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INSTRUMENTATION AND FUTURE PROSPECTS OF HPTLC- A REVIEW

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ABSTRACT

High Performance Thin Layer Chromatography (HPTLC) technique is a sophisticated and automated form of thin-layer chromatography (TLC) with better and advanced separation efficiency and the detection limits and it is an excellent alternative to GC and HPLC. Application of HPTLC includes the phytochemical and biomedical analysis, herbal drug quantification, active ingredient quantification, fingerprinting of formulations, and check for adulterants in the formulations. HPTLC useful in detecting substances 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 powerful analytical tool in the field of analysis. Experts are 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.

KEYWORDS: High Performance Thin Layer Chromatography (HPTLC), Analytical tool, Herbal, Forensic, Hyphenation.

INTRODUCTION

HPTLC is an powerful analytical method equally suitable for qualitative and quantitative analytical tasks.[1,2] 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 of HPTLC fundamentals are principle, theory, instrumentation: implementation, optimization, validation, automation and qualitative and

quantitative analysis; applications: phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, fingerprint analysis and the potential for hyphenation (HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser) have been reported.[3]

Classification of HPTLC

HPTLC techniques may be classified into four classes i.e. Classical, High performance, Ultra and Preparative thin layer chromatography.[4-6]

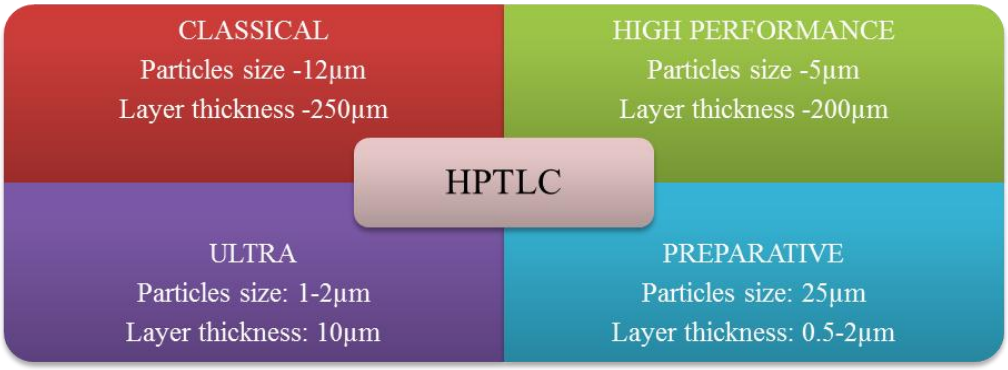


Figure 1: Classification of HPTLC.

Table 1: Differences between HPTLC and TLC.

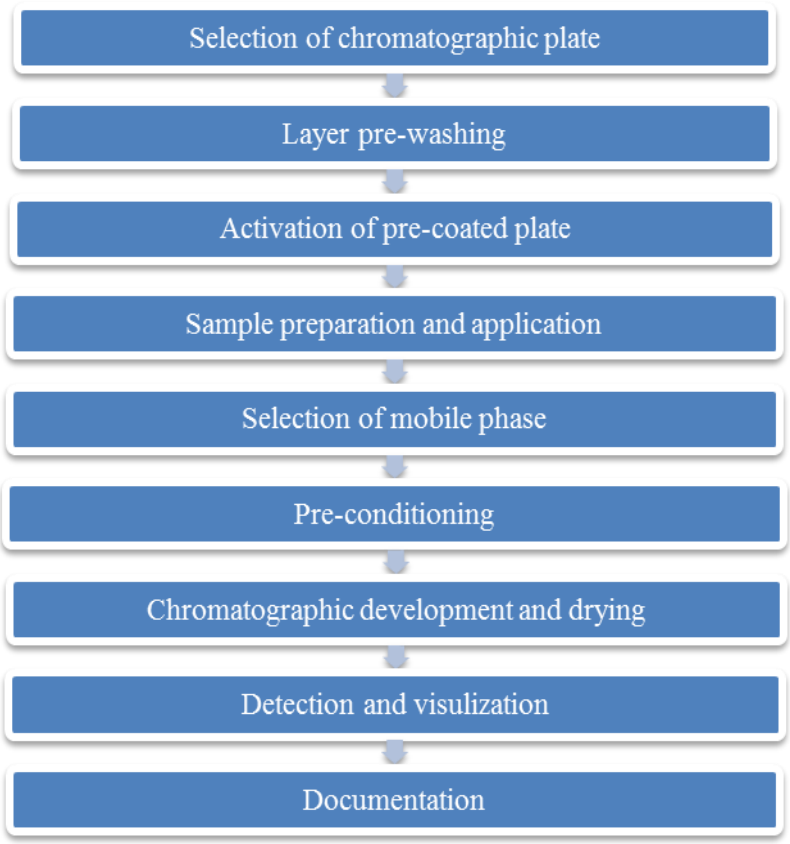
Parameters	HPTLC	TLC
Chromatographic plate used	Pre-coated	Hand made
Sorbent layer thickness	100-200µm	250µm
Pre-washing of plates	Must	Not followed
Application of sample	Automatic	Manual
Sample volume	0.2-5µl	1-10µl
Analysis time	Greatly reduced	Slow
Shape	Spot/band	Spot
Spots size	0.5-1mm	2-4mm
Efficiency	High	Slow
Development chamber	Less amount of solvent is required	More amount of solvent is required
Scanning	Densitometer	Not possible

Automation of HPTLC

Modern TLC is widely known as HPTLC, which can only be performed on precoated layers, using instrumentation and mainly for the purpose of quantification. Hence, the terminology TLC and HPTLC is used interchangeably. To teach the principle of chromatography, almost all over the world, TLC is used. The main reasons for this choice are visibility of the sample during chromatography, ultra-low-cost apparatus for demonstration, and simplicity to perform. Its approach for improving resolution under capillary flow-controlled conditions is

to use multiple developments. Its either one-dimensional or two-dimensional separations are possible in planar chromatography. Mobile-phase velocity can also be controlled by external means, such as in forced-flow development.[7]

HPTLC is the fastest chromatography method. The chromatography of samples is done in parallel. Each and every step of the procedure is performed independently, makes HPTLC not only faster but flexible enough for HPTLC System to analyse different samples in parallel. Consumption of stationary phase and mobile phase is directly proportional to the number of samples being analysed.[8]



Steps involved in HPTLC

Selection of chromatographic plate

- Handmade plates which are made up of cellulose and other materials which are not used much now a days.
- **Pre-coated plates:** The plates with different sorbent layer and support materials have different format and thickness are used for qualitative and quantitative analysis.
- Support materials used in plates: Glass, Polyester /polyethylene, Aluminium.

- The Sorbents used in plates are silica gel 60F, aluminium oxide, cellulose, silica gel chemically modified –a) Amino group (NH_2) b) CN group.
- The Smaller particle size of silica helps in greater resolution and sensitivity.

Layer pre-washing

- In this purification step, the primary purpose of the pre-washing is to remove impurities which include water vapours and other volatile substances from the atmosphere when they get exposed in the lab environment.
- Some common methods used for prewashing are-Ascending, Dipping, Continuous.
- The Solvents used for pre-washing: Methanol, Chloroform: Methanol (1:1), Chloroform: Methanol: Ammonia (90:10:1).

Activation of Pre-coated Plate

- The Freshly opened box of HPTLC plates doesn't need activation.
- If plates exposed to high humidity longer time then activation is requested.
- The plates are activated by placing in an oven at 110-120^{0}C for 30min, where this step is used to remove water that has been physically adsorbed on to the surface on sorbent layer.

SAMPLE PREPARATION AND APPLICATION

➢ **Sample preparation**

- The Sample and reference substances should be dissolved in the same solvent to ensure comparable distribution at starting zones.
- It needs a very less amount of sample with high concentrated solution need to be applied.
- After that the plates were dried and stored in dust free atmosphere.

➢ **Sample application**

- Usual concentration range recommended for HPTLC is 0.5-5µL.
- This size of sample spot applied must not exceed 1mm in diameter.
- Problem from overloading can be overcome by applying the sample in the form of a band.
- Selection of applicator to be used depends on the sample volume and number of samples to be applied.
- Some applicators used for application of sample are Micro syringes, Linomat etc.

Figure 2: Camag Linomat Applicator used in HPTLC.

Selection of mobile phase

- The selection of mobile phase is based on the adsorbent material used as stationary phase and also depends on the physical and chemical properties of the analyte.
- The peaks of interest should be resolved between R_f value of 0.15 and 0.85.
- Power of elution of the mobile phase depends on a property called eluent strength which is related to the polarity of the mobile phase components.
- The more nonpolar the compound, the faster it will elute (or the less time it will retain on the stationary phase) and the more polar the compound the slower it will elute (or more time retain on the stationary phase).
- The less amount of mobile phase is required than TLC.[11,12]

Table 2: Few examples of Common Solvents with their respective Elution strengths.

S. No	Solvent	Eluent Strength
1	N- Pentane	0.00
2	Hexane	0.01
3	Cyclohexane	0.04
4	Carbon tetrachloride	0.18
5	Toluene	0.29
6	Chloroform	0.40
7	Methylene Chloride	0.42
8	Tetrahydrofuran	0.45
9	Acetone	0.56
10	Ethyl Acetate	0.58
11	Aniline	0.62
12	Acetonitrile	0.65
13	Ethanol	0.88
14	Methanol	0.95

Table 3: Most widely used Mobile Phases in detection of few chemical compounds.

S. No	Chemical compounds	Mobile phase
1	Anthraglycosides, Cardiac Glycosides	Ethyl Acetate: Methanol: Water [100:13.5:10]
2	Lipophilic Compounds Toluene Essential oils, Terpenes, Coumarin, Napthoquinons, Velpotriate	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: Methanol: Water [70:30:4] Chloroform: Methanol [90:10] Toluene: Ethyl Acetate [70:30]
6	Lignans	Diethyl Ether: Toluene [1:1] Saturated with 10% Acetic Acid

Pre-conditioning (Chamber saturation)

- Un-saturated chamber causes high Rf values.
- Saturation of Mobile phase should be done in chamber by lining with filter paper for 30min prior to development shows uniform distribution of solvent vapours results in low Rf values.
- For low polarity mobile phases, there is no need of saturation however; saturation is needed only for high polar mobile phases.

Developmental Techniques

- The Plates are spotted with sample and air dried and placed in the developing chambers.
- The different developmental techniques used are –

- Ascending
- Descending
- Horizontal

- In general, saturated twin-trough chambers fitted with filter paper are the best reproducibility.[13]

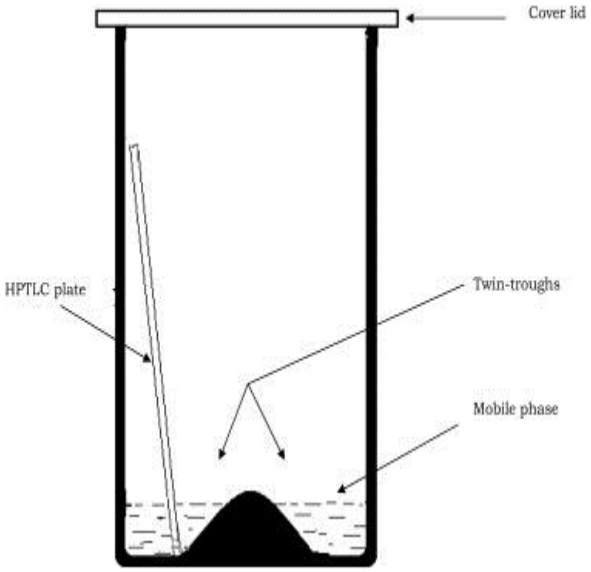


Fig. 3: Twin Trough chamber.

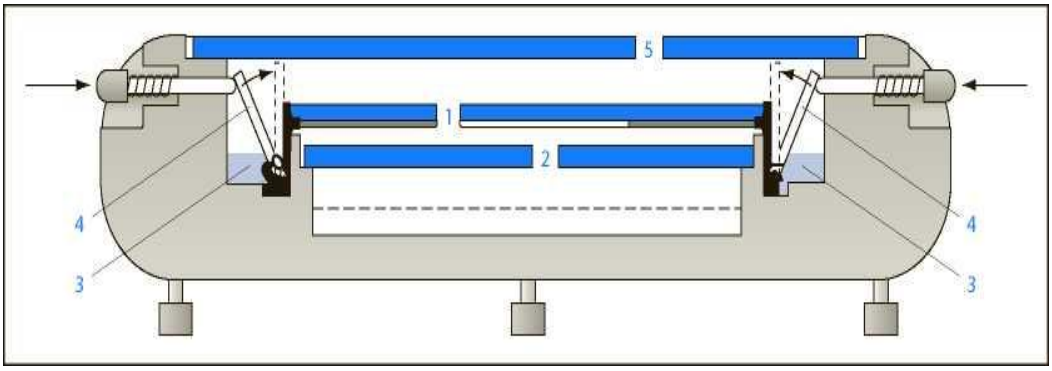


Fig. 4: Horizontal development chamber.

Detection

The 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.[14,15]

Visualization at UV 254 nm

F254 should be described as phosphorescence quenching. It has very short lived, but longer than 10 seconds. The F254 fluorescent indicator is excited with UV wavelength at 254 nm and emits green fluorescence. Compounds which absorb radiation at 254 nm reduce the emission on the layer, and a dark violet spot on a green background is observed where the compound zones are located.

Visualization at UV 366 nm

F366 should be described as Fluorescence quenching. In this instance the fluorescence doesn't remains after the source of excitation is removed. The quenching is shown by anthraglycosides, coumarins, flavonoids, Phenol carboxylic acids, some alkaloid types (Rauwolfia, Ipecacuanha alkaloids).

Visualization at white light

Zone containing separated compounds can be detected by viewing their natural colour in daylight (White light).

Derivatisation

- Derivatization can be defined as procedural technique which primarily modifies analytes functionality in order to enable chromatographic separations.
- Derivatization can be performed either by immersing the plates or by spraying with suitable reagent on the plates. For better reproducibility, immersion is preferred as derivatization technique.[16,17]

Table 4: List of few Derivatization reagents used.

S. No	Colour Reagents	Chemical compounds	Colour obtained
1	Dragendroff Reagent	Alkaloids	Red-brown Zone (vis)
2	Diphenyl boric acid -2-aminoethyl ester forms complexes with 3-hydroxyl flavones via condensation reaction	Flavonoids	Intense yellow, Orange and Green Fluorescent zones in UV 366 nm
3	Vanillin Sulphuric Acid orAnisaldehyde Sulphuric Acid	Essential Oil	Red-brown, Yellow-brown, Dark green Zone (vis)
4	Ninhydrin Reagent	Amino acids, peptides, amines and amino-sugars	Yellow, brown to pink and violet (vis)
5	Iodine: It produce iodine reaction possibly result in an oxidative products.	Indole, quinoline derivative, thiols and all organic compounds	Dark zone (UV 254)

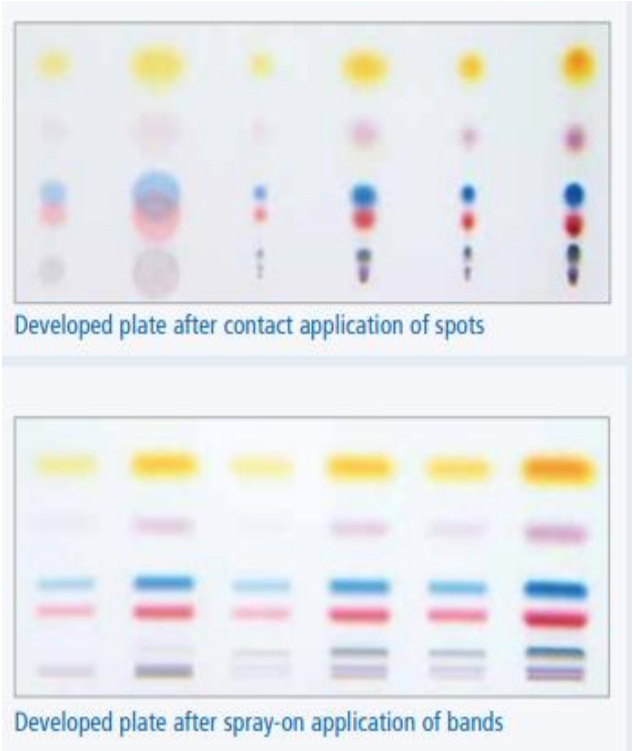


Fig 5: Developed HPTLC plates after Derivatization.

QUANTIFICATION

Scanning Densitometry

Allow measuring the absorption and/or fluorescence of underivatized or derivative substances at wavelength between 200 and 800 nm.

Wavelengths up to 800nm can be evaluated and spectra of any peak can be recorded. The Biological tests can be performed directly on the HPTLC plate.[20]

Digital Camera-Based Image Documentation

The UV Cabinets are now being replaced with improved design and UV Cabinets which are allowed digital camera to be fixed for recording images of the plate. were as in now a days, HPTLC is a primary requirement for any laboratory involved in herbal analysis for establishing the identity of plant extracts by comparison with Botanical Reference Material (BRM) extracts to detect the substitutes or adulterants and the studies of formulations etc. Forensic analysts has long ago stated that the starting points are the microscope for physical inspection and TLC for chemical inspection.

Software-Induced Scanning

This can be used for quantification in absorbance and fluorescence modes and record UV–Vis in absorbance spectra. As depending on the end-user requirements, the gradient chamber

and/or a photo documentation device and bioluminescence detector may be added or a fully automatic system could be procured. Hyphenation techniques with MS or IR or NMR can be achieved with a suitable commercially available interface. A recently available device interfaces HPTLC with MS. This interface enables the chosen fraction which is extracted from the layer and feeds it directly into the MS. The analysis output from LC–MS can be greatly increased, when compiled to TLC or HPTLC. Any specified fraction from the plate can be analyzed. Other fractions present can be ignored. Optimization of MS parameters for a particular molecule can be optimized using TLC.[21]

Fig 6: Densitometer and Obtained Chromatograms by Densitometer.

Pharmaceutical Applications 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 Department
- Purity determination
- Clinical applications: For metabolism studies, Drug screening etc.
- Forensic Science: In Poisoning investigations, assaying radiochemical impurities of radiopharmaceuticals, detection and identification of Pharmaceutical raw materials, drugs and their metabolites in biological samples.
- Cosmetic Analysis: In determination of Hydrocortisone in lanolin ointment.
- Natural products Detection: Glycosides in herbal drugs, piperine in piper longum etc.

- Analysis of drug in blood.
- HPTLC has been used for routine quality control of topiramate, dutasteride, pharmaceutical formulations.
- In herbal medicinal products, HPTLC is also used as an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability.

CONCLUSION

HPTLC method is used as important tool in qualitative and quantitative determination for Phytochemical analysis, analysis of a wide range of compounds, such as herbal and botanical dietary supplements, nutraceuticals, traditional western medicines, traditional Chinese medicines and Ayurvedic (Indian) medicines, detection of pesticides or insecticides in food; analyzing the dye composition of fibres in forensics.

Due to its vast use, it has become a powerful analytical tool in the field of Pharmaceutical Analysis. It is noteworthy to Utilize the HPTLC instrumentation toward the analysis of drug formulations, API's, Natural products, Food stuffs and much more in the future prospects.

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