Lab 5

THIN-LAYER CHROMATOGRAPHY

ANALYSIS OF CAFFEINE AND ANALGESICS[1]

Introduction

This week you will use thin layer chromatography (TLC) to determine whether you successfully isolated caffeine from tea (Lab 3), and if so, how pure it is. You will also use this technique to identify unknown commercial analgesics. The structures of common components of analgesics are shown in Figure 1.

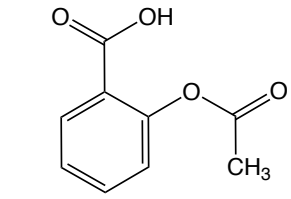
Reading Assignments

Techniques :

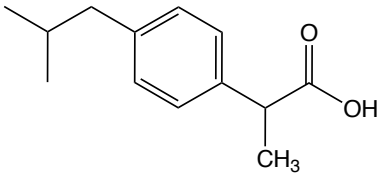
- TLC: Mohrig,[2] p. 255 - 269; video link on Lab-flow under Lab 5

New Techniques

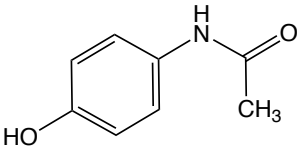
Thin Layer Chromatography. As introduced in Lab 4, chromatography is the general method used to separate mixtures of two or more compounds. Some examples include column chromatography, which you will attempt later this semester, and thin layer chromatography (TLC), the focus of today's experiment. TLC is an extremely simple, fast, and inexpensive technique involving separation of compounds based on differences in polarity, adsorptivities, and solubilities. TLC has many uses including determination of the number of components in a mixture, identification of the components in a mixture, monitoring the progress of a reaction, measuring the effectiveness of a purification, and monitoring column chromatography.



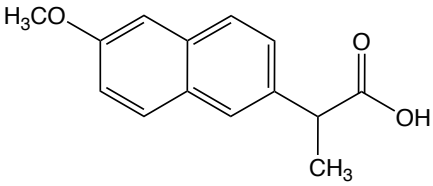
Acetylsalicylic acid (Aspirin)



Ibuprofen (Advil®)



Acetaminophen (Tylenol®)



Naproxen (Aleve®)

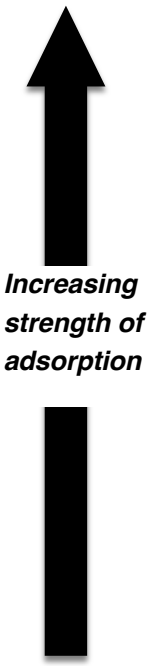
Figure 1. The active compounds in several common over-the-counter analgesics.

As you have learned from your online resources and reading Mohrig, chromatography typically involves distributing a mixture between two phases: one that is moving (**mobile phase**, or **eluent** – solvent) and one that is stationary (**stationary phase**, or **adsorbent**, usually highly polar). This technique involves:

1. Dissolving the mixture in a volatile solvent.
2. Spotting the mixture on a piece of glass or plastic (ie. the plate) that is coated with a thin layer of an adsorbent
3. Immersing the plate in a **TLC chamber** (jar/beaker containing a shallow layer of eluent).
4. The eluent will travel up the plate and through the adsorbent via capillary action. At this stage, differential partitioning occurs between the components dissolved in the eluent and those adsorbed to the

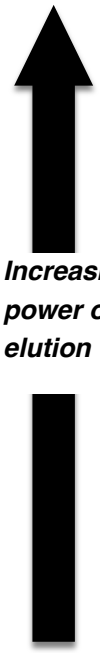
stationary phase. The more strongly a given component of a mixture is adsorbed onto the stationary phase, the less time it will spend in the mobile phase and the more slowly it will migrate up the plate. In principle, all the components will differ in solubility and in the strength of their adsorption to the stationary phase and therefore some components will be carried farther up the plate than others. As a result, you get separation!

Many solvents can be used as the mobile phase and some examples are listed below (Fig. 2). In general terms, a non-polar solvent will carry the more non-polar components up the stationary phase and leave behind the more polar compounds. A more polar solvent will carry the less-polar and the more-polar compounds further. Combinations of solvents are useful because the polarity of the mobile phase can be fine-tuned by varying the ratio of the more polar solvent to non-polar solvent. Popular combinations include 0-30% ethyl acetate/hexanes and 0-40% ether/pentane.



Increasing strength of adsorption

Group	Functionality
Carboxylic acids	RCO_2H (more polar, protic)
Alcohols	ROH
Amines	RNH_2, R_2NH, R_3N
Ketones, Aldehydes	R_2CO, RCHO
Esters	RCO_2R
Ethers	ROR
Alkyl halides	RX (X = Cl, Br, I)
Hydrocarbons	Aromatics, Alkanes (more non-polar, aprotic)



Increasing power of elution

Solvents
Acetic acid
Methanol Ethanol
Triethylamine, Pyridine
Acetone
Ethyl Acetate
Diethyl ether
Chloroform Dichloromethane
Toluene Hexane Petroleum ether

Figure 2. Relative adsorptions of general functional groups and eluting power for some common solvents.

Common adsorbents used in TLC include alumina (aluminum oxide, Al_2O_3) and silica gel (SiO_2). Both are highly polar (alumina more than silica gel).

Analyzing the Results of TLC. The number of resolved spots gives you an idea of how many compounds were present in the original sample. Remember that more than one compound may travel up the plate the same distance and therefore the number of spots you can see is the *minimum* number of compounds in the mixture.

The distance a compound travels up a plate compared to the distance the solvent front traveled is reported as the R_f value (Figure 3). Generally, more polar compounds have smaller R_f values and less polar compounds have larger values. A compound should have the same R_f value (approximately – this is not an exact science - see co-spotting below) every time a TLC experiment is run under the *exact same conditions*. Therefore, you can confirm the identify an unknown by comparing the experimentally determined R_f value with the value reported in the literature.

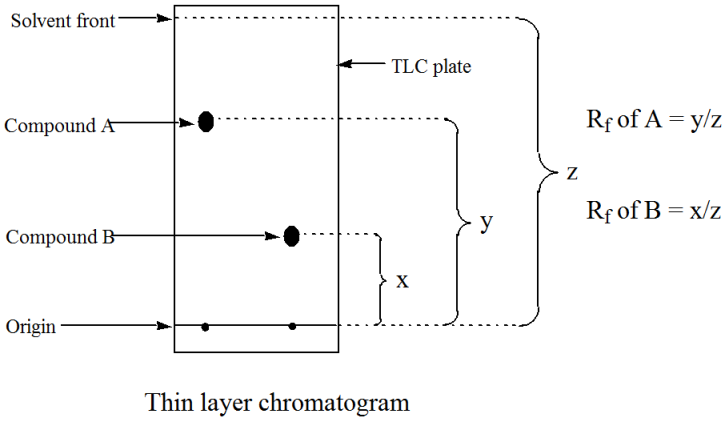
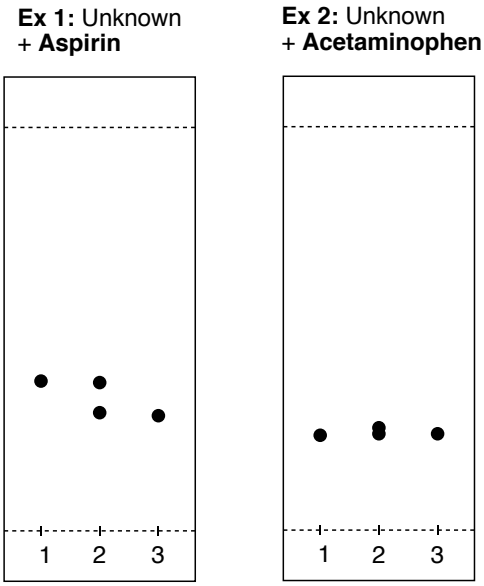


Figure 3. Example of a TLC plate and R_f value calculation.



Co-spotting. Identification of an unknown is more reliable if you co-spot, especially when two compounds have similar R_f values. On a single TLC plate your unknown is spotted in lanes 1 and 2. A known compound you suspect to be your unknown is spotted in lane 3 and directly on top of your unknown in lane 2. The plate is developed and visualized.

If two spots appear in lane 2 then the unknown is NOT the same as the known (Ex 1, Fig. 4). If one spot appears in lane 2 then the two compounds are most likely the same (Ex 2, Fig. 4). The examples in Fig. 4 shows that the unknown is acetaminophen and not aspirin.

Figure 4. Example of co-spotting results. Example 1 shows the unknown is not aspirin since the cospot shows two separate spots. Example 2 shows that the unknown is likely acetaminophen since the spots overlap in the cospot lane overlap.

See the links listed on the Sapling site under **Lab 5** and Mohrig[2] for additional information.

Developing the TLC Plate with Chemical Staining

In some cases, spots can be visualized by treating the TLC plate with a chemical "stain" following the elution process. Such stains will turn a reactive compound from clear to colored. There are a number of chemical stains that can be used and several are discussed on the following page.

- **Potassium permanganate ($KMnO_4$):** Excellent for visualizing compounds that are oxidizable (e.g. alcohols, alkenes, alkynes, aldehydes). The spots generally turn yellow to brown, while the plate itself becomes purple.
- **Vanillin & p-Anisaldehyde:** These are general-use stains good for visualizing strong or weak nucleophiles (e.g. amines, alcohol) and some aldehydes and ketones. They do not stain alkenes, aromatics, esters or carboxylic acids. The spots can appear dark yellow to purple, while the plate is typically pink.
- **Bromocresol green:** This stain is particularly good for visualizing compounds with groups having a pK_a of < 5. (e.g. carboxylic acids). Spots will appear light yellow, while the background turns blue-green.
- **Ferric chloride ($FeCl_3$):** This stain is good for visualizing phenols. The oxygen of the phenol compound is a good nucleophile and chelates to iron atoms. The complexes that form often appear blue, while the plate is pale yellow.

SAFETY ISSUES

Standard issues apply for the use of **gloves, safety glasses** and **GLASS pipettes** (see Lab 1).

Take care when handling the **solvents** (ethyl acetate, acetic acid, and ethanol). Avoid contact with your skin and eyes! Wash with cool water if exposed.

Procedure

1. Preparing the developing chamber

a. Line a 150 mL beaker **or** your glass jar with half of a piece of filter paper (see picture below).

b. Pour the eluent (99:1 ethyl acetate: glacial acetic acid) into the beaker to a depth of ~6-8 mm. Cover with a watch glass. Tilt the beaker to wet the filter paper. Allow the solvent to travel up the filter paper so that the chamber is saturated with the solvent. This is important for preventing the TLC plate from drying out as the compound travels up the plate. Note: It is important that you **keep the chamber covered at all times** to minimize the evaporation of the organic solvents. Evaporation would lead to a change in the ratio of solvents and therefore the polarity and would make repeating the TLC experiment impossible.

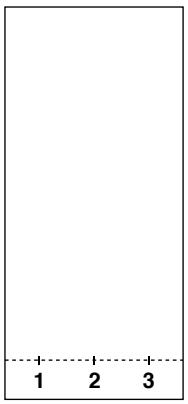
2. **Preparing the plates.** Each group should obtain 2 silica gel plates.

   a. With a **pencil**, lightly draw a baseline ~1/4 inch from the bottom of each silica gel TLC plate.

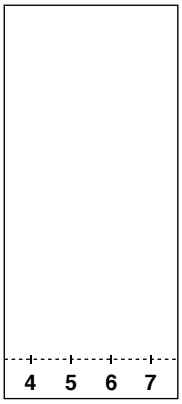
   b. Label the plates as shown to the right:

   c. Label 6 test tubes: caffeine standard, isolated caffeine, aspirin, acetaminophen, ibuprofen, naproxen and unknown.

   d. Deliver 3-5 mg (a small amount ~ a spatula tip full) of the each of the standards to the corresponding test tube. Deliver 20-30 mg of the unknown to the appropriate tube. Be sure to clean the spatula between uses by wiping with a Kimwipe moistened with acetone. Add ~1 mL of the 1:1 mixture of ethanol:ethyl acetate to each tube and shake lightly to dissolve. It is important that each solution is dilute – if they are too concentrated the spots will streak and it will be impossible to gather any useful information.



1 = caffeine standard
2 = standard + isolated caffeine
3 = isolated caffeine



4 = aspirin standard
5 = acetaminophen standard
6 = ibuprofen standard
7 = unknown sample

   Note – the unknown analgesic (obtained from your TA) will not dissolve completely because there are insoluble binders present in the tablets. Write down the letter of your unknown in your notebook.

   e. "Spot" each compound according to the pictures above by **lightly** and **quickly** touching the spotter to the plate. Capillary action will draw the solvent out. The smaller the spot the better. You may need to spot a few times to ensure enough compound has been delivered (you can check this using the UV lamps). In between "touches" gently blow on the spot to evaporate the solvent. Be sure to touch the same place each time.

3. **Developing the plate**.
   a. Carefully place the plates into the developing chamber. The solvent level should be **below** the spot on the plate (It is important not to have too much solvent in the beaker or to spot the material too low on the plate. Why?). It is also important to set the plate in straight instead of at an angle (why?).
   b. Cover the chamber and observe as the eluent travels up the plate. When the solvent reaches approximately ¼ inch from the top, remove the plate and carefully mark the solvent front lightly with a pencil.

4. **Visualizing the plate**. If the components in your mixture are possess color this is easy. However, most of the time you have to process the plate further. Visualize your plates **by each of the following methods** in today's experiment:

   a. **UV lamp**. Make sure that all of the solvent has evaporated. Place the plate under a UV lamp (hold with tweezers so as not to expose your skin to the damaging light) and turn it on. The background will be green and the spots will appear dark. Trace the outline of the spots with a pencil since they will disappear again once you turn the lamp off. Please make sure that you turn

off the lamp each time you use it! Note: some compounds do not show up with this visualization method.

b. **Iodine (I_2) Chamber**. Placing the plates in a container with a few crystals of I_2 will cause most organic compounds (spots) to turn brown as the iodine adsorbs to the molecules. This works best if you shake the chamber so that the solid sits on top of your plates. When you observe a definite change in appearance remove the plates. Immediately trace the outline of the spots because the color will disappear as the iodine sublimes. Note which compounds stain and *to what intensity*.

5. **Interpretation**. Calculate and tabulate the R_f values for each of the spots using the formula described in the introduction. Dispose of the used plates in the regular trash and TLC spotters in the broken glass bin.

Pre-lab Assignment

- **Prereading** - Be sure to read the sections pertaining to new techniques and chemistry in this lab (see the first page) and view the video links provided under Lab 5 on Labflow.
- **Pre-lab Quiz** - You will complete Prelab Quiz 5 on Labflow during the week of your lab meeting.
- **Pre-lab Notebook**:
  - ✓ **Objective** – Include main goals of the experiment, no structures necessary. Predict what the TLC plate containing the isolated and standard caffeine (lanes 1-3) will look like 1) if your caffeine is pure, 2) if your caffeine is contaminated by a non-polar contaminant. 3) if your caffeine is contaminated by a more polar contaminant.
  - ✓ **Table of Reagents and Separation Scheme** - Not required this week.
  - ✓ **Procedure** – Write a brief stepwise procedure in your pre-lab pages. It should contain enough detail that another student could read it and follow the steps.

In-Lab Notebook

- **Procedural Changes:** Write in your in-lab notebook pages any changes made to the procedure when necessary.
- **Observations:** record EXACT amounts of reagents used, drawn images of TLC plates with spots indicated, and all measurements for future R_f calculations. This should be written directly in your notebook as you complete the lab.

All information above should be written directly into your lab notebook. The duplicate pages will be turned into your TA after he/she has signed them prior to you leaving lab.

All information below will be included on the Post-Lab Report Worksheet or typed Discussion and does NOT have to be written separately in the notebook.

Post-Laboratory Report

Complete the report as directed on the next pages.

References

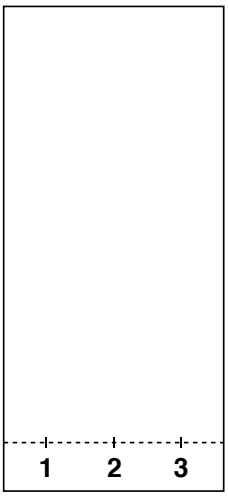
1. a) Pavia, D. L., Lampman, G.M., Kriz, G.S., Engel, R.G. *Introduction to Organic Laboratory Techniques* Saunders College Publishing: New York, 1999, pp 119-127. b) Sampath, V. *Organic Chemistry Laboratory C344*, Colorado State University, 1997.
2. Mohrig, J. R., Alberg, D. G., Hofmeister, G. E., et al. *Laboratory Techniques in Organic Chemistry*, 4th ed. W. H. Freeman & Co.: New York, NY; 2014

Chemistry 201L - **Post-lab Report for TLC - Analysis of Caffeine and Aspirin** (Lab 5)

Name:_____ **TA name & Section number:**_____

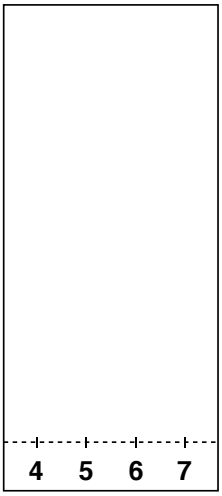
***For TLC plates below indicate solvent front, approximate location and shape of spots, and results of your visualizations (which spots appeared under UV lamp and/or with iodine chamber).*

TLC Analysis of isolated caffeine



1 = caffeine standard
2 = standard + isolated caffeine
3 = isolated caffeine

Identification of Unknown Analgesic



4 = aspirin standard
5 = acetaminophen standard
6 = ibuprofen standard
7 = unknown sample

Observed R_f values (show calculations & use correct significant figures for full credit):

Isolated Caffeine:

Standard Caffeine:

Aspirin:

Acetaminophen:

Ibuprofen:

Unknown:

What was the letter of your assigned unknown? _____

Which components did you find in YOUR unknown? (circle all those that apply)

1) Caffeine 2) Aspirin 3) Acetaminophen 4) Ibuprofen

Labflow Discussion: Answer the discussion questions on the Labflow website.

Based on the TLC results, what compound(s) did you isolate from tea in Lab 3? How have you confirmed this (i.e., what data do you have that suggests this)? For the TLC results, include a comparison of R_f values and a discussion of the co-spotting experiment.

What were the components of your unknown analgesic based on the TLC experiment? What evidence do you have to support this (including Rf value comparisons in your discussion)?

Based on the experimental observations, which chemical stain(s) might you use to confirm the presence of your unknown? (*These are the four stains mentioned before Safety Issues in the handout*.)

In addition to the above, answer the following questions.

1. What was the purpose of the 1% glacial acetic acid in your mobile phase? Which functional groups can acetic acid interact with and through what interactions? Consider the polarity and functional groups of both the compounds and the stationary phase.
2. Relative to ethyl acetate, what would be the effect on the R_f values of the compounds if the following mobile phases where used.
    a. 50% : 50% Hexane : Ethyl Acetate
    b. 100% Hexane
    c. 100% Acetone
3. Reverse-phase TLC uses silica that is modified to have octadecane (C_{18}) molecules at the surface instead of hydroxyl groups. If you repeated this experiment using reverse-phase TLC plates, in what order would you predict the compounds will elute? Provide an explanation for your prediction.

References are NOT required for this lab discussion.