

## Lab 8: Chromatographic Analysis of Analgesic Drugs

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### Objectives

By the end of this laboratory, you should have developed the skills to do the following:

- Use TLC to identify compounds in a mixture.
- Separate a mixture of compounds using column chromatography.

### Recommended Resources

- Tutorial ~ Introduction to Thin Layer Chromatography  
<https://www.youtube.com/playlist?list=PL03C01E9EB4EF6B45>
- Website ~ Thin Layer Chromatography  
<http://orgchem.colorado.edu/Technique/Procedures/TLC/TLC.html>
- Video ~ Column Chromatography  
<http://www.youtube.com/watch?v=EOJrRnpiq5s>
- Website ~ Column Chromatography  
<http://orgchem.colorado.edu/Technique/Procedures/Columnchrom/Columnchrom.html>

### Background

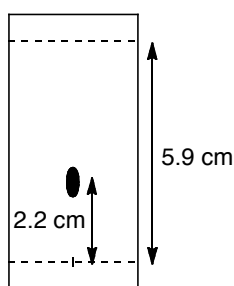
Chromatography is a method frequently used to separate mixtures of compounds. In chromatography, compounds are separated based on the way they interact with a porous material, called the adsorbent or *stationary phase*, and the solvent that moves the mixture through the adsorbent, called the eluent or *mobile phase*. In this lab, you will perform thin layer chromatography (TLC) to identify an unknown drug and then use column chromatography to separate out the components of a drug.

	Aspirin	Acetaminophen	Ibuprofen	Salicylamide	Caffeine
Advil Dual Action		250	125		
Anacin	400				32
Aspirin	325				
BC Powder	325			95	16
Excedrin	250	250			65
Excedrin Mild Headache		325			65
Tylenol		325			

Your unknown will contain one of the following drugs: Advil, Anacin, Aspirin, BC Powder, Excedrin, Excedrin Tension Headache, or Tylenol. Each drug consists of a mixture of 1-3 compounds. The table on the previous page lists the composition of each drug in milligrams per tablet.

First, you will use TLC to develop your initial hypothesis as to the identity of the unknown drug. TLC is a microscale analytical technique typically used to identify compounds, determine their purity, or determine the progress of a reaction. TLC plates are prepared by spreading a thin layer of the adsorbent on a solid support (in this case, plastic). A binder is used to help the adsorbent stick to the solid support. In this lab, we will use silica gel as our solid phase, although other adsorbents can also be used. Silica gel is a polar adsorbent that can hydrogen bond. Thus components of the mixture that are polar and/or can hydrogen bond will interact more with the silica gel and hence move less quickly up the TLC plate. Non-polar compounds will move faster and migrate further up the TLC plate. Our TLC plates also contain a fluorescent powder to aid in visualizing the spots. The solvent (mobile phase) also affects the movement of compounds on the TLC plate. As the polarity of the mobile phase increase, the compounds will move faster on the TLC plate.

To determine what your unknown drug is, you will have to determine which components it obtains. You will be provided with standards of each of the components in order to compare against your unknown. Once you have run a TLC of your unknown and all of the standards, you can visualize the spots by using ultraviolet (UV) light. Compounds that are UV active will appear when the TLC plate is exposed to UV light. To compare the spots, measure the  $R_f$  (retention factor) of each component in your unknown as shown below, and compare that value to each of the standards.  $R_f$  values are used in the event that the distance the solvent travels up the plate changes.



$$\text{Retention factor } (R_f) = \frac{\text{Distance spot traveled}}{\text{Distance solvent traveled}}$$

$$\text{Retention factor } (R_f) = \frac{2.2 \text{ cm}}{5.9 \text{ cm}} = 0.37$$

In the example shown above, the spot started at the dotted line on the bottom of the plate. As the solvent moved up the plate, it traveled 5.9 cm but the spot traveled only 2.2 cm. Thus the  $R_f$  of the spot is 0.37. Note that the distance the spot traveled is measured from the middle of the spot.

Once you have mastered TLC and developed an initial hypothesis regarding the identity of your unknown, you will perform column chromatography to physically separate the components of your unknown. The principle behind column chromatography is the same as with TLC, just on a larger scale. You will then determine the melting point of each component to confirm your initial hypothesis.

### Lab Notebook Preparation

Before coming to lab, the following items must be in your lab notebook:

1. Title of experiment & date the experiment is to be performed
2. Table or list of possible unknowns and their components
3. Structures of aspirin, acetaminophen, ibuprofen, salicylamide, and caffeine

4. Melting points for aspirin, acetaminophen, ibuprofen, salicylamide, and caffeine
5. Equation for calculating  $R_f$
6. Hazards of and appropriate precautions for the safe handling of dichloromethane, iodine, and silica gel
7. References

### Directions

1. With your instructor's permission, prepare your own custom TLC spotters. (Safety notes: Make sure that no flammable solvents are anywhere near the Bunsen burner. Do NOT wear gloves while making TLC spotters.)
  - a. Obtain a glass pipet or a melting point capillary that is open on both ends.
  - b. Setup a Bunsen burner so that it gives off a small, blue flame.
  - c. Insert the melting point capillary (or the thin part of a glass pipet) into the blue part of the flame.
  - d. As soon as the glass has started to soften, remove it from the flame and quickly, but gently, pull. You should end up with a thinner section of glass in the middle.
  - e. Next, gently twist and pull to break the capillary at the thinner section of glass.
  - f. Once they have cooled, both ends can be used as TLC spotters. Make several TLC spotters so that you have one for each unknown and standard.
2. Prepare your unknown for TLC.
  - a. Grind one tablet into a fine powder. This can be done with the flat side of a spatula or a mortar and pestle.
  - b. Add 5 mL of a 1:1 ethanol/dichloromethane solution to your unknown and mix to dissolve as much of it as possible.
  - c. If any solid remains, filter your unknown to remove the solid.
3. Prepare your TLC plate.
  - a. Use pencil to gently draw a line 1-2 cm from the bottom of your TLC plate. Make sure that you mark the side with the silica gel. (Note: No. 2 pencils work well. Pencils with harder lead tend to chip the TLC plate.)
  - b. Mark along the line where you plan to spot the unknown and the standards. Be sure to leave enough space so that the spots do not bleed into each other. You may find it helpful to label the marks so you know what they are later (i.e, "U" for unknown, "I" for Ibuprofen, etc.).
  - c. Use your TLC spotter to spot the unknown and standards on the TLC plate. This is done by dipping the narrow tip of the spotter into the solution to be spotted, and then BRIEFLY touching it to the plate. You want nice, small spots, and not large ones. It is recommended that you practice on a paper towel first until you have mastered the technique. Don't forget to use a different spotter for each solution.

- d. If you want to check and make sure you spotted everything correctly, you can check to see what your TLC plate looks like under the UV lamp before proceeding. If it looks bad, start over.
4. Prepare the developing chamber.
  - a. Obtain a jar or beaker that fits your entire TLC plate.
  - b. Add about 1 cm of a 200:1 solution of ethyl acetate/acetic acid.
  - c. Hold your TLC plate next to the chamber to make sure that the solvent level is below the level of the spots. If it is too high, remove some solvent.
5. Run the TLC plate.
  - a. Insert your TLC plate evenly into the chamber. Make sure that the line and the spots are at the bottom of the plate and that they are above the level of the solvent.
  - b. Cover the chamber with a lid or watch glass.
  - c. Do not disturb the developing chamber while the TLC plate is running.
  - d. Once the solvent is close to the top of the plate, remove the plate from the chamber and mark the solvent line with a pencil.
  - e. Allow the plate to dry.
6. Visualize the plate.
  - a. Observe the spots under short-wavelength (254 nm) ultraviolet light, and mark them with a pencil. Note their color and shape. (Safety note: Do not look directly at the light source.) If your TLC plate looks funny, you may choose to redo it. Common problems include spots that are too large and/or bleed into other spots (too concentrated), spots that do not appear (too dilute), or spots that run crooked.
  - b. If your TLC plate looks good, calculate the  $R_f$  value of each spot.
  - c. Develop a hypothesis as to the identity of your unknown.
  - d. If necessary to confirm or refute your hypothesis, you can also visualize the spots using an iodine chamber. (Make sure you do this step AFTER you have visualized the spots using UV.) This is done by inserting the TLC plate into the iodine chamber for several minutes. Note which spots react with iodine and which do not, then remove the TLC plate from the chamber.
7. Determine the optimum solvent for the analysis of your unknown. (Note: If the 200:1 solution of ethyl acetate/acetic acid works well, you can skip this step.)
  - a. Repeat steps 3-6 for a 1:3 solution of hexane/ethyl acetate with acetic acid, a 1:2 solution of hexane/ethyl acetate with acetic acid, and a 1:1 solution of hexane/ethyl acetate with acetic acid.
  - b. Use the solvent system that best separates your spots for the rest of the lab.
8. Run further TLC plates as necessary in order to confirm your hypothesis regarding the identity of your unknown. You may choose to re-run TLC plates and/or co-spot your unknown with one or more standards.

9. Prepare the unknown for column chromatography by adsorbing it onto silica gel. (If you need to re-prepare your sample, do so as described in step 2.)
  - a. Add a small amount (approximately  $\frac{1}{2}$  a gram) of silica gel to the solution of your unknown that you prepare in step 2. (Safety note: Dry silica gel must be handled in fume hood or under a snorkel.)
  - b. Evaporate off the solvent until only a white powder remains.
10. Prepare the column.
  - a. Obtain a polypropylene column.
  - b. Make sure that the column has a filter (a white disc) in the bottom on the column.
  - c. Add about 3 g of silica gel. (Safety note: Dry silica gel must be handled in fume hood or under a snorkel. To safely weigh the silica gel, weigh your column, add silica gel in the fume hood, and then weigh again. Repeat as needed.)
  - d. Clamp the column to a ring stand allowing enough space below it for a test tube. Make sure that column is as vertical as possible and that the silica gel is as horizontal as possible.
  - e. Add a small layer of sand to the top of the silica gel.
  - f. Fill the column with a 1:1 solution of hexane/ethyl acetate.
  - g. Drain the solvent through the column until the surface just disappears into the sand.
11. Add your sample to the column and elute the column. (Note: Once you have added your sample to the column, do not allow the column to run dry until it is done.)
  - a. Add your unknown/silica gel mixture from step 9 to the top of the sand in the column.
  - b. Add a small layer of sand on top of the unknown/silica gel mixture.
  - c. Run 40 mL of a 1:1 solution of hexane/ethyl acetate through the column while collecting the eluent in labeled test tubes (~10 mL per test tube). Be careful not to let the solvent layer drop below the level of the sand.
  - d. Run 40 mL of a 1:2 solution of hexane/ethyl acetate through the column while collecting the eluent in labeled test tubes (~10 mL per test tube).
  - e. Run 40 mL of acetone through the column while collecting the eluent in labeled test tubes (~10 mL per test tube).
  - f. Dispose of the silica gel and sand in the solid waste container.
  - g. Rinse the column with deionized water and return it to the cart (do NOT throw it away).
12. Isolate each component.
  - a. TLC each test tube (or fraction) to determine which component it contains and its level of purity.
  - b. Combine all of the test tubes containing that component into a single, pre-weighed container.
  - c. Evaporate off the solvent to determine how much of the component was isolated.
  - d. Repeat steps 12b and 12c for each component present in your unknown.

13. Once each component has dried, confirm its identity and purity via melting point.

### Reporting your Results

Write your report according to the guidelines described in “Topic 4: Writing an Organic Chemistry Lab Report”. Work by yourself on this report.

### References & Additional Resources

1. Lehman, J. W. *Operational Organic Chemistry: A Problem-Solving Approach to the Laboratory Course*, 3rd ed.; Prentice Hall: Upper Saddle River, NJ, 1999; pp 117-122.
2. Revell, K. D. *J. Chem. Educ.* **2011**, *88*, 1413–1415.