Asian Journal of Applied Chemistry Research

Volume 14, Issue 3, Page 23-38, 2023; Article no.AJACR.107435
ISSN: 2582-0273

Exploring the Word of Thin-Layer Chromatography: A Review

Khalid Hameed [a*], Muhammad Shoaib Khan [a], Ayesha Fatima [a], Syed Mudassir Shah [a] and Muhammad Ali Abdullah [a]

[a] Department of Chemistry, University of Education Lahore, Faisalabad Campus, Pakistan.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2023/v14i3268

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/107435

Review Article

Received: 07/08/2023
Accepted: 12/10/2023
Published: 17/10/2023

ABSTRACT

A common analytical method in chemistry for separating and identifying mixture ingredients is called thin-layer chromatography (TLC). A thin coating of stationary phase, often silica gel or alumina, is put into a sample, which is then placed on a flat substrate like a glass plate. The sample is then exposed to a mobile phase, often a solvent, that follows capillary action through the stationary phase. Thin-layer chromatography may be used to determine the chemicals that are present in a particular sample and track the development of a reaction. Additionally, similar substances in a mixture may be separated using TLC. Thin-layer chromatography is the favoured technique in many standard procedures in environmental chemistry, industrial chemistry, dye purity, plant material, and herbal analysis. Without having to read the whole report, it aids readers in rapidly grasping the size and importance of the study.

Keywords: Chromatography; TLC; stationary phase; mobile phase.

**Corresponding author: Email: khalidhameed087@gmail.com;*

1. INTRODUCTION

Chromatography Color and writing are both referred to as "chroma" and "graphy," respectively. Russian botanist Mikhail S. Tswett divided plant colour combinations into their individual pure components using a crude kind of chromatographic separation [1]. Writing is derived from the term chroma, which stands for colour. Russian botanist Mikhail S. Tswett used a rudimentary kind of chromatographic separation to separate plant colour combinations into their respective pure components [2]. His research's main finding was that plant pigments separated into bands of their individual components when they travelled through the stationary during this procedure. Both the stationary phase and the mobile phase are used in chromatography to separate the components. the terms "chroma" and "graphy," which are used to describe colour and writing, respectively. Russian botanist Mikhail S. Tswett used a rudimentary kind of chromatographic separation to separate plant colour combinations into their respective pure components. I move my feet in a certain way. Components in non-volatile mixtures may be separated using the chromatography method known as thin-layer chromatography (TLC) [3]. A thin coating of adsorbent material is placed on top of a TLC plate constructed of a non-reactive solid. The motionless phase is right now.

Chromatography method used to separate mixtures is called thin layer chromatography (TLC). The stationary phase is the adsorbent layer in chromatography. A solvent or solvent combination (referred to as the mobile phase) is pulled up onto the plate by capillary action after the sample has been deposited on the plate. Separation is accomplished because various Analytes ascend the TLC plate at differing Ts [4]. M. Tswett developed capillary motion in 1906, which enables the solvent to transport the sample up the plate and cause separation depending on changes. A sheet of glass, plastic, or aluminium foil is coated with a thin coating of an adsorbent material to be used in thin-layer chromatography (blotter paper). Often, cellulose, aluminium oxide, or silica gel are used to create this product.) differences [5] in the molecules' polarity, size, and other chemical properties. Following removal and drying of the plate, the separated materials may be seen using a number of detection methods, such as UV light, iodine vapour, or specific chemical reagents. This is helpful for qualitative analysis since it enables the identification of specific chemicals and the quantification of the purity of those molecules. This rapid and inexpensive technique is used at research centres by, among others, the pharmaceutical and food sciences industries. on differences in the molecules' polarity, size, and other chemical properties. Following removal and drying of the plate, the separated materials may be seen using a number of detection methods, such as UV light, iodine vapour, or specific chemical reagents. This is helpful for qualitative analysis since it enables the identification of specific chemicals and the quantification of the purity of those molecules. This rapid and inexpensive technique is used at research centres by, among others, the pharmaceutical and food sciences industries [6].

2. PRINCIPLE OF TLC

The principale of Separation in TLC is Adsorption.TLC is based on the idea that molecules move through a stationary phase at varying rates while being transported by a mobile phase, which is often a solvent (normally a thin layer of adsorbent material, such as silica gel). TLC requires substances that stick to the stationary phase's surface. Depending on the compounds' affinities for the stationary phase, different compounds migrate through the chromatographic process at different speeds. A small patch of the mixture was placed on a TLC plate. The stationary phase seems to be divided at the bottom [7]. Following that, the plate is put in a container with some solvent (the mobile phase). The solvent raises the sample of the plate due to capillary action. The chemicals are moved up the plate as the solvent passes through the mixture. With the stationary phase, various substances will respond differently and move at varying rates. As a result, the compounds separate along the plate's length. The TLC plate is normally dried once the separation is finished before being inspected.The components towards the stationary phase travel slower ,On the other side the components having lesser affinity towards the stationary phase travel faster.This is often accomplished by injecting chemicals, shining UV light over the plate, or just seeing the marks that the separated components have left behind [8]. The distance that each compound has travelled may be calculated using the Rf (retention factor) value [9].

3. RF VALUE

The Rf values of the separated chemicals may be compared to accepted standards or literature

values to identify the mixture's constituents [10]. With the proper calibration, TLC may be used for both qualitative and quantitative analysis, making it a versatile and quick procedure. It often serves a range of functions in the fields of chemistry, biochemistry, and pharmacology, including determining the purity of compounds, monitoring chemical processes, and determining the constituent parts of combinations. Less than 1 Rf value Because Rf values measure the ratios of solut e (analyte) migration lengths to solvent fronts, they can never be more than one [11].Because solutes need stationary phases to exhibit certain required qualities, the solvent front always travels more slowly than the solute front. By dividing the spot's travel distance by the solvent's travel distance, RF is computed. The Rf value only stays constant when all experimental circumstances are the same for each component. based on the following factors [12].

3.1 Nature of Adsorbent

The term "adsorbent" describes the solid substance that is applied in a thin layer on a flat substrate, such as a glass or plastic plate, in thin-layer chromatography (TLC). Typically, this adsorbent is made of a material like silica gel or alumina. How the different components of the sample separate depends on the sample's characteristics, notably the particle size and chemical makeup [13]. As the sample molecules pass over the TLC plate, the adsorbent interacts with them, causing them to segregate depending on how well they stick to it and the mobile phase. Depending on the particular separation needs of the experiment, a variety of adsorbents may be utilised. A natural polysaccharide called cellulose may be employed as an adsorbent in TLC [13] . Since it may be derived to create chiral stationary phases, it is very helpful for separating chiral chemicals. A non-polar adsorbent used to separate non-polar or hydrophobic chemicals is reverse-phase silica. The use of non-polar solvents with it is common. Certain TLC plates have fluorescent chemicals added to them that make it simpler to see separated molecules under UV light [14]. When choosing the best adsorbent, the kind of chemicals being separated and the suitable separation conditions will be taken into account. While non-polar adsorbents like RP silica are better at sorting out non-polar molecules, polar adsorbents like silica gel and others like them tend to interact better with polar compounds [15]. The solvent system and the breadth of the adsorbent layer both affect the TLC separation procedure.

3.2 Mobile Phase

For the mobile phase, the appropriate solvent must be employed. To properly separate the compounds in the sample, it must be suitable for the chemical composition of the sample and have the proper polarity [16]. If any of the solvents are very volatile or hygroscopic, a fresh batch of solvents must be made for every Run. One example is acetone. The mobile phase, or solvent, that runs over the TLC plate is one of the numerous variables affecting the thin-layer chromatography (TLC) method [17]. These components include the chosen solvent as well as details on its composition and polarity. How molecules may be viewed and analysed on the TLC plate depends on the mobility and segregation of compounds caused by the properties of the mobile phase. Distinct chemicals have different effects on the mobile phase, which causes them to move on the plate at various rates and facilitates their separation [18].

3.3 Temperature

These components include the solvent's polarity, make-up, and preference. The mobile phase's characteristics have an impact on the compound migration and separation on the TLC plate, which allows for compound visualisation and analysis. Distinct compounds have different interactions with the mobile phase, which causes them to move on the plate at various speeds and facilitates separation [19]. As a result of diverse chemical reactions during TLC, the rates of migration and separation may change [20].

4. DIMENSIONS OF LAYER

Typically plates The appropriate layer thickness is around 250 micrometres. The R Values have a wide range below 200. The efficacy of separation and the pace at which chemicals move through the layer are both impacted by the thickness of the stationary phase, which is generally a thin layer of adsorbent material on a solid support [20]. Although the process can take longer as a result, thicker layers might result in slower migration and better compound separation. Although thinner layers could hasten migration, separation might suffer. Additional variables affecting TLC outcomes include development distance, solvent concentration, and mobile phase thickness [21].

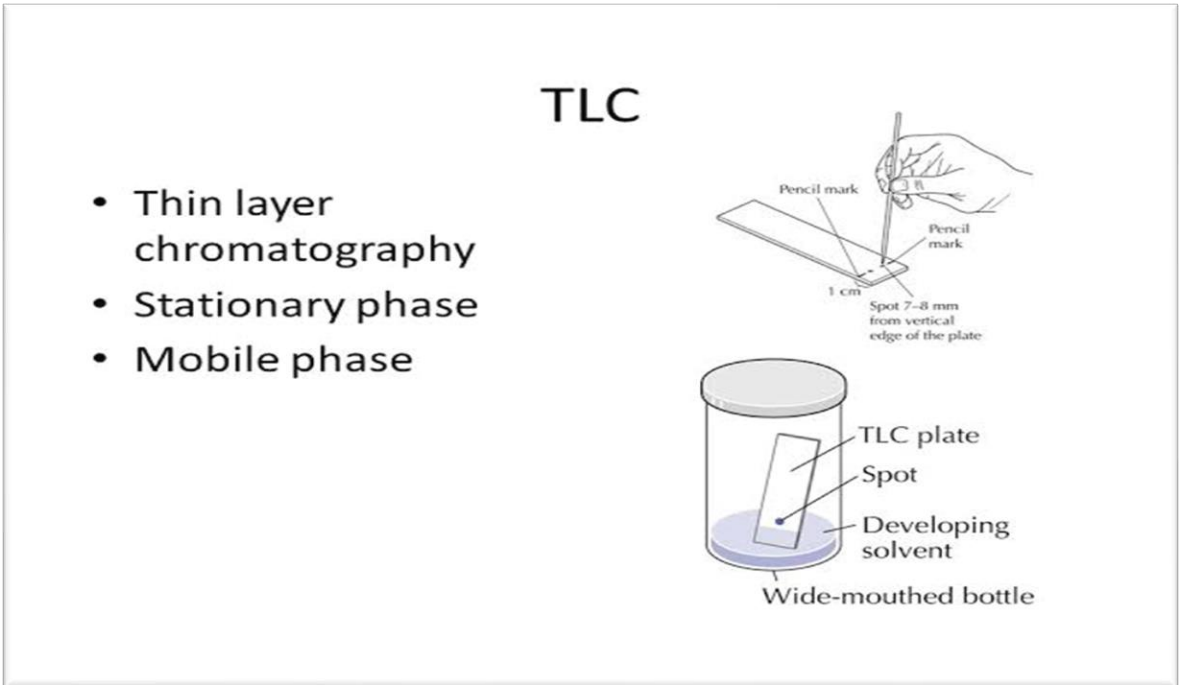

Fig. 1. Stationary phase and mobile phase in TLC plate

4.1 Developing Tank

The procedure may take longer as a consequence of the thicker layers, which may lead to slower migration and better compound separation. Although separation may suffer, faster migration may be obtained with thinner layers. The development distance, solvent concentration, and mobile phase thickness are further factors influencing TLC results [22].

4.2 Mass of Sample

The ratio of the tank's dimensions to those of the TLC plate may affect the separation's quality. More even development may be attained with a larger tank. Using little tanks is the most efficient method to do this. Use filter paper liners, and enough solvent, and wait at least 30 minutes after the tank has equilibrated before running the plates. There must be a cover that fits adequately [21].

4.3 Photographic Technique

The brightness and clarity of the picture are influenced by the length of the exposure to light. The final picture may be impacted by the development and processing of photographic materials, including the chemicals employed and their temperature [23].

4.4 Visualization

A larger sample mass may produce more intense spots, which can make them simpler to detect and quantify when you develop the TLC plate and visualise the separated compounds (often using UV light or chemical reagents) [24].

4.5 Sensitivity

The bulk of the sample in quantitative TLC may impact how sensitive the analysis is. Less intense spots may arise from smaller masses, making it more difficult to determine the compound's amount with precision. When putting a sample on a TLC plate, it's crucial to maintain equilibrium. The ideal mass to utilise allows for detection but isn't too big to interfere with separation and resolution. The type of the substances being tested and the sensitivity of the detection technology utilised will determine the sample's precise mass [25].

5. PREPARATION OF PLATE

Before usage, a TLC plate has to be well-cleaned. To do this, thoroughly clean the substance by washing it in a suitable solvent, such as acetone or methanol, to eliminate any impurities or pollutants. At the base of the TLC plate, a baseline should be created with a pencil

approximately 1 cm away. Verify that the route is clear and symmetrical. These are made by combining water with an adsorbent, such as silica gel, and a tiny quantity of inert binders, like calcium sulphate (gypsum). These are its components. Thick metal foil, thick glass, and plastic sheets are the three materials that are most often used as carriers on nonreactive surfaces [26]. The finished plate is baked in an oven for 30 minutes at 110 °C to dry and activate it. The adsorbent layer generally has a thickness of 0.1-0.25 mm for analytical TLC and a range of 0.5 to 2.0 mm for preparative TLC [27].

6. CAPILLARY SPOTTERS

TLC spotters are made using glass capillary tubes, which may be purchased from the majority of chemical or glassware providers. A capillary tube with two open ends may be used to make two TLC spotters. Capillary melting point tubes with one sealed end may be used to create one TLC spotter. A new capillary must be used for each sample. The capillary may also be cleaned by repeatedly drawing solvent into it and wiping it out with a napkin. The tiny diameter of microcapillary tubes makes them ideal for marking TLC plates or chromatography sheets since they contain relatively small amounts of liquid within each one. When a TLC plate or piece of chromatography paper is in contact with the glass tube's end, capillary action causes the liquid to be drawn into the tube and forced out. TLC spotters are made using glass capillary tubes, which may be purchased from the majority of chemical or glassware providers capillary tubes with two open ends may be used to make two TLC spotters. Capillary melting point tubes with one sealed end may be used to create one TLC spotter. A new capillary must be used for each sample. The capillary may also be cleaned by repeatedly drawing solvent into it and wiping it out with a napkin. The tiny diameter of microcapillary tubes makes them ideal for marking TLC plates or chromatography sheets since they contain relatively small amounts of liquid within each one. Capillary motion draws liquid into the tube and forces it out when a TLC plate or sheet of chromatography paper comes in contact with the glass tube's end. Glass capillary tubes, which can be acquired from most chemical or glassware suppliers, are used to make TLC spotters. Two TLC spotters may be created using a capillary tube that has two open ends. One TLC spotter may be made using capillary tubes with a sealed end and a high melting point. A new capillary must be used for each sample. The capillary may also be cleaned by repeatedly drawing solvent into it and wiping it out with a napkin. The tiny diameter of microcapillary tubes makes them ideal for marking TLC plates or chromatography sheets since they contain relatively small amounts of liquid within each one. When a TLC plate or piece of chromatography paper is in contact with the glass tube's end, capillary action causes the liquid to be drawn into the tube and forced out. The finished plate is baked in an oven for 30 minutes at 110 °C to dry and activate it. The adsorbent layer generally has a thickness between 0.5 and 2.0 mm for preparative TLC, compared to 0.1-0.25 mm for analytical TLC [28].

7. SPOTTING THE PLATE

Before choosing a TLC, dilute the material that has to be separated in a solvent first. The solvent-filled capillary is then used to cover the TLC putting medium. If everything is done correctly, the solvent should drain onto the medium and leave a moist circular area. A sample containing two chemicals would result in two separate spots, and so on, as each component should, in theory, generate a different spot in a combination. The Rf-value of each molecule is a fundamental property (retention factor). This value only represents the height to which a chemical climbs on a TLC plate [29].

8. LOCATION OF THE SPOTTING

The adsorbent material, which is often silica gel or alumina, is essential to TLC. The surface chemistry, particle size, and chemical composition of the adsorbent all have an impact on where spots are distributed. Even though finer particles provide more efficient separation, surface chemistry may have an impact on how much chemical interaction there is with the stationary phase. Spotting involves applying a small amount of this diluted solution using a micropipette to the end of a TLC plate, in this case, a plastic sheet that has been lightly dusted with silica gel powder. The spotting solution quickly evaporates, leaving behind just a tiny fraction of the original ingredient. Since UV light is non-destructive and simple to employ, it is often utilised as the initial visualisation approach on an eluted TLC plate. Any dark areas that a UV light could disclose should typically have a pencil circle drawn around them since they will vanish when the light is turned off [30]. To use the following specifically for spraying the unseen regions in TLC, see the following.

- Since the invisible patches are inorganic, corrosive agents may also be used to spray them. Potassium dichromate is diluted with concentrated sulfuric acid. In the process, most organic compounds, especially those utilised for sugars, convert potassium dichromate (yellow) to chromic sulphate (green).
- Burnt organic substances are visible as black patches when sulphur trioxide vapours, which are produced when sulfuric acid is heated and fuming, are released.
- A potassium permanganate solution is used to treat invisible regions Iodine vapours must be used to spray hidden areas.

9. DEVELOPMENT OF SOLVENTS

A small pool of a development solvent is immersed at the plate's bottom to produce a TLC plate. Then, capillary action causes the solvent to climb up the plate. The solvent leaves its initial location and moves up the plate. The developing solvent and the silica gel plate are vying for the spotted substance [31]. The extremely polar silica gel acts to hold it in place while the solvent tries to move it. it to the ring. The polarity of the plate, the developing solvent, and the spot material must all be balanced for the outcome to be effective. The spot will migrate away from its initial site if the development solvent is sufficiently polar. Different polarities from different spots' areas will split off as independent spots when the spot travels away from its starting place. The plate is taken off, the solvent is allowed to nearly reach the top of the plate before being allowed to evaporate, and the solvent front is indicated with a pencil [32].

9.1 Mobile Phase

The mobile phase, a fluid that travels through a chromatographic system and may either be liquid or gas, moves the materials to be separated over the stationary phase. The extremely polar stationary phase and comparatively nonpolar mobile phase are mixed in the "normal phase" TLC (an organic solvent or solution). Solvent (Mobile Phase) The selection of the proper solvent is perhaps the most important stage in TLC, and determining the best one may need some trial and error. Think of the analytes' chemical properties like you would while choosing a plate [25]. The mobile phase, which is made up of a solvent or mixture of solvents, is the phase that moves. There shouldn't be any garbage on this stage. Spots appear more often when purity quality rises. Acceptable TLC mobile phases include ethyl acetate, dichloromethane, and ether. In this process, hexanes may be used in place of petroleum ether, cyclohexane, or heptane. Chloroform may often dissolve more polar compounds. The best solvent may need some trial and error during the most important TLC step, the mobile phase. Consider the chemical properties of the analytes, just as you would when be selecting a plate. A common starting solvent is hexane and ethyl acetate combined at a 1:1 ratio [33]. Some other types of mobile phase which are used on TLC for experiment are given in Table 1.

If the solvent system is very non-polar so that the non-polar components of the sample will move up the TLC plate than the polar components. If the solvent system is very polar then the polar component will travel further up the TLC plate than the non-polar components. Hexaneis non-polar, dichloromethane is polar, diethyl ether is non-polar, methanol is polar and chloroform is non-polar. Hexane, chloroform and diethyl ether are non-polar, then system A,B and D are non polar. But the Methanol is a Polar due to the presence of 5.0 Chloroform in 0.4 methanol .So that decreasing the Polarity of System D having a ratio of Chloroform and methanol. For a moment, dichloromethane and methanol are polar so that System B and E are also be polar.

9.2 Developing of TLC Plate

In thin-layer chromatography, plate development—the first step—is where the real separation occurs. The developing techniques of TLC are also be One dimensional development, Two dimensional development, Horizontal development and Multiple Development. This method involves adding a solvent solution to the sample on the TLC layer to separate the combination into its constituent parts. Diverse strategies may be used to establish separation. During this typical TLC development process, the plate is placed in the proper TLC developing chamber so that the solvent may wet the TLC layer underneath the starting line. Due to capillary forces, the solvent raises the layer and carries the sample mixture. The plate is dried after it has reached the necessary height (10–15 cm for TLC and 3–7 cm for HPTLC), the solvent front is indicated with a pencil or spatula, and the solvent is removed. The plate is thereafter taken out of the chamber. The slurry for soft layer coating is made by combining water, silica gel,

and a very small quantity of gypsum (calcium sulphate). On a spotless glass plate, this combination is employed and applied as a thick slurry [27] The finished plate is baked for 30 minutes at 110°C, then dried and activated. Due to its remarkable separation properties, silica gel is often used in thin-layer chromatography as both an adsorbent and a binder. A nonpolar solvent should be used to dissolve the material before adding it since polar solvents tend to disperse the beginning site. Apply a volatile solvent to the material to dissolve it. When applying the sample, it is important to avoid touching the adsorbent's surface since doing so would distort the spots and reduce the accuracy of the quantitative data on the chromatogram that followed. The sample site's diameter should fall between 2 and 5 mm.

9.3 Visualization

Since organic compounds often appear colourless on a TLC plate's white background, scientists frequently find it difficult to pinpoint the location of compounds following a TLC. After elution, the molecules must briefly "visualise," or transform into something visible. Visualization approaches may be either destructive (the compound changes into something new after treatment) or non-destructive (the compound remains intact). Using a chemical stain may be dangerous, however, viewing a TLC plate under UV light is safe. Utilizing UV light is the technique most often employed to observe the TLC of organic compounds. The TLC plate's surface material that fluoresces at 254 nm in UV-C light gives the plate its green colour. Visualization approaches may be either destructive (the compound changes into something new after treatment) or non-destructive (the compound remains intact). When compared to seeing a TLC plate under UV light, using a chemical stain is detrimental [34]. Fluorescence, which is brought on by UV radiation, contributes to the spots' visibility. To identify the material type, specific spray reagents, detecting agents, or visualisation agents are employed. Example. ferric chloride is used to treat tannins and phenolic compounds. Analyze the amino acids using acetone or ninhydrin. Fluorescent materials that become visible when exposed to UV light may be utilised as a covering to simplify the process of seeing the separated chemicals on plates. TLC plate visualisation is critical for analysing and understanding the results of a chromatographic separation in general because it allows researchers to identify the components of a mixture and compute the concentration of each component [35]. The visualizating or detecting agents for a non specific methods in TLC Plate are shown in Table 2.

Table 1. Types of mobile phase which are used on TLC

System	Ratio	Mixture used
A	0.6:5.0	Hexane: Chloroform
B	3.0:3.0	Hexane: Dichloromethane
C	3.0:3.0	Hexane: diethyl ether
D	4.8:0.4	Chloroform: Methanol
E	4.8:0.4	Dichloromethane: Methanol

Table 2. Visualizating agents for a non specific methods in TLC Plate

Visualizating Agents	Using Method of visualizing Agents
Iodine Chamber method	It is based upon the observation that iodine has a high affinity for both unsaturated and aromatic compounds. In this method staining of a TLC plate with iodine vapor is among the oldest methods for the visualization of organic compounds.
Sulphuric Acid Spray reagent	This particular stain is excellent for functional groups which are sensitive to oxidation
UV Chamber for fluorescents	It is Suitable for inspection thin-layer chromatogram and other object in a undarkened room.
Fluorescent Stationary phase	UV indicator with efficient radiation for short-wave UV ranges. UV254manganese activated zinc silicate with absorption maximum at 254nm(green fluorescence.
Ninhydrin	Ninhydrin will react with the amino acid to produce a purple compound.

10. ASSESSMENT OF TLC

A method for dissolving mixtures into their constituent parts before the examination is thin-layer chromatography or TLC. To identify compounds, confirm their identities, and determine the purity of a chemical, TLC may be utilised. More polar molecules "climb" through the TLC plate more slowly than less polar compounds, which "climb" higher. The amount of spots on the TLC plate and where they are will be decided in the next phase. Each point in the combination represents one of the chemical elements. TLC may be used for numerical analyses. In quantitative TLC analysis, the relationship between the detector signal and the material amount is often nonlinear. Peak height and integrated area measures are related in the same way. Peak height measurement is an easy-to-use yet powerful approach [36]. By comparing the Rf values from the TLC experiment to values from recognised standards or published literature, the components of the mixture may be identified. TLC is often used in the fields of organic chemistry, biochemistry, and pharmaceuticals for tasks including chemical purification, reaction monitoring, and sample purity assessment. It's an excellent tool for rapid and inexpensive qualitative analysis. The purity of a certain ingredient present in a mixture may be ascertained using thin-layer chromatography (TLC), an analytical method often used to separate and identify the chemicals included in a specific mixture. In contrast to analyte spots, the adsorbent layer will therefore quickly glow bright green. In addition to general, non-specific colourants like iodine vapours, there are specialised colour reagents that may be dipped into the TLC plate or sprayed directly onto the plate, which can be used to snuff out fluorescence. After each region can be distinguished, the Rf value, or retention factor, may be calculated by dividing the Product's trip distance by the Solvent's overall journey distance (the solvent front). The kind of TLC plate and solvent employed dictate these values rather than physical constants [37].

11. PREPARATIVE TLC

Combinations of up to a few hundred milligrammes may be separated using TLC on a modest, semi-preparative scale. Instead of "spotting" the mixture as spots on the TLC plate, the mixture is placed on the plate as a thin, uniform layer that extends slightly above the solvent level and reaches the solvent level. The compounds separate into horizontal bands rather than horizontal spots when they are developed in a solvent. Each ring has been stripped of its backing (or a desired band). Filtration separates the component after the supporting material has been taken out using a suitable solvent (like DCM). The most inexpensive and effective approach for small-scale reactions with distinct products may not necessarily be chromatography. Chemical development of the whole plate is not possible since the results would be ruined [38] In light of this, this technique is effective when used with coloured or UV-visible items technique involves covering most of the plate and exposing a tiny portion of it to an iodine-containing chemical developer. This may either be done by covering the majority of the plate or by doing so while chopping off a little portion of the plate. As a result, this method works best with coloured or UV-visible objects. Another technique is to cover the majority of the plate and expose a tiny portion of it to an iodine-containing chemical developer. One approach to accomplish this is to either cover the majority of the plate or cut a small portion of the dish off [39].

12. APPLICATIONS OF TLC

TLC is a separation method that may be used for both qualitative and quantitative sample analysis. Furthermore, it is quite flexible. Nearly every chemical type, including lipids, nucleotides, glycosides, carbohydrates, fatty acids, alkaloids, insecticides, steroids, and glycosides, may be analysed using TLC. One of the most important applications of TLC is the division of multicomponent medicinal compositions. Colours, preservatives, sweeteners, and other cosmetic ingredients are separated and identified using the TLC technique in the food and cosmetic sectors. Several examples of this kind are shown below: The properties of the solvent and adsorbent have a big impact on the Rf values. Thin Layer Chromatography must be used to determine each amino acid's Rf values to first separate and then identify each one in a given mixture [40].

Utilize TLC to identify and separate amino acids. Small dots of the identified amino acids are placed adjacent to a drop of the mixture that has been mixed up and placed on the bottom of the thin layer plate. To identify the precise amino acids that are present in your sample, we may compare the Rf values of your amino acid spots with established standards. Although TLC may

be used to quickly and relatively easily separate and identify amino acids, correct interpretation of the data may need some ability. For more exact and thorough analyses of amino acids, high-performance liquid chromatography (HPLC) is often utilised. Analysis, quality control, and research are just a few of the uses for TLC, also known as thin-layer chromatography, that are routinely carried out in the pharmaceutical and medical industries. TLC is used to evaluate the purity of the therapeutic ingredient [41].

Analysts can assess the presence of contaminants or the degree of purity of a pharmaceutical component by comparing a sample's separation to that of a reference standard. Antibiotics come to mind. The solvents used to separate penicillin on silica gel "G" were isopropanol-methanol and acetone-methanol (1:1). (3:7). Iodine-azide reaction was used as a detection technique. by misting an iodine solution with a sodium azide content of 3.5 per cent over the dry plates. TLC is a chromatographic technique that's often used for preparative-scale isolation, qualitative rather than quantitative analysis of alkaloids, and the separation of specific compounds from mixtures of several chemicals. TLC provides a chromatographic drug and plant extract fingerprint. Thin-layer chromatography (TLC) is an appropriate technology for alkaloids qualitative studies. A vast variety of plants, fungi, and certain animals contain the complex family of chemical substances known as alkaloids. Usually, they include simple nitrogen atoms. Here is a qualitative examination of alkaloids using TLC that shows how their various polarities may separate a mixture of alkaloids into their parts [41].

A small amount of the alkaloid mixture is applied to a TLC plate, and it is then allowed to pass through a stationary phase (commonly silica gel or alumina) while being transported by a mobile phase (usually a solvent). After creating the TLC plate, alkaloids may be found using a variety of methods. Finding alkaloids that glow under UV light may be done in several ways, one of which is by using a UV lamp. Certain stains can be required for certain alkaloids to be seen. Thin-layer chromatography (TLC) is an appropriate technology for alkaloids qualitative studies. A vast variety of plants, fungi, and certain animals contain the complex family of chemical substances known as alkaloids. Usually, they include simple nitrogen atoms. This TLC study of alkaloids is qualitative. Based on the different polarity of the distinct alkaloids in a mixture, alkaloids may be separated using TLC. On a TLC plate, a small quantity of the alkaloid mixture is placed, and it is then allowed to pass through a stationary phase (often silica gel or alumina) while being moved by a mobile phase (usually a solvent). After creating the TLC plate, alkaloids may be found using a variety of methods. There are many ways to locate alkaloids that glow under UV light, one of which is by using a UV lamp. Some alkaloids can need specific stains to be seen. The relative front (Rf) values for each location on the TLC plate may be calculated. Ratios called rf values show how a material flows along the solvent front. These characteristics of specific alkaloids may be used to identify them. You may detect if there are alkaloids in a combination by comparing the Rf values of the locations where they mingled with the Rf values of known alkaloids. Although TLC is a useful tool for early identification, it is sometimes necessary to validate the identity of alkaloids using additional analytical techniques like mass spectrometry (MS) or nuclear magnetic resonance (NMR) spectroscopy. Although TLC is primarily a qualitative method, it may also be used for semi-quantitative analysis by comparing spot intensities or quantifying spot areas using densitometry. In conclusion, TLC provides a quick and inexpensive way to qualitatively analyse alkaloids. To more accurately identify and characterise the alkaloids, it enables the separation and visualisation of the alkaloids in complicated combinations [42].

It should be used in conjunction with other specialised analytical techniques for a full examination of the alkaloids. The usage of vitamins in soft drinks, unlawful German additives (such as sandalwood extract in fish and meat items), residues in vegetables, salads, and meat, and adherence to limit values may all be used to identify pesticides and fungicides in drinking water (e.g. polycyclic compounds in drinking water, Aflatoxins in milk and milk products). Food safety and allergy sufferer labelling depend on TLC's capacity to recognise allergenic ingredients in foods. It is used to confirm the authenticity of the country of origin and quality of herbal and spice goods by comparing their chemical profiles to recognised criteria. It could be able to detect any lingering pharmaceutical residues in food, assuring both its safety and compliance with regulations may spot contaminated food by checking for illegal or subpar ingredients. a versatile technique that aids in thoroughly checking food components to

ensure their quality, safety, and compliance with regulatory criteria. TLC may be used to identify compounds, confirm their identities, and evaluate a chemical's purity. As indicators of how a reaction is progressing, one may utilise both the existence of a product and the lack of a reactant. Thin-layer chromatography with flame ionisation detection (Iatroscan TLC-FID) offers a rapid and accurate method for detecting saturated and aromatic hydrocarbons as well as resin/asphaltene fractions in solvent extracts of petroleum source rocks, reservoir rocks, and crude oils. Despite the instrument's apparent simplicity, calibration and parameter modification require special attention because of challenges brought on by the detector system's operating characteristics.

However, the procedure is quicker and more precise than the standard MPLC analysis of petroleum fractions. It should be used in conjunction with other specialised analytical techniques for a full examination of the alkaloids. The usage of vitamins in soft drinks, unlawful German additives (such as sandalwood extract in fish and meat items), residues in vegetables, salads, and meat, and adherence to limit values may all be used to identify pesticides and fungicides in drinking water (e.g. polycyclic compounds in drinking water, Aflatoxins in milk and milk products). TLC's ability to identify allergenic components in food is crucial for food safety and labelling for allergy sufferers. By comparing their chemical profiles to accepted standards, it is used to verify the legitimacy of the place of origin and quality of herbal and spice products through alkaloid analysis, which should be used in concert with other specialised analytical methods. Pesticides and fungicides in drinking water may be detected by the use of vitamins in soft drinks, illegal German additions (such as sandalwood extract in fish and meat items), residues in vegetables, salads, and meat, and adherence to limit values (e.g. polycyclic compounds in drinking water, Aflatoxins in milk and milk products). TLC's ability to identify allergenic components in food is essential for food safety and labelling for allergy sufferers is used to confirm the accuracy of the country of origin and the quality of herbal and spice items by comparing their chemical profiles to approved criteria. It could be able to identify any leftover pharmaceutical traces in food, ensuring both its safety and compliance with legal requirements. ' may identify tainted food by looking for questionable or inferior components. a flexible method that assists in carefully examining food ingredients to guarantee their quality, safety, and compliance with legal requirements. TLC may be used to recognise compounds, validate their identities, and assess a chemical's purity.

A product's presence or absence may both be used as indicators of how a reaction is progressing. In solvent extracts of petroleum source rocks, reservoir rocks, and crude oils, thin-layer chromatography with flame ionisation detection (Iatroscan TLC-FID) provides a quick and precise way to identify saturated and aromatic hydrocarbons as well as resin/asphaltene fractions. The detector system's operation presents challenges, despite the instrument's apparent simplicity of use, necessitating additional attention for calibration and parameter modification. Nevertheless, the procedure is quicker and more precise than the typical MPLC analysis of petroleum fractions. However, the procedure is quicker and more precise than the standard MPLC analysis of petroleum fractions. It should be used in conjunction with other specialised analytical techniques for a full examination of the alkaloids. The usage of vitamins in soft drinks, unlawful German additives (such as sandalwood extract in fish and meat items), residues in vegetables, salads, and meat, and adherence to limit values may all be used to identify pesticides and fungicides in drinking water (e.g. polycyclic compounds in drinking water, Aflatoxins in milk and milk products) [43].

TLC's ability to identify allergenic components in food is crucial for food safety and labelling for allergy sufferers. Comparing their chemical profiles to accepted standards is used to verify the legitimacy of the place of origin and quality of herbal and spice products. It could be able to identify any leftover pharmaceutical traces in food, ensuring both its safety and regulatory compliance. ' may identify tainted food by looking for unauthorised or inferior components. is a flexible method that assists in completely examining food ingredients to guarantee their quality, safety, and compliance with legal requirements. TLC may be used to recognise compounds, validate their identities, and assess the purity of a chemical. Both the presence of a product and the absence of a reactant may be used as markers of how a reaction is developing. In solvent extracts of petroleum source rocks, reservoir rocks, and crude oils, thin-layer chromatography with flame ionisation detection (Iatroscan TLC-FID) provides a quick and precise approach for identifying saturated and aromatic

hydrocarbons as well as resin/asphaltene fractions. Despite the instrument's seeming simplicity, calibration and parameter adjustment need extra care because of difficulties brought on by the detector system's operational characteristics. The process, however, is speedier and more accurate than the typical MPLC examination of petroleum fractions. For a thorough alkaloid analysis, it should be used in concert with other specialised analytical methods. Pesticides and fungicides in drinking water may be detected by the use of vitamins in soft drinks, illegal German additions (such as sandalwood extract in fish and meat items), residues in vegetables, salads, and meat, and adherence to limit values (e.g. polycyclic compounds in drinking water, Aflatoxins in milk and milk products). TLC's ability to identify allergenic components in food is crucial for food safety and labelling for allergy sufferers. Comparing their chemical profiles to accepted standards is used to verify the legitimacy of the place of origin and quality of herbal and spice products. It could be able to identify any leftover pharmaceutical traces in food, ensuring both its safety and regulatory compliance. ' may identify tainted food by looking for unauthorised or inferior components. is a flexible method that assists in completely examining food ingredients to guarantee their quality, safety, and compliance with legal requirements. TLC may be used to recognise compounds, validate their identities, and assess the purity of a chemical. Both the presence of a product and the absence of a reactant may be used as markers of how a reaction is developing. In solvent extracts of petroleum source rocks, reservoir rocks, and crude oils, thin-layer chromatography with flame ionisation detection (Iatroscan TLC-FID) provides a quick and precise approach for identifying saturated and aromatic hydrocarbons as well as resin/asphaltene fractions. Despite the instrument's seeming simplicity, calibration and parameter adjustment need extra care because of difficulties brought on by the detector system's operational characteristics. The process, however, is speedier and more accurate than the typical MPLC examination of petroleum fractions. To do a thorough study of the alkaloid, it should be used in concert with other, more specialised analytical methods. Pesticides and fungicides in drinking water may be detected using a variety of methods, including the use of vitamins in soft drinks, illegal German additions (such as sandalwood extract in fish and meat items), residues in vegetables, salads, and meat, and adherence to limit values (e.g. polycyclic compounds in drinking water, Aflatoxins in milk and milk products).

The ability of TLC to recognise allergenic components in food is crucial for food safety and labelling for allergy sufferers. Comparing their chemical profiles to recognised standards is used to verify the legitimacy of the place of origin and quality of herbal and spice products. It could be able to identify any undetectable pharmaceutical traces in food, ensuring both its safety and regulatory compliance. By looking for illegal or inferior components, you may be able to detect tainted food. is a flexible technique that assists in carefully evaluating food ingredients to make sure their quality, safety, and compliance with regulatory requirements. TLC is a technique that may be used to recognise compounds, validate their identities, and assess the purity of a chemical. As signs of how a reaction is developing, one may look for the existence of a product or the disappearance of a reactant. To identify resin/asphaltene fractions, saturated and aromatic hydrocarbons, as we. TLC is a technique that may be used to identify different compounds, count the number of elements in a mixture, and ascertain the identities and purity of each ingredient. Monitoring the appearance of a product or the disappearance of a reactant may also show how a reaction is progressing. Quantitative analysis of resin/asphaltene fractions, saturated and aromatic hydrocarbons, petroleum source rocks, reservoir rocks, and crude oils may be done quickly and accurately using thin-layer chromatography with flame ionisation detection (Iatroscan TLC-FID(Despite the instrument's apparent simplicity, calibration and parameter setting need extra care owing to the intricate nature of the detector system's operating characteristics. The technique, however, is speedier and more accurate than the standard MPLC examination of petroleum fractions. TLC may be used in petrochemical research to track reactions and component conversion, which is essential for process improvement [44].

Impurities will result in several spots as opposed to a pure product, which only has one. TLC is often used to separate and analyse natural compounds from plants, fungi, or marine creatures. It aids in the identification and division of the many constituents contained in these intricate mixes. TLC is used to verify a sample's purity because of its high sensitivity, which makes it possible to detect contaminants in samples that are otherwise thought to be clean.

TLC may be used to determine if a reaction is complete and has proceeded as intended. The kind of byproducts may be determined using TLC. Observing the behaviour of the spots with conventional reagents may sometimes provide information for the quick identification of the products if the reaction doesn't go as anticipated or planned [45].

13. PROBLEM-SOLVING FOR TLC

Here are some of the typical TLC issues and their associated fixes. The sample was packed too full. After dilution, do TLC again on your sample. You might also simply have a sample with a range of separate components, which would produce several discrete spots that connect to create what seems to be a streak. It's conceivable that the experiment's results weren't as anticipated. On a TLC plate, compounds that are very basic or acidic (amines or carboxylic acids) may sometimes exhibit this behaviour. To achieve clearer plates, add a few drops of acetic acid (carboxylic acids) or ammonium hydroxide (amines) into the eluting solution [46]. The adsorbent has either peeled off the plate's edges as it has become bigger or the plate is contacting the walls of the container (or the paper used to saturate the container). It is more difficult to accurately measure Rf values on plates that are not straight. Make careful that nothing biological falls on the plate by mistake. If you purchase one and put it on your desk while doing an experiment, an organic compound may spill or fall on it. As it goes on, a cloud of blue specks appears on the plate. Maybe instead of a pencil, you marked the origin with an ink pen. The chemical's diluted solution may have prevented you from identifying all of it. Try to concentrate the solution or use it many times in a single spot, letting the area dry between applications [47]. If a chemical does not appear under UV light, take into account using a different technique to view the plate (such as staining or exposing it to iodine vapour). You may not have any chemicals since your experiment did not provide the outcomes you were aiming for. If the solvent level in the developing jar is lower than the origin (spotting line) of the plate, the compounds won't be able to ascend the TLC plate through capillary action. The chemicals will be dissolved into the solvent reservoir as an alternative. There won't be any stains on the plate when it is created as a result. When the developing jar is raised, these images demonstrate how the yellow chemical enters the solvent [47].

14. IDENTIFICATION OF NAPHTHODIANTHRONES

Naphthodianthrones must be separated from one another and identified using a chromatographic method to be used in TLC (Thin Layer Chromatography) for this purpose. Utilizing the right solvent, remove the naphthodianthrones from your sample material. Your sample's components may segregate when the solvent is moved up the plate by capillary action, depending on their affinity for the adsorbent and the solvent. According to their chemical makeup, naphthodianthrones travel at various speeds. To validate the identification of the naphthodianthrones, you may additionally employ further analytical methods like mass spectrometry, HPLC, or NMR. Because TLC provides a preliminary identification, it should be regarded as a qualitative approach [48]. More sophisticated techniques must be utilised for quantitative analysis. Depending on the exact naphthodianthrones you are dealing with, other solvents, TLC plates, and visualisation techniques can also be required, therefore it is crucial to adjust your strategy. TLC was used to locate Hypericum perforatum extracts that contained naphthodianthrones using silica gel plates that fluoresced. ethyl acetate, formic acid, and an indication Water (30:2:3 v/v/v) or toluene-ethyl acetate Water and formic acid are split 50:40:5:5 in the mobile phase. TLC may also be used to identify indolic alkaloids that have been obtained using stationary techniques like silica gel plates and acetone-light chromatography from a variety of Rauwolfia species. 100 % A 2:7:1 petroleum-diethyl amine solution containing ammonium cerium (IV) sulphate exhibits spots. More sophisticated techniques must be utilised for quantitative analysis. Depending on the exact naphthodianthrones you are dealing with, other solvents, TLC plates, and visualisation techniques can also be required, therefore it is crucial to adjust your strategy. TLC was used to locate Hypericum perforatum extracts that contained naphthodianthrones using silica gel plates that fluoresced. More sophisticated techniques must be utilised for quantitative analysis. Depending on the exact naphthodianthrones you are dealing with, other solvents, TLC plates, and visualisation techniques can also be required, therefore it is crucial to adjust your strategy. TLC was used to locate Hypericum perforatum extracts that contained naphthodianthrones using silica gel plates that fluoresced. ethyl acetate, formic acid, and an indication Water (30:2:3 v/v/v) or toluene-

ethyl acetate Water and formic acid are split 50:40:5:5 in the mobile phase. TLC may also be used to identify indolic alkaloids that have been obtained using stationary techniques like silica gel plates and acetone-light chromatography from a variety of Rauwolfia species. 100 % A 2:7:1 petroleum-diethyl amine solution containing ammonium cerium (IV) sulphate exhibits spots. More sophisticated techniques must be utilised for quantitative analysis. Depending on the exact naphthodianthrones you are dealing with, other solvents, TLC plates, and visualisation techniques can also be required, therefore it is crucial to adjust your strategy. TLC was used to locate Hypericum perforatum extracts that contained naphthodianthrones using silica gel plates that fluoresced. ethyl acetate, formic acid, and an indication Water (30:2:3 v/v/v) or toluene-ethyl acetate Water and formic acid are split 50:40:5:5 in the mobile phase. TLC may also be used to identify indolic alkaloids that have been obtained using stationary techniques like silica gel plates and acetone-light chromatography from a variety of Rauwolfia species. 100 % A 2:7:1 petroleum-diethyl amine solution containing ammonium cerium (IV) sulphate exhibits spots [49].

15. IDENTICICATION OF DRUGS: CAFFEINE ASPIRIN, IBUPROFEN AND ACETAMINOPHEN

Include the relevant citation. Standards were drawn using a light pencil about 1 cm from the edge of a chromatographic plate for aspirin, acetaminophen, ibuprofen, and caffeine. Placing a suitable adsorbent such as silica gel on top of TLC plates. develop a solvent (e.g., a mixture of ethyl acetate, acetone, and methanol). Caffeine, aspirin, acetaminophen, and ibuprofen standards. Examples of the medical procedures you might want to discuss. building the room with an iodine chamber or a UV light. To create stock solutions, each of the four drugs—aspirin, acetaminophen, ibuprofen, and caffeine—must be separately dissolved in a suitable solvent. Make sure the concentrations are not too high to prevent overpopulated areas on the TLC plate. Apply very small patches of the sample and the standard solutions on the TLC plate, often away from the edge and toward the bottom. Use a capillary tube or a micro syringe for precise spotting. Place the TLC plate in a developing chamber with the developing solvent (previously saturated with the vapour of the solvent mixture) [49]. When the chamber is closed, capillary action will assist in elevating the solvent to the plate. Caffeine may leave stains when exposed to UV light. Due to UV, caffeine shines. Use an iodine chamber to evaluate the other medications. As a consequence of the compounds' interaction with the iodine vapour, patches of the chemicals will become visible. A comparison between the Rf values from the locations in your sample and the standards should be done. If the Rf values are almost identical, the presence of the relevant medications in your sample is indicated. You might decide to carry out further tests employing mass spectrometry, IR, or UV absorption to identify or quantify the isolated spots with better accuracy [50]. The Substance having RF value are given in the Table 3.

16. BY TLC SEPARATION OF INORGANIC COMPOUNDS

The excellent method for identifying and detecting inorganic ions is thin layer chromatography (TLC). It makes it simple to switch the mobile phase, carry out selective separations, and carry out straightforward detection. TLC has been used to separate organic metal compounds as well as cationic, anionic, and covalent species [51]. Before TLC of groups of cations is carried out, silica gel is first washed with acid and water to eliminate impurities of sodium, magnesium, calcium, and iron. However, the calcium sulphate binder is removed during this process. Therefore, calcium sulphate must be replaced by starch or another suitable binder. After washing and drying the TLC plate, the spots of cations or anions needing to be separated are placed on this plate. The plate is then kept clean. The Plate's lower half is then immersed in a solvent in a tightly sealed chamber. The item is then removed from the space, dried, and examined. suitable spot-visualization agents [52].

Table 3. Substance with RF value

Substances	Aspirin	Caffeine	Acetaminophen	Ibuprofen
RF values	0.50	0.009	0.25	0.60

17. CONCLUSION

The thin layer chromatography (TLC) manage Successfully So that some error are found in the begging of experiment. When the problems was solved so precautions are taken. There are three steps are involved for the performance of Thin layer chromatography That is Spotting, Development and visualization. The Rf values of different samples are measured. It is more used in analytical techniques because of its low cost , Simplicity, high sensitivity and speed of separation. The analysis technique by using Thin layer chromatography was Successful performed and the Polarity of the solvent was able to identify. TLC are used in our daily lifes and food industry. Thin layer chromatography are used in separating and studying various aspects of food items life colours, Sweetness and preservation of products. It is also be used in cosmetics industry for the separation and analysis of different cosmetics products and its components.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Marston A. Role of advances in chromatographic techniques in phytochemistry. Phytochemistry. 2007; 68(22-24):2786-2798.
2. De Rijke E, et al. Analytical separation and detection methods for flavonoids. Journal of Chromatography A. 2006;1112(1-2):31-63.
3. Pauli GF, Jaki BU, Lankin DC. Quantitative 1H NMR: Development and potential of a method for natural products analysis. Journal of Natural Products. 2005;68(1):133-149.
4. Horváth C, Nahum A, Frenz JH. High-performance displacement chromatography. Journal of Chromatography A. 1981;218:365-393.
5. Consden R, Gordon AH, Martin AJP. Qualitative analysis of proteins: A partition chromatographic method using paper. Biochemical Journal. 1944;38(3):224.
6. Partridge S. Displacement chromatography on synthetic ion-exchange resins. 3. Fractionation of a protein hydrolysate. Biochemical Journal. 1949;44(5):521.
7. Partridge S, Brimley R. Displacement chromatography on synthetic ion-exchange resins. 8. A systematic method for the separation of amino acids. Biochemical Journal. 1952;51(5):628.
8. Umano K, et al. Volatile chemicals identified in extracts from leaves of Japanese mugwort (*Artemisia princeps* Pamp.). Journal of Agricultural and Food Chemistry. 2000;48(8):3463-3469.
9. Stahl E. Dünnschicht-chromatographie. Springer; 1962.
10. Wagner H, Bladt S. Plant drug analysis: a thin layer chromatography atlas. Springer Science & Business Media; 1996.
11. Tyihák E, Mincsovics E, Kalász H. New planar liquid chromatographic technique: overpressured thin-layer chromatography. Journal of Chromatography A. 1979;174(1):75-81.
12. Kumar S, Jyotirmayee K, Sarangi M. Thin layer chromatography: A tool of biotechnology for isolation of bioactive compounds from medicinal plants. International Journal of Pharmaceutical Sciences Review and Research. 2013;18(1):126-132.
13. Spackman DH, Stein WH, Moore S. Automatic recording apparatus for use in chromatography of amino acids. Analytical chemistry. 1958;30(7):1190-1206.
14. LaCourse WR. Column liquid chromatography: equipment and instrumentation. Analytical chemistry. 2000;72(12):37-52.
15. Hostettmann K, Marston A. Saponins. (No Title); 1995.
16. Sakakibara H, et al. Simultaneous determination of all polyphenols in vegetables, fruits, and teas. Journal of agricultural and food chemistry. 2003;51(3):571-581.
17. Lindon JC, et al. Directly coupled HPLC-NMR and its application to drug metabolism. Drug metabolism reviews. 1997;29(3):705-746.
18. Nguyen DTT et al. Fast analysis in liquid chromatography using small particle size and high pressure. Journal of separation science. 2006;29(12):1836-1848.
19. Chan EC, et al. Ultra-performance liquid chromatography/time-of-flight mass spectrometry based metabolomics of raw and steamed Panax notoginseng. Rapid Communications in Mass Spectrometry: An International Journal Devoted to the Rapid Dissemination of Up-to-the-Minute Research in Mass Spectrometry. 2007; 21(4):519-528.

20. Issaq HJ. Capillary electrophoresis of natural products. Electrophoresis. 1997;18(12-13):2438-2452.
21. Rabanes HR, Guidote Jr AM, Quirino JP. Capillary electrophoresis of natural products: Highlights of the last five years (2006–2010). Electrophoresis. 2012;33(1): 180-195.
22. Tomás-Barberán F. Capillary electrophoresis: A new technique in the analysis of plant secondary metabolites. Phytochemical Analysis. 1995;6(4):177-192.
23. Purcell EM, Torrey HC, Pound RV. Resonance absorption by nuclear magnetic moments in a solid. Physical review. 1946;69(1-2):37.
24. Bloch F. Nuclear induction. Physical review. 1946;70(7-8):460.
25. Sherma J. Thin layer chromatography, in Analytical Instrumentation Handbook. CRC Press. 2004;1021-1040.
26. Radwan S. Coupling of two-dimensional thin-layer chromatography with gas chromatography for the quantitative analysis of lipid classes and their constituent fatty acids. Journal of Chromatographic Science. 1978;16(11): 538-542.
27. Macala L, Yu R, Ando S. Analysis of brain lipids by high performance thin-layer chromatography and densitometry. Journal of lipid research. 1983;24(9):1243-1250.
28. Hahn-Deinstrop E. Applied thin-layer chromatography: best practice and avoidance of mistakes. John Wiley & Sons; 2007.
29. Grinberg N. Modern thin-layer chromatography. CRC Press; 1990.
30. Marston A. Thin-layer chromatography with biological detection in phytochemistry. Journal of Chromatography A. 2011; 1218(19):2676-2683.
31. Komsta L, Waksmundzka-Hajnos M, Sherma J. Thin layer chromatography in drug analysis. CRC Press; 2013.
32. Srivastava M. High-performance thin-layer chromatography (HPTLC). Springer Science & Business Media; 2010.
33. Skipski VP, et al. Separation of lipid classes by thin-layer chromatography. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism. 1965;106(2): 386-396.
34. Jeffrey S. Profiles of photosynthetic pigments in the ocean using thin-layer chromatography. Marine Biology. 1974;26:101-110.
35. Poole CF, Poole SK. Instrumental thin-layer chromatography. Analytical Chemistry. 1994;66(1):27A-37A.
36. Cimpoiu C. Analysis of some natural antioxidants by thin-layer chromatography and high performance thin-layer chromatography. Journal of Liquid Chromatography & Related Technologies. 2006;29(7-8):1125-1142.
37. Scott P, Lawrence J, Van Walbeek W. Detection of mycotoxins by thin-layer chromatography: application to screening of fungal extracts. Applied Microbiology. 1970;20(5):839-842.
38. Wilson I. The state of the art in thin-layer chromatography–mass spectrometry: A critical appraisal. Journal of Chromatography A. 1999;856(1-2):429-442.
39. Ruiz J, Ochoa B. Quantification in the subnanomolar range of phospholipids and neutral lipids by monodimensional thin-layer chromatography and image analysis. Journal of lipid research. 1997;38(7):1482-1489.
40. Shaw PD, et al. Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography. Proceedings of the National Academy of Sciences. 1997; 94(12):6036-6041.
41. Cashel M, Lazzarini RA, Kalbacher B. An improved method for thin-layer chromatography of nucleotide mixtures containing32P-labeled orthophosphate. Journal of Chromatography A. 1969;40:103-109.
42. Privett O, et al. Lipid analysis by quantitative thin-layer chromatography. Journal of the American Oil Chemists' Society. 1965;42(5):381-393.
43. Cruz-Hernandez C, et al. Methods for analysis of conjugated linoleic acids and trans-18: 1 isomers in dairy fats by using a combination of gas chromatography, silver-ion thin-layer chromatography/gas chromatography, and silver-ion liquid chromatography. Journal of AOAC International. 2004;87(2):545-562.
44. Rhee IK, Van Rijn RM, Verpoorte R. Qualitative determination of false-positive effects in the acetylcholinesterase assay using thin layer chromatography. Phytochemical Analysis: An International

Journal of Plant Chemical and Biochemical Techniques. 2003;14(3):127-131.
45. Stroka J, Anklam E. Development of a simplified densitometer for the determination of aflatoxins by thin-layer chromatography. Journal of Chromatography A. 2000;904(2):263-268.
46. Minnikin D, Alshamaony L, Goodfellow M, Differentiation of Mycobacterium, Nocardia, and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. Microbiology. 1975;88(1):200-204.
47. Sujata V, Ravishankar G, Venkataraman L. Methods for the analysis of the saffron metabolites crocin, crocetins, picrocrocin and safranal for the determination of the quality of the spice using thin-layer chromatography, high-performance liquid chromatography and gas chromatography. Journal of Chromatography A. 1992;624(1-2):497-502.
48. Guiochon G, Siouffi A. Study of the performances of thin-layer chromatography: Spot capacity in thin-layer chromatography. Journal of Chromatography A. 1982;245(1):1-20.
49. Hodisan T, et al. Carotenoid composition of Rosa canina fruits determined by thin-layer chromatography and high-performance liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis. 1997;16(3):521-528.
50. Schmidtlein H, Herrmann K. Quantitative analysis for phenolic acids by thin-layer chromatography. Journal of Chromatography A. 1975;115(1):123-128.
51. Ando S, Chang NC, Robert KY. High-performance thin-layer chromatography and densitometric determination of brain ganglioside compositions of several species. Analytical biochemistry. 1978;89(2):437-450.
52. Wollish E, Schmall M, Hawrylyshyn M. Thin-layer chromatography. Recent developments in equipment and applications. Analytical Chemistry. 1961; 33(9):1138-1142.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/107435