Thin Layer Chromatography and Melting Point

**Introduction** The main purpose of this lab is to determine the identity of an unknown organic compound. For this lab, this goal could be achieved through measuring the unknown compounds chromatographic properties and then comparing the unknown substance’s melting point to the literature values of the substances that could possibly be the unknown organic compound.   
 In order to accomplish this goal, a TLC plate would have to be prepared of approximately 5cm wide with around 10-15 cm height. A line of would be drawn above 1 cm and it would be spotted with standard compounds under its corresponding name, except for the PR that we chose, which we would have to crush and then mix it 1ml of Ethanol and then spot it onto the TLC Plate; same goes for the unknown. Although for this to work, some aspects need to be kept constant: solvent system, absorbent, thickness of the absorbent, amount of material spotted, and the temperature. Both experiments have to be done at the same time in order for the aspects to be kept constant. After TLC plate would be placed under the UV light to find the spots. Lastly, the compound would be melted in order to find its melting point to determine the identity and purity of the unknown compound.  
  
**Table of Physical Data and Hazards**

|  |  |
| --- | --- |
| *4-Acetaminophen* | *M.P = 169 – 172 C* |
| *Acetylsalicylic Acid (Asprin)* | *M.P = 135 C* |
| *Caffeine* | *M.P = 238 (anh)* |
| *Ibuprofen* | *M.P = 75 – 77 C* |

***Safety Precautions:***

* *There are toxic and poisonous substances: Acetamidophenol, Ibuprofen, and ethanol*
* *There are flammable substances: Ethanol, ethyl acetate, and hexanes.*
* *UV waves are harmful to the naked eye. Do not look directly at the UV lamp*
  + *Use goggles and gloves at all times.*

***Wastes***

* *Organic waste: Eluting Solvent and table extracts => need to be thrown under “organic waste” in the fume hood.*
* *Solid chemical wastes: Use melting point capillaries, pipettes, and TLC plates => Trash.*

**Procedure**

1. 10mg of UNK in a clean vial and dissolve it with 1ml of ethanol.
2. Pick a pain reliever, crush it and mortar it till it is in a powder form
   1. Take 10mg of it into a clean vial, dissolve it with 1ml of ethanol.
3. Cut the plate in about 5cm wide x 10-15 cm in length.
   1. Draw a light line with a pencil 1cm above the bottom edge of the TLC plate.
   2. Label the names from left to right: AC, ASP, CF, IBU, PR, UNK.
4. Obtain a microcap and rinse it several times with the same solvent and dry it by using it on a paper towel.
5. Use the microcap, fill it with AC and then gently tap it on the plate where the line is drawn above “AC”
   1. Do this for all compounds and after each one, rinse the microcap as they are reusable if rinsed.
6. Take a beaker and partially fill the beaker with a eluting solvent to the depth of 0.5 cm.
   1. There would be 2 eluting solvents, you’ll pick one and your partner will pick the other.
      1. Hexanes: ethyl acetate : acetic acid (20:80:1)
      2. Hexanes: ethyl acetate: acetic acid (50:50:1)
7. Place the TLC plate into the beaker, make sure the solvent doesn’t pass the 1cm line drawn. Then cover the beaker with a watch glass.
8. Keep it in the beaker until the solvent front is close to reaching the top of the TLC plate, take it out and mark the solvent front as it would evaporate soon.
   1. Leave it for 1 or 2 minutes to dry.
9. Take the TLC plate under the UV light and circle the spots with a pencil.
   1. By doing this you could assume which one is your unknown based on the chromatographic properties. Although one cannot be perfectly sure, that’s why the Melting Range test is done.
10. Record all data, and start the melting point test.
11. Take a sample from the unknown into the capillaries and shove the sample down to the end (as there is one open end, and one close ended {bottom of the capillary}).
12. Place it into the Melting machine, set the start temperature about 20-40 C below the melting point of your assumed unknown pain reliever. Record the ranges at which it starts to melt and when its completely liquid.
    1. This allows you to determine whether your assumption was correct or not, and if it was correct, was the unknown substance pure or impure.