



International Journal of Science, Architecture, Technology and Environment

A Review on Thin Layer Chromatography: Principle, Types, Procedure and Pharmaceutical Applications

Abu Bakkar Siddique^{1*}, Kaja Reshma²

¹Department Of Pharmaceutical Analysis Nimra College of Pharmacy Vijayawada 521456 AP INDIA

²Entrepreneurship Cell Coordinator Department of Pharmaceutical Analysis Nimra College of Pharmacy Vijayawada 521456 AP INDIA

*Corresponding author, reshmakaaja@gmail.com

DOI: <https://doi.org/10.63680/ijstate092648.42>

Abstract

Thin layer chromatography (TLC) is a simple, quick, inexpensive, and widely applicable planar chromatography technique for the separation, identification, and preliminary analysis of a range of compounds present in a mixture. Thin layer chromatography has a stationary phase that consists of a layer of an absorbent agent (such as silica gel or alumina) on a suitable substrate (the support medium) and a mobile phase that consists of an appropriate solvent or solvent mixture. Applying a sample to the plate and then developing it with a suitable solvent system leads to the different compounds moving at different rates, because of their differing adsorption and/or partition behaviors between the stationary and mobile phases. These compounds appear as individual spots and can be detected by visible observation, ultraviolet light, or suitable visualization reagents. The retention factor (R_f) is often used to compare the migration of the components under specified experimental conditions. Thin layer chromatography has important applications in pharmaceutical analysis, pharmacognosy, phytochemical screening, natural-product research, food analysis, and educational laboratories. This technique is appreciated for its simplicity, low cost, quick development, small sample requirement, and ability to analyze several samples at once.

Keywords: Thin layer chromatography, TLC, stationary phase, mobile phase, adsorption, separation, R_f value, pharmaceutical analysis, qualitative analysis.

1. INTRODUCTION

Chromatography is one of the most important analytical techniques used to separate, identify, and evaluate the individual components of a mixture. The main idea of chromatography is based on the different affinities of substances toward stationary and mobile phases, which results in their migration with different speeds and allows their separation.

Thin layer chromatography (TLC) is a type of chromatography that utilizes a thin layer of an adsorbent as a stationary phase. The stationary phase and mobile phase (a suitable solvent or a mixture of solvents) are

placed on top of a suitable substrate. A small amount of the sample is applied on the opposite side of the plate, and the plate is placed in a chamber with the mobile phase.

When the solvent moves vertically through the stationary phase (due to capillary forces), each component of the mixture migrates individually at a speed depending on the interaction with the mobile and stationary phases. Due to this, the components with a higher affinity towards the stationary phase move slower than those interacting mainly with the mobile phase and separate from each other. The separated compounds can be visualized afterwards directly or with the use of UV light, iodine vapour, or chemical reagents.

Their migration can be represented as retention factor (Rf), which is calculated based on the distance travelled by a substance. This value allows for approximately assessing the degree of similarity of the investigated substances. Thin layer chromatography is one of the most commonly used analytical techniques because it is simple, quick, inexpensive, and requires only a small amount of the sample. It is used in pharmaceutical, pharmacognostic, and phytochemical analyses, as well as in biochemical, food, and educational research. Despite being less sensitive and less informative compared to more modern methods for the separation and identification of substances, it is still widely used.

2. HISTORICAL BACKGROUND

Chromatography development began in the early twentieth century when a Russian botanist Mikhail S. Tsvet investigated plant pigment using chromatographic techniques. The basic principle of separation process based on substances' differential migration with respect to a solvent front was presented.

Later, when chromatographic science was developed, various planar chromatographic techniques have been described and improved. Thin layer chromatography (TLC) appeared as a new planar chromatographic method based on a thin layer of an appropriate adsorbent carried on a plate. It was proposed as a convenient alternative for the quick sample separation and qualitative analysis of chemical substances.

Application of such adsorbents as silica gel and alumina, improved preparation of TLC plates, wide range of solvent's systems, visualization techniques, and availability of commercial ready-to-use plates allowed TLC to become one of the most popular methods of chemical analysis. It can be noted that TLC is convenient for simultaneous analysis of several samples with a small amount of a substance.

Later it has been applied for pharmaceutical analysis, pharmacognosy, natural product research, biochemical investigation, food analysis, and teaching purposes. In spite of the fact that the latest instrumental methods are characterized by very high efficiency, TLC is applied for fast qualitative analysis, identification, and monitoring of a substance.

3. DEFINITION

Thin layer chromatography (TLC) is a technique of chromatography that is performed on a flat surface to separate and determine components present in a mixture. The stationary phase in TLC is a thin layer of an adsorbent material such as silica or alumina that has been bonded to a suitable plate. A mobile phase is made up of a solvent or mixture of solvents.

When a sample is introduced to a TLC plate near the bottom and the plate is placed in a suitable solvent system TLC plate, the mixture moves up the TLC plate due to capillary action. Components of a mixture travel at

different speeds due to the different affinities of components in the mixture for the stationary and mobile phases; this results in spots which represent the individual components.

The spots can be observed and identified by viewing the TLC plate with the naked eye and/or ultraviolet light or by using chemical reagents to spot the substances. The relative distances traveled are referred as retention factors (Rf) and can be used to identify individual substances.

4. PRINCIPLE OF THIN LAYER CHROMATOGRAPHY

The thin layer chromatography works on the principle of differential interaction and subsequent distribution of the constituents of a mixture between a stationary phase and a mobile phase. In case of a conventional normal-phase TLC, the stationary phase is usually a polar adsorbent like silica gel or alumina and the mobile phase is an appropriate solvent or a mixture of solvents.

When the sample is applied on the TLC plate near the lower end and the plate is placed in a developing chamber, the mobile phase moves up the TLC plate by capillary action. During this process, the components of the sample interact with the stationary phase and mobile phase repeatedly until they get separated.

A particular component will interact more with the stationary phase and will, therefore, move slowly down the plate. On the other hand, a component that has a higher affinity for the mobile phase will travel faster with the mobile phase and, therefore, will be farther from the point of application. Due to this differential interaction, the various constituents of the sample get separated as spots at different distances on the TLC plate.

Since adsorption plays a significant role in conventional normal-phase TLC, where the sample molecules are adsorbed on the surface of the adsorbent, the overall separation behaviour depends on the nature of the stationary phase and the mobile phase and the chemical properties of the sample.

5. MECHANISM OF SEPARATION

The separation process in TLC can be explained by the following:

A small amount of the sample mixture is loaded as a concentrated spot near the bottom of the TLC plate. The plate is then placed in a developing chamber with the sample spot facing up and the solvent level below it. As development starts, the mobile phase moves through the stationary phase by capillary action. The constituents of the sample spend varying amounts of time interacting with the stationary phase and the mobile phase. As a result, the components of the sample travel at different speeds up the TLC plate and get separated.

The components that have a stronger relative affinity towards the stationary phase stay close to the origin, while those with a stronger relative affinity towards the mobile phase get farther from it. Therefore, the original mixture is separated into distinct spots of its constituents at different distances on the TLC plate.

The spots are visualized using appropriate visualization techniques after the TLC develops fully. The distance migrated by each spot is then measured, which can be used to determine the retention factor.

6. COMPONENTS OF THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) requires several components for the application, development, separation and detection of sample constituents. The main components include the TLC plate, stationary phase, mobile phase, sample, developing chamber, sample applicator, solvent reservoir, and detection system.

6.1 TLC Plate

A TLC plate consists of a supporting material which has been covered with a thin, fairly homogeneous layer of a suitable stationary-phase material. Glass, aluminium, and plastic sheets, are common support materials. The adsorbents that are frequently used in conventional TLC practices are silica gel and alumina. Commercial pre-coated plates are widely used since they offer a relatively uniform stationary layer and ease of experimental preparation.

6.2 Stationary Phase

The stationary phase refers to the thin layer of adsorbent present on the TLC plate. Silica gel is the most common stationary phase in normal-phase TLC while alumina and cellulose may also be used for selected applications. Chemically modified stationary phases are used in reversed-phase TLC. The nature and activity of the stationary phase affect the interaction and migration of sample constituents.

6.3 Mobile Phase

The mobile phase refers to a suitable solvent or solvent mixture that runs through the stationary layer during the development. Commonly used solvents in TLC practices include hexane, toluene, ethyl acetate, methanol, ethanol, acetone, and water. The composition of the mobile phase is selected according to the chemical properties of the substances being separated and has a major influence on migration and R_f values.

6.4 Sample

The sample is the substance or mixture to be separated. It may contain pharmaceutical substances, plant extracts, amino acids, sugars, pigments, dyes or natural-product constituents. The sample is generally dissolved in a suitable solvent and applied to the plate in a small concentrated spot or narrow band.

6.5 Developing Chamber

The developing chamber refers to a closed container that is used to develop the TLC plate. It contains the mobile phase and provides a relatively controlled environment for solvent movement. The solvent level should be below the sample origin. Closing the chamber helps to reduce solvent evaporation and allows the chamber atmosphere to be enriched with solvent vapour.

6.6 Sample Applicator

A capillary tube, micropipette or suitable applicator may be used to apply the sample to the TLC plate. The sample should be applied carefully as a small, concentrated spot or narrow band to obtain good separation.

6.7 Solvent Reservoir

The solvent reservoir refers to the portion of the developing chamber containing the mobile phase. It is normally placed at the bottom of the chamber, while the sample spot is above the solvent level.

6.8 Detection and Visualization System

After development, the separated components may be detected by direct observation, if they are naturally coloured. Colourless substances can be visualized using ultraviolet light, iodine vapour or suitable chemical

reagents. Ninhydrin is commonly used for the detection of amino acids, while other visualization reagents are selected according to the chemical nature of the analyte.

7. TYPES OF THIN LAYER CHROMATOGRAPHY

Thin layer chromatography can be classified based on the types of stationary phase, mode of development, purpose of analysis, and analytical performance level. The major types of TLC include normal-phase TLC, reversed-phase TLC, two-dimensional TLC, preparative TLC, and high-performance thin-layer chromatography.

7.1 Normal-Phase TLC

Normal-phase TLC is the conventional TLC and usually involves the use of a moderately polar stationary phase and mobile phase. At the same time, separation is achieved through the differential interactions between the components. Typically, components are separated according to the relative affinity for the stationary phase. For example, for a particular compound, the lesser the interaction with the stationary phase, the higher the migration. Meanwhile, higher interactions with the mobile phase increases the migration of the component. Normal-phase TLC is commonly used for the routine qualitative analysis of pharmaceutical substances, natural products, plant extracts, alkaloids, steroids, and other compounds.

7.2 Reversed-Phase TLC

Reversed-phase TLC is different from the normal type because it involves the use of a relatively non-polar stationary phase and a moderately polar mobile phase. Commonly, reversed-phase TLC stationary phases are synthesized by grafting hydrophobic groups to the surface of silica gel. This chromatographic method is primarily applicable for cases where interactions of the components with the stationary phase are dominant, for example, when normal-phase TLC is not suitable.

7.3 Two-Dimensional TLC

Two-dimensional TLC allows separating complex mixtures based on two different chromatographic systems. This technique is commonly applied when a mixture cannot be separated by developing the plate only in one direction. This approach involves two successive developments of the plate, initially placing the sample spot location near the corner and then rotating it at an angle, usually 90° and developing it in the second direction. Thus, two-dimensional TLC can allow separating components using two different solvent systems.

7.4 Preparative TLC

Preparative TLC aims for isolating or purifying compounds rather than analyzing them. In this type of TLC, a relative large sample volume is applied to the TLC plate. A band of the separated compounds is then visualized and cut from the plate, and compounds are extracted using an appropriate solvent. Preparative TLC is commonly used to isolate small amounts of compounds in natural product and pharmaceutical analyses.

7.5 High-Performance Thin-Layer Chromatography

High-performance thin-layer chromatography (HPTLC) refers to a group of advanced planar chromatography techniques that use standardized plates, and optimized sample application and detection processes, for achieving high-resolution separations and enhanced analytical performance. Compared to conventional TLC, HPTLC can offer improved standardization and analytical performance. This technique can be used for qualitative, quantitative, and fingerprint analyses of pharmaceutical, herbal drugs, and phytochemicals, among

many other applications. It is also suitable for densitometric scans of TLC plates at different wavelengths and commonly used in quality control and pharmaceutical analysis.

8. EXPERIMENTAL PROCEDURE OF THIN LAYER CHROMATOGRAPHY

The experimental procedure of TLC deals with sample preparation, plate preparation, sample application, preparation of chamber, chromatographic development, detection and measurement of the separated components.

8.1 Materials Required

The basic materials include a suitable TLC plate, sample solution, mobile phase, developing chamber, capillary tube or micropipette, pencil, ruler, forceps and an appropriate system for visualization like ultraviolet light or chemical detection reagents.

8.2 Sample Preparation

The sample should be dissolved or extracted using a suitable solvent. The concentration should be appropriate for producing detectable spots without excessive sample loading.

8.3 Plate Preparation

A clean TLC plate with a straight baseline drawn approximately 1–2 cm from the lower edge is chosen. Pencil is preferred because the graphite does not normally dissolve and migrate with the mobile phase.

8.4 Sample Application

A small quantity of the sample solution is applied on to the baseline using a capillary tube or suitable applicator. The sample should form a small, concentrated and well-defined spot. The spot is allowed to dry beforehand and development. Small applications may be applied if required with drying between applications.

8.5 Preparation of Developing Chamber

The selected mobile phase is placed in the developing chamber. The solvent level must remain below the baseline. The chamber is closed before development to reduce solvent evaporation and allow the chamber atmosphere to become enriched with solvent vapour and is commonly referred to as chamber saturation.

8.6 Development of the Chromatogram

The TLC plate is placed inside the developing chamber with the sample spot above the solvent level. The mobile phase moves through the stationary layer mostly by capillary action. As the solvent moves, the components of the sample interact differently with the stationary and mobile phases and therefore migrate at different rates.

8.7 Removal of the Plate and Marking of Solvent Front

When the solvent has travelled an appropriate distance, the plate is removed carefully from the chamber. The solvent front is marked immediately with a pencil because the solvent may evaporate quickly after removing the plate.

8.8 Drying and Visualization

The developed plate is allowed to dry. Separated components may be detected by direct observation when they are naturally coloured or using ultraviolet light, iodine vapour or suitable chemical reagents. Ninhydrin

is frequently used for the detection of amino acids while other reagents may be chosen according to the nature of the analyte.

8.9 Measurement and R_f Determination

After visualization, the distances travelled by the individual components and by the solvent front are measured from the same origin. These measurements are used to calculate the retention factor (R_f).

8.10 Ascending Development

In ascending development, the mobile phase is moved upwards through the stationary layer by capillary action. It is a simple and commonly used method of TLC development.

8.11 Descending Development

In descending development, the mobile phase moves downwardly through the stationary layer with gravity being partly responsible for solvent movement.

8.12 Horizontal Development

In horizontal development, the TLC plate is maintained in a horizontal arrangement and the mobile phase travels across the stationary layer.

8.13 Two-Dimensional Development

In two-dimensional TLC development, the plate is first developed in one direction using a selected solvent system. After drying, the plate is rotated, usually by approximately 90°, and developed again in the second direction often using a different solvent system. This may improve the separation of complex mixtures.

9. R_f VALUE AND FACTORS AFFECTING R_f

9.1 R_f Value

Retention factor (R_f) is a value, which determines the relative position of each component on TLC plate. It is calculated by dividing the distance travelled by a particular component from the origin by the distance travelled by the solvent front from the origin.

In mathematical terms, it can be expressed as:

$$\text{Rf} = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

9.2 Importance of R_f Value

R_f values are useful in comparing components so that compounds with same R_f values can be easily identified. They can also be used to quantify components through comparison with other compounds. For instance, R_f values can be used to determine the amount of a particular component. They can also help in determining the progress of a reaction, comparing different samples, and knowing the best solvent to use.

On the other hand, R_f values of a particular substance cannot be used to identify it because R_f values are not fixed or constant for a particular substance. Therefore, R_f values of an unknown substance should not be used to identify it directly. Instead, it is better to compare it with similar standard substances using the same stationary and mobile phases as well as similar experimental conditions.

9.3 FACTORS AFFECTING R_f VALUE

There are several factors that can affect R_f values obtained from TLC analysis. These factors include:

9.3.1 Mobile Phase Composition

The mobile phase composition has a great effect on the TLC analysis results since changing the mobile phase or its ratio can significantly change R_f values.

9.3.2 Solvent Polarity

The polarity of a solvent can also affect R_f values. In case of mixed solvent, changing the solvent ratio will significantly change the R_f value.

9.3.3 Nature of Stationary Phase

The nature of the stationary phase also has a significant effect on the R_f values of most substances. The most common stationary phases used in TLC analysis include silica gel and alumina. On the other hand, different manufacturers produce different types of TLC plates. Some plates are manufactured using different materials for the stationary phase so that they are suitable for use with certain mobile phases.

9.3.4 Activity of Adsorbent

The activity of an adsorbent and its moisture content can significantly affect the R_f values of most substances. A change in the activity of the adsorbent will bring about change in how strongly it interacts with the mobile phase; hence, altering the R_f values.

9.3.5 Sample Concentration

Using highly concentrated samples can result in poor separation of spots because they may appear as one spot with R_f value of zero. On the other hand, using a large spot size can also be a problem because only a small portion of the sample may be visualized after elution. Therefore, it is better to use a spot with moderate size and high concentration.

9.3.6 Temperature

Temperature is a major determinant of elution in TLC analysis. Increasing temperatures can significantly change some physical properties of the solvent. For instance, a change in vapour pressure of the solvent can easily take place due to change in cohesive forces among solvent molecules. As a result, differences in temperatures can lead to significant changes in R_f values of different substances.

9.3.7 Humidity

Humidity can also affect TLC analysis since it is related to moisture content of a silica gel TLC plate. Therefore, changes in humidity will affect R_f values of most substances analyzed using silica gel plates.

9.3.8 Chamber Saturation

Changes in chamber saturation can lead to significant changes in migration rates of substances. Therefore, it is important to ensure that TLC chamber is saturated appropriately and consistently to obtain accurate results. A low saturation level will result in higher evaporation rates of the solvent from TLC plate.

9.3.9 pH

In a case where a substance can be ionized, changes in pH will significantly affect its extent of ionization.

9.3.10 Chemical Nature of Sample

The chemical nature of a sample determines how it interacts with stationary and mobile phases. Therefore, different substances will have varied R_f values using the same TLC set up.

9.3.11 Development Conditions

Changes in development distance and time will bring about changes in migration rates of substances; hence, altering R_f values.

10. APPLICATIONS OF THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) can be used for a variety of analytical purposes, including separation, comparison, and identification of compounds in a mixture. The versatility, simplicity, low cost, small quantity of sample required, and ability to do multiple sample analyses simultaneously make TLC valuable in pharmaceutical, biochemical, chemical, food and natural-product studies.

10.1 Identification and comparison of drugs

TLC can be used for the preliminary identification or comparison of drugs. A test sample can be spotted onto the plate with an appropriate reference standard and chromatographed side by side under similar conditions. The comparison of spot position or R_f values can help determine whether a test sample is comparable to a reference standard.

10.2 Preliminary purity testing

TLC can be used as a rapid but limited means of determining the purity of a substance. If spots are observed on the TLC chromatogram in addition to the spot from the sample of interest, it indicates the presence of various impurities, degradants, related compounds, or other analytes. However, the absence of additional spots does not guarantee the purity of the sample.

10.3 Examination of pharmaceutical formulation

For appropriate pharmaceutical formulations, TLC can be used for preliminary analysis and comparison of their active ingredients or other chemical constituents. In addition, TLC can be used to develop analytical methods and for other investigative purposes.

10.4 Analysis of herbal drugs

Herbal drugs and plant materials often have complex chemical constituents. Appropriate TLC procedures can be used to separate and identify individual compounds or classes of compounds.

10.5 Phytochemical screening

TLC is valuable in phytochemical investigation for the preliminary detection and comparison of some constituents, including alkaloids, flavonoids, tannins, terpenoids, steroids, glycosides, and other secondary metabolites. Appropriate TLC procedures can be used for their detection and comparison using suitable reagents or UV light.

10.6 Herbal drug fingerprinting

Qualitative herbal fingerprints can be obtained by appropriate TLC or HPTLC procedures for the comparison of herbal material or extract. Similar patterns can be obtained for a given herbal material to demonstrate its identity and other characteristics.

10.7 Amino acid analysis

TLC can be used for separation and preliminary identification of amino acids as described in section 8.11. The separated amino acids can be visualized using a ninhydrin reagent.

10.8 Separation of sugars

TLC can be used to separate and examine suitable sugars and related carbohydrates, depending on the nature and composition of the sample and the choice of stationary and mobile phases.

10.9 Separation of plant pigments

The technique can be used to separate and examine plant pigments, including chlorophyll, carotenoids, xanthophylls, and other related compounds, depending on their nature and the choice of stationary and mobile phases.

10.10 Monitoring of chemical reactions

Monitoring of chemical reactions is a valuable application of TLC. A reaction mixture can be analyzed at different time points, and the results can be compared to examine the progress of the reaction. The disappearance of a starting material spot and the appearance of a product spot can indicate the completion of a reaction.

11. ADVANTAGES OF THIN LAYER CHROMATOGRAPHY

1. Simple procedure
2. Rapid analysis
3. Economical
4. Small sample requirement
5. Simultaneous analysis
6. Useful for qualitative analysis
7. Easy visualization
8. Wide applicability
9. Minimal equipment
10. Useful for reaction monitoring

12. LIMITATIONS OF THIN LAYER CHROMATOGRAPHY

1. Limited sensitivity in some applications
2. Limited quantitative capability
3. Variation in R_f values
4. Limited resolution for complex mixtures
5. Manual operation
6. Spot distortion
7. Solvent evaporation
8. Compound-dependent detection
9. Limited suitability for some analyses

13. PRECAUTIONS IN THIN LAYER CHROMATOGRAPHY

- The TLC plate should be clean and handled carefully.
- The baseline should be drawn with a pencil rather than an ink pen.
- The sample should be applied as a small and concentrated spot or narrow band.
- The applied sample should be allowed to dry before development.
- The level of the mobile phase must be below the baseline.
- The sample spot should not come into direct contact with the mobile phase before development.
- A suitable and properly prepared mobile phase should be used.
- The developing chamber should be properly closed during development.
- Appropriate chamber saturation should be maintained if required.
- The stationary phase surface should not be touched with fingers.
- The plate should be placed carefully in the developing chamber.
- The developing chamber should not be disturbed during development.
- The plate should be removed at the appropriate development distance.
- The solvent front should be marked immediately after removing the plate.
- The plate should be dried appropriately before visualization.
- Suitable UV conditions or visualization reagents should be selected according to the sample.
- Experimental conditions should be kept consistent when comparing R_f values.

14. CONCLUSION

Thin layer chromatography (TLC) is a quick, facile, economic, and versatile analytical planar chromatographic technique used mainly for the separation, comparison, identification, and detection of a mixture of substances. This analytical technique is performed on a thin layer of a stationary phase, such as silica gel or alumina, bonded to an inert plate support, and a mobile phase, which can either be a solvent or a mixture of solvents.

The migration of the applied mixture is due to the differences in the components' affinity towards the stationary or mobile phases. The mobile phase travels along the stationary layer through capillary action, and the analytes are separated based on their distinct distribution coefficients. The separated spots can be visualized either by the naked eye or using ultraviolet light or chemical reagents.

The retention factor (R_f) is calculated from this chromatographic data to compare the mobility of an analyte under identical experimental conditions. Normal-phase thin-layer chromatography, reversed-phase thin-layer chromatography, two-dimensional thin-layer chromatography, preparative thin-layer chromatography, and high-performance thin-layer chromatography techniques are some of the various types of TLC.

This chromatographic method finds applications in pharmaceutical analysis, pharmacognosy, phytochemical screening, herbal drug analysis, natural-product research, biochemical analysis, food analysis, and reaction monitoring. Thin-layer chromatography is a valuable tool in analytical chemistry due to its inherent simplicity, rapid analysis, low cost, reduced sample amount, and ability to analyze multiple samples simultaneously.

However, the drawbacks of the conventional TLC technique include the dependency of R_f values on the chromatographic conditions, limited quantitative analysis, reduced reproducibility, and lesser application in highly sophisticated and complex analytical problems. Thus, depending on the purpose and analytical needs, TLC, or any other analytical technique, can be chosen for a particular analysis.

In conclusion, thin layer chromatography remains an essential analytical tool in chromatography for qualitative, screening, or comparative analysis or for laboratory exploratory research. This method continues to provide significant contributions to pharmaceutical and related industries due to its versatility and ease of use.

AKNOWLEDGEMENTS: The author is thankful to Guide Prof Kaja Reshma Professor Entrepreneurship Cell Coordinator Nimra College of Pharmacy for advice in writing this Article.

Declaration of Conflicting Interests:

The authors declare no potential conflicts of interest with respect to the research, authorship and publication of this article.

Funding:

The author received no financial support for the research, authorship and publication of this article.

15. REFERENCES

1. Stahl E. Thin-Layer Chromatography: A Laboratory Handbook. Springer-Verlag.
2. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall.
3. Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. Cengage Learning.
4. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental Methods of Analysis. CBS Publishers & Distributors.
5. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. CBS Publishers & Distributors.
6. Chatwal GR, Anand SK. Instrumental Methods of Chemical Analysis. Himalaya Publishing House.
7. Sharma BK. Instrumental Methods of Chemical Analysis. Goel Publishing House.
8. Vogel AI. Vogel's Textbook of Quantitative Chemical Analysis. Pearson Education.
9. United States Pharmacopeial Convention. United States Pharmacopeia–National Formulary (USP–NF). Rockville, MD: United States Pharmacopeial Convention.
10. World Health Organization. The International Pharmacopoeia. Geneva: World Health Organization.
11. Snyder LR, Kirkland JJ, Dolan JW. Introduction to Modern Liquid Chromatography. Wiley.
12. Touchstone JC. Practice of Thin Layer Chromatography. John Wiley & Sons.