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An Overview on High Performance Thin layer Chromatography

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Abstract - HPTLC method includes selection of stationary phases, mobile phases, detection methods, and optimization of experimental conditions to achieve high resolution, sensitivity, and reproducibility. HPTLC is one type of planner chromatography and most advanced form of instrumental TLC. HPTLC operates on the same separation by adsorption concept as TLC. HPTLC offer wide choice of stationary phase Like Silica gel for normal phase and C8, C18 for reversed phase modes where sample can be detectable in nanograms with increased precision and sensitivity. HPTLC is a great instrument for detecting adulterations and is well suited for checking stability as well as assessing and tracking the processes of cultivation, harvesting, and extraction. Furthermore, the article explores the wide range of applications of HPTLC in pharmaceutical, environmental, food, and forensic analysis by integration of HPTLC with other analytical techniques, such as mass spectrometry (MS) and spectrophotometry, to enhance its capabilities. This review aims to serve as a valuable resource for researchers and analytical chemists involved in the development and optimization of HPTLC methods for complex matrix.

Keywords: HPTLC, Resolution, Advantage, Pharmaceutical

INTRODUCTION:

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate.

The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation .

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction . Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analyses in a mixture. The two are not mutually exclusive.[1]

Principle of Chromatography:

Chromatography is based on the principle where molecules in mixture applied onto the surface or into the solid, and fluid stationary phase (stable phase) is separating from each other while moving with the aid of a mobile phase. The factors effective on this separation process include molecular characteristics related to adsorption (liquid-solid), partition (liquidsolid), and affinity or differences among their molecular weights. Because of these differences, some components of the mixture stay longer in the stationary phase, and they move slowly in the chromatography system, while others pass rapidly into mobile phase, and leave the system faster. [2]

Three components thus form the basis of the chromatography technique.

- 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".
- Separated molecules.

The type of interaction between the stationary phase, mobile phase, and substances contained in the mixture is the basic component effective on separation of molecules from each other.

Principle of HPTLC:

HPTLC High-Performance Thin Layer Chromatography operates on the fundamental principle of differential migration of compounds between a stationary phase and a mobile phase. The separation occurs on a flat surface of modified sorbent material, typically silica gel, with precisely controlled particle size and pore dimensions. The enhanced resolution in HPTLC compared to conventional TLC stems from the use of finer particle sizes (5-7 µm) and more uniform layer thickness (100-200 µm).[17]

The migration of analyses follows complex physicochemical interactions, including adsorption, partition, and capillary action, contributing to the separation efficiency.[3]

Advantage of HPTLC:

- The separation process is easy to follow, especially with colored compounds.
- Ability to analyze crude samples containing multi-components.
- Two-dimensional separations are easy to perform. Stability during chromatography should be tested using two-dimensional development.
- Several samples can be separated parallel to each other on the same plate resulting in high output, time-saving, and rapid low-cost analysis.
- Contact detection allows radiolabelled compounds to be monitored and microbial activity in spots to be assessed.[15]
- HPTLC can combine and consequently be used for different modes of evaluation, allowing the identification of compounds having different light-absorption characteristics or different colors.
- Specific and sensitive color reagents can be used to detect separated spots (Dragendroff reagent/Kedde reagent).[16]
- 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]

Instrumentation of 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 0.15 and 0.85. The elution power of the mobile phase depends on a property called eluent strength which is related to the polarity of the mobile phase components. The more nonpolar the compound, the faster it will elute (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).

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.[5]

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 254 nm: F254 should be described as phosphorescence quenching. In this instance, the fluorescence remains for a short period after the source of excitation is removed. It is very short-lived, but longer than 10 seconds. F254 fluorescent indicators are excited with UV wavelength at 254 nm and emit green fluorescence . Compounds that absorb radiation at 254nm reduce this emission on the layer, and a dark violet spot on a green background is observed where the compound zones are located . This quenching is caused by

compounds with conjugated double bonds. Anthraglycosides, coumarins, flavonoids, polyphenols in essential oils, and some alkaloid types such as indole, isoquinoline, quinoline alkaloids, etc. should be detected under 254 nm.[6]

Visualization of white light: The zone containing separated compounds can be detected by viewing their natural color in daylight (White light).[14]

Applications of HPTLC:

Many qualitative and quantitative methodological applications, such as those for herbal and dietary supplements, nutraceuticals, and a range of medications, utilize the HPTLC technique. Forensic applications include toxicity tests, assaying radio chemical contaminants in radio medicines, and detecting and identifying prescription raw ingredients, products, and their metabolites in biological mediums. Scientific usage includes metabolism tests and drug screening. Many lipids have also been examined and investigated using HPTLC; distinct lipid sub-classes were divided with repeatable and encouraging results. HPTLC in quality control of pharmaceuticals: Pharmaceutical formulations including dutasteride, nabumetone, and primates have all undergone routine quality control using HPTLC. [7]

I. 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 stabilityindicating methods were reported.

II. Developed and validated for precision, accuracy, toughness, robustness, specificity, recovery, the limit of detection (LOD), and the limit of quantification was a stability-indicating HPTLC method for the measurement of ropinirole HCL. [13]

III. 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.[12]

IV. HPTLC has been reported for the development of a quality assurance program. [44]

V. HPTLC as a biomarker in pharmacognostic research: Many plants utilized in Indian medical systems have undergone HPTLC investigation for a variety of pharmacological properties like CNS, hepatoprotective, etc.[8]

VI. The Micheliachampaca (leaves and stembark) quercetin was detected and quantified using the HPTLC method, and the estimated values show that the leaves constitute the plant's highest source of quercetin.[9]

VII. HPTLC method can be used regularly to estimate the amount of curcumin in commercial turmeric powder.

VIII. HPTLC in herbal products: provide information on the HPTLC analysis of herbal items.

IX. HPTLC in fingerprinting analysis: The details regarding HPTLC determination of fingerprinting analysis.[10]

X. HPTLC offers advantages in analyzing complex formulations, particularly herbal medications, where multiple active compounds need to be identified and quantified

XI. HPTLC complements these analyses by providing rapid visual detection of impurities and degradation products through specific derivatization techniques.[18]

CONCLUSION:

In conclusion, High-Performance Thin Layer Chromatography (HPTLC) is a powerful and versatile analytical technique widely used for the qualitative and quantitative analysis of various substances. Method development involves optimizing parameters like stationary phase, mobile phase, and detection methods to achieve accurate, reproducible results. HPTLC offers significant advantages, including cost-effectiveness, simplicity, and the ability to handle multiple samples simultaneously. Its applications span across pharmaceuticals, environmental monitoring, and food safety. Overall, HPTLC remains a valuable tool in analytical chemistry, with continued advancements enhancing its effectiveness and broadening its applications.

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