HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1. a) The smaller particle size gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This gives a much better separation.

   b) Reversed phase HPLC has long hydrocarbon chains attached to the silica surface. This makes the surface non-polar. A polar solvent is used, such as a mixture of methanol and water.

   c) In normal phase HPLC, the stationary phase is polar and the mobile phase is non-polar. A polar compound in the mixture will spend more time attached to the stationary phase, and less time in solution in the solvent – and the more polar the compound the more true that will be. So the more polar the compound, the greater its retention time in the column. (Or, the less polar the compound, the shorter the retention time.)

   d) The opposite is true in reversed phase HPLC. Here, the surface of the stationary phase is non-polar, and the solvent polar. The more polar a compound is, the more time it will spend in the solvent, and less time on the stationary phase. So the more polar the compound, the shorter its retention time. (You could also look at this in terms of the greater attractions of the less polar compounds to the stationary phase, and the fact that they will be less attracted to solvent molecules. Either way, retention time is shorter the more polar the molecules being separated.)

2. a) The pressure, the exact nature of the stationary phase (including the particle size), the exact nature of the solvent, and the temperature.

   b) UV light of a suitable wavelength (which is absorbed by the molecules you are trying to detect, but not by the solvent) is shone through the output from the chromatography column, and the amount passing through is measured on a detector. This is converted into a series of peaks - the higher the peak, the higher the proportion of the UV that has been absorbed.

   c) You can't say anything about the relative concentrations of two different substances from the areas under the peaks. The reason that Y produces a smaller peak may be that it is less concentrated, or it may just be that Y doesn't absorb as strongly as X at the wavelength you are using.

   d) If you are comparing two samples of the same substance, you *can* use the areas under the peaks, because you know that, molecule for molecule, they will absorb the same amount of UV at a particular wavelength. The ratio of the areas under the peaks tells you the ratio of their concentrations.

   e) When the detector is showing a peak, some of what is passing through the detector at that time can be diverted to a mass spectrometer. There it will give a fragmentation pattern which can be compared against a computer database of known patterns to identify the compound.