Name ___________________________

What's in that Pill?

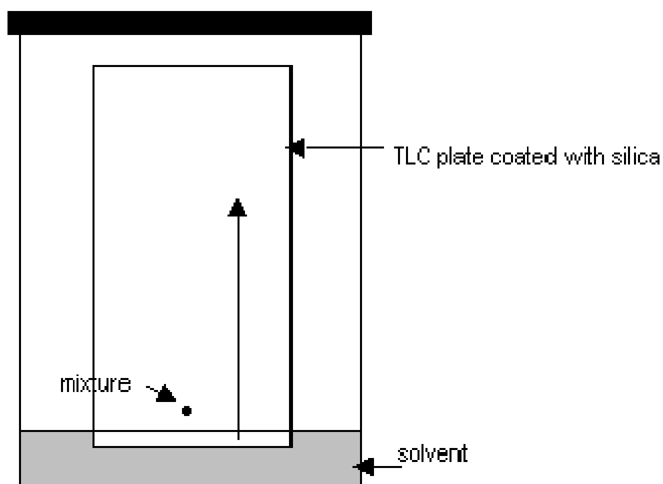
One of the challenges that chemists often have to contend with is separation of different compounds in mixtures. Forensic chemists, for example, are often asked to identify pills or powders found at the scene of a crime or a drug-overdose. Those pills or powders may be single pure substances or, more commonly, mixtures of pure substances.

In this lab you will analyze some over-the-counter pain medications to determine what types of substances they contain. You've probably already heard of some of the commonly used **analgesics** (pain relievers) – substances like acetaminophen and aspirin. The tablet, however, is not all active medication. It probably includes a "binder" to hold the medication together in tablet form and may also include a coating to protect the medication from moisture and air. Did you know that some of the analgesics you see advertised on television also include caffeine? Your challenge in this lab will be to figure out what active ingredients are present in some over-the-counter pain medications.

Thin-layer Chromatography

If you've drawn with water-soluble markers on a piece of paper and then gotten the paper wet, you have 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**. You probably remember that molecules are polar when electronegative atoms cause the molecule's electron cloud to shift, giving one side of the molecule a partial positive charge and the other a partial negative charge. You probably also remember the saying "Likes dissolve likes." This refers to the fact that polar solutes are soluble in polar solvents, but are immiscible in nonpolar solvents. This is the reason that oil and water don't mix – oil is nonpolar and water is polar.



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. ("Adsorbent" refers to a material to which other materials will stick.) Then you allow a solvent to move up the adsorbent material, carrying the substances in your mixture with it. While the solvent moves up the silica-coated TLC plate, the substances in the mixture are subjected to two forces – attraction to the solvent and attraction to the adsorbent silica. The molecules in the mixture move along, caught in a tug-of-war between the solvent and the silica. Molecules that are more strongly attracted to the solvent than the silica will move a long distance in the solvent. Molecules that are more strongly attracted to the silica will not move very far at all. When the

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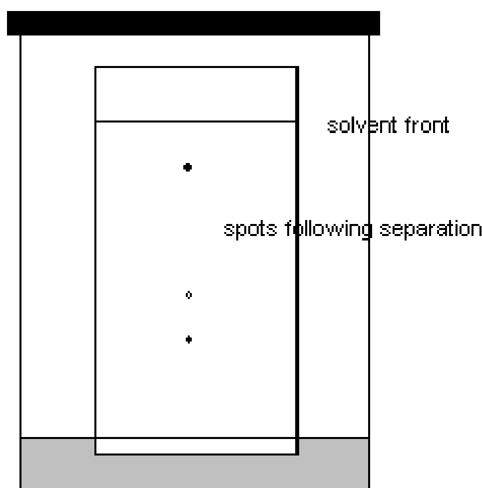
solvent has moved up the plate, molecules of different substances will make different spots on the plate.

When a particular solvent is used (hexane for example) substances in the mixture will always behave the same way. Their behavior is so predictable that we can use this technique to help identify the substances. If you separated samples of the same mixture on different TLC plates using hexane as the solvent each time, you would see the same pattern of spots each time. The only thing that might change the exact position of the spots is the length of time you run the experiment. To handle this variable, chemists express the distance that a spot moves as a ratio – the ratio of the distance the spot moves to the distance the solvent moves. The ratio is referred to as the $\mathbf{R_f}$ and the formula for calculating it is below:



$$R_f = \frac{\text{distance moved by spot}}{\text{distance moved by solvent}}$$

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.

TLC has been used by chemists for identification of unknown substances for quite a while, so many substances have been tested and their R_f values are known. Using a different solvent will change the R_f value, so when you report your R_f value, you should also indicate the solvent you are using.

Visualizing the Results

Unfortunately, unless the substances you are separating are colored, you won't be able to see them when you look at your TLC plate. In the lab, chemists may expose the TLC plates to chemicals like iodine that will react with the organic molecules they are trying to identify, producing visible brown spots. You will be using a slightly different technique to visualize your results.

The TLC plates that you are using are made of a plastic base, covered with a thin layer of silica. Mixed into the silica layer is a fluorescent material. Fluorescent materials are substances that absorb light in the UV range (invisible to our eyes) and give off light in the visible range. These materials appear to glow when they are exposed to UV or "black light."

What happens on the molecular level when **fluorescence** occurs? Imagine a relaxed molecule (in the ground state) being struck by a photon of UV light. The molecule absorbs the photon and one of its electrons immediately becomes excited. This disturbs the ground state molecule's electron cloud and the whole molecule "tenses up" and becomes excited too. Not being very comfortable in this excited state, the molecule begins to vibrate and relax a little. It continues vibrating and finally relaxes enough to be able to take one big fall back down to its ground state. In this last fall, it releases the remaining energy in the form of a photon of light. Since some of the energy of the absorbed photon was used up in vibrations, the final photon has less energy than the one that was absorbed. UV light is composed of high energy photons that we are unable to see. Visible light has lower energy, but the receptors in our eyes are able to detect it.

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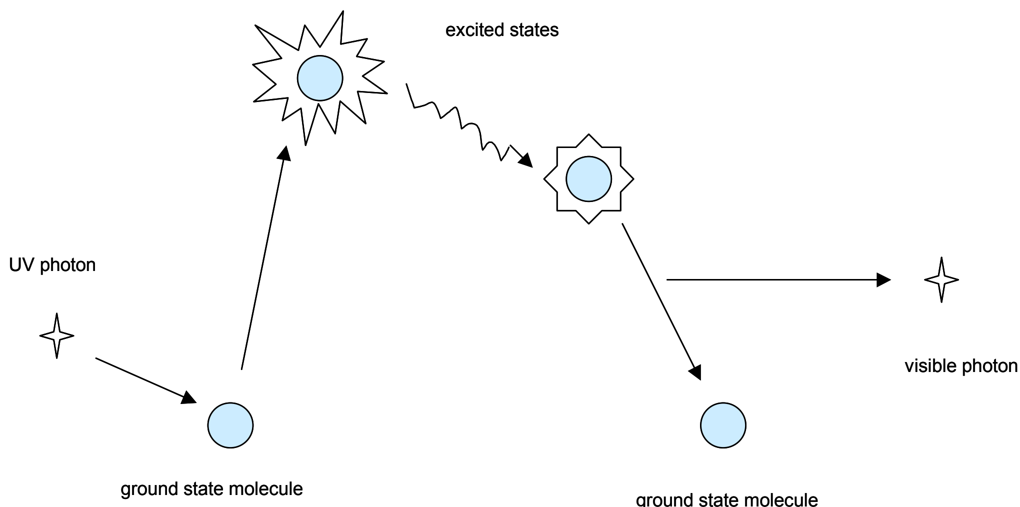
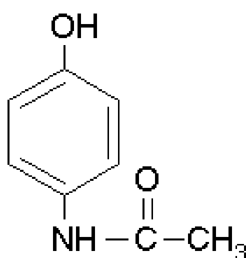


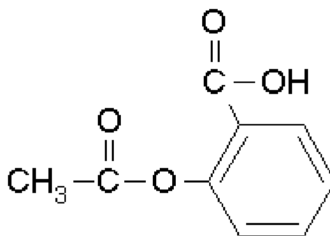
Actually, this is only one of the possible outcomes when a molecule becomes excited. In some cases, the excited molecule may bump into another molecule in its environment and transfer its energy to the other molecule. If the other molecule doesn't release the transferred energy in the form of a photon (if, for example, it just releases the energy a little at a time by vibrating) then no fluorescence will occur. Instead of glowing, the substance will behave as if it isn't fluorescent at all. This is referred to as **quenching**. If you want something to fluoresce, quenching is bad, but in the case of fluorescent TLC plates, it's good. When you put a spot of your mixture onto the fluorescent plate, the substances in the mixture will quench the fluorescence. In other words, the plate will fluoresce everywhere except where the spot is. By looking at the plate under a UV light, you will be able to tell where the initial spot is and also where the separated spots are at the end of the experiment. The spots will be dark areas where the fluorescent molecules in the plate are quenched by the substance you've put on the plate.

Analgesics

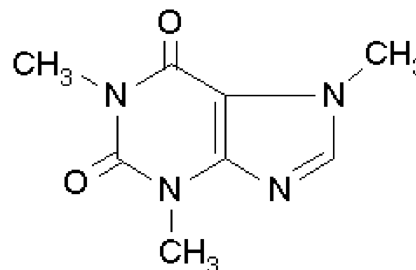
You will be examining over-the-counter medications to see if they contain one or more of the substances pictured below – aspirin, acetaminophen and caffeine.



acetaminophen



aspirin



caffeine

Notice that each of these compounds contains different atoms. Oxygen and nitrogen are considerably more electronegative than carbon and hydrogen. Since the arrangement of atoms

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is different in each case, the distribution of electrons in these molecules will also differ and they will have different molecular polarities. This also means that they will interact differently with the solvent and the silica adsorbent layer, so they will move different distances on the TLC plate.

Materials

10 mL graduated cylinder or pipette
400 mL beaker with Al foil cover (developing chamber)
TLC plate
small screw cap test tubes
pestle
standard solutions (aspirin, acetaminophen and caffeine)
medication tablets (Anacin, Tylenol, No-Doz and Excedrin)
UV light
95% ethanol
ligroin (petroleum ether)
ethyl acetate
microcapillary tubes
ruler
pencil
paper towels
safety goggles
waste containers (one for ethyl acetate and one for ligroin-ethanol)

Safety

As always, safety goggles must be worn whenever you are working in the lab area, even when you are cleaning up.

The solvents used in this experiments are safe to use in small amounts in a well-ventilated room. They are quite flammable, so make sure there is nothing that will produce a flame or spark in the work area. You can minimize the risk by keeping the solvent containers closed whenever possible.

When you are ready to dispose of wastes, pay attention to the labels on the waste containers. There are two of them, one for the solvent used to dissolve the medication sample and the other for the solvent used to run the TLC experiment. The waste containers are located in the fume hood.

Used microcapillary tubes should be placed in the broken glass container.

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. Your safety goggles protect against UV as well as chemicals, so wear them when you look at your TLC plates.

Procedure

1. Prepare the developing chamber
    a. You will share this chamber with another lab group. Fill the bottom of the beaker with enough ethyl acetate to fill it to a depth of approximately 0.5 cm. Put the foil cover on snugly and set it aside until you are ready to use it.

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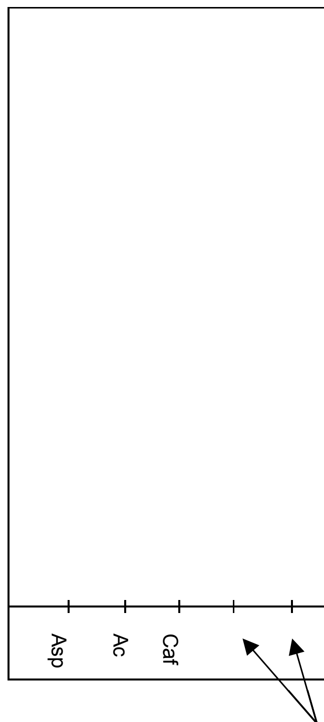
2. Prepare the TLC plate
    a. Handle the plate by the edges, being careful not to touch it with your fingers. Oil from your skin will interfere with the movement of solvent on the plate. Look at the plate and decide which edge is the straightest – use this edge as the bottom of the plate.
    b. Use a dull #2 pencil to lightly draw a line 1 cm from the bottom of the plate and make the other marks shown in the drawing. Do not press hard enough to dig into the silica layer – it's fragile.
    c. Lay the plate on a clean dry paper towel until you are ready for it.



3. Prepare the solutions
    a. Label two small, clean, dry screw cap tubes with the names of the medications you've been given. Water will affect your experiment, as will contaminants, so make sure they are clean and dry.
    b. Place the first of your medication samples between two layers of paper towel. Use the pestle to crush it to a fine powder. You will only need about one-fourth of a tablet, so if you have crushed a full tablet, share the powder with another group.
    c. Place the powder in the appropriately labeled beaker and add about 1 mL of ethanol and 1 mL of ligroin to the beaker and swirl it gently for a couple of minutes. Some of the solid may not dissolve. This is not a problem. Pour the solution into a clean, dry, labeled screw cap tube, being careful not to transfer the solid material. You will share this tube with others.
    d. Repeat steps b and c with your second medication sample.

4. Spot the practice plate
    a. Your teacher will provide a small practice plate. Obtain a microcapillary tube and dip it into one of the clear solutions you have just prepared.
    b. Briefly touch the tip of the microcapillary tube to the pencil mark on the TLC plate. A small amount of the liquid in the tube will be drawn onto the plate, making a small spot. The solvent will evaporate in a few seconds. To apply another layer of your solution, touch the end of the tube to the same spot and let it dry again. Keeping the spot small is very important. You can apply several layers in the same spot, but make sure the solvent dries between applications or your spot will begin to spread out. They should be about this size •. Save the tube to use when you spot your experimental plate.
    c. Look at your spot under UV light and have your teacher approve it.

5. Spot the TLC plate
    a. Obtain standard solutions of aspirin, acetaminophen and caffeine. Carefully spot your 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.
    b. Spot the solutions you prepared.
    c. Ask your teacher to check the spots before you move on to the next step.

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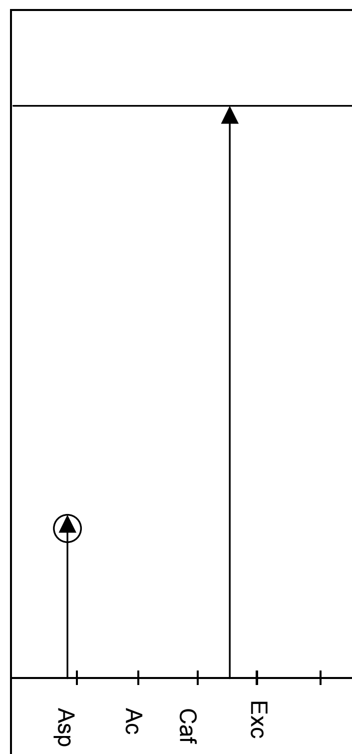
6. Develop the TLC plate
    a. Don't go any further until the lab group you are sharing the developing chamber with is ready.
    b. Place the developing chamber on your lab table in a spot where it will remain undisturbed for 15-20 minutes. Carefully remove the lid without sloshing the ethyl acetate.
    c. Handle the plates by their top edges. Lower one plate into the jar so the markings are just above the solvent level and let it lean against the side of the beaker. Lower the second plate and put it into position opposite the first. Don't let the plates touch.
    d. Put the foil cover back on the jar without disturbing the solvent or the plates and do not move the jar until development is over.

7. Dry the TLC plate
    a. When the solvent has moved to within 1 cm of the top of the plate, remove both plates and place them on a double layer of paper towel.
    b. Immediately mark the solvent front with a pencil. The solvent front is the highest level reached by the solvent. It will evaporate quickly so make sure you do this as soon as you take the plate out of the chamber.
    b. Replace the cap and ask your teacher if you should dispose of the solvent in the waste container or leave it for another class to use.

8. Visualize the results
    a. Wit your safety goggles on, look at your plate under the UV light. Outline all spots with a pencil. If you have any questions about interpreting your results, ask your teacher for help.

9. Calculate R_f values
    a. Using a pencil, place a dot in the center of each of the spots you've outlined.
    b. Measure the distance in centimeters from the staring line at the bottom of the plate to the solvent front line. Record the measurement in Data Table 1.
    c. Measure the distance from the starting line to the center of each spot of standard solution. Record the measurement in Data Table 1.
    d. Make the same measurements for the over-the-counter medications. Use the portions of the Data Table 2 that are appropriate for the meds you tested. If the meds produced more than one spot, record measurements for each spot. You can probably make tentative identifications of the spots by comparing them with the standards.
    d. Calculate the R_f value for each spot using the formula given in the introduction to the lab. Record your results in the space provided in each data table.



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Data and Calculations

First, record measurements and show your calculations for the standard solutions in the space below.

Table 1

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:

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For the over–the-counter medications, record your results in the space below. You may need to use more than one space for a medication. For example, if a medication shows more than one spot with TLC, you should record measurements and do calculations for each spot.

Table 2

Over-the-Counter Meds	Spot #1		Spot #2		Spot #3	
	Spot Distance (cm)	R_f	Spot Distance (cm)	R_f	Spot Distance (cm)	R_f
No Doz						
Anacin						
Excedrin						
Tylenol						

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Drawing Conclusions:

Compare the R_f values from the standards in Table 1 with the R_f values from the medications in Table 2. Identify the substance(s) present in each of the medications that you and your colleagues tested:

Name of Medication: ______________________

Substance(s) Present:____

Name of Medication: ______________________

Substance(s) Present:____

Name of Medication: ______________________

Substance(s) Present:____

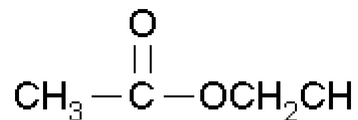
Name of Medication: ______________________

Substance(s) Present:____

1. What factors affect the distance that each spot travels on the plate?

2. What was the purpose of applying spots of known caffeine, aspirin and acetaminophen solutions?

3. Ethyl acetate has the structure shown at the right.



In comparison with other solvents used for TLC, ethyl acetate is relatively polar.

Based on this information, rank aspirin, acetaminophen, and caffeine in order of increasing polarity:

____

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4. Your eyes are not able to detect UV light. Explain what is happening when you look at a TLC plate that appears to be glowing green.

5. Explain what is happening when you look at one of the spots on a TLC plate and it appears dark.

6. Is it safe to assume that a mixture that produces two spots in your TLC experiment contains only two substances? Why or why not?

7. Knowing when a chemical reaction is complete can be difficult. In an organic chemistry lab, scientists who want to combine two different compounds in a synthesis reaction may take samples of the reaction mixture periodically and run the samples on TLC plates until they see one spot on the plate instead of two. Why does this tell them that the reaction is complete?

8. 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 that you got the first time. If, however, you used ethanol as a solvent, your R_f values would be different. Explain why this is true.

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