 minutes	4-6 minutes
7	Urgent Samples	Start analysis any time on receipt of sample, but finish 60 minutes.	1-2 hours start-up time and then analysis time
8	Analysis requiring post- chromatographic derivatization	Simple, additional 10-15 minutes requires after chromatographic separation.	Complicated, additional 1-3 hours may be required after chromatography.

Application of HPTLC :

The HPTLC technique is used in many qualitative and quantitative scientific applications, including those for nutraceuticals, herbal and dietary supplements, and a variety of pharmaceuticals. Radiological assaying, toxicity testing, and other forensic applications radioactive medication toxins, as well as identifying and detecting prescription raw components, finished goods, and their byproducts in living environments. Utilizing science involves drug screening and

metabolic testing. Numerous lipids have also been studied and looked into. different lipid sub-classes were distinguished with consistent and encouraging results using HPTLC [27]. outcomes. Numerous reports on investigations pertaining to clinical medicine have already been published in several journals. HPTLC is currently widely used in the study of medicines in serum and other tissues .[28]

HPTLC in quality control of pharmaceuticals :

Pharmaceutical formulations including dutasteride, nabumetone, and primates have all undergone routine quality control using HPTLC. [29] For the simultaneous quantitative determination of sulpiride and mebeverine hydrochloride in the presence of their reported impurities and hydrolytic degrades, whether in pure form or pharmaceutical formulation, validated sensitive and highly selective stability-indicating methods were reported. [30] Developed and validated for precision, accuracy, toughness, robustness, specificity, recovery, the limit of detection (LOD), and the limit of
quantification was a stability-indicating HPTLC method for the measurement of ropinirole
HCL (LOQ). The evaluation and monitoring of the growing, picking, and extraction processes, as well as the testing of stability, are also excellent uses for HPTLC, which is also a great tool for spotting adulterations. HPTLC has been reported for the development of a quality assurance program. [31]

HPTLC as a biomarker in pharmacognostic research :

Numerous plants used in Indian medicine have been investigated using high-performance liquid chromatography (HPTLC) for a range of pharmacological qualities, including hepatoprotective and CNS effects. Using the HPTLC method, quercetin from the Micheliachampaca (leaves and stem bark) was found and measured. The estimated values indicate that the plant's leaves are its primary source of quercetin. [32] The HPTLC method is a highly reliable and reproducible way to measure the amount of curcumin in commercial turmeric powder. [33]

HPTLC in other fields:

The developing world has recently demonstrated HPTLC as an internationally recognized, workable approach for the characterization of small compounds in quality evaluation. Steroids, insecticides, and chemical purity testing are all done with it. Moreover, it is used to analyze vitamins, water-soluble flood dyes, and pesticides in fruits and vegetables, as well as other things. [34,35,36]

Conclusion :

Manufacturing (bulk drug manufacturing units, process monitoring, fermentation broth analysis, residue analysis, and in-process materials testing); quality control (raw material assays, multi-component formulations, uniformity of content testing, impurity profiling, and method validations); formulation analyses; stability, sustained release; and bioavailability studies are among the pharmaceutical testing applications of HPTLC that have shown promise. The best instrument for identifying herbal materials is HPTLC. Furthermore, it is employed in semi-quantitative comparison to get quantitative outcomes. The use of HPTLC to check pharmaceutical compounds for antibacterial activity is becoming more common. The validation of newly arrived products and their integration into regulatory frameworks hold significant value for the future of high-performance liquid chromatography. HPTLC has been used in marine invertebrates to distinguish novel, promising.

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