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A REVIEW ON HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY

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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 future.

KEYWORDS: *High Performance Thin Layer Chromatography (HPTLC), HPTLC-Scanning Diode Laser, adulterants*

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. HPTLC is a well known and versatile separation method, which shows a lots of advantages in comparision to other separation techniques. The basic difference between conventional TLC and HPTLC is only particle size and pore size of the sorbents. It is a powerful analytical method equally suitable for quantitative analytical tasks . 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 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

PRINCIPLE

HPTLC having similar approach and employ the same physical principles of TLC (adsorption chromatography) i.e, the principle of separation is adsorption. The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent. The component with more affinity towards the stationary phase travels slower. The components with the lesser affinity towards the stationary phase travels faster. Thus the components are separated on a chromatographic plate.

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STEPS INVOLVING IN HPTLC

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 .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.. The Table 1.gives the details of mobile phase generally used in detection of some chemical compounds. Table 1. Generally used Mobile phase in detection of some chemical compounds

Table 1 Generally used mobile phases in detection of chemical compounds.

SN 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 Essentiaal oils,coumarin,terpenes , Naphthaquinons,velpotriate	Toulene,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]

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. 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 2. 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

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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 . 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 . 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.

PREWASHING

The main 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. Silica gel 60F is most widely used sorbent. The major disadvantage of this sorbent is that it contain iron as impurity. This iron is removed by using Methanol : Water in the ratio of 9:1.This is the major advantage of the step of pre-washing.

Some of the common methods involved in pre-washing

a] Ascending method
b] Descending method
c] Continous method

ACTIVATION OF PLATES

Freshly opened box of HPTLC plate does not require activation. Plates are exposed to high humidity or kept in hand for long time require activation.Plates are placed in oven at 110˚c-120˚c for 30 min before sample application.

PRE-CONDITIONING

Also called chamber saturation.
Un-saturated chamber causes high Rf values

SAMPLE APPLICATION

Sample application can be done by using

1] Capillary tubes
2] Micro syringes
3] Micro bulb pipettes
4] Automatic sample applicator

POST CHROMATOGRAPHIC STEPS

1] Detection
2] Photo Documentation
3] Densitometry Measurements

1] DETECTION

Detectionn under UV light is first choice – non destructive Non UV absorbing compunds like ethambutol, dicylomine etc- dipping the plates in 0.1% iodine solution.

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2] DENSITOMETRY MEASUREMENTS

Measure visible, UV absorbance or Fluorescence.Convert the spot\ band into chromatogram consisting of peaks.

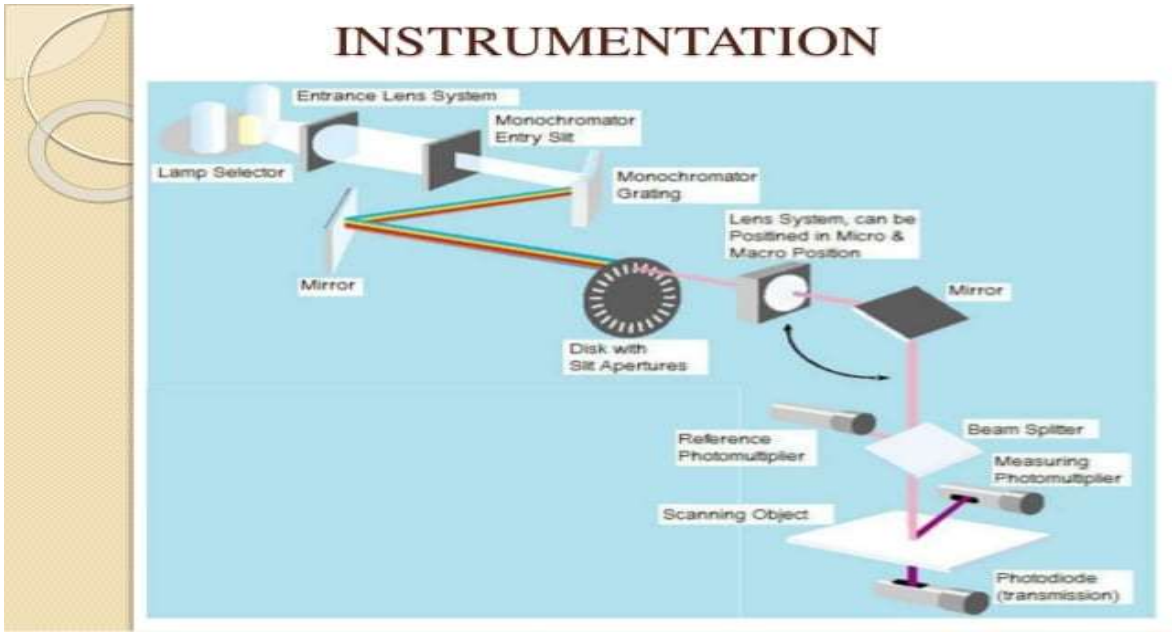

Table 3 .Differences between TLC and HPTLC

PARAMETERS	TLC	HPTLC
Chromatographic plate used	Hand made/precoated	Precoated
Efficiency particle size)	Less	High (Due to smaller
Shape	Spot	Spot/Band
Mean particle size 10-12 um 5-6 um	10-12um	5-6um
Layer Thickness 250 um	250um	100um
Plate Height 30 um 12 um	30um	12um
Prewashing of plate	Not followed	Must followed
Aplication of sample Automatic/semi-automatic		Manual/Semi-automatic
Sample Volume	1-5ul	0.1-0.5 ul
Shape of Sample × 1mm W)	Circular (2-4 nm Dia)	Rectangular (6 mm L
Separation	10-15 cm	3-5 cm
Separation Time	20-200 Min	3-20 Min
Sample tracks per plate	≤ 10	≤ 36 (72)

APPLICATIONS OF HPTLC

Pharmaceutical-industry-Quality control, identity, purity test etc.
Food analysis – Quality control, additives , pesticides, stability testing etc.
Clinical applications – Metabolism studies, drug screening, stability testing etc.
Industrial applications - Process development and optimization etc.
Forensic applications – Poisoning investigations
Biomedical Analysis – Separation of gangliosides
Environmental Analysis – Pesticides in drinking water, Selenium in water.

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Cosmetics – Hydrocortisone and cinchocaine in lanolin ointment etc.
Natural products, plant ingredients – Glycosides in herbal drugs, Piperine in piper longum etc.
Finger print analysis – Finger prints for identification of liquorice, ginseng etc.
Analysis of drug in blood.

A] QUANTITATIVE DETERMINATION

1] Biochemical research / Biotechnology – Separation of gangliosides
2] Clinical – Inorganic and organic mercury in water and human serum. Caffeine in urine.
3] Food analysis – Vitamin C in fruit juices , Aflatoxins in food stuff.
4] Doping analysis – Atenolol in urine.

B] FINGER PRINT ANALYSIS

a] HPTLC finger print of Valerian.
b] Finger print of garlic, Ashwaganda.
c] Finger prints for identification of liquorice , gingseng .

5] Identification and separation of phenyl thiohydantoin – amino acid.
6] Analysis of drug in blood .
Ex: a] separation of phenothiazine drugs like chlorpromazine, acetophenazine, perphenazine, trifluperazine, and thoridazine.
7] Identification of mycotoxins in admixture: Ex: detection of sterigmatocystin, zearalenone, citrinin, patulin, penicillic acid.
8] determination of polycyclic aromatic hydrocarbons in particulate sample. Ex: determination of chrysene, pyrene, fluoronthene etc.

CONCLUSION

Applications of HPTLC for phytochemical analysis, finger print analysis, and HPTLC future to combinatorial approach, HPTLC-MS, HPTLCC-FTIR and HPTLC a power analytical tool in the field of analysis. It is noteworthy that utilization of instrumental HPTLC towards the analysis of drug formulations, bulk drugs, natural products, clinical samples food stuffs, environmental, and other relevant samples will increase in the future.

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*The Pharma Review 2007; 11-13 High Performance Thin Layer Chromatography and Its Role Pharmaceutical Industry: Review/Bandameedi Ramu1, *, Kishore Babu Chittela2 1 Department of Pharmaceutics, Kadiyala Vijaya Kumari College of Pharmacy, Hyderabad, India*
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