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Drug Analysis Using Thin-Layer Chromatography

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Description

The majority of evidence submitted to crime labs comes from drug-related crimes. Often, this evidence includes unidentified powders that may be illegal drugs. In order to prosecute individuals for possession of illegal substances, it is necessary for forensic scientists to positively identify any suspected drugs submitted to the laboratory. In addition, forensic toxicologists must determine the identity of drugs found in the bodies of drug-overdose victims. Although illegal substances can cause overdose, people also overdose on common over-the-counter (OTC) drugs, like aspirin, when attempting to take their own lives. Thin-layer chromatography (TLC) is one technique used to identify unknown drugs. Chromatography is simple to perform, is straightforward to interpret, and works equally well for legal and illegal substances. This experiment uses TLC to identify the active ingredients in some common OTC painkillers.

OTC painkillers contain one or more active ingredients. The most common active ingredients are aspirin, acetaminophen, and caffeine. You will be supplied with standard solutions of these ingredients. Each pair of students will determine the active ingredient(s) in four OTC painkillers by comparing the R_f values obtained for the painkillers with the R_f values obtained for the three standard solutions.

Safety, Handling, and Disposal

It is your responsibility to specifically follow your institution's standard operating procedures (SOPs) and all local, state, and national guidelines on safe handling and storage of all chemicals and equipment you may use in this activity. This includes determining and using the appropriate personal protective equipment (e.g., goggles, gloves, apron). If you are at any time unsure about an SOP or other regulation, check with your instructor.

The liquids used in this experiment are nontoxic to inhale in small amounts. A hood is not needed for this experiment. However, some students may wish to work in a fume hood due to the smell of these liquids. The liquids are highly flammable. There should be no flames used during this experiment. Never look directly into an ultraviolet (UV) light, as this can cause eye damage.

Dispose of microcaps in the broken glass container. Dispose of the ethyl acetate in the waste container labeled "Ethyl Acetate Waste Container." Dispose of the other liquids in the waste container labeled "Ligroine-Ethanol-Drug Waste Container."

Procedure

I. Prepare developing chamber—Work in groups of two.

1. Obtain a large, wide-mouthed, screw-cap jar. You will share this jar.

2. Pour enough ethyl acetate in the jar to reach a height of 0.5 cm above the bottom of the jar. Screw the top on tightly.

3. Have your instructor inspect the jar.

II. Prepare plate—Work individually.

1. Obtain one TLC plate (10-cm x 7-cm plate) and a smaller "practice plate." Handle the plates by the edges. Place the plates shiny side down on clean paper. The larger plate should be exactly the size shown in Figure 1.

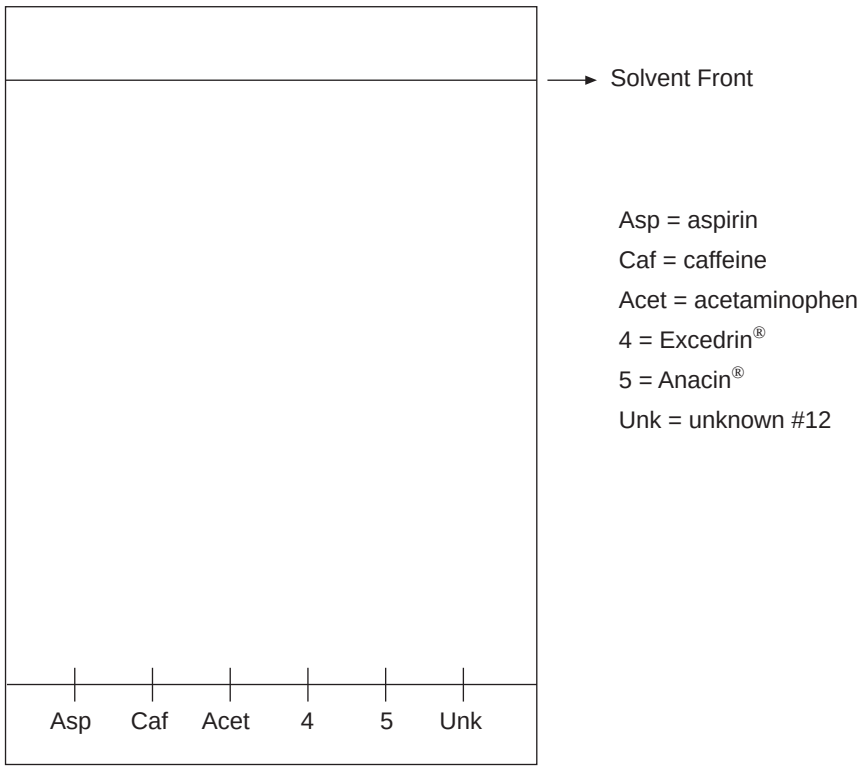

Figure 1: Preparing a TLC plate

2. Using a #2 pencil, lightly draw a line 1 cm from the bottom of the plate and make the other markings as indicated. Do not dig into the white powder on the plate.

III. Prepare solutions—Work in groups of four.

1. Label four clean, dry beakers as follows: Ana, Ty, ND, and Ex.
   If the beakers appear dirty, they should be washed and dried thoroughly with paper towels before using. Any traces of water will interfere with the experiment.

2. Obtain a tablet of Anacin®. Place it between two clean paper towels. Crush the tablet to a fine powder using a pestle.

3. Add about one-fourth of the crushed tablet to the beaker labeled "Ana."

4. Repeat steps 2 and 3 above three times using Tylenol®, No-Doz®, and Excedrin®. Use a clean paper towel each time. Use about one-eighth of the crushed Tylenol tablet and about one-fourth of the crushed No-Doz and Excedrin tablets.

5. Add about 2 mL ethanol and 2 mL ligroine to each beaker. Swirl to mix. This mixture is flammable. All of the solid may not dissolve.

IV. Prepare unknown—Work individually.

1. Obtain an unknown from your instructor. Record the unknown number.

2. Place a small amount of the unknown, equivalent to one-fourth of an aspirin, in a clean, dry beaker. Label the beaker "unknown."

3. Add about 2 mL ethanol and 2 mL ligroine to the beaker. Swirl to mix. This mixture is flammable. All of the solid may not dissolve.

V. Spot the practice plate—Work individually.

1. Obtain several clean microcaps.
   When spotting the plates, be careful to avoid cross-contaminating the solutions by reusing microcaps (i.e., use the Anacin microcap in the Anacin only, the Tylenol microcap in the Tylenol only, etc.).

2. Place several pencil spots on your "practice plate."

3. Using any one of the four solutions from Part III, Step 5, spot the practice plate following the directions given in the instructor's demonstration. Dip one end of the microcap in the top of the solution so that only the clear liquid enters the microcap by capillary action. Briefly place the tip of your microcap directly on the pencil mark. A small amount of liquid will come out of the microcap and form a spot on the plate. In a few seconds, the solvent will evaporate.

4. Visualize your practice spot using the UV light. Generally, your spots should be no bigger than this: • Have your instructor approve your spots.

VI. Spot the TLC plate—Work individually.

1. Obtain standard solutions of aspirin, acetaminophen, and caffeine. Carefully spot your TLC plate with the three standard solutions directly on top of the appropriate pencil-mark crosshatch.

2. Choose two of the four student-prepared solutions of OTC drugs. Mark your TLC plate to make it clear which spots correspond to which painkillers.
   Each pair of two students should be sure that they have at least one piece of data for each of the four OTC drug solutions.

3. Carefully spot your plate with the two OTC solutions you've chosen. Apply the solutions directly on top of the pencil-mark crosshatch.

4. Spot your plate with your unknown.

5. Visualize your plate under the UV lamp and have your instructor approve your spots. If not enough chemical was applied, your instructor will tell you to apply another spot directly over the original spot.

VII. Develop the TLC plate—Share a jar with one other student.

1. Place the prepared developing chamber jar on the lab bench where it won't be moved for about 20–30 minutes.

2. Your plate and your lab partner's plate will have to go in the jar at the same time, so make sure both are ready. Carefully open the jar without moving the liquid in the container. Pick up one plate by the top and carefully lower it into the jar so the markings are on the bottom of the plate. Add the second plate into the jar so that the shiny sides of the plates face the center of the jar. Tent the plates so that the shiny sides touch at the top and are separated at the bottom. The plates should not be buckled or curved along the edges. Screw the cap on tightly without moving the container. DO NOT MOVE THE JAR UNTIL THE DEVELOPMENT IS OVER.

3. During the development, you can dispose of the solutions in the waste container labeled "Ligroine-Ethanol-Drug Waste Container." Microcaps go in the broken glass container. Keep an eye on the solvent front. It should not be allowed to reach the top of the plates. The development will take about 20–30 minutes.

VIII. Dry the TLC plate—Work individually.

1. When the liquid has moved to within 1 cm of the top of the plates, remove the plates and place them on paper towels until dried. You must remove both plates at the same time.

2. Immediately after removing the plates from the jar, mark the solvent front with a pencil. (See Figure 2.)

3. Check with the instructor to make sure the ethyl acetate is not being saved for another lab. If it is not, dispose of the ethyl acetate in the waste container labeled "Ethyl Acetate Waste Container."

IX. Visualize—Work individually.

View your plate under the UV light. Outline all the spots with a pencil. Your instructor will help you interpret the spots on your plate.

X. Calculate R_f values—Work individually.

1. Using a pencil, place a dot exactly in the center of each spot.

2. Measure the distance in centimeters from the line on the bottom of the plate (starting line) to the solvent front.

3. Measure the distance from the line on the bottom of the plate (starting line) to each spot. Calculate an R_f value for each spot as shown in Figure 2.

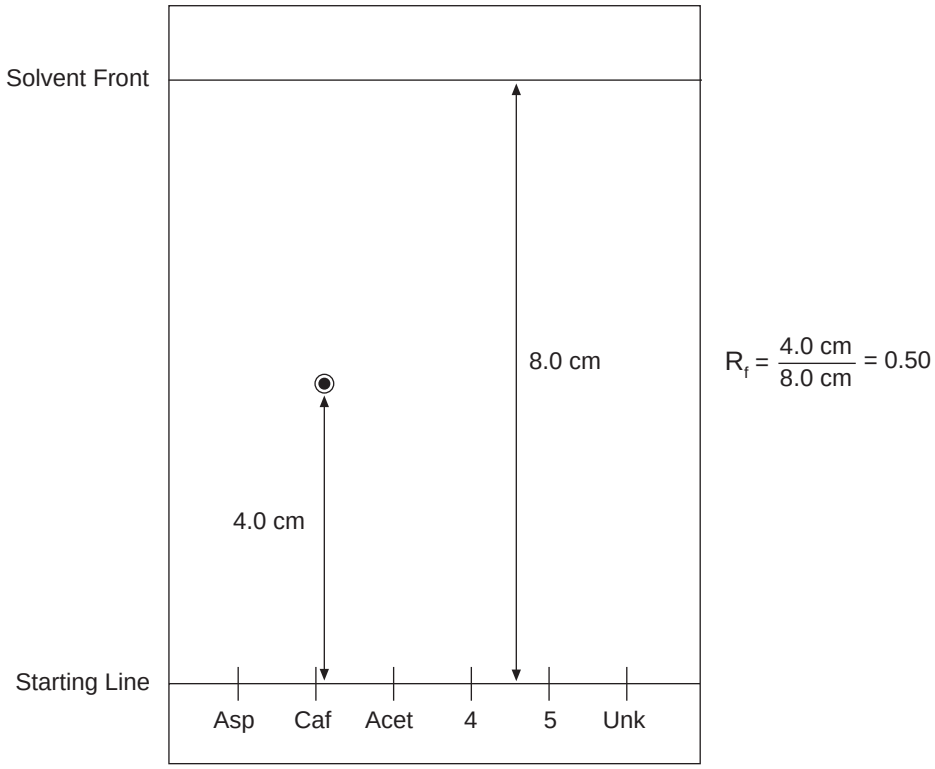

Figure 2: Calculating an R_f value

4. By comparing the R_f values obtained for aspirin, acetaminophen, and caffeine to the R_f values obtained for Anacin, Tylenol, No Doz, and Excedrin, determine what active ingredients are present in these OTC pain relievers.

5. Fill out the chart on the report page and attach your plate with tape or staples.

6. View your lab partner's TLC plate and determine what active ingredients are in the OTC drugs that your partner tested.

Name _____
Partners _____
_____
_____

ATTACH YOUR TLC PLATE HERE

Show calculations for each R_f value and report the R_f values in the data tables provided.

	Calculation	R_f
Aspirin		
Acetaminophen		
Caffeine		

Name _____

OTC DRUGS

		Caffeine	Acetaminophen	Aspirin
		R_f / Calculation	R_f / Calculation	R_f / Calculation
No Doz				
Anacin				
Excedrin				
Tylenol				
Unknown #____				

INSTRUCTOR NOTES
Drug Analysis Using Thin-Layer Chromatography

Materials

Per pair of students

- 10-mL graduated cylinder
- large, wide-mouthed, screw-cap jar for developing chamber
- 6 beakers
- 2 TLC plates with fluorescent indicator cut to size
- pestle

Per class

- 5 g aspirin (to make up standard)
- 5 g acetaminophen (to make up standard)
- 5 g caffeine (to make up standard)
- 10 tablets Anacin
- 10 tablets Tylenol
- 10 tablets No-Doz
- 10 tablets Excedrin
- UV light
- 100 mL 95% ethanol
- 100 mL ligroine
- 250 mL ethyl acetate
- 200 microcaps (microcapillary tubes)
- rulers
- pencils
- paper towels
- goggles
- waste containers (labeled “Ethyl Acetate Waste Container” and “Ligroine-Ethanol-Drug Waste Container”)

Safety, Handling, and Disposal

As the instructor, you are expected to provide students with access to SOPs, MSDSs, and other resources they need to safely work in the laboratory while meeting all regulatory requirements. Before doing this activity or activities from other sources, you should regularly review special handling issues with students, allow time for questions, and then assess student understanding of these issues.

The liquids used in this experiment are nontoxic to inhale in small amounts. A hood is not needed for this experiment. However, some students may wish to work in a fume hood due to the smell of these liquids. The liquids are highly flammable. There should be no flames used during this experiment. Never look directly into a UV light, as this can cause eye damage.

Dispose of microcaps in the broken glass container. Dispose of the ethyl acetate in the waste container labeled “Ethyl Acetate Waste Container.” Dispose of the other liquids in the waste container labeled “Ligroine-Ethanol-Drug Waste Container.”

Procedural Tips and Suggestions

1. Microcaps may be made by drawing out capillary tubes in a Bunsen burner flame and breaking the capillary in half at the narrowed region. Alternatively, microcapillary tubes can be purchased.

2. TLC plates coated with silica gel and fluorescent indicator are available from Kodak. They must be cut to size. TLC plates without fluorescent indicator may also be used. However, these must be developed using an iodine chamber.

3. Traces of water will foul the results of this experiment. All glassware must be thoroughly dried.

4. Unknowns can be made of aspirin, acetaminophen, caffeine, or any combination of these three substances. Unknowns can be distributed to students in small screw-cap vials or in small, sealed envelopes.

5. Ink and skin oils will foul the results of the TLC. Therefore, ink cannot be used on the TLC plates, and the plates should be handled by the edges only.

6. Depending on class size, it may be necessary to make more than two sets of standards. Drug standards can be made as follows:

   Aspirin Standard: Place 1 g aspirin in a beaker. Add 10 mL ethanol and 10 mL ligroine and swirl. All of the solid may not dissolve. Divide into two small vials labeled “aspirin.”

   Caffeine Standard: Place 1 g caffeine in a beaker. Add 10 mL ethanol and 10 mL ligroine and swirl. All of the solid may not dissolve. Divide into two small vials labeled “caffeine.”

   Acetaminophen Standard: Place 0.5 g acetaminophen in a beaker. Add 10 mL ethanol and 10 mL ligroine and swirl. All of the solid may not dissolve. Divide into two small vials labeled “acetaminophen.”

References

Williams, K.L. *Macroscale and Microscale Organic Experiments;* D.C. Heath: Lexington, MA, 1989.

Adapted by Annina M. Carter and Janet K. Pasco, 1996.

Name Sample Data
Partners

Show calculations for each R_f value and report the R_f values in the data tables provided.

	Calculation	R_f
Aspirin	$\frac{0.90\text{ cm}}{8.50\text{ cm}}$	0.11
Acetaminophen	$\frac{4.50\text{ cm}}{8.50\text{ cm}}$	0.53
Caffeine	$\frac{2.15\text{ cm}}{8.50\text{ cm}}$	0.25

Name Sample Data

OTC DRUGS

	Aspirin		Acetaminophen		Caffeine	
	Calculation	**R_f**	**Calculation**	**R_f**	**Calculation**	**R_f**
No Doz					$\frac{2.15 \text{ cm}}{8.50 \text{ cm}}$	0.253
Anacin	$\frac{0.75 \text{ cm}}{8.50 \text{ cm}}$	0.088			$\frac{2.10 \text{ cm}}{8.50 \text{ cm}}$	0.247
Excedrin			$\frac{4.45 \text{ cm}}{8.50 \text{ cm}}$	0.524	$\frac{2.10 \text{ cm}}{8.50 \text{ cm}}$	0.247
Tylenol			$\frac{4.65 \text{ cm}}{8.50 \text{ cm}}$	0.547		
Unknown # 12			$\frac{4.45 \text{ cm}}{8.50 \text{ cm}}$	0.524	$\frac{2.20 \text{ cm}}{8.50 \text{ cm}}$	0.259