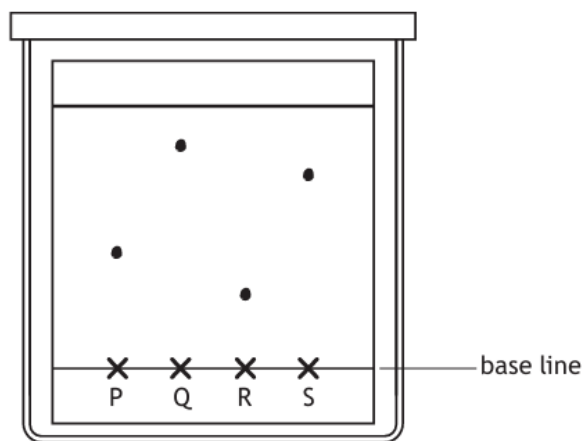


# Chromatography

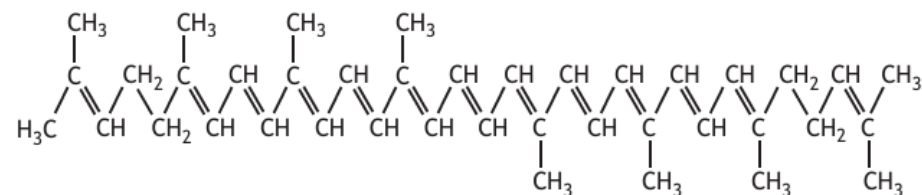
- Four amino acids, P, Q, R and S were analysed by chromatography.  
Larger molecules travel a shorter distance from the base line.  
Less polar molecules travel a greater distance from the base line.



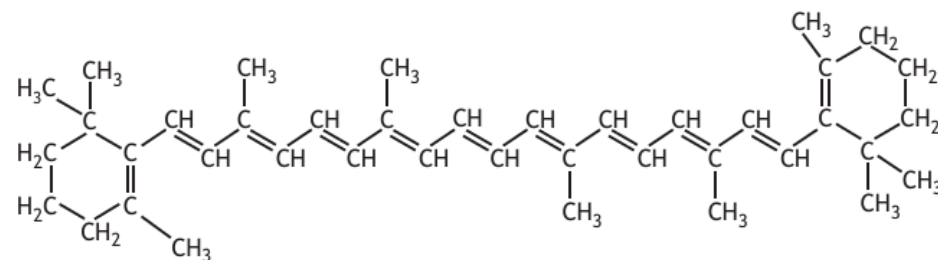
Which of the following statements is correct?

- A P is less polar than S
- B Q is a larger molecule than P
- C R is more polar than P
- D S is a smaller molecule than Q

- Tomato juice contains a mixture of terpenes including lycopene and beta-carotene.  
Terpenes can be separated using chromatography.



lycopene



beta-carotene

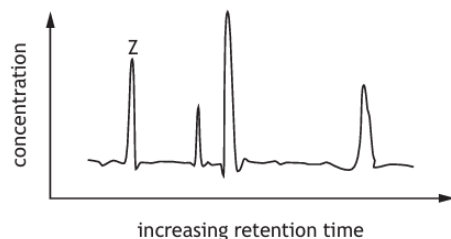
Which of the following is the most suitable solvent to separate lycopene and beta-carotene?

- A Ethanol
- B Pentane
- C Propanoic acid
- D Water

# Chromatography

3. A chemist analysed a mixture of four dyes, A, B, C and D, using gas-liquid chromatography. The time taken to travel through the column (retention time) depends on the polarity of the molecule. The more polar the molecule the longer the retention time.

The following chromatogram was obtained.



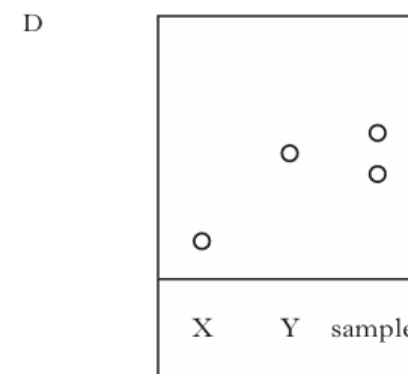
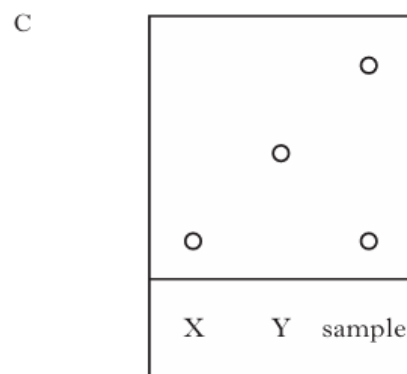
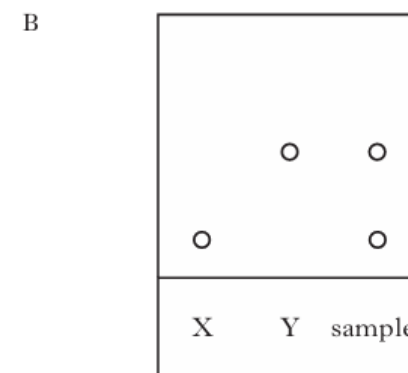
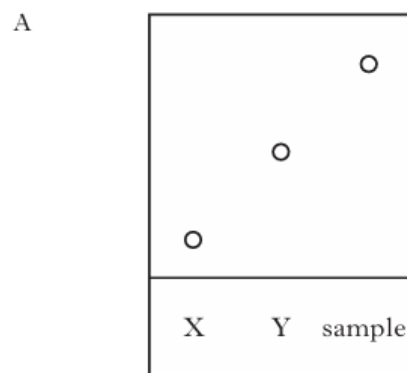
Which of the following compounds corresponds to peak Z?

Dye	Structure
A	
B	
C	
D	

4. An organic chemist is attempting to synthesise a fragrance compound by the following chemical reaction.
- $$\text{compound X} + \text{compound Y} \rightarrow \text{fragrance compound}$$

After one hour, a sample is removed and compared with pure samples of compounds **X** and **Y** using thin-layer chromatography.

Which of the following chromatograms shows that the reaction has produced a pure sample of the fragrance compound?

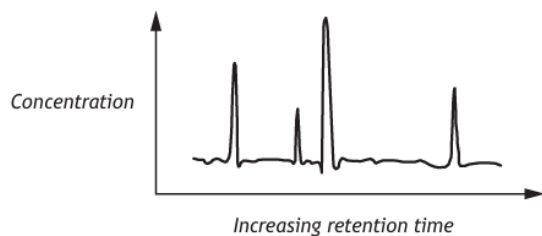


# Chromatography

5.

A chemist analysed a mixture of four dyes **A**, **B**, **C** and **D** using gas-liquid chromatography.

In this technique, compounds are separated depending on their polarity, with the most polar having the longest retention times. The chromatogram obtained is shown below.



Which of the following compounds was present in greatest concentration?

Dye	Structure
A	
B	
C	
D	

## Chromatography

1. ) Paper chromatography is often used to analyse the mixtures of amino acids produced when peptides are broken down.

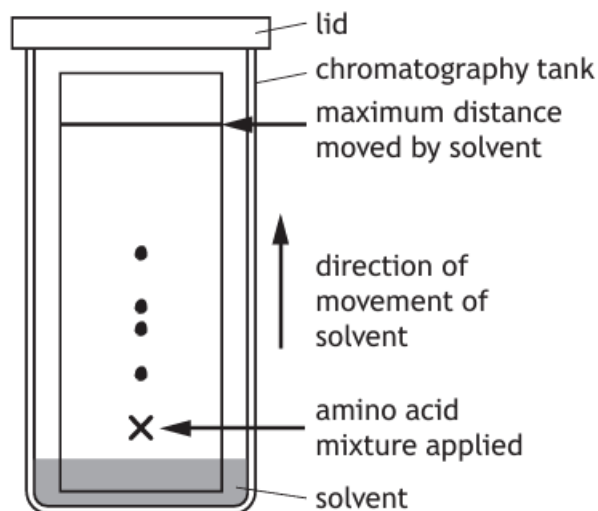
On a chromatogram, the retention factor  $R_f$ , for a substance can be a useful method of identifying the substance.

$$R_f = \frac{\text{distance moved by the substance}}{\text{maximum distance moved by the solvent}}$$

The structure of the pentapeptide methionine enkephalin was investigated.

A sample of the pentapeptide was completely hydrolysed into its constituent amino acids and this amino acid mixture was applied to a piece of chromatography paper and placed in a solvent.

The chromatogram obtained is shown below.



- (ii) It is known that this amino acid mixture contains the amino acid methionine. The  $R_f$  value for methionine in this solvent is 0.40.

Draw a circle around the spot on the chromatogram that corresponds to methionine.

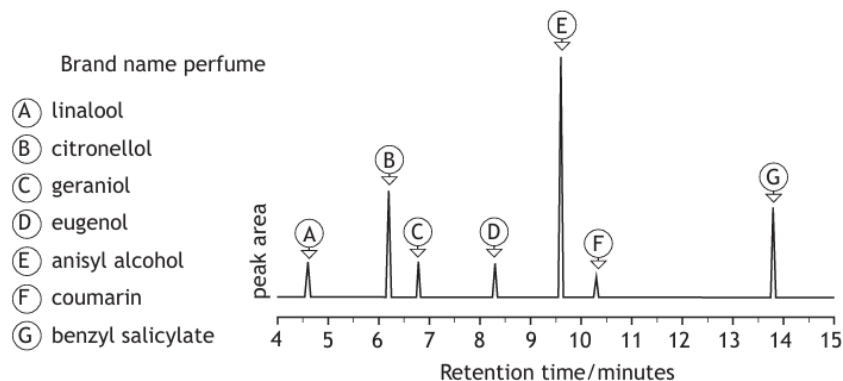
- (i) Suggest why only four spots were obtained on the chromatogram of the hydrolysed pentapeptide.

# Chromatography

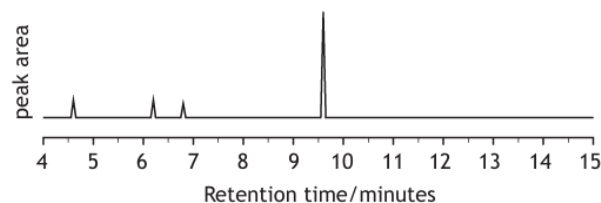
2.

Up to 10% of perfumes sold in the UK are counterfeit versions of brand name perfumes.

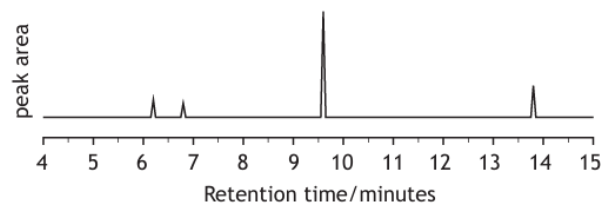
One way to identify if a perfume is counterfeit is to use gas chromatography. Shown below are gas chromatograms from a brand name perfume and two different counterfeit perfumes. Some of the peaks in the brand name perfume have been identified as belonging to particular compounds.



Counterfeit A



Counterfeit B



- (a) Identify one compound present in the brand name perfume that appears in both counterfeit perfumes.

1

- (b) Some compounds in the brand name perfume are not found in the counterfeit perfumes. State another difference that the chromatograms show between the counterfeit perfumes and the brand name perfume.

1

- (c) The gas used to carry the perfume sample along the chromatography column is helium.

- (i) Suggest why helium is used.

1

- (ii) Apart from the polarity of the molecules, state another factor that would affect the retention time of molecules during gas chromatography.

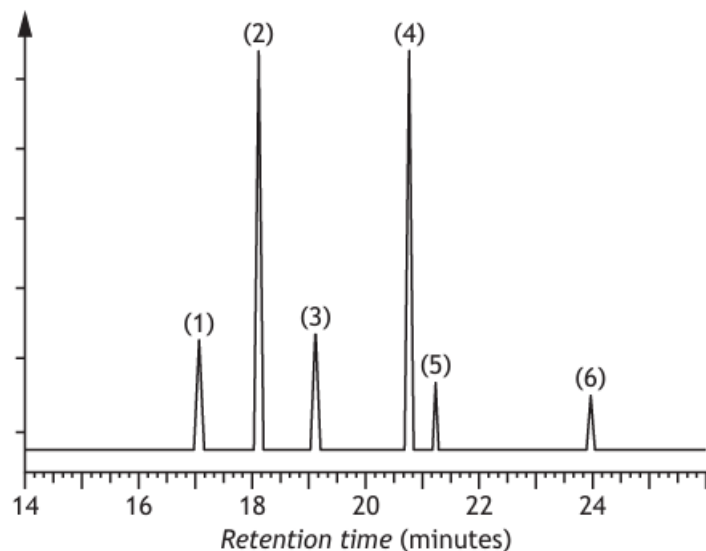
1

# Chromatography

3. Essential oils from the lavender plant are used in aromatherapy.

Gas chromatography can be used to separate and identify the organic compounds in lavender oils.

Chromatogram 1 - Lavender oil A



Peak	Component	Component peak area
1	1,8-cineole	7432
2	linalool	31 909
3	camphor	7518
4	linalyl acetate	27 504
5	geranyl acetate	3585
6	farnesene	1362

Total peak area = 79 310

The relative concentration of each component can be calculated using the following formula.

$$\text{Relative concentration} = \frac{\text{Component peak area}}{\text{Total peak area}} \times 100 (\%)$$

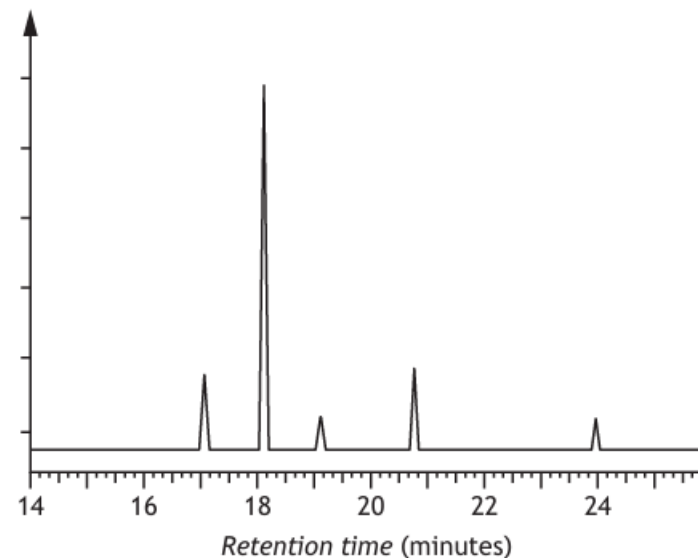
- (i) Calculate the relative concentration of linalool in lavender oil A.

1

(continued)

- (ii) Different varieties of lavender oils have different compositions.

Chromatogram 2 – Lavender oil B



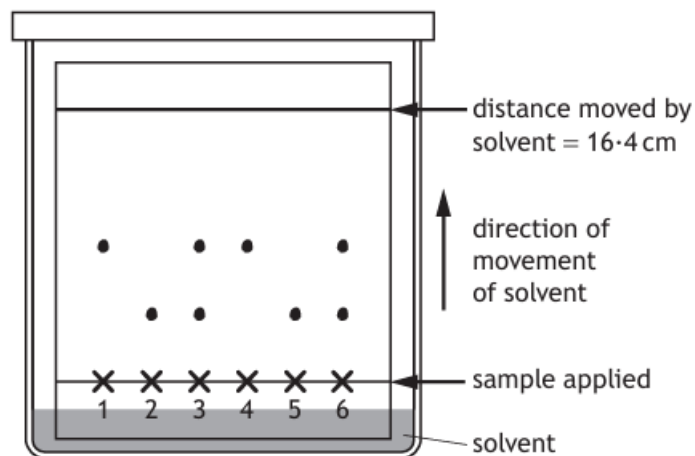
Identify the component found in lavender oil A that is missing from lavender oil B.

1

# Chromatography

4. The maturing process in cider samples can be monitored using thin layer chromatography.

Samples of lactic acid, malic acid and ciders A, B, C, and D are spotted on a silica plate and the solvent allowed to travel up the plate. The chromatogram obtained is shown below.



Number	Sample applied	Distance moved by spot(s) (cm)
1	lactic acid	8.2
2	malic acid	4.1
3	cider A	4.1, 8.2
4	cider B	8.2
5	cider C	4.1
6	cider D	4.1, 8.2

The retention factor,  $R_f$ , for a substance can be a useful method of identifying the substance.

$$R_f = \frac{\text{distance moved by the substance}}{\text{distance moved by the solvent}}$$

- (A) Calculate the  $R_f$  value of malic acid.

1

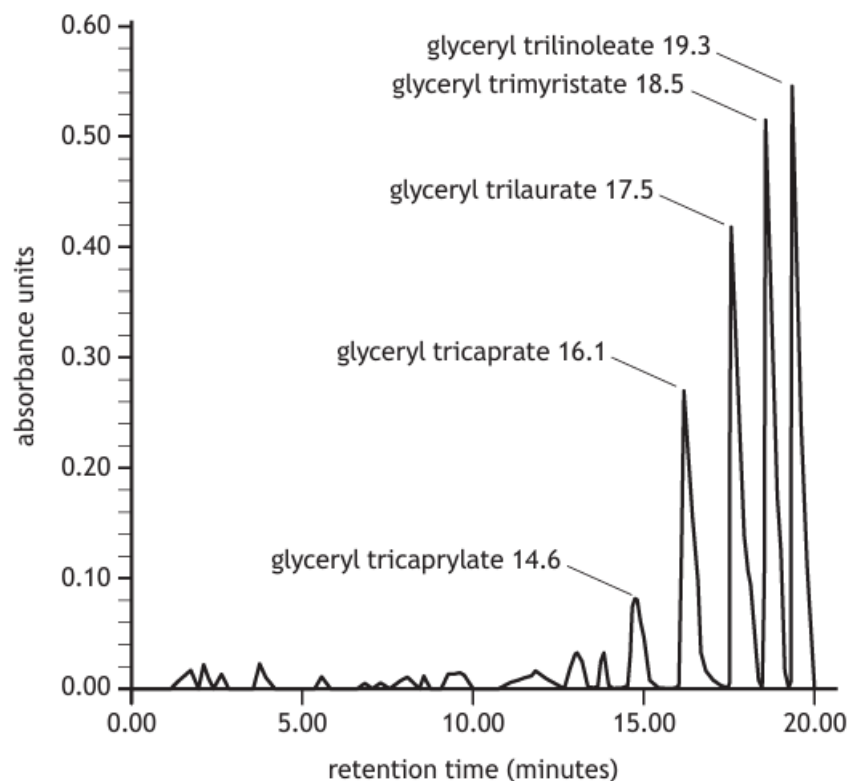
- (B) The maturing process is complete when all of the malic acid has been converted to lactic acid. The cider is now ready to be bottled.

Use the chromatogram to determine which cider is ready to be bottled.

1

## Chromatography

5. Chromatography can be used to separate the fats and oils in coconut oil. The result of a chromatography experiment is shown.



- (ii) Identify the compound listed in the table which is the most unsaturated.

1

- (i) Using the graph and the information in the table, predict the number of carbons in glyceryl trilaurate.

1

Name	Molecular formula	Melting point (°C)
Glyceryl tricaprystate	$C_{27}H_{50}O_6$	10
Glyceryl tricaprurate	$C_{33}H_{62}O_6$	31
Glyceryl trilinoleate	$C_{57}H_{98}O_6$	-5

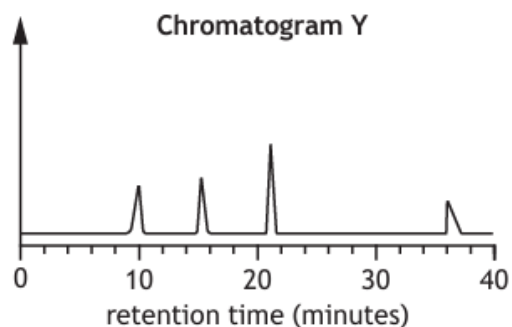
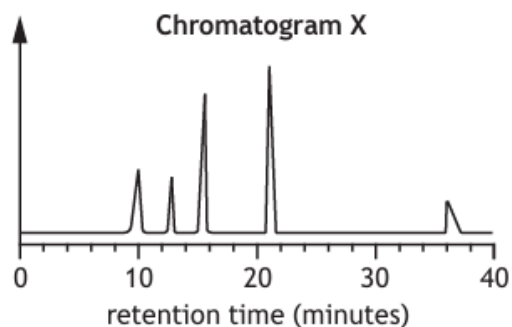


## Chromatography

6. The flavour of cheese changes over time as the concentrations of flavour molecules change.

Gas chromatography can be used to analyse the concentrations of flavour molecules.

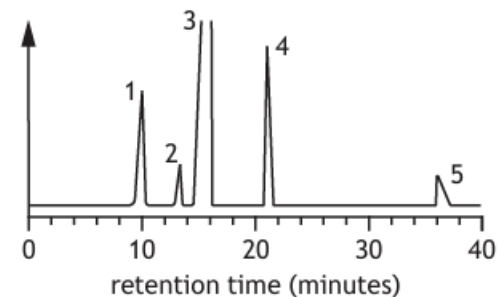
(A) Chromatograms for two samples of cheese are shown below.



Determine the retention time, in minutes, of the peak in **Chromatogram X** that is missing in **Chromatogram Y**.

1

- (B) The following chromatogram was obtained from another sample of cheese. The concentration of a flavour molecule in cheese can be determined by calculating the area under the peak that corresponds to that molecule.



The concentration of flavour molecule 3 cannot be determined from this chromatogram.

Suggest what would need to be done to the sample to allow the concentration of flavour molecule 3 to be determined.

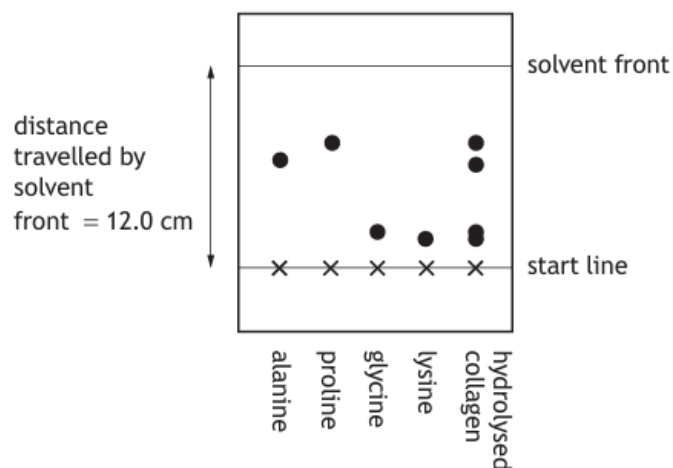
1

# Chromatography

7. Collagen is a protein that is found in skin and blood vessels.

A sample of collagen was analysed using paper chromatography to determine its amino acid composition. Amino acid samples were spotted onto paper at the start line, along with a hydrolysed collagen sample. The spots represent the distances travelled by the amino acids carried by the solvent.

The chromatogram obtained is shown.



The  $R_f$  value for an amino acid is found using the following equation.

$$R_f = \frac{\text{distance travelled by amino acid}}{\text{distance travelled by solvent front}}$$

The table shows the distances travelled by the amino acids in this sample.

Amino acid	Distance travelled (cm)
Alanine	5.2
Proline	5.6
Glycine	1.8
Lysine	1.6

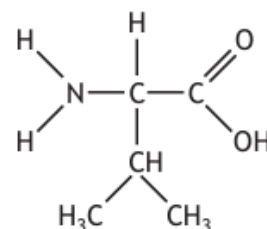
- (i) Calculate the  $R_f$  value for proline.

- (ii) The sample of collagen contained all four amino acids.

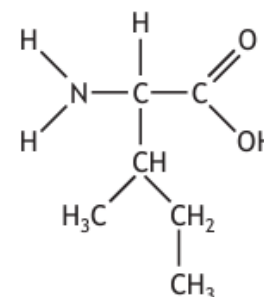
Suggest why there are only three spots in the chromatogram from hydrolysed collagen.

1

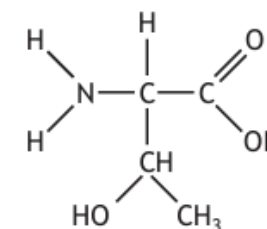
- (iii) More polar amino acids are carried for shorter distances by the solvent, as they attach more firmly to the chromatography paper.



valine



isoleucine



threonine

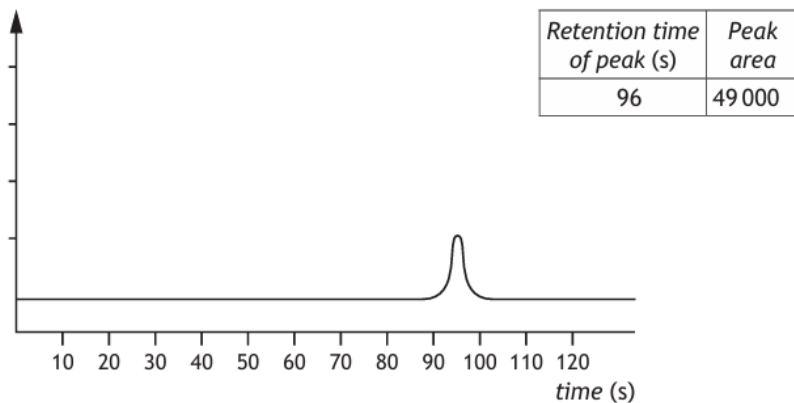
Predict which of the amino acids shown would have the smallest  $R_f$  value.

1

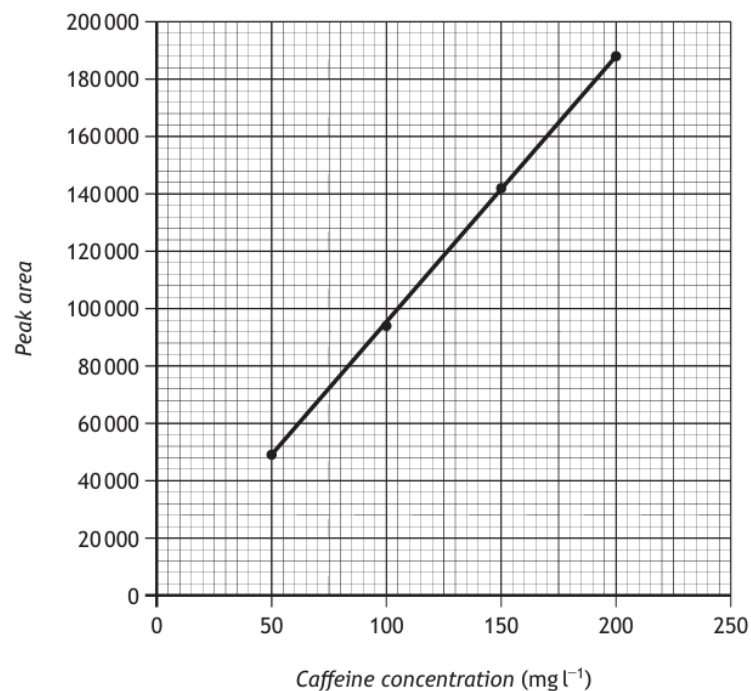
# Chromatography

8. The concentration of caffeine can be found using chromatography.

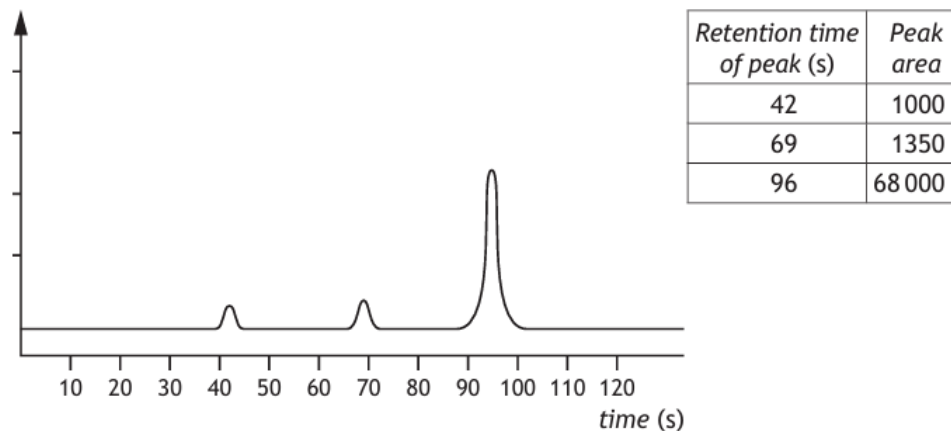
A chromatogram for a solution containing  $50 \text{ mg l}^{-1}$  of caffeine is shown below.



Results from four caffeine solutions were used to produce the calibration graph below.



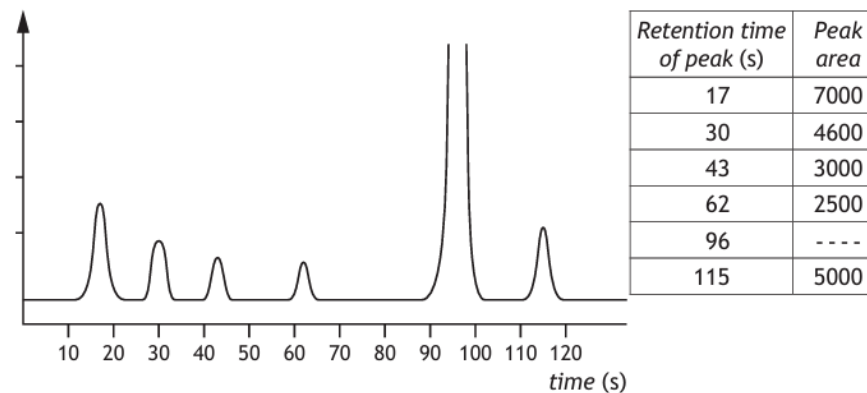
- (i) The chromatogram for soft drink X is shown below.



Determine the caffeine content, in  $\text{mg l}^{-1}$ , of soft drink X.

1

- (ii) The chromatogram for soft drink Y is shown below.



The caffeine content of soft drink Y cannot be determined from its chromatogram.

Suggest what could be done to the sample of soft drink Y so that the caffeine content could be reliably determined.

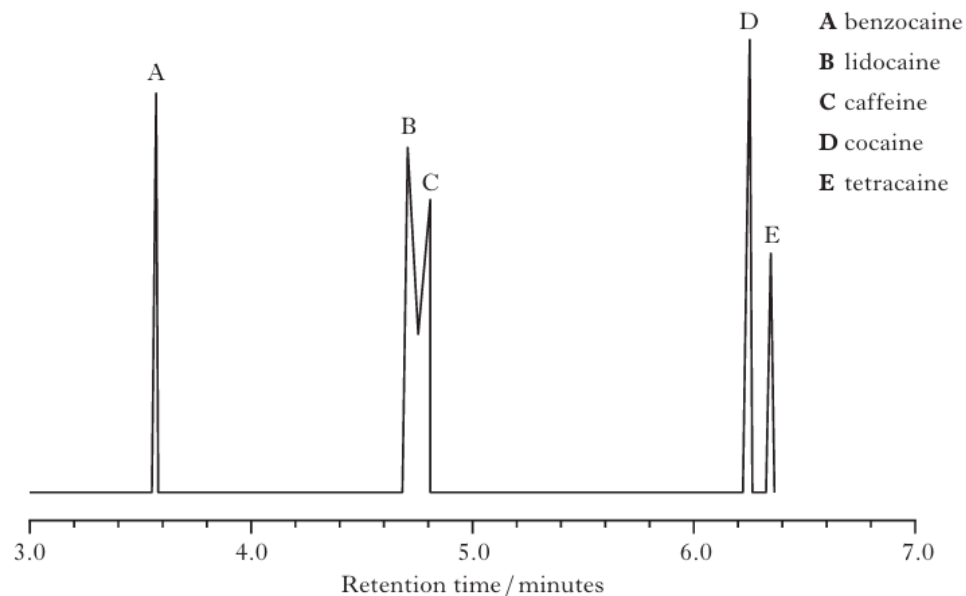
1

# Chromatography

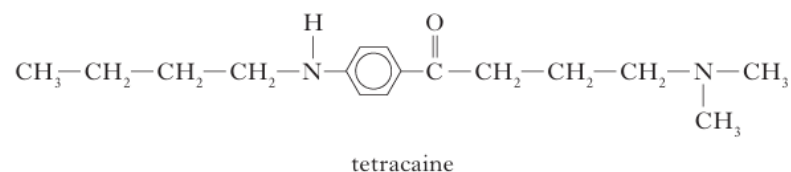
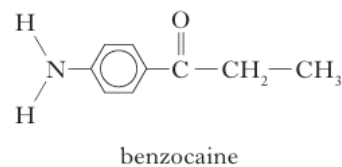
9

When forensic scientists analyse illegal drugs, anaesthetics such as lidocaine are sometimes found to be present.

The gas chromatogram below is from an illegal drug.



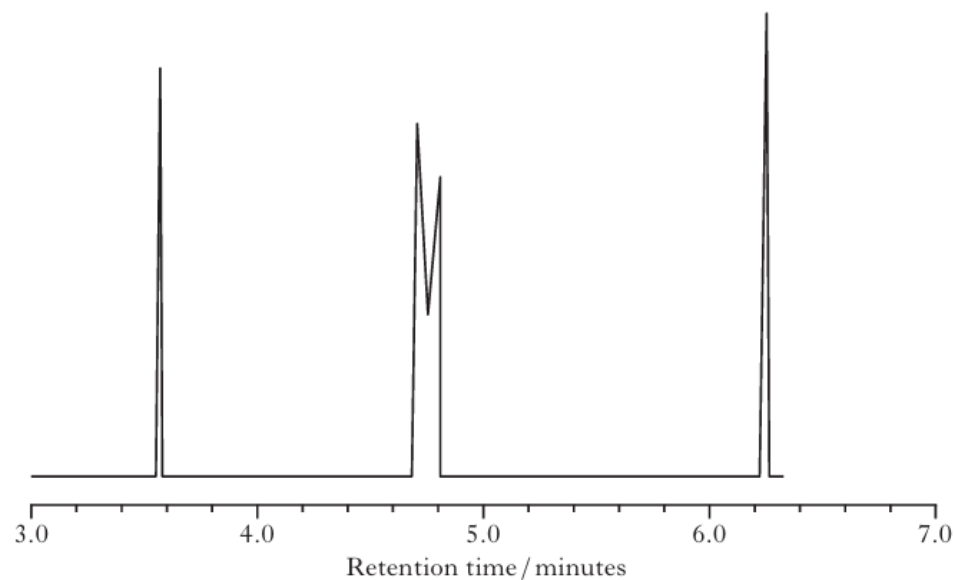
(i) The structures of benzocaine and tetracaine are shown below.



Suggest why benzocaine has a shorter retention time than tetracaine.

(ii) Why is it difficult to obtain accurate values for the amount of lidocaine present in a sample containing large amounts of caffeine?

(iii) Add a peak to the diagram below to complete the chromatogram for a second sample that only contains half the amount of tetracaine compared to the first.



## Chromatography Answers

1. C
2. B
3. B
4. A
5. D

1. The peptide molecule:  
must have contained an amino acid  
that is repeated in the sequence

OR

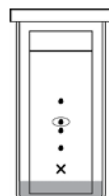
contained only four different amino  
acids (accept four different  
peptides).

OR

The peptide contains two amino  
acids:  
with the same  $R_f$  value

OR

that moved the same distance.



1

3.  $\frac{40 \cdot 23}{40 \cdot 2 + 40} (\%)$   
geranyl acetate/peak 5

4.  $\frac{0 \cdot 25}{0 \cdot 3}$   
(Cider) B or (sample) 4

5. 35 - 45 inclusive  
(ii) | Glycerol trilinoleate

6. (iv) | 12 - 13 (minutes)  
(A)

- (iv) | dilute sample/use less of sample  
(B)

2.	(a)	Any one of the common compounds correctly identified ie citronellol / geraniol / anisyl alcohol	1
	(b)	The concentration / volume of compounds (that are common to both/present in the counterfeit) is different are present in lower concentration in the counterfeit	1
	(c) (i)	Inert / will not react with the molecules (being carried through the column)	1
	(ii)	Size (mass) of molecules / temperature of column.	1

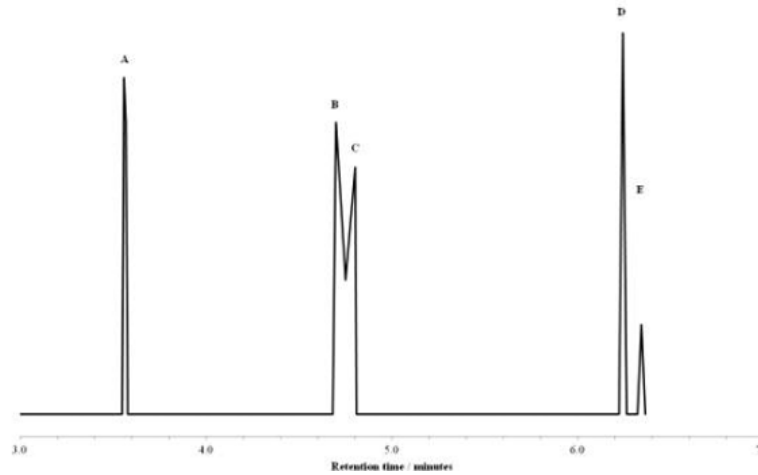
7.	(i)	$\frac{0.467}{0.47}$	1
	(ii)	The spots for glycine and lysine overlap/are close together. OR The $R_f$ values are similar/close together for glycine and lysine.	1
	(iii)	Threonine	1

## Chromatography Answers

8. (i) 69 – 70 (mg)

(ii) Sample of Y should be diluted  
OR  
Less of sample Y should be used  
OR  
Smaller sample of Y

iii Peak for tetracaine at correct RT with approximately half original height



9. i Benzocaine is a smaller/Tetracaine is bigger **(1)**  
or  
weaker London Dispersion Forces with Benzocaine **(1)**  
or  
weaker Van der Waal's forces for Benzocaine **(1)**  
or  
Benzocaine has lower b.pt **(1)**  
or  
Benzocaine more soluble/attracted in/ to mobile phase **(1)**  
or  
Benzocaine less strongly attracted to stationary phase **(1)**  
or  
Benzocaine is more polar **(1)**

ii The peaks for lidocaine and caffeine overlap  
or  
Candidate wording for idea of masking

7.