Thin Layer Chromatography (TLC) is a method used to quickly separate and analyze small amounts of material. This technique is most often used to separate components of a mixture and compare them with components of known identity. It can be useful in determining whether a reaction has reached its endpoint (are starting materials still present?), determining whether a reaction has produced more than one product, or in identifying the components of a mixture when a comparison standard is available.

A TLC plate is a plate of glass or aluminum that has been coated on one side with a thin layer of solid (stationary phase). In this experiment, the solid used is silica gel (SiO$_2$ xH$_2$O), a fine-grained, white solid. A solution of the material to be analyzed is applied to the solid layer near the bottom of the plate, the origin, in a small spot using a micropipet. Additional spots of standard materials for comparison are also applied. The plate is then placed in a developing chamber containing a small amount of solvent, the mobile phase, which then travels up the plate by capillary action. The solvent is allowed to travel to within 1-2 cm of the top of the plate then is removed from the chamber. The solvent front, or the farthest line reached by the solvent, is marked with pencil, then the plate is allowed to dry. The plate is then visualized using special lights and/or indicator sprays and the component spots are circled in pencil for purposes of comparison and analysis.

The technique of chromatography separation relies on two competing processes – the different attractions of the components to the stationary and mobile phases. The attractions to the stationary phase from strongest to weakest are: ionic > hydrogen-bonding > dipole-dipole > London (or van der Waals) dispersion forces. Therefore if a component of the spot exhibits an ionic attraction, usually seen with an acid or base, to the silica, then that component will be more resistant to moving with the stationary phase. This may be exhibited by streaking or trailing of the spot as it is dragged up the plate by the solvent yet is strongly attracted to the silica.

Attractions to the mobile phase (solvent) are based on solubility, the basis of which is “like dissolves like”. Therefore a polar solvent will exert more mobile force on a polar component whereas a non-polar solvent will exert more mobile force on a non-polar component. Thus the choice of mobile phase must be compatible with the polarities of the mixture components so that each component will move a different amount and separate from the others. For example, if the components of the mixture are of low polarity, they are most likely not soluble in water, which is very polar. Therefore the use of water as the mobile phase would be inappropriate because none of the materials would move up the plate. Chemists using the technique of thin layer chromatography need knowledge of relative polarities of solvents and mixture components combined with some trial and error to find the best solvent or solvent mixture to maximize separation. In this lab the solvent has been determined for you.

In this experiment you will use TLC to determine the two components in an unknown by comparing to three pure standard drugs. The three standards are: caffeine, acetylsalicylic...
acid (aspirin) and acetaminophen (active ingredient in Tylenol). They will be spotted alongside your unknown.

The chemical structures of the three drugs are shown in Fig 1 below. You will visualize the components on the TLC plate by using UV lamps. The silica of the plates has been coated with a special material that fluoresces when exposed to ultraviolet light. The drugs applied to the plate will not fluoresce and so will appear as a dark spot. You will determine and compare Rf values for each known and unknown. You will also learn the technique of making micropipets from capillary glass.

![Chemical structures of analgesic components](image)

Figure 1. Chemical structures of analgesic components

**Procedures:**

**Prepare Capillary Tubes:**

| Capillary tubes | Bunsen burner |

1. Using the Bunsen burner, heat the center of a capillary tube.
2. Roll the tube gently between your fingers and stretch it while it is in the flame.
3. Steadily pull the ends apart from one another.
4. Break the stretched tube to create two tiny pipets.
5. Repeat these steps with about five more capillary tubes.
6. Store pipets in paper cup as directed by your teacher.

!!Safety note: All flames must be extinguished before proceeding to the next part of the lab.
Prepare a developing chamber:

- Large wide-mouthed, capped jar
- Ethyl acetate
- Centimeter ruler

7. Remove the cap from the jar.
8. Pour enough ethyl acetate into the jar so that it reaches a height of 0.5 cm above the bottom of the jar.

Prepare Plate:

- TLC plate
- #2 pencil
- Centimeter ruler

9. Obtain one TLC plate. **CAUTION: Be sure to handle the plates by the edges because skin oils can affect results.** Place the plate shiny side down on clean paper.
10. Using a #2 pencil and ruler, draw a line across the plate 1 cm above the bottom and make the other markings as indicated in the diagram below. Write your name in pencil along the top of the plate.
Spot the TLC Plate:

Aspirin solution  
Caffeine solution  
Tylenol solution  
Unknown mixture solution  
UV lamp

11. Obtain unknown solution and record number. Unknown:_____________ (You will use only one unknown, but will spot it twice for confirmation of results.)
12. Using one capillary tube pipet for each solution, dip the tube in the solution, and dot it on the TLC on the line marked for that solution. The spot should be as small as possible yet dark enough to indicate that enough material is deposited. You may re-spot once the initial spot has dried. Be careful not to scratch the white powder of the TLC plates. Discard used pipets immediately after use so cross-contamination does not occur.
13. Using the UV lamp, have your instructor check the TLC plate to verify that the solution dots are concentrated enough for the experiment. Caution!! Do not look directly at the UV light!

Develop the TLC Plate:

Previously prepared developing chamber  
Previously prepared TLC plate  
#2 Pencil  
UV lamp

14. Place the prepared developing chamber jar on the lab bench where it won’t be moved for about 20-30 minutes.
15. Carefully open the jar without moving the liquid in the container. Pick up the TLC plate by the edges and carefully place it into the jar so that the markings are on the bottom of the plate. Place the cap on tightly without moving the container. CAUTION: Do not move the jar until the development is finished. Splashing of the solvent will affect the quality of your spots. Do not let the solvent front reach the top of the plate. The development should take approximately 20-30 minutes.
16. During the development, dispose of excess solutions and pipets in their appropriate waste containers.
17. When the development is complete, remove plate from jar and immediately draw a pencil line to indicate solvent front. This line does not have to be straight but should follow the solvent pattern. Place the plate on paper towel in the fume hood to dry.
18. Allow the solvent to evaporate, and then view your plate under the UV light. Outline all the spots with a pencil. CAUTION: Do not look directly at the UV light.
Analysis

- Reproduce a scale drawing of your plate in your lab book. Include markings for solvent front, origin, and component spots. Unknown solutions will contain 2 components.
- Calculate Rf values:
  To analyze the results of chromatography in a quantitative way, a mathematical function called retention factor, or Rf, is used. The Rf value is equal to the distance a particular component traveled divided by the distance traveled by the solvent (solvent front). Use a metric ruler to measure from the origin to the center of each spot. Divide this by the distance from the origin to the solvent front. See Fig. 2 below. Show work for your calculations. Construct a table of this information.

![Fig 2. Chromatogram distances for Rf calculation showing a 3 component mixture. Three Rf’s should be calculated – one for each spot.](image)

Lab report:

Data and Results:

- Same scale drawing of the chromatogram.
- Results table of Rf values for each spot
- Any calculations used to determine Rf values and to compare component Rf’s.

Conclusion/Discussion:

- Discuss your results. What two drug components did you determine for your unknown (be sure to give Unknown # for the solution)? How did the Rf values compare? How confident are you about your results?
- Based on your results, which component was most strongly attracted to the silica? Explain your choice.
- Hydrogen bonding between the silica (white powder on the plate) and the components is possible if the molecule has a hydrogen bonded to oxygen, nitrogen or fluorine. By looking at the chemical structures, which drugs (acetylsalicylic acid, acetaminophen, caffeine) can have hydrogen bonding attractions to the silica?
- Based on how high they moved up the plate, which drug was most soluble in the solvent? Which was least soluble in the solvent. Explain your answers.