IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
April 2023 Vol.:27, Issue:1

A Comprehensive Review of High-Performance Thin Layer Chromatography (HPTLC)

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
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Submitted: 25 March 2023
Accepted: 31 March 2023
Published: 30 April 2023

Keywords: High-performance thin-layer chromatography (HPTLC), Thin-layer chromatography (TLC), Instrumentation, Application

ABSTRACT

High-Performance Thin Layer Chromatography (HPTLC) is an enhanced and automated method of thin-layer chromatography (TLC) that offers superior separation performance and detection limits and is frequently a great substitute for GC and HPLC. The study of phytochemicals and biological substances, the quantification of herbal drugs and active ingredients, the fingerprinting of formulations, and the detection of adulterants in formulations are all applications of HPTLC. Chemicals of forensic interest can be found using HPTLC. By utilizing a tiny amount of solvent, it is more sensitive and possible to run numerous samples in a short amount of time. One of the more complex instrumental techniques, it makes use of all of thin-layer chromatography's capabilities. The sort of apparatus utilized in HPTLC, its whole methodology, and how this process is superior to TLC are briefly discussed in this study. To focus on the application of HPTLC, examples of medications, pharmaceuticals, and formulations that have undergone HPTLC analysis are provided in this article.

1. INTRODUCTION

The process of learning about a sample by some type of chemical analysis can be used to summarise the science of analytical chemistry. Any solid, liquid, or gaseous compound may make up the sample being examined, and the analysis will produce data of some sort that will be relevant to the original query posed about the sample. Some information about the sample can be gleaned from the data obtained during the study. This information could be qualitative or quantitative. [1]

Types of atoms, molecules, functional groups, or some other qualitative measure are examples of qualitative information, whereas quantitative information offers numerical information, such as the number of various chemicals in the sample. [2] Currently, an analytical chemical analysis typically involves some kind of analytical device that conducts the actual analysis, with computer software handling data processing and instrument control. In light of this, it is accurate to claim that analytical chemistry has been computerized. Also, the format of the results from analytical chemical studies has altered. Today, a single sample can yield tremendous amounts of data after only a few periods of examination. [3] Using techniques like infrared (IR) spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, Raman spectroscopy, mass spectrometry (MS) fluorescence spectroscopy, near-infrared (NIR) spectroscopy, and nuclear magnetic resonance spectroscopy (NMR), High-performance liquid chromatography (HPLC) and high-performance thin layer chromatography (HPTLC) large amounts of data on a sample can be collected in a short period.[4] Chemical analysis is a crucial part of a laboratory's ability to guarantee the regular, acceptable performance of analytical methods. Despite the significant number of significant published works on this topic, there is still variability in the approaches used because the validation of an analytical method depends on the method's particular goal. [5] This may make it difficult to comprehend the results and validate methods. We cover pertinent techniques of various parameters in quantitative high-performance thin-layer liquid chromatographic methods and validation domains in pharmaceutical analysis to aid in the planning of validation methods. Moreover, this article provides a full review of HPTLC method development that should be useful as an introduction to analytical validation for practical applications in academic research or the industrial sector.[6]

Principle: HPTLC uses the same physical principles as TLC (adsorption chromatography), i.e., adsorption is the fundamental unit of separation. Capillary action causes the solvent from

the mobile phase to pass through. According to their affinities with the adsorbent, the components migrate. The component that is more attracted to the stationary phase moves more slowly. The components that have a lower affinity for the immobile phase move more quickly. A chromatographic plate is used to separate the components as a result. [7]

Advantages/disadvantages of HPTLC over TLC: In recent years, HPTLC has been utilized as an alternative to traditional TLC and has proven to be an effective method for accurate identification. Software controls the instrument. For determining impurities using HPTLC, the most effective silica gel hydrophilic phase that satisfies the standards of the majority of pharmacopeias is utilized more frequently. [8, 9]

Table No 1: Difference between TLC and HPTLC [10-13]

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)
14	Sample tracks per plate	≤10	≤36 (72)
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

When analyzing substances, HPTLC has several benefits over other methods such as HPLC, spectrophotometry, titrimetry, etc. The following are some benefits of HPTLC: [14-15]:

- The choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step.
- The separation process is easy to follow, especially with colored compounds.
- Ability to analyze crude samples containing multi-components.
- Two-dimensional separations are easy to perform. Stability during chromatography should be tested using two-dimensional development.
- Several samples can be separated parallel to each other on the same plate resulting in high output, time-saving, and rapid low-cost analysis.
- Contact detection allows radiolabelled compounds to be monitored and microbial activity in spots to be assessed.
- HPTLC can combine and consequently be used for different modes of evaluation, allowing the identification of compounds having different light-absorption characteristics or different colors.
- Specific and sensitive color reagents can be used to detect separated spots (Dragendroff reagent/Kedde reagent).
- HPTLC method may help to minimize the exposure risk of toxic organic effluents and significantly reduces its disposal problems, consequently, reducing the environmental pollution.

Table no 2: A Comparison evaluation of HPLC and HPTLC [16-19]

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.

Common Methodology for HPTLC:

Analysis: One of the most important steps for qualitative and quantitative analysis is method development in thin-layer (planar) chromatography. A thorough literature review is always the first step when developing a new analytical process [20, 21], providing essential details regarding the physicochemical properties of the sample and its nature (structure, polarity, volatility, stability, and solubility). There are a lot of trial and error processes involved. The following are the general steps required in developing an HPTLC method:

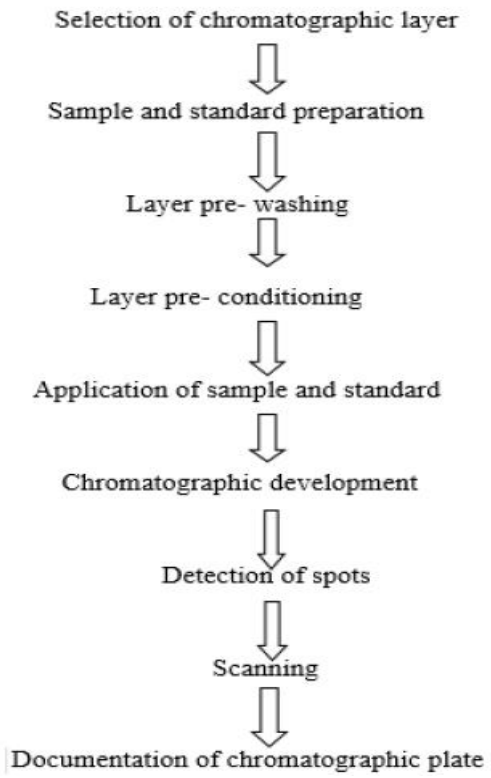

Figure no 1: Flow Chart of HPTLC

Selection of the Stationary Phase: During method development, stationary phase selections should be based on the type of compounds to be separated[22, 23]. HPTLC uses smaller plates (10*10 or 10*20 cm) with significantly decreased development distance (typically 6cm) 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. [24]

Mobile Phase Selection and Optimization: The selection of the mobile phase is based on the adsorbent material used as the stationary phase and the physical and chemical properties of the analyte. [25, 26, 27]

Sample Preparation and Application: A good solvent system moves all components of the mixture off the baseline but does not put anything on the solvent front. The peaks of interest should be resolved between Rf 0.15 and 0.85. The elution power of the mobile phase depends on a property called eluent strength which is related to the polarity of the mobile phase components. [28]The more nonpolar the compound, the faster it will elute (the less 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 following chart helps predict the order of elution.

Pharmaceutical preparation with a sufficiently high concentration of analyte is simply dissolved in a suitable solvent that will completely solubilize the analyte and leave excipients undissolved to yield a test solution that can be directly applied on an HPTLC plate [29]. It is a fact that the application of the sample is the most critical step to obtaining a good resolution for quantification in HPTLC [30, 31]. Sample application technique depends on factors such as the type of sample matrix, workload, and time constraints.

Chromatogram Development (Separation): Although chromatogram development is the most crucial step in the HTLC procedure, important parameters are generally overlooked [32]. 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. [33]

Detection- Detection of separated compounds on the absorbent layers is enhanced by quenching of fluorescence due to UV light (ranging 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 indicators are excited with UV wavelength at 254 nm and emit green fluorescence [34]. Compounds that absorb radiation at 254nm reduce this emission on the layer, and a dark violet spot on a green background is observed where the compound zones are located [35]. This quenching is caused by compounds with conjugated double bonds. Anthraglycosides, coumarins, flavonoids, polyphenols in essential oils, and some alkaloid types such as indole, isoquinoline, quinoline alkaloids, etc. should be detected under 254 nm [36].

Visualization at UV 366 nm: F 366 should be described as fluorescence quenching. In this instance, the fluorescence does not remain after the source of excitation is removed[34]. This quenching is shown by all anthraglycosides, coumarins, flavonoids, Phenolcarboxylic acids, and some alkaloid types (Rauwolfia, Ipecacuanha alkaloids). [37]

Visualization of white light: The zone containing separated compounds can be detected by viewing their natural color in daylight (White light).

Derivatization: Derivatization can be defined as a procedural technique that primarily modifies an analyte's functionality to enable chromatographic separations. Derivatization can

be performed either by immersing the plates or by spraying the plates with a suitable reagent [38-39]. For better reproducibility, immersion is the preferred derivatization technique.

Applications of HPTLC: Many qualitative and quantitative methodological applications, such as those for herbal and dietary supplements, nutraceuticals, and a range of medications, utilize the HPTLC technique. Forensic applications include toxicity tests, assaying radio chemical contaminants in radio medicines, and detecting and identifying prescription raw ingredients, products, and their metabolites in biological mediums. Scientific usage includes metabolism tests and drug screening. Many lipids have also been examined and investigated using HPTLC; [40] distinct lipid sub-classes were divided with repeatable and encouraging results. Related to clinical medicine many reports on studies have already been published in many journals. In the analysis of drugs in serum and other tissues HPTLC is now strongly recommended. [41]

I. HPTLC in quality control of pharmaceuticals: Pharmaceutical formulations including dutasteride, nabumetone, and primates have all undergone routine quality control using HPTLC. [42] 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. [43] 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. [44]

II. HPTLC as a biomarker in pharmacognostic research: Many plants utilized in Indian medical systems have undergone HPTLC investigation for a variety of pharmacological properties like CNS, hepatoprotective, etc. The Micheliachampaca (leaves and stembark) quercetin was detected and quantified using the HPTLC method, and the estimated values show that the leaves constitute the plant's highest source of quercetin. [45] With strong reliability and reproducibility, the HPTLC method can be used regularly to estimate the amount of curcumin in commercial turmeric powder. [46]

III. HPTLC applications in drug analysis: Table 3 provides information on the HPTLC analysis of pharmaceutical drugs in various formulations.

IV. HPTLC in herbal products: Table 4 provides information on the HPTLC analysis of herbal items.

V. HPTLC in fingerprinting analysis: The details regarding HPTLC determination of fingerprinting analysis are given in Table 5.

VI. 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. [27, 28, 29]

Table no 3: The details regarding HPTLC determination of pharmaceutical products in various formulations

S.	Drug	Dosage form	Technique used
1	Pregabalin and amitriptyline	Pharmaceutical dosage form	Stationary phase: silica gel F254 mobile phase : ethanol: ethyl acetate: acetone: ammonia solution (8:2:1:0.05, by volume) amitriptyline scanned at 220 nm and pregabalin scanned at 550 nm
2	Silymarin and vitamin E	Pharmaceutical dosage form	Stationary Phase: Silica gel 60F254 Mobile Phase :hexane:acetone: formic acid (7:3:0.15, v/v/v) developing system with UV detection at 215 nm.
3	Glibenclamide, rosiglitazone maleate and metformin hydrochloride	Tablet	Stationary Phase: Pre-coated RP-18 F254s aluminum sheets Mobile Phase: Methanol–tetrahydrofuran–water–glacial acetic acid (16: 3.6: 4: 0.4, v/v) DigiStore 2 Documentation System with winCATS software version 1.4.10 was used for the quantitation and photo documentation
4	Vildagliptin and metformin hydrochloride	Pharmaceutical dosage formulation	Stationary Phase: silica gel precoated aluminum plate 60 F254 Mobile Phase :hexane: methanol:acetonitrile: glacial acetic acid(2:3:5:2.5:0.2,v/v/v/ v/v)Absorbance at 217 nm
5	Diphenhydramine and naproxen sodium	Tablet	Stationary phase: Silica gel 60 F 254 Mobile Phase : toluene: methanol: glacial acetic acid (7.5:1:0.2 v/v/v) densitometry at 230 nm
6	Voriconazole	Cream formulation	Stationary Phase: Silica gel 60 F254 Mobile Phase : Acetonitrile: water (60:40 % v/v) Quantification was achieved by densitometric at 257 nm

Table no 4: The details regarding HPTLC determination of herbal products

S.	Herbal plant	Active constituent	Technique used	Uses
1	A.paniculata and E. alba	Androgphlide and wedalolactone	Stationary Phase: Silica gel, 60 F254 Mobile Phase : toluene:acetone:formic acid (9:6:1, v/v/v) Detection at 254 nm	Hepato-protective formulations
2	Phyllanthus	Phyllanthin and hypophyllanthin	Stationary Phase: Silica gel, 60 F254 Mobile Phase : hexane:acetone: ethyl acetate (24:12:8,v/v/v)Detection at 580 nm	Viral infections, liver disorders, bacterial infections.
3	Ocimum sanctum	Eugenol	Stationary Phase: Silica gel, 60 F254 Mobile Phase : toluene: Ethylacetate: formic acid (90:10:1 v/v/v)Detection at 280 nm	Cardiopathy, Blood disorders, Asthma, skin diseases.
4	Lagerstroemia speciosa	Corosolic acid	Stationary Phase: Silica gel, 60 F 254 Mobile Phase : chloroform: methanol (9:1, v/v)Detection at 20 nm	Antidiabetic activity
5	Amarathusspinosus Linn	Rutin	Stationary Phase: Silica gel, 60 F 254 Mobile phase: ethyl acetate: formic acid: methanol: water (10:0.9:1.1:1.7 v/v/v/v)	Antidiabetic, antithrombotic, anti-inflammatory, anticarcinogenic activity

Table 5: The details regarding HPTL determination of fingerprinting analysis

Drug	Technique used
DPPH Fingerprinting of honey	Stationary Phase: Silica gel, 60 F 254 in saturated (33% relative humidity), automated developed chamber (ADC2, CAMAG) detected at 366 nm
Piper Betel L. leaves	Stationary Phase: Silica gel 60 F 254 Mobile Phase : toluene: ethyl acetate: formic acid (70:30:1) Detected at 254 nm and 366 nm
Eugenol from piper betel leaf extract	Stationary phase: Silica gel, 60 F 254 Mobile phase : hexane: chloroform: methanol (4:4:2 v/v/v) detected at 254 nm and 366 nm
Flower extract of Punica granatum	Stationary phase: Silica gel, 60 F 254 Mobile phase: toluene: ethyl acetate: formic acid (5:4:1 v/v/v) detected at 254 nm and 366 nm

2. CONCLUSION

The HPTLC technique is also extremely helpful for routine analyses of pharmaceutical and clinical data, analyses of traditional medicines and medicinal plants, analyses of foods and dietary supplements, analyses of environmental factors, analyses of cosmetics and toxicology, analyses of plants and herbs, and analyses of food and food supplements. With the multiple benefits listed above over other chromatographic techniques, HPTLC can be used with safety in many scientific domains by examining the aforementioned applications.

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