REVIEW ARTICLE

An Overview on High-Performance Thin-Layer Chromatography (HPTLC)

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Abstract

High-performance thin-layer chromatography is one of the sophisticated instrumental techniques based on the full capabilities of thin-layer chromatography. The advantages of HPTLC are automation, scanning, optimization & computerization, selective detection, and minimum sample preparation. High-performance thin-layer chromatography (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. 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.

Key words: Drugs, Herbal, High-performance thin-layer chromatography, Hyphenation, Quality control, Stability

INTRODUCTION

High-performance thin-layer chromatography (HPTLC) is an improved method of TLC which is used for separation of mixtures and preparative, qualitative, and quantitative analysis of drug samples.[1,2]

WHY HPTLC

- This is an optimized analytical method which is suitable for automation and computerization. This is a highly useful method for qualitative and quantitative analysis.
- This is more rapid and sensitive than other methods.
- Sample cleanup is easy as the stationary phase is disposable and 100% sample is accountable.

HPTLC plates are generally coated with smaller, narrow size distribution of silica gel particles which lead less diffusion and more compact and concentrated spots for better sensitivity.

Since silicagel particles are smaller (5–12 um) size of particles cause smaller size of plates and requires small amount of sample solution (O.lul) for spotting gives shorter development distance which leads to utilize of less amount of mobile phase and shorter duration of time.[3,4] The difference between TLC and HPTLC is shown in Table 1.

VARIOUS STEPS INVOLVED

1. Selection of HPTLC plates and Sorbents
2. Pre chromatographic operations
3. Applications of sample
4. Separation or development of chromatograph
5. Detection and derivatization
6. Quantization.

Selection of Plates

Previously, handmade plates were used for TLC, but because some defects like non-uniformity of the layer, formation of thick layer pre-coted plates is used in HPTLC.[9-11]

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Table 1: Comparison of TLC with HPTLC[5-8]

Parameter	TLC	HPTLC
1. Plate to be used	Handmade/pre-coated	Pre coated
2. Layer thickness	200–250 um	100–200 urn
3. Particle size	5–20 urn	4–8 ^m
4.Pre washing	Not required	Necessary
5. Sample application	Manual	Automatic
6. Shape	Spot	Band
7. Size of applied area	3–6 mm	1–2 mm
8. Development time	Depends on m.p	40% less than TLC
9. Optimum development distance	100–150 mm	50–70mm
10. Diameter of separated zones	6–15 mm	2–4 mm
11. Quantitation	Manual	Instrumental

Supporting materials used in HPTLC

Glass support

Glass with 1.3 mm thickness is used.

Advantages

- Heat resistant
- Easy to handle
- Offer superior flat and smooth surface for chromatographic work.

Disadvantages

- High weight
- High cost
- Requires additional packing mater.

Poly ester or plastic sheet

With the thickness of 0.2 mm is used.

Advantages

- These are economical
- These are unbreakable
- Less packing material required
- Easy to handle.

Disadvantages

- Not heat resistant (above 120°C).

Aluminum sheet

With the thickness of 0.1 mm is used.

Advantages

- These are economical
- Unbreakable
- Less packing material required
- Temperature resistant.

Disadvantages

- Not used for high concentration of mineral acids and concentrated ammonia because the will chemically react with aluminum.

Sorbents used in HPTLC

a. Silica gel GOF → adsorption and partition chromatography.
b. High purity silica gel → AFLOTOXIN.
c. Aluminum oxide → alkaloids and steroids.
d. Cellulose microcrystalline → amino acids; dipeptides; sugars; and antibiotics.
e. Chemically modified silica gel
   i. NH_2_CQQH: _phenolic groups. Nucleotides; B_1. B_6. B_{12} vitamins.
   ii. CN:-pharmaceutical preservatives.
   iii. CH1R: resolution of enantiomeric substitutions for optical purity such as amino acids and lactones.
   iv. DJOL: hormones and steroids.
   v. Reversed phase: – Stationary phase is modified either by impregnating the normal layer with hydrophobic substances such as liquid paraffin or chemically modifying by treating silanol groups with hydrocarbons such as silanes of different chains length, vi. RP-2: RP-8; RP-18: Non-polar substances (lipids), fatty acids, carotenoids, steroids, cholesterol, vitamins, antioxidants, and cardiac drugs.
f. Hybrid plates
   In this type, certain portion of the silinol groups is chemically bonded with octadecyl chain whereas the remaining silanol groups remain non-bonded. The non-bonded toups impart wettability thus giving hybrid nature to plate.
g. Dual phase plate

In this type, a part of plate is reversed phase whereas remaining part contains normal phase coating.

Plate size

Pre-coated plates for HPTLC are available in different sizes.

- 20*20 cm
- 10* 20 cm
- 10* 10 cm

- 5* 10 cm
- 5* 7.5 cm.

Layer thickness

- Thickness of the layer also alters the efficacy of HPTLC,
- Thickness should be less.
  - 100–200 µm
  - 250 µm-conventional TLC.

Particle size of sorbents

- Particle size of a sorbent should be uniform
- Particles should be small in size.
  - 6 µm-HPTLC
  - 10 µm-TLC.

Effect of particle size on efficiency

Smaller particles provide greater surface area for adsorption of components to the sorbent particles. An adsorption is one of the principle behind the separation of the components.[12]

The separation of components can be achieved in shorter migration distance (3–5 cm) jyhen compared with TLC (10–15cm).

Due to shorter distance the time required for operation is also decreased.

Particle size for
- TLC 5–20 µm
- HPTLC 4–8.

Pre-chromatographic Operations

Pre-chromatographic operations play the main role in the efficacy of HPTLC. It includes sample preparation, washing of pre coated plates, and activation of the plates etc.

pre washing of pre-coated plates

Sorbent with large surface area absorbs water vapor as well as other impurities form atmosphere when exposed for long time.
- To avoid, it they are placed in foils.
- To avoid, any possible interference due to impurities with the chromatographic separation particularly in case of quantitative work, it is always recommended to clean the plates.

There are three ways for cleaning.
1. Ascending method
2. Dipping method
3. Continuous method.

Ascending method
In this method, pre-coated plates are allowed to run the blank performance with the suitable solvent (mobile phase).

During this operation, the solvent moves upward.

While moving, solvent carries the impurities to the top of the plate. Moreover, after this operation 10–20% of upper portion is removed.

This is the time consuming process, but this is the better one.

Dipping method
In this method, chromatographic plates are dipped in suitable solution for specified time.

In this method, the first plate is dipped in Chamber-A followed by Chamber-B, here, solvent of Chamber-A is primary washing solvent and solvent of Chamber-B is secondary washing solvent. Then, solvent of chamber-A is discarded. The next plate is firstly dipped in Chamber-B (primary solvent) followed by Chamber-C (secondary solvent).

Continuous method
In this method, the plates are introduced in to the chamber having entrance and exit slits.

Solvent is made to flow continuously through the chamber and that moves along with impurities.

The plates should dried in oven in vertical position, as in horizontal drops of solvent may fall on the plates of condensation. Washed plates should be placed in desiccators.

Solvents used for pre-washing

1. Methanol
2. chloroform: Methanol (1:1)
3. chloroform: Methanol: Ammonia (90:IO: l)
4. Ammonia solution (1%).

Activation of pre-coated
1. The plates are activated by placing them in oven at
2. 1 10–120°C for 30 min → Glass plates and aluminium sheets.
3. 1 10–120°C for 15 min → Poly ester sheets to remove water.
4. Activation at high temperature for a long time should be avoided because it may lead to very active layers which cause decomposition of sample.

Sample preparation
1. Proper sample preparation is the important thing to get good results in HPTLC.
2. For this selection of suitable solvent is very important.
3. Solvent for dissolving the component should be non-polar and volatile in nature because polar solvents may lead to loss of separation efficacy by inducing circular chromatography at origin.
4. For reverse phase, water miscible volatile solvents are used.

Application of Samples

Sample application is the most critical step for obtaining good resolution for quantification by HPTLC. Sample should completely transfer to layer, and in this process, it should not damage the layer. Automatic application is always advised for quantitative work. While using graduated capillaries, one must ensure that they will fill and empty completely.

Sample and standard solutions are applied either as a spot orjiand, but in case of HPTLC bands are preferred.

For sample application
1. Capillary tubes.
2. Micro bulb pipettes.
3. Micro syringes.
4. Automatic application devices

	TLC	HPTLC
Spotting volume	1–10 µl	0.1–2.0 µl
Spot diameter	3–6 mm	l–2 mm
Sample concentration	0.1–1 ug/ml	0.1–1 ug/m

Application of samples as bonds

Sample volume and concentration primarily depends on component under analysis and their stability and sensitivity. If more volume of sample has to be applied as a spot, it may leads to overloading which results in trailing of zones and poor resolution.[13,9]

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Advantages
- Better separation because the components move uniformly as a rectangular area.
- Equal Rf values of components of sample and standard solutions.
- Response of densitometer is superior incase at a band than that observed from an equal amount of sample applied as spot.
- Application of different volumes of as bands from one solution gives same concentration curve as by application of equal amount of sample with different concentration.
- This correlation is absent for spots.
- Less concentrated samples can be applied.

Linomat 5

Linomat 5 offers semiautomatic sample application for quantitative and qualitative as well as preparative separations. With this, instrument samples are sprayed on to the chromatographic in the form of narrows bands. This technique allows layer volumes to be applied them by spotting. During the spraying, the solvent of sample evaporates and concentrates the Sample in to a narrow band of selectable length.

Mobile phase: Mobile phase commonly called solvent system[14]
- Solvent composition is expressed by volume (v/v).
- Various components of m.p should be measured separately and then placed in the mixing vessel; this avoids contamination of solvents and avoids possible volumetric errors.
- Different compounds of m.p are mixed in a mixing vessel and then introduced in to developing chamber.
- Chamber usually containing multicomponent m.p once used is not recommended for re use.
- Chemical reaction may occur between different components of m.p such as acetic acid and amrnoma.
- Polar solvent portion of m.p may get absorbed by the layer during the exgerLment.
- Selection of m.p should be done by considering the analyzer.

For example
1. Propranolol HCI: Hydrochlorothiazide
   (0.5 m Nacl-methanol-Glacial acetic acid) and (Chloroform: Methanol: Ammonia) (12: 8: 0.01 v/v).
2. Amloride HCI; furosemide
   (Chloroform: Methanol: Glacial acetic acid) (8.5:15:0.05 v/v).
3. Atenolol; Nifedipine
   (Toluene: Propanol: Ammonia) (6.5:3.5:0.02 v/v).
4. Spironolactone: Furosemide
   (Chloroform: Methanol: Glacial acetic acid) (7:3:0.1 v/v).
5. Aspirin dipyridamol
   (Chloroform: Methanol: Glacial acetic acid) (9.5:0.5:0.04v/v).
6. Paracetamol: Ketoprofen
   (0.5 MNaCl: Methanol: Glacial acetic acid) (12: 8: 0.01 v/v).

Chambers

Different types of chambers are available.

Twin trough chamber

These are available with stainless steel lid, with glass lid, and without lid.
- 20* 20 cm
- 20* 10 cm
- 10* 10 cm
- 10* 5 cm

Flat bottom chambers

These are available with stainless steel lid, with glass lid, and without lid.

- 20* 20 cm
- 20* 10 cm
- 10*10 cm
- 10*5 cm

Horizontal chambers

Chamber saturation

- Chamber saturation influences the separation. Unsaturated chambers lead to edge effect.
- Filter paper and saturation pads are used achieves chambers saturation.

Development chambers used in HPTLC shown in Figures 1 and 2.

Development of Chromatogram

HPTLC plates are developed under optimized parameter using sophisticated devices.

The most common methods[15-19]

- Ascending method
- Descending method
- Horizontal development
- Radial method
- Two-dimensional development.

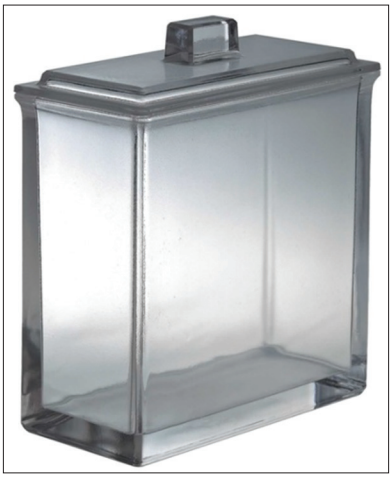

Figure 1: Twin trough chamber

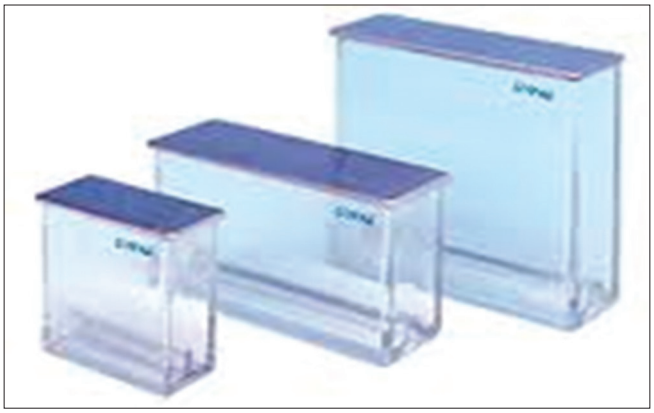

Figure 2: Horizontal chamber

After development of chromatogram, the plate is removed from the chamber and M.P is allowed to evaporate.

Detection and Derivatization

After drying, the separated components can be identified by various physical and chemical methods for quantitative and qualitative analysis.

Coloured substances and colorless substances absorbing U.Vradiations are with intrinsic fluorescence can be detected easily by using densitometer.

Derivatization by reagent treatment

Formation of colored compound

- Alkaloids-with Dragendorff's reagent
- Amino acids-with ninhydrin reagent
- Sulfa drugs-$NaNo_2$ followed by BMR.

Formation of u.v absorbing compound

Ex: Ethambutol Hcl with iodine.

(Best results are obtained by dipping the plates in 0.10–0.25% solution of \i in chloroform: Methanol (1:1).

Formation of fluorescent compound

Ex: Ethinyl estradiol with 10% v/v alcoholic H_2SO_4.

Water wetting method

On dipping on spraying with water, lipophilic substances kike steroids, fatty acids, and hydrocarbons can appear as white spots against semitransparent background.

Instead of water hydrophilic dye (methylene blue) give blue back ground non-wetted in pale (for lipophilic substances).

The reagent can be applied by spraying and dipping method.

Quantitation

After detection evaluation is important step.

Qualitative analysis

Qualitative analysis can be achieved by comparing the Rf-values.

Quantitative analysis

Direct method

Visual comparison

In this method, sample and standard solutions are applied alternatively by bracketing the sample between standard samples. However, here, the concentration of standard sample should applied increasing order but this is not an accurate method.

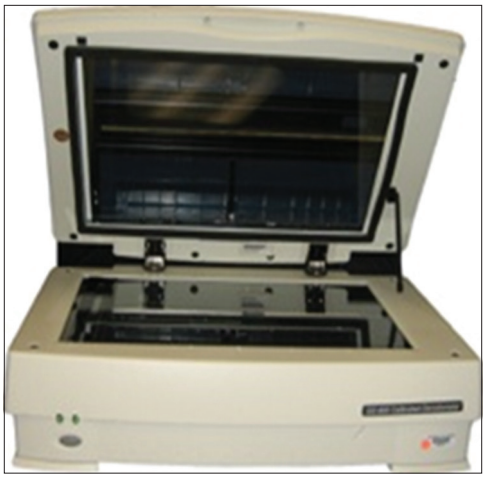

Figure 3: Densitometer with scanner

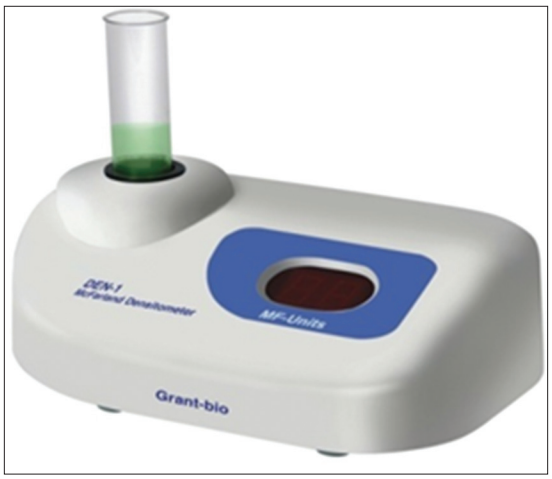

Figure 4: Densitometer

In this method, the amount of component present in the sample spot is determined by comparing the comparing the color intensity of with that of standard spots.

Insitu densitometry

HPTLC plate is introduced into the densitometer and scanned readings of absorbance are noted.

In this method, five known concentration standard samples and one test sample is used. and a calibration graph is constructed (concentration (vs)absorption)and the concentration of unknown is measured. The instrumentation densitometer is shown in Figures 3 and 4.

Indirect method

In this method, removal of analyte from the plate is followed by quantification.

Ex: Scrapping and evaluation.

Steps:

- Detection of spot/band

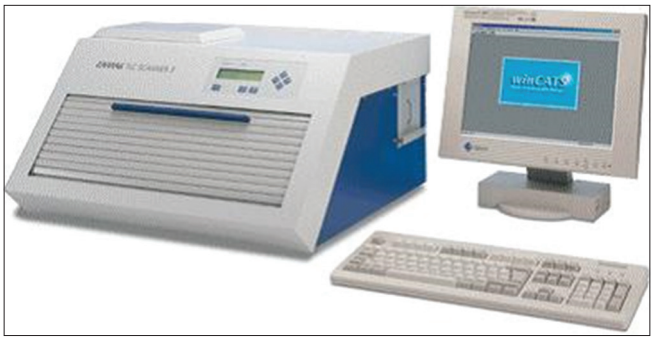

Figure 5: HPTLC Instrument

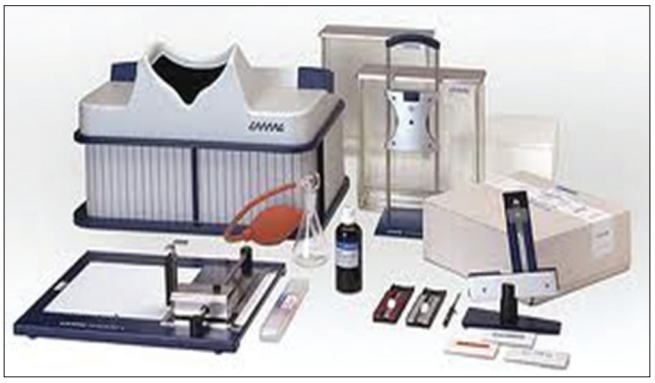

Figure 6: HPTLC Kit

- Scrap the zone
- Extract the analyte with vacuum devices
- Analyze by suitable instrument.
  a. Spectrophotometry
  b. Colorimetry
  c. Fluorimetry.

Applications of HPTLC[20-24]

HPTLC is widely used in

- Pharmaceutical industry
- Biomedical research
- Clinical analysis
- Cosmetic industry
- Environmental analysis
- Food industry.

Typical application

- To analyze the aflotoxins
- Composition of brain gangliosides
- Quantitative detection of prostaglandins in plasma
- Analysis of environmental contaminants
- Analysis of Hg in drinking water
- Analysis of human skin lipids
- Determination of sorbic acid in wine
- Characterization of industrial waste.

Instrumentation of HPTLC shown in Figures 5 and 6.

CONCLUSION

Applications of HPTLC are, they are used in the field of phytochemical analysis, biomedical analysis, clinical analysis, and environmental analysis and also used in the food industry and herbal drug quantification, fingerprint analysis, and HPTLC future, to combinatorial approach, HPTLC-MS, HPTLC-FTIR, and HPTLC-Scanning Diode Laser made HPTLC is a powerful analytical tool in the field of analysis. It is noteworthy that utilization of instrumental HPTLC toward the analysis of drug formulations, bulk drugs, natural products, clinical samples food stuffs, environmental, and other relevant samples will increase in the future. 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 toward the future of HPTLC.

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