Thin layer chromatography (TLC) is primarily a method for the rapid qualitative analysis of mixtures of organic compounds.

The term **chromatography** is derived from the original use of the method for separating yellow and green plant pigments. Chromatography has since evolved into a very general separation method for many types of mixtures. It is based on selective adsorption of compounds on a solid with high surface area. As the mixture passes over the solid, the components are adsorbed and released from the surface at different rates. They are thus continuously partitioned between the adsorbent and a moving phase, either a vapor or a solution. The process is analogous to separation by fractional distillation or by extraction, in which different compounds are partitioned between liquid and vapor, or between two immiscible liquids, respectively.

Chromatography can be carried out in several ways. In column chromatography, and in thin layer chromatography, a solution of the mixture flows over a solid adsorbent. Separation occurs as molecules are adsorbed and desorbed during passage over the surface. In paper chromatography, the mixture is partitioned between water molecules adsorbed on the paper and a solvent that moves over the paper. In gas-liquid chromatography (GLC), a mixture of volatile compounds is separated by passing the vapor over an adsorbent packing in a long, heated tube.

Chromatographic methods have high resolving power, i.e., they are capable of sharp separations of closely related compounds, particularly when very small samples are used. Both GLC and column chromatography can be carried out with instruments that detect extremely small amounts of compounds in the gas stream or liquid, respectively, as it leaves the chromatographic column. In GLC, the detector responds to the thermal conductivity of the gas stream or the ionization of the gas as it passes through a flame. In liquid (column) chromatography instruments, the detector senses changes in the refractive index of the solution. Signals from the detector corresponding to each component in the mixture and proportional to the amount of the compound are recorded automatically on a chart. These instruments thus provide powerful methods for quantitative analysis.

GENERAL PROCEDURE

TLC is carried out on glass plates or strips of plastic coated on one side with a thin layer of adsorbent. The adsorbent contains a small amount of gypsum ($CaSO_4$) which acts as a binder to give an adherent coating. For routine work, small TLC plates can be prepared by dipping microscope slides in a slurry of adsorbent in chloroform. More uniform plates are obtained by mixing the adsorbent with an equal weight of water, spreading the mixture on a glass plate and allowing it to set and dry. Precoated TLC plates are available commercially with various adsorbents in very uniform layers on a plastic backing.

To "load" the plate, very small samples of the mixture in some volatile solvent are applied as spots near one end and the solvent is allowed to evaporate. The plate is then placed, sample end down, in a closed vessel containing a shallow pool of the developing solvent. The solvent rises on the plate by capillary action, passing over the sample and causing the compounds to move at varying rates depending on their relative affinities for the adsorbent and the solvent. When the solvent has risen to the top of the plate, the plate is removed and the solvent is allowed to evaporate. The zones or spots containing the various components of the mixture are then detected at various points along the plate. If the compounds are colorless, they are made visible by treating the plate with a reagent, such as iodine vapor, that causes color to develop.

IDENTIFICATION BY TLC

The main uses of TLC are for quick observation of the number of compounds present in a sample, and for qualitative detection of a given compound in a mixture. For the latter purpose, a sample of the compound in question is placed in one position at the bottom of the plate and a sample of the mixture is placed in an adjacent position. With a plate 3 to 4 cm wide, it is possible to place samples in as many as three or four "lanes." If identical compounds are present in two or more lanes, they should appear at the same height after

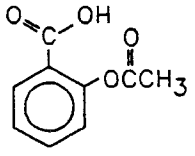
the plate has been developed with solvent. The position of the spot relative to the solvent front, called the R_f value (distance of spot/distance of solvent), depends on the thickness of coating, the amount of sample and the temperature, and may vary from one plate to the next. Comparison of two samples on the same plate is therefore essential.

In addition to the position of the known sample and that of a spot in the mixture, the appearance of the compound on the developed plate may greatly strengthen the identification if it is distinctive after visualization. If there is a reagent specific for the compound of interest, this is sprayed in a fine mist over the surface. A general reagent such as iodine will cause brown or black spots with practically all compounds; these may be slightly different shades, but the color is usually not very characteristic.

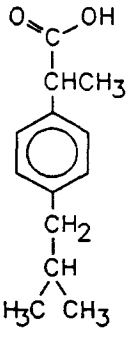
Another method for visualizing spots is illumination of the plate with an ultraviolet lamp. Many substances, particularly aromatic compounds, will show a bright fluorescence which may have a characteristic color. A further possibility is use of an adsorbent layer that contains a trace of a fluorescent dye. Compounds that are fluorescent still show up as bright spots on a light background; any others appear as a dark spot since they quench the fluorescence of the background dye.

ANALGESIC DRUGS

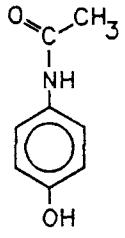
In this experiment, TLC will be used to examine the composition of various analgesic (pain relieving) drugs. The best known of these is aspirin, but several other chemically similar compounds are also used as analgesics. Among these are acetaminophen and ibuprofen. Caffeine is sometimes added to these formulations to overcome drowsiness. In addition to the active ingredients, the tablets of the drugs contain starch, lactose and other substances that act as binders and permit rapid solution, and sometimes also inorganic bases.



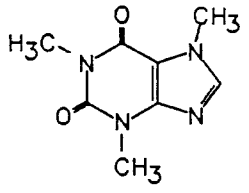
Aspirin



Ibuprofen



Acetaminophen



Caffeine

EXPERIMENT

In this experiment you will obtain as an unknown a proprietary analgesic drug. The objective is to identify the unknown drug by TLC comparison with several known compounds. The unknowns are:

Drug	Ingredients
Anacin	aspirin, caffeine
Excedrin	acetaminophen, aspirin, caffeine
Motrin	ibuprofen
Tylenol	acetaminophen (325 mg)

The known compounds to be used for reference are: aspirin (acetylsalicylic acid), acetaminophen (4-acetamidophenol), caffeine, and ibuprofen [2-(4-isobutylphenyl)propionic acid].

Capillaries for applying the samples can be prepared by heating and drawing out an open-end melting point tube. Soften a 1 cm section in the center of each tube by heating in a low burner flame, remove the tube from the flame and draw it out to a thread-like thickness. Break in the middle of the thin portion to obtain two capillaries.

Label five 10 X 75 test tubes with the following designations: 1-Asp, 2-Ace, 3-Unk, 4-Caf, 5-Ibu. Place 20 to 30 mg of each of the four knowns in tubes 1,2,4, and 5. In tube 3 place 20 to 30 mg of the unknown analgesic, assigned by your Laboratory Instructor.

To the tubes containing the reference compounds, add 0.5 mL of methanol; add 1 mL of methanol to the unknown. The concentrations should be in the range 30 to 60 mg/mL; only a small fraction of the solution will be used, and much small quantities of sample and solvent can be taken. Stir the unknown gently with a stirring rod and allow the insoluble material to settle. Swirl or stir (use a clean rod) the other samples until all or nearly all of the solid is dissolved.

Obtain a 5 X 10 cm piece of fluorescent Silica Gel TLC sheet (handle only by the edge, and do not touch the coated surface). Samples of the five solutions should be spotted on the coated side about 1 cm from one end of the sheet, and about 0.75 cm apart, with the outer two spots about 1 cm from the edge of the sheet. (You should draw a very light pencil line where you spot the samples across the bottom of the sheet.) The samples should be applied in the order of 1 to 5 from left to right, with the unknown in the middle lane. In pencil, under each spot, label the name of the compound, such as Asp, Ace, etc. It is most important to avoid applying too large an amount of sample. The spot after application should be about 1 to 2 mm (1/16 inch) in diameter. It is a good idea first to practice applying spots to a small scrap of sheet.

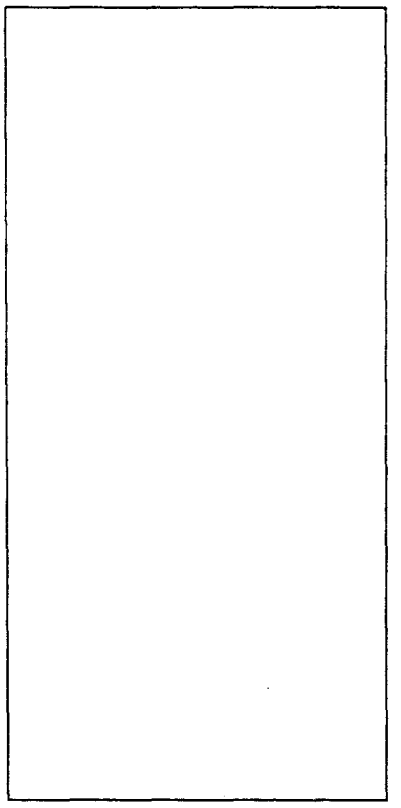
To apply the samples, touch the end of a capillary tube to the solution and then touch this gently to the plate at the proper place. When the five samples have been applied, place the sheet, spotted end down, in a developing jar containing a pool of solvent about 1/2 cm deep. The solvent system used in this experiment is ethyl acetate. Cap the jar securely and develop the chromatogram. About 15 minutes are required for the solvent to rise to within 1 to 2 cm of the top of the sheet. A wick may be added to help saturate the environment. Simple fold a piece of filter paper and stand it inside the jar, making sure it does not touch the TLC sheet.

After development, remove the sheet, and mark the solvent boundary with a small scratch. Recap the jar and allow the sheet to dry. Examine the chromatogram under a UV lamp and sketch in your report the appearance of the plate, indicating the location and approximate size of the spots and any distinctive colors.

Analysis of drugs by thin layer chromatography - Report

Name_____Section_____

1. Appearance of Developed Chromatogram



2. Identification of Compounds in Chromatogram

Identify and label the spots in the chromatogram, including as many of the spots in the unknown lane as possible (mark directly on the sketch).

3. Conclusions

From the number, positions, and appearance of the spots in the unknown lane and the compositions of the possible unknowns, which analgesic was your unknown?

4. Summary

List all the compounds that you can identify from your chromatogram (knowns and compounds identified in the unknown) in order of decreasing R_f value.

QUESTIONS

What would be the result of each of the following errors in TLC technique?

1. Too much sample applied:

2. Polarity of solvent too high:

3. Solvent pool in developing jar too deep: