**Research & Reviews: Journal of Pharmacognosy and Phytochemistry**

e-ISSN: 2321-6182
p-ISSN: 2347-2332

# A Brief Note on Thin-layer Chromatography

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**Opinion Article**

**Received:** 1-Mar-2022, Manuscript No. JPRPC- 55729; **Editor assigned:** 3-Mar-2022, PreQC No. JPRPC- 22-55729(PQ); **Reviewed:** 17-Mar-2022, QC No JPRPC-22- 55729; **Revised:** 21-Mar-2022, Manuscript No. JPRPC-22-55729 (R); **Published:** 28-Mar-2022, DOI: 10.4172/ 2321-6182.10.2.004

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## DESCRIPTION

Thin-Layer Chromatography (TLC) is a type of chromatography used to separate non-volatile mixtures. Thin-layer chromatography is carried out on a sheet of inert substrate such as glass, plastic or aluminium foil that has been coated with a thin layer of adsorbent material typically *silica* gel, aluminium oxide (alumina) or cellulose. The stationary phase refers to this adsorbent layer. Following the application of the sample to the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate by capillary action. Separation occurs because different analytes ascend the TLC plate at different rates. The properties of the mobile phase differ from those of the stationary phase. Non-polar mobile phases such as heptane are used with *silica* gel, a very polar substance. The mobile phase may be a mixture going to allow chemists to fine-tune the mobile phase's bulk properties.

The spots are visualised after the experiment. This is often accomplished simply by projecting ultraviolet light onto the sheet the sheets are frequently treated with a phosphor and dark spots appear on the sheet where compounds absorb the light impinging on a specific area. Spots can also be visualised using chemical processes anisaldehyde, for example, forms coloured adducts with many compounds and sulfuric acid chars most organic compounds, leaving a dark spot on the sheet. To evaluate the results, divide the distance travelled by the substance under

e-ISSN: 2321-6182
p-ISSN: 2347-2332

consideration by the total distance travelled by the mobile phase this ratio is known as the Retardation Factor (Rf) or retention factor in colloquial parlance. To obtain a quantitative result, the solvent absorption must be stopped before the mobile phase reaches the end of the stationary phase. In general, a substance with a structure similar to the stationary phase will have a low Rf whereas one with a structure similar to the mobile phase will have a high retardation factor. Retardation factors are typical, but they vary depending on the precise condition of the mobile and stationary phases. Thin-layer chromatography can be used to track the progress of a reaction identify compounds in a given mixture and assess the purity of a substance. Analyzing ceramides and fatty acids detecting pesticides or insecticides in food and water, analysing the dye composition of fibres in forensics, assaying the radiochemical purity of radiopharmaceuticals or identifying medicinal plants and their constituents are some examples of these applications.

The original method can be improved in a number of ways including automating the various steps increasing the resolution achieved with TLC and allowing for more accurate quantitative analysis. This technique is known as HPTLC or "High-Performance TLC." HPTLC typically employs thinner layers of stationary phase and smaller sample volumes which reduces resolution loss due to diffusion. TLC plates are typically available commercially with standard particle size ranges to improve reproducibility. They are made by combining an adsorbent such as *silica* gel with a small amount of an inert binder such as calcium sulphate (gypsum) and water. This mixture is applied as thick slurry to an unreactive carrier sheet which is typically glass, thick aluminium foil or plastic. The finished plate is dried and activated in an oven for thirty minutes at 110 °C. The absorbent layer thickness is typically around 0.1–0.25 mm for analytical purposes and around 0.5–2.0 mm for preparative TLC. The method is similar to paper chromatography but with the added benefit of faster runs better separations and the ability to use different stationary phases. TLC is frequently used for monitoring chemical reactions and qualitative analysis of reaction products due to its simplicity and speed. Plates can be labeled before or after the chromatography process with a pencil or other non-interfering or reactive implement. The following steps are taken to run a thin layer chromatography plate.

A small spot of solution containing the sample is applied to a plate using a capillary tube about 1.5 centimeters from the bottom edge. To avoid interfering with the sample's interactions with the mobile phase in the following step the solvent is allowed to completely evaporate. If the sample was applied with a non-volatile solvent the plate must be dried in a vacuum chamber. This step is frequently repeated to ensure that there is sufficient analyte at the starting point on the plate to produce a visible result. Different samples can be placed in a row of spots that are all the same distance from the bottom edge and each will move in its own adjacent lane from its own starting point. A small amount of an appropriate solvent (eluent) is poured to a depth of less than 1 centimeter into a glass beaker or any other suitable transparent container (separation chamber). A strip of filter paper (also known as a "wick") is

e-ISSN: 2321-6182
p-ISSN: 2347-2332

placed in the chamber so that the bottom of the paper touches the solvent and the paper lies on the chamber wall and almost reaches the top of the container. Close the container with a cover glass or any other lid and leave it for a few minutes to allow the solvent vapors to ascend the filter paper and saturate the air in the chamber.