HPLC CHROMATOGRAPY – TOPIC TEST 1

QUESTION 1
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.

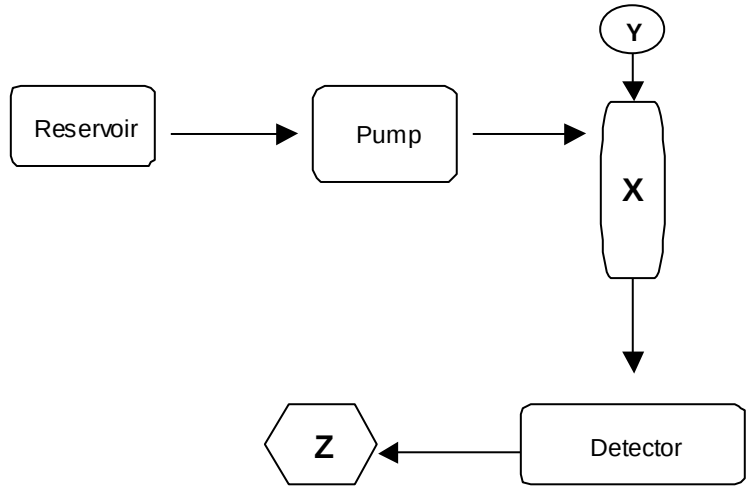
QUESTION 2
In HPLC, a substance with a high retention time:

A Has a high degree of adsorption into the stationary phase.
B Has a high degree of adsorption into the mobile phase.
C Remains in solution for long periods of time.
D Takes a long time to go through the detector.

QUESTION 3
Which of the following is not true about HPLC systems:

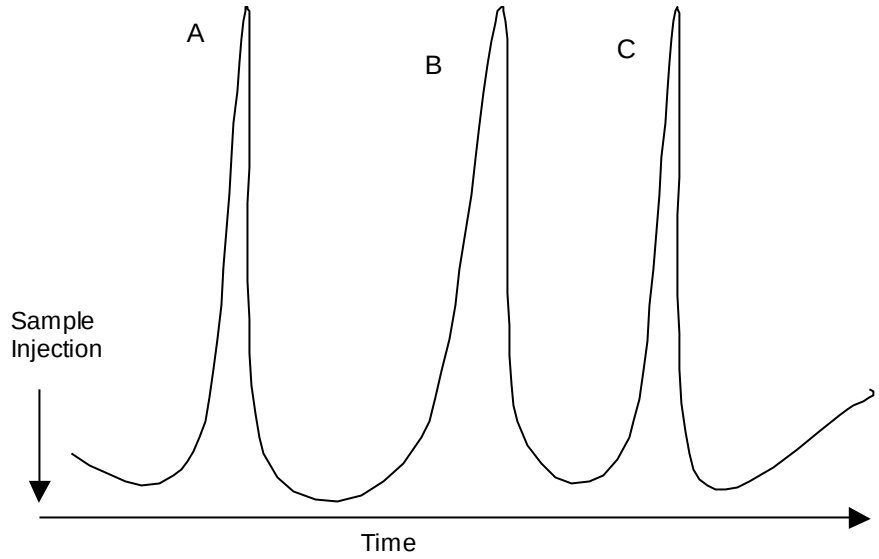
A The mobile phase is pumped under pressure.
B A variety of column packing materials is available.
C It is suitable for both heat resistant and heat sensitive substances.
D It is widely used in food analysis.

QUESTION 4
The following represents a schematic HPLC system:



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 5
The following represents a generalised chromatogram from an HPLC system:



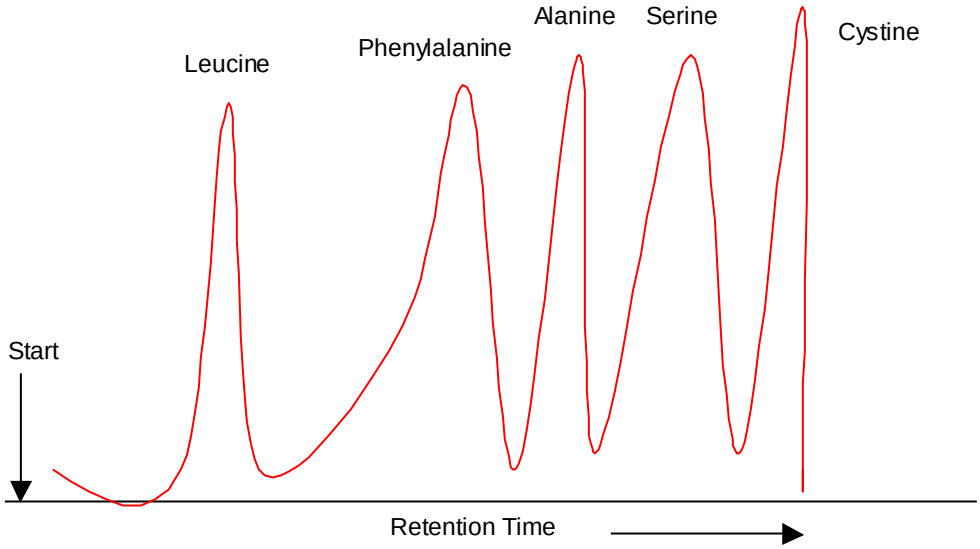
Which of the following statements is true about the above chromatogram?

A Substance A has the highest retention time.
B Substance C has the highest retention time.
C Substance B has the lowest retention time.
D Substance C has the lowest retention time.

QUESTION 6
Suggest reasons why analysis of vitamin C (ascorbic acid) is best carried out with an HPLC system and not gas chromatography.

Solution

QUESTION 7
The following is a chromatogram of the separation of five amino acids:

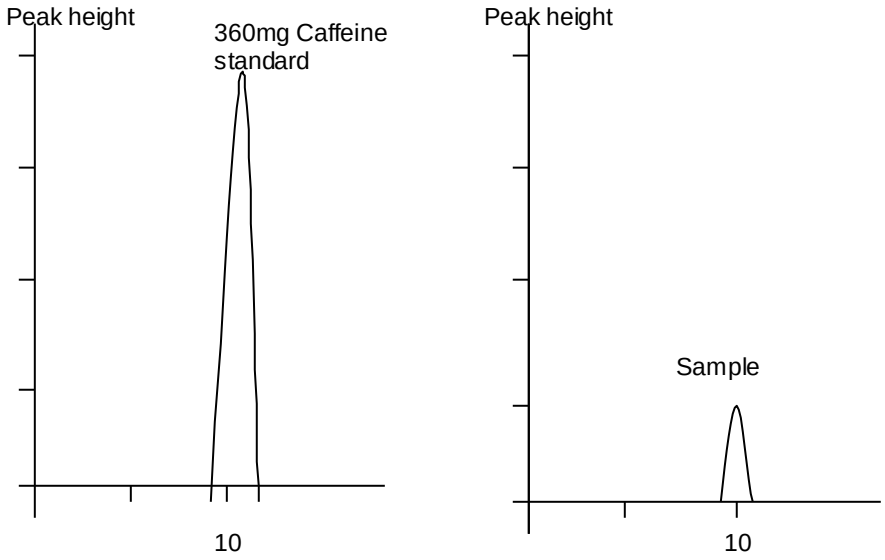


(a) Name the amino acid, which is most strongly adsorbed into the stationary phase.

(b) Name the amino acid with the highest affinity for the mobile phase.

(c) Suggest what change or changes would be necessary to alter the order and the retention times of the amino acids.

QUESTION 8
The following is a series of chromatograms depicting the peak heights and retention times of a caffeine standard and a sample from 100ml of instant coffee. The coffee sample was prepared by dissolving a level teaspoon of instant coffee in a standard 250mL cup.



(a) How can it be deduced that the sample peak is also caffeine?

(b) By comparing the peak heights, estimate the amount of caffeine in the sample.

(c) Calculate the amount of caffeine in the original 250mL cup

(d) Suggest a more accurate way of calculating the exact amount of caffeine from the peaks.

SOLUTIONS

QUESTION 1 Answer is B

QUESTION 2 Answer is A

QUESTION 3 Answer is C

QUESTION 4 Answer is D

QUESTION 5 Answer is B

QUESTION 6

Ascorbic acid is very heat sensitive and HPLC is carried out at room temperature, therefore there is less of a chance of decomposition. With GC, the sample is vaporised and also the column is heated, ascorbic acid would surely decompose at such temperature.

QUESTION 7

(a) Cystine

(b) Leucine

(c) Changes in solvent and/or column would change the retention time and eve the order of exit from the column.

QUESTION 8

(a) The retention time is around the same. *on a proper chromatogram it would be more evident.

(b) If 360mg = 4 units and sample = 1 unit. Caffeine in sample = 90mg

(c) 90mg/100mL

$\therefore$mg/250mL = 90 X 2.5 = 225mg

(d) Especially if peaks are wide, a more accurate method is to calculate the area under the peaks.