CHEM 267. Week 1. Thin Layer Chromatography (TLC). (revised 7/10).

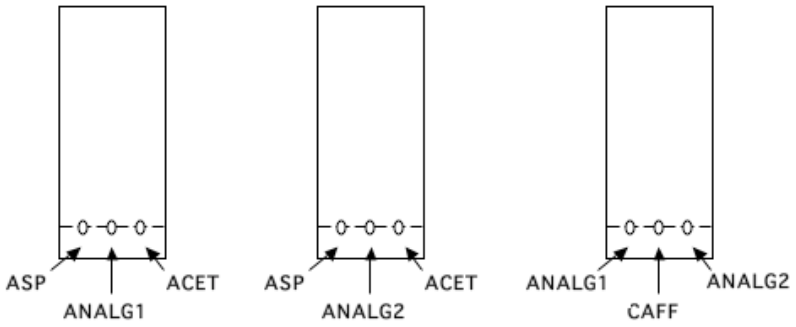
(1) TLC analysis of analgesics.

To help follow the handout, view the photos on the course website.

CAUTION: the solvents used in this experiment, ethyl acetate, methanol, and ethanol, are flammable. Never look directly at the ultraviolet (UV) lamps. Eye damage could result.

Changes: (1) ibuprofen does not work well under these conditions, so will not be an ingredient in the analgesics used in this experiment. (2) The development solvent will be pure ethyl acetate, not 95% ethyl acetate/ 5% acetic acid. (3) You will use commercial micro capillaries (pipets). You will **not** make your own micro capillaries as stated in the lab text.

Spotting. Use the commercial micro pipets to spot the plates. The powdered side of the silica gel plate, not the shiny, plastic side is the side that is spotted. Lightly draw a pencil line about 1 cm from the end of a plate. Use a pencil, not a pen, and be careful to not scratch the surface of the plate. The plates are of such a size that only three spots can be run on each. Use a fresh pipet for each spot. Make each spot as small as possible (less than about 1 mm diameter). After applying the spots check them under the UV lamp to see that they are of a reasonable size. The UV light must be held close to the plate to see the spots (CAUTION: do not look directly into the UV light.) If the spot is much too large, prepare a new plate. If a spot is too small, add more solution. Analyze two unknown analgesics in the following way: two of the three known compounds (references) plus the first analgesic to be analyzed (unknown 1) will be spotted on one plate, the same two knowns plus the second analgesic (unknown 2) will be spotted on a second plate, and both analgesic 1 and analgesic 2 along with the third known compound will be spotted on a third plate. (If the plates were wider, you could analyze each unknown on just one plate.)



To obtain a sample of a known solution, bring a micro pipet to the sample solution at the common bench, dip it into the solution to fill it, then take it back to your workspace for spotting. Try to minimize traffic at the common bench and take care to not contaminate the solutions with a micro pipet that has already been used. The purpose of the experiment is to determine which of the known compounds are contained in the analgesics that you chose to analyze. Solutions of the known compounds will already be made. You must make 1% solutions of the unknown analgesics. To do this, take a small part of a crushed tablet and add enough ethanol to make an approximately 1% solution as described in the text (very approximate - do not weigh samples). About 1 mg in several drops of solvent will produce an approximately 1% solution. The analgesic contains insoluble binders so not all of it will dissolve.

Development. The development chamber is a small screw-capped jar with a 5.5 cm filter paper placed into it to ensure that the atmosphere is saturated with vapor (see Fig 8.7). Place about 2 mL of solvent into the chamber (2 mL is a full squeeze of the bulb using a Pasteur pipet.) Watch out for the following: the depth of the solvent must be below the position of the spots or else the spotted material will dissolve in the solvent; the plate must stand vertically in the tank and the silica gel must not touch the filter paper; the solvent must not be allowed to run all the way to the top of the plate; the correct amount of material must be spotted (checking under UV light before development will help - if the spots are too large, another plate can be made - if the spots are too small, more material can be spotted); the UV light must be held close to the plate to see the spots (CAUTION: do not look directly at the UV lamp). After the solvent has almost reached the top of the plate, the plate is removed and the position of the solvent front marked before the solvent evaporates. Allow the development solvent to run almost to the top of the plate - this will use the entire plate and allow for the best possible separation. Allow the plate to dry in the hood. Development takes little time so if a plate comes out poorly another can easily be done. Because there are a limited number of bottles please use only one ethyl acetate development chamber. Each run is relatively brief so there is no need to hog the bottles.

Visualization. The plate will be visualized by two methods, first, by short wavelength UV light. (CAUTION: do not look directly at the lamp.) The light must be held close to the plate to see the spots. Some spots will be very faint. The observed spots should be outlined with a pencil. The plate can then be placed for a few minutes into a jar containing iodine crystals. These spots may appear different. They should also be circled. A comparison of the two visualization techniques can be made and may help in identifying the unknown spots. The iodine method must be done after the UV method because the compound may react with the iodine, possibly changing the results.

The Rf values should be calculated for the known compounds and for all components of the analgesic. From your results, identify which components are present in a particular analgesic. Some of the components may have very similar Rfs under these conditions. However, their spots may look different upon treatment with iodine and this may allow a distinction to be made. Note that caffeine is present in small amounts and may not show up strongly. Purposely overloading a spot may help to show a component present in small amounts.

You may choose from the following analgesics: Anacin, Extra Strength Excedrin, Extra Strength Tylenol, and CVS Super Strength Pain Reliever. You may also analyze your personally-preferred legal analgesic if you wish. Just bring a tablet to lab.

(2) Solvent Effect on Rf Values. Three known compounds, anthracene, benzil, and triphenylmethanol will be developed in two different solvents. Prepare two identical plates, spotted with each of those compounds, and develop one in ethyl acetate and the other in a mixture of 95% hexane and 5% t-butyl methyl ether. Use a second development chamber for this solvent. Calculate Rf values, and in the post-lab write-up, discuss the effect of solvent on Rf, keeping in mind the structures and polarities of the compounds and solvents.

SAFETY: For minor thermal burns, place the burned tissue immediately into cold water or if necessary ice water.

BEFORE YOU LEAVE THE LAB: put away your equipment and lock your drawer, clean up your work areas, close the fume hood sash completely, turn off all utilities and ask your TA for her or his signature. In general, please try to keep the lab in as good condition as you found it. If you see caps off of bottles, replace the caps. If you see spilled chemicals, clean them up or at least report it to your TA.

WASTE: Never dispose of glass waste in the regular trash. The custodian could become injured. Dispose of all glass pipets and glass waste in the cardboard "Glass Only" containers. Dispose of all solutions and developing solvents in the NON-halogenated Liquid waste container in the hood. When finished, pour as much of the solvent as possible into the waste container and leave the screw-capped jars open in the hood so they will dry. Leave the filter paper in the jars.

Postlab Questions

1.) Arrange the following compounds in order of increasing Rf in a TLC analysis: benzoic acid, benzaldehyde, 2-octanone, decane, and cyclohexanol.

2.) What would happen if the spot were applied to the TLC plate below the level of development solvent?

3.) What would be the result of adding too much sample to the TLC plate?

4.) In a TLC analysis of analgesics, what would be the result if a solvent of too low polarity is used to develop the plate?

5.) The Rf of ibuprofen was found to be 0.32 when t-butyl methyl ether was used as the development solvent. What effect would there be on the Rf of ibuprofen if acetone had been used to develop the TLC plate?