Lesson 1 Chromatography

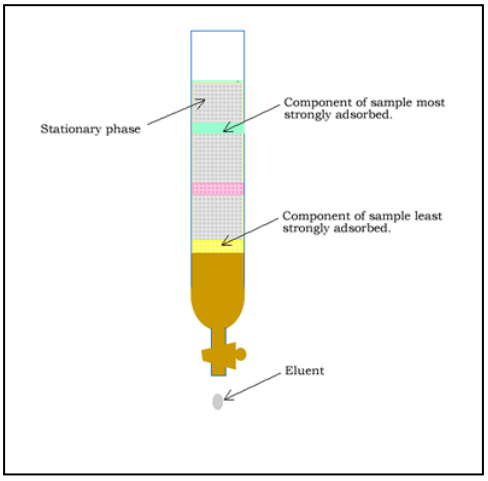
Click to revise column chromatography

Chromatography is a technique where by organic compounds can be separated and analysed.

All chromatographic techniques consist of a stationary and a mobile phase. The mobile phase is the mixture made of the solvent in which a mixture of compounds is dissolved. The mobile phase flows over the stationary phase. As the compounds in the mixture interact with the mobile and stationary phases they are slowed down to different degrees depending on the molecular structure of each compound.

The simplest of the chromatographic techniques is paper chromatography, pictured on the right. Thin layer chromatography, although similar to paper, consists of a glass plate covered with aluminium oxide. Both paper and thin layer chromatography are used for qualitative analysis only. They can identify the chemical present but are not sensitive enough to enable the calculation of concentrations.

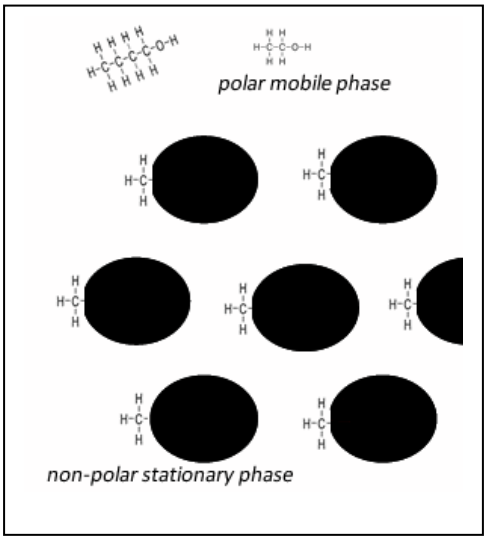
Column chromatography of HPLC is one technique that is more sensitive and can be used for quantitative as well as qualitative analysis, that is, the compound can be identified and its concentration calculated.



In column chromatography a mixture of compounds is forced through a column of chemically coated beads. The interaction between the beads and the compounds in the mixture determines the time each compound spends travelling through the column. This is known as the retention time R_t and is unique to each compound and hence can be used to identify compounds.

How does column chromatography work?

The molecules of each compound in the solvent interact with the stationary phase and mobile phase to different degrees. They ***adsorb*** (stick to) onto the stationary phase and ***desorb*** (release) from the stationary phase at different rates depending on their molecular structure.

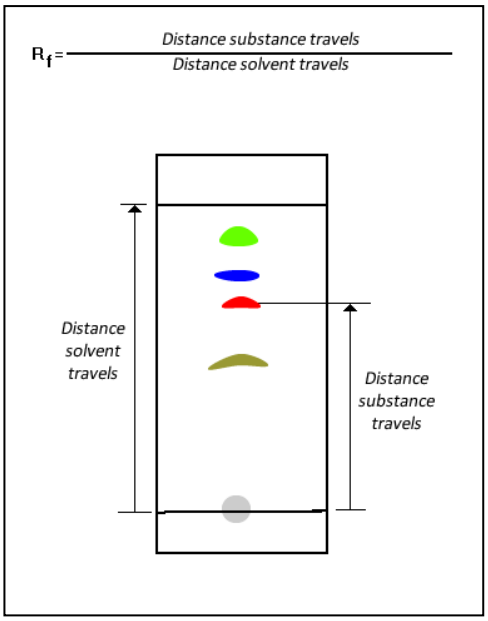


Lets look at a solution of ethanol and butanol. Water is used as the solvent and beads coated with methyl groups form the stationary phase. Butanol, having a longer hydrocarbon chain, will interact more with the stationary phase, than ethanol. Ethanol, being a smaller molecule than butanol, will readily dissolve in the mobile phase (water) and be swept down the column.

What does the chromatogram tell us?

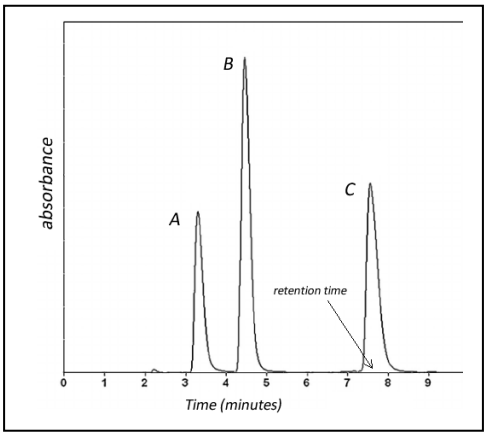
Keep in mind that TLC is not on the 2023-2027 study design but we cover it only briefly as a qualitative technique.

In TLC the chromatogram can be used to identify a specific substance by measuring the relative distance the substance travels to derive it R_f (retardation factor) value. The R_f value is unique to each compound when the chromatogram is developed using the same stationary phase and the same mobile phase. Hence, known R_f values can be compared to those of unknown substances in order to identify substances in a mixture.



In HPLC the chromatogram is made up of peaks representing individual compounds. Each peak appears along the x-axis at a unique time called the ***retention time R_t.*** This is the time it takes for the compound to appear in the eluent.

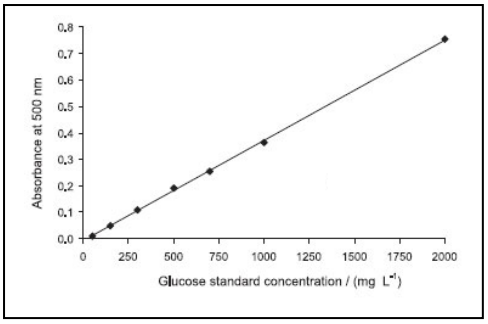
The area under the peak is proportional to the amount of substance in the sample being analysed or its concentration.



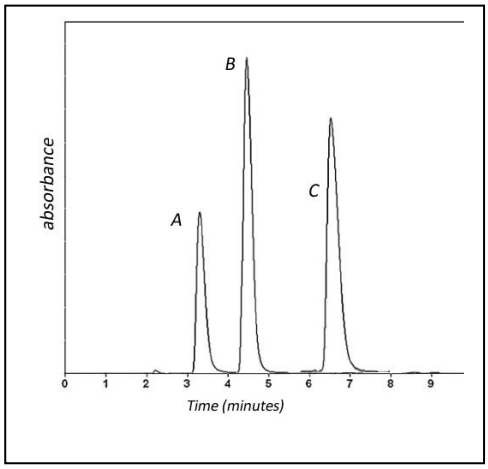
So the retention time (R_t) identifies the substance and the area under the peak indicates it's concentration. For extreme accuracy in identifying compounds, the column can be connected to a mass spectrometer where the eluent is diverted into the analysis chamber of the mass spectrometer where it will give a fragmentation pattern which can be compared against a computer database of known patterns. That means that the retention time is not needed to identity a huge range of compounds and hence the column is simply used to separate the components of the mixture.

In order for the concentration to be accurately determined a calibration curve of absorbance vs concentration must first be established using standard solutions.

An example of such a calibration curve is shown on the right. This calibration curve gives the relationship between absorbance and concentration. Once the absorbance of a peak is known the concentration of the substance can be determined by reference to the calibration curve.



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.

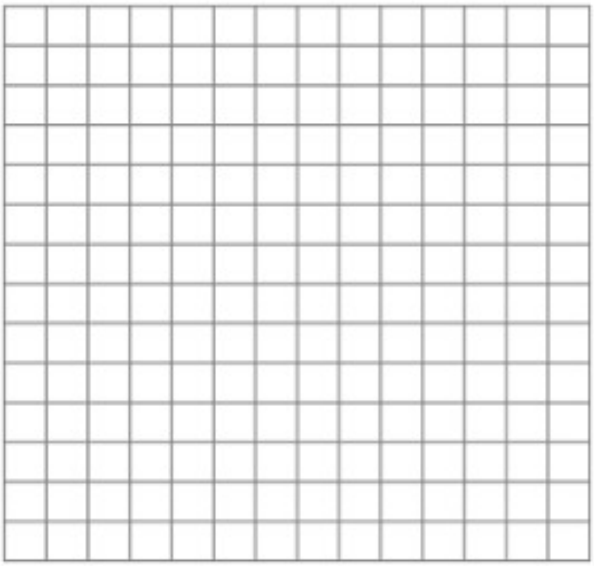


b) The initial mixture contained 0.35 mM of methanol, 0.75 mM of ethanol and 0.65 mM of butanol.
The chromatogram shown on the right was obtained.
Another mixture of methanol, ethanol and butanol was separated using the same column, under the same conditions. This mixture contained 0.70 mM of methanol, 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

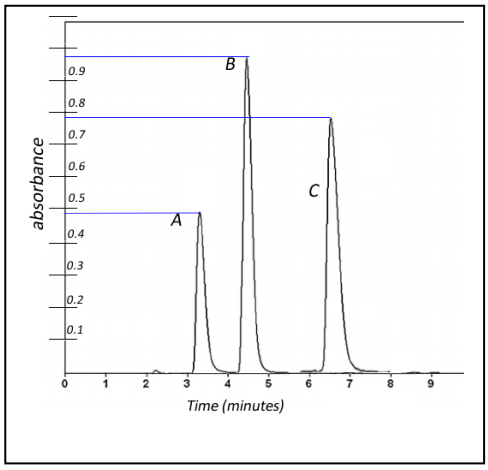
2) A drink is to be analysed for its caffeine content. A set of standard solutions were made up and their absorbance measured, the table below shows the results.

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 on the right, was produced.

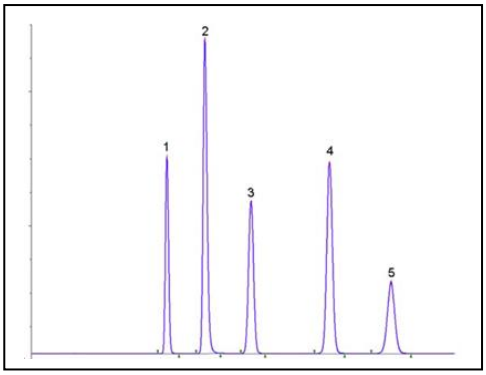


i. If the retention time of caffeine in the column was 3.3 minutes, what is the concentration of caffeine in the sample.

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

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

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.

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

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

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

v. Double the amount of the sample "A" placed in the column.

Solutions