HPLC – CONCEPT TEST

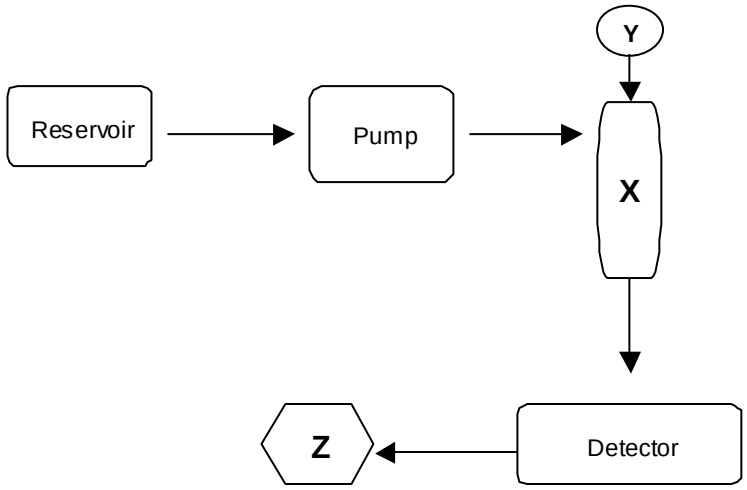
QUESTION 1
Which of the following statements is correct? In HPLC systems:

A The mobile phase can be a liquid or a gas.
B The stationary phase is inside a metal column.
C The sample needs to be vaporised before it can be injected.
D A sample cannot be recovered as it is destroyed.

QUESTION 2
Which of the following is not true about HPLC systems?

A The mobile phase is pumped under pressure.
B A variety of column packing materials is available.
C It is suitable for both heat resistant and heat sensitive substances.
D It is widely used in food analysis.

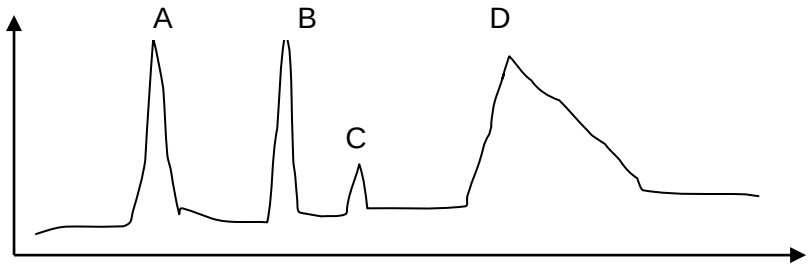
QUESTION 3
The following represents a schematic HPLC system:



Which of the following statements is correct?

A The sample is injected at Z.
B The solvent is stored at Y.
C The sample is recovered at Y.
D The chromatogram is generated at Z.

QUESTION 4
This chromatogram was produced using a high performance liquid chromatograph.



Which statement regarding this chromatogram is correct?

A Peak D corresponds to the component with the least attraction for the stationary phase.
B Peak A corresponds to the most volatile component.
C This chromatogram has very poor resolution.
D Peak C corresponds to the component present in the greatest quantity.

QUESTION 5
The factor which has the greatest influence on the retention time of a component in HPLC is

A The size of the molecules making up the component.
B The quantity of the component.
C The rate of flow of the inert carrier gas.
D The interaction of the eluent stream with UV radiation.

QUESTION 6
Which pair of variables provides qualitative information only?

A Retention time and peak area
B Retention time and retention factor
C Peak area and retention factor
D Peak area and molecular size

QUESTION 7
During HPLC, the liquid mobile phase is pumped through the column at high pressure because

A High pressure is required for the desorption of the components into the mobile phase.

B The small size of the particles in the column creates a large resistance to the flow of the mobile phase.

C The components in the mixture will not adsorb onto the column at low pressure.

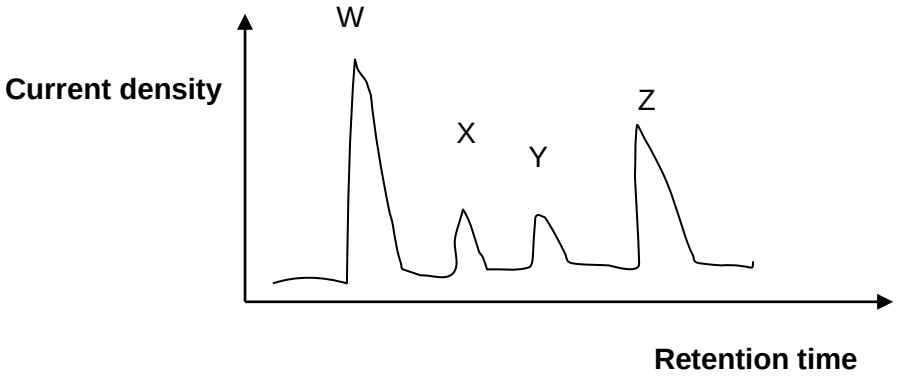
D Molecules with molar masses larger than 300 $gmol^{-1}$ will decompose at lower solvent pressures.

QUESTION 8
List one advantage and one disadvantage of using small particles in the column of a high performance liquid chromatograph.

Solution

QUESTION 9

A HPLC chromatogram produced from a mixture of alcohols is shown below. Four peaks labelled W, X, Y and Z are shown.



The formulae of the alcohols are:

$C_{30}H_{61}OH$, $C_{13}H_{27}OH$, $C_{20}H_{41}OH$, $C_{10}H_{21}OH$, $C_{15}H_{31}OH$

(a) The mixture contains 5 different alcohols but the chromatogram contains only 4 peaks.

Which alcohol does not have a peak on the chromatogram? Give a brief explanation for your answer.

(b) Excluding the alcohol you identified in part **a**., place the letters W, X, Y and Z in the appropriate spaces below so as to correctly identify the peaks in the chromatogram. Give a brief explanation for your answer.

$C_{30}H_{61}OH$ _____

$C_{13}H_{27}OH$ _____

$C_{20}H_{41}OH$ _____

$C_{10}H_{21}OH$ _____

$C_{15}H_{31}OH$ _____

QUESTION 10
Below are 5 statements relating to the procedure of paper chromatography. Each statement is incorrect. Rewrite each statement so that it contains correct information.

Statement 1
Using a felt tip pen, draw an origin line about 2 cm from the bottom of the chromatography paper.

Statement 2
Place a large concentrated drop of the sample to analysed onto the origin line.

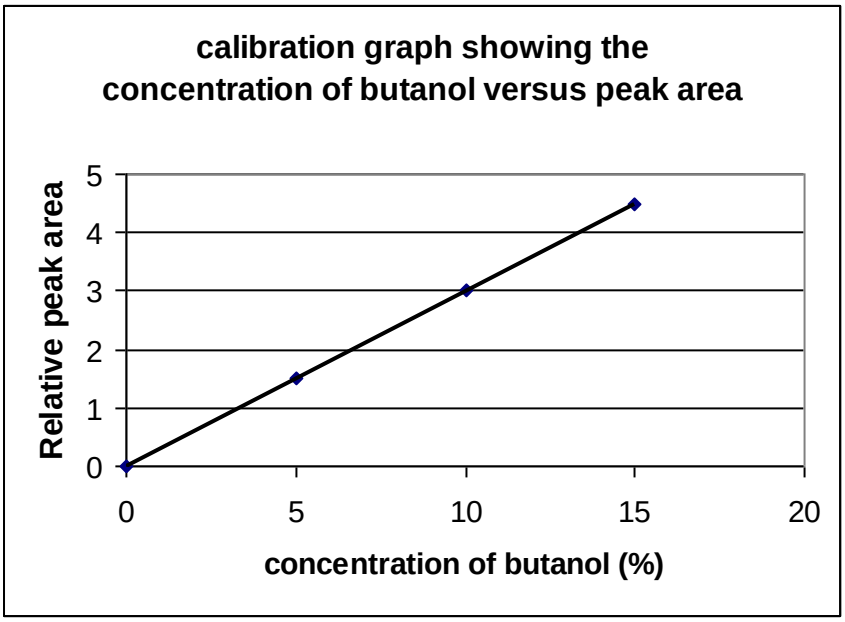
Statement 3
Immerse the paper into a suitable solvent so that the level of the solvent is above the origin line and sample.

Statement 4
Allow the solvent to rise as far up the paper as it possibly can.

Statement 5
Remove the paper from the solvent. Calculate the R_f value for each spot on the chromatogram by dividing the distance each spot travelled from the origin line by the full length of the chromatography paper.

QUESTION 11

A solution of butanol was assayed (quantitatively analysed) using HPLC. Standard solutions of butanol were analysed to produce a calibration graph of butanol concentration versus peak area. This graph is shown below:



The relative peak area of the unknown butanol sample was 2.

(a) Use the standard curve to determine the amount of butanol in the sample.

(b) Assuming that the concentration of the butanol is expressed as % v/v, what volume of butanol would be present in a 200 mL sample of this solution?

Solution

SOLUTIONS

QUESTION 1 Answer is B

QUESTION 2 Answer is C

QUESTION 3 Answer is D

QUESTION 4 Answer is A

QUESTION 5 Answer is A

QUESTION 6 Answer is B

QUESTION 7 Answer is B

QUESTION 8

One advantage – the small particle size presents a large surface area for the adsorption of compounds onto the stationary phase. This will result in better separation of the components.

One disadvantage – the small particle size creates a large resistance to the flow of the liquid mobile phase. Therefore, the added expense of pushing the liquid through at high pressure is necessary.

QUESTION 9

(a) The alcohol $C_{30}H_{61}OH$ will not have a peak on the chromatogram. This is because its molar mass is 438 $gmol^{-1}$. The high temperatures caused by the oven in GLC will cause most molecules with molar masses above 300 $gmol^{-1}$ to decompose. So the $C_{30}H_{61}OH$ molecules will decompose and cease to exist, hence their absence on the chromatogram.

(b)

$C_{30}H_{61}OH$	_____
$C_{13}H_{27}OH$	X
$C_{20}H_{41}OH$	Z
$C_{10}H_{21}OH$	W
$C_{15}H_{31}OH$	Y

Compounds with larger molecules will experience stronger interactions with the stationary phase, resulting in longer retention times as they will take the longest amount of time to pass through the column. Therefore, the alcohol with the smallest molecular size ($C_{10}H_{21}OH$) will correspond to the peak with the lowest retention time and so on.

QUESTION 10

Statement 1
Using a **lead pencil**, draw an origin line about 2 cm from the bottom of the chromatography paper.

Statement 2
Place **a small drop** of the sample to analysed onto the origin line.

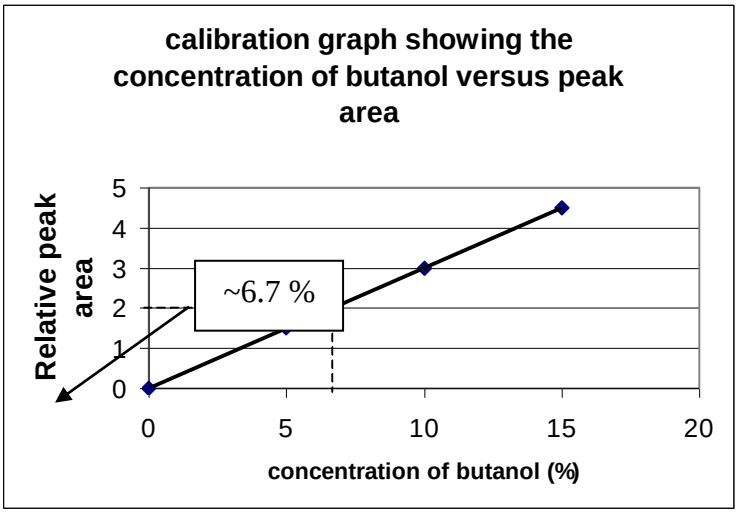
Statement 3
Immerse the paper into a suitable solvent so that the level of the solvent is **below** the origin line and sample.

Statement 4
Allow the solvent to rise to a **height that is about 1 or 2 cm from the top end of the paper**.

Statement 5
Remove the paper from the solvent. Calculate the R_f value for each spot on the chromatogram by dividing the distance each spot travelled from the origin line **by the distance that the solvent has travelled from the origin line to solvent front (the highest point on the paper that the rising solvent reached)**.

QUESTION 11

(a)



According to the standard curve, the concentration of butanol in the solution is about 6.7 %.

(b) Assuming that the concentration is 6.7 %v/v:

100 mL solution → 6.7 mL of butanol
200 mL solution → *x* mL of butanol

$x = 6.7 \times \frac{200}{100} = 13.4$ mL