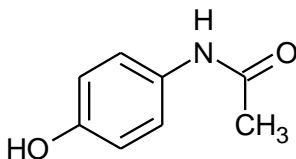# TLC Analysis of Analgesics

Introduction

Thin Layer Chromatography (TLC) is a very common and versatile analysis technique. TLC can be used to check the progress of a reaction, to determine the purity of a compound and even aid in the identification of a compound or mixture. In this experiment you will use TLC to identify the active ingredients in some commercial analgesics. The commercial analgesics will be composed of one or more of the following ingredients:
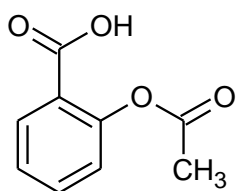
Acetylsalicylic Acid   Acetaminophen   Caffeine
(Aspirin)
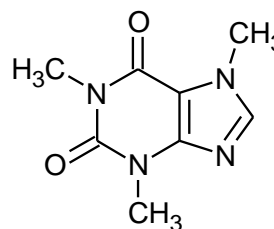
Required Reading:

Padías:

   Chromatography, general (pp. 162 – 163)

   Thin-Layer Chromatography (pp. 165 – 172)

Special Safety Notes:

   Spotting capillaries are made of very fine glass and should be handled with care and disposed of in the glass bins.

Procedure:

Preparation of the TLC Plates

(NOTE: Handle TLC plates by the edges only!)

Obtain two (2) 5 cm x 5 cm TLC plates; one will be your reference plate and one will be your sample plate. USE A PENCIL TO MAKE THE FOLLOWING MARKINGS ON EACH PLATE. Do not push down on the pencil; simply drag it across the plate to make a faint line. On each plate draw a line 0.5 cm from the bottom of the

plate (this line is called the "origin"). Draw another line 0.5 cm from the top of the plate (this line will be the "solvent front"). On the bottom line of each plate (the "origin"), starting 1 cm from the side, make 4 tick marks at 1 cm intervals. On the reference plate, label four "lanes" as shown below to designate where each standard as well as the reference mixture will be spotted. On the sample plate, label four "lanes" as shown below to designate where each commercial analgesic as well as the reference mixture will be spotted.

Reference Plate   Sample Plate
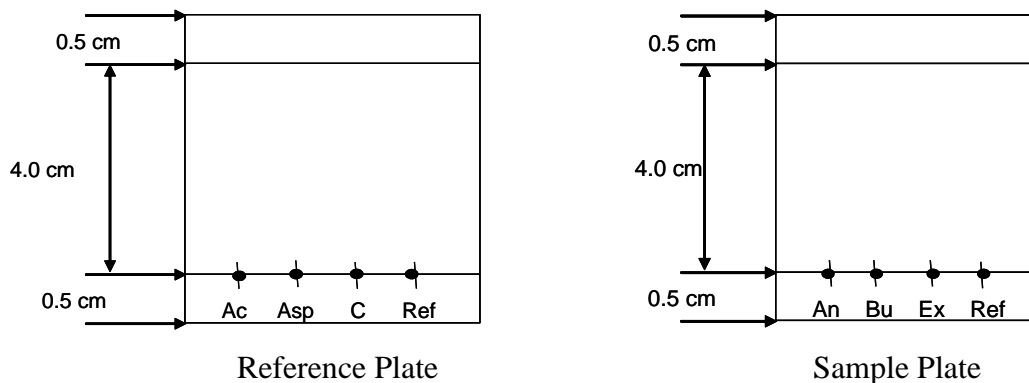
Spotting and Developing the Reference Plate

Using a 1 μL micropipet, spot 1 μL each of the individual reference solutions and the reference mixture in the proper "lanes" at the origin on the reference plate. Using your tweezers, grasp the plate at the top and wave it back and forth in the air to assist in the evaporation of the spotting solvent. Prepare the TLC developing chamber by adding 4 mL of ethyl acetate to a 400 mL beaker and cover with a watch glass. To place your reference plate in the developing solvent (eluent), remove the watch glass, and while grasping the plate at the top using tweezers, slowly lower the reference plate into the chamber so it stands more or less vertically in the chamber. Replace the watch glass to prevent solvent evaporation. Allow the eluent to rise up the plate by capillary attraction until the solvent reaches the "solvent front" line at the top of the plate. Remove the plate from the chamber using tweezers and wave it back and forth in the air to assist in the evaporation of the developing solvent from the plate. Examine the plate under UV light (254 nm) and GENTLY circle each spot with a pencil.

Analysis of the Commercial Analgesics

        Crush ¼ tablet of each commercial analgesic, and place the crushed tablet into a **clean**, **dry** 3-mL or 5-mL conical vial. Add methanol until the level is slightly above the 2.0 mL mark on the vial (anywhere between the 2.0 and 3.0 mL marks should be fine). Cap the conical vials and shake each one (shake it hard!) for 1 min. Allow each mixture to settle for about a minute (the mixture may remain cloudy, even after this settling period). Using a 1 µL micropipet, spot 1 µL each of the individual analgesic solutions and the reference mixture in the proper "lanes" at the origin on the sample plate, allow the spotting solvent to evaporate, then elute the plate using ethyl acetate as the developing solvent as described above. Allow the eluent to evaporate as described above and examine the plate under UV light (254 nm) and GENTLY circle each spot with a pencil.

Writing Your Report:

        Draw each TLC plate, label each lane, and label all spots. Calculate and report R_f values for each standard, as well as for each component in each commercial analgesic (remember to show your calculations). Identify the components in each analgesic and explain your reasoning.

Questions:

1. What would be the result of using a very polar solvent like methanol (instead of ethyl acetate) to develop your TLC plates?

2. A mixture of p-methylphenol and 4-methylcyclohexanol was run on a TLC plate using 3:1 hexane:ethyl acetate as the eluent. Only one spot was seen using a UV lamp. Does this mean that both spots have the same R_f or is there another explanation? How might you solve this problem?

3. A TLC analysis of a sample composed of *o*-xylene (1,2-dimethylbenzene), *o*-toluic acid (2-methylbenzoic acid), and *o*-cresol (2-methylphenol, or 1-hydroxy-2-methylbenzene) gave the plate below. Calculate the R_f values and match the three compounds to the three spots. Explain your reasoning.

Origin   Solvent
                                         front
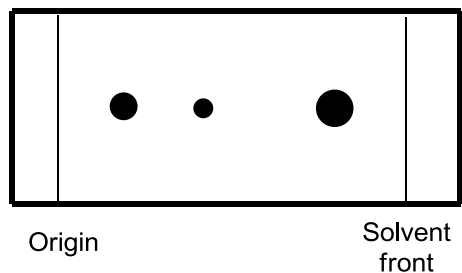
References

Pavia, D. L.; Lampman, G. M.; Kriz, G. S.; Engel, R. G. *Introduction to Organic Laboratory Techniques, A Microscale Approach;* 3rd ed.; Brooks/Cole: Pacific Grove, CA, 1999; pp. 115 – 119.