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A REVIEW ARTICLE ON A LABORATORY TECHNIQUE:- THIN LAYER CHROMATOGRAPHY (TLC)

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Abstract :- Thin Layer Chromatography (TLC) is a simple, rapid, and versatile analytical technique widely used for the separation and identification of components present in complex mixtures. This review highlights the fundamental principles of TLC, including its stationary and mobile phase interactions, capillary movement, and migration behaviour governed by polarity differences. The article discusses essential parameters influencing TLC performance such as nature of adsorbent, mobile phase composition, layer thickness, temperature, and sample mass. It also provides an overview of TLC instrumentation components including adsorbents, plate preparation, spotting, developing chambers, solvent systems, visualization techniques, and data evaluation methods. TLC remains a valuable tool across fields such as pharmaceuticals, biotechnology, phytochemistry, and natural product analysis due to its cost-effectiveness, minimal sample requirement, and ability to rapidly assess purity, monitor reactions, and support qualitative identification of compounds.

KEYWORDS:- TLC, Review on TLC, Thin Layer Chromatography, Instrumentation of TLC, Advantages of TLC, Principle of TLC.

I. INTRODUCTION:-

Chromatography is a term which is used for set of laboratory techniques for the separation of mixture into compounds.[1]The term chromatography is found from Greek word that is "chroma" and " graphing" both meaning is to write. All the chromatographic techniques work on almost same principle. All Chromatographic techniques have two phases first on is stationary and second is mobile phase. Stationary phase is consisting of Silca or Alumina and Mobile phase is consisting of Solvents.[2] Chromatography is a technique used to separate the different components present in a mixture. The separation happens because different substances move at different speeds depending on how they distribute between these two phases. Chromatography is a very popular technique and is mainly used for analysis.[12] Chromatography techniques such as TLC and HPTLC are very useful for creating characteristic fingerprints of herbal drugs. These fingerprints help in the identification and analysis of herbal raw materials as well as finished herbal products.[13] They are very Advances are continually

improving the technical performance of chromatography, allowing the separation of increasingly similar molecules.[14]

Chromatographic techniques:-

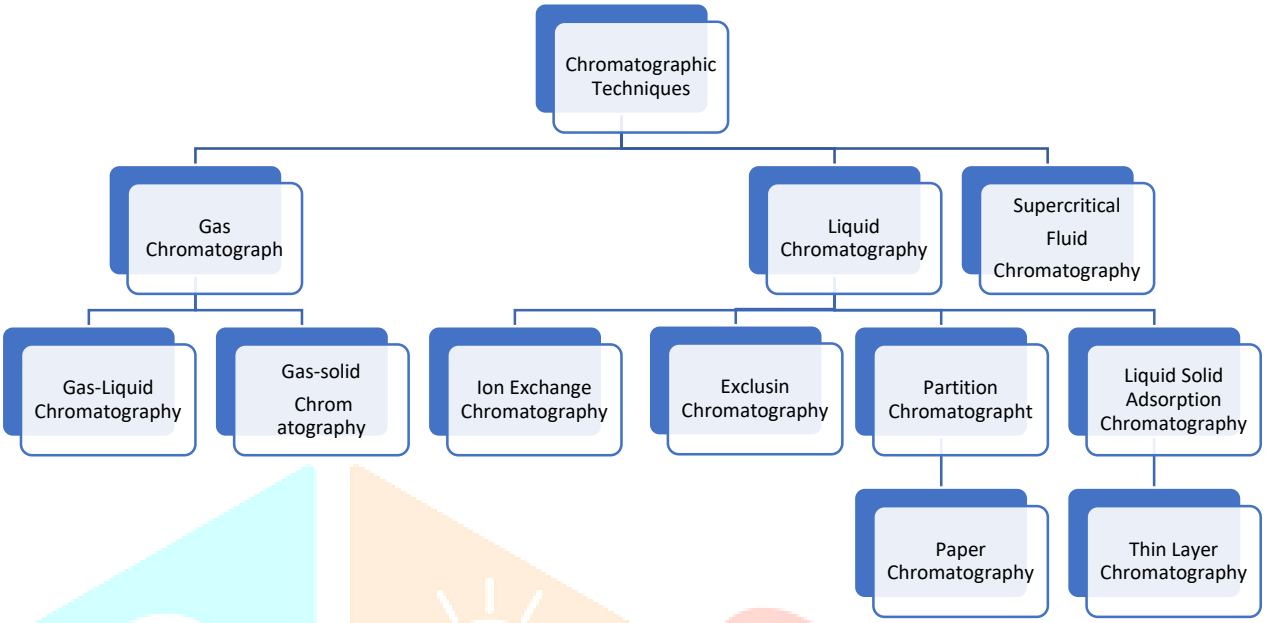

Figure 1:Techniques of Chromatography

II. Thin Layer Chromatography:-

Thin Layer Chromatography (TLC) is a simple method used to separate the different parts of a mixture. This is liquid chromatographic technique. It was discovered by M. T Swett in 1906. In TLC, a thin layer of material such as silica gel, aluminium oxide, or cellulose is spread on a glass, plastic, or aluminium plate. This layer is called the stationary phase .A small spot of the sample is placed on the plate, and then the plate is placed in a liquid solvent (called the mobile phase). The solvent moves up the plate by capillary action. As it moves, different substances in the mixture travel at different speeds, allowing them to separate from each other.[3]TLC is used to identify the compounds present in the substance, to monitor the reaction. The separation is based on the different partitioning of the mobile phase and stationary phase.[4] If the Given two compounds have different polarity, then the more polar substance will have stronger interaction with stationary phase and therefore more capable to dispel to mobile phase from the binding phase. Oppositely, less polar substance will move higher in upward direction.[3]Depending upon the solubility and rate of migration, compounds in the mixture move up on the plate at different rates resulting in separation of the compounds. There are various chromatographic techniques are developed but Thin Layer Chromatography (TLC) is widely used laboratory technique.[4]Applications of TLC are as follows – Botany, Phytochemistry and Medicine, Chemotaxonomy of plants, searching for enzyme inhibitors templates.[5]

The hydrophobic nature (water-repelling property) of a compound affects how a drug spreads in the body after it is absorbed. It also influences how fast the drug is broken down (metabolized) and removed (excreted) from the body. The partition coefficient is an important factor used in QSAR studies to understand the relationship between the chemical structure of a drug and its biological activity.[15] Thin layer chromatography (TLC) and spot tests are simple and useful methods for the routine detection of carbamate pesticides in toxicology. Different colour-producing (chromogenic) reagents and solvent systems are used, which give specific-coloured spots when they react with these insecticides. These coloured spots help in identifying the pesticides.[16] Classical thin-layer chromatography (TLC) has some advantages such as being simple to perform and having low cost. However, compared to high-

performance liquid chromatography (HPLC), TLC has some limitations like lower separation efficiency, lower productivity, and less repeatable results.[17] TLC is a useful method for identifying and estimating the amount of active compounds present in plant extracts. It is a simple method to separate different components of a mixture and makes the results easy to understand. This technique is also low-cost and easy to use.[18]

Quantative estimation of some phytoconstituents can be done examples are:- Alkaloids, Flavonoids, Glycosides, Phenols, Saponins, Sterols, etc.[19]

2.1 Advantages of TLC:-

1. TLC is much simpler technique.

2. TLC can be performed on analytical as well as on a preparative scale.

3. The separation process is faster and the selectivity for compounds is high.

4. The purity standards of the given sample can be assessed easily.

5. The TLC offers a faster and more efficient separation than paper chromatography and the majority of paper chromatographic separations have now been superseded by the TLC procedure.

6. It helps with the visualization of separated compound spots easily.

7. A good separation on inorganic adsorbent layers can be achieved rapidly as the development time is only an hour; while paper and column chromatographic techniques require several hours or days for development.

2.2 Disadvantages of TLC:-

1. There is no longer stationary available in TLC plates, therefore, its separation length is insufficient in comparison to other chromatographic techniques.

2. The results obtained are difficult to reproduce.

3. Only soluble components of the mixture can be separated.

4. It is not automatic.

5. It works in the open system, thus can get affected by temperature and humidity.

2.3 Application of Thin Layer Chromatography

1- It is also useful is analysis of mixture of amino acid and mixture of sugars.

2- It also offers a rapid method of separating and estimating sugars quantitatively; how ever the identification depends upon determination of their physical constants and formation of characteristic derivatives.

3- The technique is also useful in isolation of pair of components having sample RF values using two-dimensional paper chromatography.

4- Paper chromatography also involves inorganic applications such as separation of cations like cadmium, zinc, mercury, beryllium and calcium.

5- It technique is also helpful in identification of accelerator and anti-oxidant in rubber and is useful for determining its quality.[20]

III. Principle of Thin Layer Chromatography :-

Thin Layer Chromatography (TLC) uses a thin glass or plastic plate coated with silica gel or aluminium oxide as the stationary (solid) phase. The mobile phase is a solvent chosen based on the type of substances being separated. In TLC, a small spot of the sample is placed near the bottom of the plate. The plate is then placed in a container (developing chamber) with a small amount of solvent. The solvent moves up the plate by capillary action. As the solvent moves, it carries the components of the mixture with it. Some compounds move faster and go higher on the plate, while others move slower and stay lower. How far each compound moves depends on its structure and functional groups that is, how much it likes (or dissolves in) the solvent versus how strongly it sticks to the plate. According to the rule "Like dissolves like", compounds that are more similar to the solvent travel farther up the plate, while those that are less soluble stay closer to the starting point.[2]

3.1 Rf Value:-

The behaviour of an individual compound in TLC is characterized by a quantity known as Rf and is expressed as a decimal fraction. The term Rf associated with the migration of the solute relative to the solvent as :

Calculation of Rf value:

RF = Distance Travelled by solute form origin line/ Distance Travelled by solvent form origin line.[4]

The Rf value is a constant for each component only under identical experimental condition. It depends upon number of factors as;

Nature of absorbent, The mobile phase, Temperature, Thickness of layer , Developing Tank, Mass of sample, Chromatographic Technique

3.2 Nature of Adsorbent:-

Different adsorbents give different Rf values even when the same solvent is used. To get consistent results, the adsorbent must have the same particle size and binder. Before using, plates should be kept in a desiccator with silica gel to keep them dry, and the sample should be applied quickly so that moisture from the air doesn't get absorbed. Science the activation process can be difficult, it's better to use plates stored at room temperature without activating them.[3]

3.3 The mobile phase:-

For the mobile phase, the right solvent must be used. It should match the chemical nature of the sample and have the correct polarity to separate the compounds properly. If the solvent is very volatile (evaporates easily) or hygroscopic (absorbs moisture), a new batch should be prepared for each run for example, acetone. The mobile phase, or the solvent that moves up the TLC plate, is an important factor in the thin- layer chromatography (TLC) process. Its type, composition, and polarity affect how well the compounds separate. The movement and separation of the sample components on the TLC plate depend on the properties of the solvent. Because different compounds interact differently with the solvent, they travel at different speeds, making it possible to separate and identify them.[6]

3.4 Temperature:-

Exact temperature control is not required, but the tank should be kept away from heat sources and direct sunlight. When the temperature increases, volatile solvents evaporate faster, the solvent moves more quickly on the plate, and the Rf values usually become slightly lower.

3.5 Thickness of layer:-

A standard micrometre plate is the preferred thickness is 250 micrometres for the TLC layer. If the layer is thinner than 200 micrometres, the Rf values can change a lot. The layer thickness can be slightly increased or decreased depending on the compound being tested.

3.6 Developing Tank:-

It is important to create saturated conditions before running TLC plates. This can be done by using a small tank lined with filter paper, adding enough solvent, and allowing the tank to stand for at least 30 minutes before use. The tank should also have a tightly fitting lid to maintain these conditions.

3.7 Mass of sample :-

Adding more sample to the TLC plate usually increases the Rf value of the drug, especially if the spot normally shows tailing. However, if too much sample is applied, the spot will also tail and may appear to have a lower Rf value. You can usually tell the difference between the two situations by how dark or intense the spot looks.

3.8 Chromatographic Technique:-

Putting more sample on the TLC plate usually makes the drug move farther (higher Rf value), especially if the spot normally spreads out. But if you add too much sample, the spot will spread too much and may look like it moved less (lower Rf value). You can tell the difference by how dark or strong the spot appears.[3]

IV. INSTRUMENTATION OF TLC:-

1. Adsorbent used in TLC(Stationary Phase)
2. Mobile Phase
3. Plate Preparation
4. Activation of TLC plate
5. Capillary spotters
6. Spotting the Plate
7. Location of Spots
8. Developing Chamber
9. Development solvents
10. Developing Plate
11. Visualization
12. Data Evaluation

4.1 Adsorbent used in TLC(Stationary Phase):-

The proper choice of Adsorbent is important for separation of compounds in mixture. It is based on following:- Solubility of substance, Nature of compound, Reactivity of compound with either solvent or the adsorbent, Chemical reactivity of compounds with binders

Adsorbents are of two types:- Firstly inorganic adsorbent and second one is organic adsorbent.

Table 1- Adsorbents used in TLC

Sr. No.	**Inorganic Adsorbent**	**Organic Adsorbent**
1	Aluminium Oxide	Cellulose and Acetylated Cell Cellulose
2	Silica Gel	Charcoal and Activated Carbon
3	Calcium Sulphate	Dextran Gels
4	Bentonites	Cellulose Ion-Exchange Powder
5	Magnesium Silicate	Ion-Exchange Resins

4.2 Mobile Phase:-

In silica gel chromatography, the moving phase (mobile phase) is an organic solvent or a mixture of organic solvents. As these solvent flows over the silica gel, it carries the analyte (sample) along with it. The analyte can move forward only when it is not attached to the silica surface. The amount of time the analyte spends stuck to the silica compared to the time it spends dissolved in the solvent decides its retention factor (Rf).[4]

In chromatography, the mobile phase (solvent) is used to dissolve the target molecule (analyte) so it can move along the stationary phase. The choice of mobile phase depends on the analyte's properties and the type of stationary phase being used. In normal-phase TLC, where the stationary phase is polar (like silica gel), common solvents used in the mobile phase are: Diethyl ether, Methylene chloride ,Chloroform

In reversed-phase TLC, where the stationary phase is non-polar, the mobile phase usually contains: Tetrahydrofuran (THF),Acetonitrile ,Methanol mixed with water. These solvents help control how strongly the analyte interacts with the stationary phase, and therefore how fast it moves.[8]

4.3 Plate Preparation:-

Plate preparation is an important step in Thin Layer Chromatography (TLC). A properly prepared TLC plate ensures good separation, sharp spots, and reproducible Rf values. TLC plates may be purchased commercially, but in many laboratory settings, plates are still prepared manually using silica gel or alumina as adsorbents.

Selection of Adsorbent:- Silica gel (SiO_2) is the most commonly used adsorbent because it provides strong separation and good stability. Alumina and cellulose are also used depending on the type of sample. Adsorbents are usually mixed with a binder like calcium sulphate ($CaSO_4$) to help them stick to the plate.

4.3.1 Preparation of Slurry:-

A slurry is prepared by mixing a measured amount of adsorbent with distilled water or an appropriate solvent. The mixture should be smooth and uniform. The slurry should not contain air bubbles, as these can create uneven layers.

4.3.2 Methods of plate preparation:-

Coating the Plates:- There are three main methods to coat the slurry onto glass/plastic/aluminium plates: Manual Spreading, Mechanical Spreader / TLC Plate Coater, Dipping Method, The slurry is spread on a glass, plastic, or aluminium sheet using: A spreader or applicator (for uniform layer), Manual pouring (in basic labs). The ideal thickness of the adsorbent layer is 0.25–0.5mm for analytical TLC. A uniform layer gives better separation and prevents tailing of spots. ϖ Drying the Plate:- After coating,

the plate is allowed to air-dry. This removes most of the moisture and helps the layer settle on the plate. Uneven drying can cause cracking or poor separation.

4.4 Activation of TLC Plate:- The glass slides are first kept in air for 5–10 minutes to dry. Then they are heated at about 100°C for 30 minutes to remove any moisture and activate the TLC plate. If volatile organic solvents are used, this heating step may not be necessary. Removing moisture helps the adsorbent layer work properly during separation.[21]

4.5 Capillary spotters:- TLC spotters are made from glass capillary tubes, which can be bought from most chemical or glassware suppliers. A capillary tube with both ends open can be cut into two TLC spotters. A capillary tube with one sealed end (like a melting point tube) can be used to make one TLC spotter. A new capillary spotter should be used for each sample. If needed, the capillary can be cleaned by repeatedly drawing solvent into it and wiping the outside with a tissue. Because capillary tubes are very thin, they hold only a small amount of liquid, making them perfect for placing tiny spots on TLC plates or chromatography paper. When the tip of the capillary touches the plate, capillary action pulls the liquid into the tube and then releases it onto the plate, forming a small, controlled spot. After spotting and applying the sample, the TLC plate is usually heated in an oven at 110 °C for about 30 minutes to dry and activate it. For preparative TLC, the adsorbent layer on the plate is thicker (0.5–2.0 mm),while for analytical TLC, the layer is thinner (0.1–0.25 mm).[6]

4.6 Spotting the Plate:- Dip the thin end of the capillary spotter into the dilute sample solution. Because of capillary action, the liquid will automatically move up into the tube. Lightly touch the capillary to the TLC plate at the start line to make a small spot. Let the spot dry, then touch the same place again. Repeating this makes the spot more concentrated but still small. Do not apply too much sample, because a large spot can cause poor separation and tailing. Make sure: Spots are not too close to the edges of the plate; Spots are not too close to each other. If possible, spot the sample, the starting materials, and any intermediate compounds on the same plate. This helps compare their movements.[4]

4.7 Location of Spots:- In TLC, the adsorbent material (usually silica gel or alumina) is very important. Its surface chemistry, particle size, and chemical composition affect how the sample spots move on the plate. Smaller (finer) particles give better separation, but the surface chemistry also affects how strongly compounds stick to the stationary phase. Spotting is done by placing a tiny amount of a diluted sample on the TLC plate. A micropipette or capillary is used to touch the edge of a TLC sheet that has a thin layer of silica gel. The solvent quickly evaporates, leaving behind a small amount of the sample. To see the spots after the TLC run, UV light is commonly used first because it is simple and does not damage the sample. When dark spots appear under UV light, they should be circled with a pencil, because they disappear when the light is turned off. If some spots are still invisible, chemical sprays can be used to make them visible.

4.7.1 Examples of visualization methods:- Potassium dichromate, Sulphur trioxide fumes, Potassium permanganate solution, Iodine vapours [3]

4.8 Developing Chamber:- The developing chamber is a crucial component in Thin Layer Chromatography (TLC) used to create a controlled environment for the movement of the mobile phase (solvent) through the stationary phase (TLC plate). Its primary function is to ensure uniform development of chromatographic spots and improve reproducibility of results.

4.8.1 Functions of a Developing Chamber

1. Maintains Saturated Atmosphere: The chamber allows the solvent to evaporate and fill the space with solvent vapours. This prevents solvent evaporation from the TLC plate and ensures consistent solvent front movement.

2. Ensures Controlled Development: The enclosed system avoids disturbances from air currents, temperature fluctuations, or accidental drying.

3. Holds Solvent Reservoir: A small amount of mobile phase is placed at the bottom of the chamber to facilitate capillary rise through the plate.

4. Prevents Spot Distortion: By maintaining humidity and vapour saturation, the chamber avoids edge effects and tailing

4.8.2 Types of Developing Chambers

1. Simple chamber: A beaker or jar covered with a lid or watches glass. Easy to use but may provide less reproducible results.

2. Twin-trough Chamber:- Consists of two compartments—one for saturation and one for plate development. More accurate and widely used in analytical TLC

3. Flat-bottom Chamber:- Ensures uniform solvent distribution and more reproducible Rf values.

4. Sandwich Setup (used in HPTLC): Uses a plate cover instead of a full chamber; appropriate for high-performance TLC plates with small particle size.

4.8.3 Procedure for Using a Developing Chamber

1. Pour mobile phase into the chamber (usually 0.2–0.5 cm depth).

2. Line the chamber walls with filter paper to promote solvent vapour saturation (recommended for reproducibility).

3. Close the chamber and allow it to pre-saturate (10–20 minutes).

4. Place the spotted TLC plate inside, ensuring the spots remain above the solvent level.

5. Allow solvent to ascend to the desired height.

6. Remove the plate and dry before visualization.

4.8.4 Importance of Developing Chamber

a. Provides reproducible and accurate Rf values

b. Minimizes environmental effects

c. Prevents irregular solvent front formation

d. Ensures better separation efficiency e. Reduces diffusion and tailing of analyte spots.[9]

4.9Development solvents:-

To develop a TLC plate, a small amount of the solvent (the developing solvent) is placed at the bottom of a container. When the TLC plate is put inside, the solvent rises up through the plate by capillary action. As the solvent moves upward, it carries the sample spot with it. There is a competition:

- The silica gel (which is very polar) tries to hold the compound,
- while the solvent tries to pull the compound upward, Good separation happens only when the polarity of
- the TLC plate (silica),
- the solvent, and

• the sample are properly balanced.

If the solvent is polar enough, it will pull the spot away from the starting point. As the spot moves, compounds with different polarities separate into individual spots. The TLC plate is removed when the solvent is about 1 cm below the top. The solvent front (the highest point the solvent reached) is marked with a pencil, and the plate is allowed to dry.[6]

4.10Developing Plate:-

A TLC plate can be developed inside a beaker or a closed jar. First, a small amount of solvent (the mobile phase) is poured into the container. Then a tiny spot of the sample solution is placed on the TLC plate about 1 cm above the bottom. The plate is placed in the container with a solvent like hexane or ethyl acetate, making sure the solvent level is below the spot, so the sample does not wash off. The solvent climbs up the plate by capillary action. As it moves upward, it dissolves the components of the sample and carries them along. Different compounds in the mixture move at different speeds because they: Stick differently to the stationary phase (silica), Dissolve differently in the solvent. By changing the solvent or using a mixture of solvents, you can adjust the separation of components (seen in their Rf values).

As the solvent travels upward, it moves each compound at its own rate. Non-polar solvents carry non-polar compounds higher on the plate because they dissolve well and do not stick strongly to the polar silica. Let the solvent rise until it is about 1 cm from the top of the plate. Then remove the plate and immediately mark the solvent front. Do not let the solvent reach or overflow the top edge. Finally, allow the plate to dry so the solvent completely evaporates.[2]

4.11Visualization:- Since most organic compounds do not have any colour, it is difficult to see where they are on a TLC plate after running the experiment. Therefore, scientists need to visualize the spots that means making the invisible spots visible. There are two types of visualization methods

1. Non-destructive methods – the compound stays the same after visualization

2. Destructive methods – the compound gets changed into something else to make it visible

A very common and safe method is viewing the TLC plate under UV light. Most TLC plates contain a fluorescent material that glows green under 254 nm UV light. When a compound absorbs this light, it appears as a dark spot against the glowing background. Chemical stains can also be used, but they are sometimes 20 harmful or dangerous. Stains may also destroy the compound, which is why they are called destructive methods. For example: Ferric chloride spray, Ninhydrin spray :- used to detect amino acids. Some TLC plates are coated with fluorescent materials to make it easier to see the separated spots under UV light. In short, TLC visualization is very important because it helps researchers locate the separated compounds, identify what they are, and estimate how much of each compound is present.

4.12Data Evaluation:-

After developing and visualizing a TLC plate, the collected information must be evaluated to understand the separation efficiency, identity, and purity of the compounds.

Rf Value Calculation:- The retention factor (Rf) is calculated using:-

Rf= Distance travelled by compound/ Distance travelled by the solvent

Comparison with Standard Compounds:- To identify an unknown compound, the sample is run alongside a known reference standard.

Spot Shape, Size, and Intensity:- Spot appearance helps evaluate sample purity:

Qualitative Interpretation:-TLC allows:-Determination of number of components

Semi-Quantitative Evaluation :-Approximate concentration can be estimated:

Documentation :-Drawing the plate outline, Marking baseline and solvent front **,**Recording Rf values

Final Interpretation:-Using all observations Rf values, number of spots, intensity, and pattern one can identify the compounds, judge purity, and decide whether further purification is required.[11]

V. CONCLUSION:-

Thin Layer Chromatography continues to be one of the most practical and widely employed chromatographic techniques in laboratory analysis. Its simplicity, low cost, and rapid operation make it suitable for routine qualitative testing, reaction monitoring, and preliminary analysis of complex mixtures. The effectiveness of TLC greatly depends on factors such as choice of adsorbent, mobile phase, plate preparation, and proper handling during spotting and development. Visualization methods and proper evaluation of Rf values further enhance its reliability in identifying compounds and assessing purity. Despite limitations such as limited separation length, difficulty in result reproducibility, and environmental sensitivity, TLC remains a fundamental analytical tool. Its broad applicability in pharmaceuticals, biotechnology, botany, and natural product research underscores its continued importance in modern scientific analysis. With advancements in adsorbents, solvent systems, and instrumentation, TLC continues to evolve as a powerful technique for rapid and efficient chromatographic separation.

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