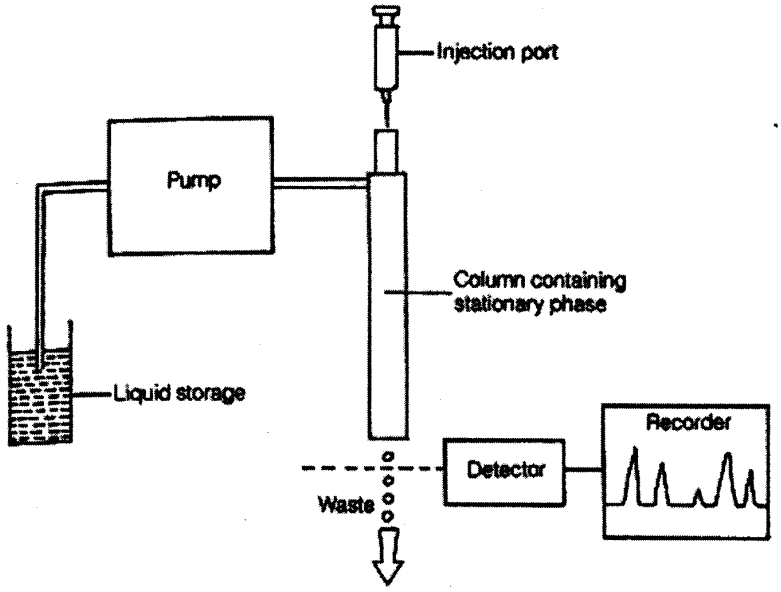
HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (A QUALITATIVE AND QUALITATIVE TECHNIQUE)

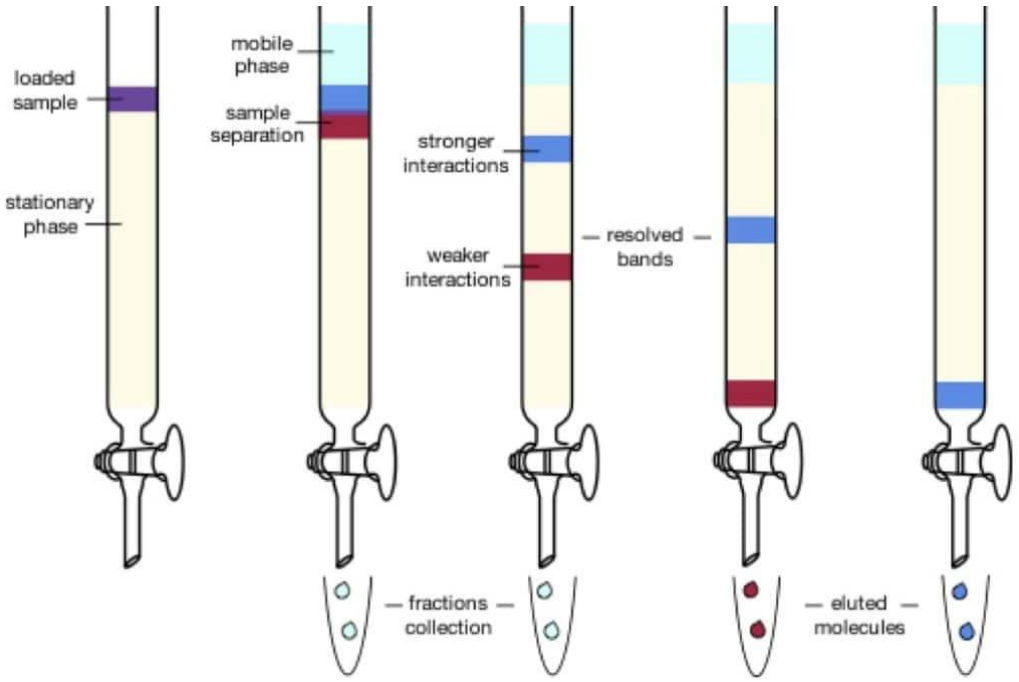
High performance liquid chromatography (HPLC) is a much more sensitive and useful technique than paper and thin layer chromatography. The instrument used for HPLC is called a **high performance liquid chromatograph**. A cross-section of such an instrument is shown below:



The main design features of this instrument are:

- **Liquid storage:** Holds the solvent which is going to act as the mobile phase.
- **Pump:** Pushes the solvent through to the column at high pressure.
- **Injection port:** The region where the sample that is to be analysed enters the instrument. The sample which is in the form of a solution or liquid is collected in a syringe and injected into the column.
- **Column:** Acts as the stationary phase. It is packed with very small particles that provide a large surface area for the adsorption of the molecules from the sample. The small particle size creates a large resistance to the flow of the mobile phase so the solvent needs to be pushed through at high pressure.
- **Detector:** Detects individual components that came from the eluent stream. The eluent stream is what the sample and solvent are referred to once they have passed through the length of the column.
- **Recorder:** Presents the information obtained from the detector in the form of a graph. Individual components are identified as different peaks on the graph. The area under each peak indicates the quantity of the component and the retention time identifies the component.

How does a high performance liquid chromatograph work?



In principle, HPLC and paper chromatography are very similar processes. They both rely on the different rates of adsorption onto a stationary phase and desorption into a mobile phase to separate the components in a mixture. The following table shows the features that the 2 processes have in common:

Paper chromatography	**Equivalent feature in HPLC**
Solvent and beaker	Solvent and liquid storage
Paper (stationary phase)	Column (stationary phase)
Origin line	Sample injection
R_f values	Retention times

HPLC is used to analyse mixtures that contain compounds with molecules of various sizes. A mixture is injected into the column. The liquid solvent is pumped through the column at high pressure. The molecules of the compounds in the mixture interact with both the solvent and the column. Some of the molecules will be more strongly attracted to the column than they are to the solvent. These molecules will take a long time to pass through the whole length of the column before they reach the detector. Other molecules will be more strongly attracted to the solvent than they are towards the column. As a result, these molecules will spend most of their time moving with the solvent and will consequently pass through the column quickly.

Once the molecules of a compound reach the detector, their **retention time (R_T)** for that compound is recorded. This is the time that a component takes to pass through the column. No two compounds will have the same retention time so R_T can be used to identify a compound by comparing it with the retention times of known compounds that were passed through the instrument under the same conditions.

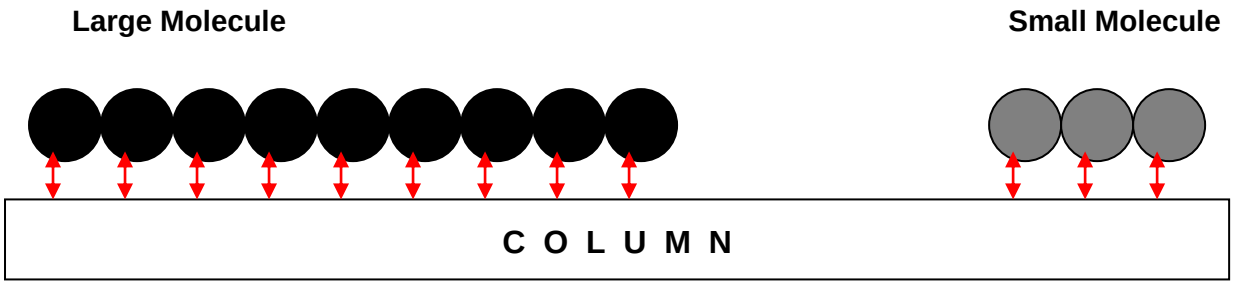
Why do different compounds have different retention times?

The chemical and physical properties of the molecules introduced into the column will determine how strongly they will attract to the mobile and stationary phases of the instrument. The most significant physical property is the size of the molecules. The greater the molar mass of the molecules the greater the attraction for the column stationary phase. Therefore, the retention time of a compound is directly proportional to the size of its molecules.

When molecules interact with the column they do so by forming bonds with the particles lining its surface. Molecules form bonds with the column over the whole length of their surface. Therefore, it takes long molecules more time to attach and detach from the stationary phase because there are more interactions involved compared to smaller molecules. Consequently, the larger the molecule the longer the retention time.

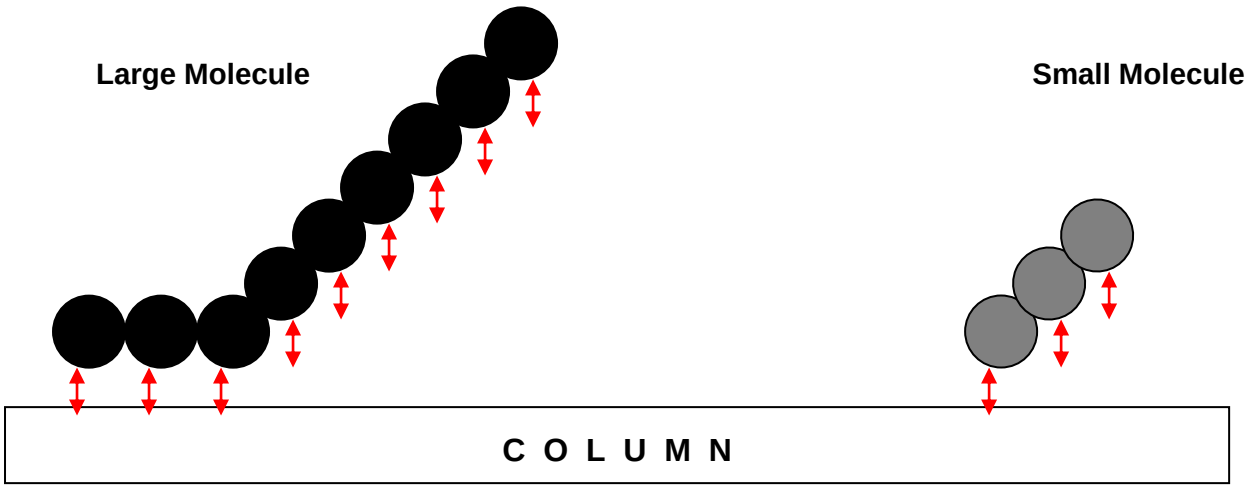
MOLECULES ADSORBED ON COLUMN

The red arrows show the attractive forces that are holding the molecules to the column.



MOLECULES DETACHING FROM COLUMN

- The larger molecule has more attractive forces (shown by the red arrows) with the column that need to be overcome compared to the smaller molecule.

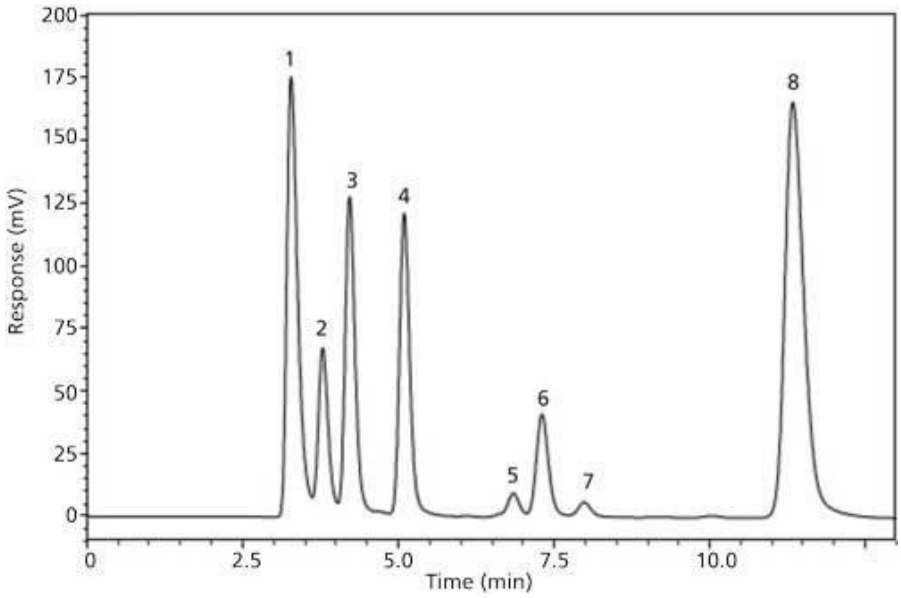


Besides retention time, which provides qualitative information only, the actual quantity of the compounds that have passed through to the detector can be determined. UV light is shone onto the eluent stream which contains a component with a particular retention time. The amount of UV light absorbed by the component is proportional to its quantity. This information is presented in the form of a graph which shows a peak for each component that passed through the instrument. The greater the area under each peak the greater the quantity of the component. A compound of unknown concentration can be analysed by having the area of its peak compared with those of known standards.

Applications of HPLC

- Suited to substances with molar masses greater than 300 $gmol^{-1}$.
- Can be used to analyse the contaminants in food.
- Used in hospitals to detect and measure the concentration of chemicals in blood.

QUESTION 1



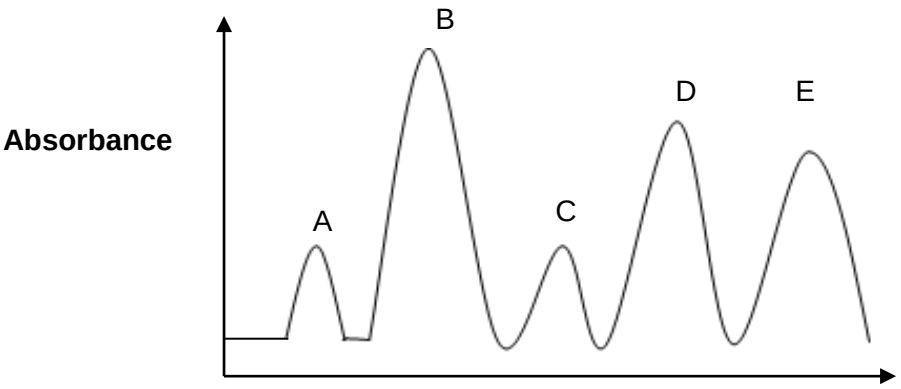
Referring to the above graph:

(a) Identify the peak which corresponds to the component which took the shortest amount of time to pass through the column.

(b) Identify the peak corresponding to the component present in highest quantity.

Solution

QUESTION 2
A mixture of hydrocarbons is introduced into a high performance liquid chromatograph. The chromatogram of this mixture shows that there are 5 different hydrocarbons present. These have been labelled as A, B, C and D.



Analysis of the chromatogram reveals that the hydrocarbons present are C_7H_{16}, $C_{15}H_{32}$, $C_{11}H_{24}$, C_9H_{20} and $C_{13}H_{28}$.

(a) Give the name of the hydrocarbons that are responsible for peaks A, B, C, D and E.

(b) Which hydrocarbon is present in the greatest quantity?

Solution

QUESTION 3

HPLC is a technique that can be used to analyse the organic compounds found in food. A food Chemist wishes to determine the amount of riboflavin found in a popular breakfast cereal. She dissolves 10 g of crushed cereal in 100 mL of 0.05 M sulfuric acid. Riboflavin standards were prepared. Each standard and the sample were injected into the HPLC and the area of each peak produced was measured. The results are shown in the table below:

Riboflavin standards (ppm)	Peak Area
0	0.00
2	25,000
4	49,000
6	76,000
8	100,000
10	127,000

The cereal sample produced a peak area of 17,000.

(a) For the above results construct a calibration graph of peak area versus concentration.

(b) What is the concentration of riboflavin in the cereal? State your answer in mg / 100 g of cereal.

(c) Would the standards and unknown cereal sample have peaks with the same retention times? Give a reason for your answer.

SOLUTIONS

QUESTION 1

(a) Peak 1.

(b) Peak 8 corresponds to the component present in highest quantity. This is because the area underneath its peak is the highest.

QUESTION 2

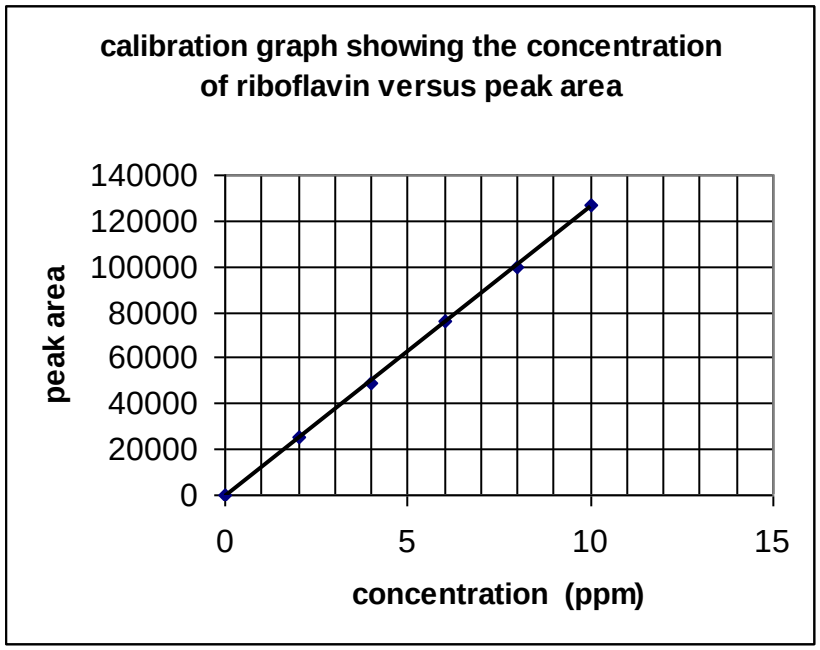
(a) The larger the hydrocarbon molecule the greater the number of interactions formed with the column stationary phase and hence the longer the retention time. Therefore:

A = C_7H_{16}
B = C_9H_{20}
C = $C_{11}H_{24}$
D = $C_{13}H_{28}$
E = $C_{15}H_{32}$

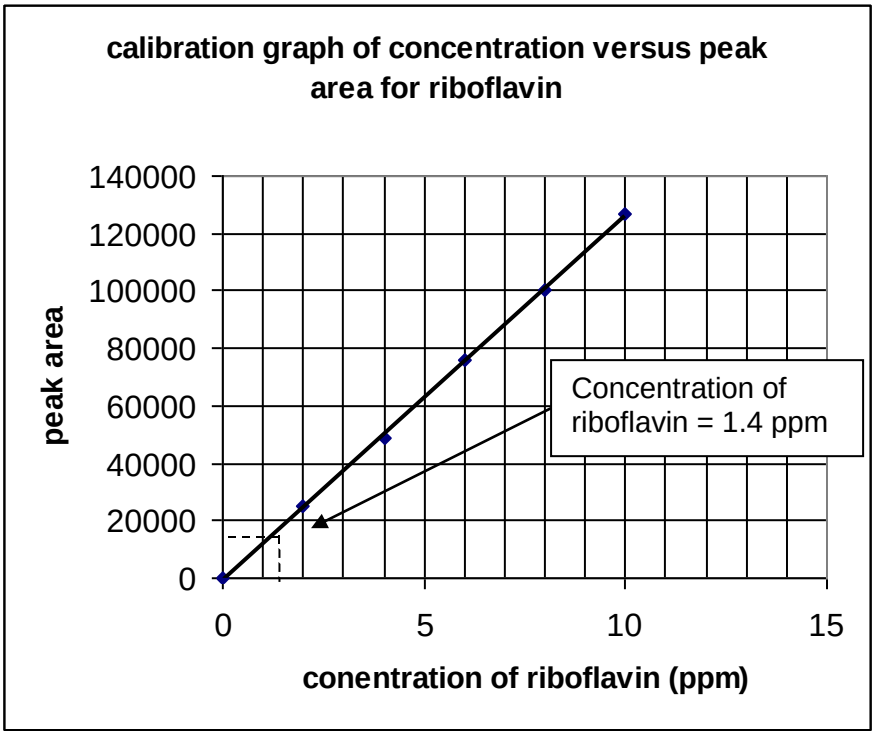
(b) The hydrocarbon at peak B (C_9H_{20}) has the greatest peak area. Therefore, it is present in the greatest quantity.

QUESTION 3

(a)



(b) Compare the peak area of the cereal sample with that of the standards:



10 g breakfast cereal

↓ add 100 mL of acid

100 mL of solution (peak area = 17,000)

Concentration of riboflavin is 1.4 ppm, therefore

10^6 mL of solution → 1.4 g
100 mL of solution → x

$x = \frac{1.4 \times 100}{10^6} = 1.4 \times 10^{-4}$ g in 100 mL of solution

Therefore 10 g of cereal also contains 1.4×10^{-4} g of riboflavin.

$1.4 \times 10^{-4} \times 10^3 = 0.14$ mg

10 g cereal → 0.14 mg
100 g cereal → x mg

$x = \frac{0.14 \times 100}{10} = 1.4$ mg/100 g

(c) As the unknown cereal sample has riboflavin in it, a peak will be produced at the same retention time as the standards.