

## Organic Chemistry Laboratory

### #7 - Thin-Layer Chromatography

This experiment will demonstrate the technique of chromatography to separate two or more compounds in a mixture. Chromatography is a very valuable technique for organic chemists and can be used for qualitative analysis of a mixture or the purification of compounds from a reaction mixture. Chromatography also has many everyday applications of chemistry including medicine and forensics.

Among the many varied techniques of chromatography there are three that are used frequently in an organic lab: column chromatography, thin-layer chromatography (TLC), and gas chromatography. Column chromatography is used to separate mixtures of compounds into the individual components and can be performed on samples ranging from milligrams to 20 or more grams. TLC is used to detect the presence of compounds and their identity. Gas chromatography can be used in either capacity but on very small scale, from nanograms to milligrams.

All types of chromatography work in essentially the same way. There is a stationary phase and a mobile phase. The stationary phase is usually made up of one of several compounds including  $\text{SiO}_2$ ,  $\text{AlO}_3$ , cellulose or waxy hydrocarbons. The mobile phase can be any combination of solvents or gas (usually helium). The stationary phase and solvent system chosen will depend greatly on the nature of the compounds to be separated.

In today's lab you will be using TLC to separate and identify an unknown analgesic (pain killer) by comparing it to known samples. You will be using prepared TLC plates that have a UV indicator mixed in with the silica gel ( $\text{SiO}_2$ ). You will also have an opportunity to experiment with different solvent systems to see what effect they have on the separation of the analgesics. Carefully read pages 188 - 198 of your lab textbook for information regarding the technique of TLC.

The analgesic you will be given has been found in the hand of an unconscious person. You need to properly identify the compound so that the right medical measures will be taken to save this person's life. Each analgesic attacks different parts of the body so that one will affect the kidneys while another will affect the liver, or nerves. Therefore the right procedure must be done according to the analgesic, if not the person's life will be at stake.

#### **Pre-lab Reading:**

Read Chapters 27 & 28 of the Zubrick text. You will not be making your own TLC plates during this experiment.

#### **Pre-lab Assignment:**

Look up the toxicities of the analgesics and caffeine that you are using today. What is the target organ for each compound?

**Procedure:**

Obtain an unknown analgesic sample and record the number in your notebook. Take five small test tubes and label them for each known; aspirin (asp), acetaminophen (act), caffeine (caf), and ibuprofen (ibu) and the unknown. Transfer a small amount (~5 mg) of each compound to the appropriate test tube. Add a small amount of methanol to each test tube to dissolve the sample and then spot a TLC plate as instructed in the lab text. If you are careful when spotting the TLC plates you will be able to spot all five samples on a single plate.

Prepare a developing chamber using the solvent system supplied and develop your TLC plate. Make certain that the methanol has completely evaporated from the TLC plate before placing in the chamber.

When the TLC plate has been developed, remove it from the chamber and using a pencil mark the plate to indicate where the solvent front is located. Allow the solvent to evaporate from the plate and use a UV lamp to find the compounds on the plate. Use a pencil to circle each compound on the plate and draw a picture of your developed plate in your notebook. Determine the  $R_f$  values of each compound and record this information in your notebook. If you cannot clearly identify your unknown, you will have to repeat the procedure with a fresh TLC plate.

Obtain a fresh TLC plate and spot the compounds on the plate and set aside to dry. Empty your developing chamber and replace the filter paper. Use one of the other solvent systems available and develop the when dry. Again use the UV light to locate the different compounds on the plate. Compare this plate with the first plate and record your observations.

Obtain one more TLC plate and repeat the above procedure but use the other remaining solvent system. Compare this plate with the other two and record your observation.

**Report:**

Report the  $R_f$  values of each compound and the unknown. Identify the unknown.

**Questions:**

1. Define the following terms; a.) eluent, b.) adsorbant, c.) reverse-phase chromatography
2. In normal-phase chromatography, which solvent has the greater eluting power, petroleum ether or ethanol?
3. A mixture containing the following compounds was separated by TLC. Which compound would you expect to have the lowest  $R_f$ ? The highest?

