

THIN LAYER CHROMATOGRAPHY- TLCⁱ

INTRODUCTION

Thin-layer chromatography (TLC) is used to identify compounds and determine their purity. It can be used to determine if a substance is a mixture (2 or more compounds) and to identify compounds when *standards* are available.

Prepared plates are made of a porous adsorbant, (silica gel or alumina) which is adhered to a thin piece of glass or plastic. The chromatography process works by differences in polarity. The more polar the mixture is, the more tightly it is held by the adsorbant. One chooses a solvent that will cause the different components of the mixture (which hopefully all have different polarities) to move across the TLC plate at different rates.

In this lab you will identify the analgesics used in some over the counter pain medications.

PREPARING STANDARDS AND UNKNOWNNS

The standards and commercial painkillers will be dissolved in a **50/50 Ethanol/Ethyl Acetate solution**. The standards will be provided by the lab. You need just a few drops of each for this experiment.

Crush one tablet of unknown between paper towels. In labeled (A or B) **clean test tube** dissolve the crushed tablet of an unknown in 2-5 mL of 50/50 Ethanol/Ethyl Acetate solution (the tablet contains fillers and not all the solid will dissolve).

SPOTTING THE PLATE

Clean gloves must be worn while preparing the TLC plates.

Obtain 2 TLC plates. Draw a light pencil line about 1 cm from the end of each chromatographic plate.

Pour two drops of each solution into **clean spot plate wells** and place the thin end of a clean capillary spotter into the solution.

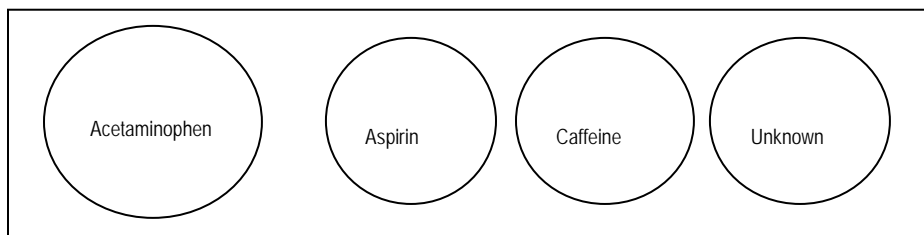


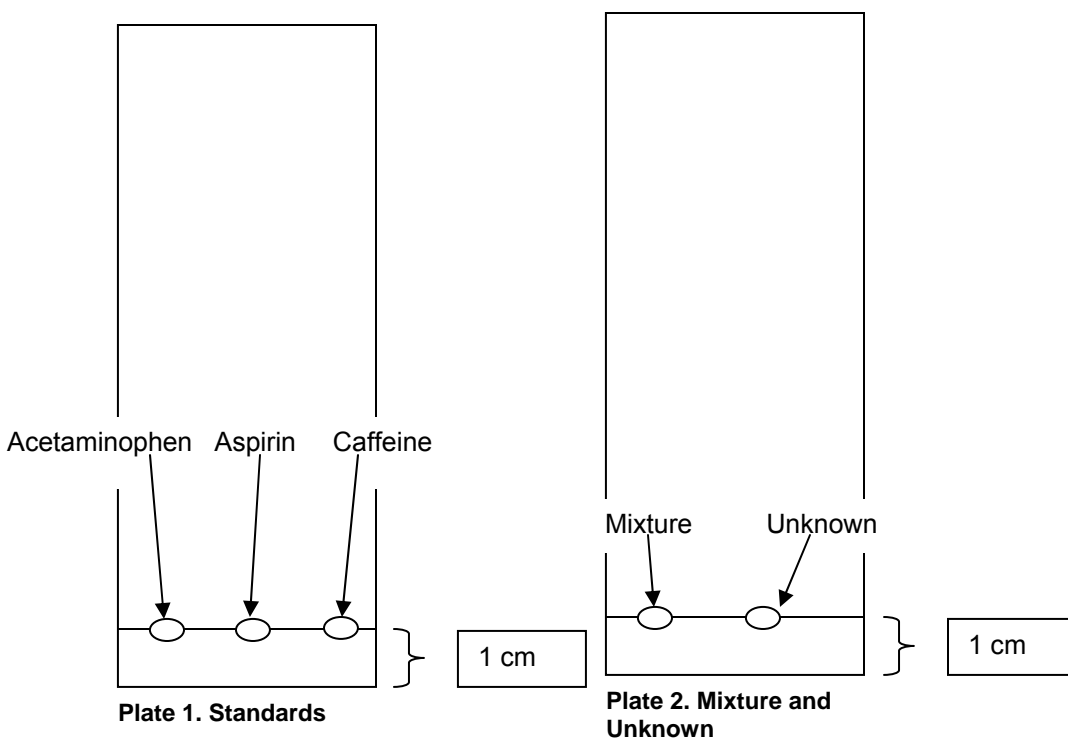
Figure 1. Spot plate wells

Using separate capillary tubes for each standard and unknown solution:

Plate 1. Spot the 3 reference standards (Acetaminophen, Aspirin, and Caffeine). Repeatedly touch the capillary to the plate briefly to form a **small** spot and build up the concentration of the compound.

Plate2. Spot the unknown commercial painkiller and a spot that is a mixture of the three standard painkillers.

Use a separate capillary tube for each standard and unknown. Make each spot as small as possible, preferably less than 0.5 mm in diameter. Examine the plate under the **ultraviolet (UV)** light to see that enough of each compound has been applied; if not, add more.



DEVELOPING A PLATEⁱⁱ

Using a large beaker as the chamber, place a half-piece of filter paper inside, and use foil or plastic wrap to cover the top. Pour the eluting solvent, (1:2 hexane-ethyl acetate), into the beaker to a depth slightly less than 1 cm. Place the prepared TLC plates in the developing chamber.

Allow the solvent rise to near the top of the plate (about 1 cm from the top), then remove the plate and mark the **solvent front** with a pencil. Transfer the plates in the hood until the majority of the eluting solvent has evaporated. Examine the plate under UV light to see the components as dark spots against a bright green-blue background. Outline the spots with a pencil and note anything distinctive about any of the compounds.

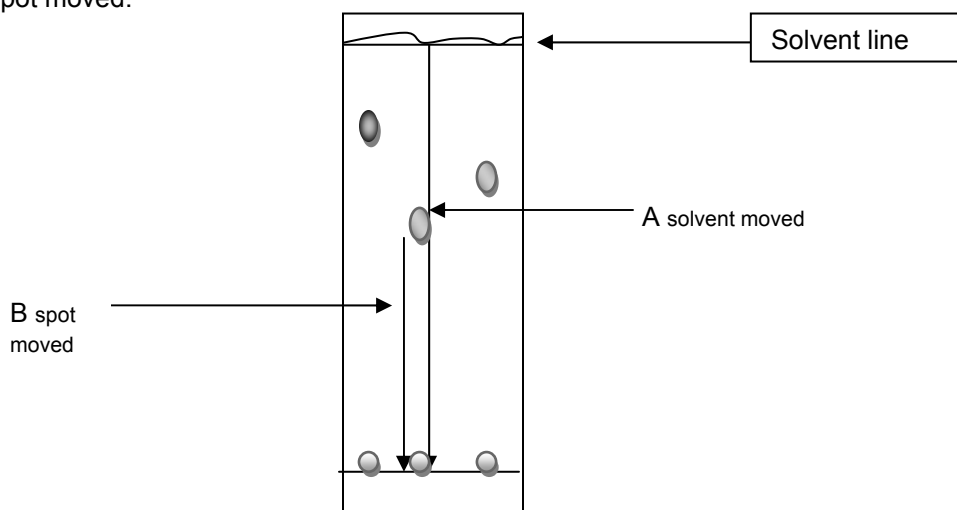
The spots should also be visualized by putting the plate in an iodine chamber. The iodine chamber is pre-made and contains a few crystals of iodine in the bottom of a capped jar. More than 2 plates can be placed in the iodine chamber at one time. Remove the plates when a definite change in appearance takes place on your plates. Note which compounds stained with iodine and to what intensity. The iodine stains will dissipate over time.

DETERMINING THE R_f VALUE

For each spot:

Measure the distance from the solvent line (drawn on the plate) to where the spot started. (A) This is the distance the solvent moved.

Measure the distance from where the spot stopped to where the spot started. (B) This is the distance the spot moved.



Divide the distance the solvent moved into the distance the spot moved. This ratio is the R_f value.

$$R_f \text{ value} = \frac{B}{A}$$

Calculate the R_f values for each spot. Unknowns can be identified using R_f values, fluorescence in UV light, changes due to iodine exposure, the reference spot and Table 1.

	Acetaminophen	Aspirin	Caffeine
Anacin		■	■
Bufferin		■	
Excedrin (extra-strength)	■	■	■
Tylenol	■		

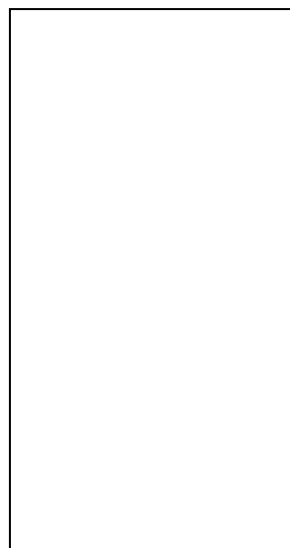
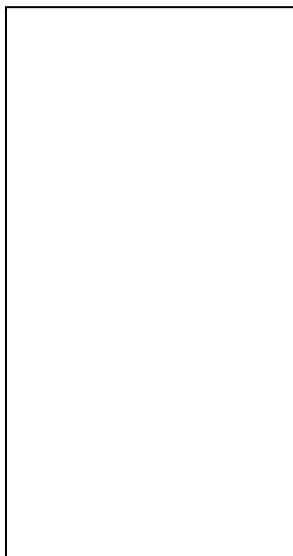
Table 1. Composition of Over the Counter Analgesics

CLEAN UP

Discard used capillary spotters in the broken glass boxes. Wash the glass stirring rod, spot plates and test tubes. Place the spot plates on the cart (or lab bench); place the test tubes in the dirty test tube bin by the sink.

REPORT

Draw a labeled picture of each plate, indicating the names of each standard, the mixture spot and unknowns A and B.



Report the R_f value and appearance of each spot:

	A	B	R_f value/s	Spot appearance	Identity of medication
Acetaminophen					
Aspirin					
Caffeine					
Unknown A					
Unknown B					

PREP ROOM GUIDE

1. STANDARDS (IN PLASTIC DROPPING BOTTLES.)
2. ELUTING SOLVENT (99/1 mixture of Ethyl Acetate/Glacial Acetic Acid).
3. SOLVENT (2-5 mL of 50/50 Ethanol/Ethyl Acetate solution.)
4. UNKNOWNNS A and B (Pills).
5. GLOVES.
6. TEST TUBES.
7. PENCILS.
8. RULERS.
9. TLC PLATES. (24 per section)
10. SPOT PLATES (12 per section)
11. CAPILLARIES (60 per section)
12. FOIL
13. FILTER PAPER.

BURNS, A., & LEVY, R. (1992). *CLINICAL DIVERSITY IN LATE ONSET ALZHEIMER'S DISEASE*. OXFORD: OXFORD UNIVERSITY PRESS.

ⁱ (adapted from: Wellesley College Online Chemistry Lab Manual
http://www.wellesley.edu/Chemistry/chem211lab/Orgo_Lab_Manual/Appendix/Techniques/TLC/week2_Table1.html)

ⁱⁱ Williamson, K., Minard, R., Masters, K. (2007). *Macroscale and Microscale Organic Experiments* 5th edition. Boston: Houghton Mifflin.