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OVERVIEW ON HPTLC: HIGHLY SOFISTICATED ANALYTICAL INSTRUMENT FOR QUALITATIVE AND QUANTITATIVE ANALYSIS

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ABSTRACT

Planar chromatography includes High Performance Thin Layer Chromatography (HPTLC), the most sophisticated version of instrumental Thin Layer Chromatography (TLC). One of the most effective analytical techniques available today, HPTLC is equally effective for qualitative and quantitative applications. HPTLC is more practical than both TLC and High performance Liquid Chromatography (HPLC). HPTLC is not only instrumental TLC but an entire concept that includes widely defined technique based on established approach. This is because HPTLC is independent of sample application, chromatogram generation, detection, etc. It is a software-controlled gadget. In this review post, we covered its basics, the instrument types utilized in HPTLC, the full HPTLC methodology, applications, and how HPTLC is superior to TLC.

KEYWORDS: HPTLC, analytical techniques, Chromatogram, HPLC, TLC.

1. INTRODUCTION

High Performance Thin Layer Chromatography (HPTLC) is a sophisticated and automated variant of thin-layer chromatography (TLC) with improved and advanced separation efficiency and detection limits.[1] It is sometimes referred to as Flat-bed chromatography, High Pressure Thin Layer Chromatography, or Planar Chromatography. HPTLC is currently ready to become one of the best technologies for ensuring product quality, purity, stability, and identification, or, to put it another way, validating the botanical product's complex composition.[2,3] Due to new, specialized equipment, it is able to carry out high-quality HPTLC analytical determinations and so ultimately attain the required dependability, reproducibility, and flexibility. Flexibility and sophistication are HPTLC's two primary

characteristics. A chromatographic fingerprint, or specific sequence of peaks that fully represent the studied sample, can be produced by HPTLC. The goal is to use a computerized digital data bank to create resultant fingerprints that can be compared with regard to the quantity, order, location (Rf), and color of the separated zones, as well as in reference to a data bank. The NMR fingerprint of the entire extract or individual sections can be used to acquire analogous and complimentary data due to straightforward processing. The fingerprint method is excellent for analyzing complicated mixtures.

An effective tool for quick comparison of similar products is HPTLC. HPTLC is a concept that includes both the use of established methodologies for qualitative and quantitative analysis and a widely defined methodology founded on scientific facts. To improve resolution and permit more precise quantitative measurements, HPTLC satisfies all quality standards for today's analytical labs.[4]

1.1 Advantages of hptlc[5,6]

- Practically, it is straightforward to understand and use.
- The system is being worked on by multiple analysts at once.
- Shorter analysis times and cheaper analysis prices.
- Low cost of maintenance.
- Visual detection is possible because the system is open.
- A wide selection of stationary phases with distinctive selectivity for mixed components are readily available.
- Sensitivity limits of analysis are typically at nanogram (ng) to pictogram (pg) levels.
- Accuracy and precision of quantification is high.
- The HPTLC approach may lower the danger of exposure to harmful organic effluents and greatly lessen the difficulties associated with disposing of them, hence reducing environmental pollution.

1.2 Regulatory acceptance of hptlc

- USP chapter 203, official from 2018 “HPTLC Methodology and fingerprint identification of materials of botanical origin”.
- USP chapter 621: Scanning Densitometry for Quantification.
- USP chapter 202: identification of fixed oils.
- USP chapter 2251: synthetic API in herbals (HPTLC-MS)
- USP 1064: Parameter selection explanation

2. Principle

It is an effective analytical approach that may be used for both qualitative and quantitative tasks. Depending on the type of the adsorbents utilized on the plates and the solvent system used for development, separation may be caused by partition, adsorption, or both phenomena.[7] Due to capillary action, the mobile phase solvent passes through. The components move in accordance with their propensities to interact with the adsorbent. The part that has a stronger affinity for the immobile phase moves more slowly. The component that moves more quickly has a lower attraction for the stationary phase. On a chromatographic plate, the components are so separated.

The schematic diagram of HPTLC is shown in the "**Fig. 1"**

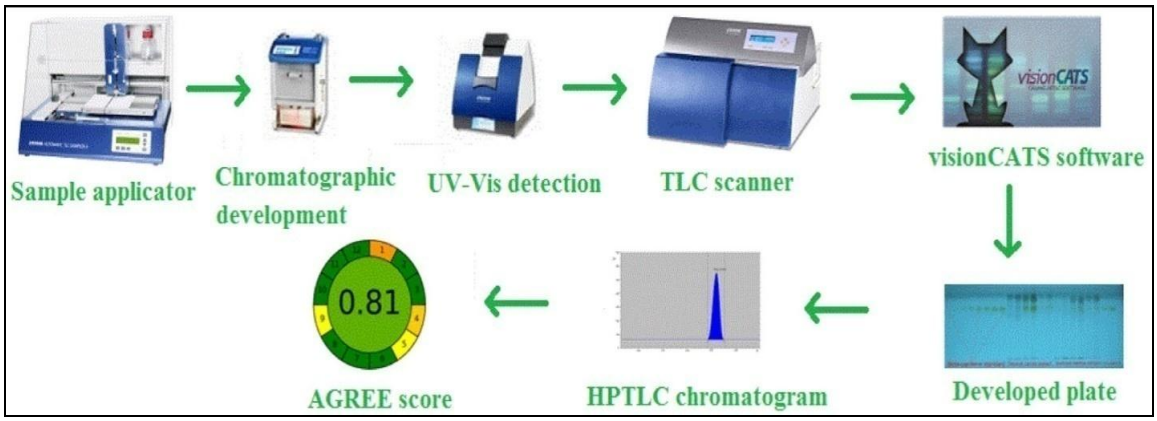

Fig. 1: Workflow of HPTLC instrumentation.

3. Methodology

Before starting HPTLC analysis of drugs in multicomponent dosage forms, it is important to set an analytical objective. This may be the determination of a specific parameter, such as concentration, or the qualitative identification of components or mixtures. Another option would be the optimization of analysis time by separating two component/mixture types. Method development usually involves many trial and error procedures, with the most difficult problem typically being determining where to start with mobile phase selection.[8]

3.1 Stationary phase

HPTLC is the most advanced form of modern TLC. It makes use of HPTLC plates that contain tiny particles with a specified size distribution to produce homogeneous layers with a smooth surface. HPTLC uses smaller plates (10X10 or 10X20 cm), and its development distance (usually 6 cm) and analysis time are greatly reduced (7–20 min).[9] HPTLC plates

are utilized for industrial pharmaceutical densitometric quantitative analysis because they offer better resolution, greater detection sensitivity, and superior in situ quantification.[10,11] Following are stationary phases that are mostly used:[4]

Silica gel GF - with a less polar mobile phase, such as chloroform– methanol

Pre-coated plates - different support materials - different Sorbents available like;

- Aluminum oxide - Basic substances, alkaloids and steroids
- Cellulose - Amino acids, dipeptides, sugars and alkaloids
- RP2, RP8 and RP18- Chemically bonded octa decyl silane (ODS), an n-alkane with 18 carbon atoms, is the most frequently used stationary phase. C8 and shorter alkyl chains provide other alternatives. These are used for separation of non-polar substances like fatty acids, carotenoids, cholesterol, etc.

3.2 Mobile phase

The adsorbent substance utilized as the stationary phase, along with the physical and chemical characteristics of the analyte are used to select the mobile phase.[12] Diethyl ether, methylene chloride, and chloroform are common mobile-phase systems that are employed based on their various selectivity properties as the strength-adjusting solvents for normal-phase TLC and methanol, acetonitrile, and tetrahydrofuran mixed with water for strength adjustment in reversed-phase TLC. "**Table no.1"** show the various mobile phase used in HPTLC method development.[13]

Table no. 1: Examples of various mobile phases used in HPTLC.

Sr no.	Identified compounds	Mobile phase
1	Alkaloids	Toluene: Ethyl Acetate: Diethyl Amine [70:20:10]
2	Lipophilic Compounds	Toluene: Ethyl Acetate [93:7]
3	Flavonoids	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water [100:11:11:26]
4	Saponin	Chloroform: Glacial Acetic Acid: Methanol: Water [64:32:12:8]
5	Coumarin	Diethyl Ether: Toluene [1:1] Saturated with 10% Acetic Acid
6	Essential Oil	Toluene: Ethyl Acetate [93:7]
7	Cardiac Glycosides	Ethyl Acetate: Methanol: Water [100:13.5:10] OR [81:11:8]
8	Terpenes	Chloroform: Methanol: Water [65:25:4]
9	Triterpenes	Ethyl Acetate: Toluene: Formic Acid [50:50:15]

3.3 Activation of pre-coated plates

- A newly opened box of plates doesn't need to be activated.
- Place plates that have been in contact with high levels of humidity or that have been on hand for a long period in an oven set to 110 to 120 °C for 30 minutes before spotting.
- Aluminum sheets should be placed between two glass plates and heated for 15 minutes at 110–120°C[1]

3.4 Preparation of Sample and Its application on plate

The preparation of sample is done by dissolving the particular sample in suitable solvent. Methanol is most communally used solvent because most of the substances are soluble in methanol. Methanol is also known as universal solvent.

Take a 100 mg sample and dissolve in 1 ml or 10 ml of solvent (Methanol). Sonicate the mixture of better mixing/ enhance the solubility. After that, centrifuge the sample solution at 5000 RPM. At the last step, use the supernant of analysis.

The most critical step to obtain good resolution for quantification in HPTLC is sample application.Because concentrations over this range lead to poor resolution in samples; the typical concentration range for samples is 0.1–1 ug/ml.[14] there are two methods for sample application. A) automatic technique B) manually technique (spot technique). Now days automatic technique is most widely used in pharmaceutical industry.There are automated tools for applying samples; particularly for quantitative HPTLC, use the Automatic TLC sampler (ATS) 4 or Linomat 5 to apply samples using the spray-on method. Typically, a syringe holding the sample is evacuated by a motor. Electronic controls are used to manage delivery volume and speed.[12]

3.5 Chamber saturation

Generally, saturated twin-trough chambers are utilized for the optimum repeatability when developing HPTLC plates in chambers (flat-bottom chambers, twin-trough chambers, or horizontal-development chambers). The procedure for chamber saturation is as follows: Filter paper lines the sides of the development chamber on three sides. A suitable amount of mobile phase is prepared, and it is gently poured into the chamber to thoroughly wet the filter paper and cause it to stick to the chamber's back wall. To level the solvent volume in both troughs, tip the chamber to the side (by roughly 45 degrees). After replacing the lid, give the chamber 20 minutes to re-adjust.

3.6 Chromatogram development

Even though the chromatogram development stage is the most significant one in the HPTLC process, crucial parameters are frequently missed.[14] On the right edge of the plate, a pencil is used to mark the desired development distance, which is 70% from the lower edge of the plate.Plates are taken out of the chamber after development and dried for 5 minutes in a stream of cold air while oriented vertically towards the chromatograph.[12]

3.7 Detection

Under either white or ultraviolet light, the chromatogram is examined. Options include scanning densitometry for quantitative measurements or visual assessment of electronic images.Using a chromatogram spectrophotometer (densitometer or scanner) with a fixed sample light beam in the shape of a rectangular slit that measures the zones of samples and standards, quantitative analysis is carried out on-site. The chromatogram is often scanned by reflectance, transmittance, absorbance, or fluorescence mode utilizing TLC Scanner 3 and win CATS software. You can choose a scanning speed up to 100 mm/s. Spectrum recording happens quickly. Calculating the concentration of analyte in the sample involves taking into account both the initial sample and dilution effects.

Fluorescence quenching might be a better way to characterizevisualization at UV 366 nm. When the source of excitation is withdrawn in this case, the fluorescence disappears. All anthraglycosides, coumarins, flavonoids, phenol carboxylic acids, and several alkaloid types exhibit this quenching (Rauwolfia, Ipecacuanha alkaloids).

3.8 Derivatization

Derivatization is a procedure that mainly alters an analyte's functionality to allow for chromatographic separations. Derivatization can be carried out by either immersing the plates in the solution or by spraying the plates with the appropriate reagent. Immersion is the recommended derivatization method for greater reproducibility.[15,16]

3.9 Quantification

The zones of the samples and standards are measured using a chromatogram spectrophotometer (Densitometer or scanner) with a fixed sample light beam in the shape of a rectangular slit.

4. Applications

The HPTLC method is used in both qualitative and quantitative analytical applications, including those for nutraceuticals, herbal and dietary supplements, and many types of drugs. Applications for HPTLC in food and feed products include quality control, additives (such vitamins), pesticides, stability tests (expiration), and more.[17]

4.1 Compositional assessment

Due to its simplicity, adaptability for quick and simultaneous analysis of several samples, and cheaper equipment cost than normal HPLC, high performance thin-layer chromatography (HPTLC) has remained an important and popular approach for the analysis of carbohydrates. Silica gel has traditionally been the main stationary phase for the separation of carbohydrates. Silica gel plates have frequently been reported to be pre-treated with suitable buffers, such as phosphate or borate, in order to improve resolution of sugar analysis. Meanwhile, acetonitrile: water (85:15 v/v) is frequently the first choice as a developing solvent, and in order to improve resolution, it is frequently modified by addition of organic solvents or acids.[18]

Analyzing the product's quality, stability, and provenance requires the fingerprinting of amino acids (both free and protein-bound amino acids). Historically, fuorescamine or ninhydrin have been used as staining agents to detect and quantify proteins on HPTLC plates.[19]

4.2 HPTLC in quality control of pharmaceuticals

HPTLC has been used for routine quality control of topiramate, dutasteride, nabumetone in pharmaceutical formulations. For the examination of ropinirole HCl, a stability-indicating HPTLC technique was created and validated for precision, accuracy, toughness, robustness, specificity, recovery, limit of detection (LOD), and limit of quantitation (LOQ).. HPTLC has been reported for development of a quality assurance program.[20]

4.3 Herbal Medicines and Botanical dietary supplements

Numerous scientists have utilized HPTLC to separate, identify, and quantify bioactive components.The identification of the several plant components is the most common use of HPTLC in botany. Testing the stability of pharmaceuticals and herbal dietary supplements is another goal of HPTLC.

4.4 Forensics

High performance thin layer chromatography is used for detecting document forgery, investigating poisoning, and analyzing dyestuffs, among other things.[21]

5. CONCLUSION

Due to the recent achievement of high automatization, the application of HPTLC can be seen as an effective instrument in the investigation of complicated mixes of natural goods, such as those now available on the market. Nonetheless, there are certain areas that need to be improved, such as the instrument cost and sensibility, which are still not on par with HPLC.The approaches might be used to various foods and food products for compositional evaluation, quality control, source determination, and so on.HPTLC is an ideal tool for identification of herbalmaterials The HPTLC method has been proposed for fast food labelling control in food inspection or for low-cost quality control in food production and drug quality control tests.

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