CH 223 – Organic Chemistry Laboratory I

Experiment # 5 - Column Chromatography of an Excedrin Tablet

The goal of this experiment is to separate the three analgesic components: Aspirin (Acetylsalicylic acid), Acetaminophen (4 – Acetamidophenol), and Caffeine in an Excedrin tablet by liquid column chromatography. Students are allowed to work in a group of three.

A. Equipments: Items listed below are some of the equipment that one may need in this experiment. More equipment may be needed depending on individuals.

1 mortar and pestle
½ extra strength Excedrin tablet
1 glass column
3 clean and weighed 400 mL beakers
1 clean and dried 150 mL beaker
1 glass stirring rod
2 test tube racks
40 or more small disposable test tubes
5 or more TLC plates
1 small long stem funnel
1 10 mL graduated cylinder
1 100 mL graduated cylinder
2 micro-3-pong clamps
1 250 mL separatory funnel and stopper
capillary tubes
developing chamber
UV-lamp
Marker pen
Cotton ball

B. Procedures and Theories

1. Prepare a slurry-packed column.

Weigh approximately 25 g of silica gel (record the mesh number) in a 150 mL clean and dried beaker. To make a slurry, add enough ethyl acetate hexanes solution (ethyl acetate : hexanes = 10 : 1) to cover the weighed silica gel. Stir the mixture thoroughly with a glass rod until no more bubbles evolve (about 2 min).[1] Obtain a

[1] This allows silica gel to be packed tightly and avoids channeling. Channeling occurs when part of the band (a band contains a separated single component) front advances ahead of the major part of the band and results in poor separation (see Figure 3).

column and clamp it securely to a stand by using two micro three pong clamps so that it is **as nearly vertical as possible**.[2] Place a 250 mL Erlenmeyer flask underneath the column to collect eluent[3] during packing and loading. Pour Ethyl acetate : Hexanes = 10:1 mixture into the column until it is half filled. Insert a tiny piece of cotton to the bottom of the column and pack it tightly (it is used as a filter to prevent sand to pass though and clog up the stopcock.) Using a long stem funnel, pour in enough sand to form a 1-cm thick layer at the bottom of the column. Tap the column **gently** with your fingers or a rubber hose so that the sand layer is level and uniform. Stir the slurry again and slowly pour it into the column, with continuously tapping, through the funnel. Rinse the beaker with additional **ethyl acetate and hexanes solution** (ethyl acetate : hexanes = 10 : 1) until most of the slurry is transferred into the column. Use a **plastic** pipet to rinse down any silica gel attached on the side of the column. **Keep on adding eluting solvents and never let the solvent level get below the top of silica gel during *packing* and *eluting*!** After all the silica gel settles and forms a level and uniform layer, add another 1-cm thick layer of sand (lever and uniform) on top of the silica gel layer. Again do not let the solvent level get below the sand layer.

2. Loading sample

As one student is adding the final layer of sand into the column, another student should grind **half** of an Excedrin tablet in 1 mL of **ethyl acetate**. Let the solvent level in the column drain down to the top of the sand layer, but not pass beyond the layer. Then introduces the heterogeneous mixture (grinded Excedrin tablet in 1 mL ethyl acetate) carefully (try not to disturb the sand layer if possible) and quickly onto the sand layer (do not allow the eluent to drain below the top of the silica gel layer). Immediately add 1mL of **ethyl acetate hexanes solution (10 : 1 mixture)** to the mortar, and then transfer it into the column once the solution level gets down near the sand layer. Repeat this procedure three times. Then fill the entire column with the 10 :1 ethyl acetate hexane solution. Place a 250 mL separatory funnel right above the column as shown in Figure 1, and fill it with about 20 mL of the 10 : 1 ethyl acetate hexanes solution. (Recycle the 10:1 ethyl acetate hexanes solution previously collected in the Erlenmeyer flask under the column.) Place the tip of the funnel inside the column, stopper the funnel and open up the stopcock. Then start collecting the eluent[3] coming out of the column in a small numbered test tube.

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[2] To perform separate a mixture of compounds effectively by column chromatography, packing of the column is the most critical operation. Silica gel must be evenly packed and free of irregularities, air bubbles, and gaps. As a compound travels down the column it will do so in a band. It is important that the front of the band be horizontal as shown in Figure 4. If two bands are close together and do not have horizontal band front, it is impossible to collect each band to the exclusion of other.

[3] A eluent acts as a solvent to differentially remove molecules of solute from the surface of adsorbent (e.g. silica gel).

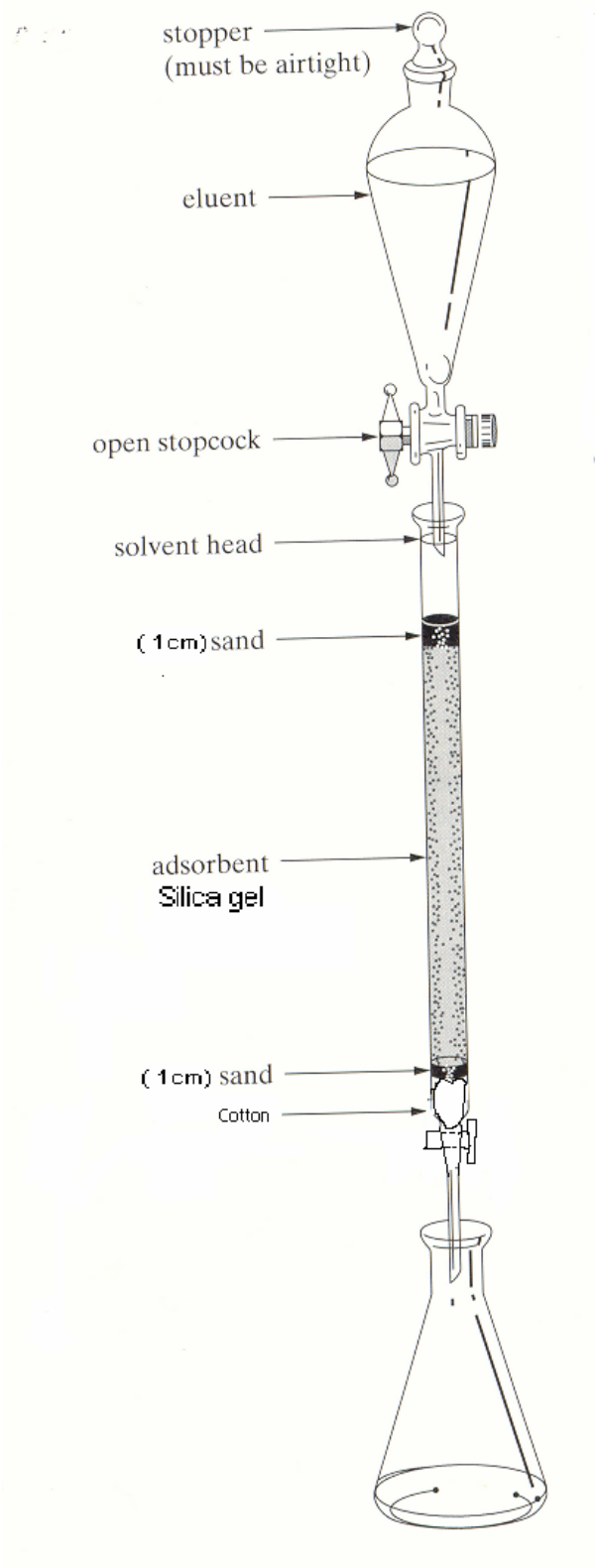
stopper
(must be airtight)
eluent
open stopcock
solvent head
(1cm) sand
adsorbent
Silica gel
(1cm) sand
Cotton

Figure 1

3. Elution

Collect the eluent in test tubes and label the test tubes as 1,2,3,…so on. Fill each test tube nearly full and consider each one as a fraction (i.e. test tube # 1 is fraction 1, test tube # 2 is fraction # 2 and so forth). Remember there are three compounds we are trying to separate. The first compound, A, (one of the three) will be likely eluted out (washed out) by the least polar solvent mixture, which is 10 : 1 ethyl acetate hexanes mixture, within the first ten fractions (result may vary depending upon how well a column is packed). To test if a test tube contains either one of the three components, place a small drop from each test tube (use a new capillary tube each time to avoid contamination) onto a TLC plate. Since all the components in this experiment are UV-active, we can visualize them under a UV lamp (not all compounds can be seen under UV). Blow dry the plate to remove the solvent **completely** and look at the spot under a UV lamp (never look at a UV lamp directly, otherwise God bless you). The presence of a component will be revealed by a dim or dark spot. A very dark and large spot indicates that that particular fraction contains a high concentration of compound(s), while a very faint and small spot shows that the fraction contains low concentration of the UV-active compound(s). Once ten fractions are collected, prepare a TLC plate by spotting each fractions. Look at the plate under a UV lamp before developing it. If the initial spot is too faint and small, spot it several more times until a distinct spot is observed. Then develop the plate in a 10 : 1 ethyl acetate hexanes solution (Figure 2).

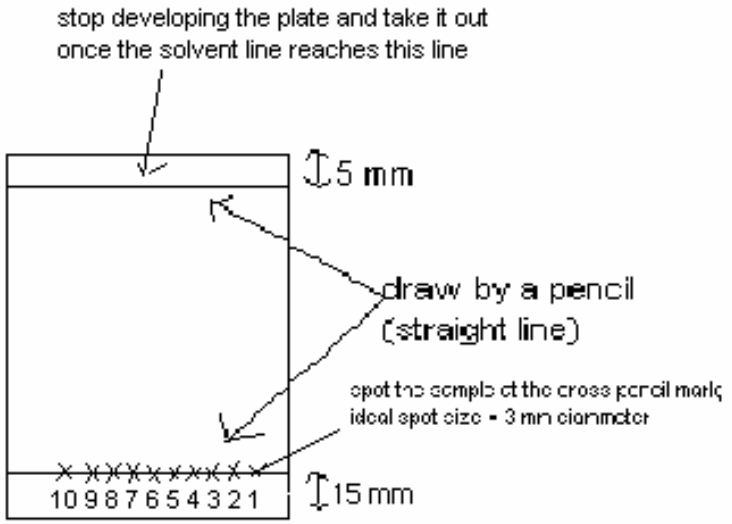

Figure 2

Once compound A is eluted out, change the eluting solvent to pure ethyl acetate (about 40 mL or more is needed; again results may vary) to elute compound B. After compound B is eluted out, change the eluting solvent to pure ethanol (the most polar solvent among all three) to elute compound C (identities of A, B, and C are for you to determine). This elution technique is called Gradient Elution. Gradient Elution is one of the most common techniques employed in liquid column chromatography. This technique starts with using the least polar solvent and gradually increasing the solvent's

polarity by either adding a more polar solvent, or switching to a more polar solvent. By doing so, it reduces separation time and improves resolution.

4. Combine fractions and sample identification.

Test all fractions by TLC. Combine those fractions containing the exact same component into a pre-weighed 400 mL breaker. Discard those fractions containing more than one component. To identify your samples, spot the combined fractions (total of 3) and the provided standards (standards are prepared from each individual compounds) side by side. Develop the plate, again, in 10 : 1 ethyl acetate hexanes solution. By comparing R_f values, one should have a clear idea about the identity of each combined fraction. To verify their identities, a co-spot experiment should be performed. To perform a co-spot experiment, spot both the standard and the combined fraction at the exact same location (on top of each other) on a plate and monitor each spotting operation by a UV lamp (several experiments can be done on the same plate in a single operation). After developing the plate, if the plate shows only **one (not two)** spot without significant **size enlargement**, one may conclude the sample and the standard are probably the same compound (there is no guarantee on this issue unless you know what exactly is in the mixture; there are compounds which share exact same R_f vaules and m.p.). In this experiment, however, it is safe to assign the unknown's identity based on R_f value since the content in each tablet is known.

Let solvent to evaporate overnight. Obtain weigh, m.p., R_f values, etc. of each individual compounds.

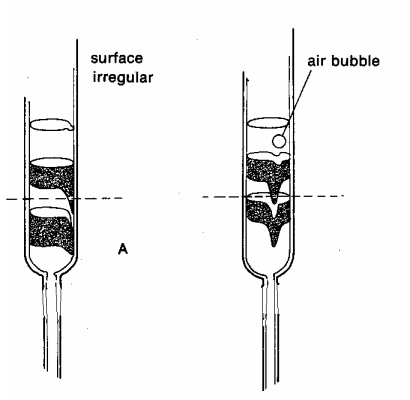

Figure 3

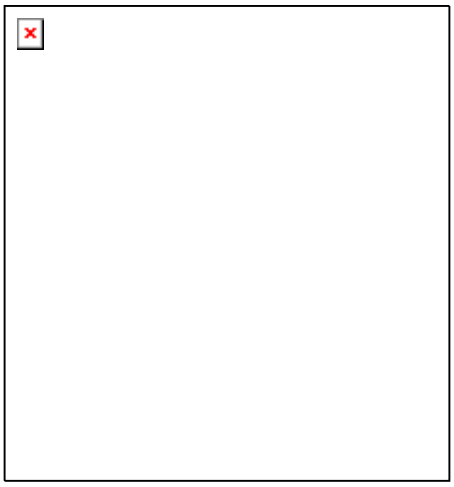

Figure 4

Experiment 5
Data sheet
TA initial:_____

Caffeine
mass yielded:_____

melting point:_____

R_f value: _____

Aspirin (Acetylsalicylic acid)

mass yielded:_____

melting point:_____

R_f value: _____

Acetaminophen (4 – Acetamidophenol)

mass yielded:_____

melting point:_____

R_f value: _____

Pre-lab question(s)

1. Describe the differences between silica gel column and reverse phase column in terms of polarity, initial solvent (polarity) choice, and order of solutes with different polarity eluted out of the column?