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A Systematic Review on High Performance Thin Layer Chromatography (HPTLC)

Komal N. Pawar*, Sandhya P. Kadam , Dr.VivekkumarK.Redasani
YSPM's Yashoda Technical Campus, Faculty of Pharmacy, Wadhe, Satara.

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ABSTRACT
High-Performance Thin Layer Chromatography (HPTLC) is a sophisticated analytical tool that has transformed the science of separation science through its greater sensitivity, resolution, and reproducibility over the conventional Thin Layer Chromatography (TLC). This systematic review delves into the concept, instrumentation, methodological innovation, and vast applications of HPTLC in pharmaceuticals, herbal drugs, food, forensic science, and biotechnology. The review highlights the most important innovations in automation, detection, derivatization, and hyphenation with mass spectrometry and FTIR that have greatly widened the application potential of HPTLC. The review highlights validated methods for quantifying drugs like Piperine in herbal cough syrup and Ondansetron Hydrochloride in tablet formulations, demonstrating the technique's robustness and accuracy in routine pharmaceutical quality control.

I. INTRODUCTION

Chromatography is a separation process that is achieved by distributing the components of a mixture between two phases, a stationary phaseand a mobile phase. Stationary phase: This phase is always composed of a "solid" phase or "a layer of a liquid adsorbed on the surface a solid support". Mobile phase: This phase is always composed of "liquid" or a "gaseous component.[2]Chromatography is probably the most powerful and versatile technique available to the modern analyst. In a single step process it can separate a mixture into its individual components and simultaneously provide anquantitative estimate of each constituent.[1]High-Performance Thin Layer Chromatography, a refinedversion of conventional TLC, has witnessed substantial advancement since its inception in the 1970s. The technique incorporates sophisticated instrumentation and standardized procedures, marking a significant leap from traditional planar chromatography[6]

WHY HPTLC?

HPTLC is the most advanced form of modern TLC [3] It is an advantageous modern analytical technique based on the principles of thin-layer chromatography(TLC).[4]HPTLC allows fast, inexpensive method of analysis in the laboratory as well as in field. It is a valuable tool for reliable identification because it can provide chromatographic fingerprints that can be visualized and stored as electronic images.[8]
It has more resolution and sensitivity and good rep roducibility as compared with Thin-Layer Chromatography.
It finds widespread applications for qualitative and quantitative analysis of the complex mixtures in pharmaceutical, herbal drugs, food, and environmental sciences.
This provides the most advanced tools that are controlled by coordinated software programming which promises improvedutility, reliability, and reproducibility of the information produced.
HPTLC is a flexible screening procedure with which bothqualitative and quantitative analyses can be done.
Instruments can be validated easily the instruments are fully valid with GMP.The HPTLC technique is vastly used in the pharmaceutical industry The HPTLC instruments are computer controlled and can therefore offer improved reproducibility of the analytical result. At the same time, HPTLC is still just as flexible and user-friendly.[9]

Automation of HPTLC

HPTLC is probably the most important and widely used analytical technique for quantitative analysis of organics and biomolecules. HPTLC is one type of planner chromatography and most advanced from of instrumental TLC. Planar chromatography is a mode of chromatography in which the stationary phase is spread on a flat,planar surface. HPTLC is physical separation technique in which a sample dissolved in a liquid is injected into a column

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packed with small particles and it is separated into its constituent components Now a day, HPTLC is more useful than TLC and HPLC. HPTLC is independent of sample application, chromatography development, etc. It is not only instrumental TLC but entire concepts that include widely standardize methodology based on validated method. It is instrument controlled by software . HPTLC is therefore the term for a method that meets all quality requirements of today analytical labs even in a fully regulated environment. Initial costs for an HPTLC system as well as maintenance, and cost per sample still remain comparatively low and all advantages derived from the planar separation principle are certainly maintained. The possibility of visual evaluation of separated samples on the plate is one the most valuable aspects of TLC. It reaches a completely new dimension in HPTLC through the use of modern techniques for generating and evaluating digital images.the use of modern apparatus such as video scanners, densitometers, and new chromatographic chambers, and more effective elution techniques, highresolution sorbents with selected particle size or chemically modified surface, the possibility of combining with other instrumental methods, and development of computer programs for method optimization[10-12]

Principle of HPTLC

HPTLC uses the same physical principles as TLC (adsorption chromatography),i.e., adsorption is the fundamental unit of separation.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.[13-15]

Separation can alsooccur due partition or by both phenomenon,depending on the nature of adsorbents employed on plates andsolvents system used for development.The mobile phase solvent flows through because of capillary action. The components move according to their affinities towards the adsorbent.[7,15,16]

HPTLC vs TLC : A Step Ahead in Chromatography[16-18]

PARAMETER	TLC	HPTLC
Technique	Manual	Instrumental
Plates	Lab made/ Precoated	Precoated
Plate Height	30 µm	12 µm
Stationary Phase	Silica gel, Alumina &Kiesulguh	Wide choice of stationary phases like silica gel for normal phase and C8, C18 for reversed phase modes
Analysis time	20-200 min	1-3 min
Mean Particle size	10-12 µm	5-6 µm
Efficiency	Less	High due to smaller Particle size
Sample Volume	1-10 µl	0.5-5 µl
Application of Sample	Manual	Automatic
Shape of sample	Circular(2-4 nm dia)	Rectangular (6mm L X 1mm W)

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Validation,Quantitative analysis	**No**	**Yes**
Scanning	**Not Possible**	**Use of UV, FluroscenceScanner,Densiometer**
PC Connectivity	**No**	**Yes**
Sample holder	**Capillary/pipette**	**Syringe**

Fig :HPTLC

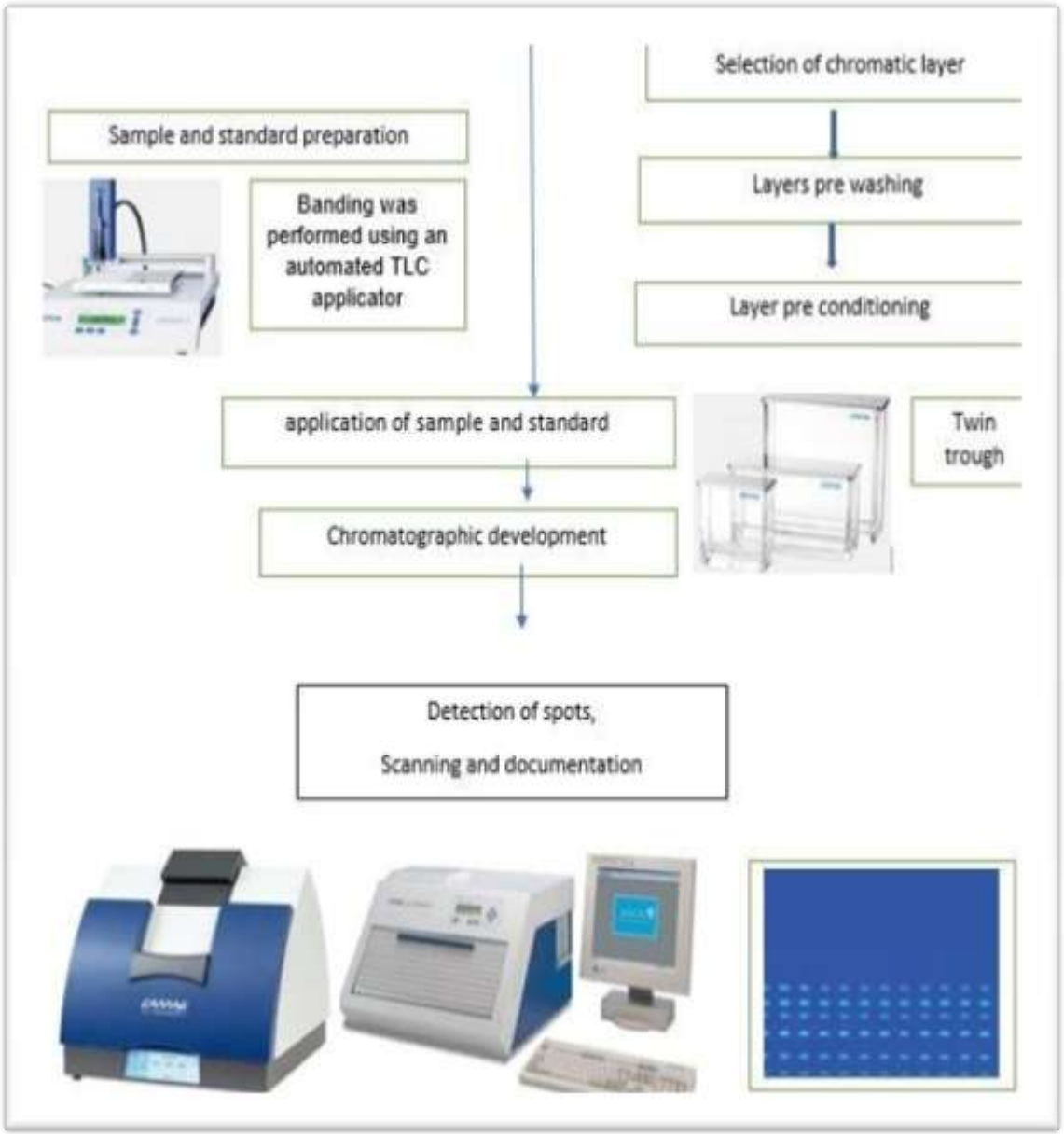

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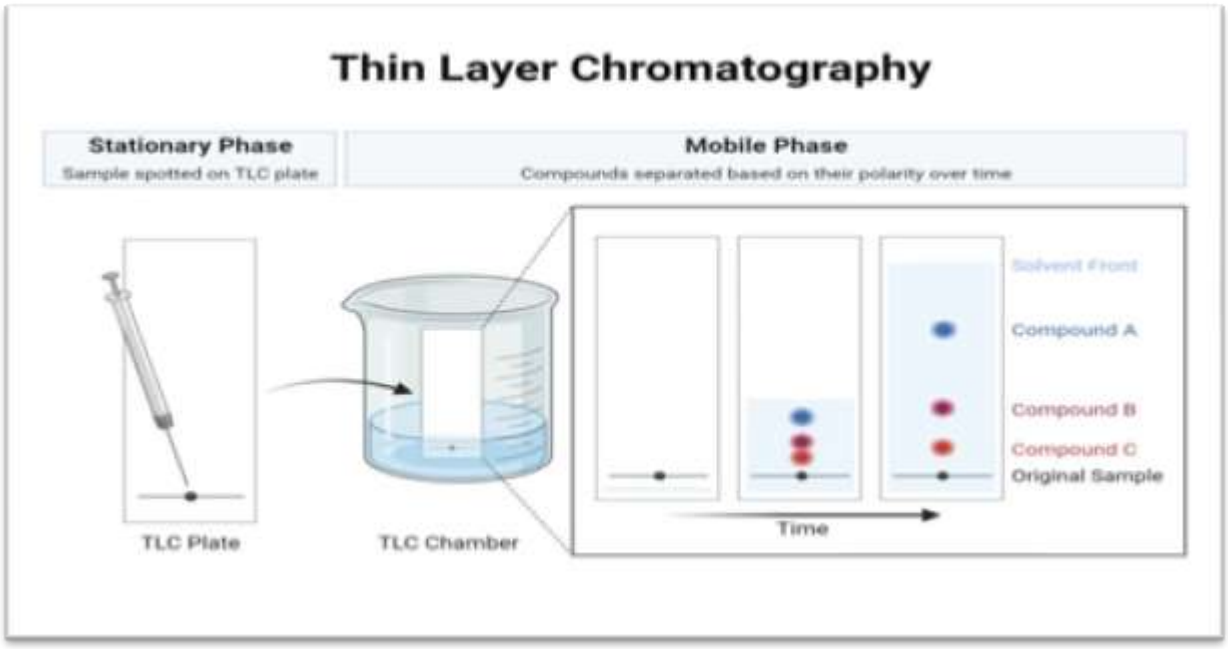
FIG :TLC



Instrumentation

Various steps involved in HPTLCChromatography:

- Selection of HPTLC plates and sorbent.
- Sample preparation including any clean up and pre-Chromatographic derivatization.
- Optimization of mobile phase
- Sample and standard preparation
- Application of sample
- Chromatographic Development (separation)
- Detection including post-chromatographic derivatization

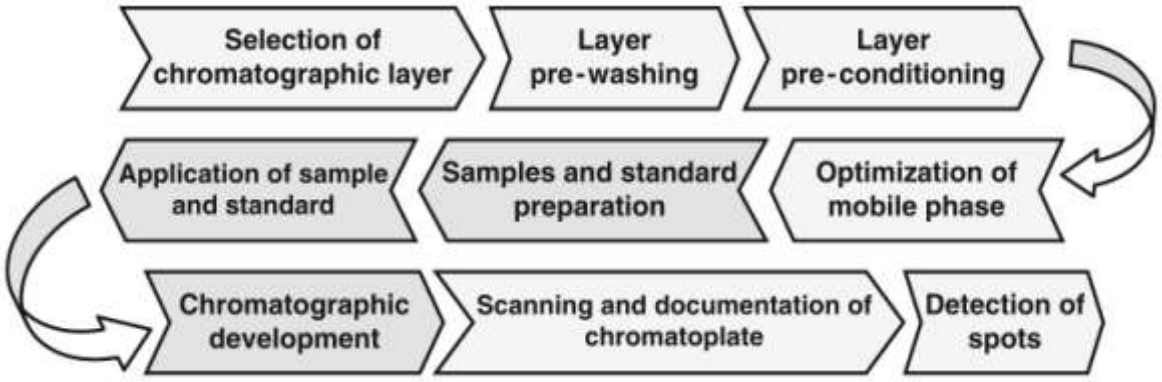


Fig : Schematic procedure for HPTLC method development

Selection of chromatographic layer:[19-27]

Selection of the Stationary Phase:

This phase is always composed of a solid phase or a layer of a liquid absorbed on the surface a solid support.[25]During method development, stationary phase selections should be based on the type of compounds to be separated.[13] It employs HPTLCplates consisting of small particles with a closely distributed size. Consequently, homogenous layers with a smooth surface can be achieved. HPTLC employs smallerplates (10 × 10 or 10 × 20 cm) [23].To separate various pharmaceutical active ingredients, whichapparently have different chemical properties, a number of stationary phases such as silica, alumina, kieselguhr,

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cellulose, gypsum and polyamides are available

Stationary Phases	Type of sample
Silica gel	All classes of compounds
Aluminum oxide	Basic compounds (alkaloids, amines, etc.), steroids, terpenes, aromatic and aliphatic hydrocarbons
Amino phase	Sugars, carboxylic acids, sulfonic acids, phenols, purines, pyrimidines, nucleotides
Cyno phase	All classes of compounds, PHB esters
Silica gel impregnated with silver nitrate	Poly cyclic aromatic hydrocarbons (PAHs), number of diol groups (boric acid), number of isolated double bands
Polyamide	Phenols, flavonoids, nitro compounds
Chiral phase	Enantiomer

Supporting materials used in HPTLC[21]

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.

Polyester 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.

Disadvantage

Not heat resistant (above 120°C).

for HPTLC analysis.[24]

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.

Mobile Phase and Optimization

The selection of mobile phase is based on adsorbent Material used as stationary phase and physical and Chemical properties of analyte.[26, 28]The peaks of interest should be resolved between Rf 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 onThe 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 in HPTLC.

Solvent	Eluent Strength
N -Pentane	0.00
Hexane	0.01
Cyclohexane	0.04
Carbontetrachloride	0.18
Toluene	0.29
Chloroform	0.40
Acetone	0.56
Ethyl Acetate	0.58
Ethanol	0.88
Methanol	0.95

Precise volumetric measurement of the components of the mobile phase should be done separately and accurately in suitable volumetric

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glassware and mixed by shaking to make sure that mixing of the contents is proper. Volumes less than 1 ml are measured by a suitable micropipette. Volumes up to 20ml are measured using a graduated volumetric pipette of appropriate capacity.Volumes more than 20 ml are measured using a suitable graduated cylinder. Toreduce volume mistakes, preparation solvents are made in a volume that is adequate for a day of work.

Sr no	Chemical compoud	Mobile Phase
1	Polar compounds Anthroaglycosides, arbutin, alkaloids, cardiac glycosides, bitter principles, flavonoids, saponin.	Polar compounds: Ethyl acetate:Methanol:Water [100:13.5:10]
2	Lipophiliccompounds Essential oils, terpenes, coumarin, Naphthoquinone, 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	Essential oils	Toluene: ethyl acetate [93:7]
6	Cardiac Glycoside	Ethyl acetate:methanol:Water [100:13.5:10] or [81:11:8]
7	Bitter drugs	Ethyl acetate: methanol: water [77:15:]
	Synthetic drug	
8	Rivaroxaban	toluene: ethylacetate:methanol [6:3:1]
9	Metolazone and Spironolactone	n-propanol: triethylamine (7:3)
10	Paracetamol and Flupirtine Maleate	Toluene: Acetone:Triethylamine(6:4:0.5).

Table :Mobile Phase generally used in detection of some chemical compounds and some synthetic drug[33, 3]

Layer Prewashing[34, 33,21,16]

Generally, methanol is used as a prewashing solvent; however, a mixture of Chloroform: Methanol (1:1), Ethyl acetate: Methanol (1:1), Chloroform: Methanol: Ammonia (90:0:1), Methylene chloride: Methanol (1:1), 1% ammonia or 1% acetic acid or even mobile phase of the method may also be used. Layer washing is done to avoid interference from impurities and water vapours. Itimprove the reproducibility and robustness of the results.

- To prevent, it they are kept in foils.
- Plates are handled at the upper edge to avoidContamination.
- Dry the plates and keep in dust free environment.
- In order to prevent, any potential interference caused by impurities with the chromatographic separation especially in the case of quantitative work, it is always advisable to clean the plates.

There are three ways for cleaning.

- Ascending method
- Dipping method
- Continuous method.

Ascending method:

In this process, pre-coated plates are permitted to run the blank performance with the appropriate solvent (mobile phase).

In the course of this operation, the solvent travels upward. While traveling, solvent takes the impurities to the top of the plate. Additionally, after this operation 10–20% of upper portion is discarded.

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This is the lengthy process, but this is the better one.

Dipping method

In this process, chromatographic plates are immersed in appropriatesolution for designated time.In this process, the initial plate is dipped in Chamber-Athen Chamber-B, here, solvent of Chamber-A is primary washing solvent and solvent ofChamber-B is secondary washing solvent. Then, solvent of chamber-A is drained. The subsequent plate is first dipped in Chamber-B (primary solvent) then Chamber-C(secondary solvent).

Continuous method

In this process, the plates are fed in to the chamber with entrance and exit slits. Solvent is designed to pass continuously through the chamber and that travels with impurities. Plates must dried in oven in vertical position, position in horizontal drops of solvent can fall on plates of condensation. Plates washed should be kept in desiccators.

Activation of Pre-coated Plate[35, 11,18]

Freshly open box of plates do not require activation[11]Plates are exposed to high humidity or kept in hand for long time require activation.[35]
The plates are activated by placing them in oven at
110–120°C for 30 min → Glass plates and aluminium Sheets.
110–120°C for 15 min → Polyester sheets to remove Water.
Activation at high temperature for a long time should be avoided because it may lead to very active layers which cause decomposition of sample.[18]

Sample Preparation and Application[34, 36,37]The diameter of the sample spot or band should not exceed 1 mm. In HPTLC, there are a number of methods for sample spotting.One permits the deposition of minute volumes of samples on the HPTLC plate by a Hamilton micro-syringe. It has been shown that confined, homogeneous bands of defined length subjected to application deliver superior chromatographic results than spot application.

The main distinction between TLC andHPTLC is that the sample application section. In TLC,You'd place the sample manually on a silica plateusing a capillary. An HPTLC auto-sampler,however, can be a precision device thatapplies a known quantity of sample by spraying itwith nitrogen gas. Also, a computer systemattached to the auto-sampler is used to feedinformation regarding the amount of sample to be used and hence the number, width, and location of thebands of the samples can be identified. [34,36]
Sample application position[37]
Distance from left and right side edges – 15 mm.
Distance from bottom edge – 10 mm.
Length of bands – 6 mm.
Center to center distance between bands – 12-15 mm.

Development TechniqueChember
These are the following types of chember

- Flat-bottom chambers
- Twin trough chambers (TTC)
- Horizontal-developmentchambers.

TTC chamber design facilitates development with minimal low volumes of mobile phase and easy pre-equilibrium of the layer with mobile phase vapors or other conditioning liquid. During development, there is a gas phase in addition to stationary and mobile phase that can significantly influence the result of the separation. This gas phase depends on the type, size, and saturation state of the chamber during development. Hard separations may be performed with enhanced resolution by two-dimensional development, wherein the sample mixture is spotted on one corner of the HPTLC plate. Apart from the typical capillary flow development of plates, forced flow over pressured layer chromatography (OPLC), in which the mobile phase is forced mechanically through the layer under a pressure membrane, has been utilized.[38]

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Fig 1 Twin trough chember[39]

Fig 2Horizontal Developing Chember[40]

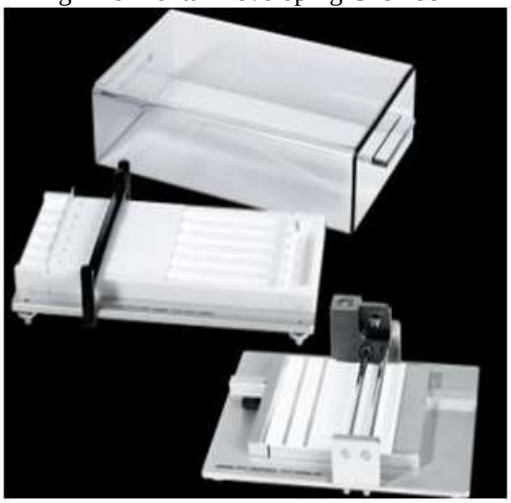

Fig 3Flat bottom chember [41]

rezitavireD dnA ecived margotamorhC

This technique consists of a gas phase alongside stationary and mobile phases.The result of the separation may be heavily influenced by this gas phase.

Processes: Once the bottom of the plate is immersed, the developing solvent will rise up the layer by capillary action until the distance that is needed is achieved, whereupon the chromatography will be halted.Silica gel as a stationary phase andadvancements, which can be describedas adsorption chromatography, are the primaryemphasis of the succeeding discussions. Thereare four types of processes:

There are fourvarious types of procedures that occur.

1. The stationary phase will take in moleculesfrom the gas phase when dry. Thepolar constituents will be pulled outof the gas phase and transferred onto thesurface of the stationary phase duringthisprocess, which is referredto as adsorptivesaturation.
2. The gas phase interacts with the previously wetted part of the layer by the mobile phase. As a consequence, the less polar components of the liquid are released into the gas phase.
3. Unlike (1), adsorption forces instead of vapor pressure govern this process.
4. Secondary fronts can occur during migration if the stationary phase separates the components of the mobile phase under certain conditions.

The Derivatizer comes with an automatedspraying device which applies a new "microdroplet" spraying technology to establish a newbenchmark for uniformity of reagenttransfer onto TLC plates The Derivatizer ensures consistent andrepeatable delivery of all typical reagents. To support the distinct physicochemicalproperties of the different reagents, forexample, acidity or viscosity, four discretecolor-coded spray nozzles are utilized, and the user has six spraying levels to choose from. Apart from much more homogeneous reagent dispersion, the derivatizer has the following benefits over hand spraying:

1. Eco-friendly and safe handling in a closed system
2. Simple handling and cleaning
3. Efficient operation means low reagent consumption (4 mL for 20 x 20 cm plates and 2 mL for 20 x 10 cm plates).
4. Reproducible and user-independent results

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Derivatization is a method that procedurally changes an analyte's character to facilitate easier chromatographic separation. Derivatization is required for certain compounds which do not react to UV light and includes reaction with a particular reagent

Immersing: Immersion device tank is filled with 200 ml of reagent and plate is set in holder of the immersion device, parameters must be adjusted as per method to initiate the process. The plate is taken out of plate holder and dried in cold air stream.

Spraying: Fill the sprayer bottle with a maximum of 50 ml of reagent. Position the plate in the spray cabinet facing a filter paper. Spray the plate with a horizontal and vertical motion until covered uniformly with the reagent. Cold air is used to dry the plate.

Heating may be necessary to induce or enhance the derivatization reaction.[7,16, 17]

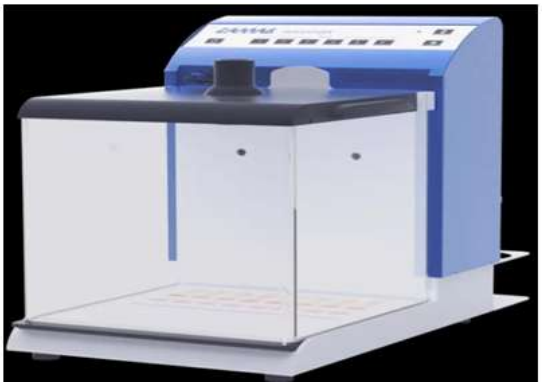

Fig 4 Derivitizer

Detection[13]:-Detection under UV light is first preference due to the fact that it isnon-destructive. regions with fluorescence or quenchfluorescence are observed in cabinets which utilizeShort-wave (254 nm) and long-wave (366 nm)

UV 254 nm Visualization: F254 can be labeled as phosphorescence quenching. For this case, the fluorescence exists briefly after excitation source is withdrawn. It is extremely transient but more than 10 seconds. F254 fluorescent markers are excited with the UV wavelength at 254 nm and emit fluorescence in green color. Chemicals which absorb 254nm radiation decrease this emission on the layer, and a dark violet mark on a green background is seen where the compound zones are present. This quenching is due to compounds containing conjugated double bonds. Anthraglycosides, cumarins, flavonoids, polyphenols in essential oils, and certain alkaloid types like indole, isoquinoline, quinoline alkaloids, etc. must be detected under 254 nm.

Visualization at UV 366 nm: F366 must be termed as fluorescence quenching. In this case, Fluorescence doesn't persist after the excitation source is withdrawn. This quenching is exhibited by all anthraglycosides, coumarins, flavonoids, Phenolcarboxylic acids, and some alcohol alkaloid types (Rauwolfia, Ipecacuanha alkaloids).

Visualization of white light: The zone with separated compounds can be observed by seeing their natural color when observed in daylight (White light).

Water wetting method[21]

In High-Performance Thin Layer Chromatography (HPTLC), the method of water wetting is applied for the identification of lipophilic compounds such as steroids, fatty acids, and hydrocarbons based on water or hydrophilic dye interaction.

Principle

After development of HPTLC plate is sprayed or dipped in water, hydrophilic regions retain the water, thus creating a clear background, and lipophilic compounds are repellent towards water, looking white spots.A hydrophilic dye like methylene blue instead of water. This gives a blue background, while lipophilic compounds are pale (white or light color).

Quantification[7]Quantification refers to the process of determining the amount or concentration of a compound after it has been separated and identified.

Scanning Densitometry

May measure the absorption and/or fluorescence of the compounds with or without derivatization from 200 to 800 nm wavelength.The evaluation of up to 800nm and recording the spectrum of any peak at HPTLC is possible.Tests of biological activity can be done on the HPTLC plate directly.

Image Documentation Using Digital Cameras

The recently introduced design replaces the UV Cabinet with improved style. Now, digitalThe ability to fix digital camera for photographing the plate images was as now, HPTL used in laboratories dealing with herbal analysis for verification of identity of plant extracts by comparison against Botanical

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Reference Materials for BRM extracts to find out whether there are substitutes or adulterants and the studies of formulations etc.Forensic analysts have long ago stated the staring points are microscope for visual examination and physical TLC for chemical examination.

Software-Induced Scanning

They can be employed for quantification in fluorescence and absorbance mode and capture UV–Vis in the absorbance spectrum. Depending on the end-user application, a gradient chamber anda photo documentation and bioluminescence detector can be included or a fully automatic system can be purchased. Hyphenation methods with MS or IR or NMR can beobtained with an appropriate commercially available interface. A recently released deviceinterfaces HPTLC with MS. This interface allows the selected fraction which is removefrom the layer and injects it directly into the MS. The output of the analysis from LC–MS can be considerably enhanced, when summarized to TLC or HPTLC. Any given fraction from the platecan be analyzed. Other fractions available can be disregarded. Optimization of MS parameters for aspecific molecule can be tuned using TLC.

Documentation

Every developed plate is recorded under UV light at 254 nm, UV light at 366 nm, and white light using digital documentation system if a form of light fails to generate usable information, that should be recorded. If a plate is derivatized, photographs are taken before and after derivatization.[8]

Advantage[16]

HPTLC is a modern adaptation of TLC with better and advanced separation efficiency and detection limits.

HPTLC allows a parallel separation and quantitative determination of many samples at the same time

HPTLC is simpler, more flexible, more accessible, and cheaper than other commonly used methods.

Sample clean-up can be avoided or significantly minimized,because the plate is disposable and the chromatographyItself is efficient solid phase purification

Planar chromatography is an optimum tool for national and global standards to maintain analysis affordable Particularly today

It requires less sample like in Nano-gram range.

Disadvantage

Plate length is limited and, hence, separation takes place only up to a certain length

Costly & requires large quantities of expensive Organics.

HPTLC are semi-quantitative for precision and not ideal for trace analysis.

Hyphenated Techniques[36]

The potential for hyphenation of two or more instrumental analysis methods to extract the maximum amount of information possible in a single run is termed hyphenation.The coupling is expected to yield an information-rich detection for identification as well as quantification compared to one analytical method. In the analysis of biological, chemical, and toxicological compounds, hyphenated analysis methods couple chromatographic and spectroscopic or spectrometric technique. The separated components of the mixture from the chromatographic method will then proceed to the spectroscopic method via an interphase.

High-Performance Thin-Layer Chromatography– Mass Spectrometry (HPTLC–MS)

Chromatography is employed in the separation of mixtures of volatile and non-volatile substances. This chromatography is performed on a sheet of aluminium foil, glass, or plastic coated lightly with an adsorbent material, usually silica gel, cellulose, or aluminium oxide. An analytical technique known as mass spectrometry (MS) produces spectra of the masses of the atoms or drug molecules which constitute a sample of material.Chemical compounds are ionised by mass spectrometry to yield charged molecules or molecular fragments. These ions are subsequently separated based on their mass-to-charge ratio by applying an electric or magnetic field or more commonly by accelerating them. Ion fragments with the same mass-to-charge ratio will deflect the same. An electron multiplier, which is an instrument capable of detecting charged particles, is employed to locate the ions. The atoms or molecules in the sample can be recognized by relating known masses to the recognized masses or by means of a distinctive fragmentation pattern.154 Due to the high sensitivity of mass spectrometry, rapid analysis, and ability to characterize structure, direct coupling of HPTLC with MS through TLC/MS Interface can be brought together. The compounds resolved in the

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form of HPTLC/TLC zone on glass plates as well as on aluminium foils are extracted directly from the plate by an appropriate solvent provided by the HPLC pump transferring them into a mass spectrometer for identification or elucidation of structure. HPTLC-MS is an adaptable method that may be utilized for separation as well as identification of pharmaceuticals and phytopharmaceuticals.155

High-PerformanceThin-Layer Chromatography–Fourier Transform Infrared Spectroscopy (HPTLC–FTIR)

High-performance thin-layer chromatography (HPTLC) and spectrometric techniques are being more and more merged. After the separation of samples with HPTLC, it is necessary to use detection methods that enable sample identification and determination.Infrared (IR) is one of the spectroscopic methods that has been integrated with HPTLC for the characterisation and identification of unknown sample. The greatest advantages of IR spectroscopy's are that minimal to no sample preparation is required prior to its use and results can be achieved rapidly without the requirement of reagents.HPTLC and FTIR spectroscopy are employed in the direct hyphenated HPTLC-FTIR method, an online coupling technique of planar chromatography.The direct on-line coupled HPTLC-FTIR has several advantages over previous hyphenated methods (HPTLC-Raman spectroscopy, HPTLC-PA, and HPTLC-MS) such as ease of use and on-line coupling's better operating properties. In modern labs, the HPTLC-FTIR combined method is being widely applied to qualitative and quantitative analysis. This method has revealed its potential in various analytical sciences, such as drug, forensic, food, environmental, and biological analysis, among others. The hyphenated HPTLC-FTIR method will be developed in the future in order to exploit the full potential of this method.

Application[42-46]

HPTLC inPharmaceuticalproducts

HPTLC is also used in analyzing the purity and efficacy of many pharmaceutical preparations and dosage forms. It would be possible to run many current pharmaceutical limit tests with a much higher degree of accuracy and precision if HPTLC methods were used. A HPTLC assay for rifampicin, isoniazid and pyrazinamide in a fixed dose form has been communicated. HPTLC technique was successfully employed for the analysis of samples of fixed-dose tablets of lamivudine, Stavudine, and nevirapine.Two Easy, Accurate, and Exact HPTLC methods have been made for the determination of Mexiletine hydrochloride, an antiarrhythmic drug in Mexicord capsules. The set procedures are in agreement in linearity, accuracy, precision, sensitivity, and specificity. Pharmaceutical Research, biochemistry, and pharmacokinetic studies are only a few of the areas where HPTLC is quickly gaining grip. Pharmaceutical quality control, content uniformity, uniformity test, identity and purity of active ingredients, preservatives in marketed formulations that may be synthetic or herbal, analysis of medicinal plants and herbs, analysis Of pesticide mixtures.

In food analysis:

Foods are usually botanical items, so they are naturally variable and complex. HPTLC can both confirm the identities of complex mixtures and detect adulteration. HPTLC is quick, risk-free, effective, and inexpensive, and it can analyze a lot of samples per day without generating a lot of waste. It is also used for the estimation of isopropylthioxanthone in milkand yogurt, mushrooms, egg yolk lipids.

In forensic analysis:

Forensic research is a multidisciplinary approach to analyzing crime-related materials and Biological samples that employs scientific expertise and advanced instruments. The detection of unknown poisonous substances in lethal intoxication cases is a common yet difficult feature of forensic toxicology. For toxic compounds, HPTLC provides identification, as well as qualitative and quantitative analysis. For example estimation and identification of heroin in forensic samples, new perspectives in the use of ink evidence in forensic science

HPTLC IN NATURAL PRODUCTS

A simple HPTLC method has been developed for the Simultaneous determination of isoorientin, isovitexin, Orientin, and vitexin, both pure and in commercial Samples of bamboo-leaf flavonoids. A fingerprint is a conventional image, such as a photograph, that represents the phytochemical composition of a plant extract or formulation. Fingerprinting may also be used to track batch-to-batch accuracy and stability studies of Herbal medicines, dietary supplements, and other products.

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In biotechnology:
By combining HPTLC with an MS or other appropriate methods such as NMR, FTIR, ESI, and MALDI, one may classify and/or confirm the chemical structures of analytes under investigation during research in biotechnology

Typical application [38, 21]

- 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.
- Determination of Pharmacokinetic studies.

Case study

Quantitativeestimation ofPiperine inherbal cough syrup by HPTLC[47]

Piperine is an alkaloid found naturally in plants belonging to the Piperaceae family, such as Piper nigrum L1, commonly known as black pepper and Piper longum L, commonly known as Long pepper. Piperine is widely used in various herbal cough syrups for its potent anti-tussive and Bronchodilator properties.

Instrument used

- Application mode: CAMAG Linomat IV Sample applicator
- Scanner mode: CAMAG TLC Scanner
- Development mode: CAMAG Twin trough chamber

Chromatographic conditions:

- Stationary phase: Pre-coated Silica gel plate 60 F254 pre-washed with Methanol Mobile phase: Ethyl acetate: Hexane (60:40 v/v)
- Distance between bands: 7mm
- Separation technique: Ascending development
- Scanning mode: Absorbance
- Lamp: Deuterium
- Wavelength: 330nm

Preparation of Standard Solutions:

Stock Solution: 50 mg of piperine dissolved in chloroform and diluted to 50 ml, resulting in 1 mg/ml concentration.

Working Standard: Diluted stock solution to obtain 15 µg/ml concentration.

Preparation of Sample Solution:10 g of cough syrup was extracted with dichloromethane until no alkaloids were detected in the aqueous layer.The pooled extracts were dried using sodium sulfate, filtered, and evaporated.Residue was dissolved in acetonitrile and diluted tomatch standard concentration.The solution was ultrasonicated, centrifuged, filtered by using whatmanfillter paper and used for estimation.

Estimation Method:

Piperine was analyzed via chromatography using LinomatIV.Peak areas were compared to standard for quantification.

II. RESULT

The solvent system of the mobile phase having Ethyl acetate: Hexane (60: 40 v/v) gave dense, Compact and well separated spots of the drug from the mixture at the wavelength of 270nm.The good average recovery values obtained in recovery studies indicate that the proposed method is accurate for estimation of drug in syrup formulation. thus the developed method was found to be accurate, precise, suitable and cost effective for the estimation of piperine in syrup formulation.

Development and Validation of HPTLC Method for Estimation of Ondansetron Hydrochloride in Bulk Drug and Tablet Dosage Form[48]

Ondansetronhydrochlorideis a serotonin 5-HT3 receptor antagonist used mainly as an antiemetic to treatnausea and vomiting, often following chemotherapy. Its effects both peripheral and central nerves by reducingthe activity of the vagus nerve which deactivates thevomiting center in the medulla oblongata and also blocksSerotonin receptors in the chemoreceptor trigger zone.

Estimation

- Mobile Phase: Methanol:Triethylamine:Glacial Acetic Acid (9.5:0.5:0.1 v/v/v)
- Stationary Phase: Silica gel 60 GF254 TLC plates
- Detection Wavelength: 309 nm using densitometric scanning
- Rf Value: 0.77 ± 0.01
- Linearity Range: 300–1100 ng/spot ($R^2 = 0.998$)
- LOD & LOQ: 54.60 ng/spot and 165.46 ng/spot respectively

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III. CONCLUSION

HPTLC is a new, efficient, and reproducible analytical method with high resolution, sensitivity, and reproducibility. It is suitable for pharmaceutical, herbal, food, and forensic analysis due to its capability to analyze multiple samples and compatibility with sophisticated detection systems. As it is increasingly being adopted in regulated environments, HPTLC remains a useful tool in research and quality control.

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