Friday Worksheet **Name: …….**
Analytical chemistry revision 4

1) The urine of an athlete, named Bob, was examined for the presence of anabolic steroids. A steroid, code named "A" is of particular interest. The area under the peak representing the protein "A" is marked as having a value of 800 units.

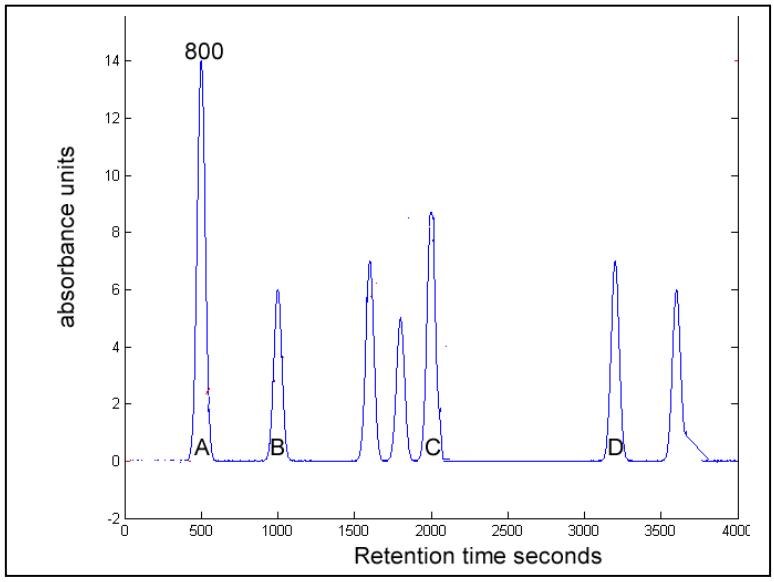


Fig 1

The column is packed with beads covered in a hydrophobic oil while the sample of urine is diluted with water before being injected into the column

a) Which compound is the most polar? Explain
"A" is the most polar. It has the lowest Rt, meaning that it is swept through the column quickly by the polar solvent.

b) What information can be derived from the spectrum shown above?
Each compound can be identified by its Rt value. The area under the peak can also indicate the amount of substance present. Using a calibration curve the exact amount of compound can be calculated.

c) Explain how each of the following will influence the rate at which a compound moves through the column.
1) The compound's solubility in water
Polar compounds will dissolve in water readily and be carried with the mobile phase, hence will have a lower retention time.
2) The compounds concentration
No impact on Rt
3) The temperature of the solvent moving through the column.
The greater the temperature the greater the kinetic energy of the particles and hence the less time the molecules will spend adsorbing to the stationary phase. The solubility of some solutes increases with temperature increase and so will be carried quicker through the column.

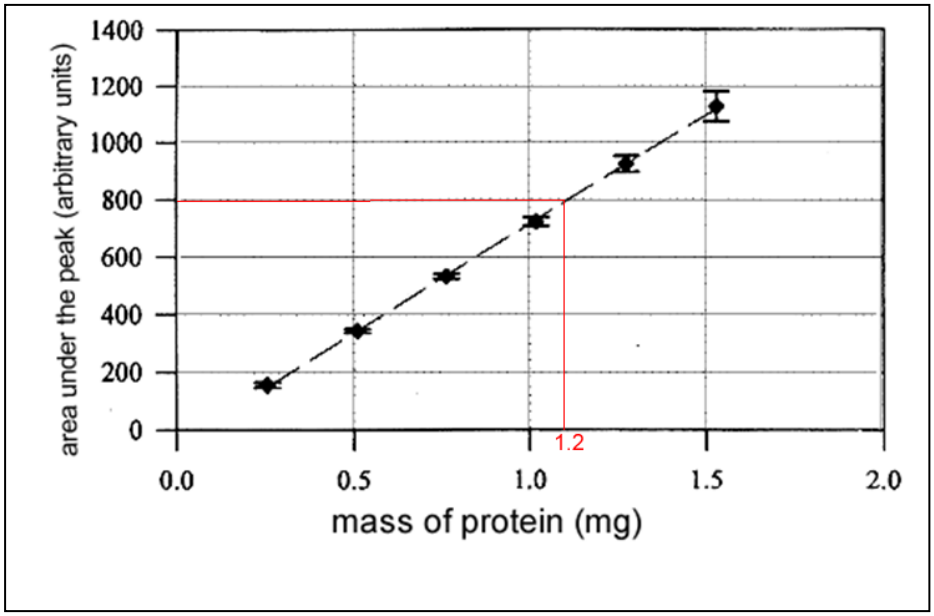
4) The size of the beads forming the stationary phase.

The smaller the beads the greater the surface area of the stationary phase in contact with the mobile phase. This increases the number of interactions between molecules in the mobile phase and the stationary phase. This will slow the molecules' passage through the column.

5) Its molecular mass

The molecular mass of a compound influences its solubility in water. The higher the mass the less soluble it is, hence will be moved along the column at a slower speed when compared to low molecular substances.

d) A calibration curve was constructed of the relationship of area of peak in the chromatogram and the mass of protein "A".

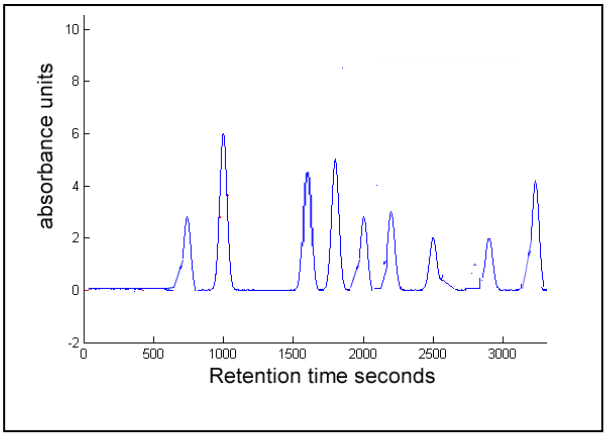


If 10.0 mL of urine was used what is the concentration, expressed as %(m/v), of protein "A" in the urine

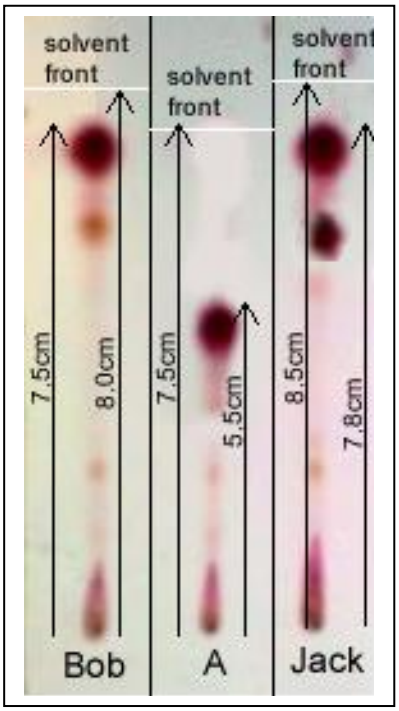
1.2 mg or 0.0012g of protein is discovered in 10.0 mL of urine.

=> (0.0012 / 10) X 100 = 0.012%(m/v)

e) Another athlete, Jack, was tested using HPLC and the chromatogram is shown on the right. Conclude which of the following steroids A, B, C and D are also found in this athlete's urine? Explain how you arrived at your conclusion. B, C and D. The Rt values match in both chromatograms.



f) In another investigation thin film chromatography was used to analyse the urine of the same athlete mentioned above. The chromatogram is shown on the right. The image on the right shows the chromatograms of two athletes and a control for protein A shown in the middle.



Do the results confirm the findings shown in figure 1, above of the athletes?

Explain.

The Rf value for protein "A" is 5.5/7.5 = 0.733

The Rf value of the protein seen in Bob's sample is 7.5/8.0 =0.938

The Rf value of the protein seen in Jack's sample is 7.8/8.5 =0.918

The results suggest that the protein found in both athlete's urine samples is not protein "A".