

# THIN LAYER CHROMATOGRAPHY OF ANALGESICS

## Introduction:

Over-the-counter analgesics typically contain one or more of the following active ingredients: acetaminophen, aspirin, caffeine, ibuprofen or naproxen. Thin layer chromatography (TLC) offers a simple method of analysis for these products.

Chromatography is a term that is widely used to describe a family of closely related separation methods. There are many separation methods, but the feature that distinguishes chromatography from other physical and chemical methods of separation is both a stationary and mobile phase; the sample to be separated interacts numerous times with these phases. The sample is carried through the system via the mobile phase, and the interactions that occur are due to the differences in the physical and chemical properties. These differing affinities govern the rate at which the individual components of the sample pass over the stationary phase under the influence of the mobile phase.

Thin layer chromatography (TLC) is one type of chromatography where the stationary phase is a thin layer of adsorbent particles attached to the solid plate. A small amount of sample is applied (spotted) near the bottom of the plate, and the plate is placed in the mobile phase. This solvent is drawn up by capillary action to a predetermined height. Each component, being different in chemical and physical composition, will interact with the stationary phase at a different time (retention time), thereby creating the individual bands on the plate. The retention time or retention factor ( $R_f$ ) is used to characterize and compare components of various samples.

$$R_f = \frac{\text{distance from origin to center of spot}}{\text{distance from origin to solvent front}}$$

## Materials:

UV Lamp	Acetylsalicylic Acid	Unknown analgesics
Empty Vials	TLC Plates	Developing Solution 95% Ethyl Acetate 5% Glacial Acetic Acid
100% Ethanol	Pencil & Ruler	Filter Paper
Salicylic Acid	Micro capillary Droppers	Jars with lids

## Safety:

- Always wear safety goggles on the MCL.
- Wear gloves and wash thoroughly with soap and water if you get solvent on you.
- Only shine the UV lamps toward the tabletops. UV light is harmful to eyes and skin.

## Procedure:

1. After massing the acetylsalicylic acid you synthesized in the first part of the lab, transfer it to a small vial. Add 5 ml 100% ethanol to the vial, cap the vial, and shake to dissolve your sample. Not all samples will dissolve.

2. Standard samples of salicylic acid and acetylsalicylic acid have been prepared in advance for your use.

*Spotting of the sample:*

1. Obtain a TLC plate. Handle the plate by the edges only, making sure to avoid touching the white silica layer.
2. With a pencil (not pen), use the ruler to *gently* draw a line about 1.5 cm from the bottom of the plate. Be careful not to chip off the silica layer. Beginning about 0.5 cm from the side edge of the plate, draw 9 evenly spaced crosshairs—one for each sample (see diagram on following page).
3. Immerse the capillary tube into the sample tube until some of the liquid is drawn into the capillary.
4. Very gently press the small end of the capillary tube at a crosshair on the TLC plate. Keep the spot small by touching the capillary quickly to the plate and then removing it immediately. Allow the spot to dry and repeat the spotting *directly* over your original spot.
5. Use the UV-lamp to ensure you have some sample on the plate. The plate should glow green, except where you used the capillary tube to deposit the sample. If no spots appear, use the capillary to apply more samples directly over the original spot.
6. Record which spot represents which tablet on the diagram on the following page.
7. Repeat steps 3-6 for the unknown sample and the other analgesics prepared by the other students.

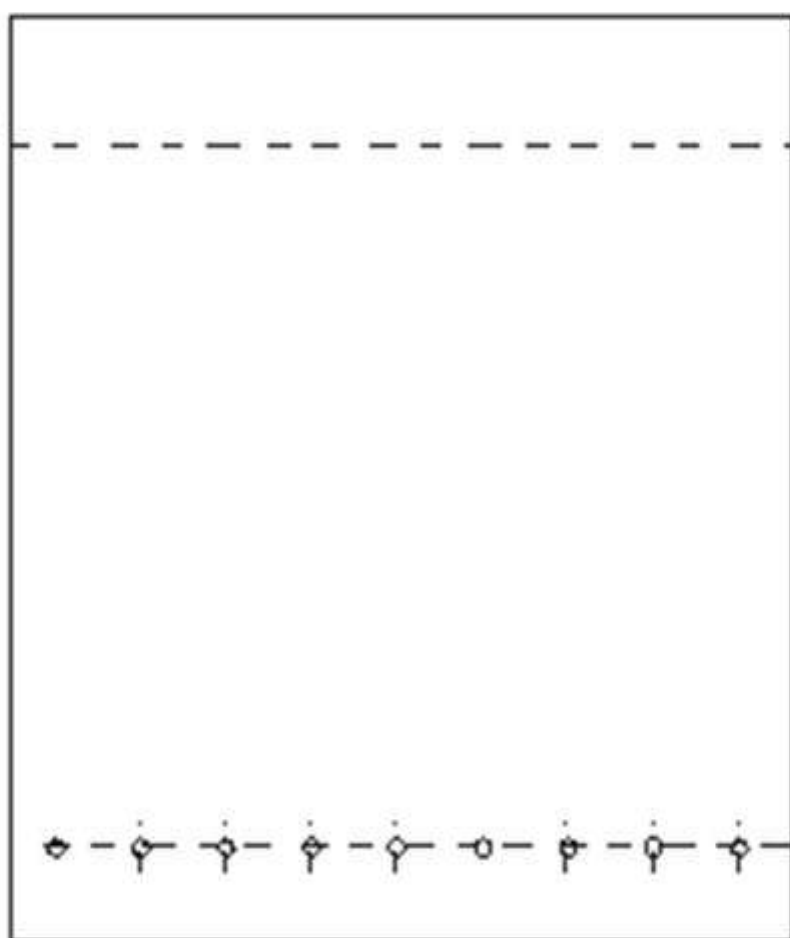
*Development of the TLC plates*

1. Prepare a developing chamber by adding about 0.5 cm of developing solution to the chamber. Carefully tilt the chamber so the filter paper gets saturated with the solvent.
2. Place the TLC plate in the chamber so that your spots are aligned along the bottom and so the plate is not touching the sides of the chamber. The plate should also be facing *but not touching* the piece of filter paper. Allow the solvent to rise to within one cm of the top of the plate (5-10 min). **WATCH**—don't let the solvent rise to the top of the plate!  
Remove the TLC plate and immediately mark where the solvent stopped rising with a pencil. This is called the solvent front. Allow the TLC plate to dry.
3. Visualize the spots by illumination under the UV lamp.
4. Mark the center of each spot with a pencil and then measure the distance traveled by each component. Using the TLC plate diagram below, draw what your plate looks like. In the table on the following page record the distances from the origin (original location of the spot) to the solvent front and the distance from the origin to the center of each spot.

*Clean-up*

1. Thoroughly rinse and dry the mortar, pestle, and spatula.
2. Dispose of all liquids as instructed.
3. Wash your hands with soap and water.

solvent  
front



1 2 3 4 5 6 7 8 9

Name of Medication

This image shows a blank sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins or other markings on the paper.