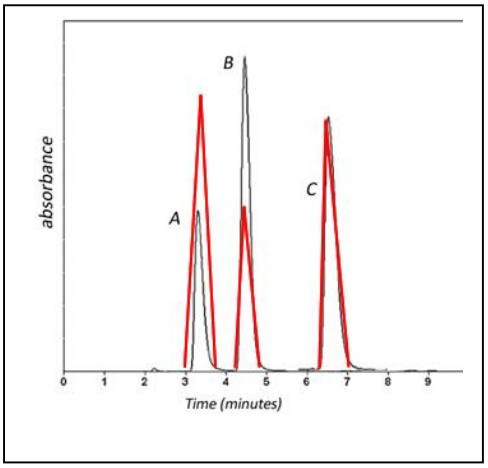
Lesson 1 Chromatography solutions



1) In one particular HPLC setup a non-polar stationary phase and polar mobile phase are used to separate a mixture of methanol, ethanol and butanol.

a) From what you know so far, identify the compound that may have formed each peak. Give reasons.

Methanol(CH_3OH) is a small polar molecule that interacts more with the polar mobile phase than it does with the stationary non-polar phase, hence, it will be removed first from the column and have the lowest R_t time. Ethanol(CH_3CH_2OH) is the next most polar substance and butanol is the least polar of the three, having a significant part of the molecule($CH_3CH_2CH_2CH_2OH$) non-polar.

b) The initial mixture contained 0.35 mM of methanol, 0.75 mM of ethanol and 0.65 mM of butanol.The chromatogram shown on the right was obtained. Another mixture of methanol, ethanol and butanol was separated using the same column, under the same conditions. This mixture contained 0.70 mM of methanol, 0.37 mM of ethanol and 0.65 mM of butanol. Draw, on the chromatogram above, the peaks formed by each substance. Explain your reasoning

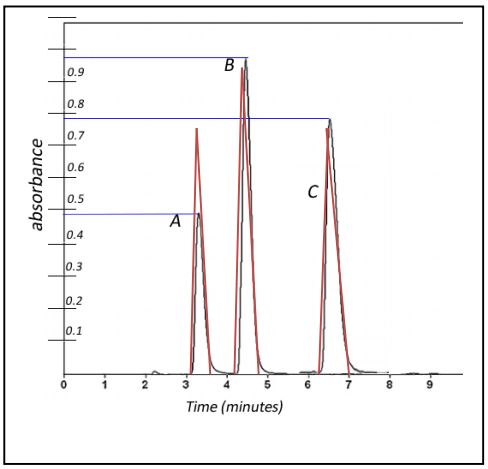
Since the R_t value identifies each compound the peaks in the second chromatograms should be at the same R_t value for each substance. The size of the peak at R_t =3.3 (methanol) should be twice as tall as the original chromatogram, ethanol has halved in concentration so its peak will reduce by 50% and the peak at R_t = 6.8 (butanol) will remain the same. The size of each peak reflects the concentration of the substance.

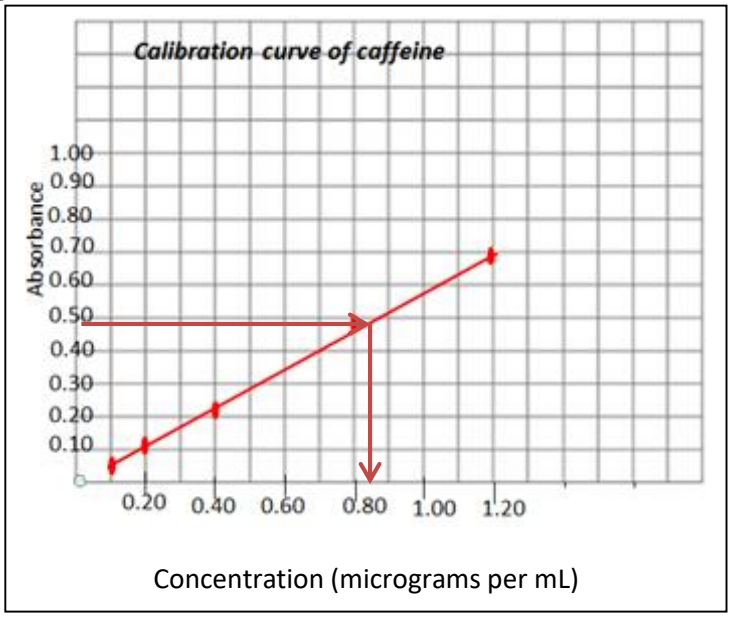
2) A drink is to be analysed for its caffeine content. A set of standard solutions were made up and their absorbance measured, the table below shows the results.

Caffeine micrograms/mL	absorbance
0.10	0.05
0.20	0.11
0.40	0.23
0.80	0.47
1.20	0.70

a) Draw a calibration curve using the graph paper shown below.

b) A sample of the drink was analysed using HPLC and the chromatogram shown below was produced.





i. If the retention time of caffeine in the column was 3.3 minutes, what is the concentration of caffeine in the sample.

Refer to the calibration curve. From the calibration curve we derive a concentration of 0.85 micrograms/mL

ii. How many different compounds are present in the drink?

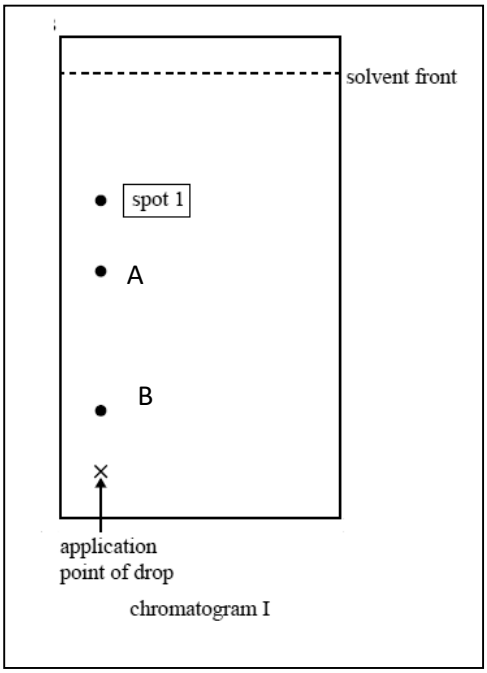
Three peaks indicates three compounds.

iii. A second drink had exactly the same ingredients in the same concentrations as the first drink except for caffeine. Its caffeine concentration, however, was 1.30 micrograms per mL. Explain how the chromatograms of the two drinks will differ?

The possible chromatogram of the second drink is shown above in red.

All peaks remain the at the same R_t value but and the heights of the peaks, excluding caffeine, will also be at the same height. Caffeine will have a peak height of 0.48 X (1.30/0.85) = 0.73 for absorbance.

2) A sample of water was analysed for organic compounds using TLC. The chromatogram produced is shown on the right.



a) How many different compounds are present?

3

b) The organic compound that produced spot 1 has an R_f value of 0.75 and has moved 4.5 cm from the application point.

If "A" has moved 4.0 cm from the application point calculate its R_f value .

The R_f value is calculated by dividing the distance travelled by pigment A by the distance travelled by the solvent layer.

Step 1 calculate the distance travelled by the solvent.

=> 4.5 / x = 0.75 (Where x is the distance travelled by the solvent)

=> x = 4.5 / 0.75 = 6.0 cm

Step 2 calculate the R_f value of A

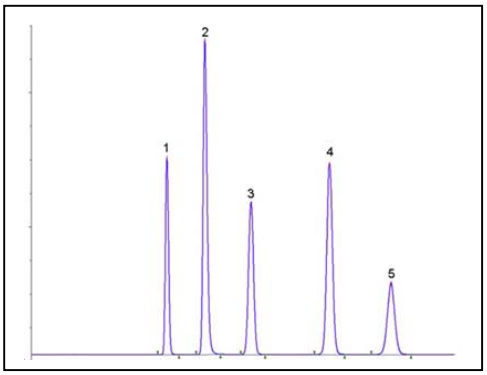
=> 4.0 / 6.0 = 0.67

c) Which of the compounds is adsorbed most strongly to the stationary phase?

B, as it travels less distance in the mobile phase.

3) An unknown sample "A" of a mixture of compounds was analysed using HPLC to produce the chromatogram shown on the right. Another sample "B", of the same mixture, was analysed under the same conditions but with some modifications, as mentioned below.

Discuss how the chromatogram of sample "B" will differ from the chromatogram shown on the right, with the following changes to the conditions.



i. The concentration of substance “2” in the sample is halved.

The height of the peak labelled 2 will be reduced by 50% all other peaks stay the same height. All peaks remain at the same R_t value.

ii. The temperature at which the column is run is increased.

All peaks move to the left indicating a decrease in R_t for each compound. Increase in temperature will increase the average kinetic energy of all compounds and will reduce the time that compounds adsorb to the stationary phase. Hence all compounds will come out form the column in less time.

iii. Smaller, more tightly packed beads, are used.

Smaller beads increase the surface area and hence the rate at which compounds are adsorbed to the stationary phase. This will have an impact on the R_t value of each compound. R_t values will increase .

iv. Increasing the pressure at which the mobile phase is forced through the column.

Increasing the pressure simply increases the force by which the solvent (mobile phase) is pushed through the column. This will result in a decrease in R_t.

v. Double the amount of the sample “A” placed in the column.

No change.

Increasing the volume of the sample should:

- have no impact on the concentration of each compound. Hence no impact on the height of each peak.

- not change the compound mixture so has not changed the position and number of peaks.