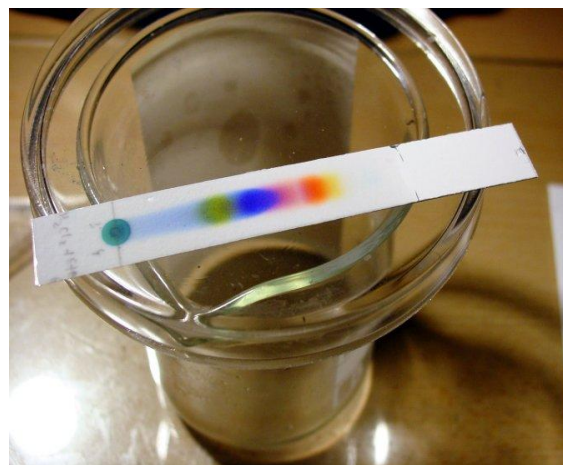
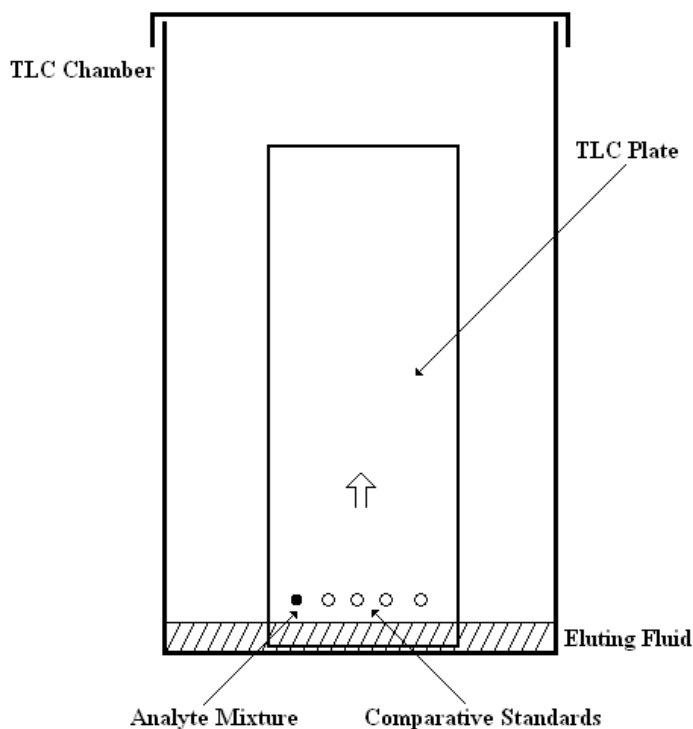


Laboratory Exercise: Chromatographic Separation

As we have discussed, chromatographic separations employ a system with two phases of matter; a mobile phase and a stationary phase. Thin layer chromatography uses a stationary phase that involves a very thin layer of solid silica deposited on a glass or plastic plate with a liquid mobile phase that wicks up the plate carrying the mixture to be separated with it.

In this exercise we will analyze a commercial pain reliever to determine the identity of the active analgesic in the reliever's prescription. We will limit ourselves to non-narcotic analgesics that contain Ibuprofen, Acetaminophen, Aspirin or Caffeine. We will separate the active ingredient of the analgesic from the binders holding the tablet together using Thin Layer Chromatography. Then, since the distance the analgesic runs along the chromatogram depends on the chemical properties of the analgesic, the distance it runs can be compared with results for standards (above list of compounds) run on the same chromatogram in order to identify it.



TLC Chromatogram of Black Ink

We will dissolve the analgesic tablet into an appropriate solvent and then apply the resulting liquid solution as a small dot near the bottom of the TLC plate. Something similar will be done for our standards. The chromatogram will then be run by dipping it in an eluting liquid in a TLC chamber. When the elutant has reached to near the top of the plate, the plate will be removed from the eluting chamber and dried. Then, because all the analgesics are whitish in color and

cannot be distinguished from the white silica, it will be visualized under UV light. The silica has been impregnated with a fluorescing compound and so will glow with a greenish color under UV light. Because the compounds run on the chromatogram absorb the light's energy, regions of the silica that contain any of the compounds run will appear as a dark spot under UV light. The spot produced by the analgesic can be compared with those of the standards run simultaneously in order to determine its identity.

Procedure

1. Prepare your chromatography chamber. Obtain a 250 mL beaker and cover it with a piece of Aluminum foil. **In the fume hood**, add eluting solvent to a depth of about 5 mm in the chamber. We will use a solvent composed of Ethyl Acetate:Acetic Acid (95:5). Set the chamber aside while you prepare the chromatography plate.
2. Prepare the TLC plate. We will use Whatman Polyester backed plates coated with Silica Gel to a thickness of 250 μm . These plates have been activated to fluoresce under UV_{254} radiation. Handle the plates only by the edges. Cut a TLC plate to the size indicated by your instructor. Using a pencil, very, very, lightly draw a line about 1 cm from the bottom edge of the plate. Mark off 5 five 1 cm intervals. Lightly label each: Ace, Asp, Caff, Ibu, Unk.

Your instructor will demonstrate how to draw out a melting point capillary tube into a micropipetter. Using a separate micropipetter, spot your unknown analgesic and each standard analgesic onto the TLC plate. Do this by touching the tip of the micropipetter filled with sample to the plate's surface four or five times. It is important the spot be reasonably concentrated and small. Do not allow the tip of the micropipetter to touch the surface for more than a fraction of a second. If it does, it will produce a spot that is too large. And, allow the spot to dry before spotting it again.

Each standard solution has been prepared by dissolving the standard in a 1:1 Ethanol:Dichloromethane mixture.

3. To prepare the unknown analgesic, obtain roughly one-half an analgesic tablet. Prepare a 9 cm pipette with a small cotton plug for filtration. Crush the sample using a pestle in a mortar. Collect the powder in a small beaker or Erlenmeyer flask. Dissolve in 5 mL of 1:1 ethanol/dichloromethane. Pass the sample thru the pipette containing the cotton plug. This will separate any insoluble material from the material soluble in the ethanol/dichloromethane mixture. Collect in a medium test tube. Rinse the beaker or Erlenmeyer flask with another 5 mL of 1:1 ethanol/dichloromethane and pass the solution thru the pipette containing the cotton plug.
3. Develop the chromatogram. Add the TLC plate to the developing chamber. Allow it to run until the solvent front is a reasonable distance (~1 cm) from the top of the plate. Remove the plate from the TLC chamber and allow it to dry. Be sure to mark the position of the solvent front.
4. Observe the plate under a UV lamp. **Do not look directly into the lamp as UV radiation can cause blindness.** Lightly circle each spot with a pencil.
5. Accurately sketch the thin-layer chromatograms in your notebook.

Data Sheet

Obs. of Analgesic Tablet:

Sketch of TLC Chromatogram:

Identity of Analgesic: _____

Name: _____

Date: _____

Signature: _____

Post Lab Questions

1. Separation of the components in the ink of an ink pen via chromatography is a favorite example. (See example) What is the composition of India Ink?
2. Suppose a mixture being separated by TLC produces two spots on the chromatogram. Is it possible the mixture contained three substances? Explain.
3. How far will a compound run along a chromatographic plate if it has a very low solubility in the eluting solvent?