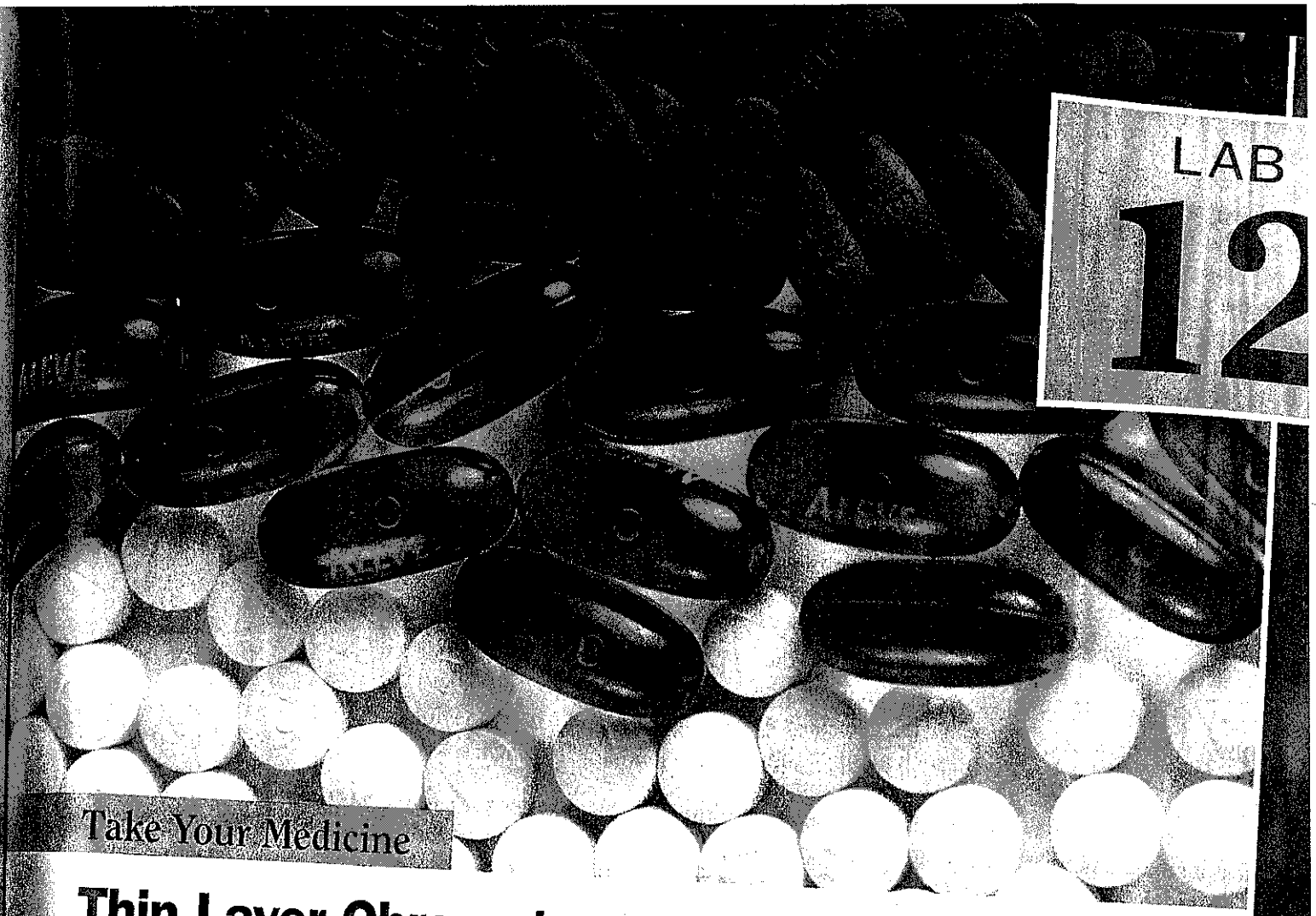


LAB 12

Take Your Medicine

Thin Layer Chromatography of Analgesic Drugs

Objectives

After completing this lab you should be able to:

- Describe how compounds may be separated using thin layer chromatography (TLC).
- Properly set up and run a TLC experiment.
- Predict the relative order in which compounds will separate on a TLC plate.
- Calculate R_f values for a TLC experiment.
- Identify the components of a mixture by comparing R_f values of the sample components to those of standard samples.
- Identify experimental factors that may determine the outcome of TLC experiment.

Most substances we encounter on a daily basis are mixtures, and often it is useful to separate the components of these mixtures in order to obtain pure substances. Many of the medicines you are familiar with were first isolated by purifying cellular extracts taken from plants. One of the most common and powerful purification techniques is **chromatography**, which literally means "color writing." The name was chosen by Russian botanist Mikhail Tswett, who discovered this technique while studying ways to separate plant pigments.

There are many different forms of chromatography, but they all operate on the same principle: compounds with similar structures or polarities tend to attract each other. Chromatography takes advantage of this fact by supplying two different phases, a **mobile phase** and a **stationary phase**, with which a molecule might interact. As the name implies, the mobile phase in a chromatographic system is allowed to flow over or through the stationary phase. The compounds in a mixture will have different affinities for the two phases, and they will pass through the system at a rate that depends upon the relative strength of their interactions with each phase.

Consider this analogy: If two people fall into a river, the one with longer arms may be able to grab hold of some rocks or nearby tree limbs (Fig. 12.1). The person with shorter arms may be unable to grab any stationary objects. Who would be carried farther downriver by the current? The individual with shorter arms, of course!

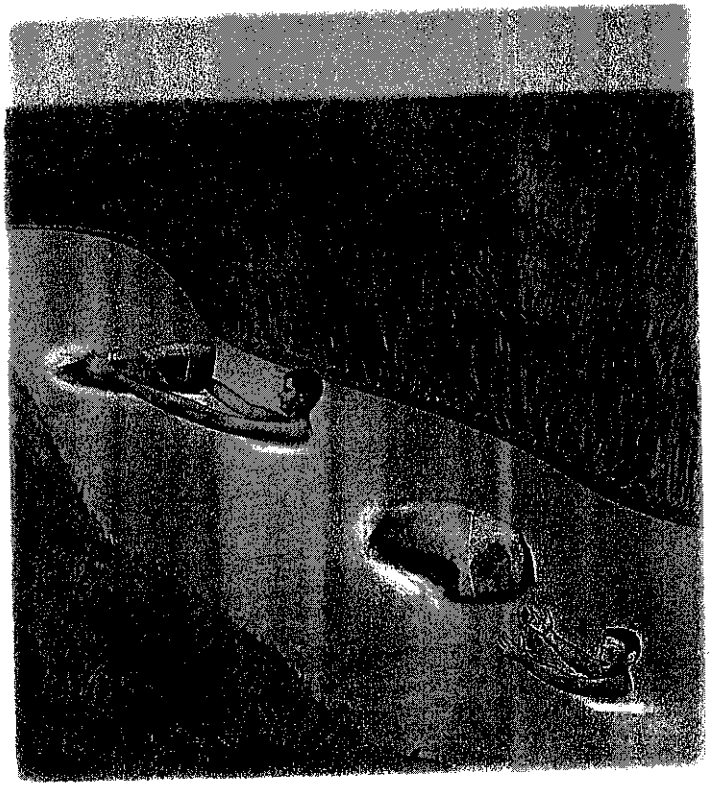


FIGURE **12.1** Analogy for chromatography.

In the same way, compounds that have stronger interactions with the stationary phase (i.e., the individual with longer arms in our analogy) will spend less time moving with the mobile phase and therefore travel less distance. On the other hand, compounds that have stronger interactions with the mobile phase (i.e., the individual with shorter arms in our analogy) will spend more time moving and therefore travel farther faster (Fig. 12.2). In order to achieve good separation, the mobile and stationary phases in a chromatographic system must be complementary. That is, a polar stationary phase should be paired with a relatively nonpolar mobile phase.

Thin layer chromatography (TLC) is one of the simplest, fastest, and least expensive chromatographic techniques. The stationary phase in TLC is usually a very thin layer of polar adsorbent, such as silica (SiO_2), coated on a rectangular plate. The sample mixture is applied to the adsorbent as a small spot near one end of the TLC plate. After the sample has dried, the TLC plate is placed in a sealed chamber containing a small amount of solvent, which serves as the mobile phase (Fig. 12.3).

The mobile phase slowly rises up the TLC plate by capillary action. As it reaches the sample spot, the compounds in the mixture will begin traveling with the mobile phase at different rates. When the solvent nearly reaches the top of the plate, it is removed from the chamber. Ideally, the components of the mixture will have traveled different distances up the plate and now appear as separated spots. Note that a paper wick (usually filter paper) is added to the TLC chamber prior to performing the separation in order to ensure that the atmosphere is saturated with the mobile phase solvent. This prevents the mobile phase solvent from evaporating off of the TLC plate, which would inhibit efficient separation. It is also important that the sample spots start out above the level of the solvent in the chamber. If not, they may simply dissolve off the plate into the layer of solvent.

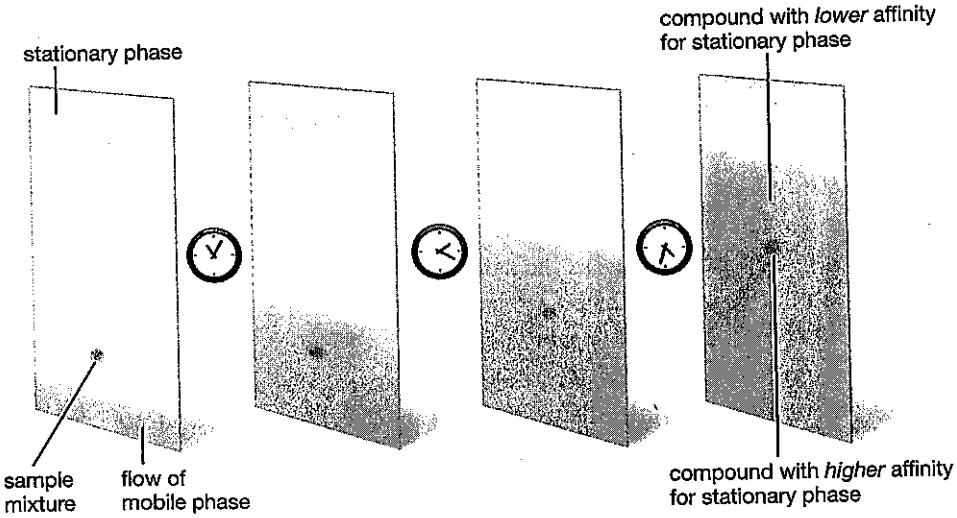


FIGURE **12.2** Overview of separation by chromatography.

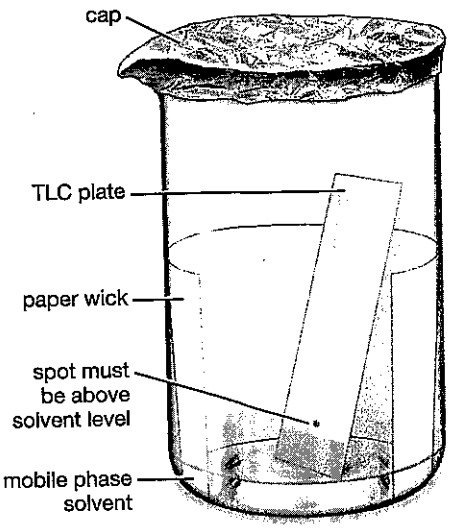


FIGURE **12.3** Overview of a thin layer chromatography experiment.

The TLC plate in Figure 12.4 is an example of an effective separation of the constituent compounds in the hypothetical sample labeled 1. In a TLC experiment, it is important to use a pencil to mark the starting line, or **origin**, where the sample was first spotted. It is also necessary to mark the farthest distance traveled by the mobile phase immediately after removing the plate from the chamber before all the solvent evaporates. The line demarking the farthest edge of the mobile phase from the bottom of the plate is called the **solvent front**. Finally, any visible spots in the sample should be circled with a pencil as soon as the plate dries because spots may fade over time. Many compounds are not colored and will not be visible to the naked eye on the TLC plate. Most modern TLC plates are coated with a compound that fluoresces when exposed to ultraviolet (UV) light. Any sample compounds present on the plate mask the fluorescence from this compound, resulting in a dark spot where the sample is located.

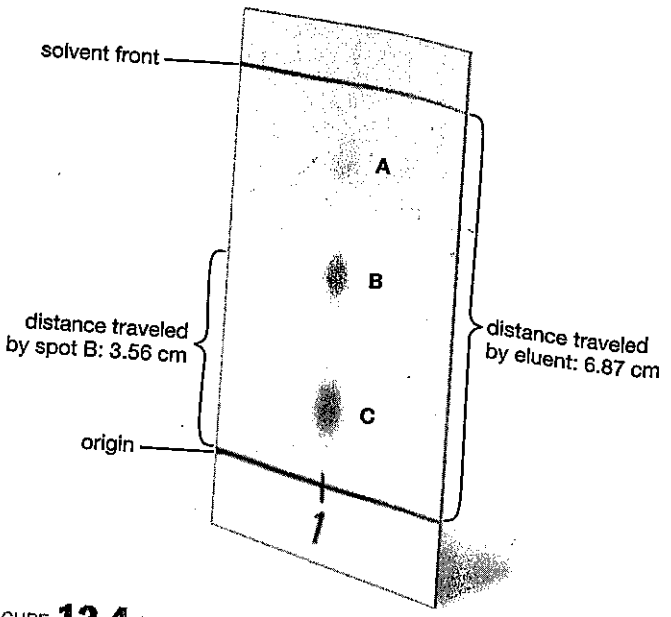


FIGURE **12.4** Important features of a TLC plate with effective separation of the compounds in hypothetical sample 1.

To quantify the distances traveled by each compound on the TLC plate, the distance from the origin to the center of each spot is measured with a ruler. Similarly, the distance from the origin to the solvent front is also measured. The distance traveled by each compound is reported as the **retention factor** (R_f), which is calculated as shown in Equation 1. For example, the R_f for spot B in Figure 12.4 would be 0.518.

Equation 1

$$R_f = \frac{\textit{distance traveled by sample}}{\textit{distance traveled by solvent}}$$

The R_f value is a unitless physical constant for a given compound under the specific experimental conditions used (mobile phase, adsorbent type and thickness, quantity of spotted material, temperature, etc.). As such, it may help in the identification of a compound in a sample. For example, if two different samples are run next to each other on the same TLC plate, spots that share the same R_f value may be the same compounds, although this is not definitive proof that they are identical. However, if two spots have different R_f values, then they must be different compounds.

Consider the TLC plate shown in Figure 12.5. Two different sample mixtures were run on this plate—one in the lane labeled 1 and the other in lane 2. Both samples separated into two individual spots. Spots A and C share the same R_f, indicating that these might be the same compound. Spots B and D have different R_f values and therefore must be different compounds. Thus, we may conclude from this TLC experiment that both samples are mixtures of at least two compounds. These two mixtures probably share one compound in common.

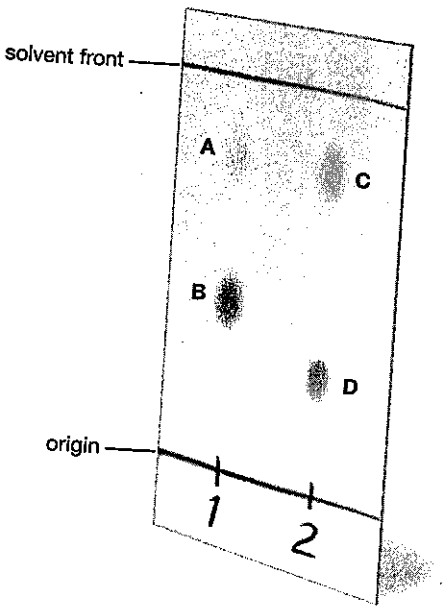
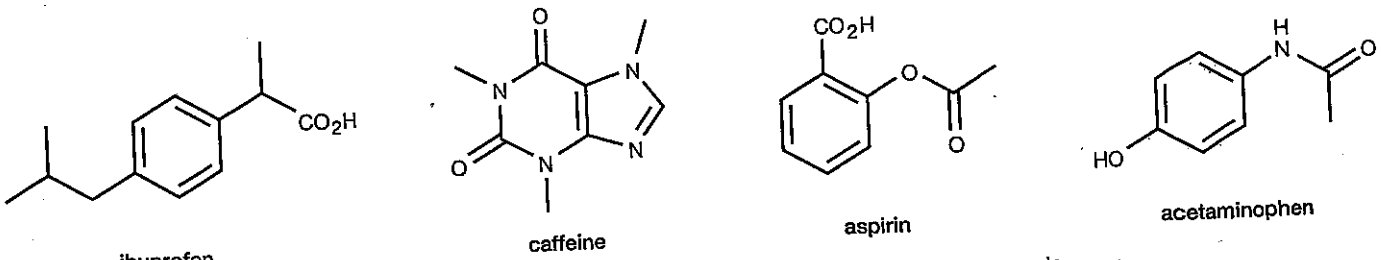


FIGURE **12.5** TLC results after separation of two different mixtures. One mixture was spotted in lane 1 and the other was spotted in lane 2.

In this lab, you will carry out TLC experiments in order to identify a sample of medication by determining its active ingredients. Medicines used to alleviate pain are collectively referred to as



ibuprofen caffeine aspirin acetaminophen

FIGURE **12.6** Chemical structures of common analgesic compounds.

analgesics. The chemical structures of common active ingredients found in over-the-counter analgesics are shown in Figure 12.6. Some analgesic brands are made of pure active ingredient while others are mixtures of two or more of these compounds. The analgesics found in five common brands of medication are given in Table 12.1.

TABLE **12.1** Active Ingredients in Some Common Over-the-Counter Medications

Brand Name	Components
Anacin®	aspirin, caffeine
Tylenol®	acetaminophen
Excedrin®	acetaminophen, aspirin, caffeine
Advil®	ibuprofen
Bayer® aspirin	aspirin

To determine the identity of your assigned analgesic sample, you will extract the active ingredients from the drug and then perform a TLC analysis on your extract. On your TLC plate, you will spot your sample along with authentic samples of the four compounds in Figure 12.6. The authentic samples should help you identify the active ingredients in your sample by a comparison of R_f values. You will perform the TLC analysis in two separate solvent systems (Solvent System 1: 20% ethyl acetate in hexanes; Solvent System 2: ethyl acetate) to examine the effect of solvent on the efficiency of separation in a TLC experiment.

12

Procedure

MATERIALS

- ☐ 250 mL beakers (2)
- ☐ Filter paper
- ☐ Aluminum foil
- ☐ Mortar and pestle
- ☐ Forceps
- ☐ Test tube
- ☐ Glass stir rod
- ☐ TLC plates (2)
- ☐ Pencil
- ☐ Ruler
- ☐ Spotter (5)
- ☐ Pasteur pipets (2)
- ☐ Wooden applicator stick
- ☐ Piece of cotton
- ☐ Scissors

Part A: Prepare TLC Chambers

1 Obtain two 250 mL beakers to use as your TLC chambers. Cut two pieces of filter paper to provide one flat edge on each. Place one piece of filter paper in each TLC chamber with the flat edge on the bottom of the beaker.

2 Use Solvent System 1 (20% ethyl acetate in hexanes) in one TLC chamber and Solvent System 2 (ethyl acetate) in the other. In each chamber, add enough of the appropriate solvent system to form a layer 0.5–1.0 cm deep.

3 Cover the chamber tightly with aluminum foil. Allow the chambers to equilibrate for at least 15 minutes while you prepare your mystery sample and your TLC plates (see Parts B and C). During this time, the solvent will travel up the filter paper by capillary action and saturate the air in the chamber with solvent vapors.

Part B: Preparing the Mystery Analgesic Sample

1 Record the sample number for your mystery analgesic pill or powder on the data sheet, page 179.

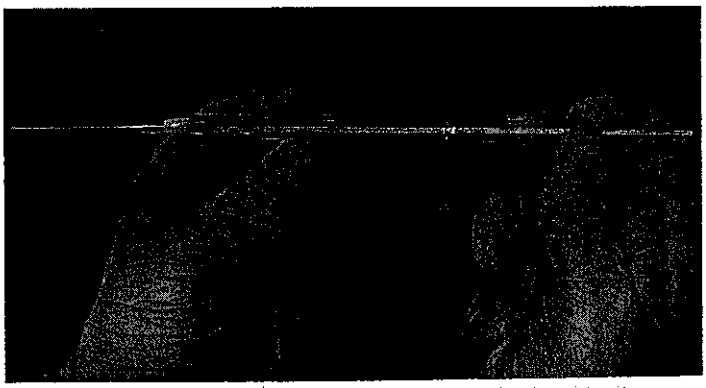
2 If your mystery analgesic sample is a pill, place it in a mortar and grind it with a pestle until you have a relatively homogenous powder.

3 Transfer the powdered sample to a test tube and add about 1 mL of the extraction solvent (50:50 ethanol:dichloromethane).

4 Stir the mixture vigorously with a glass rod. Do not be concerned if some of the material does not dissolve. Insoluble binders are often used to hold together commercial analgesic tablets, and enough of the analgesic will dissolve in the extraction solvent for a TLC analysis. The solid material will be removed in step 5.

5 Prepare a microscale filter by plugging the tip of a Pasteur pipet with a small piece of cotton. Use another pipet to add about 1 mL of your analgesic solution to the microscale filter. Collect the extract solution in a clean test tube as it filters through the cotton plug.

6 Save your mystery analgesic sample for TLC analysis.



TECHNIQUE TIP

Prepare a microscale filter by using a wooden applicator stick to *gently* fit a cotton plug into the tip of a Pasteur pipet. A loose fit of the cotton in the pipet is sufficient and will allow the solution to flow easily. If the cotton is pressed too tightly, the solution will flow very slowly. Filter the analgesic sample through the cotton plug and collect the extract.

5 Once you have spotted each of your samples and all spots are dry, use forceps to pick up the TLC plate near the top edge (Fig. 12.8). Place one TLC plate in the chamber containing Solvent System 1 and the other in the chamber containing Solvent System 2. Be sure you position the plates with the origin toward the bottom and such that the side edges are not touching the filter paper wick.

6 Immediately cover the chambers with the aluminum foil and allow the solvents to rise up the plates.

7 When the solvent is approximately 0.5 cm from the top edge of the TLC plate, use forceps to remove it from the chamber. Be sure to handle the plate above the solvent front.

8 Quickly mark the position of the solvent front with a pencil before the solvent evaporates (Fig. 12.9).

9 Allow the TLC plate to dry, and then hold it under a UV light to visualize any analgesic spots that are not visible to the naked eye. Use a pencil to circle each compound on the TLC plate.

10 If your instructor advises, you may also visualize the compounds on the TLC plate by placing it in an iodine chamber for several minutes. The iodine will color the spots a dark brown.

11 Illustrate your results on the TLC plate templates provided on your data sheet, page 179.

12 On each TLC plate, use a ruler to measure the distance from the origin to the solvent front and the distance from the origin to the center of each spot. Record the distances on the data sheet, page 179.

13 Calculate the R_f of each spot on the TLC plates and use your results to identify the components in your mystery analgesic. Use your results and Table 12.1 (pg. 172) to determine the brand identity of the mystery sample. Record the analgesic(s) present and the identity of your mystery sample on the data sheet, page 180.

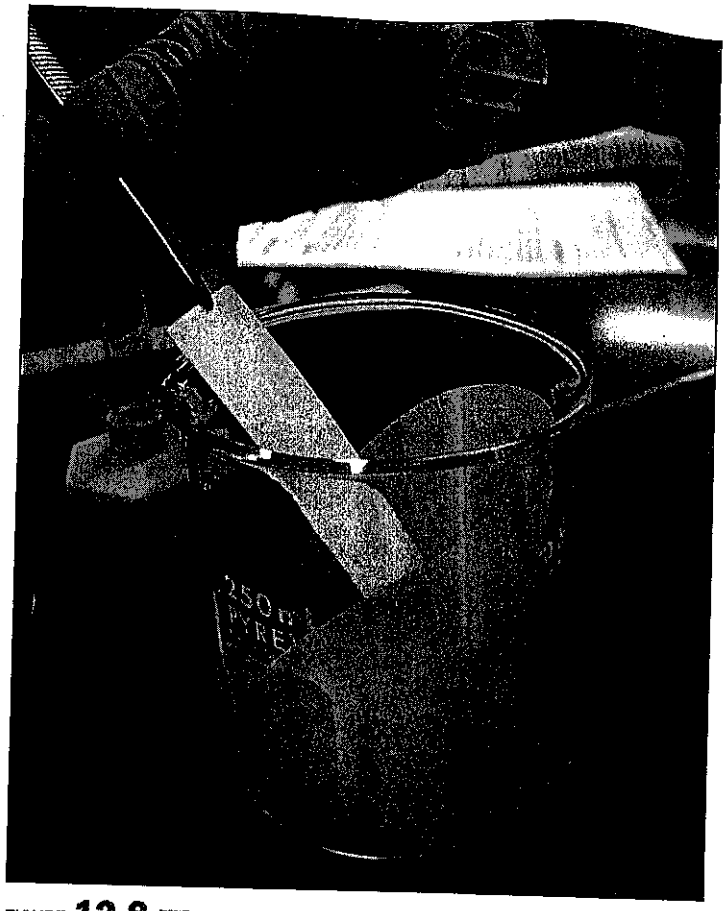


FIGURE **12.8** Use forceps to insert the TLC plates into the appropriate chamber. Ensure that the origin is above the mobile phase and that the edges of the plate do not touch the filter paper wick.

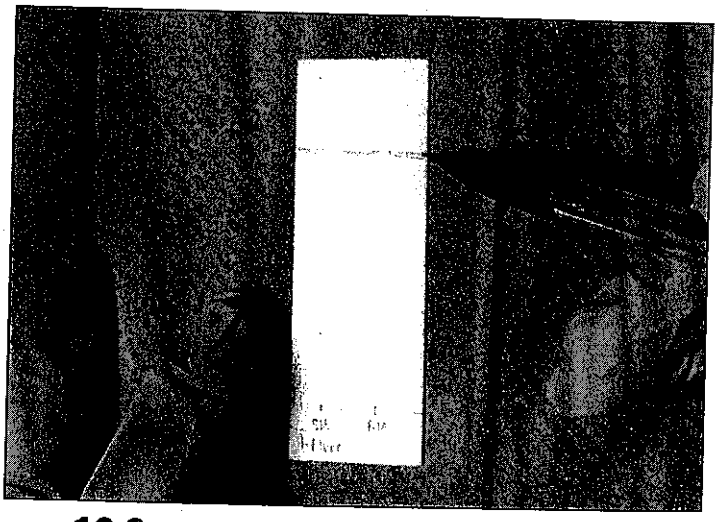
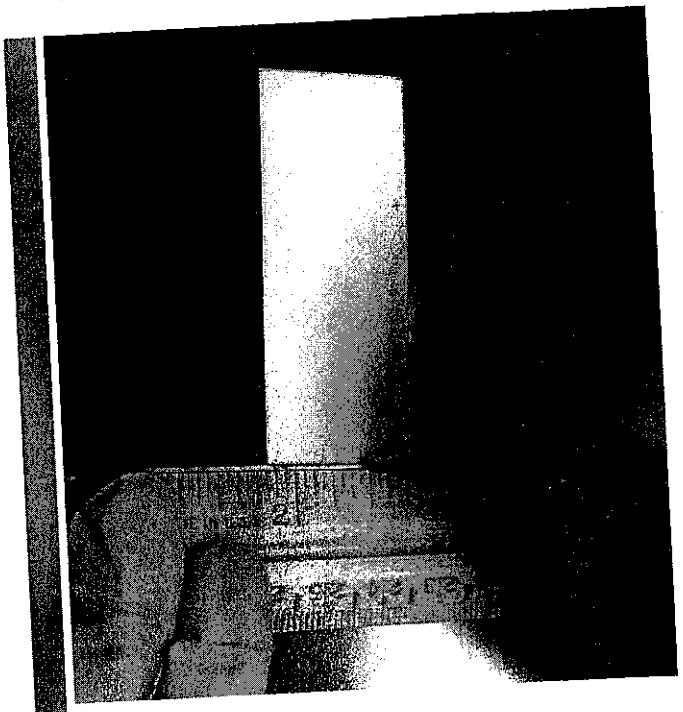


FIGURE **12.9** Mark the position of the solvent front on the TLC plate before it evaporates.

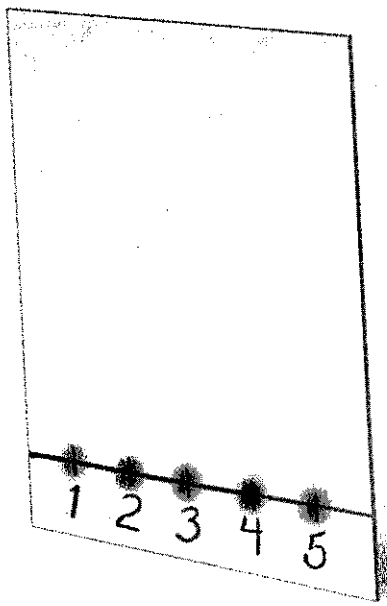
Part C: TLC Analysis

1. Obtain two TLC plates. Handle these plates carefully and by the edges to prevent contamination or scraping the silica surface.
2. Orient your rectangular plate so that the long side is vertical. Use a pencil to mark a light starting line (origin) approximately 1 cm from the bottom of the plate. The origin must be above the solvent level in the TLC chamber when your TLC plate is standing in the solvent. Do not press too heavily! You do not want to gouge a trench into the silica surface.
3. On each TLC plate, use a pencil to label five lanes in which you will spot your five samples (four analgesics and one mystery sample) as illustrated in Figure 12.7. Sample lanes should not be too close to the edge of the TLC plate and should be separated from each other by approximately 0.5 cm.
4. Spot each of the five samples in the corresponding lane on the TLC plate by following the individual steps outlined below. Correct application of the sample spot is critical to the success of a TLC experiment. You should practice these steps on a paper towel several times before applying a sample to your TLC plate.
   - Dip a spotter into the appropriate sample solution.
   - Quickly and gently touch and remove the tip of the spotter to the surface of the TLC plate on the starting line at the appropriate position. Try to make as small a spot as possible, and be careful not to grind the spotter into the surface.
   - Wait until the spot dries and then reapply the sample to the same spot 2 to 3 more times. If you do not wait for the spot to dry, reapplication will enlarge the spot and decrease the efficiency of your separation.
   - Hold your TLC plate under a UV lamp. You should see dark spots at the origin in each lane. Make sure that none of the sample spots are overlapping or too close (<0.5 cm) to the edge of the plate. If any spots are missing or considerably lighter than the others, reapply the sample 2 to 3 more times and then recheck the plates to ensure that the spots are visible.



TECHNIQUE TIP

Use a ruler and pencil to help you draw a straight line for the origin near one end of the TLC plate. Press lightly with the pencil to prevent gouging a trench in the silica surface.



1 = ibuprofen
2 = caffeine
3 = aspirin
4 = acetaminophen
5 = mystery sample

FIGURE **12.7** Example TLC plate with five analgesic samples spotted in individual lanes.

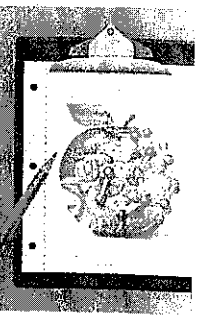
Name _____

Lab Partner _____

Lab Section _____ Date _____

PRELABORATORY EXERCISE

12



1 Provide a term that matches each description below.

a Class of compounds that act as pain relievers _____

b Immobile phase in a chromatography system _____

c Chromatography that uses a thin layer of adsorbent on a rectangular plate _____

d Starting line on a TLC plate _____

e Distance traveled by the solvent on a TLC plate _____

2 Think about what happens when you put a drop of food coloring in a glass of water. Keeping that in mind, why do you think it is important for the spots applied to a TLC plate to be as small as possible?

_____

_____

_____

3 A student set up the TLC experiment illustrated here. Identify two errors in this setup, and describe how these errors might affect the results.

Error 1

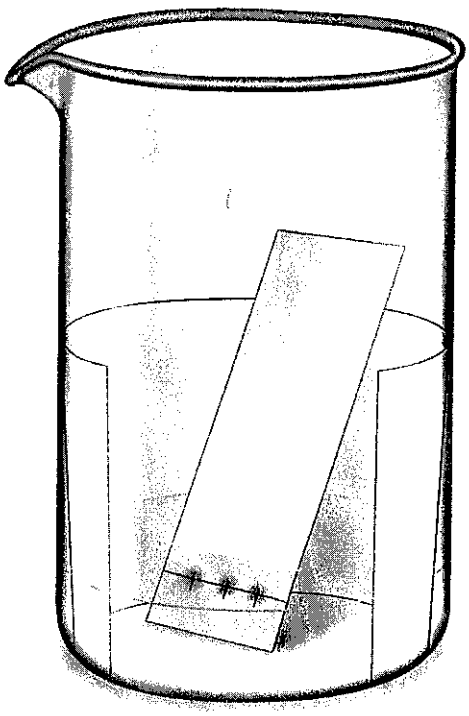
_____

_____

_____

_____

_____



Error 2

_____

_____

_____

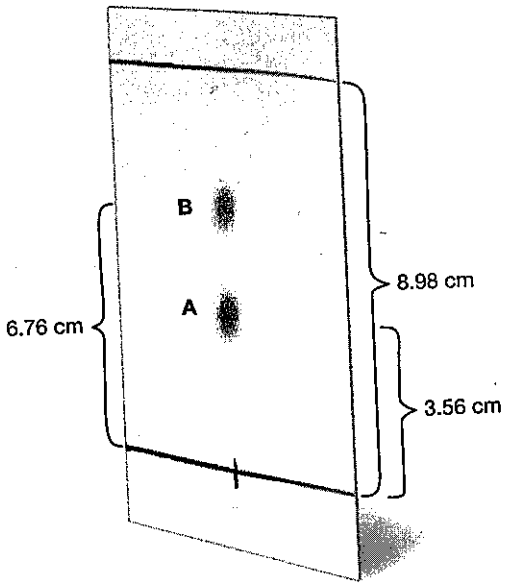
_____

_____

4 Consider the TLC plate illustrated below and calculate the R_f values for spots A and B.

R_f of Spot A _____

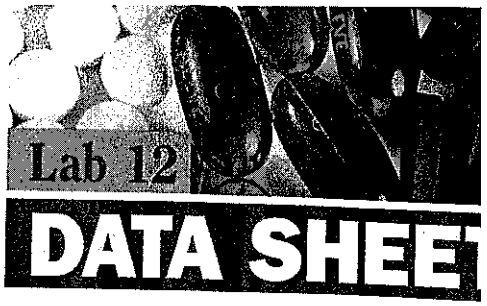
R_f of Spot B _____



Name _____

Lab Partner _____

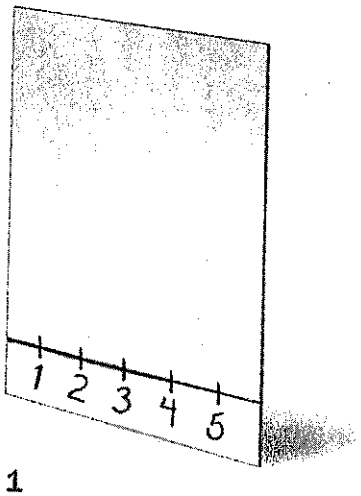
Lab Section _____ Date _____



Part B: Preparing the Mystery Analgesic Sample

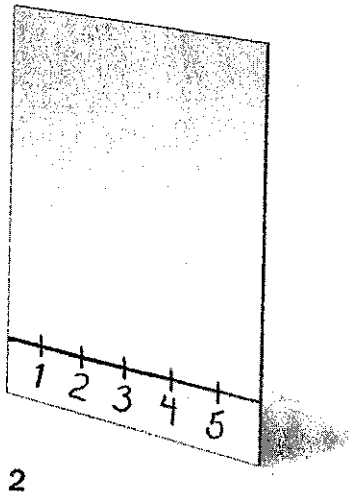
Mystery analgesic sample ID # _____

Part C: TLC Analysis



Solvent System 1

Distance traveled by solvent _____



Solvent System 2

Distance traveled by solvent _____

TLC Data: Solvent System 1

Lane Number	Distance Traveled	R_f (Show Calculation)	Identity
1			
2			
3			
4			
5			
5 (if additional spot present)			
5 (if additional spot present)			

Data: Solvent System 2

Lane Number	Distance Traveled	R_f (Show Calculation)	Identity
1			
2			
3			
4			
5			
5 (if additional spot present)			
5 (if additional spot present)			

Analgesic(s) present in mystery sample _____

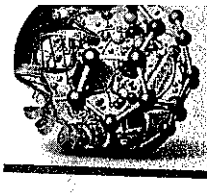
_____

_____

Identity of mystery sample _____

Lab Partner _____

Lab Section _____ Date _____



LAB 12 REFLECTIVE EXERCISES

1 Did you use the TLC plate run in Solvent System 1 or 2 to determine the identity of your analgesic sample? Explain your choice.

2 Explain how your TLC data allowed you to determine the identity of your mystery analgesic sample.

3 Determine each of the following from the TLC data you used to identify your analgesic sample.

a The analgesic ingredient that has a polarity most similar to the stationary phase _____

b The analgesic ingredient that has a polarity most similar to the mobile phase _____

4 Why is it not possible to have an R_f value greater than 1.00?

5 A student used a pen rather than a pencil to mark the origin on a TLC plate. Explain how this could be a problem for the student's TLC experiment.

6 Why do we allow the solvent to rise nearly to the top of the TLC plate rather than removing the plate when the solvent is only half-way up the plate?

7 After performing a TLC experiment, a student was surprised to find that the plate appeared blank under a UV light. The instructor suggested dipping the plate in a chemical reagent, and the samples suddenly appeared as bright pink spots. What might account for this result? What does this suggest about the reliability of using only one visualization method (such as exposure to a UV light) to interpret the results of a TLC experiment?

8 If a student performed a TLC experiment and failed to get a good separation of the compounds in a sample of medication, which of the following would be the best choice of experimental factors to change in order to achieve a better separation? Explain your answer.

a Mobile phase solvent

b Size of TLC chamber

c Temperature

d Length of TLC plate

12

Explanation