



Review Article

High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery

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ABSTRACT

HPTLC is an advanced type of planar chromatography used extensively in the recent years for fingerprinting of medicinal plants, products and for screening lichen substances, quantification of active ingredients and herbal drugs, phytochemical and biomedical analysis and also used to check of presence of adulterants in the formulation. It is more sensitive and possible to run more sample in a short period of time, by using small amount of solvent. It is one of the sophisticated instrumental techniques based on the full capabilities of thin layer chromatography. In this paper, a brief discussion of the type of instrument used in HPTLC, its entire approach, and how this procedure is superior to TLC is presented. This article attempts to concentrate on the use of HPTLC by including examples of medications, medicinal drugs, and formulations that have been analyzed using HPTLC.

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1. Introduction

Chromatography has grown in significance and popularity to become a leading type of analysis in instrumental analytical chemistry. The term chromatography is derived from Greek chroma meaning “colour” and graphien meaning “to write”. MichailTswett (1996) a Russian botanist used this technique to separate various plant pigment. Chromatography is the science of separation used either for identification or quantification of chemical substances. HPTLC is the improved method of TLC in more optimized way. It is also known as planar chromatography or Flat-bed chromatography.¹ High performance thin layer chromatography (HPTLC) basically depends upon the full capabilities of thin layer chromatography (TLC). As it is useful in analysis of qualitative method and it combines the art with quickness at a moderate cost

of chromatography.² Modern TLC is widely known and practical as HPTLC, which can only be performed on precoated layers, using instrumentation and mainly for the purpose of quantification. Hence, here the terminology TLC and HPTLC is used interchangeably. To teach the principal of chromatography, almost all over the world, TLC is used.³ HPTLC began around 1975 with the introduction of high efficiency, commercially precoated plates, which are smaller (10 x 10 or 10 x 20 cm), have a thinner (0.1-0.2 mm) layer composed of sorbent with a finer mean particle size (5-6 μm) and a narrower particle size distribution or classification (4-8 μm), and are developed over shorter distances (about 3-7 cm) compared to classical TLC plates, which are generally 20 x 20 cm with a 0.25 mm layer containing particles with an average size of 10-12 μm (3-20 μm range). HPTLC plates provide improved resolution, shorter analysis time, higher detection sensitivity, and improved in situ quantification.⁴ Among the modern analytical tools HPTLC is a powerful analytical method equally suitable for

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qualitative and quantitative analytical tasks, because of its suitability for high-throughput screening, sensitivity and reliability in quantification of analytes at nanogram levels. It is one of the most applied methods for the analysis in pharmaceutical industries, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis, environment analysis, and other areas.⁵

HPTLC is the most simple separation technique available today, because of following advantages:

1. Enormous flexibility.
2. Parallel separation of many samples with minimal time requirement.
3. Unsurpassed clarity and simultaneous visual evaluation of all samples and sample components.
4. Simplified sample preparation due to single use of the stationary phase.
5. Possibility of multiple evaluations of the plate with different parameters because all fractions of the sample are stored on the plate.
6. Required less mobile phase and sample amount.

2. Principle and Working

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, depending upon the nature of adsorbent used on plates and solvents system used for development.⁶

The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent. The components with more affinity towards the stationary phase travels slower and the components with lesser affinity towards the stationary phase travel faster.

HPTLC take place with high speed capillary flow range of the mobile phase. There are three main steps that includes,

1. Sample to analyzed to chromatogram layer volume precision and suitable position are achieved by use of suitable instrument.
2. Solvent migrates the planned distance in layer by capillary action in this process sample separated in its components.
3. Densitometer is used for scanning separation tracks with light beam in visible or UV region.

2.1. Protocol used for drug analysis

1. Selection of Chromatographic Layer
2. Sample and Standard Preparation
3. Activation of Precoated plates
4. Application of Sample and Standard
5. Selection of Mobile Phase
6. Preconditioning (Chamber Saturation)

2.2. Steps involved in HPTLC

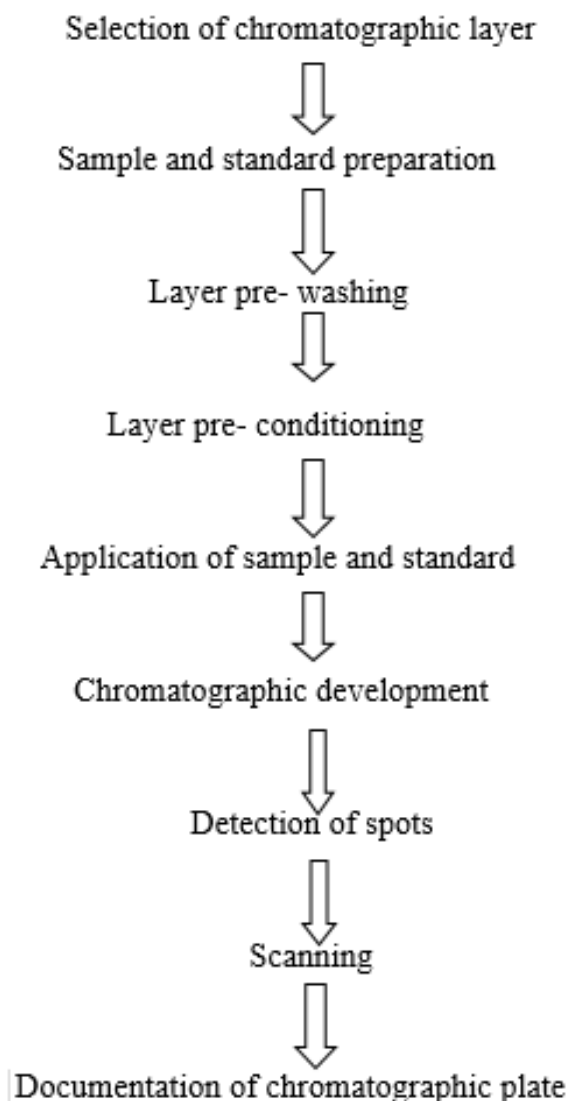


Fig. 1: Sample and standard preparation: Methylene chloride

2.3. Selection of chromatographic layer

Precoated plates — different support materials — different sorbents available.80 % of analysis — silica gel 60F — Basic substances, alkaloids and steroids- Aluminium oxide amino acids, dipeptides, sugars and alkaloids — cellulose. Non-polar substances, fatty acids, carotenoids, cholesterol- RP2, RP8 an RP18. Preservatives, barbiturates, analgesic and phenothiazine-hybrid plates- RPWF24s.

2.4. Sample and standard preparation: Methylene chloride

To avoid interference from impurities and water vapours. Low signal-to-noise ratio — Straight base

Table 1: Difference between TLC and HPTLC

Feature	TLC	HPTLC
Technique	Manual	Instrumental
Plates	Lab Made/ pre-coated	Pre-coated
Plate height	30 μm	12 μm
Layer of sorbent	250 μm	100 μm
Stationary phase	Silica gel, alumina & kieselguhr	Wide choice of stationary phase like silica gel for normal phase and C8, C18 for reversed phase modes
Analysis time	20-200 min	1-3 min
Separations	10-15 cm	3-5 cm
Mean particle size	10-12 μm	5-6 μm
Efficiency	Less	High due to smaller particle size
Sample holder	Capillary/ pipette	Syringe
Sample spotting	Manual spotting	Auto sampler
Size of sample	Uncontrolled/ solvent dependent	Controlled solvent Independent
Shape of sample	Circular (2-4 mm dia)	Rectangular (6mm L X 1mm W)
Sample tracks per plate	≤ 10	≤ 36 (72)
Vol. range	1 to 10 μL	0.1 to 500 μL
Development chamber	More amount	New type that require less amount of mobile phase
Wavelength range	254 or 366 nm, visible	190 or 800 nm, monochromatic
Detection limit (Absorption)	1-5 pg	100-500 pg
Detection limit (Fluorescence)	50-100 pg	5-10 pg
PC connectivity, method storage, validation	No	Yes
Quantitative analysis	No	Yes
Scanning	No	Use of UV/ Visible/ fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and the scanner is an advanced type of densitometer
Analysis Judgment	By analyst	By machine

Table 2: A Comparison evaluation of HPLC and HPTLC

Criteria	HPTLC System	HPLC System
Validation	Relatively simple	Relatively Simple
Documentation	Meets all the requirements	Meets all the requirements
Photo documentation	Possible	Not possible
Sample preparation	Very simple and fast; dissolve; centrifuge and supernatant for application	Expensive, time consuming, complex, extraction, filtration is essential prior to chromatography
Number of CUT'S (content uniformity test) system handled at a time	Up to 5 tests of 17 samples each	Maximum of 1 sample and 1 test at a time
Chromatography time of each CUT (content uniformity test)	45-60 minutes	4-6 minutes
Urgent Samples	Start analysis any time on receipt of sample, but finish 60 minutes.	1-2 hours start up time and then analysis time
Analysis requiring post chromatographic derivatization	Simple, additional 10-15 minutes requires after chromatographic separation.	Complicated, additional 1-3 hours may be required after chromatography.

Table 3: The details regarding HPTLC determination of pharmaceutical products in various formulations

Drug	Dosage form	Technique used	Reference
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	Ibrahim A Naguib, nesma A ali, fadwa A elroby, Mohamed R elghobashy, 2021
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.	Maimana A magdy, Rehab M abdelfatah, 2020
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 photodocumentation.	Swati D Bhende, muraliBalaram varanasi, konde abbulu, 2020
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	Atul R. bendale, R.P. singh, G. vidyasagar, 2017
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	R.P. bhole, S.S. shinde, S.S. chitlange and S.B. wankhede, 2015
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	Manali W. Jain, Atul A. Shirkhedkar, Sanjay J. Surana, 2012

Table 4: The details regarding HPTLC determination of herbal products

Active constituent	Herbal plant	Technique used	Uses
Andrographide and wedalolactone	Andrographispaniculata and Eclipta alba	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
Phyllanthin and hypophyllanthin	Phyllanthus	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 disorder, bacterial infections.
Eugenol	Ocimum sanctum	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.
Corosolic acid	Lagerstroemia speciosa	Stationary Phase : Silica gel, 60 F 254 Mobile Phase : chloroform: methanol (9:1, v/v) Detection at 20 nm	Antidiabetic activity
Rutin	Amarathusspinosus Linn	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, and anticarcinogenic activity

Table 5: The details regarding HPTLC 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

line- Improvement of LOD. Solvents used are Methanol (1:1), Ethyl acetate: Methanol (1:1), Chloroform: Methanol: Ammonia (90:10:1), Methylene chloride: Methanol (1:1), 1% Ammonia or 1 % Acetic acid. Dry the plates and store in dust free atmosphere. A good solvent system is one that does not put anything on the solvent front, but moves all components of the mixture off the baseline. Between R_f 0.15 and 0.85 range, the peaks of interest should be resolved. The elution power of the mobile phase depends on a property called eluent strength.⁷

2.5. Activation of precoated plates

Freshly open box of plate do not require activation. Plates exposed to high humidity or kept on hand for long time to be activated. By placing in an oven at 110-120°C for 30 minutes. Prior to spotting Aluminium Sheets should be kept in between two glass plates and placing in oven at 110-120°C for 15 minutes.⁸

2.6. Application of sample and standard

Usual concentration range is 0.1 $\mu\text{g}/\mu\text{l}$ above this causes poor separation. Automatic applicator- nitrogen gas sprays sample and standard from syringe on TLC plates as bands.⁹ Band wise application better separation high response to densitometer. With sufficiently high concentration of analyte, Pharmaceutical preparation is simply dissolved in a suitable solvent that will solubilize the analyte.¹⁰ It is a critical step of application of the sample and to obtain good resolution for quantification in HPTLC. Sample application techniques depend on factors like the type of workload, sample matrix and time constraints.¹¹

2.7. Selection of mobile phase

Poor grade of solvent used in mobile phase preparation was found to decrease resolution, R_f reproducibility and spot definition. It also based on one's own experience and literature. Taking into consideration, sorbent layer mobile

phase and the chemical properties of the analyte should be chosen. Using 3 or 4 components in mobile phase should be avoided as it is often difficult to get the reproducible ratios of different components.¹²

2.8. Preconditioning (Chamber saturation)

Chamber saturation has a pronounced influence on the separation profile. Time required for the saturation depends on the mobile phase. If plates are introduced into the unsaturated chamber, during the course of development, the solvent evaporates from the plate mainly at the solvent front; hence it result in increased R_f values. If tank is saturated prior to the development, solvent vapours soon get uniformly distributed throughout the chamber. As soon as the plate is kept in such a saturated chamber, it soon gets pre-loaded with solvent vapours hence less solvent is required to travel a particular distance, resulting in lower R_f values. But in some cases depending on their polarity saturation and non-saturation of chambers are required. Filter paper lining for 30 min prior to development in saturation chamber leads to uniform distribution of solvent vapours and less solvent require for the sample to travel.¹³

2.9. Chromatographic development and drying

Different types of chambers used:

1. Twin trough chamber
2. Rectangular chamber
3. V- shaped chamber
4. Sandwich chamber
5. Horizontal development chamber
6. Automatic development chamber

The different methods used for development of chambers are- ascending, descending, 2-dimensional, horizontal, multiple overrun, gradient, radial anti-radial, multimodal, forced flow planar chromatography. Plates are spotted with sample and air dried and placed in the developing chambers. After the development plate is removed from chamber and

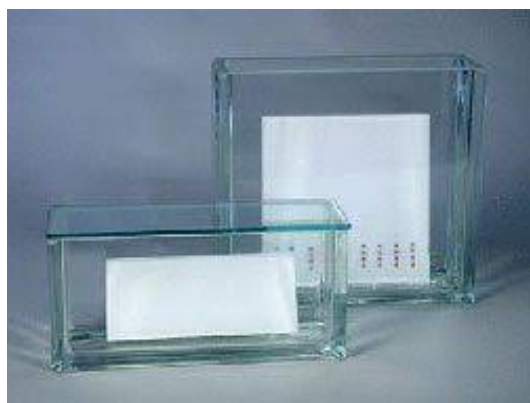


Fig. 2: Fig : Rectangular chamber



Fig. 3: Twin trough chamber

mobile phase is removed under fume cup-board to avoid contamination of laboratory atmosphere. The plate should be always laid horizontally because when mobile phase evaporates the separated components will migrate evenly to the surface where it can be easily detected.

2.10. Drying

Drying of chromatogram should be done in vacuum desiccators with protection from heat and light. If hand dryer is used there may be chances of contaminating plates. Evaporation of essential volatile oils if any present in the spot or compound sensitive to oxygen may get destroyed due to the rise in temperature.^{14,15}

2.11. Detection and visualization

Under detection of UV light is first step and is non-destructive. Spots of fluorescent compounds can be seen at 254 nm i.e. short wave length. Non-UV absorbing compounds being visualised by using 0.1% iodine solution. If individual component does not respond to UV, then derivatization is needed with visualizing agent. By quenching of fluorescence due to UV light (200-400 nm) detection of separated compounds on the sorbent layers is

enhanced. This process is commonly known as fluorescence quenching. Visualization at UV 254 nm: F254 should be described as phosphorescence quenching. In this instance, after the source of excitation is removed the fluorescence stays for a short period. It is longer than 10 seconds but, very short lived. Anthraglycosides, coumarins, flavonoids, propyl phenols in essential oils, some alkaloid type such as indole, isoquinoline and quinoline alkaloids etc. should be detected under 254 nm.¹⁶ Visualization at white light: By viewing their natural color in daylight (white light) zone containing separated compounds can be detected.¹⁷

2.12. Derivatization

Derivatization is a procedural technique that modifies functionality of an analyte's to enable chromatographic separations. Derivatization can be performed either by spraying the plates with a suitable reagent.^{18,19}

2.13. Quantification

Sample and standard sample should be chromatographed on same plate and after development, chromatogram is scanned. Densitometry is a simplest way of quantifying the desired sample components directly applied on the plate. The resolution of compounds to be separated on the chromo-plate is followed by measuring the optical density of the separated spots directly on the plate. The evaluation of original data using the conventional methods of scanning by measuring the transmitted light is delivered on stationary phase. The scanning densitometer is an advanced workstation for evaluation of TLC/HPTLC and by measuring the objects by absorbance of fluorescence.²⁰

3. Pharmaceutical Applications of HPTLC

The HPTLC approach is used in a variety of qualitative and quantitative methodological applications, including herbal and dietary remedies, nutraceuticals and a variety of medicines.

It's used in quality management and purity checks; scientific uses include metabolism tests and medication screening; and forensic applications include toxicity trials, assaying radio chemical impurities in radio pharmaceuticals, and detecting and identifying prescription raw materials, products, and their metabolites in biological media. In HPTLC, many lipids have also been analyzed and studied; 20 different lipid sub classes were separated with the reproducible and promising 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.²¹

3.1. HPTLC in quality control of pharmaceuticals

HPTLC has been used for routine quality control of to primate, dutasteride, nabumetone in pharmaceutical formulations.²² 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 degrades whether in pure form or in pharmaceutical formulation.²³ 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 quantification (LOQ). HPTLC is also an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes testing of stability. HPTLC has been reported for development of quality assurance program.²⁴

3.2. HPTLC as biomarker in pharmacognostical research: *Micheliachampaca*

HPTLC analysis of many plants used in Indian systems of medicine has been performed for various pharmacological activities like CNS, hepatoprotective etc. HPTLC method has been used for detection, and quantification of quercetin in *Micheliachampaca* (leaves and stem bark) and the estimated values indicate that the leaves are the richest source of quercetin.²⁵ HPTLC method for estimation of curcumin in marketed turmeric powder can be used routinely with good reliability and reproducibility.²⁶

3.3. HPTLC applications in drug analysis

The details regarding HPTLC determination of pharmaceutical products in various formulations are given in Table 3

3.4. HPTLC in herbal products

The details regarding HPTLC determination of herbal products are given in Table 4

3.5. HPTLC in fingerprinting analysis

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

3.6. HPTLC in other fields:

In recent years, the developing world shows HPTLC as a globally accepted practical solution for characterization of small molecules in quality assessment. It is used for steroids, pesticides and purity control of chemicals.²⁷ and also used for analysis of vitamins, water-soluble food dyes, pesticides in fruits and vegetables and also in other stuff.^{28,29}

4. Conclusion

HPTLC technique is also extensively useful to routine analysis of pharmaceutical and clinical analysis, analysis of medicinal plants and traditional medicines, analysis of food and food supplements, environmental, cosmetic, toxicological by forensic aspects, herbal and plant analysis. In view of the numerous advantages as enumerated above against other chromatographic techniques HPTLC can safely be adopted for several fields of science, analyzing the applications as mentioned above.

5. Source of Funding

None.

6. Conflict of Interest

None.

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