

Name:
Lab Partners:

Date:

Lab # 12: What's in that Pill? Applying Intermolecular Forces to Thin-Layer Chromatography Accelerated Chemistry 1

Introduction

A challenge often facing a chemist is to determine the chemical components of a mixture. For example, a forensic chemist might have to identify a pill found at a crime scene of a drug-overdose. Most often, pills are mixtures of various substances.

In this lab you will analyze two over-the-counter pain medications – Excedrin and Anacin- to determine what types of **analgesics** (pain relievers) they contain. You will compare the TLC of two medications to standards of common analgesics – acetaminophen, aspirin (acetylsalicylic acid), and caffeine- to determine which analgesics each contain.

Thin-layer Chromatography – How does it work?

If you've drawn with water-soluble markers on a piece of paper and then gotten the paper wet, you've seen chromatography in action. The colors in the ink spread out in the water layer, and pretty soon you can see that what originally looked like green ink was actually a mixture of blue and yellow.

Every molecule has properties that depend on the atoms it's made of; the chief property that thin-layer chromatography (TLC) depends on is **polarity**. Remember that bonding a highly electronegative atom to another atom causes polarity. The electronegative atom causes a shift in the bonding atoms' electron clouds toward itself, creating a partial negative end around the electronegative atom, and a partial positive end around the other atom. When you use TLC, you put a tiny spot of the mixture you want to separate onto a thin layer of a solid adsorbent material, like silica (SiO_2), called the stationary phase. (Adsorbent refers to a material to which other materials will "stick.") The silica is polar. Then, you allow a less polar mobile phase to move up the adsorbent material, carrying the substances in the mixture with it. While the mobile phase moves up the silica-coated TLC plate, the substances in the mixture are subjected to two forces - attraction to the mobile phase and attraction to the stationary phase. The molecules in the mixture move along, caught in a tug-of-war between the mobile phase and the stationary phase. Molecules that are more strongly attracted to the silica (molecules that are highly polar) will not move very far up the plate.

When a particular solvent, such as hexane or ethyl acetate ($\text{CH}_3\text{CH}_2\text{OCOCH}_3$), is used with the same stationary phase, substances in a mixture will always behave the same way because the chemical interactions between the stationary and mobile phases are the same. Their behavior is so predictable that you will observe the same patterns of spots each time. The only thing that might change the exact placement of the spots on the plate is the amount of time that the experiment is run. To handle this variable, chemists express the distance that a spot moves as a ratio – the distance the spot moves to the distance the mobile phase moves up the plate. This ratio is referred to as the R_f value. Since the solvent always moves at least as far as anything dissolved in it, the value of the R_f is always less than 1.

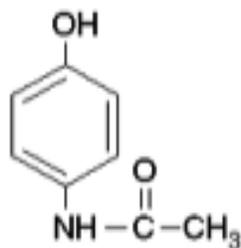
$$R_f = \frac{\text{distance spot traveled}}{\text{distance solvent traveled}}$$

Safety

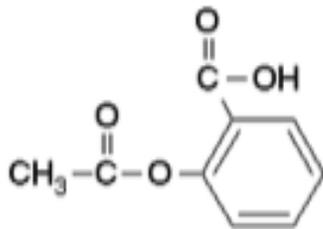
- As always, safety goggles must be worn when you are working in the lab.
- The solvents used in this lab are quite flammable; so make sure there is nothing that will produce a flame or spark in the work area.
- UV light can cause eye damage, so never look directly into the light. Use the light to locate and mark your spots and then turn it off.
- When you are ready to dispose of wastes, pour the using ethyl acetate mobile phase in the waste container on the front lab bench. Do not pour the ethyl acetate down the drain.

Pre-lab

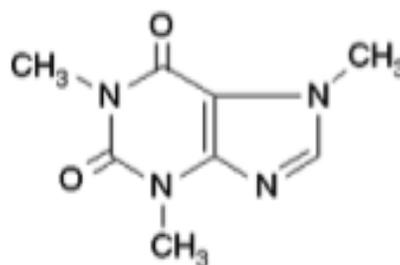
1. You will be examining the following analgesics to determine if they are contained within the two over-the-counter medications, Excedrin and Anacin. Examine the chemical structures of the three analgesics. Circle the polar covalent bonds in each.



acetaminophen



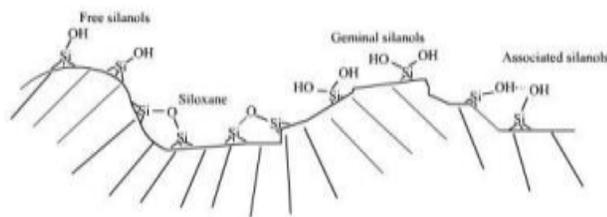
aspirin



caffeine

Figure 1. Structures of the three standard analgesics

2. Which analgesic has the most number of polar bonds?
3. The stationary phase of the TLC plate used in this lab is made of silica (SiO_2), which is a polar compound. Which of the following molecules will be most attracted to the stationary phase? Why?
4. Which compound do you expect to move the farthest up the TLC plate with the mobile phase, i.e. have the largest R_f value? Explain.
5. Which compound do you expect to have the smallest R_f value? Explain?
6. A grain of sand is a silica particle. Examine the structure of silica shown in **Figure 2**. Si and O form a network covalent structure with a tetrahedral geometry. But, the structure of the silica is different at the surface of the particle. Why is silica polar?



Silica particle

Figure 2.

Procedure

Part 1. Prepare the developing chamber

1. Fill the bottom of a 400-mL beaker to a depth of about 0.5 cm with ethyl acetate.
2. Take a small piece of filter paper and gently rest it against one side of the beaker, dipping the edge of the paper into ethyl acetate and allowing the paper to become saturated.

Part 2. Preparing the TLC plate

1. Handle the TLC plate by the edges, being careful not to touch it with your fingers. Oil from your skin will interfere with the movement of the solvent on the plate. Look at the plate and decide which edge is the straightest – use this edge as the bottom of the plate.
2. Use a dull #2 pencil to lightly draw a line 1 cm from the bottom of the plate. Do not press hard enough to dig into the silica layer, it is fragile and large divots in the surface of the plate will interfere with developing.
3. Make five, evenly spaced, marks on the line. Label each – one for the aspirin standard, one for the acetaminophen standard, one for the caffeine standard, and one mark for each of the two trial medications (See Figure 3.)

Part 3. Prepare the solutions of the analgesics

1. Obtain a TLC spotting well plate.
2. Place a small amount of each analgesic into separate wells of the spotting well plate, as labeled. Scatter the wells you use for each analgesic so that the solvent from one does not contaminate the well of another.
3. Fill each well containing an analgesic with ethyl acetate and stir to dissolve. (Not all of the sample will dissolve. The tablets used contain binders that are not soluble in ethyl acetate. The important analgesic will dissolve.)

Part 4. Spot the practice plate

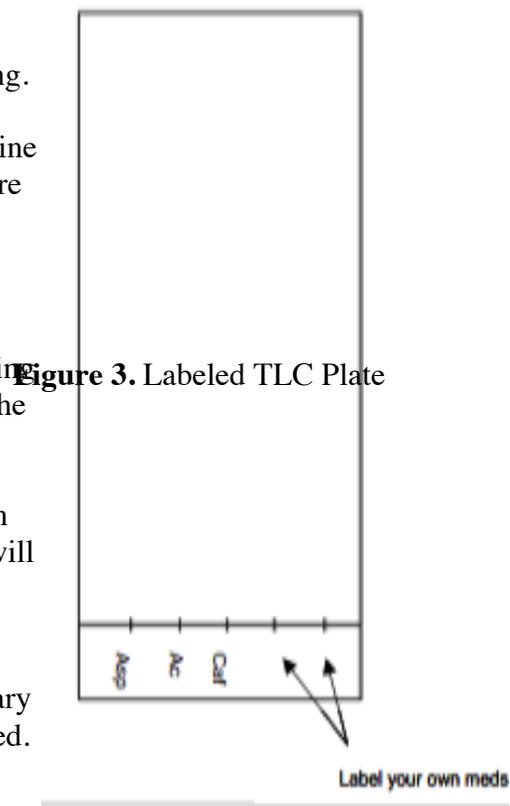
1. Your teacher will provide a small practice plate. Obtain a microcapillary tube and dip it into one of the analgesic solutions you have just prepared.
2. Briefly touch the tip of the microcapillary tube to the pencil mark on the practice TLC plate. A small amount of liquid in the tube will be drawn onto the plate, making a small spot. The solvent will evaporate in a few seconds.
3. Repeat step 2, four times. Keeping the spot small is very important so that one spot does not develop into the spot of another analgesic. However, you have to have enough of the substance present on the TLC plate to be detected.
4. Take your plate over to the UV light and visualize the spot. It should be about this big: ● Have your teacher approve the spot.

Part 5. Spot the TLC Plate

1. Carefully spot your prepared TLC plate directly on the pencil marks in the same way you spotted the practice plate. Use a different microcapillary tube for each solution you spot.
2. Visualize the spots to make sure they are the proper size. Have your teacher approve your plate before you move on.

Part 6. Develop the TLC Plate

1. Place the developing chamber on your lab table in a spot where it will be undisturbed for 15-20 minutes.



- Carefully remove the lid without sloshing the ethyl acetate
- Handle the plate by its top edge. Lower the plate into the jar so that the markings are above the solvent level and let the plate lean against the side of the beaker. Put the foil cover back on the jar without disturbing the solvent or the plate. Do not move the jar until developing is over.
- When the solvent has moved to within 1.0-1.5 cm from the top of the plate, remove the plate and place it onto the surface of a paper towel.
- Immediately mark the solvent front with a pencil as shown in Figure 4. The solvent front is the highest level reached by the mobile phase. It will evaporate quickly, so make sure you do this as soon as you take the plate out of the chamber.

Part 7. Visualize your results and calculate R_f values

- With your safety goggles on, look at your plate under the UV light. Outline all spots with a pencil.
- Using a pencil, place a dot in the center of each spot that you outlined
- Measure the distance, in cm, from the starting line at the bottom of the plate to the solvent front line. Record this measurement in Data Table 1.
- Measure the distance from the starting line to the center of each spot of standard solution. Record the measurements in Data Table 1.
- Make the same measurements for the over-the-counter medications. Use the portions of Data Table 2 that are appropriate for the meds you tested. If the meds produced more than one spot, record the measurements for each spot.
- Calculate the R_f values for each spot using the formula given in the Introduction. Record your results.

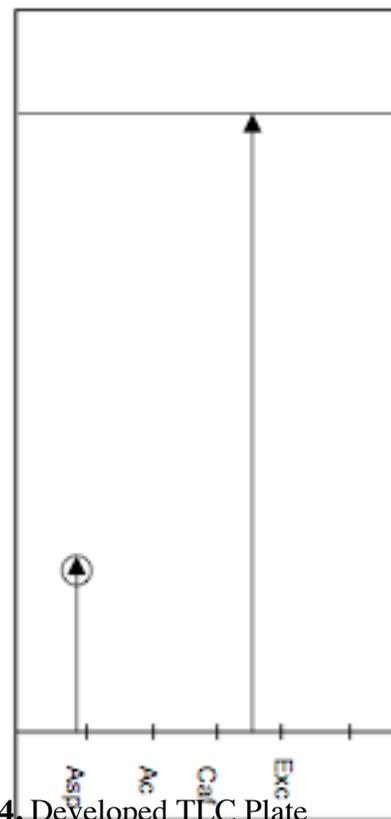


Figure 4. Developed TLC Plate

Data and Calculations

Data Table 1. Standard Solutions' R_f Values

Standard Solutions	Solvent Front (cm)	Spot distance (cm)	R_f Value
Aspirin			
Acetaminophen			
Caffeine			

In the space below, show your calculations for the standard solutions.

- Aspirin R_f calculation

- Acetaminophen R_f calculation

- Caffeine R_f calculation

Data Table 2. Medication R_f values

Medications	Spot 1		Spot 2		Spot 3	
	Spot Distance (cm)	R_f	Spot Distance (cm)	R_f	Spot Distance (cm)	R_f
Excedrin						
Anacin						

Analysis and Conclusions

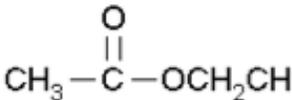
1. Compare the R_f values from the standards in Table 1 with the R_f values from the medications in Table 2.
2. Identify the substance(s) present in each of the medications.

Name of Medication:

Substance(s) Present:

Name of Medication:

Substance(s) Present:

2. What factors affect the distance each spot travels on the plate?
3. What was the purpose of applying spots of known caffeine, aspirin and acetaminophen solutions?
4. Ethyl acetate has the structure shown at the right. Is ethyl acetate polar? In comparison to silica, which is more polar? Why can ethyl acetate be used to separate the analgesics?

5. Is it safe to assume that a mixture that produces two spots on a TLC plate contains only two substances? Why or why not?

6. If you were to run this experiment a second time using the same chromatography solvent (ethyl acetate), you would expect to get the same R_f values you got the first time. If, however, you used ethanol instead, your R_f values would be different. Explain why this is true. (Compare the polarity of ethanol to that of ethyl acetate.)