Experiment 6
Thin-Layer Chromatography (TLC)

OUTCOMES

After completing this experiment, the student should be able to:

- explain basic principles of chromatography in general.
- describe important aspects of TLC.
- identify the composition of an unknown drug mixture by using TLC.

DISCUSSION

Chromatography is one of the most important and widely used analytical techniques known to chemists. It is a technique that is used for separating, purifying, and identifying certain chemical compounds. Chromatography means literally, "written in color", since it was a technique originally used to separate colored materials, like the pigments in flowers. Today it is used to separate very complex mixtures, often containing several hundred compounds. Modern instruments are equipped with various sensitive detectors that allow chromatographic techniques to be carried out with colorless compounds, often with very minute quantities. Chromatography is used in the separation of petroleum, natural and artificial flavorings, amino acids, perfumes, and many others. It is also used to identify components in these mixtures or drugs that may be present in a urine or plasma sample.

There are many different types of chromatographic techniques used today. These include high-pressure liquid chromatography (HPLC), gas chromatography (GC), column chromatography, paper chromatography, and the technique you will be using in this experiment — TLC. All types of chromatography involve a stationary phase and a mobile phase.

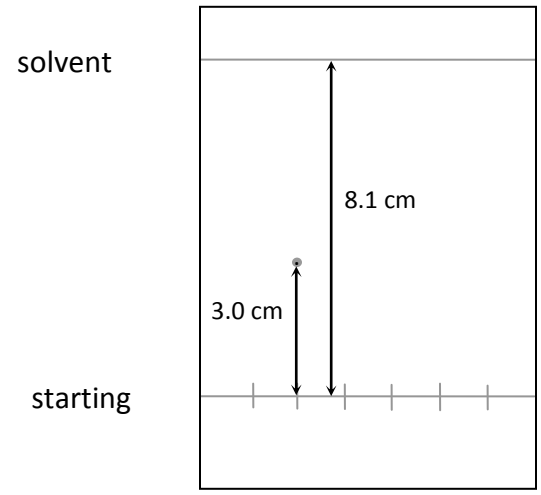
In TLC, the mixture to be analyzed is dissolved in a solvent. The resulting solution is then spotted near the bottom of a rectangular sheet of glass or plastic that has been coated with a powder such as silica, and then allowed to dry. The sheet is then lowered into a sealed chamber containing a small amount of a solvent or mixture of solvents, keeping the spots above the surface of the solvent. Once lowered into the chamber, the solvent begins to wick up the sheet through capillary action. The coating on the sheet is considered the stationary phase, since it does not move, while the solvent is considered the mobile phase, since it moves up the sheet.

As the solvent moves up the sheet, compounds are carried up the sheet at different rates. Compounds that have a greater affinity for the solvent than for the coating are carried further up the sheet, while those that have a greater affinity for the coating than for the solvent will move more slowly. This allows one to determine the minimum number of components in a sample and often leads to the identity of those components.

The identity of a component is confirmed through the calculation of its retention factor, R_F. The retention factor of a substance is constant under uniform experimental conditions. The retention factor is a ratio of the distance traveled up the paper by the component spot to the distance traveled by the solvent:

$$R_F = \frac{\text{distance traveled by component spot}}{\text{distance traveled by solvent}}$$

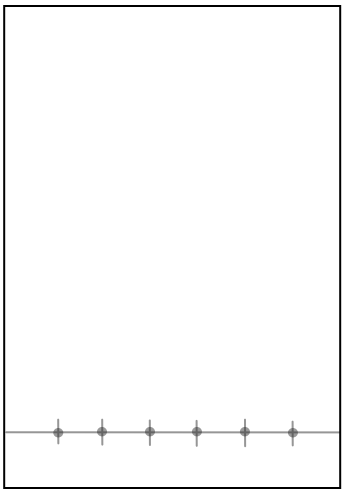
Refer to the figure to the right for a sample calculation of the retention factor. As you can see, the retention factor is dimensionless, i.e. it has no units. If a spot were to have $R_F = 0.50$, that means the spot traveled halfway up the sheet. If a spot were to have $R_F = 0.25$, that means the spot traveled one-quarter the distance up the sheet.



$$R_F = \frac{3.0\ \text{cm}}{8.1\ \text{cm}} = 0.37$$

There are many variations possible for this experiment. One may substitute the TLC sheets with filter paper or blotting paper. Instead of using drug samples to spot the sheets or paper, one may substitute felt-tip pens, or food colorings, or grind some flowers into some alcohol or fingernail polish remover (which is acetone or ethyl acetate). These materials may be spotted onto the sheet or paper using a capillary tube. One may also try different solvents or combinations of solvents in varying ratios. The only requirement is that the solvents must be miscible with each other. Experiment and have some fun! Report any interesting findings to your instructor.

PROCEDURE



1. This experiment may be performed in pairs or as a table, as per your instructor's directions. Goggles are required.

2. While under the hood, pour some developing solvent (a 200:1 mixture of ethyl acetate & acetic acid, already prepared) into a 1000 mL beaker to a depth of 0.5 cm. Cover the beaker with plastic wrap and secure it into place with a rubber band. Alternatively, a large watch glass can be used to cover the chamber. This process should saturate the beaker with vapors of the solvent.

3. Obtain a rectangular TLC sheet and place it onto a paper towel. Be careful to touch the TLC sheet only on the edges, without touching your fingers to the surface. Using a pencil, draw a faint line on the powdery side of the plate, about 1 cm from the bottom. The line

should be parallel to the bottom edge. Do not allow the pencil to dig into the coating on the sheet at all. Then faintly draw six small hash marks, evenly spaced onto the pencil line. See the figure at the bottom of the previous page.

4. Obtain one of the four different *known* drug samples from your instructor. If you are given whole tablets, place one tablet between the sheets of a clean paper towel. Crush the tablet into a powder with a pestle. Using a spatula, place a small amount of the powder into a *clean* 50 mL beaker. (1-2 mm of the powder on the tip of your spatula should be enough. If you were given ibuprofen as your known sample, use about 4-5 mm of powder on the tip of your spatula). (*Note: The other groups should use tablets of the other drug samples so the class will obtain a single set of four samples.*)

5. Under the hood, add about 2 mL of ethanol and 2 mL of hexanes to each of the five beakers. Mix the contents of the beakers with a *clean* stirring rod to get as much of the tablet to dissolve as possible. Some solid will remain undissolved.

6. Label the beakers with the name of the drug that was used. Place two small capillary tubes into each of the beakers. Do not allow the capillary tubes to be placed into more than one beaker to avoid cross-contamination of the samples.

7. Obtain an unknown in a vial from the instructor. Record the unknown number on the vial. Add about 2 mL of ethanol and 2 mL of hexanes to the vial while under the hood. Place the cap onto the vial and shake or swirl to get as much of the solid to dissolve as possible. Obtain a clean capillary tube to be used only for your unknown sample.

8. Onto a scrap TLC piece, practice spotting small samples of a drug onto the plate. To do this, dip one end of the capillary tube into the top of the solution so that only the clear portion of the liquid enters the capillary tube. Then lightly and quickly touch the capillary tube to the surface of the TLC sheet. Try to keep the spots as small as possible, preferably around 1 mm, and all spots roughly the same size.

9. Once you have refined your spotting technique, move on to the TLC sheet you prepared earlier. Spot the known drug samples and your unknown sample onto the hash marks on your TLC sheet in the following order (from left to right):

    1. Unknown
    2. Acetaminophen
    3. Aspirin
    4. Caffeine
    5. Ibuprofen
    6. Unknown

    Your unknown sample gets spotted in two places on your TLC sheet to simplify the comparisons later. Make sure each person in your group gets a chance to spot at least one sample. Once you've spotted onto all of your hash marks, check your plate under the UV

light to make sure the spots are satisfactory. They should be clearly visible and of similar intensity. You may spot any of the hash marks again (up to a total of 5 times) to ensure that there is enough of each drug applied to the TLC sheet.

10. Lift the plastic wrap from the beaker and quickly lower the TLC sheet into the beaker. Make sure that the drug samples on the pencil line do not go below the surface of the developing solvent. Position the plate so that it is not touching the sides of the beaker, and make sure it does not curve or buckle in the beaker. Reseal the beaker with the plastic wrap. As the solvent migrates up the TLC sheet, do not disturb the beaker.

11. Remove the TLC sheet once the solvent front has migrated to 1-2 cm from the top of the sheet and place it onto a paper towel (while still under the hood). Using a pencil, *immediately* mark the solvent front with a pencil, before the solvent evaporates.

12. View your TLC sheet under UV light. You will need to do this in a darkened room. Lightly circle each of the spots on the sheet. Some spots may appear faintly near the solvent front. If your spots are not distinguishable, your instructor may have you prepare and develop a new TLC sheet. After you have circled each of the spots, return to the lab. Use your pencil to mark the center of each of the spots that you circled. Place your name on the front or back of your TLC sheet and turn it in with your report.

13. Once your instructor announces the known samples will no longer be needed by any groups, dispose of all chemicals and capillaries as directed by the instructor.

Name_____ Lab Section_____

PRELAB QUESTIONS

1. Briefly describe chromatography. Why do compounds separate in chromatography?

2. Why should you not use a capillary tube for more than one of the drug samples?

3. Which safety precautions, if any, must be observed during this experiment?

Name_____ Lab Section_____

Partner's Name_____

DATA AND QUESTIONS

Unknown Vial Number _____

1. Measure and record the distance from the starting line to the solvent front.

2. a) Prepare a neat table that organizes the information requested in (b), (c), and (d) below.
   b) Measure the distance from the starting pencil line to the center of each of the spots for the six samples you tested (including your unknown). Record the distances.
   c) Calculate the retention factor for each of the spots (see the discussion section).
   d) Identify each of the spots of the six samples for which you calculated the retention factors in #2 above as: aspirin, acetaminophen, ibuprofen, or caffeine.
   e) Tell which drug or drugs were in your unknown.

3. Why must the spots not be lowered into the solvent? Why was a pencil used to draw the line instead of an ink pen?