TLC Analysis of Analgesics Drugs Experiment

3.0 Introduction:

Thin layer chromatography (TLC) is a common chromatographic technique used by organic chemist.

- This technique can be used to purify lesser amounts of material (between 50mg to 100mg) in the case of preparative TLC.
- To determine the number of components in a mixture.
- To determine the identity of two substances.
- To monitor the progress of a reaction.
- To determine the effectiveness of a purification.
- To determine the appropriate conditions for a column chromatography.

TLC works the following way. A sample is spotted in a silica gel (Si_2O) coated sheet. Organic chemist refers to this sheet as the ***stationary phase***. Not all the stationary phases, in TLC, are silica based. Others include alumina (Al_2O_3) and Carbon 18, also known as reverse phase. Once the sample is spotted using a capillary tube the sheet is inserted in a developing chamber. This developing chamber will contain a ***liquid or mobile phase*** which, through capillary action, is going to move up the silica coated sheet carrying your sample with it. Separation of each component in your mixture will be accomplished based on the polarity of the molecules present in the mixture. Try to envision the following. The spotted material has the choice of spending time or partitioning in the mobile phase or stationary phase (Si_2O). The mobile phase is a non-polar liquid, and the stationary phase is a polar one. Therefore, the mobile phase is not going to move all the components of the mixture at the same rate. Polar material will be retained in the stationary phase for a longer time that non-polar materials which will prefer to travel with the mobile phase. That is the theory behind TLC and how the separation of single components in a mixture is achieved ***based on differences in polarity***. In today's lab you will be using TLC to identify the single components of a mixture of analgesics found in several pain killers.

You will be given two commercially prepared TLC plates with a flexible backing and a silica gel coating with a fluorescent indicator. This fluorescent indicator will allow you to visualize the material spotted in the plate by shinning UV light on it. Only substances that have UV active functional groups or chromophores will be visible under UV light. If you do not

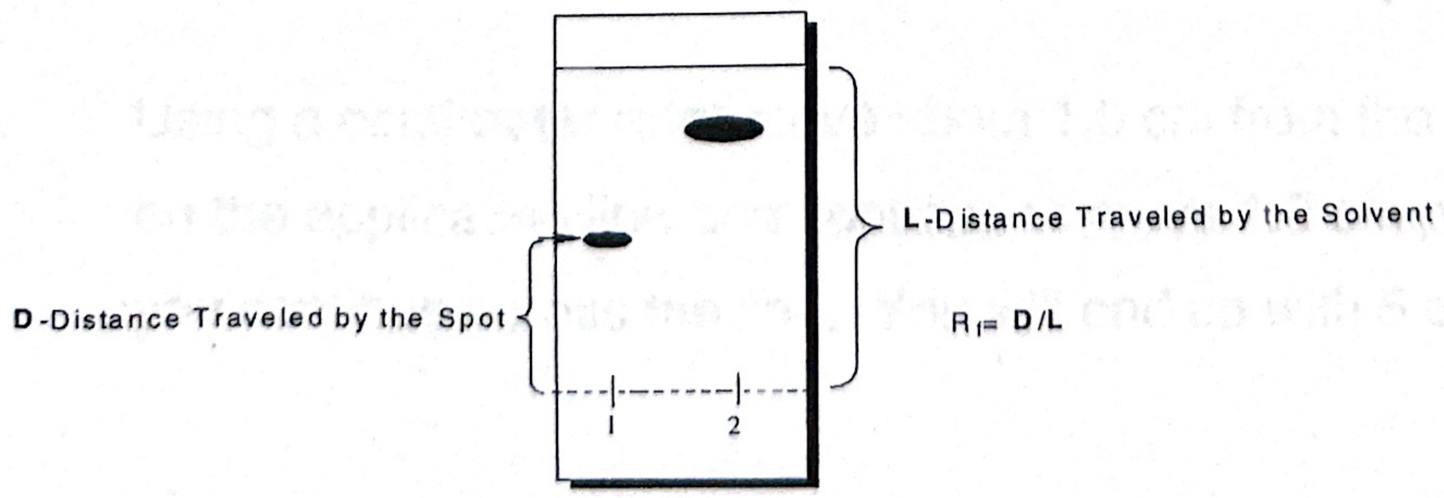

Figure 3.0: A typical TLC plate

have any in your analyte, you must use an indicator or a reagent to help you visualized the spotted material. On one TLC plate you will spot five compounds often found in analgesic formulations. These compounds are Acetaminophen (Ac), Aspirin (Asp), Caffeine (Caf), Phenacetin (Phe), Salicylamide (Sal). They will all be available as solutions dissolved in 20 ml of 50:50 mixture of methylene chloride and absolute ethanol. You will use this plate as a reference and the purpose of running the first plate is to establish a Rf, ***rate of flow***, value for all five known analgesics. Eventually you will use these Rf values as a reference and compared them to the values obtained on your second plate consisting of your analgesic mixture. How you spot your material in the sample is very important. ***Your instructor will show you how to do it.*** You will notice that once you place your plate in the developing chamber the liquid will rise through capillary action carrying your spotted material with it. The rate at which each spot moves or ***rate of flow Rf*** will help us identify each component in the mixture. How do we calculate Rf's values? You will notice that the solvent along with your spot will move up the plate. For our purpose we will call the distance traveled by the spot in our TLC plate **D**, which will be divided by the distance traveled by the solvent **L**. This ratio is what organic chemist refers to as the ***Rf value*** (Figure 3.0).

When measuring D, you must do it from the line of origin to the center of the spot. On a second plate several solutions prepared from commercial analgesic tablets will be spotted and allowed to run. The tablets will be crushed and dissolved in a 50:50 methylene chloride-alcohol mixture. Both plates will be visualized by UV and by the colors that are produced during UV fluorescence. This difference in appearance under UV illumination will help to distinguish the substances from one another. It is important to outline very lightly in pencil the spots observed and to note the color of those that have fluoresced. Doing this will enable one to establish which, of the reference standard analgesic used on the first TLC plate, are present in the commercial analgesics run on the second plate.

3.1 Procedure:

1. Obtain two TLC plates from your instructor. These plates should be handled carefully, or the absorbent may fall off.

2. Using the first plate, **lightly** draw a pencil line across the plate about 1.5 cm from the bottom of the plate. This is called the point of application line.

3. Using a centimeter ruler move about 1.0 cm from the edge and place a dot on the application line and continue to move 1.0 cm placing another dot as you continue across the line. You will end up with 5 evenly spaced dots.

These are the points (dots) at which the samples will be spotted. On the first plate, spot Ac, Asp, Caf, Phe and Sal. Spot each sample at least twice, allowing the spot to dry in between each spot. Make the spots small.

4. Obtain a 16 oz wide-mouthed screw-cap jar for use as a development chamber. Place 20 ml of the eluting solvent, ethyl acetate, in the chamber. The solvent level must be below the application (pencil) line when the plate is inserted in the chamber.

5. Insert the plate in the chamber and cap the jar. **DO NOT MOVE THE JAR.** The eluting solvent will transverse the plate pulling the dissolved analgesics with it.

6. When the solvent is approximately 1 cm from the top of the plate immediately remove the plate from the chamber and mark the solvent front line with a pencil.

7. While the first plate is still in the chamber prepare the second plate. Prepare it the same as the first plate, however, there are SIX solutions of commercially available analgesics tablets/capsules. Space the points ¾ cm, apart. The ultimate purpose of the experiment is to identify from the known TLC what substances present on this plate are also present in the tablets that were run on the second plate.

8. Crush one whole tablet using a mortar and pestle. Mix 10 ml of absolute ethanol and 10 ml of methylene chloride in a 50 ml beaker. Transfer the crushed tablet to this mixture and warm on a hot plate for a few minutes. The entire tablet will dissolve because of starches/fillers in the tablet.

9. These 6 analgesic tablet solutions will be spotted on the second plate. It will be necessary to double, or triple spot them. Dry in between each spotting. Use a new chamber and solvent to run the second plate. Once

again remove the second plate when the solvent is approximately 1 cm from the top. Be sure to mark the height of the solvent front.

10. Observe the TLC plate using a UV light. Using a pencil circle any the spots and note their color under UV fluorescence.

11. Using the first plate, measure the distance the solvent traveled from the origin to solvent line – this is known as the "L" value. Measure the distance to the center of five spots on this plate and record. These are known as "D" values. Calculate Rf by dividing each D by the L value.

12. Using the second plate measure the L value for this plate. Then measure all the D values for each tablet solution that was run on the plate. Now calculate all the Rf values.

13. State what standards from the first plate are present in each analgesic tablet on the second plate.

RESULT PLATE #1

Analgesic Name	L(cm)	D(cm)	Rf (Rate of flow)	Fluorescence (Y/N)
Ac				
Asp				
Caf				
Phe				
Sal				

RESULT PLATE #2

Tablet Letter	L(cm)	D_1	D_2	D_3	Rf_1	Rf_2	Rf_3	Fluorescence(Y/N)
1.								
2.								
3.								
4.								
5.								
6.								

Name each analgesic(s) which are present in each analgesic tablet on plate #2.

1.

2.

3.

4.

5.

6.

Experiment-Write Up-Grading Rubric

TLC Analysis of Analgesics Drugs Experiment

Title and entry in Table of Contents- 1 point

Purpose- 1point

Introduction- (pre-laboratory notes)-2 points

Observations and Experimental log- 3 points, should include:

- TLC plates (actual plates stapled to the notebook)
- Complete Summary Tables including Rf calculations…etc.

Discussion- 5 points

- Define Chromatography.
- Describe how thin layer chromatography was used to identify the compounds in this lab.
- Explain why different compounds travel at different rates along the TLC plate? Relate your answer to the single components in your analgesic pills.
- What is the Rf value?
- Provide a rationale for your identification of each of the single components of your mixture found in the analgesic pills.
- **Laboratory Techniques and Results** - 3 points-Points will be deducted from not wearing the appropriate eye protection, improper disposal of reagents, breaking of glassware and unsafe behavior in the lab.