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A Comprehensive Working, Principles and Applications of Thin Layer Chromatography.

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ABSTRACT

In this present article, we address the basic aspects such as idea, mechanism and working of Thin layer Chromatography (TLC) in analytical as well as preparative preparation methods. We have gone through diverse journals for gathering complete package of TLC and found that TLC is very simple, easy, less time consuming, cost-effective and multiple samples could be run in one go hence, is constantly the first choice for varied application in qualitative analysis of pharmaceutical products. In this modern scientific world were HPLC and HPTLC technology has developed still TLC holds good promise for identification and analysis of different bioactive compounds, secondary metabolites, Vitamins and amino acids. It is a very preliminary analytical method done prior to HPLC and reaction progress can be monitored easily. It can be used for separating compounds from crude extracts and separating impurities from a compound. Identical compounds from the mixture can be easily separated by analytical and further by preparative TLC. Many standard methods in industrial chemistry, environmental toxicology, steroids, food chemistry, water, inorganic pesticide, dye purity, cosmetics, plant materials, and herbal analysis rely upon TLC as the preferred approach. Hope this review article will help in understanding principal and working of TLC in the field of research.

Keywords: TLC, analytical TLC, preparative TLC, solvent system, capillary action.

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INTRODUCTION

Plants are the human best friend providing natural valuable herbal medicine for curing various ailments [1] and a very established field that covers a study of plants is called as botany. Plants had never discriminated any global nations for their potent efficiency of compounds such as secondary metabolites, alkolides, flavonoids,quinines, terpenoids, tannins, saponins, carbohydratesphytoalexin, amino acids, compounds with tumor inhibition and anti-inflammatory property [2]. Study of these important compounds comes under organic chemistry and plant biochemistry. Recently, phytochemistry has been the word used to define work undertaking study of these pharmaceutical compounds. Scientists and researchers have constantly been using plant species for identification and extraction of phyto constituents with enhance curing and medicinal ability. In order to obtain these compounds in the pure form from the raw extract they need to be separated from raw extract by using available techniques such as super critical fluid extraction and soxhlet. The extracted crude samples are then needed to be further analysis using analytical techniques. TLC is most common and efficient techniques used for detection and analysis and separation of phytoconstituents compound, and it is estimated that 60% of analyses are performed based on TLC over worldwide. Thus, it is very important to know basic working and functioning of TLC [3]. Initially, TLC optimization of the solvent system for development and purification of secondary metabolites or other compounds from plants is a very crucial step [4]. thereby in this article we try to assemble some of the important aspects such as principle, apparatus and working of TLC.

Chromatography

In order to produce best possible source paper for TLC, we have performed immense literature and journal's search such as Journal of Planar chromatography modern TLC, Journal of chromatographic science, World journal of pharmacy and pharmaceutical sciences, Journal of lipid research, google scholar search engine, NCBI and in certain cases assess to article was denied there we used abstract content to unpack creamy valuable information. We concluded that there are large numbers of research article published for TLC and which confirms that TLC is immensely used for the experimental purpose in pharmaceutical, drug, cosmetology, analysis of natural, synthetic steroids, heavy petroleum products, amino acids and phospholipids [5], herbal products, environmental pollutants, dyes [6] and food industries. In the current, upgraded name of TLC is planar chromatography. The exact definition of chromatography is “the separation of two or more compounds by distribution between two phases. One is stationary and other is a mobile phase." Chromatography was discovered by M. Tswett in 1906 and in 1938, Izmailov and Shreiber introduced TLC with some modification. It is a very preliminary, microscale technique that is used prior to advanced complex techniques and historical known before 100 years [7], In 1951, Kirchner et al modified and applied absorbent on glass plate and Shahl in 1956 uses a spreader for preparation of uniform layer of TLC [8]. Even nowadays in this modern world of technology TLC remains 1st choice for various compound analyses [23] TLC may be used to separate single constituents from mixtures of compounds that may be a future potent drug.

TLC has been important for separation of secondary metabolites, Polyphenol, alkaloids, saponin, flavanoid, flavanone, amino acids, aromatic amines, acids, alcohols, glycols, amides, proteins, peptides, antibiotics, porphyrins, bile acids, pesticides and vitamins in soft drinks. Steroids such as sterols, sterone, cholesterol, hormones, estrogen, progesterone, bile salt and synthetic steroids such as mifepristone, oxandrolone [9] Other Steroids, such as nandrolone, dromostanolone, stanozolol, are defined illegal and are banned in sports of Olympic games are also analyzed by using TLC. Simple, rapid and sensitive thin-layer chromatography (TLC) assay has been developed by [10] for analyzing bacterial cultures for the presence of auto inducers. This assay works on signals that hold separation by thin-layer chromatography for detection with *Agrobacterium tumefaciens* harboring *lacZ* fused to a gene that is regulated by auto induction. The Review describes by [11] gives an overview of the static phases used in TLC assuming attractive forces between the mobile and the static phase. For TLC, a Traditional and modern sample preparation method is explained in detail by [12]. Several influential aspects that need to be taken into construction for formulating a solvent system for separation and isolation of various compounds explain by [4] Research studies by [13] shows that TLC is an important investigative method for analysis of hydrophobic vitamins. Vitamins, A, D, E, and K have been successfully quantified using TLC. A quick, economic, and reliable thin layer chromatography (TLC) method for rapid screening of tuberculosis pharmaceuticals has been given in details by [14]. Comprehensive details of TLC systems and stationary phases for analysis of natural and synthetic steroids from plant product, blood serum and urine has been given by [9]. Use TLC for separation of plant photosynthetic pigments [15]. Describe shows that thin-layer chromatographic video imaging may be useful for analysis of sample along with other analytical techniques. They study 20 species of Salvia genus for composition of essential oils, phenolic acids, and flavonoids. They found that six species have the highest contents of acidsic acids and five species with the highest contents of flavonoids. In the year, 2011, TLC has been used successfully for detection of endosulfar poisoning [16] in Aurangabad Regional Forensic Science Laboratory [17]. Describes those porphyrins which naturally occur in meat Identified using TLC, for example, hemin, protoporphyrin IX (PPIX), and zinc (II) protoporphyrin IX TLC,(II)PPIX) [18]. Explains principal and steps for performing TLC with details of prepration and tips and troubleshooting solution.

TLC is a primary, easy to use and solvent used are unhazardus with no requirement of sophisticated instruments [18-20]. Usually It is composed of stationary phase and mobile phase, which are performed on a sheet of solid surface such as glass, plastic, aluminum foil that is coated with absorbent material such as silica powder, aluminum oxide and cellulose, which is called as stationary phase [11]. Mobile phase may consist of single or mixture solvents depending on extracts to separate. This mobile phase is drawn up through the stationary phase by capillary action allowing separation of various compounds on the basis of their solubility and retardation in stationary phase and mobile phase. Separation is achieved by competition of the solute molecules and the mobile phase for binding places on the stationary phase. The most common stationary phase used is a silica gel which is polar in nature, in case if two compounds in extracts have different polarity, highly polar compound will have strong interaction with silica and separated out initially in no time. Fewer polar compounds will separate in second position

that has little interaction with stationary phase. On contrary, non polar compound will separate last, which would have non interaction with the stationary runs a longer distance on the plate.

The complete understanding of handling TLC is handy for designing research to analysis, separate medicinal significant compounds. Optimization of the solvent system for TLC profiling for identification of amino acid, amines, alkaloids and secondary metabolites of cureable plants is highly useful for production of medicinally prominent medicines and novel pharmaceutical products.

In this paper, we have discussed the principle, working, techniques, advantage's disadvantages and applications of TLC technology that will certainly help to identify, quantify and purify future novel pharmaceutically importance compound of therapeutic purpose.

Principle of TLC

TLC principle works on a solubility rule "Like Dissolves Like" and is followed on separation of mixture of polar, non polar, mid polar compounds from the extracts on a static phase (silica gel) and movable phase or combination of movable phase such as ethyl acetate, chloroform, hexane, and methane that runs on static phase. 2ul-5ul crude extract (mixture of compounds) is spotted at 1cm position from the bottom of TLC plate using a capillary spotter. The plate allowed to develop in developing chamber with a suitable movable phase or combination of movable phase, which is of appropriate level well below the spotted sample applied. The mobile phase is drawn up through the stationary phase by capillary action [18]. Few compounds in mixture would dissolve in mobile phase and goes up the plate conversely some compound in mixture will remain on the stationary phase. The movement of compounds from the mixture relies on the physical properties, molecular structure, and functional groups. Suppose the physical property of compound from mixture is similar to the mobile phase the compound will remain longer in mobile phase and will travel a longer distance on TLC plate [11]. The compounds that are not as much soluble in mobile phase will have an affinity for stationary phase and will travel to a smaller extent than the soluble compounds [21].

An Rf value is "retardation factor" or "ratio to front" which can be calculated by using the formula.

$$Rf = \frac{\text{Distance traveled by compound.}}{\text{Distance traveled by solvent front}}$$

These Rf values can be calculated by observing spots on TLC plates under UV transilluminator at 365nm [22]**.** The compounds travel from origin spotting position and distance traveled by solvent front is noted. Then the given formula would give the R*f* value for the compound. Identical molecules will invariably travel the equivalent distance under similar temperature, solvent system and stationary phase. However, the molecules traveled at same position always may no longer be the identical compound. Supplementary supporting data is

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needed before coming to the conclusion. Figure 3 shows various bands of secondary metabolites separated on TLC by using solvent system chloroform: ethyl acetate: benzene: glacial acetic acid (25: 15: 2: 10). TLC chamber design may play a vital role in identifying bioactive metabolites, which ranges from 100ml to 100ml closed chamber [23]. An Rf value occurs between 0 – 1 and depends upon following factors, which determine the efficiency of a chromatographic separation.

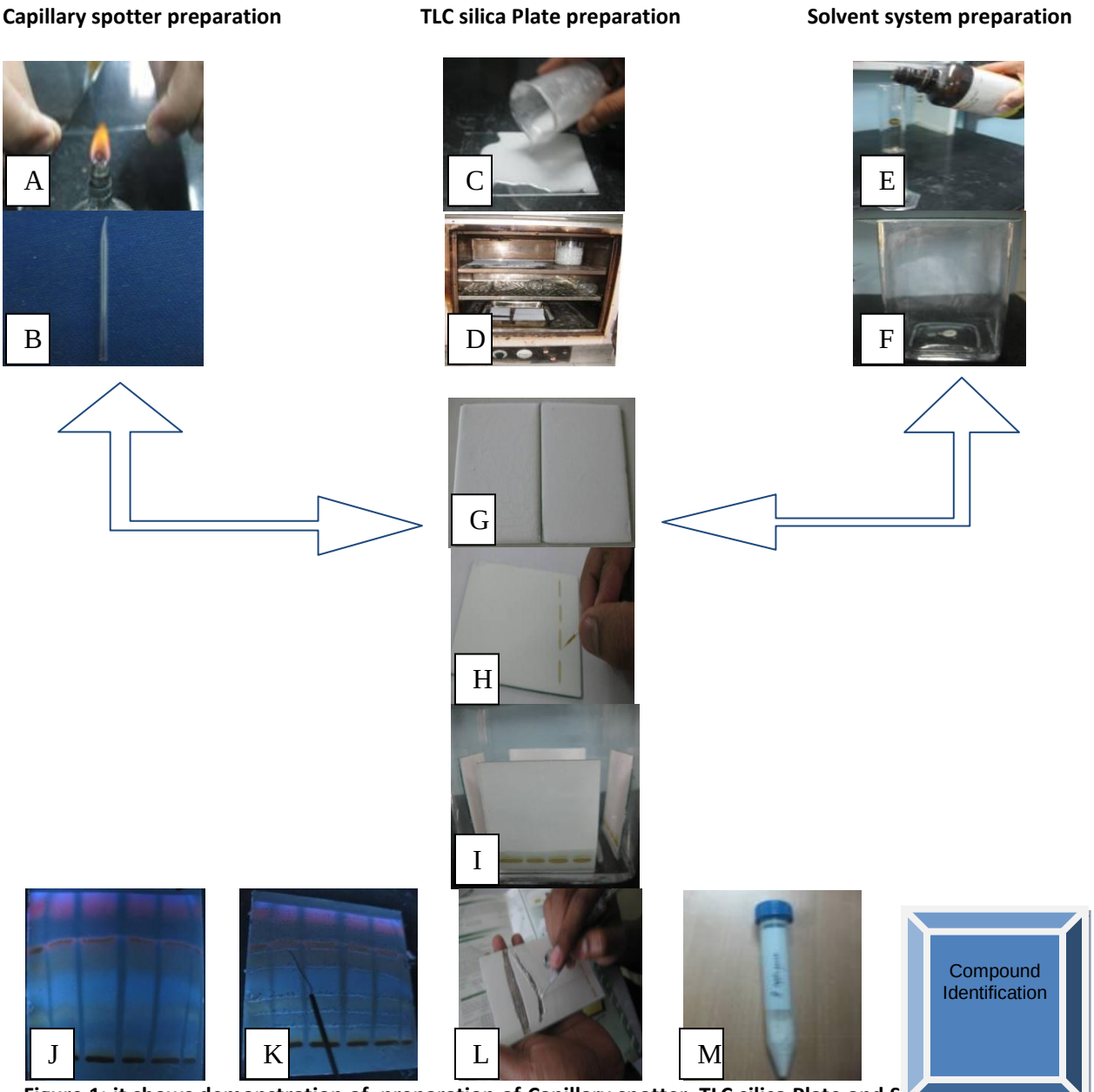

Figure 1: it shows demonstration of preparation of Capillary spotter, TLC silica Plate and S Preparation of capillaries. C-D: Preparation of TLC plate using Silica. E-F: Preparation of selected Solvent system. G: Silica plate. H: Spotting of sample. I: Developing of TLC plate in developing chamber. J: TLC plate under UV transilluminator at 365nm. K: Marking of the specific band from TLC under UV transilluminator. L: Scraping of the specific band from TLC under transilluminator at 365nm. M: Separated compound obtained from TLC.

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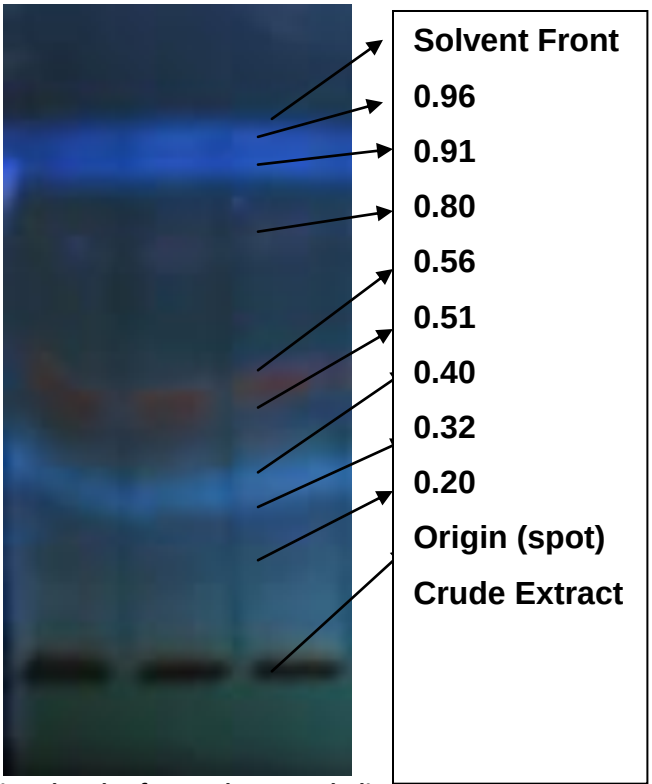

Figure 3: It shows various bands of secondary metabolites separated on TLC by using solvent system chloroform: ethyl acetate: benzene: glacial acetic acid (25: 15: 2: 10), Rf values of all bands are marked with solvent front and sample spot.

Nature of absorptive: silica gel with 60 -120 mesh size is one of the most common absorbent used. This absorbent usually is polar in nature and used for separation of compounds [21], while other absorptive are companies made aluminum TLC plates. All available distinctive absorbent will fetch alike compounds in the same solvent system with different R*f* value [24]. If absorbent is a polar, then compound with polar nature will travel fewer distances as it will have polarity for polar absorbent. Absorbent must dry in oven at 110°C for 1 hr to remove water vapors so that water will not interfere with separation of sample.

Mobile phase: Mobile phase must be without contamination and in appropriate volume for producing reproducible results. All the time fresh mobile phase/ solvent system must be prepared for running every new sample [24]. Concentration of a mixed solvent system, for example, ethyl acetate: chloroform: methanol must be mixed in appropriate volume. TLC can be run at room temperature. However, developing chamber must be kept away from direct sunlight and heat. Temperature may cause sudden evaporation of volatiles solvents such as methanol, acetone, ethanol, chloroform, ethyl acetate, benzene [21]. If the solvent system gets direct contact with heat, its volatile solvents may be vaporized and their concentration in the system may decrease that will ultimately hamper separation of compounds.

Thickness of layer: The layer thickness must be of approximate 250 micrometers. For analytical TLC, the thickness is less and for preparative thickness is more.

Developing tanks: Developing tanks are of varying size example 250ml, 500ml, 1000ml beakers with closing caps from top [24]. A simple beaker of varying size cover with aluminum foil can also be used. The developing tanks used must be allowed for saturation for 15 min by using a selected system for efficient separate.

Mass of sample: Mass of sample effect on compound separation by reducing R*f* values. If higher mass of sample is loaded on TLC, then Rf value will decrease and the separated compound will form smear. Thus, sample must be loaded of minimum quantity.

Apparatus and working

TLC consists of TLC glass plates, stationary phase, solvent system, aluminum foil, chromatographic chamber. It is very important to run the identical sample in duplicate in same TLC plate, in same concentration and in the same chromatography chamber. This will eliminate the chance of confusion for identification of unknown compound with the known compound. Thus crude samples are always loaded in duplicate for constant and reproducible results. This will help to sort precise comparison of movement of various compounds that when run along a marker compound.

TLC plate Prepration / Stationary phase TLC plate is prepared by using approximate silica powder (polar) in appropriate ml of distilled water and is continuously stirred using glass rod to form a slurry [11]. Thin slurry is good for analytical TLC and silica layer on the glass plate must be thick for preparative TLC analysis [21]. When stationary phase is silica, which is polar then it is known as standard, and if it is non polar, then it is known as reverse phase [24].

TLC plate size: truly, the size of TLC plate is not an important issue, and it doesn't have any relation with resolution of compounds. Conversely, it will affect the developing time of TLC. Large-size plate will take more time and solvent to complete run on TLC and small time will have reverse effect. 6.5 cm length by 2.5 - 5 cm width is enough to produce good results in a minimum amount of time. A plate larger than 6.5 cm length will require more time to run. "Preparative separation of bile acids by adsorption chromatography, has been achieved successfully using the plates of dimensions 5 x 20 size in measuring jar of 550 cm3" [25].

Developing Silica gel, TLC plate: Silica TLC plate is developed by pouring slurry of silica with water, once slurry is viscous and thin it is poured on TLC glass plates. These plates may vary in size. It is then allowed to solidify at room temperature. Further it is allowed to dry in hot oven incubator at 110° C for 1 hrs. This developing of plates is essential step as it removes excess of water used to make silica plate. If these droplets are not removed they may interfere with the compound separation and regularized pours will not obtain, which may hamper separation of compounds [21]. Silica gel is having electropositive silica and electronegative oxygen, which make silica strong polar stationary phase. Thus, the more polar the molecules in mixture they will exert more attractions to stationary phase and non polar will have fewer attractions to stationary phase.

Capillary spotter preparation: Capillary spotter is prepared by holding the capillary tube from both the ends in the blue flames of Bunsen burner and when the capillary is softly melted is taken off from burner and is polled in appositive direction to form micro thin tipped capillary spotter for sampling.

Spotting of sample: Depending upon the requirement of an experiment, the sample is dissolved, in particular, solvent to dissolve it completely and is spotted on a TLC silica gel plate. Figure 1H indicates spotting of crude extract on TLC and TLC running in the solvent system with moving solvent front is observed in Figure 1. I. In order to spot the sample the capillary end is touched to the silica gel with several repetitions. If it is analytical, 2-5µl of crude sample is enough to spot on a silica plate of TLC [26] (**2)**. Conversely, if it is preparative 15-20 µl of crude extracts is loaded on silica TLC for maximum isolation of a potential band (color) by Visualizing under UV transilluminator [22]. Small sample application will result in good separation, were it will minimize overlapping of spots. However, if the sample is diluted, repeated application of sample on the same spot is applied with a second paused in next application in same place. This will allow the solvent, to evaporate in which extract is dissolved and adjacent spot is not overlapped. While spotting the sample care is taken for not disturbing a stationary phase absorbent which will result in an uneven flow of solvent as well as a sample.

Position of spotting at baseline of TLC plate: crude sample that is obviously dissolved, in particular, solvent, is pulled up in the capillary spotter by capillary action. Crude sample is spotted above 1 cm from baseline of silica TLC plate. The spotting of sample must be enough above such that spotted sample should not get wet in the running solvent or mobile phase of a solvent chamber [26].

Development Chamber: Once the crude sample is spotted on TLC plate. The plate is ready to transfer in developing chamber [27]. This developing chamber previously equilibrated with suitable solvent system and its vapors. TLC previously equilibrated chamber in vertical position such that stationary phase is in contact with mobile phase [24]. Figure 1 F show desktop developing chamber. Small, medium, large size large-size 0ml, 500ml, and 1000ml may be used as developing chamber. To the top of beaker aluminum foil is wrapped and cover it with glass plate.

Running solvent/ mobile phase: Depending upon the compounds in a solvent tract the choice of a solvent system is made. Water (polar) is universal solvent dissolving most of the compounds in it. Chloroform is non polar. Ethyl's acetate is mid polar. Separating chamber is usually allowed to get saturated with vapors of solvent / mobile phase. The phenomena of like dissolve like applied here. Compound that is polar will be dissolved in polar solvent, compounds that are non polar will dissolved in non polar solvent system and most of the compound, which are polar and non polar has an ability to get dissolved in mid polar solvent such as ethyl acetate. Sometime combinations of mobile phase are used to separate polar, nonpolar, mid polar compounds. Solvent system used for separation of stress induced metabolites is chloroform: ethyl acetate: benzene: glacial acetic acid (25: 15: 2:10) has been optimized by [28] for medicinal important *passiflora foetida*. There are many reports acknowledging the separation

of compound performed by using combinations of a solvent system [21]. Optimizing good solvent system is very important and the most difficult level of TLC. Basically start optimizing with non polar solvent and observe the separation if compounds dosent move to fast add polar solvent. Now compare this plate with the previous plate. If the spot stays at its original site add more of the polar solvent conversely if the spot runs with solvent front try adding non polar solvent. Once solvent front reaches the maximum end of TLC plate this means sample are fully run on TLC. TLC plates are removed from developing chamber and are allowed to dry of solvent/ mobile phase completely. Polarity of solvents depends upon the dielectric constant more the dielectric constant more polar the solvent system. Some examples of solvent system along with dielectric constant are given below.

Solvent	Notation	Dielectric Const
Hexane	H	1.9
Petroleum Ether	PE	2.0
Cyclohexane Cy		2.0
Carbon Tetrachloride		2.2
Benzene	B	2.3
Toluene		2.4
Diethyl Ether	DE	3.4
Chloroform	Ch	4.8
Ethyl Acetate	EA	6.0
Acetic Acid	AA	6.2
Isopropyl Alcohol		18.3
Acetone		20.7
Ethanol		24.3
Methanol	M	32.6
Water		78.5

Visualization: Crude extract is initially dissolved in suitable solvent in which extract dissolved completely. Test crude sample (10 µl) spotted onto the silica plates. Plates were developed in the saturated vertical chromatographic chamber saturated by 1-100ml solvent system (as per requirement) [12]. After 30-90, min plates were removed from developing chamber and immediately visualized under 365 nm in (UV) Ultra-violet transilluminator. Numbers of distinctive colors spotted on a plate, each indicates distinct compound. If extract contains to dye or inks, then the visualization would be very easy, however, organic compounds are color less. In this case, the plate is kept in jar containing iodine crystal's most organic compounds absorb iodine vapors within 2 minutes and become visible.

Analysis: Individual band traveled distance is measured under UV transilluminator and R*f* factor retardation factors (R*f*) were measured against solvent front and compared with the standard retardation factor values given in literature [29]**.** Alternatively, the standard compound with known R*f* value is applied along with the separating samples. Further screening & selective separation for antimicrobial activity on TLC may be performed.

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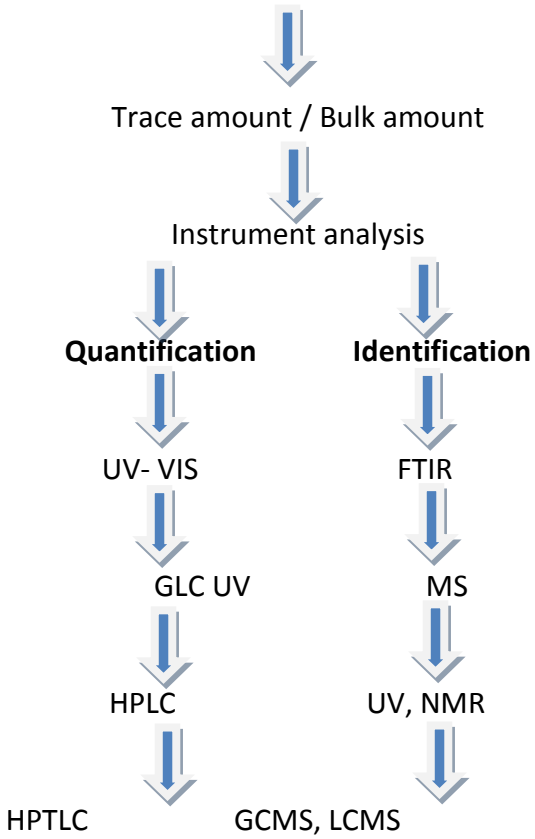
Analytical TLC: It is used for analysis and identification of various compounds that are run on TLC plate observed under UV transilluminator. Quantity of sample loaded is very less (5µl-10µl) due to preliminary analysis of identification of secondary metabolites and other compounds [18].

Preparative TLC: It has been used for isolation of identified specific compound that was previously observed under UV transilluminator [18]. Column chromatography would be used for obtaining a compound in grams. In case of preparative TLC, sample is spotted in maximum quantity (30ul-100ul) on the TLC silica plate (coated 1-3mm silica) for scraping out of the interest band from the separated bands. Secondary metabolite isolated in large quantity is further processed for UV spectrophotometer, GCMS, FTIR, NMR and mass spectroscopy. Figure 2 shows schematic possible steps for identification of novel drugs and some of the bioinformatics tool for analysis of drug efficiency.

Figure 2: It shows a schematic diagram of possible steps for identification of novel drugs



Bioinformatics Tools for possible effectiveness and efficacy of drug to target molecule

Preadmit
Schrodynger
Discovery Studio

AutoDock

NOTE: FTIR (Fourier transform infrared spectroscopy), UV (Ultraviolet visible spectroscopy), MS (Mass spectroscopy), GLC-UV (Gas liquid chromatography), HPLC (High Pressure liquid chromatography), NMR (Nuclear magnetic resonance), HPTLC (High-Performance Performance thin layer chromatography), GCMS (Gas chromatography mass spectroscopy), LCMS (Liquid chromatography mass spectroscopy).

Applications

TLC has been used for various analyses in pharmaceutical and drug industries. **Table 1 gives detail of** thin layer chromatographic analysis of various synthetic, natural, plant and animal products indicating stationary phase and mobile phase used for their separation along with references. Some of the few examples are a detail below.

TLC of secondary metabolites: plant secondary metabolites are naturally producing compounds, which are not of earliest importance to plants, on other sides, they are subordinate means they are not involved in original metabolism. Secondary metabolites are produced on external stimuli, abiotic stress [30-31] biotic stress, injury, heat, cold, etc. some of the important secondary metabolites are alkaloids, esters, flavonoids [32], isoflavonides, Phytoalexins, Tannins, Salicylic acid, lignin. All these metabolites are identified from plant explants by using TLC. Stress conditions on plant's results in production of secondary metabolites from plant such as amino acid example proline, quaternary amines such as poly amine, different sugar and alcohol [33]. All these secondary metabolites could be analyzed using TLC. Different kinds of the metabolites are used for formulation of medicines and cosmetology. Secondary metabolites isolated and purified may be used for antimicrobials test, compound structure identification, MS, FTIR, and GCMS. It can also be used for improving organic farming for better crop results.

TLC of amino acid: Various chromatographic systems were developed for analysis amino acids that were discussed in various articles and few were presented by [9]. TLC of secondary metabolites is easy as compare with amino acids because secondary metabolites are colored, however, amino acids are colorless making them tougher to be visualized by naked eyes. The ninhydrin or the black-light visualization techniques is basically used for observing amino acids [34]. Several amino acids, proteins and peptides have been successfully separated and isolated from urine using silica gel plates. All these substances were found to be ninhydrin positive. The developments were carried out first with chloroform-methanol-20%ammonium hydroxide (2:2:1) and then with phenol-water [35] describes a simple fast procedure for extraction, separation, and quantitative estimation of amino acid from plant tissue.

TLC of phospholipids: Phospholipids and glycolipids from plants have been analyzed by using TLC with solvent system chloroform-methanol-water (75:25:2.5) from plant [36]. Same solvent system with varying ratio has been used to analyses Phospholipids pooled rat livers. Other common mobile phases are Triethylamine, ethanol, hexane and isopropanol. To improve the separation of phospholipids a couple of chemical modifications can be made to the silica gel. Many reagents can be used to detect phospholipids such as 3% Copper acetate, Iodine, 8%

phosphoric acid and Molybdenum blue [37] Method is simple, rapid and reliable for determination of phospholipids with average recovery of 100-8%.

TLC of prostaglandins: TLC has been used for separating prostaglandins derivatives, for example, prostaglandin E1, (PGE1) and prostaglandin FI, (PGF1,) from sheep vesicular glands and Prostaglandins El. E2, and EI were obtained from sheep vesicular glands and are separated using TLC [38].

Pharmaceutical and drugs: Antibiotics Penicillin's have been separated on silica gel 'G' [12,3] by using the two solvents, acetone- methanol (1:1) and iso-propanol-methanol (3:7). As the detecting agent, the iodine-azide reaction could be employed by spraying the dried plates with a 0.1 % iodine solution containing 3.5% of sodium azide. Several other drugs have been separated and isolated using TLC.

Cosmetology: In the identification of dye raw materials and end products, preservatives, surfactants, fatty acid, constituents of perfumes TLC has been used in cosmetology.

Clinical chemistry and Biochemistry: A detail application of TLC in clinical chemistry is given in paper by [39] for the determination of active substances and their metabolites in biological matrices, diagnosis of metabolic disorders. TLC serves as a useful tool in analysis of the urinary constituent derived from lipids in analysis of many urinary constituents such as steroids, amino acids, porphyrins and bile acids. Urinary analysis by TLC is most effective when done in conjunction with other chromatographic processes, so that minor metabolites can be detected and resolved completely free of other components.

Food analysis: TLC used for the determination of pesticides [12] and fungicides in drinking water, residues in vegetables, salads and meat, vitamins in soft drink [34] sandalwood extract in fish and meat products) aflatoxins in milk and milk products [40] describes a good review explaining TLC analysis of products, foods, beverages, and plant constituents explaining in details of a solvent system used and mode of detection.

Analysis of heavy petroleum products: Thin-layer chromatography (TLC) is commonly used for analysis petroleum products and coal products. In particular, For such a data, TLC is the simplicity and economy [41].

Separation of aromatic amines: Aromatic amines have the main role in formation of variety of dyes used in textile, leather, plastic and paper products. Cationic and non-ionic surfactant-mediated systems shall be use as mobile phases in thin-layer chromatographic for separation of aromatic amines on silica gel layers [42].

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Table 1: It shows Thin Layer Chromatographic analysis of various synthetic, natural, plant and animal products indicating stationary phase and mobile phase used for their separation along with references.

Sr no	Analyte	Stationary phase	Mobil phase	Remarks	Reference
1	Androgens and gestagens	Silica	Cyclohaxane/ethylacetate/ethanol (24:16:1). chloroform/benzene/ethanol (36:4:1) in one direction; chloroform/acetone (9:1) . hexane/dichloromethane/acetronitrile (4:3:2) in second direction for androgens and gestagens respectively	HPTLC separation of anabolic Androgens. Detected by fluorescence after immersion in a 5% sulfuric acid-ethanol solution for 30 sec and viewed under UV366nm	(43)
2	Androsterone, epiandrosterone, testosterone, etc.	Silica RP-18W	Methanol/water and acetonitrile/w ater(in Different compositions)	Lipophilicity of selected steroids Was determined by RPHPTLC. Lipophilicity values were estimated by Computational methods. Detected by spraying with sulfuric acid/methanol(1:9) and heating at 120oC for 15 min.	(44)
3	Bile Acid	Silica	Diethyl oxalate-dioxane, (40:10) Diethyl oxytlaite-isopropyl alcohol (48:8) Cyclohexane-ethylacetate: aceticacid (10:15:4) Benxene-isopropyl alcohole- acetic acid (30:10:1)	acid solvent systems are superior to basic and neutral solvents for analyzing completely unknown mixture of bile acids	(25)
4	Flavonoid glycosides	Silica	t-butanol-acetic acid-water (3:1:1) n-butanol-acetic acid-water (4:1:5) Water-methanol-ethylmethyl ketone-acetylacetone (13:3:3:1) Ethyl acetate-pyridine-water-methanol (80:20:10:5); best for flavones C-glycosides		(32)
5	Nonpolar flavonoid aglycone (dihydroflavonoids, isoflavones and methylated flavones)	Silica	Acetic acid (10%-30%) Chloroform-methanol (15:1 to 3:1)		(32)

6	phospholipids and glycolipids from plant	Silica	chloroform-methanol-water (75:25:2.5)	separated by first developing the plate in chloroform-methanol-water (75:25:2.5, by volume) in the first direction. After allowing sufficient time for drying, the plate is developed, at right angles	(36)
7	Phospholipids from pooled rat liver	Silica	chloroform-methanol-acetic acid-water (25:15:4:2, by vol.).	Forty g. of Camag (Muttenz, Switzerland) silica gel, without calcium sulphate binder, was slurried with 90 ml. of 1 mM-Na2CO, solution and transferred to the applicator. The plates (200 mm. x 200 mm.) were prepared in the usual manner and The spots were detected with iodine vapour	(37)
8	Polar flavonoid aglycone (flavones, flavonols)	Silica	t-butanol-acetic acid-water (3:1:1) Methanol- acetic- acid water (18:1:1) Toluene-pyridine-formic acid (36:9:5) Chloroform-acetic acid-water (35:15:2)		(32)
9	Progesterone, testosterone, testosterone hydrogen sulfate sodium salt, etc.	Silica	Methanol/ethylacetate/chloroform/ methylenechloride (first inverse gradient program) and methanol/chloroform (second inverse gradient program)	Programmed multiple development of analysis of steroids. Detected under UV 254.Densitometry was used for the quantification.	(45)
10	prostaglandins and derivaties such as PGE1-278, PGE2-278, and PGEs-278), PGE1-278-Me, PGE2-278-Me, and PGE3-278-Me are methyl esters of compounds	Silica	Benzene-dioxane 5 :4, Ethyl acetate-methanol- water 8:2:5, Ethyl acetate-methanol- water 16: 25:10		(38)
11	Secondary metabolites	Silica	Chloroform: ethyl acetate: benzene: glacial acetic acid (25: 15: 2: 10)	Separates approximates 7-9 stress induced secondary metabolites on TLC profiling	(28)*
12	Steroids	Silica	Chloroform/ethanol/water (188:12:1)	Detection under UV.Quantification by radioimmunoassay	*(46)*
Note: (28) reference is unpublished data*					

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Advantages of TLC

- **Microscale techniques:** only few milligrams of extracts is enough to run on TLC plate for analysis and identification.
- **Rapid identifier:** Sample identification in short time.
- **Easy to monitor**: a chromatography separation reaction.
- **Easy determination:** Number of compounds in a mixture could be easily determined.
- **(Bioautography):** Identification of antimicrobial compounds can be done readily of TLC profiled plates. Here pouring potential test microbes (E.coli, S. aureous) cultures along with agar on resolved TLC plates can yield the inhibition band on TLC this will confirm an antibacterial activity.
- **Spray reagents:** The developed, dried TLC plates are used for spraying. 5 – 10 ml solution of spray is sprayed from 10- 15 cm distance in the even manner over the surface of TLC. Excessive spraying of reagent is not recommended.
- Resolved TLC plates can be stored for long duration time.
- Separated compounds may be subjected to FTIR, IR, MS GCMS, LCMS, NMR.

Problems in TLC

- **Large spots** huge spotting of crude sample causes inappropriate separation of compounds that will result in smear formation and smudging.
- **Bands pungent curves:** many times compounds on TLC plate are separated with a very pungent curving instead of proper straight bands. This may be due to concentrated sample or may be due to previously used solvent system.
- **Uneven leveling of spots:** results in unevenly distribution of sample on stationary phase.
- **Uneven advancement of solvent front:** TLC plates that are not made evenly, and silica gel is not spread evenly then the solvent front run in disturb manner affecting movement and separation of compounds.
- **Smear formation:** a developed TLC plate sometimes shows smear formation this may be due to the unequal distribution of molecules between stationary and movable phase or may be due to mix solvent/ mobile phase.
- **Plate position:** it is very important to keep the plate in vertical position in the chromatography chamber. Slight dash may led to fall of TLC plate in solvent system spoiling whole plate and sample.
- **Uneven level of developing chamber:** the baseline of developing chamber must be a plane, if it is unlevel the solvent front will not travel in equal level over TLC affecting separation of molecules.

CONCLUSION

- TLC is rapid, cost effective, inexpensive and multi compound loading system makes a reliable universal technique use to determine animal, plants and synthetic products.

- TLC globally is the first most solutions for analysis of herbal, potent constituent, drug, steroids, amino acid and secondary metabolites.
- Numerous optimized systems have been standardized for effectively screening of qualitative pharmaceutically important compounds.
- With the advancement of technology, TLC has been successfully integrated with other techniques for precise and quality detection of drug potential compounds.

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