Chromatography
Marking Guide

Procedure

Optimum Solvent is CH_2Cl_2:hexanes 6:4.

Place a piece of cotton in the bottom of the column (Figure 4). The cotton should be just tight enough to hold the sand in position without affecting the flow of the column.

The cotton holds in the sand

Add approximately 1 cm of sand to the top of the cotton.

Sand holds in the silica

Add some of your solvent to the column and gently tap the burette (using your hand or a cork ring only) while draining a small amount of solvent to remove any air bubbles from the sand. Close the stopcock and add solvent as necessary until there is approximately 10 cm of solvent above the sand.

Draining column packs sand and removes air bubbles. These bubbles degrade separations because they create channels for the mobile phase thereby avoiding the stationary phase.

Having 10 cm of solvent over the sand prevents the sand from being disturbed when the silica slurry is added.

In an Erlenmeyer flask slurry 15 g of silica with your solvent (CAUTION: silica gel is harmful if inhaled. Weigh the silica in the hood and be careful not to spill the dry silica). Slowly pour the slurry of silica into the column being careful not to disturb the sand. Wash the inside of the burette with your solvent and allow solvent to drain from the column to compact the silica. Stop the flow when the silica is compacted (don't let the silica run dry) and if necessary add more solvent so that there is approximately 8 cm of solvent above the silica column.

Adding silica as a slurry removes air bubbles, and protects glass column from any heat generated when solvent and silica are mixed. 8 cm of solvent above packed column protects it from disturbance.

Add 1cm of sand of the column and wash the inside of the burette with solvent. The column is now packed.

Sand provides a convenient way to load column. It also keeps the silica level during loading.

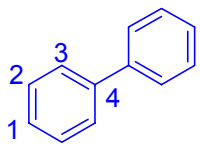
To load the column, drain the solvent until the sand just runs dry but the silica does not. Never let the column run dry! If air bubbles form in the silica poor separation will result. Dissolve your sample in a <u>minimum</u> of solvent. The best solvent for this purpose is the solvent system you will run your column with. If your mixture is not soluble enough in this mixture, use benzene or CH_2Cl_2. Benzene is better as it does not perturb the polarity of the mobile phase. Using a pipette, apply the sample to the top of the column. Rinse any residual sample with a <u>small</u> amount of the solvent you used to load the sample. Your goal during the loading process is to apply the sample, dissolved in solvent, to the top of the column using the smallest volume of solvent possible. For this experiment, 1 or 1.5 mL is a good volume.

Add the sample trying not to disturb too much the polarity of the solvent system. Local increases in solvent polarity will degrade the separation. A narrow sample band is required to ensure good separation of components.

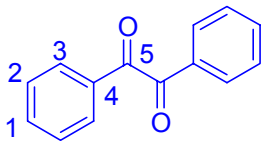
Drain the column until the sand is partly dry but the silica is wet. The sample is now adsorbed to the top of the silica. Add a small amount of solvent (the one you will use to run the column) to the top of the column. The total volume added should be about the same as that used to load the sample. Drain the column until the sand is partly dry but the silica is wet. <u>Carefully</u> fill the column with your solvent. It is important to avoid disturbing the sand layer. Open the stopcock to run the column. When eluting, keep the column full of solvent for a faster run. Collect fractions as you do this. Elute until both compounds are off the column (10 to 15 fractions). Using TLC, you can monitor the progress of the separation. Spot every fraction on a plate. Depending on the size of plate used, you can easily spot 5 to 10 fractions (figure 5). Once the separation is complete combine the fractions containing the same compound together in a round bottom flask and evaporate the solvent using the rotary evaporator. Do not combine mixed fractions unless your separation was poor and you need to re-run the column.

Running the column, testing fractions and combining them provides the pure samples. (2)

Spectra



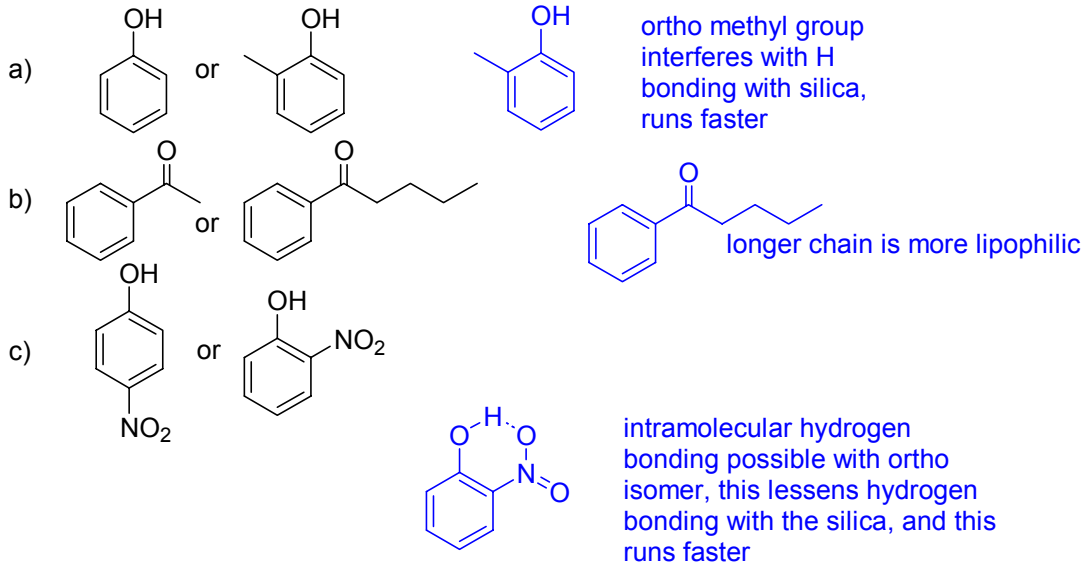
No	^1H	^{13}C
1	7.39 – 7.33 (m, 1H)	127.2
2	7.49 – 7.43 (m, 2H)	128.7
3	7.63 – 7.59 (m, 2H)	127.2
4		141.2



No	^{1}H	^{13}C
1	7.67 - 7.62 (m, 1H)	134.9
2	7.52 – 7.47 (m, 2H)	129.0
3	7.98 – 7.95 (m, 2H)	139.8
4		132.9
5		194.5

Questions

2. Which of the following sets of compounds is likely to have the largest R_f on a typical silica gel TLC? Give a reason for your choice.



3. You are given a mixture of biphenyl, benzyl amide and benzyl amine. The sample is spotted on a TLC plate and eluted. Predict the relative R_f values for the three components.

   Benzyl amine most polar (most hydrogen bonding possible), it will have the smallest R_f. Biphenyl is the least polar and has the highest R_f. Benzyl amide is in between.

4. A yellow compound was placed on the top of a chromatography column and solvent was immediately added to fill the reservoir. The entire volume of solvent in the reservoir turned yellow. No separation was realized during elution of this column. What went wrong? How could this be corrected?

   Compound was not adsorbed onto the silica before solvent was added. Instead of sticking to the column, it dissolved in the eluant. Adding solvent diluted the compound into the whole solvent reservoir. This created a situation in which the yellow compound was constantly loaded onto the column, and so no separation was possible.

   Correct by loading properly - adsorbing onto the column before filling the reservoir.

5. A green compound is placed on a column and the column is eluted with hexanes. After a large volume of solvent has been collected but the green band is still at the top of the column. What should be done to recover the red compound? How could this experiment have been done better?

   Green compound is polar and does not partition well into the hexanes. Instead it sticks to the solica. Increase polarity of the solvent by adding EtOAc until compound elutes.

   Correct this in the future by testing the R_f first using TLC.

6. Indicate how the following errors could be corrected.

   a) A mixture of 1,2-dimethylbenzene and 1, 3-dimethylbenzene shows one spot with an R_f of 0.96 when eluted with ethyl acetate.

   Solvent is too polar. Try a less polar solvent. This can be done by increasing the proportion of hexanes. This will make the components run slower and give better chance to separate.

   b) A mixture of adipic acid and benzoic acid gives one spot with an R_f or 0.03 when eluted with hexanes.

   Solvent not polar enough, and so products simply stick to the solica. Increase solvent polarity by increasing the proportion of EtOAc. This will make the components run faster and give better chance to separate.