

Experiment 5

Thin Layer Chromatography and Melting Point: Identification of Analgesics

Reading: Handbook for Organic Chemistry Lab, sections on Writing Lab Reports (Chapter 6), Melting Point (Chapter 10), and Thin Layer Chromatography (Chapter 7).

The identification of unknown compounds is an important task of the organic chemist. The identity of a particular compound is usually determined by measuring more than one of its physical properties, for instance, by measuring its melting or boiling point and its spectroscopic characteristics. Chromatography can also be used to support a compound's identification if its chromatographic properties are compared with those of known compounds.

Thin layer chromatography (TLC) is the chromatographic method most commonly used to separate mixtures of compounds. It is generally applied as a qualitative analytical tool rather than as a means of purification; however, TLC can also be used to separate small quantities of compounds from mixtures. TLC is simple, inexpensive, and gives the user a quick answer as to how many components are in a mixture. Sometimes TLC can be used to identify the compounds in a mixture when comparisons are made with known materials. The most important measurement is retention factor, or R_f .

Melting point (MP) is another technique used to identify compounds. If the melting point of an unknown sample is the same as the published melting point of another compound, then the unknown sample may be the same compound. However, it may not be – perhaps it is only a coincidence that the two melting points are the same. The best way to make sure is to take a mixed melting point. This involves mixing up equal amounts of the unknown compound and the known compound, and taking another MP measurement. If the mixed melting point is lower than either of the individual compounds, then they are not the same. Usually, if a compound is impure, its melting point will be lower than the published value and will cover a broader range of temperatures instead of being a sharp, defined point.

Identification of Analgesics and Unknowns

Your goal in this lab is to identify an unknown compound - one of the analgesics shown in Figure 5-1 - by measuring its melting point and its chromatographic properties by TLC. You will also use TLC to verify the ingredients of the “over-the-counter” pain relievers which will be provided.

You will work in pairs, and you and your partner should each measure the melting point of the unknown compound that your TA gives you. This allows both of you practice in melting point determination and illustrates the reliability of the method. Compare the melting point with those of the analgesics.

The identity of both the unknown compound and the pain reliever ingredients are verified by comparing their TLC R_f values with those of authentic samples, or standards. Each member of the pair will use a TLC eluting solvent of different polarity. At the conclusion of the experiment, exchange data with your partner to compare the effect of solvent polarity on R_f values.

Current pain reliever preparations generally contain one or more of the following compounds: acetaminophen, aspirin, caffeine, and ibuprofen (Figure 5-1). In some analgesics you may find other ingredients listed on the label besides the four mentioned above, such as an antihistamine or a mild sedative.

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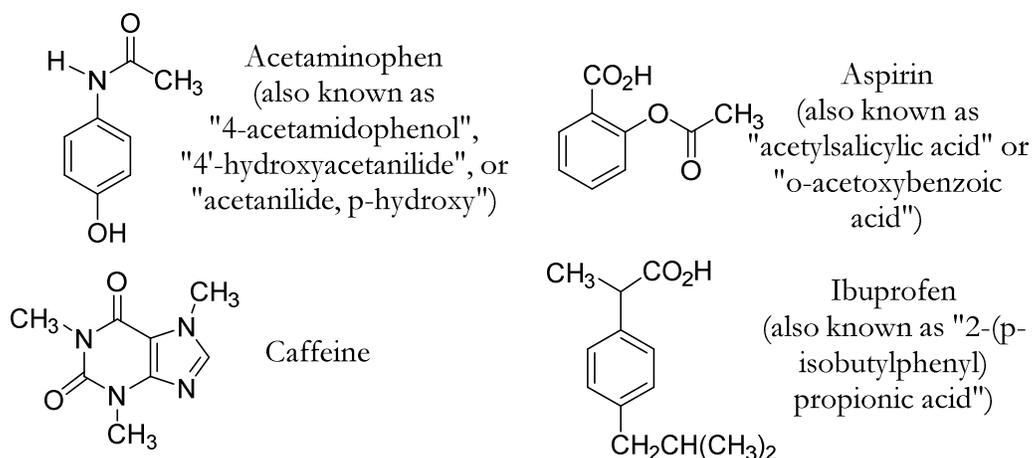


Figure 5-1: Compounds commonly found in over-the-counter analgesics

Safety Precautions

Acetaminophen, ibuprofen, and ethanol are toxic or poisonous. Ethanol, ethyl acetate, and hexanes are flammable.

UV radiation is harmful to your eyes and skin; do not look directly at the UV light and always wear your safety goggles while viewing the TLC plates, as they have a UV filter in the lens material. Anyone whose skin is highly sensitive to UV radiation (i.e., anyone who sunburns easily) should wear protective clothing and gloves while viewing the TLC plates under the UV lamp.

Procedure

Work with a partner for this lab. Each pair will receive one unknown compound in a vial from their TA. You should choose one solvent mixture while your partner chooses the other; the two possible solvents are 20:80:1 hexanes/ethyl acetate/acetic acid and 50:50:1 hexanes/ethyl acetate/acetic acid.

First, make up about 20 mL of the solvent mixture you will use. From your drawer, choose an Erlenmeyer flask and a rubber stopper that fit together. Using your graduated cylinder (for hexanes and ethyl acetate) and a syringe (for acetic acid; the syringe will be attached to the acetic acid bottle), measure out these solvent volumes and add them to the flask:

- 4 mL hexanes + 16 mL ethyl acetate + 0.2 mL acetic acid (if you are making 20:80:1), or
- 10 mL hexanes + 10 mL ethyl acetate + 0.2 mL acetic acid (if you are making 50:50:1).

Stopper the flask and swirl gently to mix. Label the flask with the solvent ratio you are using. You should leave this flask stoppered when not in use, so that the solvent ratio does not change due to evaporation.

Make up five TLC spotting vials of the four standards plus your unknown. You can share these vials with your partner, so you don't each need to make up all the spotters. To make up a vial, place about 0.01 g (10 mg) of a compound into a clean vial and then dissolve it in 1 mL of ethanol; make sure to label each vial clearly. You do not need to weigh out exactly 10 mg of each compound. You can weigh out one compound, and then use that as a visual guide to put approximately the same amount of compound into each vial.

Next, prepare the TLC spotter of your pain reliever sample by crushing a tablet in a mortar and pestle until it is a fine powder. Place a small portion (~10 mg) in a clean vial, and then dissolve it in 1 mL of ethanol.

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Now you are ready to prepare your developing chamber, following the directions in the TLC section of the Handbook. Add enough of your solvent mixture to fill the chamber to a depth of about 0.25 cm, then cover it with a watch glass.

You can also prepare your TLC plate, again following the directions in the Handbook. For this lab, you are spotting six samples on the same plate, so you should cut a plate about 3.5 – 4 cm wide. Prepare your TLC plate by drawing a light pencil line 0.5 cm from the bottom of the plate and writing lightly (in pencil) AC, ASP, CF, IB, PR, and UNK below the line as illustrated in Figure 5-2. **Be extremely careful not to write so hard that you scratch off the silica** – this will cause your plate to develop improperly, and you will have to prepare and run a new plate.

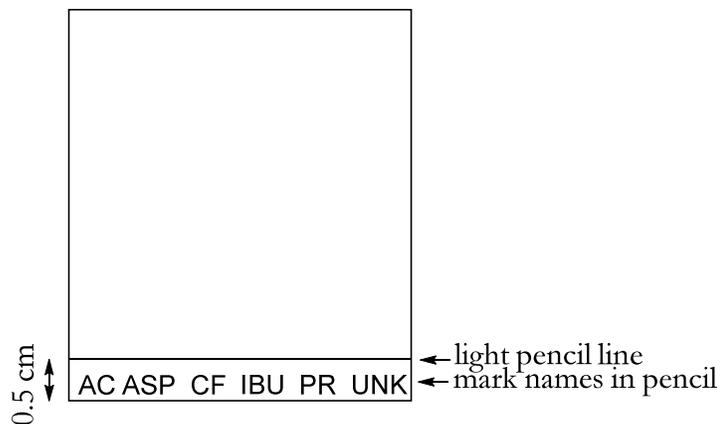


Figure 5-2: A prepared TLC plate, with labels for each of the samples.

From left to right, spot your TLC samples in the following order: acetaminophen (AC), aspirin (ASP), caffeine (CF), ibuprofen (IBU), your chosen pain reliever (PR) and the unknown compound (UNK). Note that this order is alphabetic to avoid confusion. Rinse the microcap between each sample by dipping it into a vial of clean ethanol and then spotting it on a Kimwipe; repeat these steps a few times to make sure it is clean. Pre-check all of your spots with UV.

Once all of your compounds have been spotted satisfactorily, the TLC plate is ready to be developed. Lean your TLC plate face-first against the wall of the beaker. Make sure the TLC plate is not touching the filter paper at all, and make sure that the solvent level is lower than the spots on the plate. Develop the TLC plate until the solvent almost reaches the top of the plate. Remove the plate from the beaker and mark the solvent front with a pencil, then give the plate a minute to air dry.

Visualize your developed spots under UV. With a pencil, carefully circle *all* the spots you observe. Measure the distance each spot has travelled from the baseline and use this to calculate the R_f .

Determine the melting point of your unknown solid using a melting point apparatus. You and your partner must each take a melting point of the unknown compound. By this point you should have a good idea of which compound your unknown is. Verify this by taking a mixed melting point of your compound combined with the standard compound that you believe it is. Using a mortar and pestle, grind together a small quantity of each compound, then take a melting point of the mixture. If you guessed correctly, the melting point of this mixture should be similar to that of your unknown. If the mixture's melting point is significantly lower, then you guessed wrong, and you should repeat this step with a different standard compound.

Write down the unknown vial number in your lab notebook, then return the vial, along with the mortar and pestle, to where you found them. **Do not keep them in your lab drawer! If you do, other students may be unable to perform this experiment and you may be docked technique points on your lab.**

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Wastes

Organic Waste: Eluting solvents and tablet extracts.

Solid Chemical Waste: Used melting point capillaries, pipets and TLC plates.

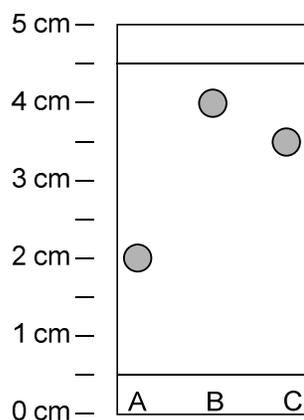
Lab Report

This lab report (and all lab reports you write for this class) should follow the guidelines given in the “Writing Lab Reports” chapter of the Handbook. In addition to these guidelines, most experiments will specify additional topics to discuss. For this particular experiment, your conclusions should include:

- Report which of the unknown analgesics you were given, based on TLC and melting point.
- Calculate R_f s of all spots, and explain the differences in results of the two different solvent systems.
- List the four known analgesics in order of polarity.
- Address how pure your compound was, based on its melting point and TLC.
- Report whether or not the pain reliever tablet had the analgesic reported on the label.

Study Questions

1) Consider the following silica gel TLC plate of compounds A, B, and C developed in hexanes:



- Determine the R_f values of compounds A, B, and C.
 - Which compound is the most polar?
 - What would you expect to happen to the R_f values if you used acetone instead of hexanes as the eluting solvent?
 - How would the R_f values change if eluted with hexanes using an alumina TLC plate?
- What could happen if you used ink to draw in your base line and letters on the TLC plate?
 - What could happen if you spot too much of a compound on the TLC plate?
 - What would happen if your solvent level is above the level of the initial spots?
 - The CRC lists the melting point for a compound as 182–183°C. You observe a melting point for this same compound isolated in your experiment as 177–181°C. What can you conclude about the compound isolated in your experiment?