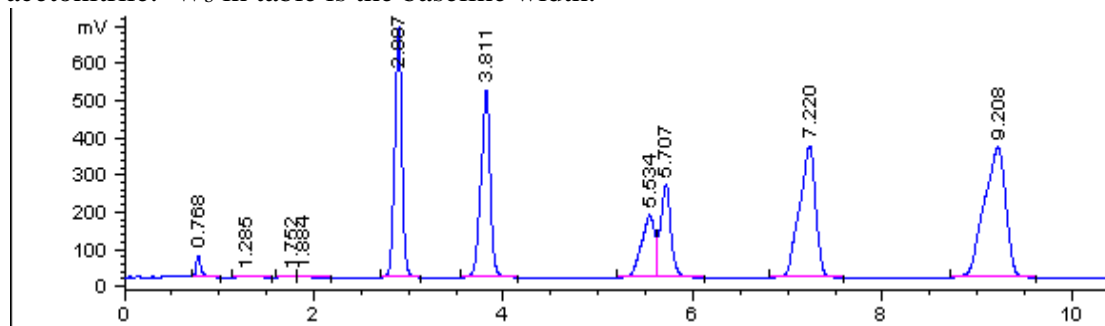


CHEMISTRY 31
Summer, 2016 - Dixon
Homework Set 3 – Additional Problem 3.1 Solutions

The following chromatogram and data table show the separation of linear fatty acids (C18:3, C18:2, C18:1, C16:0, C17:0, and C18:0 – where the first number gives the number of carbons in the fatty acids and number after the colon gives the number of double bonds – all in *cis* isomer). All of the fatty acids have pK_a values of around 4.8. The separation was performed on a C18 (reversed phase) column using HPLC with an eluent of 0.001 M trifluoroacetic acid (TFA = strong acid) in water (8%) and 92% acetonitrile. W_b in table is the baseline width.



Compound	Retention (min.)	Area	W_b (min.)
Unretained	0.768	NA	NA
C18:3	2.887	3611.5	0.127
C18:2	3.811	3389.3	0.156
C18:1	5.534	1656	0.222
C16:0	5.707	1908.9	0.180
C17:0	7.22	4182.1	0.266
C18:0	9.208	5552.7	0.358

a) Calculate the retention factor (k) of C16:0.

$$k = (t_r - t_m)/t_m = (5.707 - 0.768)/0.768 = \mathbf{6.43}$$

b) Based on the elution order, what effect does the number of double bonds have on the compound's "polarity"?

*Since the more double bonds a fatty acid has the sooner it comes out, we know that the fatty acids with more double bonds are more like the mobile phase than like the stationary phase. Since this is a reversed phase column, this means **the double bonds make the fatty acids more polar.***

c) Without the TFA present, what would happen to the retention times?

*Without TFA, the fatty acids will exist as ions and will be **less (or even un-) retained** (as ions are not soluble in non-polar media)*

d) Calculate the N value C17:0 (using equations for assumed Gaussian peak shapes).

$$N = 16(t_r/w)^2 = \mathbf{11800}.$$

e) What is the resolution between the two least well resolved peaks?

C18:1 and C16:0 are the least well resolved

$$R = \Delta t_r / w_{ave} = (5.707 - 5.534) / [0.5(0.222 + 0.180)] = \mathbf{0.86}$$

- f) Would decreasing the % acetonitrile be expected to improve the resolution of the overlapping peaks? Explain your answer.

Yes – it should. *Decreasing the % acetonitrile will increase retention and will improve resolution. However, because k is large to start with (6.43 for C16:0), it will not improve the resolution to full baseline resolution (assuming no changes in the relative retention).*