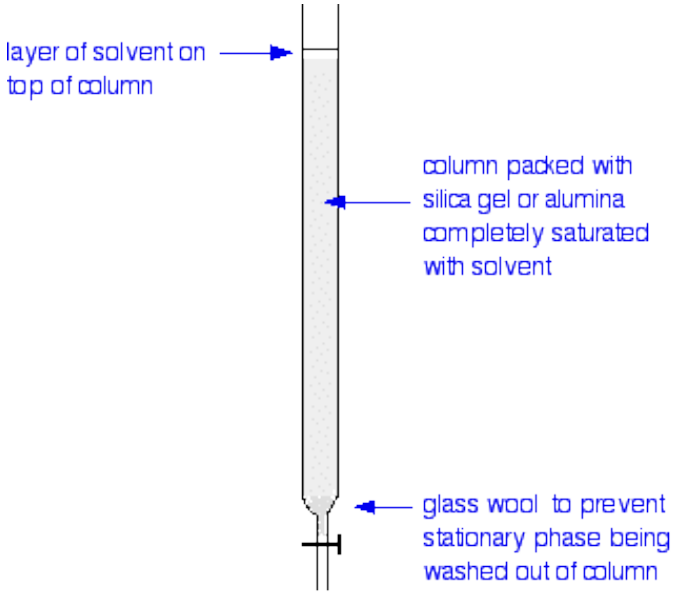
Isolation and Purification of Organic Compounds
Thin Layer Chromatography (TLC)

Chromatography is a term that describes a number of separation methods that rely on the equilibrium distribution of the components of a mixture between a solid or liquid stationary phase and a liquid or gaseous mobile phase. Commonly used chromatographic methods for organic separations are listed.

- **High pressure liquid chromatography (HPLC): components of a mixture are distributed between a stationary liquid phase chemically bonded to 1-5 µm beads packed in a column and a liquid mobile phase pumped under high pressure (10-40 MPa). Reverse phase chromatography in which the stationary phase is non-polar (C_8 to C_{18} linear hydrocarbon chain) and the mobile phase is polar (H_2O/MeOH, H_2O/ACN mixtures) is most common.**
- **Gas-liquid chromatography (GLC or GC): the stationary phase is a liquid film adsorbed on a solid support packed in a column. The mobile phase is an inert carrier gas such as N_2 or Ar. Device is housed in an oven for temperature control. Separation is largely based on vapor pressure differences among components. H-bonding, dipole-dipole interactions, and van der Waals forces between components and the stationary phase also affects the rates at which components are eluted from a GC column. Limited to materials with significant vapor pressure at 30-250 °C.**

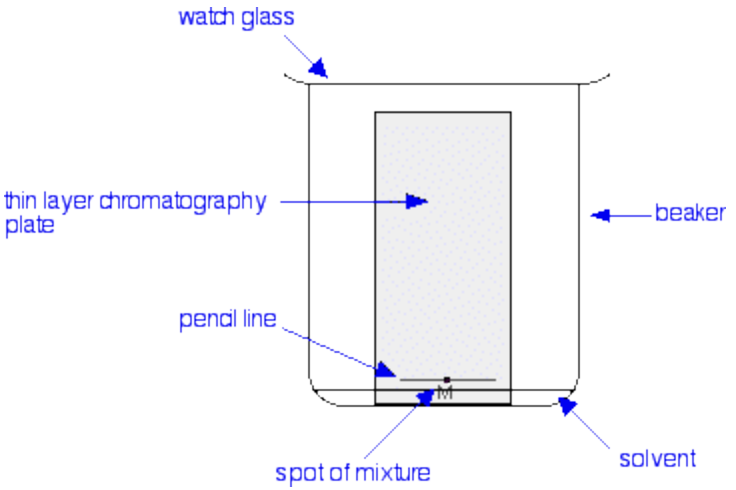
Chromatographic methods continued

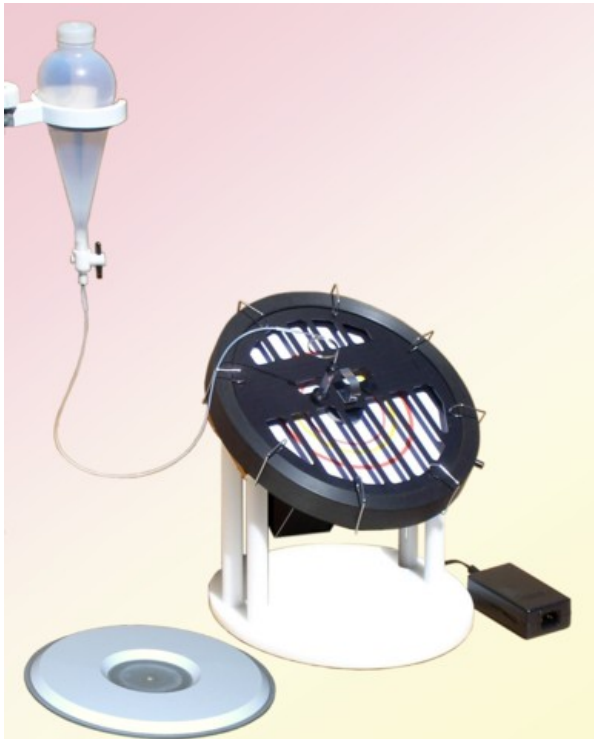
- **Column chromatography: The stationary phase is usually a finely divided (100-300 μm) highly polar silica or alumina bed supported in a glass column. The mobile phase is typically a low to moderately polar organic liquid. Hexanes/EtOAc mixtures are very commonly used because the polarity of these mixtures can be adjusted dramatically by changing the solvent composition. Dichloromethane/acetonitrile is also popular, but more expensive. Flow rates are controlled by gravity, particle size, and a stopcock. Components typically elute in polarity order, least polar compounds eluting first. Fractions are evaporated to obtain components, so the method is limited to components with low vapor pressure and to low boiling mobile phase solvents. A variant of the method called flash chromatography speeds up the process by applying pressure to the top of the column.**



Chromatographic methods continued

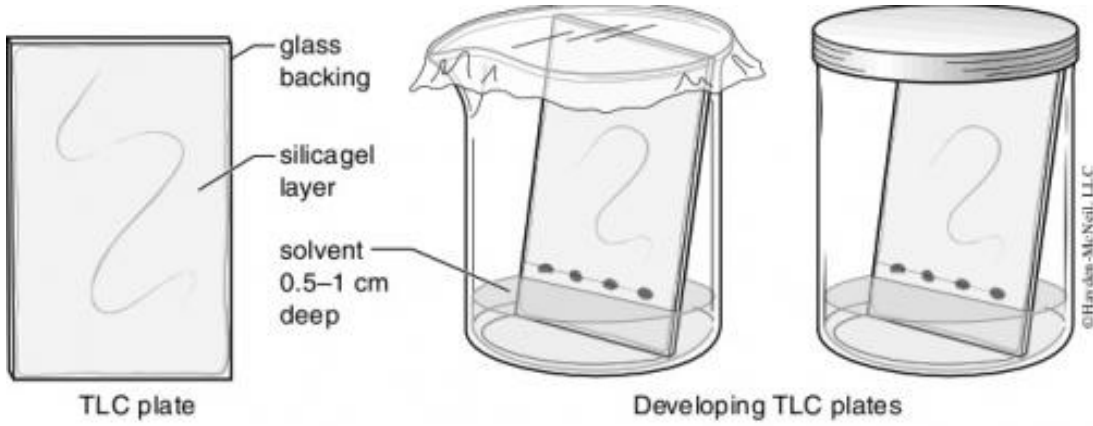
- **Thin layer chromatography (TLC): The stationary phase is a thin layer (ca. 0.25 mm) of finely divided material (< 50 µm) typically composed of silica or alumina with a gypsum binder coated on a glass or plastic plate. Other stationary phases such as cellulose are often used. The mobile phase is an organic solvent or solvent mixture of low to moderate polarity that travels up the plate by capillary action. Solvent mixtures are similar to those used in column chromatography with hexanes/EtOAc being particularly popular. The sample is applied as a ”spot” near the bottom of the plate before development. TLC is typically used as an analytical method to assess purity and to predict the results of a column chromatography separation. Variants, including centrifugal radial thin layer chromatography (right), are used for 100-300 mg scale separations.**

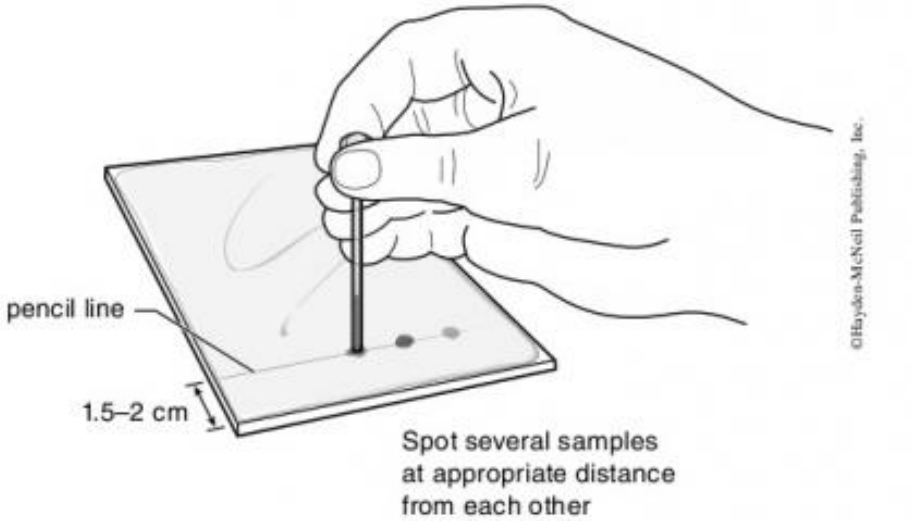




TLC Methodology

This diagram illustrates the method for spotting and developing TLC plates. Draw a pencil line 1.5 cm above the bottom of the plate with a ruler, and apply the samples as solutions in a volatile solvent on the pencil line. Most commercial plates are wide enough to apply at least three spots. The solvent level in the developing chamber must be low enough that the spots are not directly exposed to the solvent. If they are, the spots will dissolve in the solvent and will be lost from the plate.

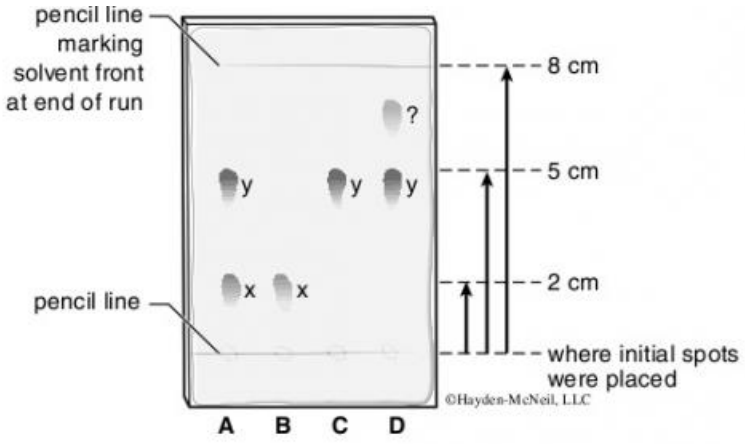




Visualization and analysis

Unless all mixture components absorb visible light, the developed plate must be visualized in an indirect way. The commercial plates that you will use are impregnated with a fluorescent indicator dye that has a light green glow under a compact UV light source. Compounds that absorb UV light or quench the fluorescence appear as dark spots against the green background.

The R_f values, calculated as shown in the diagram on the right, are approximately constant for a given compound developed on a particular type of plate with a constant solvent mixture. These values indicate the relative polarity of the materials (more polar materials have smaller R_f values) and can help to identify compounds.



Developed TLC plate
- A: Standard containing two components x and y
- B: Sample 1 containing x
- C: Sample 2 containing y
- D: Sample 3 containing y and another, unknown compound

Solvent front at 8 cm—

$$R_f \text{ for x} = \frac{2 \text{ cm}}{8 \text{ cm}} = 0.25$$

$$R_f \text{ for y} = \frac{5 \text{ cm}}{8 \text{ cm}} = 0.625$$

This Week's Experiment

The experiment is composed of two parts. In part 1 you will work in groups of four to examine the effects of analyte and solvent polarity in the TLC method. You will work as a group to characterize the TLC behavior, by R_f measurement, of 8 different compounds of variable polarity with two different hexanes/EtOAc solvent mixtures.

In part 2 you will use TLC to examine the crude samples of acetylsalicylic acid, acetaminophen and caffeine that you obtained from the extraction experiment (Expt #2) and the pure samples of acetylsalicylic acid and acetaminophen that you obtained by recrystallization (Expt #3). In this part of the experiment you will have the opportunity to adjust the solvent composition to optimize your results.

Experimental Hints

1. **Do not contaminate the TLC spotters by using them for more than one type of sample.**
2. **Make a sketch in your notebook of each plate that you prepare so that you know the identity of the samples applied to the plate.**
3. **Make sure that the solvent level is below the pencil line that marks the positions of the spots when you immerse your plate in the developing jar.**
4. **Cover the developing jar (beaker) with a watch glass or aluminum foil to minimize evaporation, and make sure that the solvent front is parallel to the surface of the solvent as the plate develops.**
5. **Be sure to mark the position of the solvent front immediately after removing the plate from the developing jar. Solvent evaporates quickly.**
6. **Since the solvent evaporates and can change composition replace the solvent each time you run a TLC experiment even if you are keeping the same solvent mixture. Used solvents should be placed in the solvent waste container in the hood.**