

DRUG IDENTIFICATION BY T.L.C.

LAB FORENSICS.1

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INTRODUCTION

In This activity, you will identify a drug. Drug identification is a routine procedure for a medical technician working in a hospital trying to identify the source of drug overdose, or the law enforcement investigator trying to identify a suspected controlled drug, or for the medicinal chemist employed by a pharmaceutical manufacturer trying to discover why a competitor's product is more effective.

Drugs can be identified in a varied of ways: characteristic colors when treated with special reagents, formation of crystalline solids with characteristic color and crystal structure when treated with appropriate reagents, or thin layer chromatography (TLC). In TLC, a drug is identified by its R_f value and by its color when treated with various reagents or when observed under ultraviolet light.

PURPOSE

To determine the composition of several over-the-counter (OTC) drug formulations using TLC. The drugs to be tested are aspirin, acetaminophen, caffeine, and salicylamide.

SAFETY

1. Wear protective goggles throughout the laboratory activity.
2. Ethyl acetate and iodine vapors can be irritating.
3. Do not look at the ultraviolet light.

MATERIALS

4-Small bottles (25-mL size) containing the "known" compounds dissolved in (50:50 v/v methylene chloride and absolute ethanol).	4-Hydroxyacetanilide (acetaminophen) (1 g per 20 mL solvent)
Caffeine (1 g per 20 mL solvent)	Ibuprofen (1 g per 20 mL solvent)
Acetylsalicylic acid (0.5 g per 20 mL solvent)	1-Small bottle, 20 mL of a combined standard reference mixture of the four compounds at the same concentration. (1 g of each of the above in 20 mL of solvent. Stir to dissolve.)
Methylene chloride, 100 mL Dichloromethane	
Ethyl acetate, 100 mL (TLC Solvent)	Thin-wall. open-end capillary tubing (1mm) for micropipets; alternative, use toothpicks
Filter paper, 12 squares for liners	24-Wide mouth, clear screw cap jars, 16 oz., or 600-mL beaker with aluminum foil cover
UV light box with UVA, UV B, UV C	

PROCEDURE

1. Obtain two precut (10 x 6.6 cm) TLC sheets. Do not touch the white surface and handle carefully only by the edges.
2. Using a lead pencil draw a *light* line across the shorter dimension 1 cm from the bottom. Using a ruler as guide on the light line, mark off five equally spaced intervals on the line as shown in Figure 1.

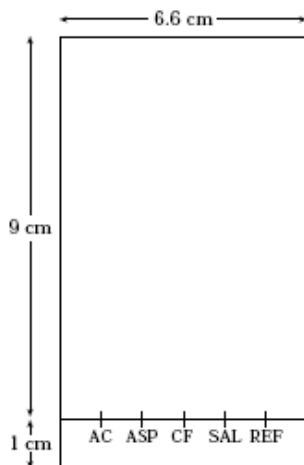


Figure 1. TLC plate.

3. Spot the sheet at each mark with the appropriate known solution: AC = Acetaminophen, AS = Aspirin, CF = Caffeine, SA = Salicylamide, and RF = Reference (Figure 2). These known solutions can be found in small reagent bottles. Using a melting point capillary tube or a wooden toothpick to spot the plate by dipping the tip of the tube (or toothpick) in the known solution and then touching the tip against the sheet at the appropriate mark. The applied spot should be no bigger 1-2 mm. (Practice spotting on a piece of filter paper before spotting the

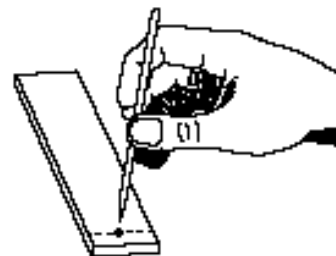


Figure 2. Spotting the TLC plate.

TLC sheet.)

4. Obtain a one-pint (16 oz) canning jar with a screw-cap lid for the development chamber. It should contain a filter paper liner as shown in Figure 3. Add ethyl acetate to the jar to a depth of about 0.5 cm and swirl to moisten the paper liner.

(*REMEMBER*: The level of the solvent must be below the spots on the sheet – 5 mL solvent is usually enough). Place the spotted sheet in the development chamber so that it rests in the solvent and against the jar wall. Replace the jar's lid and allow the sheet to develop (about 20 min).

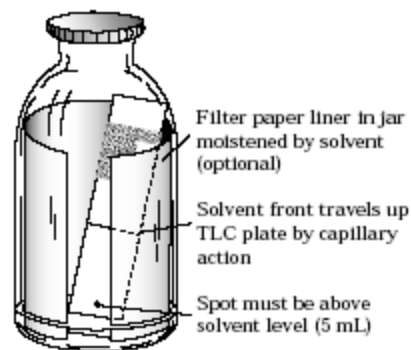


Figure 3. Development jar.

5. When the solvent has risen on the sheet to within 1 cm of the top, remove the sheet from the jar. Using a lead pencil, mark the position of the solvent front.
6. Allow the sheet to air dry and then observe it under a short-wavelength ultraviolet lamp. Lightly mark any observable spots with a pencil and note the color each spot.
7. Using a millimeter ruler, measure the distance that each spot (use center of spot for consistency) has traveled relative to the solvent front. Calculate its R_f value.

$$R_f = \frac{\text{Distance Traveled by substance}}{\text{Distance traveled by solvent front}}$$

8. Obtain one-half tablet of one of the following: Anacin™, Bufferin™, B.C. Powder™, Excedrin™, Tylenol™, generic aspirin, generic acetaminophen, or an OTC analgesic of your choice. Crush the tablet on a sheet of paper with a spatula. Transfer the crushed tablet to a small test-tube and add 3 mL each of absolute ethanol and methylene chloride [*CAUTION: Avoid breathing methylene chloride. It is toxic and can have a narcotic effect in high concentration.*]. Using a stirring rod dissolve as much of the table as possible. It will not completely dissolve.
9. Spot a TLC sheet with the reference solution and unknown solution from Step 9.
10. Develop the spotted sheet in the jar as before (Steps 4 and 5).
11. Observe the sheet under ultraviolet light and mark visible spots (Step 6).
12. Observe the sheet in the iodine jar and record visible spots (Step 7).
13. Calculate R_f values for any spots (Step 8).
14. Identify the components of your unknown analgesic.

15. Thoroughly wash your hands before leaving the laboratory.

DATA ANALYSIS

1. Can ultraviolet light be used to distinguish the substances from one another?
2. Can iodine be used to distinguish the substances from one another?
3. Can the R_f value be used to distinguish one substance from another?
4. Is it important that the sheets be examined under ultraviolet light before the iodine visualization?
5. Why do some substances move along the TLC plat at a rate different from other substances?