International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

Analytical Method Development and Validation of Hptlc

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Date of Submission: 15-10-2020 Date of Acceptance: 05-11-2020

ABSTRACT: The analysis of pharmaceutical and natural compound and newer drugs is commonly used in all the stages of Drug discovery and development process. High performance thin layer chromatography (HPTLC) it is automatic and sophisticated form of Thin Layer chromatography TLC. Due to vast chemical diversity compounds has gaining importance. This led to phenomenally increase in demand of Herbal drug medicines and need for ensuring quality, safety and efficacy of herbal drugs. By using this modern analytical technique, it needs small amount of sample for detection. This review is to focus on application of HPTLC technique and the validation parameter test.

Keyword: -HPTLC, TLC, HPLC, Application

I. 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.[1]

Analysis of pharmaceutical compounds and newer drugs is usually utilized altogether the stages of drug discovery and development process. These analytical techniques provide more accurate précised data, not only supporting drug discovery and development but also post market surveillance. [22] Thin layer chromatography studies are among the key identity tests in most pharmacopeial monographs. Pharmacopeial standards are typically employed by industry as a basis for meeting QC requirements and current good manufacturing practices (cGMPs). An extension of TLC is high-performance thin layer chromatography (HPTLC) is strong, simplest, rapid, and efficient tool in quantitative chemical analysis of compounds. HPTLC is employed for the identification of constituents, identification and determination of impurities, and quantitative determination of active substances. HPTLC is an analytical technique supported TLC, but sort of the enhancements a bit like the use of upper quality TLC plates with finer particle sizes within the stationary phase which enable better resolution. HPTLC is that the foremost powerful advanced kind of Thin Layer Chromatography (TLC) and consists of chromatographic layers of utmost separation efficiency and thus the appliance of sophisticated instrumentation for all steps within the procedure include accurate sample application, standardized reproducible chromatogram development and software-controlled evaluation.[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.

2.1 Principle

International Journal of Pharmaceutical Research and Applications
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HPTLC happen with high speed capillary flow range of the mobile phase. Mainly there are 3steps involved that has,[1]

• Sample to analysed to chromatogram layer volume precision and suitable position are achieved by use of suitable instrument.

• Solvent Migrates the planned distance in layer by capillarity during this process sample separated in its components

• Densitometer is used for scanning separation tracks with beam in visible or UV region.

HPTLC Instrumentation

Fig 1: - Instrumentation Parts of HPTLC

(i) Auto-Sampler [3]

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. Also, a computing system connected to the auto-sampler is employed to feed data about the quantity of sample to be applied and therefore the number, width, and position of the bands of the samples.

Note that unlike TLC, you want to filter the sample employing a syringe filter before applying it on the TLC plate, alternatively there's a high chance that the undissolved sample or precipitate, might clog the HPTLC syringe (these syringes may cost you a reasonably penny).

(ii) Developing Chamber [3]

Usually, an oblong glass chamber is employed for developing HPTLC plates, almost like TLC. Here, you saturate the chamber for a minimum of 20-30 minutes by pouring a solvent and covering it. you'll also place a paper dipped within the solvent system to permit even distribution of the vapours. After saturation, you want to place the plate vertically within the chamber. you want to confirm that the sample spots remain above the extent of the solvent.

There are two main sorts of vertical developing chambers viz. flat bottom chamber and a twin trough chamber (this one features a ridge at the bottom of the chamber dividing it into two troughs). The solvent consumption is a smaller amount in twin trough chambers as compared to the flat bottom ones.

Experts who use HPTLC also recommend you employ an Automatic Developing Chamber (ADC) where the plate is immersed during a pre-saturated chamber and is conditioned there for a specific period of your time then dipped within the solvent. a bit like the sample application part, this procedure is additionally fully automated. After the solvent

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

front reaches a specific height, the plate is automatically removed. Thus, there's no human interference, and therefore the results are comparatively more accurate and reliable.

(iii) Chromatogram Immersion Device and Derivatizer [3]

For all my experiments thus far, I even have always used 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. This manual method of immersing the plate within the derivatization agent are often used for TLC also as HTPLC, as long because the speed, and therefore the time that the plate is immersed within the derivatization agent is kept as constant as possible, throughout your experiments. This manual way of derivatization are often semi-automated by employing a chromatogram immersion device, which immerses and withdraws the plate from the derivatization agent with uniform vertical speed and immersion time.

In contrast, the derivatize is fully automated, where it uniformly sprays the derivatization agent on the HPTLC plate. Also, the derivatization assembly may be a closed system and hence no aerosols/spraying reagents leak. Thus, the derivatize offers advantages like homogeneity, accuracy, and convenience over the manual and semi-automated methods.

(iv) TLC Scanner for Chromatogram Evaluation [3]

When you're scanning your compound for the primary time, you'll choose a multiwavelength scan (ranging from 190 to 900 nm) to work out the simplest wavelength for your compound. the simplest wavelength would show sharper peaks as compared to others.

Once you've got found an appropriate wavelength for your compound, you'll select that specific wavelength whenever you scan your plate. The quantification of your compound is then only a matter of a couple of mouse-clicks. 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. you'll prefer to display the info (areas) or the peaks (3D view) either individually or as an overlaid diagram. Moreover, you'll even assign substance names to the peaks.

2.2 Features [1, 2,23,24]

• Analysts work simultaneously.
• Lower analysis time and fewer cost per analysis.
•No Cost effective
• Several prior treatments for solvent.
• Accuracy and precision of quantification is high.
• Per sample low mobile-phase consumption.
• Visual Detection possible-open system.
• No interference from previous analysis-fresh stationary and mobile phase-no contimation.
• Low maintenance cost

2.3 Stationary Phases [4]

HPTLC is that the foremost advanced quite modern TLC. It uses HPTLC plates featuring small particles with a narrow size distribution which lands up in homogenous layers with a smooth surface to be obtained. HPTLC uses smaller plates (10 × 10 or 10 × 20 cm). HPTLC plates provide improved resolution, higher detection sensitivity, and improved in place quantification and are used for industrial pharmaceutical densitometric quantitative chemical analysis. Normal phase adsorption TLC on colloid with a less polar mobile phase, like chloroform–methanol, has been used for quite 90% of reported analysis of pharmaceuticals and medicines.

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.

Other precoated layers that are used include alumina, magnesium silicate, periclase, polyamide, cellulose, kieselguhr, ion exchangers, and polar modified colloid layers that contain bonded amino, cyano, diol, and thiol groups.[5]

2.4 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 supported their diverse selectivity properties are ether, dichloromethane, and chloroform combined individually or in conjunction with hexane because the strength adjusting solvent for normal-phase TLC and methanol, acetonitrile, and tetrahydrofuran mixed with water for strength adjustment in reversed phase TLC. Separations by ion pairing on C-18 layers are through with a mobile phase like methanol–0.1 M acetate buffer

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

(pH 3.5) containing 25 mM sodium pentane sulfonate (15.5:4.5).

2.5 Classification [1]

Fig 2: -Classification of HPTLC

2.6 Advantages [1,3]

- Shorter analysis time
- Require lower amount of Solvent
- Enormous Flexibility
- Better accuracy and Sensitive
- Sample can be detected in nano gram
- Multiple Sample Handling
- Wide decision of stationary phase
- Simple representation of isolated mixes.

2.7 Disadvantages[1]

- Require large space
- Bulky instrumentation
- Technically skilled person requires.

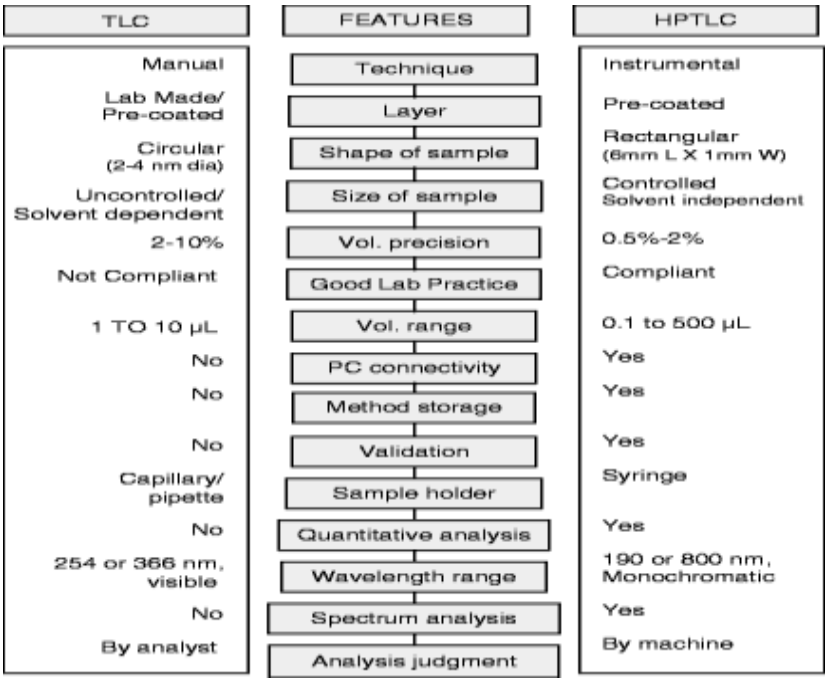

Fig 3: -Difference between TLC & HPTLC

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

Parameters	HPLC	HPTLC
Type	Reverse Phase Chromagraphy	Straight Phase Chromagraphy
Stationary phase	Liquid	Solid
Conditioning phase	None	Gas
Separation by	Partition	Adsorption
Results	By machine	By machine + eyes
Analysis	On - line	Off - line
Resolution	Very high	Moderate to high
Chromatography System	Closed	Open
Separating medium	Tubular column	Planar layer (plate)
Strongly Retarded Fractions Seen As	Broad peaks	Sharp Peaks
Analysis in parallel	No. Only 1 at a time	Yes. Upto 100 samples.
High temp. / pressure	High pressure	None
Time per sample	2- 60 min	1-30 min
Data obtained from chromatography	Limited to very high	High to very high

Fig 4: -Difference between HPLC & HPTLC

II. METHOD OF PREPARATION

Steps involved in HPTLC method Step involved in HPTLC/HPTLC Methods: -

3.1 Sample preparation.
3.2 Selection of Chromatographic layer
3.3 Plate, prewashing, conditioning
3.4 Sample application
3.5 Pre-conditioning.
3.6Mobile phase.
3.7 Chromatography development
3.8 Detection spot.
3.9 Scanning & documentation

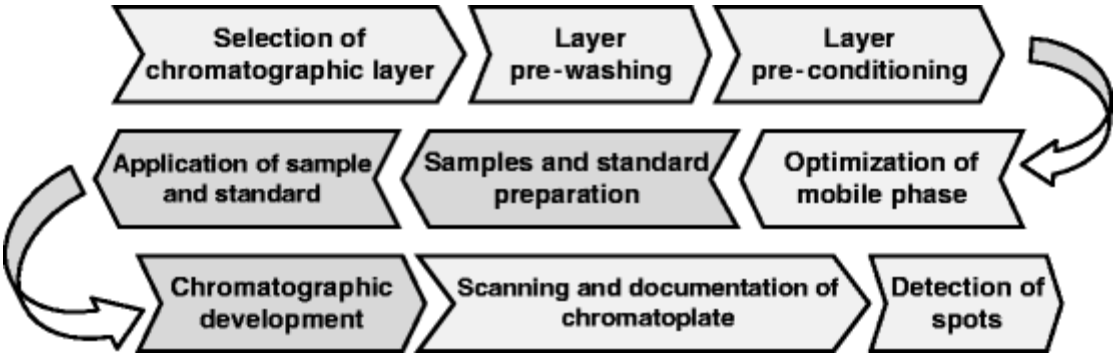

Fig 5: - Steps involved in HPTLC

3.1 Sample Preparation [1]

• Solvents used for sample preparation are ammonia, methanol, chloroform, ethanoic acid.
• an honest solvent system is one that doesn't out anything on solvent front but moves all components of the mixture off the baseline. the height of interest should be resolved when the Rf range is between 0.15 and 0.85. The elution power of the mobile phase depends on a property called eluent strength. • More non-polar compound, faster it'll elute than that of polar.

3.2 Selection of Chromatographic layer [1]

• With different supporting materials i.e. plastic, aluminium, glass, precoated plates are available in several thickness and formats.
• Usually plates with solvent thickness of 100-250mm are usually available for qualitative and quantitative chemical analysis.
❖ Commonly available precoated plates are: -
• quite 80% of study is completed on colloid 60F plates.
• Aluminium oxide: Basic substances, alkaloids and steroids are available.

International Journal of Pharmaceutical Research and Applications
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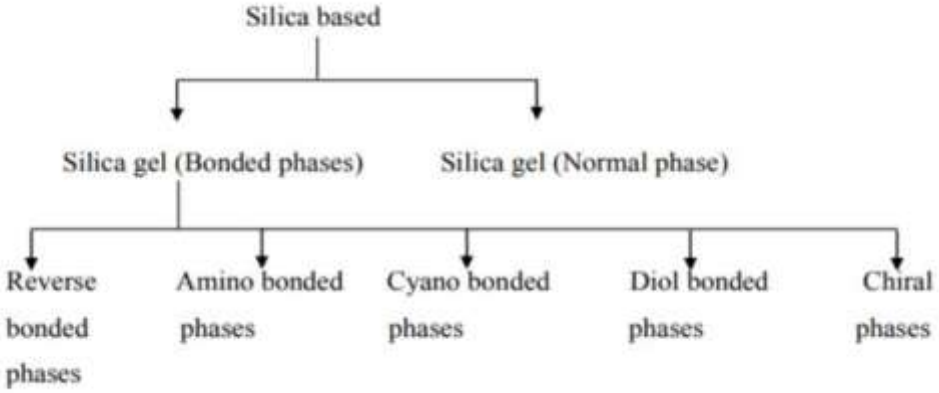

Fig 6: -Silica Based

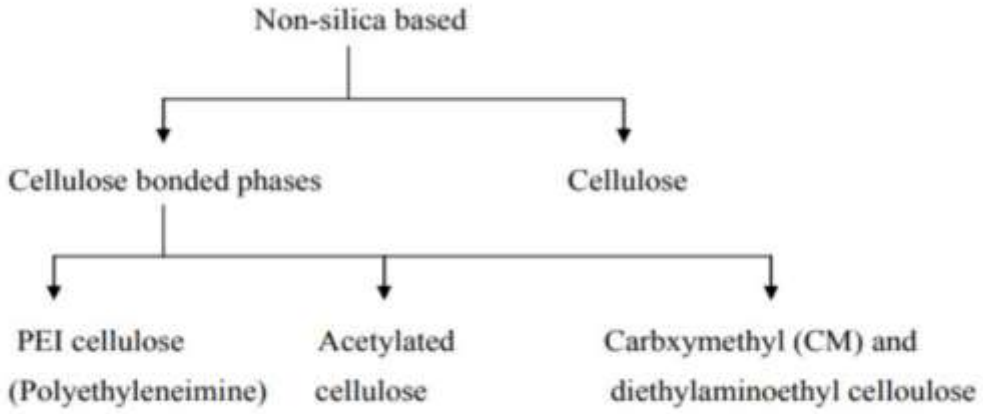

Fig 7: -Non-Silica Based

3.3 Plate, prewashing, conditioning [4,21]

• Plates are handled at the upper edge to avoid contamination. Plates are used without pre-treatment unless chromatography produces impurity fronts because of contamination of the plate. For reproducibility and quantitative qualitative analysis, layers are often prewashed using 20 ml methanol. The methanol is used as a prewashing solvent, a mixture of methanol and ester or even mobile phase is used, per trough during a 20 × 10 cm twin trough chamber (TTC). the two 20 × 10cm or four 10 × 10 cm plates are often developed back-to-back in each trough of the TTC. Remove the plate and dry it for 20 min during a clean drying oven at 120°C. Equilibrate plate with laboratory atmosphere (temperature, relative humidity) during an appropriate container providing protection from dust and fumes.

Activation of Pre-coated Plates: - [21]

Freshly open box of plates does not require any activation. Plates which are kept on hand for long time requires activation. The plates is activated by placing the plates in an oven 110-120 ℃ for 300 prior to spotting. Aluminium sheets should be placed in in between two glass plates and kept in oven at 110-120℃ for 15 min.

Preparation of Plates: -

• TLC plates are often made with suitable apparatus. such layers don't adhere well to the glass support. Precoated plates use small quantities of very high relative molecular mass polymer as binder overcomes most limitations of a home-made layer. Precoated layers are reasonably abrasion resistant, very uniform in layer thickness, reproducible, preactivated, and ready to use. They are available with glass or aluminium or polyester support. Aluminium foil plates are less expensive to buy, cheaper, are often cut, and thus easy to

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

carry around or transport or mail. Glass plates are the only for highest quality of results. most often, layers containing a fluorescent indicator F254 are used. this allows the visualization of samples during a UV cabinet very simply, instantly, and during a non-destructive manner. Commonly used size of plates in TLC is 20×20 cm and in HPTLC 20 × 10 cm or 10 × 10 cm is widespread.

3.4 Sample application

• The concentration range is 0.1-1 mg/ml; above it ranges it cause poor separation. Sample and standard are often sprayed or applied nitrogen gas sprays on TLC plates as bands or spot by using automatic sample applicator.
• With sufficiently high concentration of analyte, pharmaceutical preparation is simply dissolved in suitable solvent which can solubilize the analyte.
• Sample application techniques depend on factor a bit like the type of workload, sample matrix and time constraints.

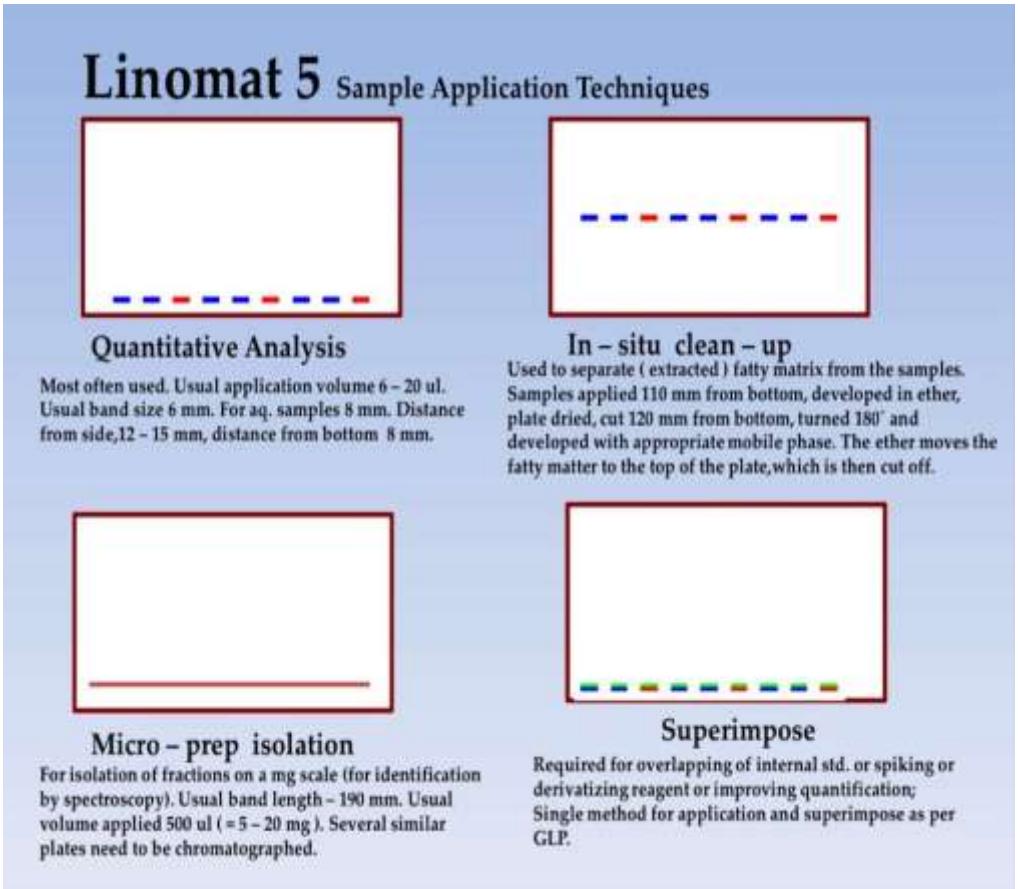

Fig 8: -Linomat 5 Sample Application

3.5 Pre-conditioning (Chamber Saturation) [1]

• On separation profile, chamber has pronounced influence.
• Higher Rf values are causes by unsaturated chamber.
• Filter paper lining for 30 min before development in saturated chamber results in uniform distribution of solvent vapours and fewer solvent requires for the sample to travel.

3.6 Mobile Phase [1]

• Poor grade of solvent utilized in mobile phases preparation was found to decreases resolution, Reproducibility and spot definition. It also supported one's own experience and literature.
• Using three or four components in mobile phase should be avoided because it is usually difficult to get the reproducible ratios of inauspicious components.

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

Mobile phase selection &optimization: -

Sr. No	Chemical Compound	Mobile Phase
1	Polar compounds: Anthraglycosides, arbutin, alkaloids, cardiac glycosides, bitter principles, flavonoids, saponin.	Ethyl acetate: methanol: water [100:13.5:10]
2	Lipophilic compounds: Essential oils, terpenes, coumarin, Naphthoquinone, velpotriate	Toluene: ethyl acetate [93:7]
3	Alkaloids	Toluene: ethyl acetate: diethyl amine [70:20:10]
4	Flavonoids	Ethyl Acetate: Formic Acid: Glacial Acetic Acid: Water [100:11:11:26]
9	Essential oils	Toluene: ethyl acetate [93:7]
7	Cardiac glycosides	Ethyl acetate: methanol: water [100:13.5:10] or [81:11:8]
8	Bitter drugs	Ethyl acetate: methanol: water [77:15:]
13	Terpenes	Chloroform: methanol: water [65:25:4]
14	Triterpens	Ethyl acetate: formic acid: formic acid [50:50:15] Toluene: chloroform: ethanol [40:40:10]

Table no.1: -Mobile Phase generally used in detection of some chemical compounds

3.6.1 Normal phase [2]

Stationary phase is polar then Mobile phase is non-polar.

Non-polar compounds eluted first due to lower affinity with stationary phase.

Polar compounds retained due to higher affinity with the stationary phase.

3.6.2 Reversed phase [2]

Stationary phase is non-polar then Mobile phase is polar.

Polar compounds eluted first due to lower affinity with stationary phase.

Non-Polar compounds retained due to higher affinity with the stationary phase.

3.7 Chromatography development

In this system additionally to stationary and mobile phases, a gas phase is present. This gas phase can significantly influence the results of the separation.[4]

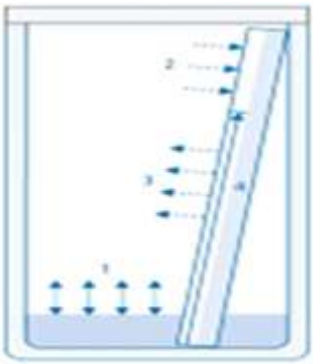

Fig 9: -Process in Developing Chamber

Processes [4]

The lower end of the plate should be immersed and act by capillarity the developing solvent moves up the layer until the specified distance is reached and chromatography is stopped. The following considerations primarily concern

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

colloid as stationary phase and developments, which can be described as adsorption chromatography.

Four sorts of Processes occur: -

The four sorts of processes occur they are

1. When it's dry, the stationary phase adsorbs molecules from the gas phase. This process, adsorptive saturation an equilibrium during which the polar components are going to be withdrawn from the gas phase and loaded onto the surface of the stationary phase.
2. A part of the layer which is already wetted with mobile phase interacts with the gas phase. Thereby especially the less polar components of the liquid are released into within the gas phase.
3. Unlike (1) this process isn't the maximum amount governed by vapour pressure as by adsorption forces.
4. During migration, the components of the mobile phase are often separated by the stationary phase under certain conditions, causing the formation of secondary fronts.

3.8 Detection spot [2,6]

• Under detection of UV light is initiative and is non- destructive. Spots of fluorescent compound scan be seen at 254 nm i.e. radio wavelength.
• Fluorescent stationary phase like colloid GF, spots of non-fluorescent are often used.
• If individual component doesn't answer UV, then derivatization is required with visualizing agent.
• Visualization at white light Zone containing separated compounds are often detected by viewing their natural colour daylight (White light).

3.8.1 Derivatization

Derivatization are often defined as a procedural technique that primarily modifies an analyte's functionality to enable chromatographic separations. Derivatization are often performed either by immersing the plates or by spraying the plates with an appropriate reagent.[2]

Sr.no	Colour reagent	Chemical compound	Colour
1.	Dragendroff Reagent It forms complex reaction with some nitrogen containing compounds	Alkaloids	Red-brown Zone (vis)
2.	Vanillin Sulphuric Acid OR Anisaldehyde Sulphuric Acid	Bitter Principle	Red-brown, Yellow-brown, Dark green Zone (vis)

Table no.2: -List of common derivatizations

3.8.2 Quantification

Generally quantitative evaluation is performed by measuring the zones of samples and standard using a densitometer or scanner with a hard and fast sample beam within the sort of an oblong slit. Sample and standard should be chromatographer on same plate-after development chromatogram is scanned TLC scanner III scan the chromatogram in reflectance or in transmittance mode by absorbance or by fluorescent mode. Scanning speed is selectable up to 100 mm/s. [2,18]

3.8.3 Scanning & documentation [2]

• Plates with imprinted identification code supplier name. Item number, batch number and individual plate number - avoid manipulation of data at any stage - coding automatically gets recorded during photo documentation.

• Allows measuring the absorption and/or fluorescence of underivatized or derivative substances at wavelengths between 200 and 800 nm. Up to 31 wavelengths are often evaluated and spectra of any peak are often recorded. Following integration densitometric data are often quantitatively evaluated. Biological tests are often performed directly on the HPTLC plate. Bacteria, enzymes, yeast, fungi, etc. are often used as test organisms.

- Scanning has been done by two methods i.e. Slit Scanning and Video Scanning.[19]

➢ **Slit Scanning Slit: -**

Scanning densitometry is now relatively mature and although limited to absorption and fluorescence detection in the UV–visible range. It consists of fibre optic bundle for illumination of sample zones and collection of reflected light (or fluorescence).

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

Photodiode-array detector is used for simultaneous length detection and spectral recording.[20]

- **Video Scanning: -**

Video densitometry is fast and simultaneous data acquisition from the entire plate. during which optical scanning takes place electronically, employing a computer with video digitizer, light, monochromators, and appropriate optics to illuminate the plate and focus the image onto a charge-coupled device (CCD) video camera. it's also useful in two-dimensional separations for thin-layer chromatography with image analysis.[20]

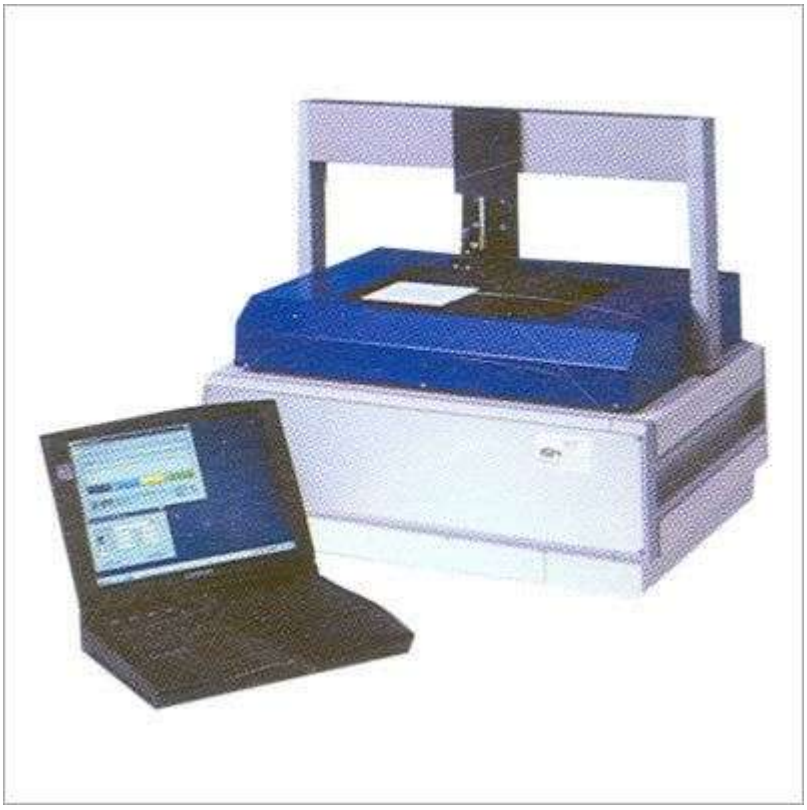

Fig 10: -Scanning instrumentation of HPTLC

III. VALIDATION PARAMETER [7,8]

There are various sorts of validation parameter which are as follows: -

4.1 Linearity and Calibration Curve

Linear relationship between peak area and concentration of the drugs was evaluated over the concentration range expressed in ng band−1 by making five replicate measurements within the concentrations containing the concentrations of the drugs respectively. All the solutions were injected and thus the chromatograms were recorded at 255 nm.

4.2 Precision

Precision of the developed method was studied by performing repeatability and intermediate precision studies. The sample application and measurement of peak area decided by performing six replicate measurements of an equivalent band employing a sample solution containing each. The repeatability of sample application and measurement of peak area were expressed in terms of relative variance (%RSD) and was found to be very low.

4.3 Limits of Detection (LOD) and Quantification (LOQ)

The limits of detection and quantification of the developed method were calculated from the quality deviation of the y-intercepts and slope of the calibration curves of medicine using the formulae as given below.

In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank methanol was spotted thrice and thus the sample was run within the solvent system. Limits of detection (LOD) and quantification (LOQ) were found. Lod

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

was calculated as 3 times the background level, and LOQ was calculated as 10 times the background level. Lod and LOQ were experimentally verified by diluting the known concentrations of drug until the quality responses were approximately 3–10times the quality deviation (SD) of the responses for 6 replicate determinations.
Limit of detection=3α/s
Limit of quantification=10α/s

4.4 Specificity

The specificity of the developed method was established analysing the sample solutions and marketed tablets regarding interferences from formulation ingredients. The spot for drug within the sample was confirmed by comparing retardation factor (Rf) values of the spot thereupon of the quality.

4.5 Sensitivity

Sensitivity of the developed method decided regarding limit of detection (LOD) and limit of quantification (LOQ). Noise decided by scanning blank spot (methanol) six-fold. Series of concentrations of drug solutions were applied on plate and analysed to figure out LOD and LOQ.

4.6 Accuracy

Accuracy of the method was evaluated by carrying the recovery study at three levels. Recovery experiments were performed by adding three different amounts of ordinary drug, that is, 80%,100%, and 120% of the drug, to the preanalyzed MME formulations, solution, and traditional tablets, and thus the resultant was reanalysed six-fold.

4.7 Robustness

By introducing small changes in mobile phase composition, its volume, chamber saturation time, and slight change within the solvent migration distance, the results on the results were examined. Robustness of the method decided in triplicate at a degree level.

VALIDATION PARAMETERS

Type of Analytical Procedure Characteristics	Identification	Testing for impurities		Assay-Dissolution (measurement Only)-Content/Potency
		Quantitation	Limit	
Accuracy	-	+	_	+
Precision	-			
Repeatability	-	+		+
Intermediate Precision		+[a]	_	+[a]
Specificity[b]	+	+	+	+
Detection Limit	-	-[c]	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	_	+

Fig 11: -Validation Parameters of HPTLC

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

Parameter	HPTLC
Linearity Range ng/spot	50-800
r^2±%RSD	0.9992±0.034
Slope±%RSD	7.9758±1.12
Intercept±%RSD	134.02±0.86
LOD (ng spot/spot)	30.191
LOQ (ng spot/spot)	91.697

LOD = Limit of detection, LOQ = Limit of quantitation, RSD = Relative standard deviation.

Fig 12: -Statistical evaluation of calibration data

IV. APPLICATIONS OF HPTLC [1,2,4]

It is most generally applied method for the analysis in pharmaceutical industry, Forensic chemistry,biochemistry,food and drug analysis and other area.[2]Due to numerous advantages another than this it includes low cost, high sample capacity, rapid results and multiple deviations.[1]

5.1 HPTLC IN PHARMACEUTICAL PRODUCTS

HPTLC is additionally utilized in analysing the purity and efficacy of the various pharmaceutical preparations and dosage forms. This method was found to be simple, precise, accurate, selective, and rapid and will be successfully applied for the determination of pure laboratory prepared mixtures and tablets. Similarly, HPTLC method was successfully used to analyse fixed-dose tablets samples.

5.2 HPTLC IN NATURAL PRODUCTS

The HPTLC technique is rapid, comparatively simple, robust, and versatile. HPTLC not only confirm but also establish its identity. it is also an ideal screening tool for adulterations and is extremely suitable for evaluation and monitoring of cultivation, harvesting, and extraction processes and testing of stability.

5.3 HPTLC in control of pharmaceuticals

HPTLC has been used for routine control of topiramate, 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 forms or in pharmaceutical formulation.[56]

Pharmaceutical applications: -

- ➢ Quality control
- ➢ Content Uniformity Test (CUT)
- ➢ Identity and purity checks
- ➢ Stability tests, etc.

5.4 Clinical applications

- ➢ Lipids
- ➢ Metabolism studies
- ➢ Drug screening
- ➢ Doping control, etc.

5.5 Cosmetics

- ➢ Identity of raw material
- ➢ Preservatives, colouring materials, etc.
- ➢ Screening for illegal substances, etc.

5.6 Herbal medicines and botanical dietary supplements

- ➢ Identification
- ➢ Stability tests
- ➢ Detection of adulteration
- ➢ Assay of marker compounds, etc.

5.7 Food and feed stuff

- ➢ Quality control
- ➢ Additives (e.g. vitamins)
- ➢ Pesticides
- ➢ Stability tests (expiration), etc.

International Journal of Pharmaceutical Research and Applications
Volume 5, Issue 2, pp: 343-361 www.ijprajournal.com ISSN: 2249-7781

5.8 HPTLC application in Drug analysis

Sr.no	Drug	Dosage Form	Technique Used	References
1.	Rivaroxaban	Pharmaceutical Dosage Form	Stationary phase: -pre-coated silica gel 60F254. Mobile phase: -toluene: ethyl acetate: methanol [6:3:1] A component spot was obtained with Rf value of 0.44±0.02.	10
2.	Ezogabine	Pharmaceutical Dosage Form	Stationary phase: - Aluminum plate pre-coated with silica gel Mobile phase: - Toluene: ethyl acetate: formic acid [3:6:1] Quantification was achieved with HPTLC detection at 254nm.	11
3.	Teleligliptin Hydrobromide hydrate& Metformin HCL	Combine dosage Form	Stationary phase: - plate pre-coated with silica gel F254 precoated TLC plates. Mobile Phase: - Toluene: methanol: GAA: TEA (5:4:0.5:0.5v/v/v/v). Well resolved peak were obtained for MET &TENE at Rf 0.57 & 0.79.	12
4.	Sitagliptin phosphate and Simvastatin	Combine dosage Form	Spectro densitometric method was developed by the separation of SIM & SITA on silica gel $60F_{254}$. Mobile phase: -toluene: methanol: acetic acid (5:4:1). Spectro densitometric measurement of the bands at 255nm. Rf values for both the drugs were found to be 0.5241 for SITA and 0.7865 for SIM respectively.	7
5.	Saroglitazar	Bulk Dosage Form	Stationary phase: - Aluminum plates precoated with silica gel 60F254. Mobile phase: - n-butanol: ammonia (7:3). Rf value=0.55 ± 0.02.	13

			The spot was densitometrically analyzed at 294 nm.	
6.	Metolazone and Spironolactone	Combine dosage Form	Chromatographic separation was carried out on Merck HPTLC aluminum plates of silica Gel. Mobile Phase: - n-propanol: triethylamine (7:3). Rf value for Metolazone=0.60 & Rf value for Spironolactone=0.69	14
7.	Montelukast sodium and fexofenadine hydrochloride	Combine dosage Form	Separation carried on Merck HPTLC aluminum plates of silica gelG60F254, (20 × 10 cm) with 250µm. Mobile phase: -ethyl acetate: methanol: ammonia (7:3:0.5). Separation of the two-drug followed by densitometric measurement was carried out in the absorbance mode at 215 nm. Drugs were resolved satisfactorily with Rf value of 0.84 ± 0.01 and 0.24 ± 0.01 for MONT and FEXO respectively.	15
8.	Ramipril and Metolazone	Combine dosage Form	Stationary phase: - aluminum plates precoated with silica gel 60 F254. Mobile phase: -toluene: ethyl acetate: methanol: glacial acetic acid (4:4:1:0.2). Densitometric evaluation of the separated zones was performed at 223 nm. The two drugs were satisfactorily resolved with RF values 0.33 ± 0.02 and 0.59 ±0.02 for Ramipril and Metolazone respectively.	16
9.	Metformin Hydrochloride, Atorvastatin and Glimepiride	Bulk Dosage Form	Stationary phase: - aluminum plates with precoated with silica gel 60F254. Mobile phase: - water:	17

			methanol: ammonium sulphate (1: 1: 4). Densitometric evaluation of the separated zone was performed at 237 nm. Rf values=0.37 ± 0.02 and 0.59 ± 0.02, 0.75 ± 0.02 for MET, ATV, GLM respectively.	
10.	Zonisamide	Bulk Dosage Form	Separation carried out by silica gel 60F_{254}. Mobile Phases: -Ethyl Acetate: Methanol: Toluene (4:1:5). Densitometric detection carried out at 254nm. LOD=1.07.	25
11.	Paracetamol and Flupirtine Maleate	Combine dosage Form	Stationary phase: - aluminum plates pre-coated with silica gel 60 F254. Mobile phase: -Toluene: Acetone: Triethylamine (6:4:0.5). Chromatographic analysis was carried out in the reflectance/absorbance mod at 250 nm. Rf value=0.27 ± 0.02 and 0.47 ± 0.02 for Paracetamol and Flupirtine Maleate, respectively.	26
12.	Alogliptin Benzoate	Bulk Drugs and Tablet Dosage Forms	Stationary phase: - Merck HPTLC aluminum sheets of silica gel 60F254. Mobile phase: - Acetonitrile:1% Ammonium acetate in Methanol (4.5:5.5). Densitometry analysis at 277 nm. The reliability of the method was assessed by the Evaluation of linearity 500-5000ng/spot for Alogliptin Benzoate	27
13.	Diphenhydramine Hydrochloride and Naproxen Sodium	Tablet Dosage Forms	Stationary phase: - aluminum plates pre-coated with silica Gel60F254. Mobile phase: -Toluene: Methanol: Glacial acetic acid (7.5:1:0.2).	28

			the rf value for DPH is 0.20 ± 0.05 and for NPS is 0.61 ± 0.06. % recoveries of DPH and NPS was in the range of 99.70%–99.95% and 99.63%–99.95%, respectively. Limit of detection value for DPH was 13.21 ng/band and for NPS was 8.03 ng/band. Limit of quantitation value For DPH was 40.06 ng/band and For NPS was 24.34 ng/band.	
14.	Alfuzosin, Terazosin, Prazosin, Doxazosin and Finasteride	Pharmaceutical Dosage Form	This work presents a simple, sensitive and fast generic HPTLC method for the simultaneous determination of five drugs prescribed for the treatment of BPH. These drugs include the a1-Adrenergic blockers; Alfuzosin Hydrochloride (ALF), Terazosin Hydrochloride (TER), Prazosin Hydrochloride (PRZ) and Doxazosin Mesylate (DOX) in addition to the 5a-reductase inhibitor; Finasteride (FIN). The cited drugs were separated on TLC-silica plates. Mobile phase: -Methylene chloride: n-Hexane: Methanol (8.8:0.3:0.9). Densitometric analysis was carried out at 254 nm for the a-blockers while fin was measured at 220 nm. Rf values = 0.26, 0.36, 0.45, 0.59 and 0.69 for ALF, TER, PRZ, DOX and FIN, respectively.	29
15.	Piperine in Sitopladi Churna - An Ayurvedic Formulation	Bulk Dosage Form	Alcoholic extract of Sitopladi churna&Pippali fruit sample were applied on TLC aluminium plates pre coated with silicagel60GF254.	30

			Mobile phase: -Toluene: Ethyl Acetate (9:1). plates were sprayed with Anisaldehyde-Sulphric acid followed by heating at 110°c for 10 min & detection & quantification were carried out densitometrically using an Uv detector at wavelength of 254nm.	

V. CONCLUSION: -

A simple, accurate, precise method based on HPTLC method has been developed for routine analysis of the samples. And this method was validated for linearity, precision, accuracy, robustness and specificity. HPTLC method has considerable advantage over the other analytical technique like require small mobile phase, larger sample capacity, multiple or repeated times. This method can be used for therapeutic drug monitoring in order to optimize drug dosage on an individual basis and are able to measure bioavailability studies and dose regulation.

VI. FUTURE PROSPECTIVE

Utilization of instrumental HPTLC toward the analysis of Drug formulation, Natural Products, clinical samples, food stuff, environmental & other relevant samples will increase in thefuture.

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