Gas Chromatography
Experiment 6A: Examination of an Unknown Mixture

Week 3 – September 8 – 14, 2009
From Bell , Clark and Taber, pages 64, 65

1. Choose one of the unknown liquid alcohol mixtures (**Unknown 1 or 2**) for GC analysis and place 5 drops in a small labeled vial.
    a. Each unknown mixture will be composed of two of the following alcohols:
        i. Methanol
        ii. 1-Propanol
        iii. 1-Butanol
        iv. 1-Pentanol
2. Obtain and record in your notebook the retention times (R_t) which have been determined for the pure alcohols (shown above) by the TA's specifically for your class. You can obtain the R_t values for the known alcohols from the gas chromatogram charts displayed in the instrument room. **Be sure that the R_t's you obtain are for the GC machine that you actually use for your analysis.**
3. Also record in your notebook the GC oven temperature, gas flow rate and column used for obtaining the above R_ts.
4. Have a TA inject your unknown sample into the GC.
5. Use the gas chromatography chart of your unknown to deduce which liquids are in your unknown by comparison of R_t's of the known compounds with those of the unknown mixture.
6. Place a copy of your chromatogram in your notebook. Be sure to record oven temperature, gas flow rate and column used.

Thin Layer Chromatography
Experiment 8B: TLC of Analgesic Drugs; Determination of an Unknown

Bell, Clark and Taber, pages 76 - 78

1. In the community hood, there are solutions of 4 reference compounds that may be ingredients in the unknown analgesic preparations. The reference compounds are:
    a. Aspirin (acetylsalicylic acid)
    b. Salicylic acid
    c. Acetaminophen (Tylenol ©)
    d. Caffeine
2. Place 2-3 drops (you don't need much material for your analysis!!) of each of the above reference compounds into a small labeled test tubes or vials and take to your bench.

3. Also in the community hood, there are solutions of 4 unknown commercially available analgesic preparations. The commercially available unknown analgesics include:
    a. Anacin
    b. Aspirin
    c. Tylenol
    d. Vanquish
4. You will be assigned one (**Unknown A, B, C, or D**) of the unknown analgesic preparations for your analysis. Place 2-3 drops of your chosen unknown in a small labeled vial or test tube and take to your bench.
5. READ THIS ENTIRE SECTION BEFORE SPOTTING YOUR TLC PLATE! Using TLC spotting capillaries, spot your unknown analgesic preparation and each of the 4 known reference samples at the bottom of a silica gel TLC plate. A spotting order is suggested as illustrated on the diagram on page 4.
    a. Tips for obtaining a good TLC Chromatogram:
        i. Remember to place the spots on the plate so that they will be above the level of the developing solution when the plate is placed in the TLC chamber.
        ii. Do not place the two end spots too close to the edges of the plate; spots too close to the end of the plate will be distorted.
        iii. Use a clean spotting capillary for each sample.
        iv. Label (lightly, with a pencil, not a pen!!) the location of each of the known and unknown spots at the top of the plate as illustrated on the diagram.
        v. Try to keep the spots small in diameter by lightly touching the spotting capillaries to the TLC plate two to three times in quick brief motions. The tighter and smaller the spots, the better the resolution of the developed plates.
        vi. Before developing the plate, check the spots under short wave UV light to see if the spots are sufficiently visible and are of approximate equal intensity. If any of the spots are very faint, you may re-spot them to increase their intensity.
6. From the back lab bench, obtain one of the large jars with lid and prepare a TLC Developing Chamber as follows:
    a. Insert into the jar one of the pre-cut filter papers (located on the back lab bench) so that the flat edge of the filter paper rests on the jar's bottom; the filter paper should be lining the side of the chamber. See the assembled TLC chamber on the cart in 385 if you have questions on the setup.
    b. Pour 25 - 30 mL of the developing solvent [Ethyl Acetate: Ethanol: Acetic Acid (25:1:1)] into the TLC chamber. When pouring in the solvent, make sure that you pour it over the filter paper so that the paper is completely saturated with the solvent. (The solvent saturated filter paper helps to saturate the TLC chamber with solvent vapors so that the TLC plate will develop faster). There should be enough solvent in the TLC jar to form a pool which is just less than 0.5 cm deep.

7. Insert your prepared spotted TLC plate (spots on the bottom!) containing the unknown and reference compounds into the TLC chamber. Make sure that the TLC plate does not touch the filter paper when you rest it inside the TLC Chamber. Also, be sure that the developing solvent level does not exceed the height of the spots that you applied to the plate.
8. Immediately screw on the lid of the TLC chamber and allow the plate to develop until the solvent front has moved about ½ to ¾ up the plate. This may require 20 minutes or more to occur.
9. Once the solvent front has reached an appropriate level on the TLC plate, remove the plate from the chamber and quickly mark on the plate the height of the solvent front with a pencil.
10. In the hood, allow the solvent to evaporate from the plate.
11. After the solvent has evaporated, use UV light (try both short and long wave length) to examine the chromatogram; lightly mark the outline of the spots with a pencil. Note any visualization differences between long wave and short wave UV light.
12. Sketch the appearance (under both short and long wave UV light) of the plate in your notebook, indicating the approximate retention factor (R_f = Distance traveled by the compound / Distance traveled by the solvent front) values, approximate spot size and any distinct colors.
13. Identify the spots on your chromatogram including as many of the spots of the unknown as possible.
14. Based on the identity and number of spots of your unknown, deduce the identity of the unknown proprietary analgesic preparation by using the **Ingredients Table** on page 4.

How to clean up the TLC chambers when you are finished:

1. Pour the developing solvent from the TLC chamber into the waste container in the hood.
2. Remove the filter paper from the TLC chamber and put in a hood to allow the solvent to evaporate from the paper. Dispose of the dry paper in the trash.
3. Allow the inverted TLC chamber to sit **opened and vented** in the hood to evaporate any remaining solvent.
4. Dispose the small vials in the metal trash containers for glass.
5. Place the dry **opened** TLC chamber and lid in the storage bin for the next class.