HOW TO DO FLASH COLUMN CHROMATOGRAPHY IN 15 MINUTES

Many students spend a lot of time doing column chromatography, and in many cases they describe the process as “difficult”, “time-consuming”, or “tedious”. Often, they would collect numerous fractions, use large amount of solvents even for scales ~100 mg, and routinely spend more than 1 hr, or many more, just on one column.

The truth is that column chromatography should take about 15 min, including fraction collection and evaporation. On scales ranging from 1 mg to 10 g. For particularly difficult separations when you need to separate and collect 4 or more compounds with close R_f’s for a couple of them, add 10 min to your total column time. And, if the flash column chromatography requires more time, it is usually not worth it (at normal pace, we see maybe one or two exceptions per student per year).

Here is how to do it.

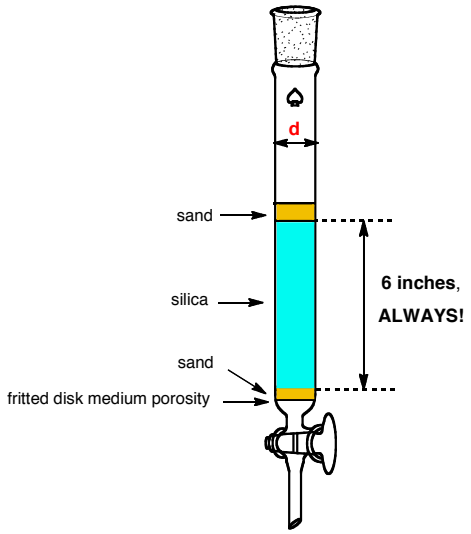
One important trick is to adhere to a standardized protocol faithfully. There are two major considerations:

1. The correlation of scale to the size of the column diameter and volume of fractions

2. The correlation of R_f of the target compound and the eluent polarity/composition

Here is our lab-specific protocol

1. Selection of the column. Use the following table. It is important to have a consistent protocol for column preparation

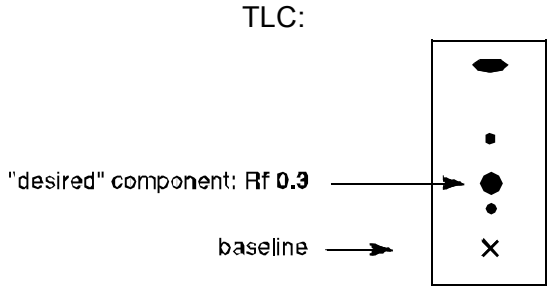


scale (total mass)	0.5-10 mg	10 – 99 mg	0.10 – 0.30 g	0.30 – 0.9 g	~1 – 2~5 g	5 - >30 g
column diameter (**d**)	10 mm	10 mm	20 mm	25 mm	50 mm	70 mm
fraction size[1]	 (1 ml)	 (~1.5 ml)	 (~3.5 ml)	 (~ 6 ml)	 (~16 ml)	 (~28 ml)
silica column height	2-3 inches	**6 inches ONLY**				6+ inches

1 We have two standard test tube sizes: A) 10 ml, VWR cat B)

Your column should be complete once you have collected fraction #28. Your compound will be somewhere between fractions #5 and #15.

2. Eluent selection: this is simple; your target compound should have an R_f of ~0.3 in the eluent you use for its chromatographic purification.[1] No gradients allowed – use only isocratic systems; however, occasionally different isocratic systems maybe used in one purification step.[2]



3. When we first pack a column, we dry pack it with silica gel, then do one wash with 100% EtOAc, then one wash with 100% hexanes. The volume of eluent is ~10-20% larger than the volume of silica. Now you are ready to start your chromatography.

4. If you did not have any baseline impurities in your initial mixture; you can reuse the column multiple times after washing with 100% EtOAc followed by 100% hexanes. Repack column with fresh silica only when actually needed – you should know what you put on your column previously.

5. Never apply your mixture on top of the column with a solvent that is more polar than your eluent.

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[1] For generally more polar compounds (>50% EtOAc/hexanes), you can have R_f up to 0.5; for non polar compounds (~5% EtOAc/hexanes), decrease polarity, targeting ~0.2 R_f

[2] Specific examples for the use of a “gradient” system is described in an Appendix. “Gradient” actually means a combination of two isocratic elutions.

6. If your mixture is poorly soluble, you can use a solvent of lower polarity than your eluent, and mixtures thereof

NOTE: Any slight deviation from these simple guidelines will result in exponential deterioration in efficiency. If you do it exactly as outlined here – you are done in 15 minutes!

For a video tutorial on packing a column, please click the following link:

http://web.chem.ucsb.edu/~zakariangroup/column-packing-tutorial.mov