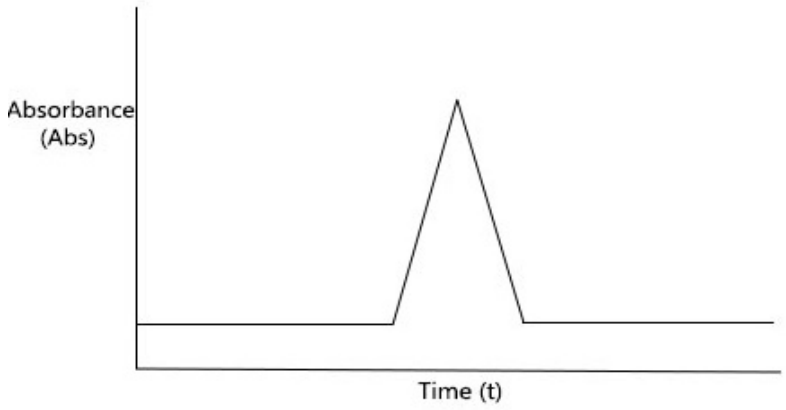
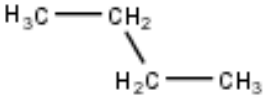
HPLC Supplemental Questions:

Often times, an HPLC column will be attached to a detector which can be used to measure the concentrations of your separated compounds (called elutions) after they pass through the column. If you have just one compound, the Chromatogram will look like this;

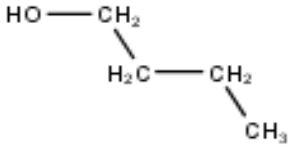


For these questions assume the peak heights represent how much of each compound there is (ie. The concentration).

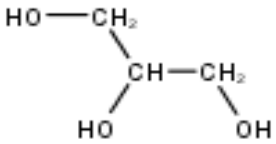
1.a Compounds Butane, Butanol, and Glycerol will be run through a nonpolar column media in Reverse Phase Chromatography.



(Butane)

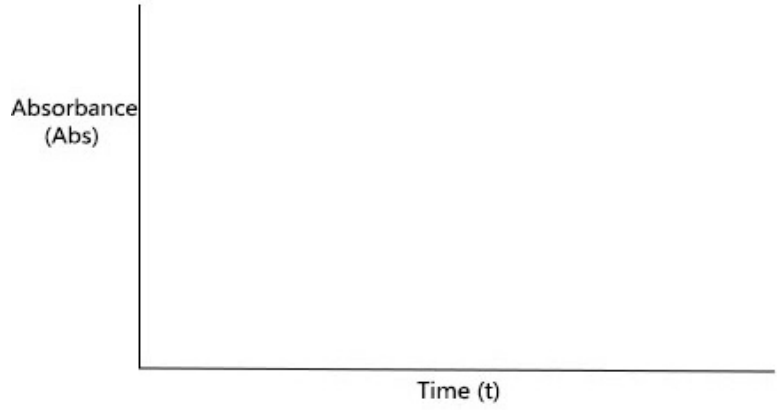


(Butanol)



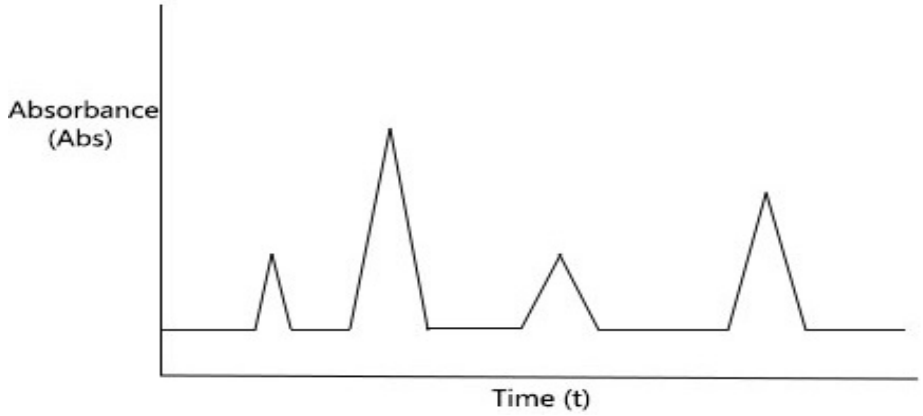
(Glycerol)

Assuming there is no difference in the peak height, and each compound is in equal concentrations; What would you expect the Chromatogram to look like?



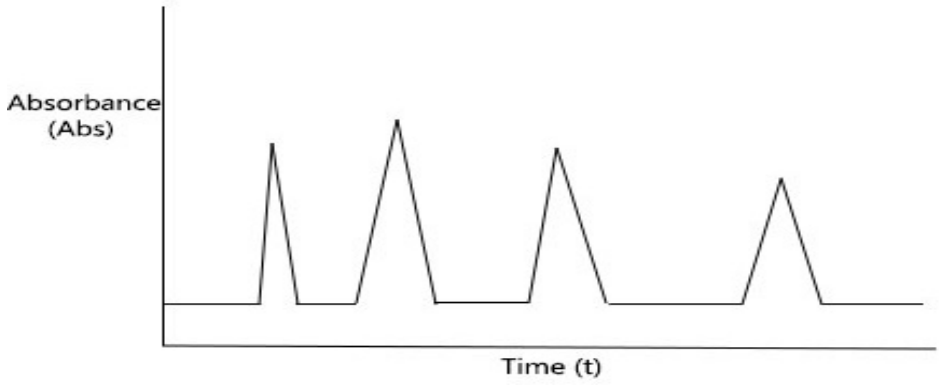
1b. Why did you not need a detector for the purple Kool-aid lab? When do you think a detector would be useful in HPLC experiments? What can the detector do that the human eye can't?

2a. Now you aren't sure which elutions belong to which peaks in Figure #4, but you have an idea how to identify them. In science there is a technique called “Sample Spiking”, where a known reference material is added to your sample in the HPLC column. If an elution peak is larger then that peak likely belongs to the known material. You think the compounds are (A, B, C, and D) and you have pure samples of each to sample spike.



How many times (at least) do you have to perform a chromatography run? Why?

2b. In one such chromatography run you sample spike with compounds A and D, and your Chromatogram looks like this;



Can you tell which specific peak is A and which specific peak is D? Explain your reasoning.