OBJECTIVES

Upon completion of this exercise, appropriate discussion and related readings, the student will be able to:

1. Understand and perform one-dimensional TLC.
2. Stain a thin layer chromatograph.
3. Calculate relative mobilities (Rf).

PRINCIPLE

Thin layer chromatography (TLC) is used in specialty areas of the clinical laboratory. Tests include 1) screening for drug overdoses, 2) screening for aminoacidurias, 3) determination of L/S ratios, and 4) screening for galactosuria or fructosuria. In the present exercise, we are interested in detection of the drug pseudoephedrine, a CNS stimulant used as a nasal decongestant.

Drugs can be separated by silica TLC. The mobility of each drug is a function of its relative affinity for each of the two phases (the solid support and the mobile solvent) which is related to the chemical nature of each drug. In general, the more polar the drug the more mobile it will be in a polar solvent. The mobile phase is manipulated by changing the polarity of its components and the pH. Changing the pH will, in turn, change the mobility of each drug since organic acid groups become more ionized while amino groups become less ionized as pH is increased.

MATERIALS

- Urine unknown (spiked with Drug standard a single drug)
- Silica gel plates
- Solvent prepared fresh (60 F-254, EM for each exercise Science)
- Applicator capillary tubes TLC developing solution: ethylacetate:methanol:NH₄OH, 85:10:5, V/V
- Hair dryer
- Developing tank capable of holding 20 x 20 glass plates

GLOSSARY

**Application point** - the place on the thin layer plate (or other stationary phase) where the sample is applied.

**Developing chamber** - the chromatography vessel in which the thin layer plate is placed until the solvent front reaches a desired place (is “developed”).

**V/V** - an abbreviation for the ratio of volumes of solvents or solutions used to prepare a solution.
PROCEDURE

1. Obtain an unknown and a standard from your instructor.

2. Obtain a silica plate that has been “preconditioned” by heating at 70°C for one hour.

3. Prepare solvent chambers by placing 100 mL of the chromatography solvent in the bottom of the developing tank that has been lined with sheets of filter paper.

4. Draw a line with a pencil 2 cm from the bottom of each chromatographic plate; label sample application positions as control, standard, or sample. Draw a line 15 cm from the application line.

5. Apply a small amount (3 \( \mu \text{L} \)) of standard, control, or sample to the silica gel at each application point. Do not allow the wet area to exceed 5 mm in diameter. Dry the sample with the hair dryer. Repeat the application and drying steps. Apply a total of 20-30 \( \mu \text{L} \) of each sample (diameter \( \leq 5 \text{ mm} \)).

6. Transfer the plate, application end at bottom, to the developing tank and allow the solvent to migrate to the 15 cm line marked on the plate (approximately 45 mm.).

7. Remove the plate from the chamber and lay it face-up on a paper towel to air dry. Mark the end of the solvent front with a pencil before the plate dries. A gentle flow of warm air from the hair dryer directed against the plate will increase the rate of drying.

8. Spray the dried chromatogram ninhydrin solution. Blot the strip face up onto a paper towel to drain excess solution. Apply heat, using the hair dryer, to dry.

9. Outline the spots with a pencil, and record the color and appearance on the data sheet.

10. Record the distance the solvent front and the pseudoephedrine migrated (in mm) on the data sheet.

DATA SHEET, EXERCISE 27
NAME: ___________
DATE: ___________

RESULTS

Chromatography time: ______________ minutes.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>Color</th>
<th>Distance Migrated (mm)</th>
<th>( R_f )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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<tr>
<td>2</td>
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<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
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</tr>
</tbody>
</table>
The unknown drug is: ______________________________

CALCULATIONS

Calculate the Rf of drug and unknown using the formula:

\[ R_{f} = \frac{\text{distance spot migrated (mm)}}{\text{Distance solvent front migrated (mm)}} \]

Discussion Questions

1. If the solvent front migrated only half-way up the strip rather than 2/3 of the way, would the Rf values change?
2. If the solvent front migrated only half-way up the strip rather than 2/3 of the way, how would the resolution of the drug be affected?
3. What is the advantage to keeping the application spot very small? What would happen to the Rf values and resolution if the application spots were much larger?
4. If you detected the drug pseudoephedrine by a TLC drug screen, would you report the result as positive?

Attach photocopy of your TLC plate here.