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A Review : Method of Development of HPTLC and Application in Pharma Industry

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Abstract: *This review provides knowledge on the development and validation parameters of HPTLC-based analytical methods in line with actual evaluation. Meet the criteria and minimize errors and investigations. This review assists in the selection of mobile thermal phases and provides guidelines for understanding the steps of good validation practices and analytical procedures.*

Keywords: Method Development, Validation, HPTLC.

I. INTRODUCTION

High Performance Liquid Chromatography (HPLC) was developed in the late 1960s and 1970s. Today, it is a separation technology that is widely accepted in both sample analysis and purification in various fields such as pharmaceuticals, biotechnology, environment, polymers, and food industry. HPLC has enjoyed a steady increase in equipment sales and publications describing new and innovative applications. Recent growth areas include miniaturization of HPLC systems, analysis of nucleic acids, intact proteins and protein digests, carbohydrate analysis, and chiral analysis.

chromatography is a technique in which solutes are separated at various elution rates as they pass through the chromatography column. Their separation is determined by the distribution between the mobile and stationary phases. To use liquid chromatography (LC) for a particular problem, various operating conditions such as column filling and mobile phase type, column length and diameter, mobile phase flow rate, column temperature, sample size, etc. are appropriate. Must be combined with. Choosing the optimal combination of chromatographic conditions requires a basic understanding of the various factors that affect LC separation.

High Performance Thin Layer Chromatography (HPTLC) is the most powerful and advanced form of thin layer chromatography (TLC), a chromatographic layer with the highest separation efficiency and a process containing accurate samples. Every step is configured with advanced equipment. Application, standardized reproducible chromatogram development, and evaluation of software control "HPTLC is a mostly scientifically standardized methodology, and qualitative and quantitative that enables separation and more accurate quantification. A concept that involves the use of validated methods for qualitative analysis.

II. PRINCIPLE

This technique is based on the same separation mode as column chromatography. H. However, adsorption, partitioning (including reverse phase partitioning), ion exchange, and gel permeation differ from column chromatography in that the mobile phase is pumped to the packed column under high pressure. The main advantages of HPLC over column chromatography are improved separability of separated substances, shorter separation times, and improved accuracy, accuracy and sensitivity of quantification of separated substances

III. HPTLC METHOD

3.1 The Stationary Phase

HPTLC is the most advanced form of modern TLC. It uses a small particle HPTLC plate with a narrow size distribution, resulting in a smooth and homogeneous layer on the surface. HPTLC uses smaller plates (1010 or 10 x 20 cm). HPTLC plates provide improved resolution, higher detection sensitivity, and improved in situ quantification and

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are used for industrial pharmaceutical densitometry quantitative analysis. Over 90% of reported drug and drug analyzes use forward phase adsorption TLC on silica gel using a less polar mobile phase such as chloroform-methanol

1. A simple and accurate HPTLC method has been developed to measure two anti-inflammatory drugs (curcumin and galangin) at the same time. The procedure was tuned to analyze both drugs in over-the-counter dosage forms (capsules) without interference with the ingredients. Chromatographic separation is performed on a precoated TLC plate (60 F254, 20 cm x 10 cm, 250 µm thick, Merck, Darmstadt, Germany) using n-hexane, ethyl acetate, acetic acid, and methanol as mobile phases. Performed by the linear increase method. Detection and quantification was achieved with 404 nm spectroscopic densitometry analysis (5)
2. A report on the densitometry TLC method developed and validated for the quantification of stigmasterol from petroleum ether extracts of Bryophyllumpinnatum leaves and stems. Separation was performed on a TLC aluminum plate precoated with silica gel 60F254. Good separation was achieved in the mobile phase using chloroform: ethanol (9.8: 0.2 v / v). Determination and quantification was performed by a reflection / absorption mode 1 densitometry scan at 490 nm
3. Describes a simple, accurate and accurate HPTLC method for estimation in bulk and tablet dosage forms. Chromatographic separation was performed. A mixture of methanol and toluene (4: 3% v / v) was used as the mobile phase and spot densitometry readings were performed at 235 nm on a precoated silica gel 60 F254 aluminum plate.
4. Lipophilic C-18, C-8. C-2: A phenyl chemically modified silica phase and a hydrocarbon impregnated silica plate developed with a more polar aqueous mobile phase. B. Use methanol-water or dioxane-water for reverse phase TLC.
5. A new high performance thin layer chromatography (HPTLC) method has been established to measure minocycline in human plasma. Chromatography was performed on an aluminum plate coated with silica gel 60F, and the mobile phase was methanol: acetonitrile: isopropanol: water 5: 4: 0.5: 0.5 (v / v). Densitometry analysis was performed at 345 nm
6. Simple, accurate, accurate, and powerful thin-layer chromatography methods have been developed and validated to estimate the simultaneous dosage form of olmesartan medoxomil and hydrochlorothiazide. Precoated silica gel 60F was used as the stationary phase and the mobile phase was a mixture of acetonitrile: chloroform: glacial acetic acid (7: 2: 0.5, v / v / v). The spot was detected at 254 nm
7. A simple, accurate, accurate, and rapid high-performance thin-layer chromatography method has been developed and validated for the measurement of tenoxicam in microemulsion gels. Tenoxicam was chromatographed on a silica gel 60F254TLC plate as a stationary phase. Toluene: Ethyl acetate: Formic acid (6: 4: 0.3 v / v / v) was used as the mobile phase [10]
8. Simple, accurate, specific and accurate high performance thin layer chromatography for simultaneous measurement. Developed a graphics method. Separation of sinitaprid and omeprazole in pharmaceutical dosage form on a 250 µm thick Merck HPTLC aluminum plate made of silica gel G60F254 (20 x 10 cm) Chromatography: Ethyl acetate: Methanol (7.3: 2: 0.7, v / v / v) As mobile phase: HPTLC separation of the two active substances followed by densitometry measurements were performed in absorption mode at 277 nm.
9. 8.Describes a thin-layer liquid chromatography (TLC) method developed and validated for simultaneous measurement of telmisartan and ramipril in a combined dosage form. This method does not require you to pre-separate the component from the sample. Telmisartan and ramipril were measured by high-speed thin-layer chromatography (HPTLC) in tablet form. The procedure was performed on TLC precoated silica gel (10 cm x 10 cm, pre-washed with methanol and activated at 60 ° C for 5 minutes prior to chromatography) on a 60 F 254 aluminum plate. The solvent system was acetone: benzene: ethyl acetate: glacial acetic acid with a ratio of 5: 3: 2: 0.03 (v / v / v / v).

Other precoat layers used include aluminum oxide , magnesium silicate and magnesium oxide. ,polyamide. A polar modified silica gel layer containing cellulose, diatomaceous earth, an ion exchanger, and bound amino, cyano, diol, and thiol groups. Optical isomer separation performed on a chiral layer prepared from C-18.

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3.2 Mobile Phase

The mobile phase selection is based on the physical and chemical properties of the adsorbent used as the stationary phase and the object to be analyzed. The mobile phase system used for different selectivity uses diethyl ether, methylene chloride, and chloroform individually or in combination with hexane as a strength control solvent for normal phase TLC and methanol. Acetonitrile and tetrahydrofuran are mixed with water to adjust the strength of the reverse phase TLC and a mobile phase such as methanol-0.1 M acetate buffer (pH 3.5) containing 25 mM sodium pentanesulfonate (pH 3.5) is used. -Perform ion pair separation in 18 layers. 15, 5: 4.5).

1. A simple and accurate HPTLC method has been developed to measure two anti-inflammatory drugs (curcumin and galangin) at the same time. The procedure was tuned to analyze both drugs in over-the-counter dosage forms (capsules), subject to interference from the ingredients. Precoated TLC plate (60 F254, 20 cm x 10 cm, 250 mm thick, Merck, Darmstadt, Germany) by linear increase method using n-hexane, ethyl acetate, acetic acid , and methanol as mobiles. , Chromatographic separation was not performed. step
2. Simultaneous quantification with lamivudine Zidovudine in tablets by the HPTLC method was developed It has been verified. Chromatogram Toluene: Ethyl acetate: Methanol (4: 4: 2, v / v / v) Pre-coated silica gel GF aluminum TLC plate Plate and quantify in densitometry absorption mode276 nm .
3. A new high-performance thin-layer chromatographic (HPTLC) method has been established for determination minocycline in human plasma Chromatography was performed on aluminium plates coated with silica gel 60F the mobile phase was methanol: acetonitrile: isopropanol: water 5:4:0.5:0.5 (v/v)
4. A new and simple HPTLC method was developed and validated for the quantitative estimation of Eugenol in muscle and joint pain relaxant herbal oil TLC aluminium plates precoated with silica gel 60F-254 (0.2 mm thickness) were used. The linear ascending development was carried out in twin trough glass chamber saturated with mobile phase Tolune: Ethyl acetate (9.3:0.7) ratio followed by densitometric determination was carried out by TLC scanner (CAMAG) at 560 nm in reflectance/absorbance mode 14
5. A sensitive, fast, and reproducible high performance thin layer chromatographic method has been developed for simultaneous analysis of diosgenin and quercetin from fenugreek seeds, using TLC aluminium plates precoated with silica gel G60F254. Among the different combinations of mobile phases used, best separation was achieved in Toluene-ethyl acetate-formic acid (5:4: 1. v/v/v). The plate densitometry scan was performed directly at 275 nm. Used for analysis of quercetin
6. A new, simple, fast, high-performance thin-layer chromatography method has been developed and validated for the quantitative analysis of carbamazepine. Carbamazepine was chromatographed on a silica gel 60F254TLC plate using ethyl acetate: toluene: methanol (5.0: 4.0: 1.0 v / v / v) as the mobile phase. Carbamazepine was quantified by densitometry analysis at 285 nm
7. A new simple, accurate, accurate, specific and selective high performance thin layer chromatography (HPTLC) method for simultaneous measurement of terbinafine hydrochloride (TH) and mometasone flotazone (MF) in cream (HPTLC) method (HPTLC) was developed. Dosage form. Chromatographic separation was achieved on Merck's precoated silica gel aluminum plate 60F254 using toluene: ethyl acetate: glacial acetic acid (8: 4: 0.1 v / v) as the mobile phase.
8. A thin layer chromatography (TLC) method for qualitative and quantitative analysis of diclofenac sodium tablets has been developed and validated according to ICH and USP guidelines. This method was developed using a mobile phase prepared with an environmentally friendly solvent. Toluene, acetone and glacial acetic acid (10: 15: 0.2 v / v / v) are placed on a precoated TLC silica gel 60F254 glass plate with a saturation time of 25 minutes. Densitometer detection wavelength 284nm with reflection absorption model
9. Accurate, sensitive, accurate. We have developed a reliable and rapid method for measuring cholesterol levels using high performance thin layer chromatography. In this method, a precoated silica gel 60 F254 plate covered with aluminum was used as the stationary phase and the sample was sprayed using the CAMAGLinomat 5 sample applicator. Chromatograms were obtained using a mobile phase consisting of chloroform: methanol (9.5: 0.5, v / v).
10. A new simple and powerful thin layer chromatography method for measuring mycophenolate mofetil in bulk and tablet form. The drug was separated on an aluminum plate precoated with silica gel 60F25 using toluene,

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acetone, and methanol as eluents in a ratio of 6: 2: 2 (v / v / v). Quantitative analysis was performed by a densitometry scan at 254 nm .

11. Simple, accurate and accurate HPTLC method duloxetine hydrochloride for estimation in bulk and tablet form. Chromatographic separation was performed on precoated silica gel 60F254 aluminum plate using a mixture of chloroform: methanol (8: 1 v / v) as the mobile phase and spot densitometry evaluation was performed at 235 nm 121.

3.3 Pre-cleaning

The plate is treated at the top to avoid contamination . Plates are used without pretreatment unless chromatography produces an impurity front due to plate contamination. For reproducibility and quantitative analysis, the layers are often prewashed with 20 mL of methanol. Methanol is used as a pre-cleaning solvent, a mixture of methanol and ethyl acetate, or even the mobile phase, used per trough in a 20 x 10 cm double trough chamber (TTC). Two 20x10 cm or four 10x10 cm plates can be developed back-to-back with each TTC trough. Remove the plate and allow it to dry in a clean drying oven at 120 ° C for 20 minutes. Equilibrate the plate in a laboratory atmosphere (temperature, relative humidity) in a suitable container protected from dust and steam. [1]

3.4 Plate Preparation

The TLC plate can be prepared with the appropriate equipment. Such layers do not adhere well to the glass substrate. Precoated panels use a small amount of very high molecular weight polymer as a binder. This overcomes most of the limitations of homemade coatings. The pre-coated layer is fairly wear resistant and the layer thickness is very uniform and reproducible. It is pre-activated and ready to use. They are available with glass or aluminum or polyester support. Aluminum foil sheets are cheap to buy and can be cut, making them easy to carry, transport and ship. Glass plates are the best choice for the highest quality results. The usual. Layer with fluorescent indicator F254. This makes it very easy, instant, and non-destructive to visualize the samples in the UV cabinet. The typical plate size for TLC is 20 x 20 cm, and HP TLC commonly uses 20 x 10 cm or 10 x 10 cm.

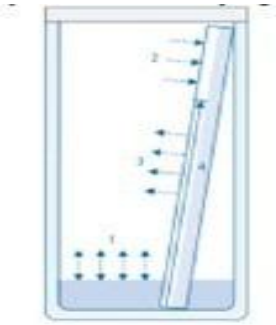
3.5 Sample Application

Sample application plays an important role and its techniques are spot application and spray-sample application to sample is the first step in chromatography and the final result of the process. Affects the quality of. The choice of application technology and device depends on your requirements. The point sample application with a fixed capacity capillary is the easiest way. Sample volumes of 0.5-5 L can be spotted on conventional layers without drying. In the HPTLC layer, the maximum is L per spot. The best resolution that can be achieved with the chromatographic system of choice is to spray the sample as a larger, narrower band. Large samples or samples with high matrix content can be sprayed in a rectangular shape and focused in a narrow band.

3.6 Chromatogram Development

In this method, there is a gas phase in addition to the stationary and mobile phases. This gas phase can have a significant impact on the outcome of the separation.

Figure 1: Process in developing chamber



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IV. PROCESS

Soak the bottom of the plate and move the developing solvent onto the bed until the capillarity reaches the desired clearance and stops chromatography. The following considerations are primarily related to silica gel as a stationary phase and developments that can be described as adsorption chromatography.

Four types of processes occur.

4 kinds of processes occur.

1. When dry, the stationary phase adsorbs molecules from the gas phase. In this process, adsorption saturation represents the equilibrium in which the polar components are extracted from the gas phase and loaded onto the surface of the stationary phase.
2. The part of the layer that is already wet in the mobile phase interacts with the gas phase. In particular, the less polar component
3. of the liquid is released into the gas phase
4. In contrast to (1), this process is determined by vapor pressure rather than adsorption force.
5. During the transition, mobile components may separate from the stationary phase under certain conditions, leading to the formation of a secondary front

The following points should be considered:

The developing solvent and mobile phase are not the same. Their composition changes with chromatography. The terms treatment solvent, mobile phase are often used interchangeably, the liquid in the chamber should be called the treatment solvent, and the liquid moving on the floor is the mobile phase. Only the composition of the developing solvent upon introduction into the chamber is clearly known. Processes (1) and (2) can be experimentally affected by: After introducing the developer into the chamber, wait for a certain period of time until the filter paper soaked in the developer is almost completely loaded into the chamber. The beginning of chromatographic chamber saturation. Prior to chromatographic development, that is, the plate interacts with the gas phase without contact with the developing solvent-pretreatment. Steps 2 and 3 can be avoided by placing the backing plate at a distance of one or a few millimeters from the chromatography layer. This is called a sandwich composition. Equilibrium predominates in steps 1 and 2, and the smaller the difference in adsorption behavior of the mobile phase components, the less pronounced in step 4 in the formation of the secondary front. Secondary fronts are often not observed in fully saturated chambers or pretreated layers. During chromatography, the components of the developing solvent loaded into the dry layer through the gas phase according to (2) are extruded in front of the actual invisible solvent front. The exception is very polar components such as water. Methanol, acid or base. This results in lower RF values for saturated chambers, especially pretreated sheets, than unsaturated chambers and sandwich configurations. Due to solvent separation and beta-front potential, development in sandwich configurations or unsaturated horizontal development chambers works best with multi-component solvents that act like single-component or single-component solvents

4.1 Consequences

In most cases, thin layer chromatography occurs in an imbalance between the stationary, mobile, and gas phases. For this reason, it is very difficult to correctly describe the state inside the developing chamber. Keeping all parameters as constant as possible will give you reproducible chromatographic results. The shape and saturation of the chamber play an important role. Unfortunately, this means that the chromatographic results will be different for each chamber

4.2 Development Chamber Selection

Chamber selection is made during the development of Method and considers which chambers are available and which chambers need to be used. Attention should also be paid to economic aspects such as time requirements and solvents. consumption. Horizontal deployment chambers have proven to be extremely economical, flexible and reproducible in operation. Designed for applications where plates are developed from both sides. It is also suitable for single-sided development in unsaturated, saturated, sandwich configurations for pretreatment of HPTLC plates. Development uses a traditional 20 x 10 cm double trough chamber. In this way, the chamber shape and chromatographic conditions of existing analytical methods can be maintained, but the environmental and operational impacts can be standardized.

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4.3 Derivatizer

Derivatizer introduces a automatic atomizer that sets a new standard for reproducibility of reagent transfer to TLC plates by utilizing proprietary "microdroplet" atomization technology (patent application). During). The derivatizer ensures a uniform and reproducible application of all common reagents. Four different color-coded spray nozzles are used to explain the different physicochemical properties of different reagents, such as acidity and viscosity, and the user can choose from six spray intensities. In addition to the significantly increased uniform distribution of reagents, the derivatizer offers other advantages over manual spraying. Environmentally friendly and safe handling thanks to the closed system Intuitive handling and easy cleaning Efficient operation (4mL). Reproducible and user-independent results (less reagent consumption for 2 mL 20 x 10 cm plates for 20 x 20 cm plates and).

4.4 Evaluation

A. Detection

The detected chromatogram is rated under white or UV light. Options range from visual inspection of electronic images to quantitative measurements using video or scan densitometry.

B. Thin-layer chromatography on traditional densitometry:

chromatogram evaluation instruments should be able to rely on both traditional densitometry and electronic imaging. Densitometry uses monochromatic light and selectable length and width slits to scan chromatogram traces and measure diffuse reflected light. The TLC scanner uses the entire spectral range of 190-900 nm and has high spectral selectivity for data acquisition. Within this range, absorption spectra can be recorded for substance identification and selection of the optimum measurement wavelength. The strength of traditional densitometry is the spectral resolution of the light source and the high reproducibility of quantitative measurements.

C. Requirements for improving the accuracy of evaluation

- Use of HPTLC plates. Small layer thickness, narrow particle size distribution and the homogenous packing of the HPTLC layer result in less fraction broadening and low background noise.
- Automatic spray-on sample application technique .Only by spraying the size of the starting zone does remain independent of the application volume and the sample is homogenously distributed across the application position. Data acquisition can be based on larger substance amounts.
- Use of a chamber providing good reproducibility of chamber conditions.
- Choosing a working range for calibration according to the absorption/fluorescence behavior of the substances. The evaluation software offers suitable calibration functions.
- Optimization of light and measurement parameters, such as slit dimensions, measuring wavelength, scanning speed for the substances to be analyzed. Suitable baseline correction to maximize the signal to noise ratio.
- Derivatization can contribute to the overall error of the determination. The more homogenous the reagent is applied the smaller the error.

V. HPTLC APPLICATIONS IN PHARMA INDUSTRY

5.1 HPTLC in the Pharma Industry Pharmaceutical Products

HPTLC with densitometry is used to determine the amount and purity of active ingredients and preservatives in over-the-counter formulations that may be synthetic or herbal. This technique is used, for example, in post-manufacturing quality control of batches of chemotherapeutic agents and to regulate caffeine levels in herbal formulations. The HPTLC also demonstrates the ability to analyze drugs without an adjuvant that interferes with the results of many studies. Over the years, researchers have developed accurate and simple HPTLC methods for measuring several active ingredients such as paracetamol, diclofenac, and famotidine Development and quantification of biomarkers The HPTLC method is useful not only for therapeutic purposes, but also for quantifying biomarkers that indicate exposure to environmental hygiene hazards such as nicotine

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5.2 Purity Control

The HPTLC test for the presence of pesticides, water, and chemicals in fruits, vegetables, vitamin supplements, and foods is globally recognized for their ability to characterize small molecules.

A. Protein Analysis

Morschheuser L. et al. Recent studies by. HPTLC has been shown to be usable in combination with aptamers to estimate proteins. HPTLC apta staining opens up a variety of possibilities, including the measurement of lysozyme (an enzyme that causes severe allergic reactions in some people) in foods and beverages.

B. Pharmacokinetics Study

HPTLC is used in bioavailability studies to evaluate over-the-counter formulations and new therapies. For example, Abdelwahabetal. It has been shown that HPTLC can be used to monitor drug-drug interactions, as observed when thalidomide and dexamethasone are co-administered to rat plasma. This study used a development method validated by the FDA.The shelf life of medicines is Researcher concerns. HPTLC Use a plate of pre-coated silica gel 60F254 Solvent mixture of methanol and ethyl Acetate and ammonia developed by Bober K. have succeeded in providing a more substantive one. Information about the decomposition products of Diphenhydramine Common spoofing in many dietary supplements and soft drinks, such as food certification sildenafil (phosphodiesterase inhibitor) and its analogs, sibutramine, can be detected by HPTLC.

C. Forensics

HPTLC has been used to investigate cases of chemical warfare, drug and alcohol abuse, and to detain suspects involved in misdemeanor and heinous crimes. Concentration levels in the picogram range can also be detected by HPTLC. HPTLC was particularly helpful in detecting cannabis, the most commonly abused recreational drug.

D. Takeaway-Message

Pharmaceutical industry Increased use of HPTLC alone or Combination with other technologies such as MS, FTIR for bulk drug analysis and pharmaceutical formulation. HPTLC is also used Successfully applied in the field of biomedicine, Growing biochemistry Application example to modern agriculture: Estimating pesticide residues in fruits vegetable.

VI. CONCLUSION

Applications of HPTLC for phytochemical analysis, biomedical analysis, herbal drug quantification, analytical analysis, finger print analysis, and HPTLC future to combinatorial approach, HPTLC-MS, HPTLC-FTIR and HPTLC-Scanning Diode Laser made HPTLC a power analytical tool in the field of analysis. It is noteworthy that utilization of instrumental HPTLC toward the analysis of drug formulations, Bulk drugs, natural products, clinical samples food stuffs, environmental, and other relevant samples will increase in the future.

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