ANALYSIS OF DRUGS BY THIN LAYER CHROMATOGRAPHY

EXPERIMENTAL TECHNIQUES REQUIRED

Chromatography (thin-layer) (T 13)

EXPERIMENTAL PROCEDURE (individual work)

- **Avoid exposure of eyes and skin to the UV lamps.**
- **Be careful with the spotting capillaries, they can be sharp**
- **Be careful if making the spotting capillaries, glass heated in a flame is hot**
- **Minimise solvent vapours by keeping the developing jar covered and working in the fumehood**

Label six 1 small test tubes with the following designations: 1-Asp, 2-Ace, 3-Unk, 4-Caf, 5-Ibu, 6-Sal. Place 20 to 30 mg of each of aspirin, acetaminophen, caffeine and salicylic acid in vials 1, 2, 4, and 6 respectively. In test tube 3, place a spatula tip of your *unknown drug* preparation (make sure to record your unknown number in your laboratory notebook). The ibuprofen sample has already been prepared and **does not require further dilution or filtering**.

Obtain 4mL of methanol in a small test tube. To each of the test tubes containing the solid compounds, add 0.5 mL of methanol. The concentrations should be in the range 30 to 60 mg/mL; only a small fraction of the solution will be used, and in principle, much smaller quantities of sample and solvent can be taken. If needed, crush the unknown sample gently with a stirring rod to help it dissolve, and allow the insoluble material (the insoluble "fillers") to settle. Swirl the other samples until all or nearly all of the solid has dissolved.

Obtain a 5 x 10 cm piece of fluorescent Silica Gel TLC plate (handle carefully, by the edge, and do not touch the white coated surface). Prepare the TLC plate based on the thin layer chromatography technique instructions with the pencil origin line on the coated side 1.5 – 2.0 cm from one end of the plate Apply spots of the samples of each of the six solutions on the origin line using a fine capillary. The spots should be about 0.5 cm apart, with the outer two spots about 0.5 cm from the edges of the sheet. The samples should be applied in the order 1 to 6 from left to right, with the unknown (3) in a middle lane. Remember that it is important to avoid applying too large an amount of sample. Check to make sure you can see the spots under a UV lamp. If they are too weak, apply a couple more spots of sample and then check again (if you can't see them before running the plate, there is no way that you'll be able to see them after developing the plate!).

When the six samples have been applied, allow all the spots to dry. While the spots are drying, prepare your developing jar by lining it with a trimmed filter paper (flat edge down) and adding about 0.5 cm deep pool of the developing solvent (ethyl acetate : acetic acid, 10:1). With the lid on, turn the jar to ensure the solvent saturates the filter paper and then stand the jar back upright and make sure the still

enough solvent depth (but not too deep). Keep the lid on the jar at all times unless you are adding or removing a plate from the jar otherwise volatile solvent can evaporate and change the composition of the solvent mixture.

Now use clean forceps to carefully place the TLC plate, spotted (origin) end down, in the developing jar. It is important that the origin line and sample spots are above the initial solvent level. Cap the jar securely and allow the chromatogram to develop. If you watch the plate and you will see the solvent front rise. It typically takes about 5-10 minutes for the solvent to rise to about 1cm from the top of the sheet but it depends on the solvent polarity.

When the solvent front is about 1 cm from the top of the plate (note that closer to the top is better, you made need to shade the jar with you hand to be able to see the solvent front) remove the plate from the jar, and quickly mark the limit of the solvent travel with a small scratch or a pencil line. Remember to recap the developing jar after you've removed the plate. Place the TLC plate horizontally on a paper towel in the fumehood with the coated side up and then allow the sheet to dry for a few minute (you should be able to see the solvent evaporate. Examine the chromatogram under the UV lamp (short wave), circle the spots with a pencil, or use a line is you see "bands". Sketch the appearance of the plate in your report, indicating the location and approximate size of the spots and any distinctive colours. After this examination, place the sheet in a jar of iodine vapour for about 30 seconds, remove and again record the appearance.

Identify and label the spots on the chromatogram, including as many of the spots in the unknown lane as possible. You should remember that in some of the mixtures, certain components are present in very low concentration and so the spots on the plate may be very small. If your sample contains aspirin this may appear as two spots with very similar R_f values due to the presence of salicylic acid.

For each spot record a) the distance traveled from the origin, b) the R_f value and c) the colour of the spot. From the number, positions (Rf values, and appearance of the spots in the unknown lane and the compositions of the "standards", identify your analgesic.

CLEAN UP

- Dispose of the developing solvents in the organic waste container (red tape) in the fume-hood.
- Empty the solution samples into the organic waste container (red tape) in the fume-hood.
- Place the unused samples in the container provided.

REFERENCES

1. Aspirin, H.O.J. Collier, Scientific American, 209, 96 (Nov. 1963)
2. Analysis of APC Tablets, V.T. Lieu, J. Chem. Ed., 48, 478 (1971).
3. TLC of Drugs, R.L. Newman, J. Chem. Ed., 49, 834 (1972).
4. TLC Separation of Common Analgesics, D.F. Roswell and N.M. Zaczek, J. Chem. Ed., 56, 834 (1979).

REPORT

Before writing any Chem 351 laboratory report, we strongly recommend that you review section 8 in the introductory section of the student laboratory manual that discusses how to write reports and/or from "writing reports" on the course website. Students often don't get the grades they would like because they make errors that are addressed in that section of the manual. These are avoidable errors.

The report for this experiment is to be completed in the 2 page template WORD provided. Remember that more it not necessarily better. It is important to be accurate and concise rather than verbose and vague. Proper English should be used and it should be written in your own words.