CHROMATOGRAPHY

Chromatography is a technique that is used to **separate** the components of a mixture by using two separate phases:

- A **stationary phase** (a solid or liquid substance that is coated onto a solid surface).
- A **mobile phase** (a gas or liquid that moves through the stationary phase).

Separation of sample components may be achieved in a variety of ways, the most common being via **adsorption chromatography**.

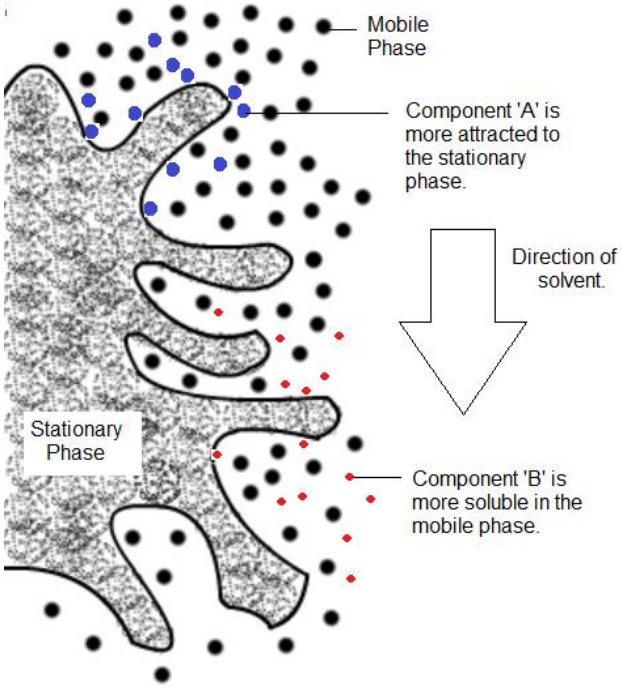
When the sample is applied to the stationary phase, it is subjected to two opposing influences:

(a) Each component in the mixture will be attracted to the stationary phase via intermolecular forces of attraction. This attraction will cause each component to be adsorbed onto the surface of the stationary phase.

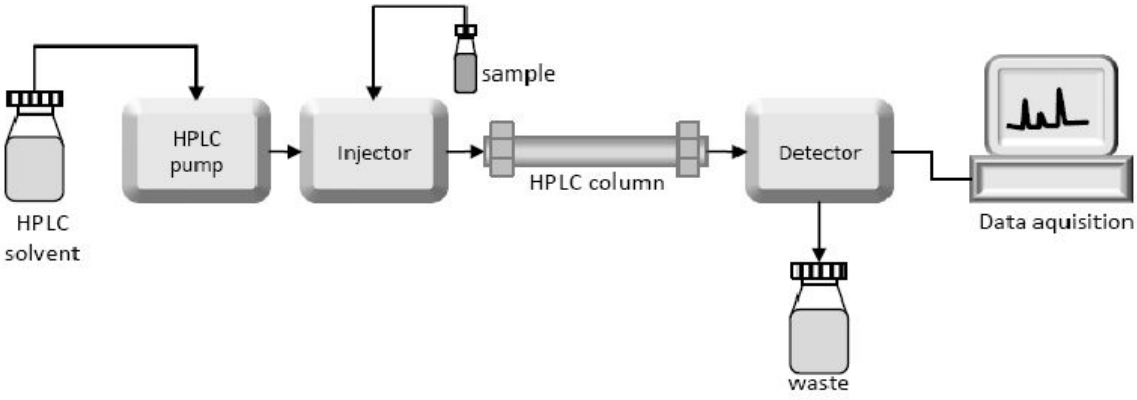
(b) Each component will also be soluble to some extent in the mobile phase due to intermolecular bonding. Hence, components will desorb from the stationary phase back into the mobile phase.

As each component in the mixture moves through the column, they are **repeatedly** adsorbed onto the stationary phase and then dissolve back into the mobile phase.

As different components have different degrees of attraction to the two phases, some components of the mixture will spend more time adsorbed onto the stationary phase while others will spend more time dissolved in the solvent. This is the basis of their separation.



HIGH PERFORMANCE LIQUID CHROMATOGRAPHY



High performance liquid chromatography (HPLC) is a specialised form of column chromatography. It allows organic molecules at low concentrations to be separated from a mixture. These components can then be quantitatively analysed.

In HPLC:

- The stationary phase consists of a solid that has been coated in a viscous liquid. The solid material is made from very fine particles (10 to 70 times smaller in surface area than the particles used in traditional column chromatography). The small particle size allows for more frequent adsorption and desorption of components, resulting in better separation of components.

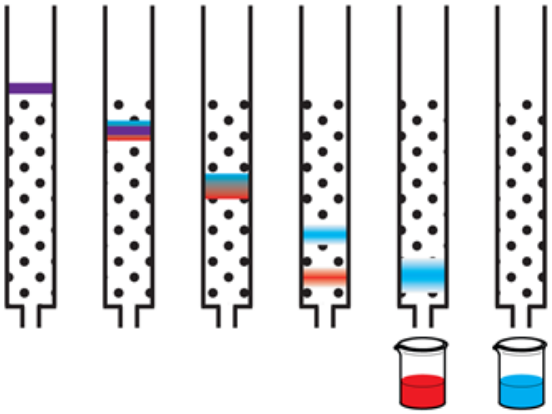
- The mobile phase is pushed through the column using very high pressures. This is due to the extremely small size of the stationary phase particles which causes high resistance to flow.

- The eluent (mobile phase that leaves the column), is collected and analysed using techniques such as UV-visible spectroscopy. This allows a chromatogram to be generated.

- Different components of the mixture will travel through the column at different rates which causes them to separate.

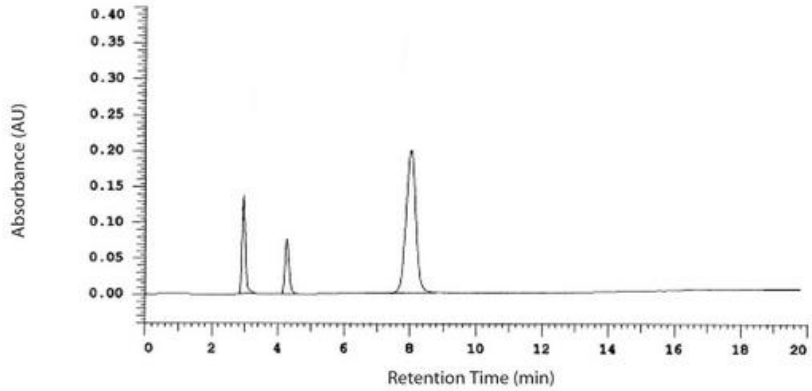
  Components more attracted to the stationary phase will take longer to pass through the column.

  Components more soluble in the mobile phase will pass more quickly through the column.



INTERPRETING CHROMATOGRAMS

As each component of a sample leaves the HPLC column, it is analysed. If this is done via some type of spectroscopy (e.g. UV-visible spectroscopy). The results of the analysis will be in the form of a graph where the y axis is absorbance and the x axis is the time taken for each component to leave the column.



A chromatogram provides information about:

- The identity each component of a sample (qualitative analysis).
- The concentration of each component of a sample (quantitative analysis).

Qualitative Analysis

- Each peak on a chromatogram is generated as different components of the mixture leave the column.
- The time taken for the component to leave the column is called its retention time (R_T).
- Each component will have a unique retention time. Therefore, components can be identified by comparing their retention times to that of known substances analysed under identical conditions. If the retention times match, then the peaks are due to the same substance.
- Those components that are not strongly attracted to the stationary phase reach the end of the column more quickly, and hence their retention times will be shorter.

Quantitative Analysis

The spectrometer also analyses the area under each peak. Peak area is proportional to the amount of each component in the sample.

To quantitatively analyse a known component of a sample, a set of standard solutions are analysed so that a calibration curve can be generated.

The position of a peak (R_T value) is used to determine the nature of each component.

The area under each peak represents the relative amounts of each of the sample components.

Note:

- Longer columns provide a better resolution of sample components.
- The R_T value is NOT influenced by the concentration of the sample components.
- The Retention Time (R_T) is characteristic of the component under the conditions that the chromatographic process is performed.

QUESTION 35
Chromatography is used to

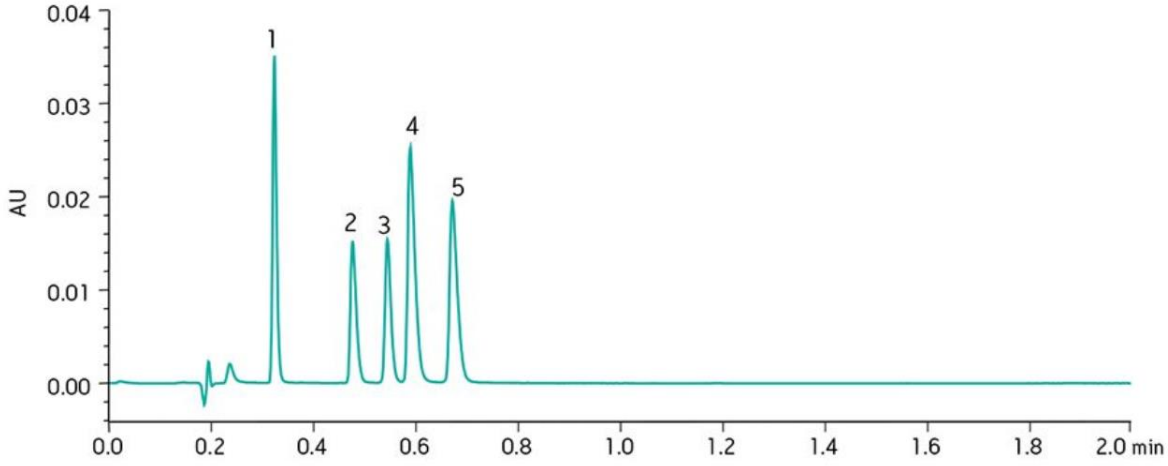
A Separate two or more compounds based on their polarities.

B Separate two or more compounds based on their masses.

C Separate two or more compounds based on how strongly they interact with other compounds.

D More than one of the above.

QUESTION 36
The chromatogram obtained from a mixture of five compounds is given below.



(a) Which compound has the lowest retention time?

(b) Which compound showed the greatest affinity for the stationary phase?

(c) Which compound is the largest?

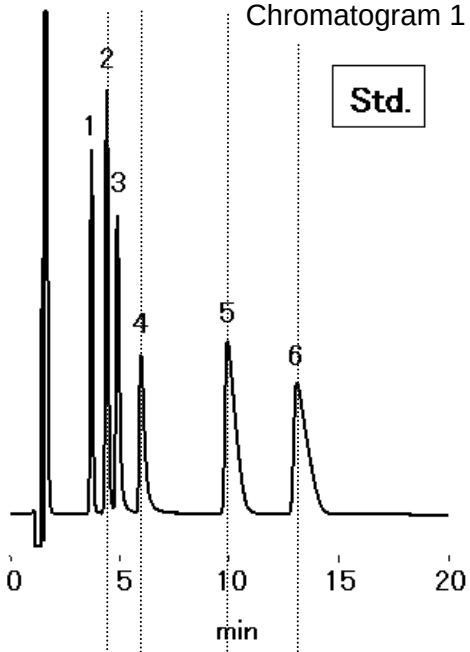
(d) Which compound is present in the highest concentration?

QUESTION 37

The table below contains ions that are commonly found in water samples. A solution containing all of these ions was analysed using HPLC. The results are shown in chromatogram 1.

Peak Number	Ion
1	Li^+
2	Na^+
3	NH_4^+
4	K^+
5	Ca^{2+}
6	Mg^{2+}

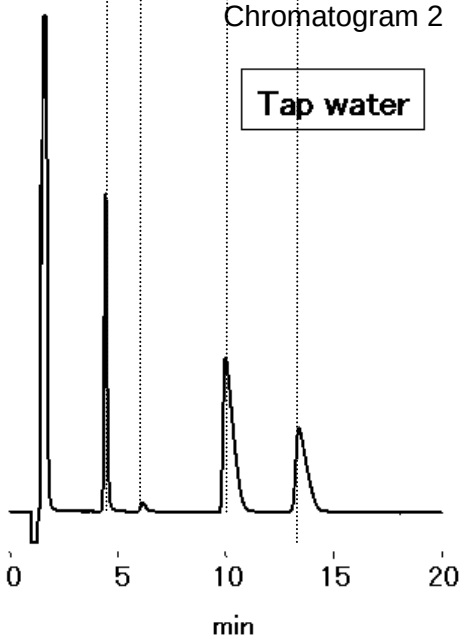
Chromatogram 1



Chromatogram 2 shows the results of a Melbourne water sample that was tested under identical conditions.

Identify which ions can be found in Melbourne's water.

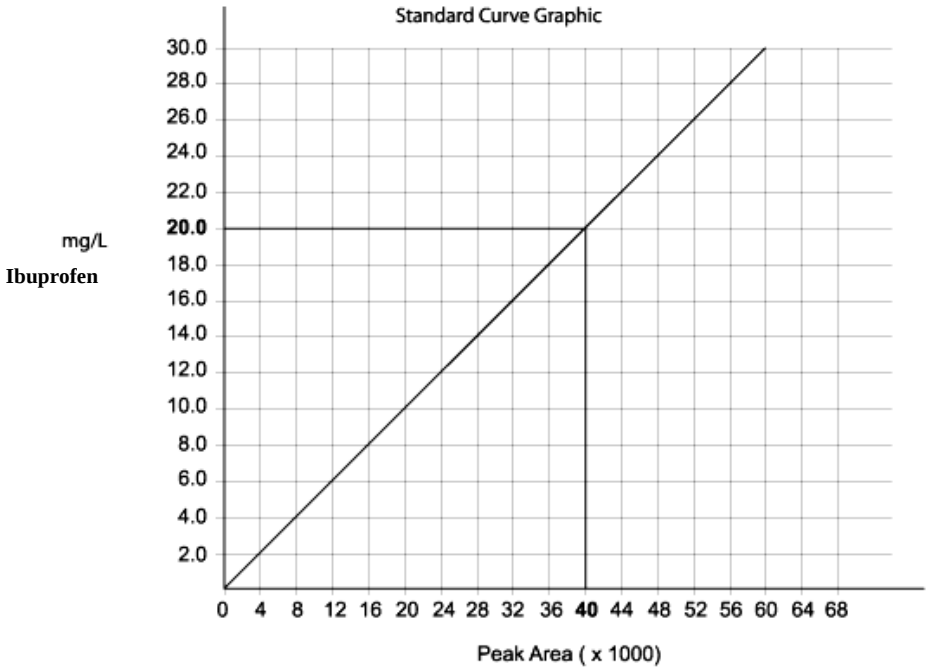
Chromatogram 2



QUESTION 38

The concentration of ibuprofen in pet medication was determined by HPLC. A $50.00\,mg$ tablet was crushed and dissolved in $100.00\,ml$ of deionised water. A $1.00\,ml$ aliquot was added to $4.00\,ml$ of mobile phase and then applied to the top of the column. A peak area of $50,000$ units was obtained.

Each ibuprofen standard was applied to the same column under identical conditions and the area of each peak produced was also measured. The results are shown below.



(a) Calculate the concentration of ibuprofen in the $100.00\,ml$ solution in mg/L.

(b) Calculate the amount, in mg , of ibuprofen in each $50.00\,mg$ tablet.

PREDICTING ELUTION ORDER

Which component of a sample is eluted first depends upon the polarity of the stationary phase and mobile phase used, and the relative affinities of the sample components to both phases.

More specifically, the patterns in elution can be predicting by comparing the net strength of bonding that occurs between each sample component and the stationary phase.

Note:

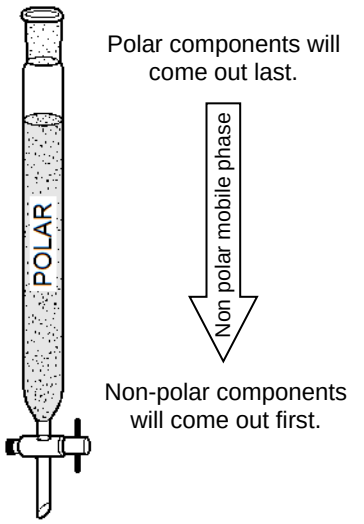
Molecules will not only form H bonding and ionic interactions with the stationary phase – they are able to interact with the stationary phase by means of dispersion forces as well.

NORMAL PHASE CHROMATOGRAPHY

(Polar stationary phase and a non-polar mobile phase)

The more **polar** the molecule, the greater the net strength of interparticle bonding between the component and the stationary phase, and hence the greater the degree of adsorption to the stationary phase. These particles will therefore be retained in the column for longer periods, hence **retention times will increase**.

Non-polar components will be less attracted to the polar stationary phase and more attracted to the non-polar mobile phase. They will have the **shortest retention times**.

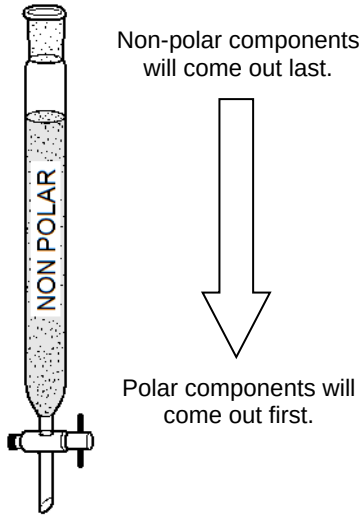


REVERSE PHASE CHROMATOGRAPHY

(Non polar stationary phase and a polar mobile phase)

The more **non-polar** a molecule, the greater the net strength of interparticle bonding between the component and the stationary phase, and hence the greater the degree of adsorption to the stationary phase. These particles will therefore be retained in the column for longer periods, hence **retention times will increase**.

Polar components will be less attracted to the non-polar stationary phase and more attracted to the polar mobile phase. They will have the **shortest retention times**.



GIVEN A MIXTURE OF COMPOUNDS OF THE SAME CHAIN LENGTH FROM DIFFERENT HOMOLOGOUS SERIES

	Normal Phase (Polar Stationary Phase)	Reverse Phase (Non-polar Stationary Phase)
Order of Elution (First to Last)	Alkanes *(Alkenes)** Esters Alcohols Carboxylic Acids	Carboxylic Acids Alcohols Esters *(Alkenes)** Alkanes
	Most non polar component eluted first	Most polar component eluted first

GIVEN A MIXTURE OF COMPOUNDS FROM THE SAME HOMOLOGOUS SERIES (DIFFERENT CHAIN LENGTHS)

The shorter chains are eluted first. This applies to both normal phase and reverse phase chromatography.

The longer a chain within a homologous series, the more dispersions forces that are formed between the chain and the stationary phase and hence the stronger the net interparticle bonding between the chain and the stationary phase. Retention times will therefore increase as chain length increases.

QUESTION 39
A mixture containing butanoic acid, hexanoic acid and methanoic acid was separated using HPLC. Which of the following statements is most correct? Give a reason to support your answer.

Statement 1: The order in which the molecules would be eluted in normal phase chromatography is hexanoic acid, butanoic acid followed by methanoic acid.

Statement 2: The order in which the molecules would be eluted in reverse phase chromatography is methanoic acid, butanoic acid followed by hexanoic acid.

Solution