Chromatography Types

A Level

Chromatography is a versatile technique that may be used to separate and identify compounds.

Part A Dissolved solids

Name the type of chromatography that can be used to separate and identify dissolved solids on a plate coated with silica gel.

Part B Identifying dissolved solids

State what quantitative value may be determined from the chromatogram to identify the solids present in the solution: [ ] [ ]

Items:

F L R X f l r x

Part C Gases and vapours

Name a type of chromatography that could be used to separate and identify gases and vapours.

Part D Identifying gases and vapours

Different components in the mixture have different [ ] [ ], and by integrating (finding the [ ]) each peak, alongside a [ ] curve, the concentration of the components can be estimated. Rather than relying on [ ] [ ] alone for identification, nowadays the chromatography technique is often coupled to [ ]: this is known as GC-MS.

Items:

circumference of **area under** **NMR** **mass spectrometry** **times** **retention** **reduction** **line height of** **calibration** **infrared spectroscopy**

Adapted with permission from UCLES, A Level Modular Sciences, December 1993, Methods of Analysis and Detection, Question 1

Raffinose

A Level

A sugar named raffinose was reacted with dilute hydrochloric acid. The resulting solution, Y, together with four known sugar solutions for reference, was analysed by chromatography. The following chromatogram was obtained.

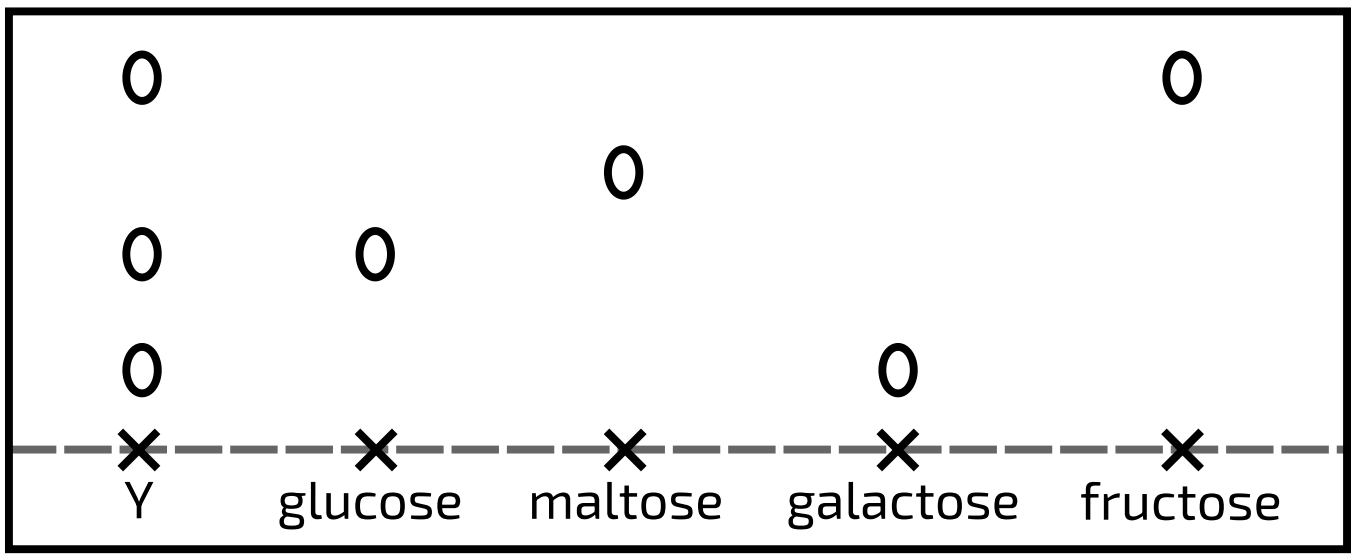

Figure 1: Chromatogram

The chromatogram shows that dilute hydrochloric acid breaks down raffinose into

- only two sugars: glucose and maltose.
- glucose, maltose and galactose.
- glucose, galactose and one sugar not among the reference sugars.
- glucose, galactose and fructose.
- glucose, maltose and fructose.

Adapted with permission from UCLES GCSE Chemistry June 1990, Paper 1, Question 3.

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Food Additive

A Level

This question is about chromatography of colourings in food additives.

- **S_1**, **S_2** and **S_3** are three colourings which are safe to eat.
- **P_1**, **P_2** and **P_3** are three colourings which are poisonous.
- **X** is a food additive which is under test to see if it is safe to eat.

The diagram shows the chromatogram obtained.

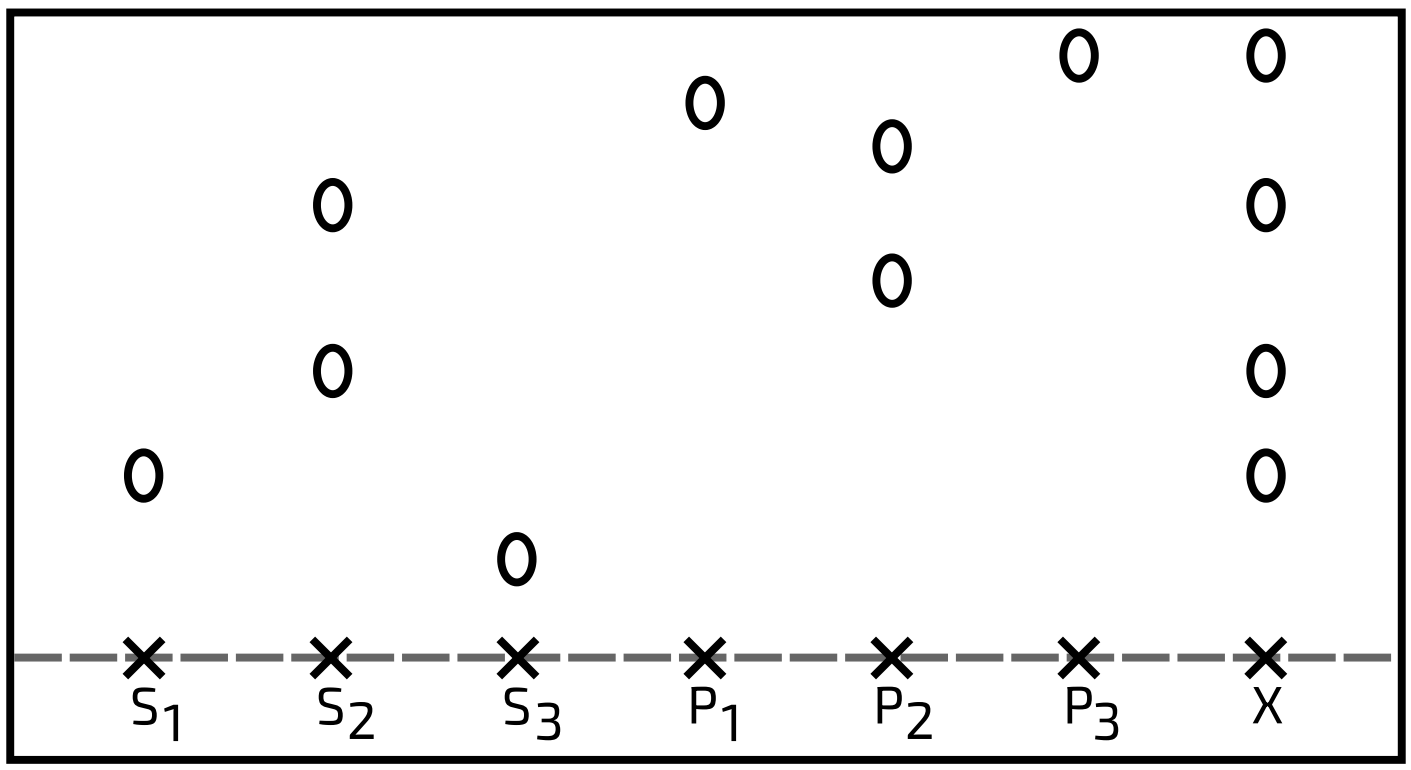

Figure 1: Chromatogram

Using this information, which statement is correct?

- X is safe to eat.
- S_3 contains the colouring which is most soluble in the solvent used.
- X appears to contain S_1, S_2 and P_3.
- P_3 contains the colouring which is least soluble in the solvent used.
- S_2 and P_2 are the only mixtures of colourings tested.

Improving Separation

A Level

A student tried to separate a mixture of food dyes by chromatography. Separation was poor, as shown in the chromatogram below.

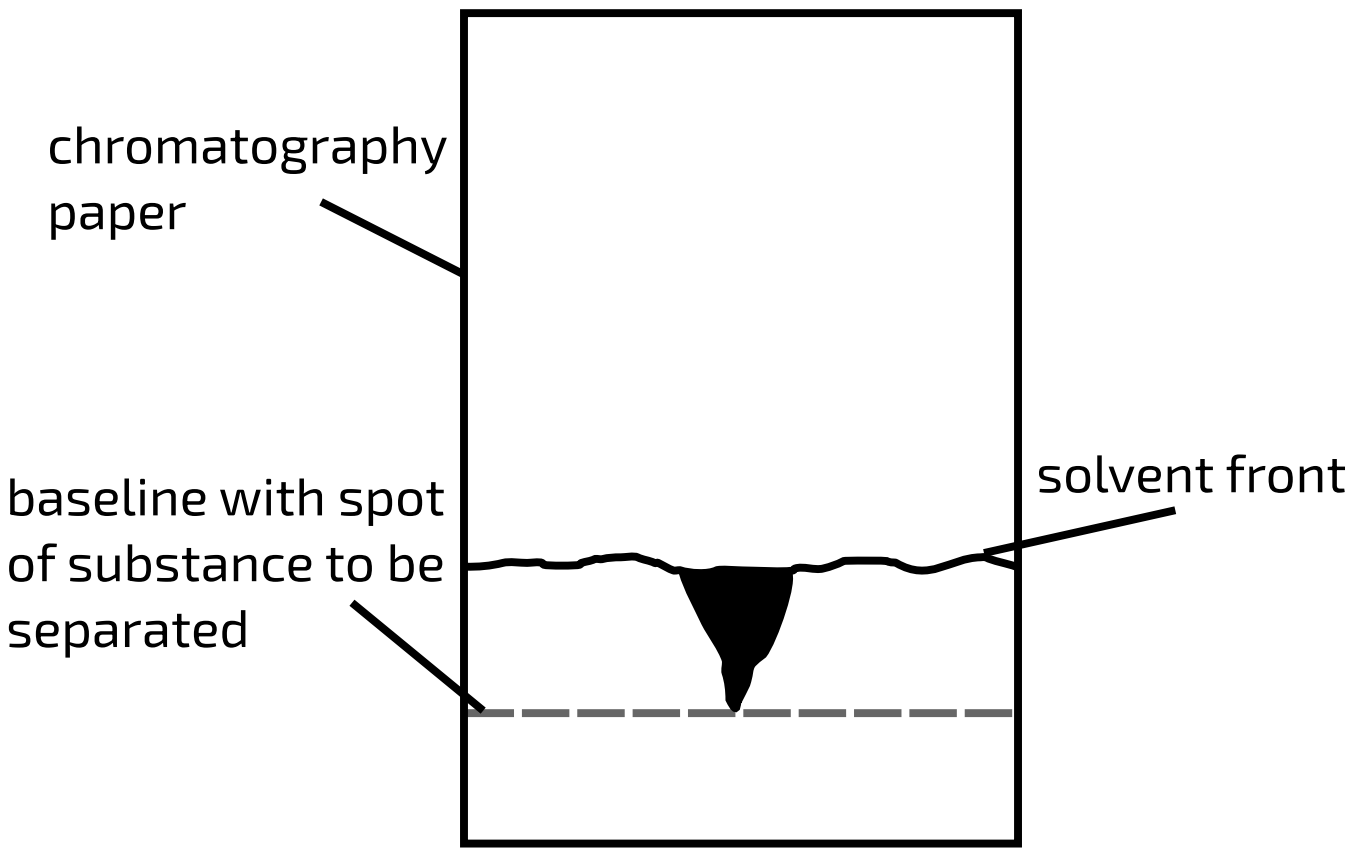

Figure 1: Chromatogram

Which change to the process would improve the result?

- allowing the solvent to rise further up the paper
- putting a larger spot of the dye mixture onto the paper
- using a smaller piece of chromatography paper
- using a larger piece of chromatography paper
- using enough solvent to cover the baseline

Adapted with permission from UCLES GCSE Chemistry June 1994, Paper 1, Question 4.

TLC

A Level

Chromatography may separate substances because they have different affinities for a mobile and a stationary phase.

Part A Phases in TLC

Name the physical state of the mobile phase used in thin layer chromatography, TLC.

Name the physical state of the stationary phase used in thin layer chromatography, TLC.

Part B Speed of travel

Some molecules travel faster than others in TLC. This is based on their different ability to interact with the solid and mobile phases. A more [ ] substance forms [ ] interactions with the solid phase, and this [ ] adsorption is harder to overcome, meaning it travels less far up the plate and has a [ ] R_f value. The solvent used cannot be too [ ] as all components will then travel up too far up the plate without separation being achieved.

Items:

light **polar** **weaker** **stronger** **lower** **higher**

Adapted with permission from UCLES A-Level Structured Science June 1997, Analytical Chemistry Paper, Question 4.

R_f Values

A Level

In chromatography, an R_f value is defined as

$$R_f = \frac{\text{distance travelled by a substance from start}}{\text{distance travelled by solvent front from start}}$$

In the following chromatogram, calculate the R_f value of each substance.

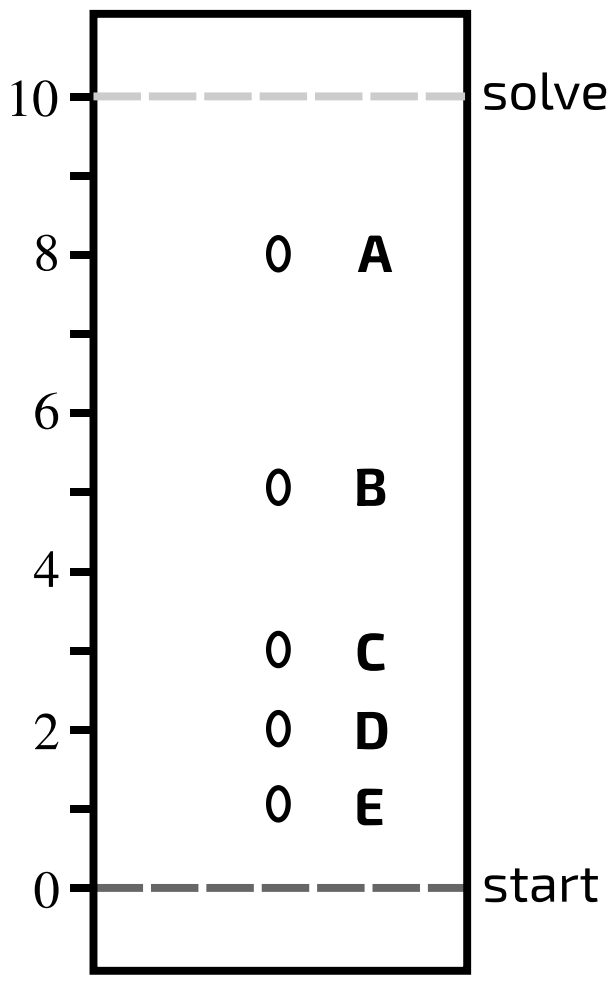

Figure 1: Chromatogram

Part A Substance A

To 1 significant figure, what is the R_f value of substance **A**?

Part B Substance B

To 1 significant figure, what is the R_f value of substance **B**?

Part C Substance C

To 1 significant figure, what is the R_f value of substance **C**?

Part D Substance D

To 1 significant figure, what is the R_f value of substance **D**?

Part E Substance E

To 1 significant figure, what is the R_f value of substance **E**?

Adapted with permission from UCLES GCSE Chemistry June 1994, Paper 1, Question 4.

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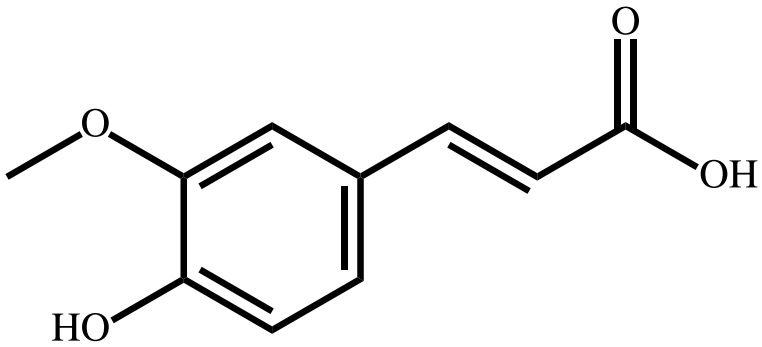
Onion Chromatogram

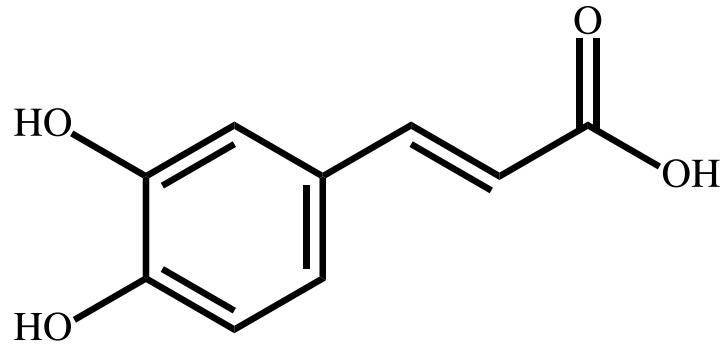
A Level

Ferulic acid is a plant derivative. It is a natural antioxidant because it terminates free-radical chain reactions. It is used commercially to give photo-protection in skin lotions and sunscreens as well as a range of medical applications. Ferulic acid is an active ingredient in many ancient Chinese herbal remedies.

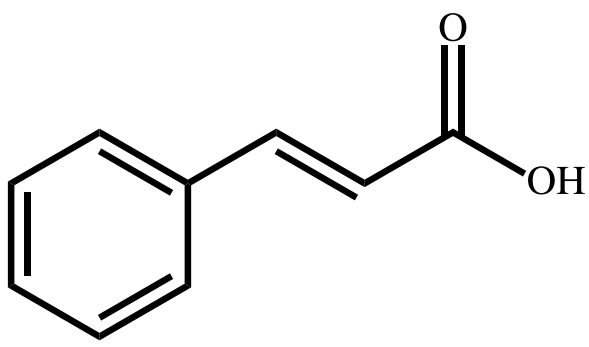
Ferulic acid occurs in onions with related acids having the structures given below.



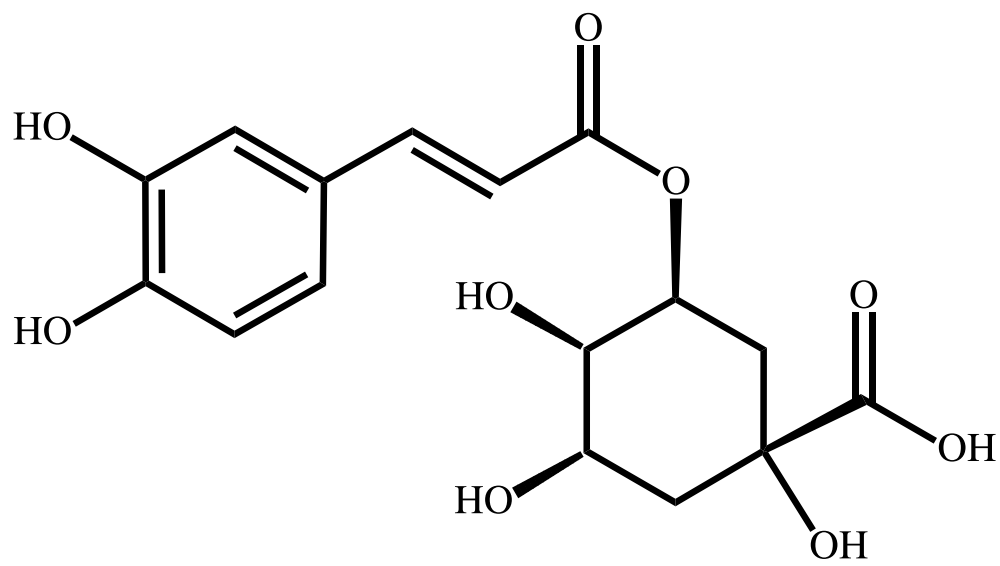
A: ferulic acid



C: caffeic acid



B: cinnamic acid



D: chlorogenic acid

Figure 1: Structures of acids found in onions.

Part A Water solubility

Rank the four acids in order of *decreasing* water solubility.

Available items

- A (ferulic acid)
- B (cinnamic acid)
- C (caffeic acid)
- D (chlorogenic acid)

Part B pH

An extract from onions is subjected to chromatographic analysis. The mobile phase is water and the stationary phase consists of small beads made from inert silane macromolecules to which $\mathrm{C_{18}H_{37}}$ alkyl groups are attached.

The water is kept at a pH of 2.0 for reproducibility of retention times. Suggest a chemical reason why a higher pH is not used by filling in the missing word in the following statement:

At a higher pH, the molecules could be _____.

Part C Water volume

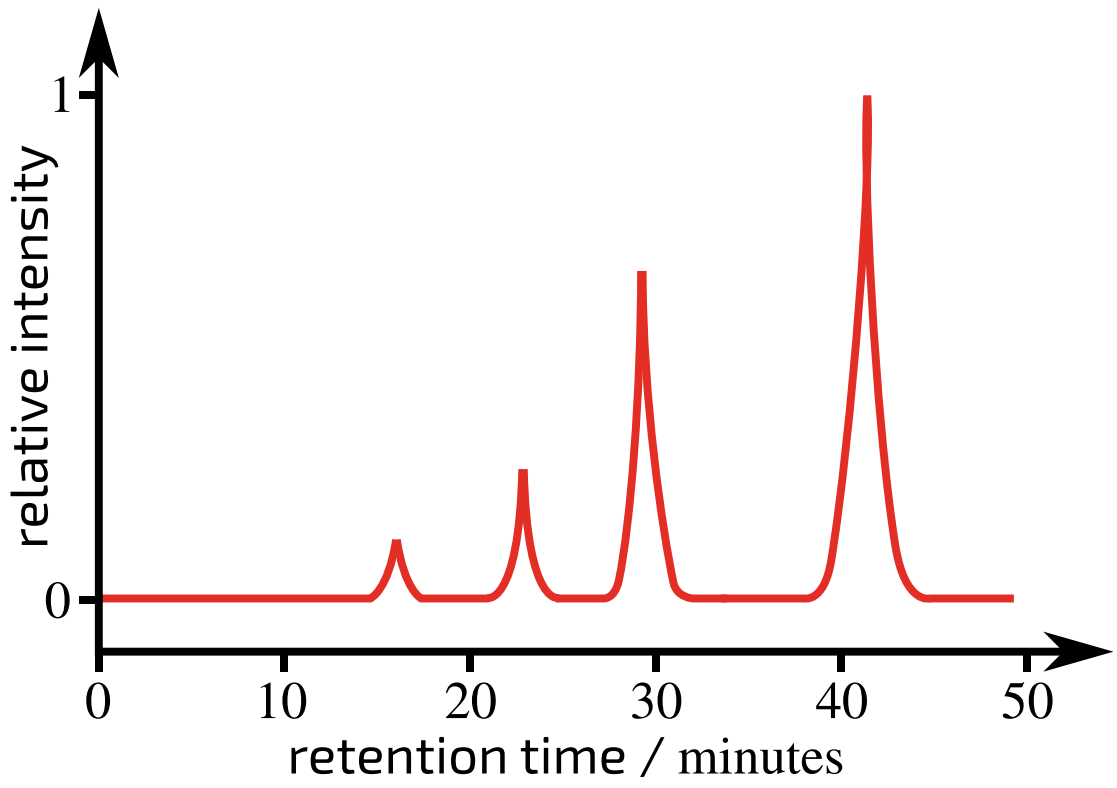


Figure 2: Onion chromatogram

The flow rate of the water is $0.5\,\mathrm{cm^3\,min^{-1}}$. Calculate the volume of water which flowed through the chromatograph before the acid with the longest retention time was detected by the recorder.

Part D Retention times

The [ ] water-soluble acid will have the longest retention time in the chromatograph. This is because it will show an increased preference for being [ ] the [ ] phase as opposed to being [ ] the [ ] phase. This means it travels through the chromatograph more [ ].

Items:

most **least** **dissolved in** **adsorbed to** **stationary** **mobile** **quickly** **slowly**

Adapted with permission from UCLES A-Level Chemistry June 1998, Paper 1, Question 24.

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Gas-liquid Chromatography

A Level

The diagram below represents an apparatus used for gas-liquid chromatography (also known as gas chromatography).

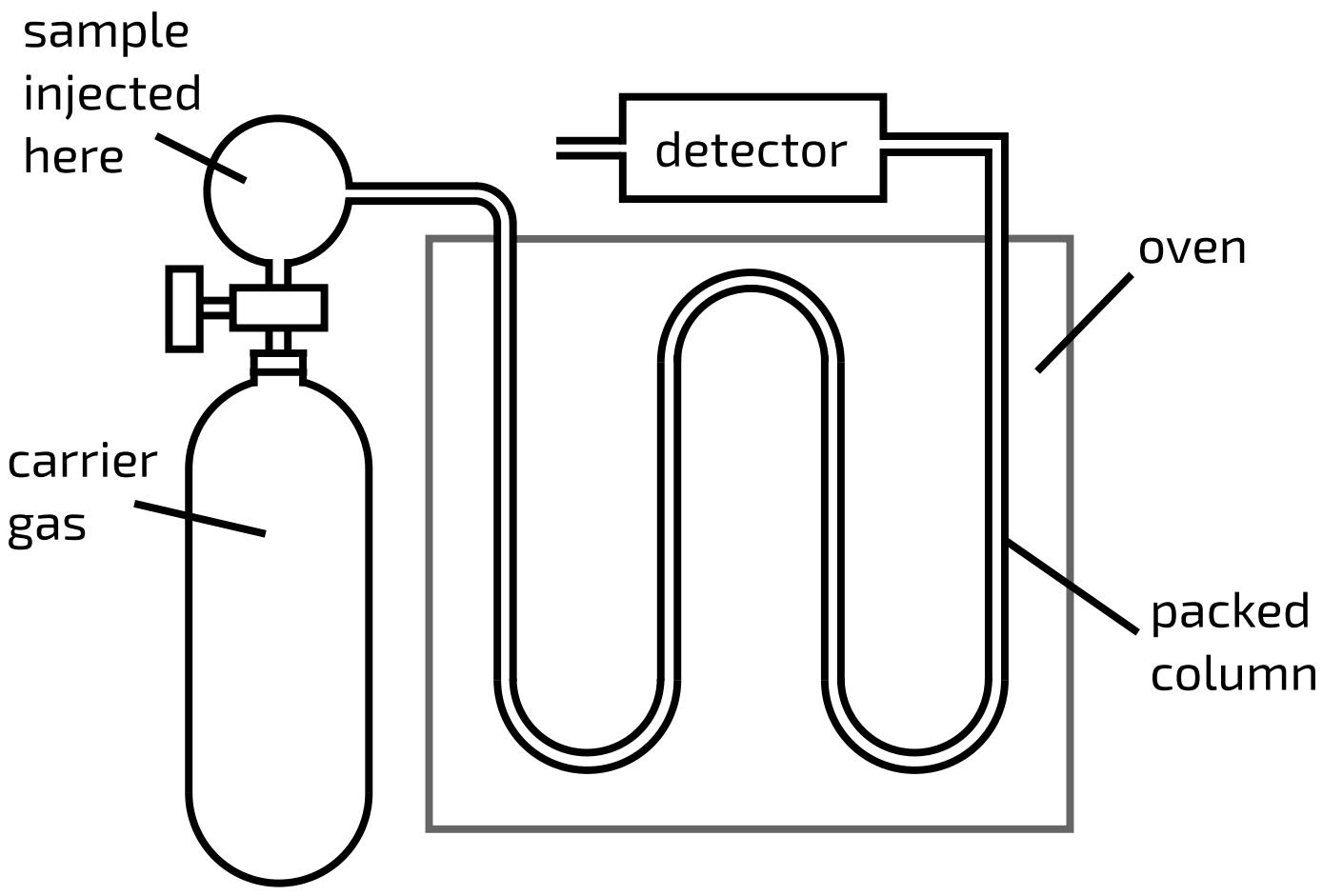

Figure 1: Gas-liquid chromatography instrument

Part A Carrier gas

Which of these would be suitable carrier gases for gas-liquid chromatography?

- [ ] helium
- [ ] oxygen
- [ ] fluorine
- [ ] argon

Part B Whisky chromatogram

The gas chromatogram of a sample of whisky (an alcoholic beverage) is given below.

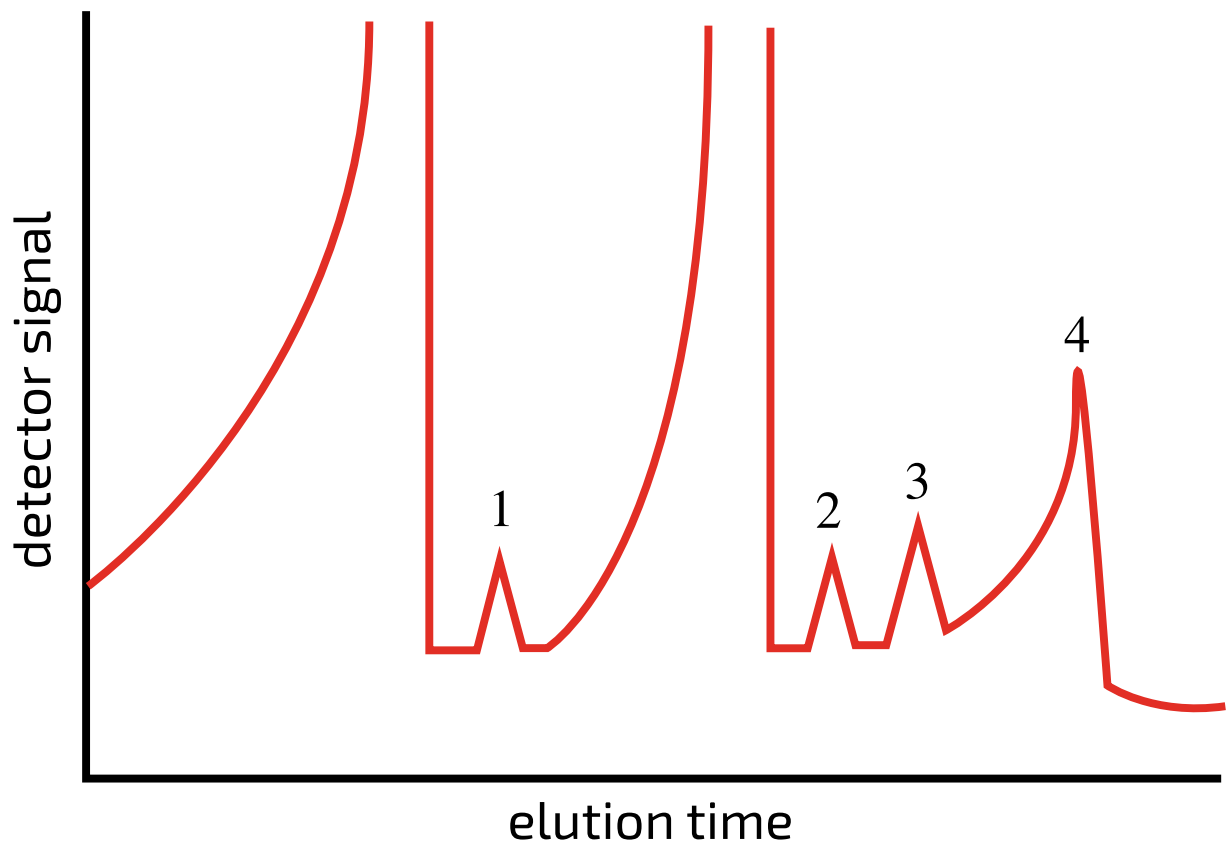

Figure 2: Gas chromatogram of whisky

The small peaks 1, 2, 3 and 4 are due to propan-1-ol, ethyl ethanoate, methanol and ethanal, respectively.

The elution time on the above diagram increases from [ ] to [ ]. The compound with the lowest elution time is [ ], because it forms the [ ] interactions with the stationary phase as well as having the [ ] boiling point.

Items:

left **right** **propan-1-ol** **ethyl ethanoate** **methanol** **ethanal** **strongest** **weakest** **lowest** **highest**

Part C Whisky components

Suggest the identity of the substance responsible for the left major peak (to the left of 1).

Suggest the identity of the substance responsible for the right major peak (between 1 and 2).

Adapted with permission from UCLES A-Level Chemistry June 1991, Paper 4, Question 6.

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Peptide Chromatogram

A Level

C C C

The table below gives data about a number of amino acids which occur in proteins.

name	relative molecular mass	R_f value in Solvent I	R_f value in Solvent II
alanine	89	0.43	0.38
aspartic acid	133	0.13	0.24
glycine	75	0.33	0.26
leucine	131	0.66	0.73
lysine	146	0.62	0.14
phenylalanine	165	0.64	0.68
serine	105	0.30	0.27
valine	117	0.58	0.40

A small polypeptide was hydrolysed with concentrated acid and, after neutralisation, the resulting amino acids were separated by two-way chromatography. The chromatogram is shown below.

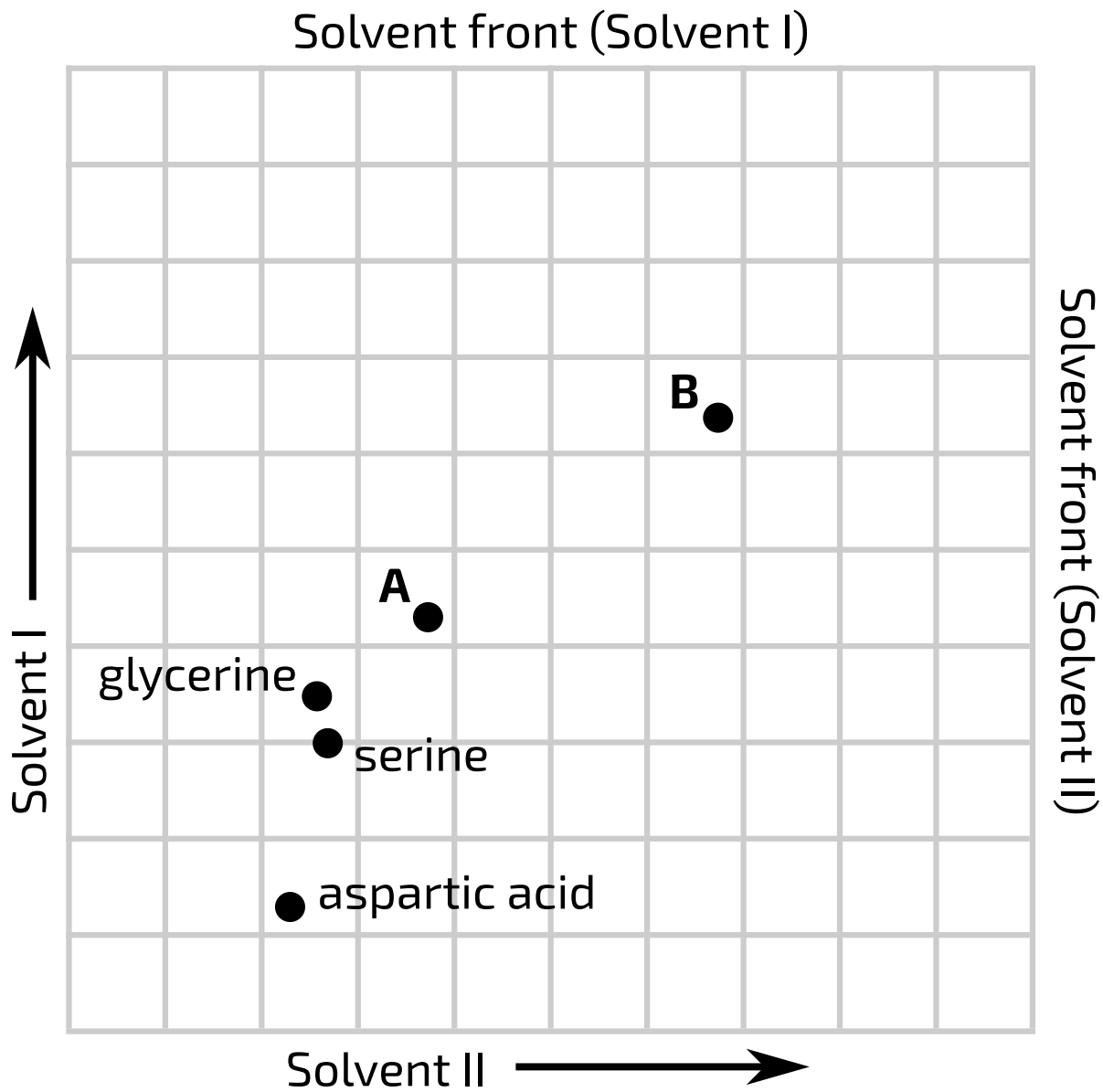

Figure 1: Two-way chromatogram showing spots for five amino acids.

Part A Identifying A

By determining the R_f values of **A** in both solvents, identify the amino acid.

Part B Identifying B

By determining the R_f values of **B** in both solvents, identify the amino acid.

Part C Methodology

It would be difficult to reliably identify all 8 amino acids using chromatography in one solvent alone. In Solvent I, while a naive reading of an R_f value of 0.64 might suggest the amino acid is [ ], allowing for a small (0.02) uncertainty in this measured value, it could be [ ] or [ ] instead. If we only used Solvent II, there is similarly a cluster of three amino acids with R_f values within 0.03 of one another that could be easily confused, with [ ] on the low end, [ ] 0.02 higher, and [ ] another 0.01 higher according to the table.

However, by using the combination of R_f values in both solvents, we can tell many of the amino acids apart more reliably: out of the Solvent I cluster, [ ] has a much lower R_f value than the others in Solvent II, while from the cluster in Solvent II, [ ] has a much lower R_f value than the others in Solvent I.

Items:

alanine **aspartic acid** **glycine** **leucine** **lysine** **phenylalanine** **serine** **valine**

Adapted with permission from UCLES A-Level Modular Sciences June 1993, Biochemistry Paper, Question 1.

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Cypermethrin

A Level

C C C

One technique used to accurately determine the concentration of substances in food is liquid chromatography followed by mass spectrometry (LC-MS). Here, the chromatography is used to separate different compounds and mass spectrometry to identify and quantify them.

The calibration line showing the peak size of the molecular ion peaks for five different concentrations of cypermethrin ($M_r = 416.30$) in pureed blueberries is shown below. The equation for the line of best fit for the data is:

$$\text{Peak area} = 44.547 \times \left(\text{Concentration of cypermethrin}/\mu\text{mol dm}^{-3}\right) + 2.403$$

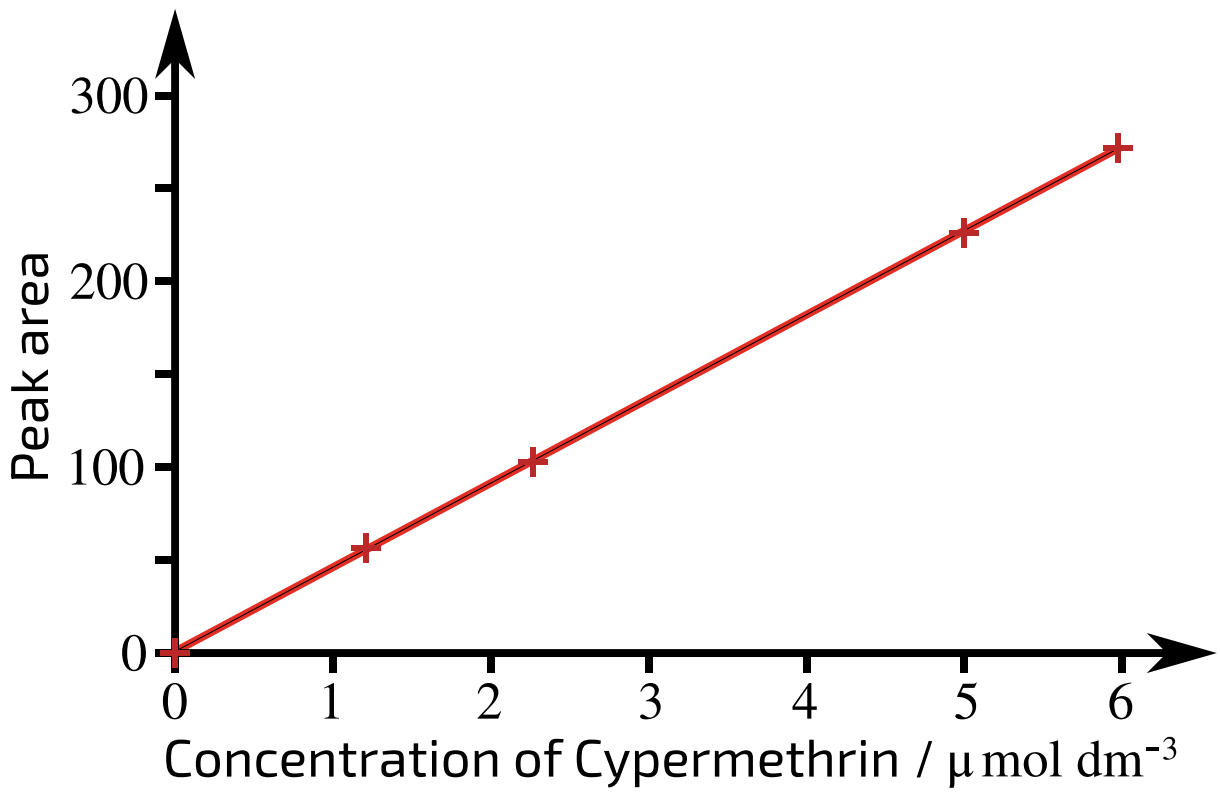

Figure 1: Calibration curve for cypermethrin quantification

The amount of cypermethrin that can be consumed without risk, the MRL (minimum risk level), is $0.020\,\text{mg kg}^{-1}\,\text{day}^{-1}$. Four blueberries were ground into a sample that had a volume of $15\,\text{cm}^3$ and the peak area was observed to be 4.8.

Part A Concentration

What is the concentration of cypermethrin in the sample in $\mathrm{mol\,dm^{-3}}$?

Part B Mass

Calculate the mass of cypermethrin in the sample.

Part C Safe number

How many blueberries can a $15\,\mathrm{kg}$ toddler consume per day without exceeding the MRL?

Adapted with permission from the Cambridge Chemistry Challenge 2016, Question 2