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Application Of Hptlc In Pharmaceutical Analysis

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Abstract:

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) (1). High Performance Thin Layer Chromatography (HPTLC) technique is a sophisticated and automated form of the thin-layer chromatography (TLC) (6). High-performance thin-layer chromatography (HPTLC) is an advantageous, modern analytical technique based on the principles of thin-layer chromatography (TLC) (3). The advantages of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, and so on enable it to be a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, food stuffs, and so on(2). Applications of HPTLC in some other areas are also briefly highlighted.(5)

Keywords:

High Performance Thin Layer Chromatography HPTLC), Thin Layer Chromatography (TLC),Instrumentation, Application(4).

Introduction:

Chromatography is that the group technique used for the separation of a complicated mixture of compounds by their distribution between two phases was invented by Russian botanist Mikhail SemyonovichTswetin 1901, during his research on plant pigment. Other separation method isn't as powerful & applicable as in chromatography. it is the foremost simple, versatile technique which is employed in analytical chemistry and pharmaceutical analysis. HPTLC basically depends upon the capabilities of TLC.As it useful in analysis of qualitative method.(10).HPTLC is an analytical technique based on TLC, but with enhancements intended to increase the resolution of the compounds to be separated and to allow quantitative analysis of the compounds(2).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 (6).

Despite the variety of analytical techniques available for identification, TLC remains important for the pharmaceutical industry.

Many TLC tests for the identification of pharmaceuticals or impurities are included in U.S.

Pharmacopoeia and European Pharmacopoeia monographs.(3). HPTLC offers better resolution and lower Limit of Detection (LODs).The usage of HPTLC is well appreciated and accepted all over the world. Many methods are being established to standardize the assay methods. HPTLC remains one step ahead when compared with other tools of chromatography(2).

The parameter that affect the separation of individual constituent present during a complicated mixture during which it includes: partition, coefficient, retention factor, capacity factor, selectivity of the mobile phase and stationary phase to the solutes and thus the resolution of the individual constituents with a complicated mixture.[1]

- The partition coefficient is that the analytes molarity in stationary phase thereto within the mobile phase.

- Rf, a fundamental qualitative value & is expressed because the ratio of migration distance mix relative to the mobile phase

- Capacity factor k.a fundamental characteristic that determine is qualitative chromatographic behaviour & it show retention time in stationary phase thereto in mobile phase & is influenced by chemical nature of two phases.(10)

Principle:

HPTLC uses the same physical principles as TLC (adsorption chromatography), i.e., adsorption is the fundamental unit of separation. Capillary action causes the solvent from the mobile phase to pass through. According to their affinities with the adsorbent, the components migrate. The component that is more attracted to the stationary phase moves more slowly. The components that have a lower affinity for the immobile phase move more quickly. A chromatographic plate is used to separate the components as a result (4)

Advantages:

- The separation process is easy to follow: especially with coloured compounds
- Several samples can be separated in parallel on the same plate resulting in a high through-put, and a rapid low-cost analysis,
- Two-dimensional separations are easy to perform,
- Specific and sensitive colour reagents can be used to detect separated spots,
- HPTLC can combine and consequently use different modes of evaluation, allowing identification of compounds having different light-absorption characteristics or different colours
- The choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step.
- Ability to analyze crude samples containing multi-components.
- HPTLC method may help to minimize the exposure risk of toxic organic effluents and significantly reduces its disposal problems, consequently, reducing the environmental pollution.(4)

Disadvantages:

1. **Limited Sensitivity**: Compared to other chromatographic techniques like HPLC (High-Performance Liquid Chromatography), HPTLC may have lower sensitivity, making it less suitable for trace analysis.

2. **Sample Size Requirement**: It typically requires larger sample sizes compared to other chromatographic methods, which can be a limitation when sample availability is limited.

3. **Limited Resolution**: While HPTLC offers good separation power, it may not achieve the same level of resolution as some other chromatographic methods, impacting the ability to separate closely related compounds.

4. **Manual Handling**: HPTLC plates often require manual handling, which can introduce variability and errors in the analysis process.

5. **Limited Automation**: Automation options for HPTLC are limited compared to techniques like HPLC, which can hinder efficiency and throughput in high-throughput analysis scenarios.

6. **Quantitative Accuracy**: Achieving accurate quantitative results with HPTLC can be challenging, especially for complex samples, due to factors such as band broadening and variability in application techniques.

7. **Limited Mobile Phase Selection**: The choice of mobile phase in HPTLC is often more limited compared to other chromatographic techniques, which can restrict its applicability to certain types of compounds.(chatGPT)

Comparision between TLC and HPTLC:(4)

S.No	Feature	TLC	HPTLC
1	Technique	Manual	Imstrumental
2	Plates	Lab made	Precoated
3	Plate height	30 micrometer	12 micrometer
4	Stationary phase	Silica gel, alumina& kiesulguhr	Wide choice of stationary phase like silica gel for normal phase and C8, C18 for reversed phase modes.
5	Mean particle size	10-12 micrometer	5-6 micrometer
6	Effiency	Less	High due to smaller particle size
7	Sample holder	Capillary/pipette	Syringe

8	Sample spotting	Manual spotting	Auto sampler
9	Size of sample	Uncontrolled/solvent dependent	Controlled solvent indepedent
10	Shape of sample	Circular(2.4nm dia)	Rectangular(6mm L X 1mmW)
11	Volume range	1-10 microlitre	0.1-500 microlitre
12	Wavelength range	254 or 366 nm, visible	190 or 800 nm, monochromatic

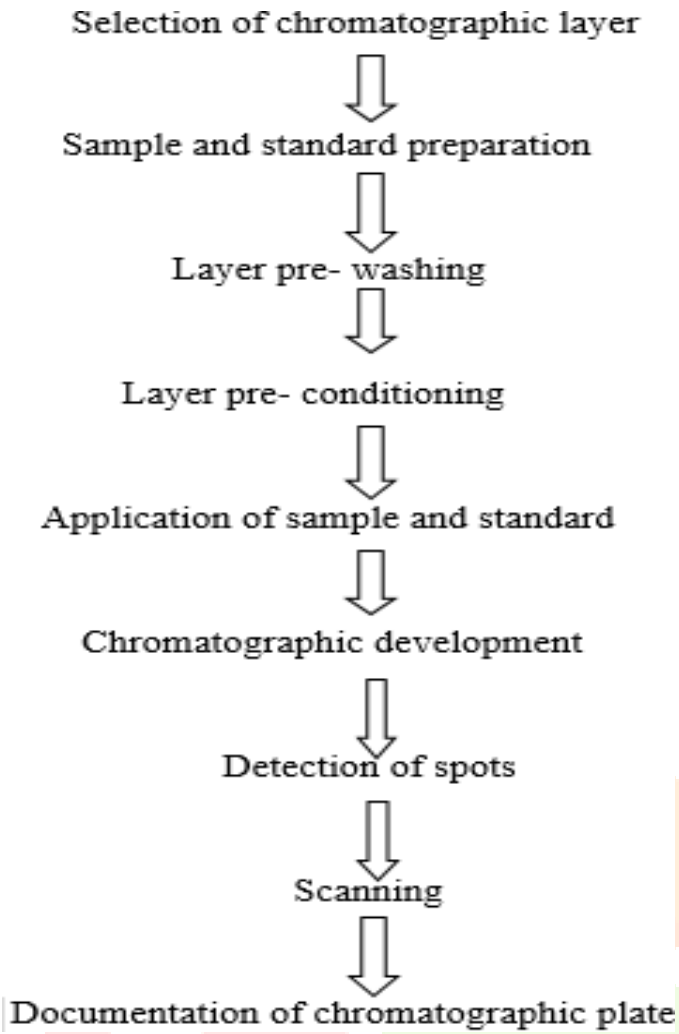


Fig. no. 1 Flow chart of HPTLC (4)

Common Methodology for HPTLC

Selection of the Stationary Phase: During method development, stationary phase selections should be based on the type of compounds to be separated. HPTLC uses smaller plates (10*10 or 10*20 cm) with significantly decreased development distance (typically 6cm) 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 the mobile phase is based on the adsorbent material used as the stationary phase and the physical and chemical properties of the analyte. **Sample Preparation and Application**: A good solvent system 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 0.15and 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 (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 helps predict the order of elution. Pharmaceutical preparation with a 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 an HPTLC plate . It is a fact that the application of the sample is the most critical step to obtaining a good resolution for quantification in HPTLC. 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 [. 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 absorbent layers is enhanced by quenching of fluorescence due to UV light (ranging normally at 200-400 nm). This process is commonly called Fluorescence quenching.

Visualization at UV 254nm: Compounds that absorb radiatiaon at 254nm reduce this emission on the layer, and the dark violet spot on a green background is observed where the compound zones are located [35]. This quenching is caused by compounds with conjugated double bonds.

Visualization at 366 nm : F 366 should be described as fluorescence quenching. In this instance, the fluorescence does not remain after the source of excitation is removed[34]. This quenching is shown by all anthraglycosides, coumarins, flavonoids, Phenolcarboxylic acids, and some alkaloid types (Rauwolfia, Ipecacuanha alkaloids)

Visualization of white light : The zone containing separated compounds can be detected by viewing their natural colour in daylight (White light).

Derivatization: Derivatization can be defined as a procedural technique that primarily modifies an analyte’s functionality to enable chromatographic separations. Derivatization can be performed either by immersing the plates or by spraying the plates with a suitable reagent [38-39]. For better reproducibility, immersion is the preferred derivatization technique.

INSTRUMENTATION:

(i) Auto-Sampler :The primary difference between TLC and HPTLC is that the sample application part. In TLC, you'd manually spot the sample on a silica plate employing a capillary. An HPTLC auto-sampler, however, may be a precision instrument that applies a specified amount of sample by spraying it using nitrogen gas.

(ii) Developing Chamber : an oblong glass chamber is employed for developing HPTLC plates Here, you saturate the chamber for a minimum of 20-30 minutes by pouring a solvent and covering it.

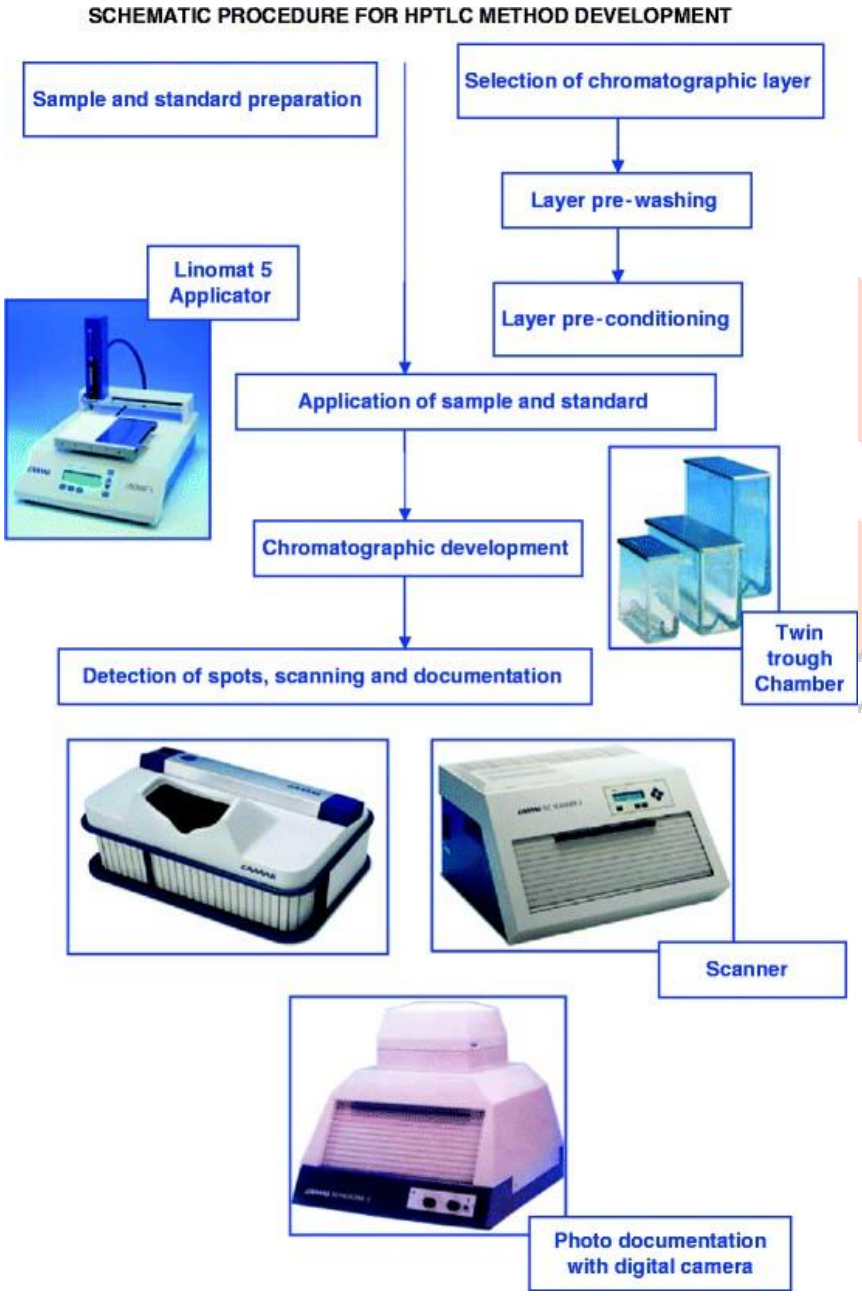
(iii) Chromatogram Immersion Device and Derivatizer : a glass chamber that holds a derivatization agent then performed the derivatization by manually dipping the plate in it for a couple of seconds before pulling it out In contrast, the derivatize is fully automated, where it uniformly sprays the derivatization agent on the HPTLC plate.

(iv) TLC Scanner for Chromatogram Evaluation : The simplest wavelength would show sharper peaks as compared to others. the number of the compound is directly proportional to the height area. Just select the height and you'll obtain data for all the peaks within that Rf value. (10)

(v) Stationary phase: HPTLC plates featuring small particles with a narrow size distribution which lands up in homogenous layers with a smooth surface to be obtained. Lipophilic C-18, C-8, C-2; phenyl chemically-

modified colloid phases; and hydrocarbon impregnated colloid plates developed with a more polar aqueous mobile phase, like methanol–water or dioxane–water, are used for reversed-phase TLC.(10)

(vi) Mobile phase: Mobile Phases [4]The selection of mobile phase is predicated on adsorbent used as stationary phase and physical and chemical properties of analyte. The mobile-phase systems are used (10).



Sr.No	Drug	Dosage form	Technique used
1	Pregabalin and amitryptiline	Pharmaceutical dosage form	Stationary phase: silica gel F254 mobile phase : ethanol: ethyl acetate: acetone: ammonia solution (8:2:1:0.05, by volume) amitriptyline scanned at 220 nm and pregabalin scanned at 550 nm
2	Silymarin and vitamin E	Pharmaceutical dosage form	Stationary Phase: Silica gel 60F254 Mobile Phase :hexane:acetone: formic acid (7:3:0.15, v/v/v) developing system with UV detection at 215 nm.
3	Glibenclamide,rosiglitazone Maleate and metformin hydrochloride	Tablet	Stationary Phase: Pre-coated RP-18 F254s aluminum sheets Mobile Phase: Methanol–tetrahydrofuran–water– glacial acetic acid (16: 3.6: 4: 0.4, v/v) DigiStore 2 Documentation System with winCATS software version 1.4.10 was used for the quantitation and photo documentation
4	Vildagliptin and metformin hydrochloride	Pharmaceutical dosage form	Stationary Phase: silica gel precoated aluminum plate 60 F254 Mobile Phase :hexane: methanol:acetonitrile: glacial acetic acid(2:3:5:2.5:0.2, v/v/v/ v/v)Absorbance at 217 nm
5	Diphenhydramine and naproxen sodium	Tablet	Stationary phase: Silica gel 60 F 254 Mobile Phase : toluene: methanol: glacial acetic acid (7.5:1:0.2 v/v/v) densitometry at 230 nm
6	Voricanazole	Cream formulation	Stationary Phase: Silica gel 60 F254 Mobile Phase : Acetonitrile: water (60:40 % v/v) Quantification was achieved by densitometric at 275 nm

2) HPTLC in fingerprint analysis :

DPPH Fingerprinting of honey Stationary Phase: Silica gel, 60 F 254 in saturated (33%relative humidity), automated developed chamber (ADC2,CAMAG) detected at 366 nm.(4)

Application of HPTLC:

1)HPTLC application in drug analysis: Table provides information on the HPTLC analysis of pharmaceutical dosage in various formulation. High-Performance Thin-Layer Chromatography (HPTLC) is indeed used in fingerprint analysis, especially in forensic science and pharmaceutical analysis. It's a technique that separates mixtures into their individual components for identification and quantification. In fingerprint analysis, it can be employed to detect and analyze various compounds present in fingerprints, aiding in criminal investigations or pharmaceutical quality control.(ChatGPT)

3)HPTLC in Herbal product:

High-Performance Thin-Layer Chromatography (HPTLC) is extensively used in the analysis of herbal products due to its effectiveness in separating and identifying compounds present in complex mixtures.

Authentication and consistent quality are the basic requirement for Indian traditional medicine (TIM), Chinese traditional herbal medicine (TCHM), and their commercial products, regardless of the kind of research conducted to modernize the TIM and TCHM.

Referring too many unknown factors existed in TIM and TCHM, it is impossible and unnecessary to pinpoint qualitatively and quantitatively every single component contained in the herbal drug. Chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to TIM and TCHM.

It can serve as a tool for identification, authentication, and quality control of herbal drugs. In this chapter, attempts are being made to expand the use of HPTLC and at the same time create interest among prospective researcher in herbal analysis.(15).

Its applications in herbal products include:

Active constituents	Herbal plant	Technique used	Uses
Eugenol	Ocimum sanctum	Stationary Phase : Silica gel, 60 F254Mobile Phase : hexane: acetone:ethyl acetate (24:12:8, v/v/v) Detection at 580 nm	Cardiopathy, Blood disorder, Asthma, Skin diseases
Phyllanthin and hypophyllanthin	Phyllanthus	Stationary Phase : Silica gel, 60 F254 Mobile Phase : hexane: acetone:ethyl acetate (24:12:8, v/v/v) Detection at 580 nm	Viral infections, liver disorder, bacterial infections.
Rutin	Amarathusspinosus Linn	Stationary Phase : Silica gel, 60 F 254 Mobile phase : ethyl acetate: formic acid: methanol:water (10:0.9:1.1:1.7 v/v/v/v)	Antidiabetic, antithrombotic, anti-inflammatory, and anticarcinogenic activity

4) HPTLC in quality control of pharmaceuticals :

HPTLC has been used for routine quality control of to primate, dutasteride, nabumetone in pharmaceutical formulations. 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 degrades whether in pure form or in pharmaceutical formulation.

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 quantification (LOQ). HPTLC is also an ideal screening tool for adulterations and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes testing of stability.

HPTLC has been reported for development of quality assurance program.Pharmaceutical formulations including dutasteride, nabumetone, and primates have all undergone routine quality control using HPTLC. For the simultaneous quantitative determination of sulpiride and mebeverine hydrochloride in the presence of their reported impurities and hydrolytic degrades, whether in pure form or pharmaceutical formulation, validated sensitive and highly selective stability-indicating methods were reported. The evaluation and monitoring of the growing, picking, and extraction processes, as well as the testing of stability, are also excellent uses for HPTLC, which is also a great tool for spotting adulterations. HPTLC has been reported for the development of a quality assuarance programme.(13)

5) HPTLC in narcotics :

Forensic analysis is the multi-disciplinary application of scientific knowledge and sophisticated instruments for investigating crime related materials and biological samples. A frequent but challenging aspect of forensic toxicology is the identification of unknown poisonous substances in lethal intoxication cases. HPTLC offers identification as well as qualitative and quantitative analysis for toxic substances, CAMAG HPTLC offers rapid identification of such toxins for antidote administration. HPTLC forensic applications include detection of forged documents by analyzing the ink used and narcotic substances, viscera samples, etc. (11)

6) HPTLC in Bio- marker :

HPTLC as a biomarker in pharmacognostic research : Numerous plants used in Indian medicine have been investigated using high-performance liquid chromatography (HPTLC) for a range of pharmacological qualities, including hepatoprotective and CNS effects. Using the HPTLC method, quercetin from the Micheliachampaca (leaves and stem bark) was found and measured. The estimated values indicate that the plant's leaves are its primary source of quercetin. [32] The HPTLC method is highly reliable and reproducible way to measure the amount of curcumin in commercial turmeric powder.(13)

7) HPTLC in Protein analysis:

Protein analysis using high-performance thin-layer chromatography (HPTLC) is not commonly used but can complement traditional electrophoretic and mass spectrometric approaches in a unique way. Due to various detection protocols and possibilities for hyphenation, HPTLC protein analysis is a promising alternative for e.g., investigating posttranslational modifications. This study exemplarily focused on the investigation of lysozyme, an enzyme which is occurring in eggs and technologically added to foods and beverages such as wine. The detection of lysozyme is mandatory, as it might trigger allergenic reactions in sensitive individuals. To underline the advantages of HPTLC in protein analysis, the development of innovative, highly specific staining protocols leads to improved sensitivity for protein detection on HPTLC plates in comparison to universal protein derivatization reagents. This study aimed at developing a detection

methodology for HPTLC separated proteins using aptamers. Due to their affinity and specificity towards a wide range of targets, an aptamer based staining procedure on HPTLC (HPTLC-aptastaining) will enable manifold analytical possibilities. Besides the proof of its applicability for the very first time, (i) aptamer- based staining of proteins is applicable on different stationary phase materials and (ii) furthermore, it can be used as an approach for a semi-quantitative estimation of protein concentrations.(14).

8) HPTLC in cosmetic analysis:

Sunscreen products are widely used across the globe due to their benefits of preventing skin from tanning and sunburns. The UV filters used in such formulations protect the skin from harmful UV rays by forming a thin, protective layer on the skin thereby blocking or absorbing UV rays from penetrating the skin. The safety of such products is of vital importance since they tend to interact with the biological matrices. In order to shield the consumer health from any harmful effects, HPTLC technique can be employed for the detection of such filters, the identity of which can be further confirmed by mass spectrometer.(12).

Sunscreens like 4-tert-butyl, 4-methoxydibenzoylmethane (BMDM) exhibit poor photostability,whwn exposed to stabilizers sunlight. A rapid, easy screening, and potentially high-throughput high-performance thin-layer chromatographic (HPTLC) method for quantifying the photostability of BMDM in the presence of known /other chemical ingredients. The method is based on spotting the silica gel coated HPTLC plates (in duplicate) with the sunscreen molecules of interest along with a known photostabilizer. This method also involves separation of sunscreens and their photodegraded products on the HPTLC plate. The results obtained with this method were compared well with those obtained with the conventional method.(16).

9) HPTLC in chemical analysis:

High Performance Thin Layer Chromatography (HPTLC) is a valuable tool to check purity, impurity of any non-volatile organic industrial materials such as dyes, surfactants, pesticides, perfumery compounds, intermediates etc. It is far simpler, cheaper and easier to understand than other similar methods of analysis. HPTLC can be used very effectively in reverse engineering because it enables rapid comparison with e.g. standard provided by a buyer or other similar products in the market. Chemical reactions can be studied very quickly e.g. within 2 hours. Complex mixtures like biological samples, reaction mixtures, fermentation broth can be easily chromatographed without much sample preparation.(12).

10) HPTLC in bioanalytical field:

I) Quantification of Suvorexant in Human Urine Using a Validated HPTLC Bioanalytical Method:

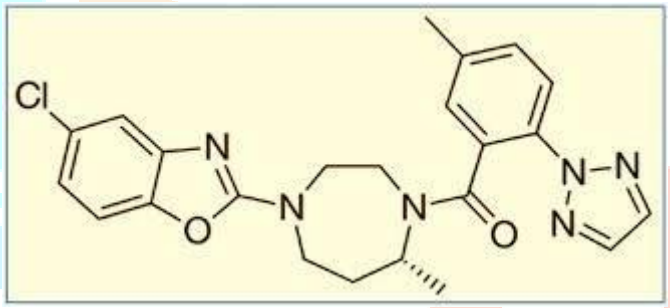
Sedative/hypnotic medicines belong to the central nervoussystem (CNS) class of medicines.1Due to their frequent usage,ability to interact with other CNS depressants to produceadditive e ects, impairment-

causing • e ects, from and misusepotential, these medications are significant a

forensicperspective.2Belsomra (Merck, Rahway, NJ) is the brandname for a relatively new class of sedative/hypnoticmedications known as suvorexant (SUV). Physicochemically, it has been found to be sparingly soluble in water and highlysoluble in organic solvents.

SUV had high oral bioavailability(82%) after oral administration. Various bioanalytical methods have been reported for the measurement and detection of SUV in numerous biologicalsamples such as urine, plasma, and blood.

Due to improvements in the stationary phases and the development of densitometers as detection tools, high- performance thin-layer chromatographic (HPTLC) bioanalytical methods attain precision and accuracy for the assessment of medicines in contrast to LC-based bioanalytical methods. However, HPTLC is only occasionally used toassess medications in biological samples.



Molecular structure of Suvorexant (SUV)

By changing the makeup of the eluent systems, it is possible to vary how much SUV is measured in samples of human urine. The retardation factor (Rf) valuesof SUV and IS using di erent eluent systems are included inTable 1. The findings indicated that when SUV was measured. utilizing various eluent systems such as, acetone and cyclohexane, cyclohexane and ethyl acetate, and ethyl acetate and methanol, the Rf value was shifted toward the upper side(>0.85). An internal standard (IS) is used in a popular bioanalyticalmethod to control the severity of measurement errors whenmeasuring medicines in biological samples, including blood,plasma, and urine.

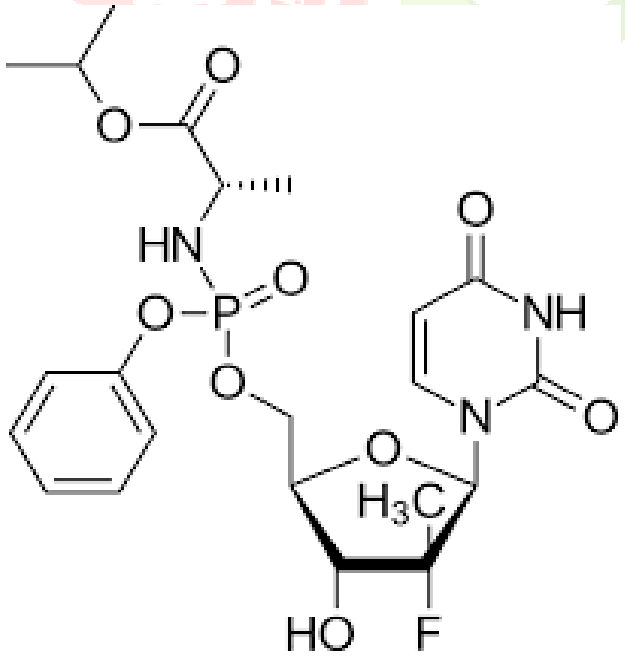
Rf Values of Suvorexant (SUV) and IS Were Recorded Using Different Eluent Systems (Mean ±SD; n=3)

Eluent system	**IS**	**SUV**
acetone/cyclohexane (90:10 v/v)	0.18 ±0.01	0.91 ±0.03
acetone/cyclohexane (97.5:2.5 v/v)	0.17 ±0.01	0.90 ±0.03

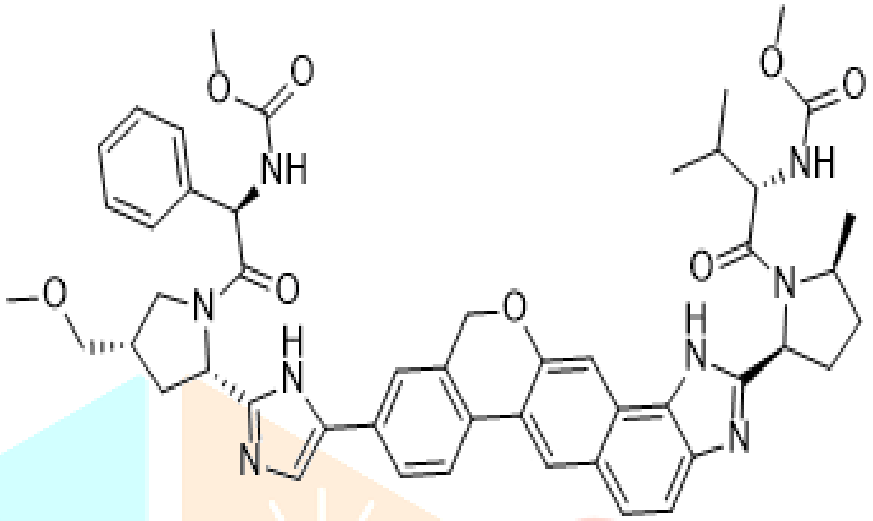
cyclohexane/ethyl acetate (90:10 v/v)	0.15 ±0.01	0.89 ±0.02
cyclohexane/ethyl acetate (97.5:2.5 v/v)	0.14 ±0.01	0.88 ±0.02
ethyl acetate/methanol (90:10 v/v)	0.12 ±0.01	0.87 ±0.02
ethyl acetate/methanol (97.5:2.5 v/v)	0.11 ±0.01	0.86 ±0.02
chloroform/methanol (90:10 v/v)	0.10 ±0.00	0.46 ±0.01
chloroform/methanol (97.5:2.5 v/v)	0.09 ±0.00	0.45 ±0.01

II) A rapid and sensitive HPTLC method for the simultaneous estimation of sofosbuvir and velpatasvir:

A rapid and sensitive high-performance thin-layer chromatography (HPTLC) bioanalytical assay was developed and validated for the simultaneous estimation of sofosbuvir (SBV) and velpatasvir (VLP) in rat plasma. Simultaneous densitometry estimation of SBV and VLP as well as internal standard (IS; cabzantinib) was carried out on glass coated silica gel 60 F254 TLC plates utilizing the combination of chloroform/methanol (90:10, v/v) as the solvent system. The entire analysis was performed at 264 nm.(21)



Molecular structure of sofosbuvir



Molecular structure of velpatasvir

11) HPTLC in Ayurvedic formulations:

I) Green HPTLC-method to estimate trans-Cinnamaldehyde in Ayurvedic formulation Sitopaladi Churna :

Traditional medicine is a preferred home remedy for common cold, cough, allergic conditions, etc. in South East Asia. Ayurveda, originated in India, has a long history of treating upper respiratory disorders**.** Sitopaladi churn (powder) is one of the most sold "over-the-counter" herbal medicine for upper respiratory ailments cure. This study was conducted to estimate and validate for quantitative study of trans- Cinnamaldehyde in Ayurvedic medicine Sitopaladi Churna (SPC) through high-performance thin-layer chromatography (HPTLC) method. The quantitative study was performed at the wavelength 294 (22)

II) HPTLC Qualification of alpha-Glucosidase Inhibitor Mangiferin

Salacia oblonga Wall. and Salacia reticulata Wight. (Family Celastraceae) are commonly known as Saptarangi and used in Ayurvedic medicine as potent antidiabetic agent. Chemical constituent

presents in both species of Salacia are working as α-glucosidase inhibitor for diabetes management. Many chromatography techniques like LC-MS, HPLC are available to quantify mangiferin but no proper data available for simple, rapid, precise, economic HPTLC method. So, HPTLC method was developed with mobile phase ethyl acetate: formic acid: water (4:0.5:0.5 v/v/v) which confirmed the presence of mangiferin at 0.38±0.01 Rf value observed under 254nm. Mangiferin was present in 1.02% in Salacia oblonga root extract, 0.94% in Salacia reticulata stem extract and 0.42% in polyherbal formulation. (20)

12) HPTLC in amino acid analysis:

The separation and identification of amino acids is important because of their increasing industrial, pharmaceutical, toxicological, and pesticidal applications. Almost all separations of amino acids are performed in normal phase (NP) systems. The four most common stationary phases are silica gel, cellulose, impregnated adsorbents, and ion exchangers, although impregnated silica gel, silanized, or octadecyl modified silica gels are used for some separations and metal ions are used as impregnating agents to modify silica surfaces.

Chiral HPTLC plates have been used for the resolution of amino acids (methionine, valine, leucine, serine, and isoleucine) using methanol-water-acetonitrile (1:1:4). They were detected by plate immersion in 0.3% ninhydrin in acetone, followed by heating at 110oC for 10 min. A new topological index for predicting the resolution of D and L amino acids was developed [13]. Chiral plates in combination with acetonitrile-methanol-water were used in the quality control of L-tryptophan as well as for the separation of thyroxine enantiomers [14,15]. Amino acid racemates were separated on impregnated silica gel plates.

13) HPTLC to differentiated among the crude polysaccharides in six traditional Chinese medicines:

A simple, rapid, and effective high-performance thin-layer chromatographic (HPTLC) method has been established for differentiating among the polysaccharides present in six traditional Chinese medicines (TCM), Cordyceps sinensis, Ganoderma lucidum, Astragalus memberanaceus, Panax ginseng, Panax quinquefolii, and Panax notogiseng. Acid hydrolyzates of the polysaccharides were analyzed by HPTLC with two detection reagents, aniline diphenylamine phosphoric acid and ninhydrin, and scanning densitometry.

The compounds were separated on silica gel plates with chloroform-n-butanol-methanol-acetic acid

water 4.5:12.5:5:1.5:1.5 (v/v) as mobile phase. Seven monosaccharides and two glucuronic acids were used as reference compounds. The results showed that hydrolysis of polysaccharides can release specific molecules present in the herbal species in addition to the monosaccharides present. This is useful for distinguishing the origins of the polysaccharides in Chinese medicines.

Separation and detection of polysacccharides by HPTLC

No.	Samples	Hydrolysis	Mobile phase	Stationary phase
1	Polysaccharides from seven species of Cordyceps	TFA (0.5 mol/L)	1-Butanol /isopropanol/acetic acid/water, 7:5:1:2 (v/v/v/v)	Silica gel plates 20×10
2	Galactomannan of the Locoweed (Oxytropis lanata)	TFA (2 mol/L)	p-Propanol /ethanol/water, 7:1:2 (v/v/v)	Chromatographic plates
3	Galactomannan of Caesalpinia pulcherrima	2 N HCL (2 mol/L)	Acetone/ Water, 9:1 (v/v)	HPTLC plates precoated with 200 micrometer layer of Si- gel Si60F254

4	Polysaccharides from G. lucidum and G.sinense	TFA (5 mol/L)	Chloroform/n-butanol/methanol/acetic acid / water, 5.5:11.0:5.0:1.5:2.0(v/v/v/v)	0.2mm Nano-silica gel 60 HPTLC plates
5	Galactomannan from the coral endophytic fungus Aspergillus ochraceus	HCL (1 mol/L)	Triethylamine/ N-butanol/water,0.7:60:30, (v/v/v)	HPTLC Silica gel plate (2cm X 4.5cm)

14) Determination of Antioxidant

The HPTLC analysis, antioxidant, and antigout activity of Asparagus racemosus, Withania somnifera, Vitex negundo, Plumbago zeylanica, Butea monosperma and Tephrosia purpurea extracts were investigated. The chemical fingerprinting were carried out by high performance thin layer chromatography (HPTLC), antioxidant activity by ABTS, DPPH, FRAP radical scavenging assays, and antiogout activity by cow milk xanthine oxidase. The HPTLC fingerprint qualitatively revealed predominant
amount of flavonoids.

These plant root extract will be subjected for further extensive studies to isolate and identify their active constituents which are useful for against inflammation and gout.

The objective of this work was to describe and develop HPTLC analysis (fingerprint and densitometry) for the determination of flavonoids in plant extracts (Asparagus racemosus, Withania somnifera, Vitex negundo, Plumbago zeylanica, Butea monosperma and Tephrosia purpurea), and to determine the validity of plant remedies used for gout by examining their antioxidant and xanthine oxidase inhibitory activity.

Plant name	Stationary phase	Mobile phase	Detector
Antioxidant: Rutin and Luteolin	Silica gel 60F254	Ethylacetate: methanol:formic acid:water(20:2.5:0.5:2)	Linomate IV automated spray

15) Determination of Heavy metals

Inorganic mercury and some organo-mercury species have been separated as dithizonates by HPTLC and determined in situ at the subnanogram level by densitometric automatic scanning of the plate. The extraction efficiency of the dithizonates was tested at pH 1, 7, and 10. The Rf values for HgD, CH3HgD, C2H5HgD, and C6H5HgD are 0.15, 0.32, 0.35, and 0.28 respectively.

The recovery of the tested mercury species in simulated and real samples has been investigated.

Conditioning of the plates with NH3 prior to

elution eliminates interference by many other metal ions. Some examples of real sample analysis (tap water, sea water, human urine) are reported.

16) Others applications of HPTLC :

I. assaying radiochemical impurities of radiopharmaceuticals.

II. HPTLC has been used for routine quality control of topiramate, dutasteride, nabumetone in pharmaceutical formulations.

III. quantitative determination of sulpiride and mebeverine hydrochloride in presence of their reported impurities and hydrolytic degradates whether in pure forms or in pharmaceutical formulation.

IV. 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).

V. 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.

VI. HPTLC analysis of many plants used in Indian Systems of Medicine has been performed for various pharmacological activities like CNS, hepatoproctative etc.

VII. 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.

VIII. HPTLC method for the estimation of curcumin in marketed turmeric powder can be used routinely with good reliability and reproducibility.(6)

CONCLUSION:

This review reveals that most of the HPTLC methods developed and validated comply with the general procedures pertaining to the quantitative mode of this technique.The HPTLC technique is also extremely helpful for routine analyses of pharmaceutical and clinical data, analyses of traditional medicines and medicinal plants, analyses of foods and dietary supplements, analyses of environmental factors, analyses of cosmetics and toxicology, analyses of plants and herbs, and analyses of food and food supplements. With the multiple benefits listed above over other chromatographic techniques. In addition, it is used for semi- quantitative comparison to provide quantitative results. HPTLC use for screening pharmaceutical compounds for the antimicrobial Factivities is emerging. In marine invertebrates, HPTLC has been utilized to separate new promising pharmaceutical therapeutants which could be used in pharmaceutical industries. The use of HPTLC to check pharmaceutical compounds for antibacterial activity becoming more common. Manufacturing (bulk drug manufacturing units, process monitoring, fermentation broth analysis, residue analysis, and in-process materials testing); quality control (raw material assays, multi-component formulations, uniformity of content testing, impurity profiling, and method validations); formulation analyses; stability, sustained release; and bioavailability studies are among the pharmaceutical testing applications of HPTLC has been reviewed.

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