

Exp 1. Thin-Layer Chromatographic Analysis of Drug Components (Exp 15 in Lehman)*Equipment in the drawer*

150ml beaker for TLC chamber
10ml graduated cylinder
test tube for the unknown sample
test tube rack

Equipment from the stock-keeper

Aluminum foil
6cm (width) by 9cm (height) TLC paper
Sample of the unknown
TLC spotting tube for the unknown

*Standards on the bench in the lab**Solvents and Reagents in the fume-hood*

200:1 ethyl acetate/acetic acid
1:1 ethanol/dichloromethane

Waste Container in the fume-hood

Preparation of the developing chamber and unknown

In a 150ml beaker measure 10ml of the developing solvent which is ethyl acetate/acetic acid (200:1). All the chemicals are in the fumehood. Read the labels carefully.

Cover the beaker with aluminum foil to allow the solvent vapours to fill the chamber.

Clean and dry a test tube. From the stock-keeper obtain a sample of your unknown.

Add 2.5ml of 1:1 ethanol/dichloromethane and mix with the solid until dissolved. Place the test tube in the test tube rack.

Prepare the TLC

Obtain a TLC paper from the stock-keeper. The size is approximately 6cm (width) by 9cm (height). Use the 6cm side to spot the standards and the unknown.

Put your TLC paper against the solvent chamber containing the solvent and mark the height of the solvent in the beaker on the TLC with a pencil. Draw a horizontal line 3mm above the marked sign on the dry TLC paper.

On the line at a distance of approximately 1.2cm from the edge start spotting, keeping the same distance between the spots, approximately 1cm.

Spotting

Make sure you wear gloves and that they are clean. The vials containing the standards are already displayed on the bench in the lab. There are two sets of standards for your convenience. Each standard vial contains a capillary TLC spotting tube. **Keep each spotting tube in its place/vial to avoid contamination.** Obtain from the stock-keeper a capillary TLC spotting tube for your unknown.

Take the capillary tube out of the vial and spot gently on the TLC paper making sure that you do not put too much liquid.

Note: Practice spotting on a Kimwipe in order to obtain uniform and small spots from the TLC spotting capillary. When ready spot your TLC plate. Spot three times, giving enough time to the spots to dry between the 3 sets of spotting.

To ensure this, look at the level of the liquid in the capillary tube. If too much liquid travelled up the tube, tap the tube on a clean Kimwipe to aspire some of the liquid out and then proceed to spotting on the plate.

Developing the TLC plate

Carefully place the TLC in the developing chamber, in the most upright position as possible to encourage the front of the solvent to travel all at once with the same rate along the TLC paper.

Observe the solvent front travelling up the plate and when the front is about 1 cm from the end of the TLC paper remove the TLC from the solvent chamber. Mark the front quickly with the pencil before it dries. When the TLC paper is completely dry put it under the UV lamp (254nm) and circle the spots with pencil by marking the center of the greatest intensity and the shape of each spot

Calculate R_f value for each spot and identify your unknown by comparison with the R_f values of the known spots.