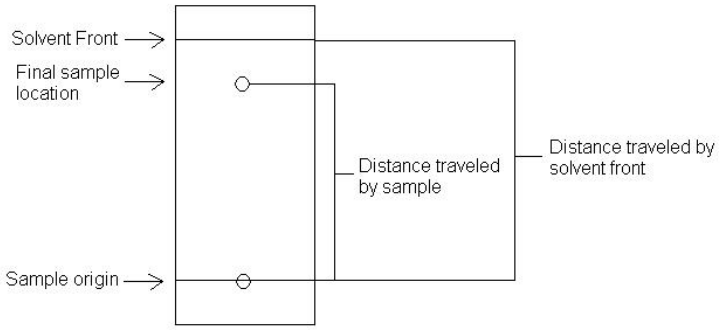
18.0 THIN LAYER CHROMATOGRAPHY

18.1 Application

Thin layer chromatography is a separation technique that utilizes ion mobility and solvent polarity to differentiate similar compounds by their retention factors (Rf). The Rf is calculated as the distance traveled by the sample divided by the distance traveled by the solvent front as demonstrated in the diagram below.

$$\text{Retention factor} = \frac{\text{Distance traveled by sample}}{\text{Distance traveled by solvent front}}$$



18.2 Equipment, Reagents, and Standards

- Silica gel plates specifically designed for thin layer separation (type and manufacturer may vary)
- Capillary tubes or micropipettes
- Glass tank or container with appropriate sealing mechanism capable of accommodating the silica plate
- Mobile phase solvent
- Working standard of the suspected analyte
- Visualization solution appropriate for the suspected analyte
- Propellant system for visualization solution

18.3 Method

An origin line is drawn in pencil on the TLC plate approximately 2 cm from the bottom of the plate. The procedural blank(s), sample(s), and standard(s) are spotted on this line. Each spot must be at least 2 cm from the side of the plate and 1 cm or more from each other. The procedural blank will consist of a separate aliquot the same solvent(s) used to extract the sample. A capillary tube or micropipette may be used for spotting as a small, tight spot is important for best results.

The spotting solvent must be volatile and relatively non-polar to minimize the wandering of the initial spot. The spot must be dried prior to being placed into the mobile phase solvent. Label the plate with pencil as necessary before spotting to avoid confusion when interpreting the results.

A developing chamber and appropriate solvent(s) will be assembled and allowed to equilibrate for approximately 30 minutes before adding the spotted plate. The solvent(s) for the mobile phase will vary depending upon the analyte(s). Mobile phase systems outlined *Clarke's Isolation and Identification of Drugs* should be used for the analyte of interest.

After spotting the silica plate, place the TLC plate into the chamber in a vertical position with the origin line above the mobile phase solvent. The solvent will be allowed to migrate from the bottom of the plate to within 5 -10 cm of the top of the plate. A shorter migration distance may be appropriate depending on the analyte. After the solvent has traveled the required distance, the plate is removed and dried either through evaporation or heated air.

The plate is then placed in a ventilated area and sprayed with an appropriate visualizing compound for the analyte and the standards. Any developed spots are circled immediately if they are not clearly visible once the plate has dried.

18.4 Interpretation

The working standard must be run on the same silica gel plate as the unknown for confirmation. The analyst will calculate the Rf values for the sample(s) and standard(s) for comparison.

A positive result is when the sample and reference standard have the same color spot and have the same Rf value. The results will be documented in the case file as outlined in the Documentation chapter of this manual.

Any spots appearing in the procedural blank will invalidate all results obtained from the analysis. The test should be repeated using new solutions if this occurs.