

DRUG ANALYSIS BY THIN LAYER CHROMATOGRAPHY

INTRODUCTION:

Over-the-counter (OTC) analgesics typically contain one or more of the following active ingredients: acetaminophen, aspirin, caffeine, and ibuprofen. In this experiment, thin layer chromatography (TLC) will be used to identify the active ingredients in some of these products.

PURPOSE:

The purpose of this experiment is to determine the active ingredient(s) in selected over-the-counter analgesics.

EQUIPMENT/MATERIALS:

Analgesic tablets	Ibuprofen
Mortar and pestle	Acetaminophen
Metric ruler	Acetylsalicylic acid
Capillary tubes	Caffeine
Developing chambers	Ethanol
Pasteur pipettes	Dichloro methane: 95% Ethanol (chromatography solvent) (50/50)
Small test tubes and rack	TLC plates
UV light box-UVC	

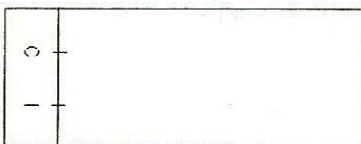
PROCEDURE:

Preparation of sample

1. Using a mortar and pestle, crush approximately $\frac{1}{4}$ of an analgesic tablet.
2. Transfer the crushed tablet to a small test tube and add 1 mL of ethanol.
3. Vigorously shake the test tube for 1 minute.
4. Place the test tube in a test tube rack and allow the insoluble material settle to the bottom.

Spotting of the sample

1. Draw a line in PENCIL ~ 1 cm from the bottom of a TLC plate. Draw lightly on the flaky side of the plate – do not scrape off the coating. Draw 2 tic marks on the line (~1cm) from each other. See the diagram below.



TLC of Analgesics

2. Immerse the pointed end of a capillary tube into the test tube containing the sample until some of the sample is drawn into the tube. Wipe outside dry.
3. Very gently press the small end of the tube onto the plate at one of the tic marks. Keep the spots on the TLC plate small and concentrated by applying the sample at least 3 times and allowing the spot to dry between applications.
4. Visualize your spots using the UV light. The spots should not be bigger than the one shown here.

Ideal spot size: ·

5. Repeat steps 2- 4 for each of the standards available.

Notes: More than 2 samples may be applied to a plate if they are kept 1 cm apart. Mark the position of the spots lightly in PENCIL, and be sure to keep a record of what sample or standard each spot represents! Use letters from data tables- write below spot in pencil.

Development of the TLC plates

1. Prepare a developing chamber by pouring enough solvent (ethyl acetate/hexanes/acetic acid (60/39/1)) into the jar to reach a height of ~ 0.5 cm above the bottom of the jar. ~10ml.
2. Place the spotted TLC plate in the chamber spot side down. The pencil line near the bottom of the plate should not touch the solvent in the jar.
3. Tightly screw on the top of the chamber and allow the solvent to rise to within 1 cm of the top of the plate.
4. Remove the plate,* mark the solvent front with a pencil, and allow it to dry.
5. Visualize the spots by illumination under a UV lamp. Trace around each spot that appears on the plate with a pencil.
6. Measure the distance in centimeters from the line on the bottom of the plate (starting line) to the solvent front. Record this distance on your Data Sheet.
7. Measure the distance in centimeters from the line on the bottom of the plate (starting line) to the center of each spot. Record these distances on your Data Sheet.
8. Calculate the R_f of each spot by dividing the distance traveled by the spot by the distance traveled by the solvent front. The R_f value is characteristic of each substance and may be used for identification of the substance.
9. Determine what active ingredients are present in the OTC analgesics by comparing the R_f values obtained for ibuprofen, acetaminophen, acetylsalicylic acid, and caffeine to the R_f values obtained for the analgesics.
10. Combine class data for the R_f values of the 4 standards and obtain a mean R_f for each standard.
11. Calculate the %error in your unknown R_f compared to the mean standard R_f value.

Name _____
Name _____
Date _____
Period _____

THIN LAYER CHROMATOGRAPHY OF ANALGESICS

Draw or attach your TLC plate(s) here.

Standards	Distance traveled by spot, cm	Distance traveled by solvent front, cm	R _f	
				Mean
a.				
b.				
c.				
d.				
e.				

Analgesic Spot #	Distance traveled by spot, cm	Distance traveled by solvent front, cm	R_f	Active Ingredient	% Error
1.					
2.					
3.					
4.					

12. What error do you observe in your data and what are the probable causes?

13. How can the controls procedure be improved?