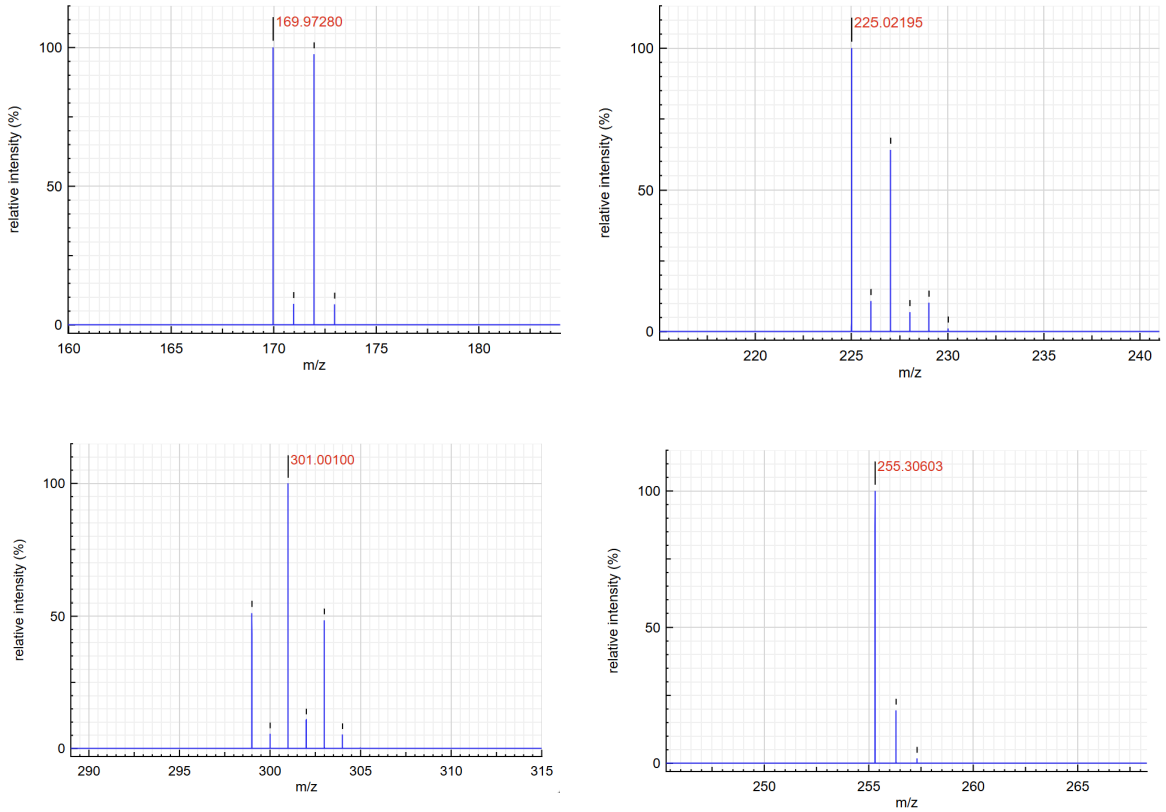
*The following questions cover analytical methods such as Mass Spectrometry, IR and UV-VIS. Please refer to **chapter 4** in Organic Chemistry with a Biographical Emphasis from Timothy Soderberg to obtain a good introduction of the methods.*

3.1 Mass Spectrometry (MS)

Match the following molecular formulas to the MS spectra bellow:

1. C7H7Br
2. C10H10Cl2
3. C18H38
4. c10H20Br2



There are two ways of doing this exercise, either by looking at the molecular ion peaks and comparing them with the molecular masses, or by looking at the isotope distributions for Br, Cl and Br2.

***Top left C7H7Br**: Presence of an M+2 peak of the same size as the molecular ion peak.*

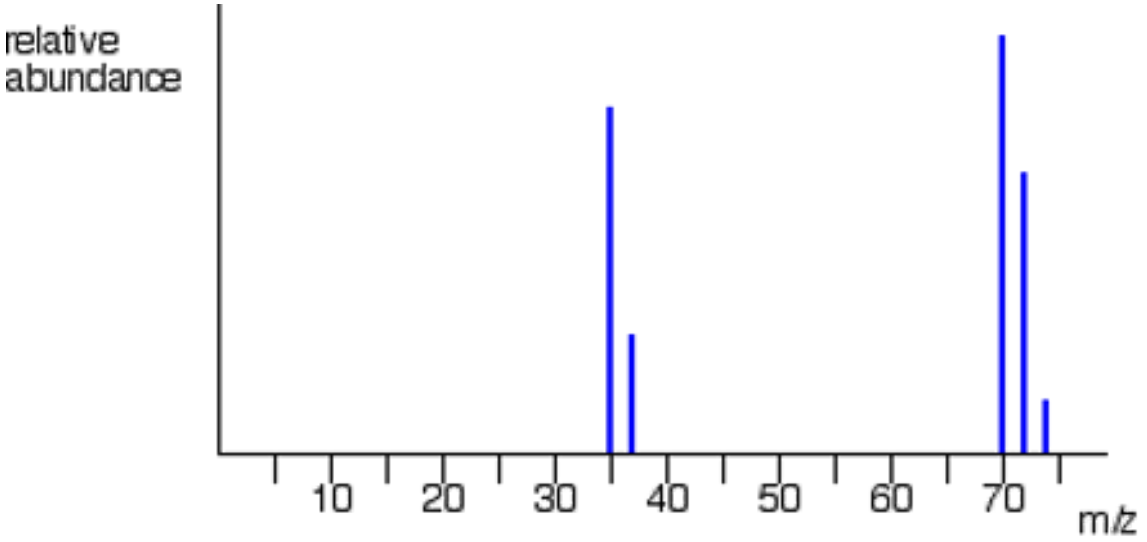
***Top right C10H10Cl2** (see ex 3.3)*

***Bottom left C10H20Br2**: here there are 3 peaks that are relevant: M (Br79,Br79), M+2 (Br79, Br81), M+4 (Br81, Br81).*

***bottom right C18H38:** only one without M+2 peak. Can't contain Cl or Br.*

3.2 Mass Spectrometry (MS)

Consider an MS spectrum of the molecule Cl_2. Explain how each of the different peaks are formed and which the monoisotopic peak is.



Cl2 has four different molecular combinations resulting in three different weights:

35Cl - 37Cl → 72 Da

37Cl - 35Cl → 72 Da

35Cl - 35Cl → 70 Da

37Cl - 37Cl → 74 Da

This will result in three peaks. at 70, 72 and 74 m/z.

The peaks at 35 and 37 are two different isotopes of Cl which occur, indicating that 35Cl is the more common isotope. This means that the peak at 70 m/z is the monoisotopic peak of Cl_2.

3.3 UV- vis

Guanosine has an extinction coefficient of 8400 [1/M*cm] at 270nm and the path length used is 1 cm. Using a spectrophotometer, you find the that A275=0.70

What is the concentration of guanosine?

To solve this problem, you must use Beer's Law.

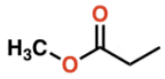
$A = \epsilon l c$

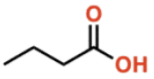
where $l = 1$, $\epsilon = 8400$ and $A = 0.70$

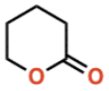
$c = 8.33 \times 10^{-5}$ mol/L

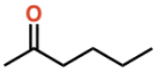
3.4 MS + IR spectroscopy

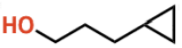
You notice a strange odour coming from a mislabelled tube in your laboratory. You know that it is one of the following molecules. You decide to do a mass spectrometry followed by an IR spectroscopy experiment to identify the culprit.

a) 

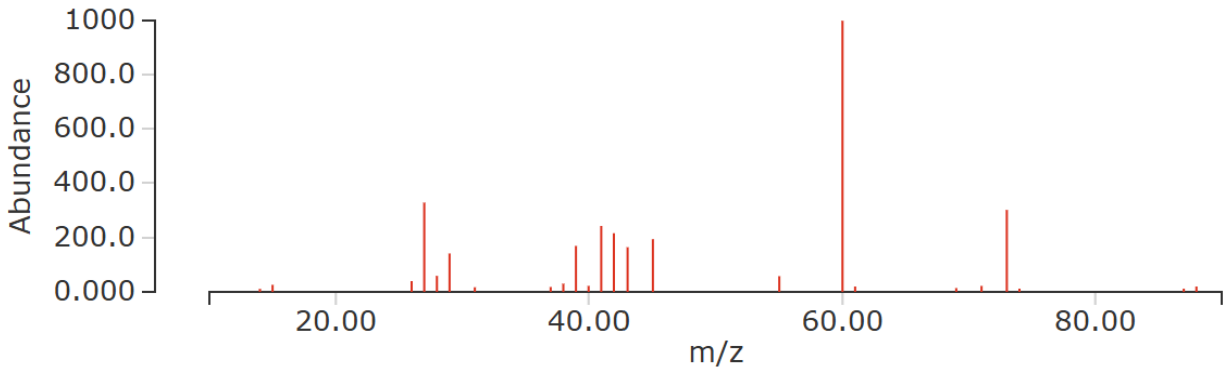
b) 

c) 

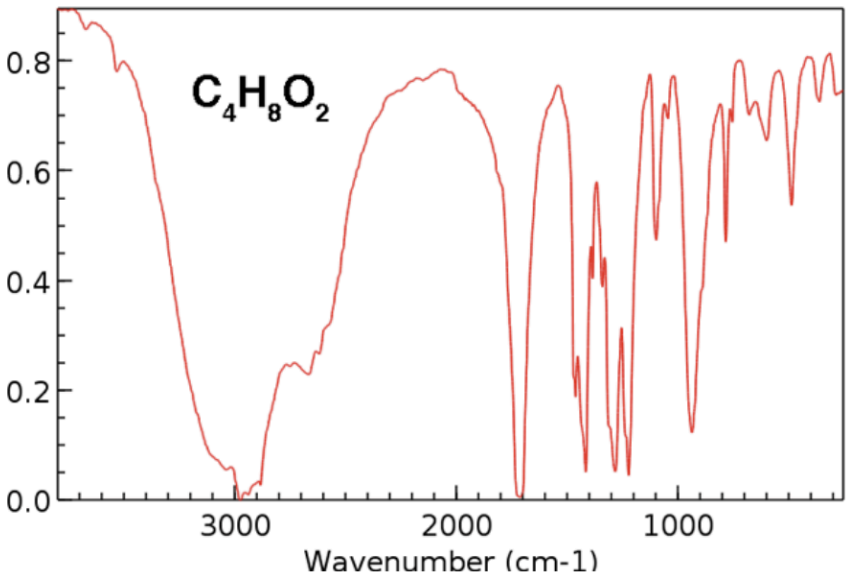
d) 

e) 

MS spectrum of compound X:



IR spectrum of compound X:

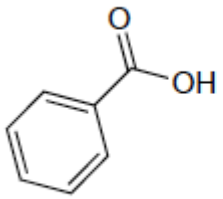


Given the spectra can you identify which compound is the culprit for the bad smell?

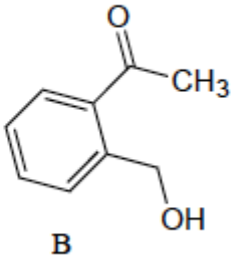
using the mass spec we can identify the molecular weight of the unknown molecule to be around 88 g/mol (small peak all the way at the end). This lets us rule out c) d) and e). To differentiate between a) and b) we use the IR spectrum. We see a huge peak in the 3300-2600 cm-1 region this is characteristic for an OH group making the correct answer b) butyric acid.

3.5 IR

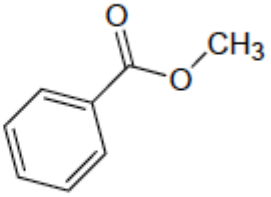
Explain how you could use IR spectroscopy to distinguish between compounds A, B, and C. How would the main peaks in their IR spectra differ?



A



B



C

All three spectra will have a strong carbonyl stretching peak, but the ester (compound C) carbonyl peak will be observed at a shorter wavelength compared to the ketone (compound B) and the carboxylic acid (compound A). In addition, Compound A will show a broad absorbance centered at approximately 3000 cm^{-1} due to carboxylic acid O-H stretching, whereas in the spectrum of compound B we should see the broad absorbance centered at approximately 3300 cm^{-1} from stretching of the alcohol O-H bond. Compound C will have no broad O-H stretching absorbance.

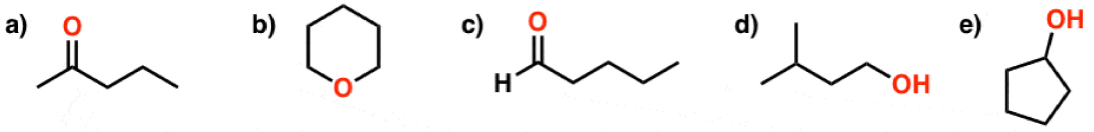
Characteristic IR absorbances

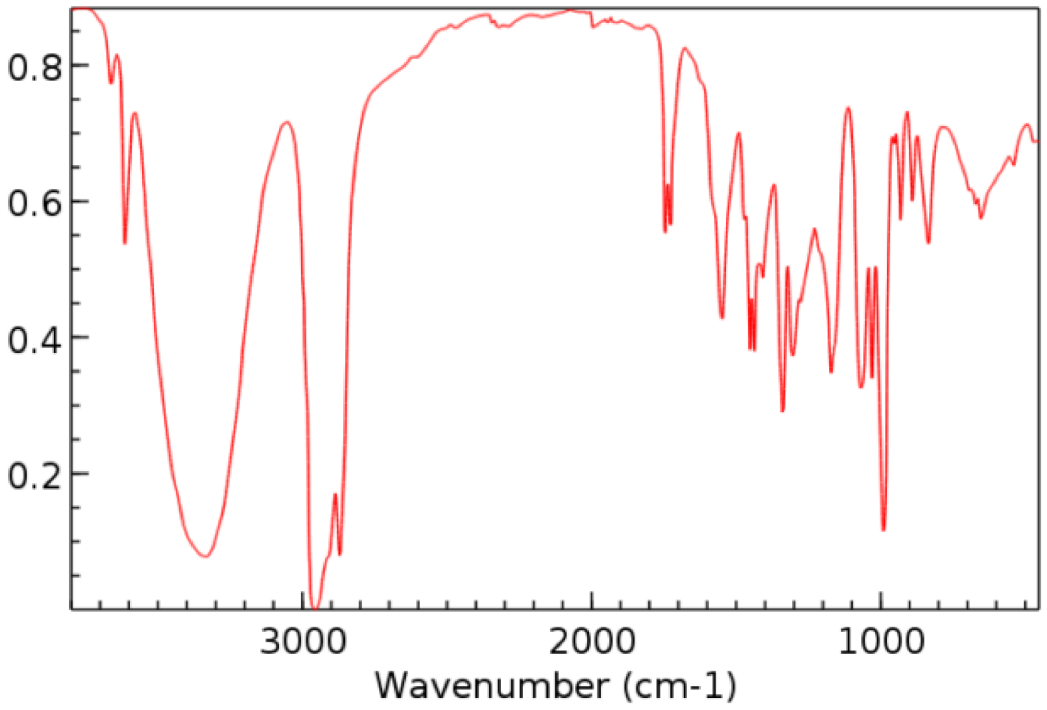
Functional group	**Characterisic IR absorbance (cm^{-1})**	**Source of signal**
carbonyl	1650-1750 (strong)	C=O strecting
alcohol	3200-3600 (broad)	O-H stretching
carboxylic acid	1700-1725 (strong)	C=O strecting
	2500-3000 (broad)	O-H stretching
alkene	1620-1680 (weak)	C=C stretching
	3020-3080	vinylic C-H stretching

alkyne	1620-1680 (weak)	triple bond stretching
	3250-3350	terminal C-H stretching

3.6 IR

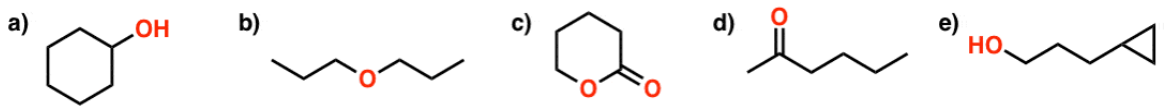
I) We have a molecule of which we know the chemical formula is $C_5H_{10}O$. In order to determine its structure we perform IR spectroscopy. Which of the following compounds corresponds to this IR spectrum?

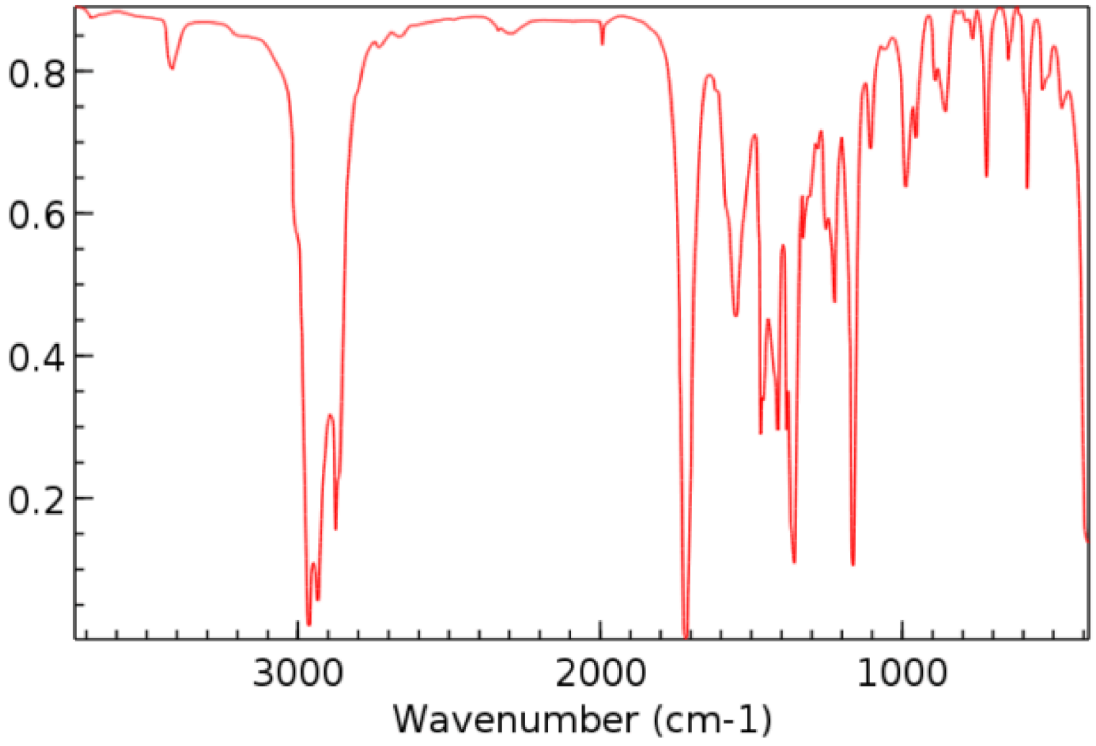




- broad peak around 3300 cm^{-1} indicating the presence of an alcohol (OH) group
- This leaves d or e. Since d has 12 hydrogens it only leaves e

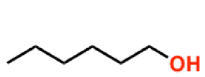
II) Which of the following compounds corresponds best to the following IR spectrum of $C_6H_{12}O$?

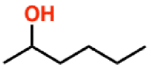


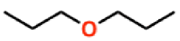


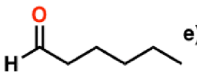
- Strong peak between 1650 and 1750 cm^{-1} indicating the presence of a carbonyl group (C=O). So its either c or d.
- c only has 5 carbons so it can only be d.

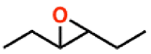
III) Which of the following compounds corresponds best to the following IR spectrum of $C_6H_{14}O$?

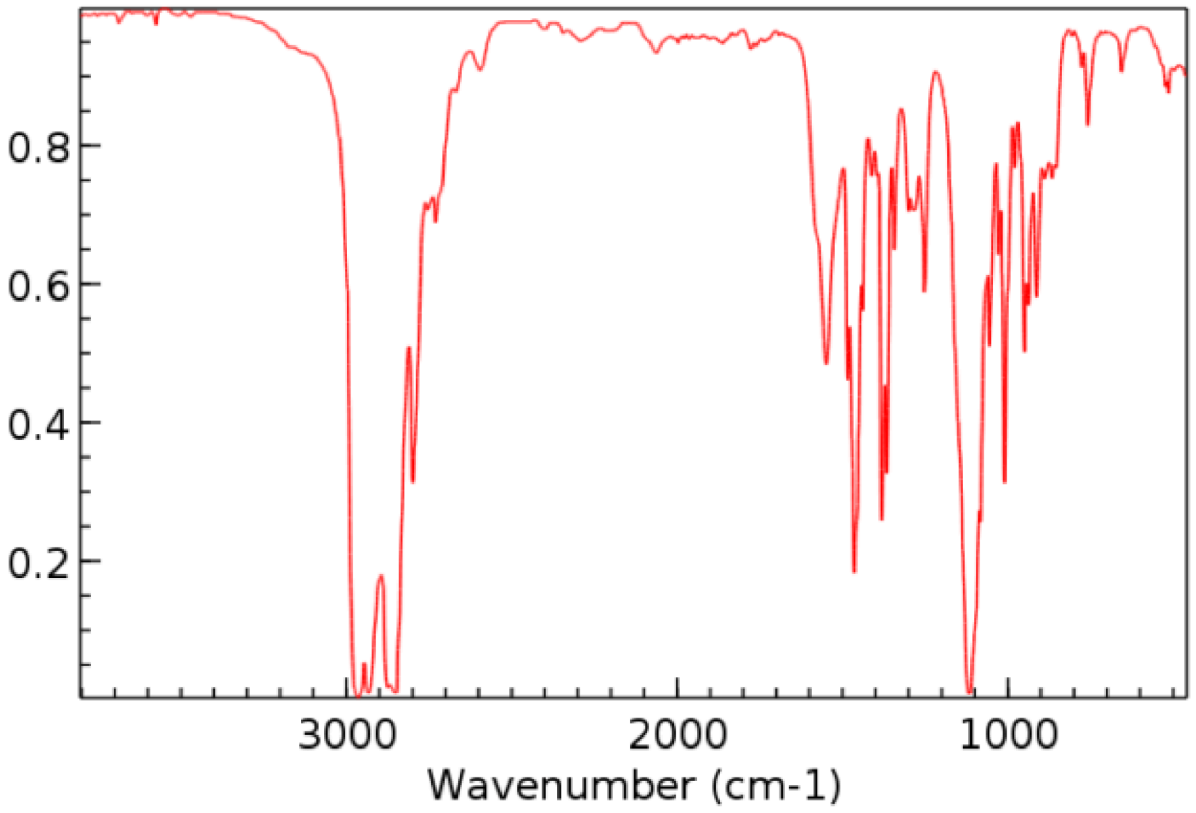
a) 

b) 

c) 

d) 

e) 

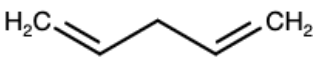


- No broad 3200-3600 cm-1 peak ruling out the presence of an alcohol (OH) group. So a and b do not correspong to this spectrum.
- No strong peak round 1650-1750 cm-1 ruling out a carbonyl group (C=O). So our spectrum also doesnt correspond to d.
- It cannot be e since it has only 12 hydrogens, leaving c as the only valid option.

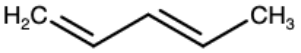
3.7 UV-VIS

Which of the following molecules absorbs in a longer wavelength in the UV-range. Explain why.

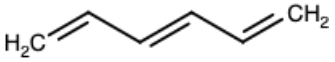
I)



A



B



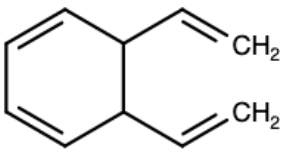
C

We expect the molecules to absorb in the following order:

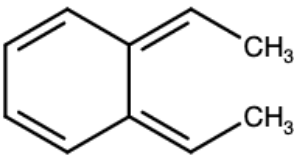
C > B > A

In general the longer the system of conjugated π--bonds the longer the wavelength it absorbs, as the energetic HOMO-LUMO gap shrinks. C has the longest conjugated system, followed by B, while A does have two isolated, non-conjugated double bonds.

II)



A



B

We expect the molecules to absorb in the following order:

B > A

In B we have a more extended conjugated π-system while in A the two π-systems are isolated from each other.

3.8 UV-vis

You want to know the concentration of your isolated DNA, by first diluting 100uL of an aqueous sample of double stranded DNA in 900uL of water. You then measure an absorbance of 0.45 at 260 nm. Calculate the concentration of your original sample using Beer-Lambert law and an extinction coefficient of 0.02 $uLng^{-1}cm^{-1}$ for double stranded DNA. Assume the path length of the light through the sample is 1cm.

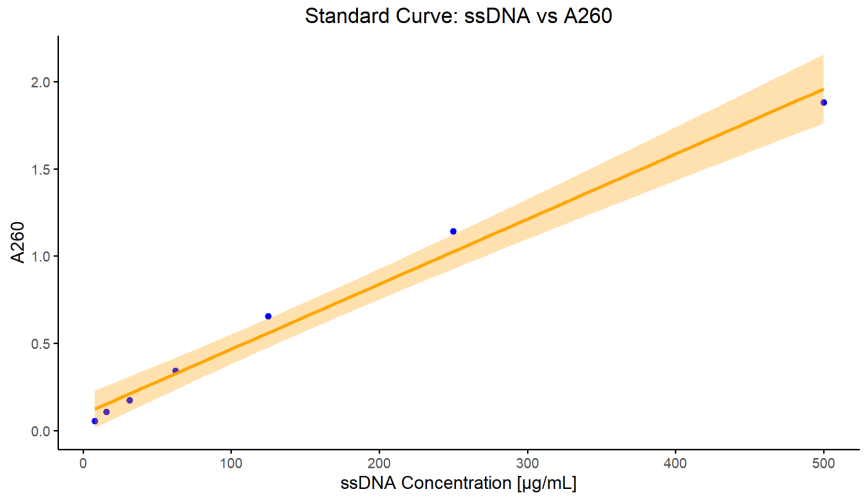
Using the Beer-Lambert law we calculate:

$$c = A \div \varepsilon l = 0.45 \div (0.02\, uL/(ng * cm) \times 1cm) = 22.5\, ng/uL$$

This we multiply by 10 as our original sample was 10 fold diluted. This gives us the final concentration of 225 ng/uL.

3.9 Calibration curves and UV- vis:

You want to calibrate your spectrophotometer to determine the concentration of an unknown DNA sample. To do this, you measure the absorbance of known concentrations of DNA and plot the calibration curve shown below:



Using this information, approximate the concentration of a DNA sample that has A260 = 1.5?

Could you use this calibration plot to find the concentration of a sample that has an A260 = 4.7?

Using the calibration curve you created in excel, the concentration of DNA can be estimated from the best fit line. The graph shows that an absorbance of 0.6 corresponds to approx 150ug/mL.

No, in general calibration curves should only be used within the range of measurements taken, as extrapolating the linear relationship too far will lead to accumulation of errors and unreliable measurements. (In practice, there are always measurement errors due to pipetting errors and the general precision of the instruments).