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HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY AND IT'S ROLE IN PHARMACEUTICAL INDUSTRY

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ABSTRACT

High-Performance Thin-Layer Chromatography (HPTLC) is an advanced version of TLC, sharing the principle of thin separation through adsorption, but with added involvement of a gas phase alongside the stationary and mobile phases. Referred to as planar or flat-bed chromatography, HPTLC is a potent analytical method suitable for qualitative and quantitative tasks. The technique employs various chromatographic plates with support materials such as glass, polyethylene, aluminum, and sorbents like silica gel 60F, aluminum oxide, and cellulose. Several factors influence HPTLC, including the type of stationary phase, mobile phase, temperature, and sample quantity. HPTLC offers numerous benefits, including improved sample application, faster and better separation, and reduced mobile phase usage, making it a valuable alternative to Gas Chromatography (GC) and High-Performance Liquid Chromatography (HPLC). Its applications span across phytochemical and biomedical analysis, active ingredient quantification, and formulation fingerprinting to check for adulterations. Moreover, it proves useful in detecting chemicals of forensic concern. The technique has witnessed remarkable advancements like hyphenations In HPTLC-MS (Mass Spectrometry), HPTLC-FTIR (Fourier Transform Infrared Spectroscopy), and HPTLC Scanning Diode Laser, turning it into a powerful analytical tool. In summary, HPTLC stands as an efficient and versatile chromatographic approach in various scientific fields.

INTRODUCTION

High Performance Thin Layer Chromatography (HPTLC) is a sophisticated and automated form of the thin-layer chromatography (TLC) with better and advanced separation efficiency and detection limits. 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, phenomenon's depending upon the nature of adsorbents used on plates and solvents system used for development. Different aspects on HPTLC fundamentals: principle, theory, understanding; instrumentation: implementation, optimization, validation, automation and qualitative and quantitative analysis; applications: phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis and potential for hyphenation (HPTLC-MS, HPTLCFTIR and HPTLC-Scanning Diode Laser) have been reported .

Advantages/Disadvantages of HPTLC over TLC: Most recently HPTLC is used as alternative to classical TLC and it is a

valuable tool for reliable identification. It is instrument controlled by software. HPTLC, which is used for impurity determination, relies more on plain but the most potent silica gel hydrophilic phase meeting the criteria of most of the pharmacopoeias.

There are several advantages of using HPTLC for the analysis of compounds as compared to other techniques, like HPLC, spectrophotometry, titrimetry, etc. Some of the advantages of HPTLC are i. Ability to analyze crude samples containing multi-components. Ii. The separation process is easy to follow especially with colored compounds. Iii. Several samples can be separated parallel to each other on the same plate resulting in a high output, time saving, and a rapid low-cost analysis. Iv. Choice of solvents for the HPTLC development is wide as the mobile phases are fully evaporated before the detection step. V. Two-dimensional separations are easy to perform. Stability during chromatography should be tested using two dimensional development.

Specific and sensitive colour reagents can be used to detect separated spots (Dragendroff reagent/Kedde reagent). Vii. HPTLC can combine and consequently be used for different modes of evaluation, allowing identification of compounds having different light-absorption characteristics or different colours. Viii. Contact detection allows radiolabelled compounds to be monitored and microbial activity in spots to be assessed. Ix. HPTLC method may help to minimizes exposure risk of toxic organic effluents and significantly reduces its disposal problems, consequently, reducing environment pollution.

Common Methodology for HPTLC Analysis Method development in thin-layer (planar) chromatography is one of the most significant steps for a qualitative and quantitative analysis. During establishing a new analytical procedure, always starts with wide literature survey . primary information about the physicochemical characteristics of sample and nature of the sample (structure, polarity, volatility, stability and solubility). It involves considerable trial and error procedures.

General steps involved in HPTLC method developments are as follow:

Basic Steps:

i. Selection of the stationary phase
ii. Mobile phase selection and optimization
iii. Sample Preparation and Application
iv. Chromatogram Development (separation)
v. Detection Quantitation: HPTLC method validation for pharmaceutical analysis

i. Specificity
ii. Linearity
iii. Range iv. Accuracy
vi. Detection Limit, Quantitation Limit
V. Precision
vii. Robustness

Basic Steps

1. Selection of the Stationary Phase:

During method development, stationary phase selection should be based on the type of compounds to be separated [15]. HPTLC uses smaller plates (10*10 or 10*20 cm) with significantly decreased development distance (typically 6 cm) 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 densitometry quantitative analysis [16].

2. Mobile Phase Selection and Optimization

The selection of mobile phase is based on adsorbent material used as stationary phase and physical and chemical properties of analyte.

3.Sample preparation and application.

A good solvent system is one that 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. [20] The more nonpolar the compound, the faster it will elute (or the less

time it will remain on the stationary phase) and the more polar the compound the slower it will elute (or moretime on stationary phase . Pharmaceutical pre with sufficient 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 HPTLC plate [21]. It is a fact that application of the sample is the most critical step to obtain good resolution for quantification in HPTLC [22]. Sample application technique depends on factors such as the type of sample matrix, workload and time constraints. 4.4. Chromatogram Development (Separation) Although chromatogram development is the most crucial step in the HTLC procedure, important parameters are generally overlooked [28]. 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 [23]. Detection- Detection of separated compounds on the sorbent layers is enhanced by quenching of fluorescence due to UV light (ranged 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 indicator is excited with UV wavelength at 254 nm and emits green fluorescence [24]. Compounds that absorb radiation at 254 nm reduce this emission on the layer, and a dark violet spot on a green background is observed where the compound zones are located [25]. This quenching is caused by all compounds with conjugated double bonds. Anthraglycosides, coumarins, flavonoids, propylphenols in essential oils, some alkaloid type such as indole, isoquinoline and quinoline alkaloids etc. should be detected under 254 nm [26]. Visualization at UV 366 nm F 366 should be described as Fluorescence quenching. In this instance the fluorescence does not remains after the source of excitation is removed [24]. This quenching is shown by all anthraglycosides, coumarins, flavonoids, Phenolcarboxylic acids, some alkaloid types (Rauwolfia, Ipecacuanha alkaloids) . Visualization at white light Zone containing separated compounds can be detected by viewing their natural color in daylight (White light) . Derivatization Derivatization can be defined as a procedural technique that primarily modifies an analyte‟s functionality in order to enable chromatographic separations. Derivatization can be performed either by immersing the plates or by spraying the plates with a suitable reagent]. For better reproducibility, immersion is the preferred derivatization technique. HPTLC vs. HPLC 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 .

Pharmaceutical application of HPTLC:

HPTLC is method deals with qualitative and quantitative analytical application such as herbal and dietary supplements, nutraceutics, and various types of medicine. It is used in quality control ,purity check, clinical application (metabolism studies , clinical studies etc), forensic: poisoning investigation, assaying radiochemical impurities of radio pharmaceuticals, detection and identification of pharmaceuticals, detection and identification of pharmaceuticals raw materials,drugs and their metabolites in biological media.

A.HPTLC in quality control of pharmaceuticals:

HPTLC has been used for routine quality control of topiramate, , deutosteriods, 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 depredates whether in pure form or in pharmaceutical formulations. Stability indicating HPTLC method for analysis of ropinirole HCl was developed and validated for precision , accuracy, ruggedness, robustness, specificity , recovery, limit of detection , and limit of quantitation . A significant difference of. Rf when drug was subjected to acidic, alkaline, oxidative, dry heat, wet heat and photo degradation stress is observed. In herbal medicine products, HPTLC is also an ideal screening tool for adulteration and is highly suitable for evaluation and monitoring of cultivation, harvesting, and extraction process and testing of stability . HPTLC has been reported for development of a quality assurance program.

B. HPTLC application in drug analysis:

The detail regarding HPTLC determination of pharmaceutical products in various formulation.

C. HPTLC as biomarker in pharmacognostic la research

HPTLC analysis of many plants used in India system of medicine has been performed for various pharmacological activities like CNS, hepatoprotactive etc . The HPTLC may be used rapid as rapid method by which to control the quality of raw materials and formulation based on the*lawsonia* plant . *Michelia Champa* L. Popularly know aa Champa is reservoir of numerous bio marker. HPTLC method has been used for detection, and quantification of quercetin in *michelia Champa* and the estimated values indicates that he leaves are the richest source of quercetin.

CONCLUSION:

Proven application of HPTLC in the pharmaceutical testing include: manufacturing, qc , the analyses of formulation, stability , sustained release and bioavailabilitystudies. In addition, it is used for semi quantitative comparison to provide quantitative results . In marine invertebrates, HPTLC has been utilizes to separate. New promising pharmaceutical therapeuants which could be used in pharmaceutical industries.

REFERENCES:

1. Amit j. , Sunil, vimal k, anupam p, phytosomes : A revolution in herbal drugs, the Pharma review 2007;11-13
2. BagulM , S.l rajini M. phytochemical evaluation of classical formulation : A case study Indian drugs 2005
3. Saraswathy A., Rukamani S., Ariyannathan: analysis of hinguvvacadicurma. Indian j. Trad, knowledge 2009
4. WanM.Sc. herbal medicine and it's standardization,Pharma info.2007