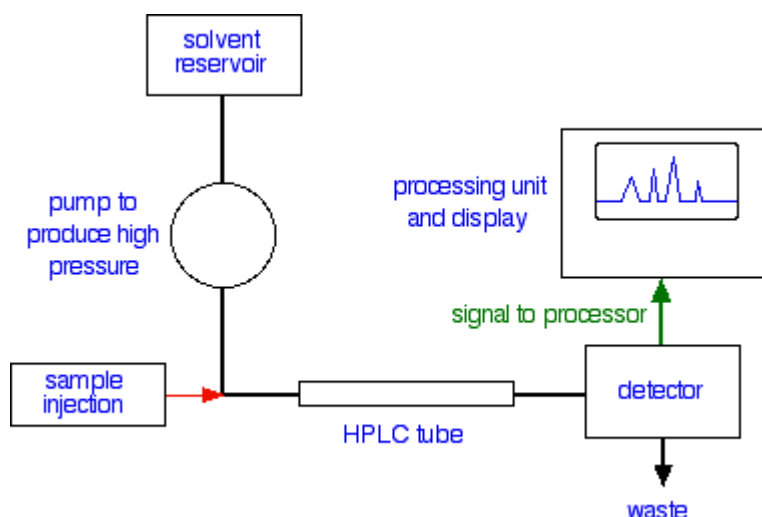


## Chemguide – questions

### HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

- HPLC is an advance on column chromatography where the solvent is forced through the column at a very high pressure of up to 400 atmospheres. This makes the whole process much faster, but also allows you to use a much smaller particle size in the column. Why is that an advantage?
  - There are two forms of HPLC – normal phase HPLC and reversed phase HPLC. In normal phase HPLC, the stationary phase is silica and the mobile phase is a non-polar solvent like hexane. What are the stationary and mobile phases in reversed phase HPLC?
  - In normal phase HPLC, how will the retention time of a component of a mixture vary with the polarity of its molecules? Explain your answer.
  - In reversed phase HPLC, how will the retention time of a component of a mixture vary with the polarity of its molecules? Explain your answer.
- A flow diagram for HPLC looks like this (diagram taken from the Chemguide page):

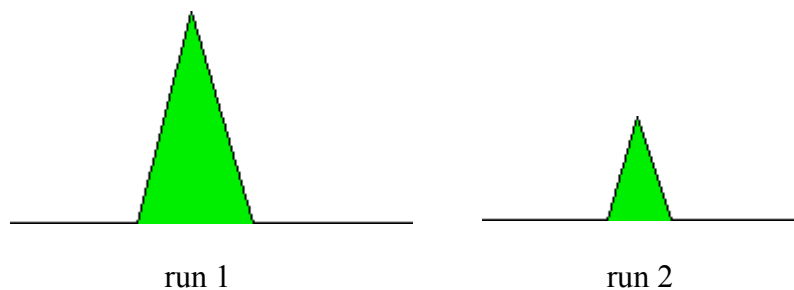


- The retention time of a particular component of a mixture is measured from the time of injection to its maximum peak height in the detector. What factors govern the length of the retention time?
- One way of detecting components of a mixture coming out of the column is by using UV light. Explain briefly how this works.
- The diagram shows a simplified output from the detector with peaks for two compounds X and Y. The areas under the peaks are shown in green. What, if anything, can you say about the relative concentrations of X and Y in the mixture? Explain your answer.



## Chemguide – questions

d) The next two diagrams show a peak for the same compound in two different runs. What, if anything, can you say about the concentrations of solutions of the compound in each of these runs? Explain your answer.



e) Explain briefly how a mass spectrometer can be used to identify the compounds producing the peaks in high performance liquid chromatography.