e-ISSN : 2455-5258

Research in Pharmacy and Health Sciences

Review Article

High Performance Thin Layer Chromatography: A Mini Review

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ABSTRACT

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 useful in detecting chemicals of forensic concern. Various advance techniques in reference to HPTLC like hyphenations in HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser have made HPTLC a power analytical tool in the field of analysis. Experts are of the opinion that HPTLC future to combinatorial approach and the utilization of instrumental HPTLC toward the analysis of drug formulations, bulk drugs, and natural products will increase in the future.

Received: 25-06- 2016

Revised: 21-07-2016

Accepted: 28-08-2016

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Mr. SV Saibaba
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Funding: Nil

Competing Interests: Nil

Keywords: High performance thin layer chromatography (HPTLC), Stability, Drugs, Herbal, Quality control, Hyphenation.

INTRODUCTION:

High Performance Thin layer Chromatography (HPTLC) 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 [1,2]. Separation may result due to adsorption or partition or by both, phenomenons'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 Steps involved in HPTLC are shown in Fig.1.

quantitative analysis; 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 reported [3].

Advantages/disadvantages of HPTLC over TLC

Most recently HPTLC is used as alternative to classical TLC and it is a valuable tool for reliable identification. It is instrument controlled by software. HPTLC, which is used for impurity determination, relies more on plain but the most potent silica gel hydrophilic phase meeting the criteria of most of the pharmacopoeias. [4,5]

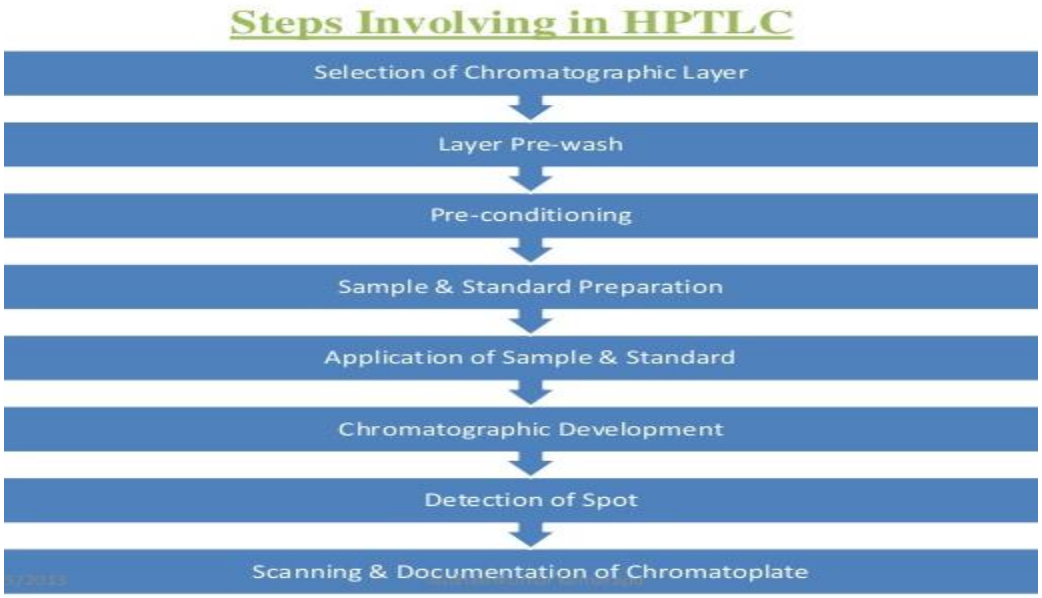

Table 1: Difference between TLC and HPTLC [6-9]

PARAMETER	TLC	HPTLC
Technique	Manual	Instrumental
Efficiency	Less	High (Due to smaller particle size)
Layer	Lab Made/ Pre-Coated	Pre-coated
Mean particle size	10-12 um	5-6 um
Layer Thickness	250 um	100 um
Plate Height	30 um	12 um
Solid Support	Silica Gel, Alumina, Kiesulguhr	Silica Gel- Normal Phase C8 and C18- Reverse phase
Sample Spotting	Manual Spotting (Capillary/	Auto sampler (Syringe)
Sample Volume	1-5 ul	0.1-0.5 ul
Shape of Sample	Circular (2-4 nm Dia)	Rectangular (6 mm L × 1mm W)
Separation	10-15 cm	3-5 cm
Separation Time	20-200 Min	3-20 Min
Sample tracks per plate	≤ 10	≤ 36 (72)
Detection Limits (Absorption)	1-5 pg	100-500 pg
Detection limits (Fluorescence)	50-100 pg	5-10 pg
PC connectivity, Method Storage	No	Yes
Validation, Quantitative Analysis, Spectrum Analysis	No	Yes
Analysis Time	Slower	Shortage Migration Distance and the analysis time is greatly reduced
Wavelength Range	254 or 366 nm, Visible	190 or 800 nm, Monochromatic
Scanning	Not possible	Use of UV/ visible/ fluorescence scanner scans the entire chromatogram qualitatively and quantitatively and scanner is an advanced type of Densitometer

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[10-12]:

- Ability to analyze crude samples containing multi-components.
- The separation process is easy to follow especially with colored compounds.
- Several samples can be separated parallel to each other on the same plate resulting in a high output, time saving, and a rapid low-cost analysis.
- Choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step.
- Two-dimensional separations are easy to perform. Stability during chromatography should be tested using two-dimensional development.
- Specific and sensitive colour reagents can be used to detect separated spots (Dragendroff reagent/Kedde reagent).
- HPTLC can combine and consequently be used for different modes of evaluation, allowing identification of compounds having different light-absorption characteristics or different colours.
- Contact detection allows radiolabelled compounds to be monitored and microbial activity in spots to be assessed.
- HPTLC method may help to minimizes exposure risk of toxic organic effluents and significantly reduces its disposal problems, consequently, reducing environment 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[13] 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:

- Selection of the stationary phase
- Mobile phase selection and optimization
- Sample Preparation and Application
- Chromatogram Development (separation)
- Detection

Quantitation: HPTLC method validation for pharmaceutical analysis [14]:

- Specificity
- Linearity
- Range
- Accuracy
- Precision
- Detection Limit, Quantitation Limit
- 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[15].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[16].

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. [17,18]. 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 [19]

S. No.	Chemical Compounds	Mobile Phase
1	Polar Compounds Anthraglycosides, Arbutin, Alkaloids, Cardiac Glycosides, Bitter Principles, Flavonoids, Saponin	Ethyl Acetate: Methanol: Water [100:13.5:10]
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 and Application-

A good solvent system is one that 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. [20] The more nonpolar the compound, the faster it will elute (or 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 is helpful in predicting the order of elution.

Table 3: Common Mobile Phases listed by Increasing Polarity

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

Pharmaceutical preparation with 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 HPTLC plate [21]. It is a fact that application of the sample is the most critical step to obtain good resolution for quantification in HPTLC [22].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.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 [23].

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 [24]. 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 [25]. 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 [26].

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.42 This quenching is shown by all anthraglycosides, coumarins, flavonoids, Phenolcarboxylic acids, some alkaloid types (Rauwolfia, Ipecacuanha alkaloids). [29]

Visualization at white light Zone containing separated compounds can be detected by viewing their natural color in daylight (White light) [27].

Derivatisation

Derivatization can be defined as a procedural technique that primarily modifies an analyte‟s 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 [28-31]. 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 4.

Table 4: Differences between HPLC and HPTLC [32]

Parameters	HPLC	HPTLC
Type	Reverse Phase Chromagraphy	Straight Phase Chromagraphy
Stationary phase	Liquid	Solid
Conditioning phase	None	Gas
Separation by	Partition	Adsorption
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)
Strongly Retarded Fractions Seen as	Broad peaks	Sharp Peaks
Analysis in parallel	No. Only 1 at a time	Yes. Upto 100 samples.
High temp. / pressure	High pressure	None
Time per sample	2- 60 min	1-30 min
Data obtained from chromatography	Limited to very high	High to very high
Post chromatography	Limited possibilities.	Simple. Possible for every

derivatisation	Cumbersome.	sample. Gives additional information
Fraction collection / micro preparative chromatography	Requires prep. scale chromatograph & fraction collector	Simple. No special requirements
Sensitivity	High to ultra	high Moderate to ultrahigh
Fluorescence data	Possible, optional	Possible, built-in
Detectors	UV, Fluor, electrochem Light scatter , MS	UV - Vis, bioluminescence , MS
Chromatogram image documentation	No	Yes. At 254 & 366 nm & visible
Sample clean-up	Through Column reusable	Not so imp. Layer disposable
Chromatographic fingerprint	Yes, but limited	Yes. Comprehensive
Cost per analysis	Very high	Low
Eqpt. maintenance	Very high	Low
Analyst‟s skills required	High to very high	Low (TLC) to high
Post chromatography derivatisation	Limited possibilities. Cumbersome.	Simple. Possible for every sample. Gives additional information

Pharmaceutical application of HPTLC

HPTLC method deals with qualitative and quantitative analytical applications such as herbal and dietary supplements, nutraceuticals, and various types of medicines. It is used 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.

a. HPTLC in quality control of pharmaceuticals

HPTLC has been used for routine quality control of topiramate, dutasteride, nabumetone in pharmaceutical formulations[33]. Validated sensitive and highly selective stability indicating methods were reported for simultaneous quantitative determination of sulpiride and mebeverine hydrochloride in presence of their reported impurities and

hydrolytic degradates whether in pure forms or in pharmaceutical formulation[34] 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[35].

b. HPTLC applications in drug analysis

The details regarding HPTLC determination of pharmaceutical products in various formulations are given in Table 2.

c. 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, hepatoproctative 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 stembark) and the estimated values indicate

that the leaves are the richest source of the quercetin [36]. HPTLC method for the estimation of curcumin in marketed turmeric powder can be used routinely with good reliability and reproducibility [37].

Conclusion

Proven applications of HPTLC in the pharmaceutical testing include: manufacturing (the manufacturing units of bulk drugs, process monitoring, fermentation broth analysis, residue analysis and in process materials testing); quality control (for raw material assays, multi-component formulations, uniformity of content testing, impurity profiling and methods validations); the analyses of formulations, stability, sustained release and bio-availability studies. 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. In marine invertebrates, HPTLC has been utilized to separate new promising pharmaceutical threatens which could be used in pharmaceutical industries.

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Cite this article as: Saibaba SV, Pandiyan S. High Performance Thin Layer Chromatography: A Mini Review. Res Pharm Healt Sci. 2016;2(4):219-226.