# Chromatography worksheet

# **A Vocabulary**

stationary phase	column	retention time	microsyringe	device	eluate	
analyte	mobile phase	theoretical plate	chromatogra	m		
eluent	sample	njector	flow volume			

1	characteristic time it takes for a particular analyte to pass through the system
2	in many separation processes is a hypothetical zone or stage in which two phases,
	such as the liquid and vapor phases of a substance, establish an equilibrium with each other.
3	substance fixed in place for the chromatography procedure
4	phase that moves in a definite direction
5	substance to be separated during chromatography. It is also normally what is needed
	from the mixture
6	the visual output of the chromatograph
7	the mobile phase leaving the column
8	the solvent that carries the analyte
9	the matter analyzed in chromatography
10	a device used in conjunction with injecting samples
11	a glass tube with a diameter from 5 mm to 50 mm and a height of 5 cm to 1 m with a tap
	and some kind of a filter
12	an object or a piece of equipment that has been designed to do a particular job
13	a small pump with a plunger that fits tightly in a tube
14	amount of eluate passing through the column

## **B** Reading

The aim of <u>chromatographic techniques</u> is to separate the sample into its components to quantify or produce a pure fraction. To obtain such separation, it is important to understand a few basic principles, which would help to improve and speed up the separation.

#### Speed of analysis

The <u>retention time</u> of a peak (i.e., how long it is held on the column before it is eluted) is a reflection of the speed of analysis.

#### Efficiency

The efficiency (i.e., how good a column is for separating different compounds) is described mathematically by the "theoretical plate number".

#### Retention

Compounds have been retained on the <u>column</u> to separate by chromatography. Most of the sample components have interacted with the <u>stationary phase (i.e., need to be retained)</u>.

In chromatographic techniques a solute (<u>analyte</u>) is preferentially distributed between two phases: a stationary (fixed) and a <u>mobile</u> (moving) <u>phase</u>.

#### High-performance Liquid Chromatography (HPLC)

This is the most popular chromatographic technique used in clinical laboratories. It offers rapid and sensitive separation with accurate quantification. It can be applied for the analysis of a wide range of compounds.

# The main components of modern HPLC are pump, injector, column, detector, and data recording device.

The sample prepared in a liquid is usually introduced on the column through the <u>injector</u> using a glass <u>microsyringe</u>.

The column is the most important part of HPLC. It is packed under controlled conditions with very small but uniform particles. Silica-based particles, most commonly used, give good separation.

To push the mobile solvent (mobile phase) through the small particles of the column, a pump capable of high pressure is used. Some of these offer very low <u>flow volumes</u> suitable for using very narrow columns.

Ultraviolet absorption is the most commonly used detection technique in HPLC (UV/VIS detector). Other detectors used are fluorescence and electrochemical detectors, which produce better sensitivity for certain types of compounds.

*Read the text and answer the questions:* 

1 What is the aim of chromatographic techniques?

2 What is determines how quickly the analysis is done?

3 How do you measure efficiency?

4 What are the two main phases in chromatographic techniques?

5 What is the most crucial part of HPLC?

6 How do you put samples into the column?

7 In which phase do you use silica-based particles? Why is silica used?

8 What is the standard HPLC detection technique?

## **C** Listening

Listen and complete each gap with one word.

1 The letters HP in HPLC stand for '\_\_\_\_\_' or 'high-performance'.

2 HPLC is used to analyse a mixture or to \_\_\_\_\_\_ a required product from others in a reaction mixture.

3 HPLC works on the same principle as \_\_\_\_\_ chromatography.

4 A liquid, called the \_\_\_\_\_\_, moves past the solid, the \_\_\_\_\_\_ phase.

5 In paper chromatography, the \_\_\_\_\_ consists of water molecules bound to the cellulose in the paper.

6 The \_\_\_\_\_\_ carries different components of a mixture, called the \_\_\_\_\_\_, along with it at different rates.

7 The speed of \_\_\_\_\_\_ depends on their relative affinity for the \_\_\_\_\_\_

8 If the \_\_\_\_\_ is more polar than \_\_\_\_\_, the more polar components of a mixture move more quickly than the less polar ones.

9 In HPLC, the \_\_\_\_\_\_ is a solid packed into a \_\_\_\_\_\_.

10 Unlike in paper chromatography, the (solvent) liquid is forced through the column by \_\_\_\_\_ pumps.

11 Two \_\_\_\_\_ can be mixed in any proportions to give a mixture, the liquid phase, of suitable polarity for the separation.

12 Water is more \_\_\_\_\_ than ethane nitrile ( $CH_3CN$ ).

13 The pumps produce a \_\_\_\_\_ of 15,000 kPa

14 A single \_\_\_\_\_\_ is injected into the solvent stream in the injection port via a hypodermic

15 Several \_\_\_\_\_\_ can be run in succession, by loading them into this auto-sampler which will run them in order without any human intervention.

16 The pumps force the mixed solvent through the \_\_\_\_\_\_. The solvent emerging from the column and carrying the separated components of the mixture passes into the detector.

17 In the detector, UV light of specific wavelength passes through the \_\_\_\_\_\_ and is absorbed by all the components to be separated.

#### **D** Grammar

4 P	ut the verb into the more suitable form, present continuous or present simple.
1	I'm going (I / go) to the cinema this evening.
2	Does the film start (the film / start) at 3.30 or 4.30?
3	(we / have) a party next Saturday. Would you like to come?
4	The art exhibition
5	(I / not / go) out this evening.
	(I / stay) at home.
6	(you / do) anything tomorrow morning?' 'No, I'm free. Why?
7	(we / go) to a concert tonight.
	(it / start) at 7.30.
8	(I / leave) now. I've come to say goodbye.
9	A: Have you seen Liz recently?
	e: No but (we / meet) for lunch next week
10	You are on the train to London and you ask another passenger:
10	Evoluse me. What time (this train / get) to London?
11	Excuse the what time
	rou are taking to Helen:
	Heten,
10	with me?
12	You and a friend are watching television. You say:
	I'm bored with this programme. What time(it / end)?
13	<ul><li>(I / not / use) the car this evening, so you can have it.</li></ul>
14	Sue (come) to see us tomorrow.
	(she / travel) by train and ber train (arrive) at 1015
2	(rain) It's going to rain. It is 8.30. Tom is leaving his house. He has to be at work at 8.45, but the journey takes 30
	(rain) It's going to rain.
2	It is 8.30. Tom is leaving his house. He has to be at work at 8.45, but the journey takes 30
	minutes.
	(late) He
3	There is a hole in the bottom of the boat. A lot of water is coming in through the hole.
	(sink) The boat
4	Lucy and Chris are driving. There is very little petrol left in the tank. The nearest petrol station is
	a long way away.
	(run out) They
3	Which is correct?
	'Did you phone Lucy?' 'Oh no. I forgot. +phone / I'll phone her now.' (I'll phone is correct)
	Can't meet you tomorrow. I'm playing / Helay tennis. (I'm playing is correct)
-	I meet / I'll meet you outside the botel in half an hour OK2' 'Ves that's fine '
	<u>Theet 7 it meet you outside the note: In hair an nour, OK</u> : Tes, that sine.
	I need some money. OK, <u>imighting / illiend</u> you some. How much do you need?"
	<u>I'm having / I'll have</u> a party next Saturday. I hope you can come.
(	5 'Remember to get a newspaper when you go out.' 'OK, Idon't forget / I won't forget.'
7	7 What time does your train leave / will your train leave tomorrow?
1	3 I asked Sue what happened, but she <u>doesn't tell / won't tell</u> me.
9	Are you doing / Will you do anything tomorrow evening?' 'No, I'm free. Why?'
10	) I don't want to go out alone. Do you come / Will you come with me?
Co	omplete the sentences using will ('ll) or going to.
1	A: Why are you turning on the TV?
	B: I'm going to watch the news. (I / watch)
2	A: Oh, I've just realised. I haven't got any money.
	B: Haven't you? Well, don't worry you some. (I / lend)
3	A: I've got a headache.
	B: Have you? Wait a second and an aspirin for you. (I / get)
4	A: Why are you filling that bucket with water?
	B: the car. (I / wash)
5	A: I've decided to repaint this room.
	B: Oh, have you? What colour it? (you / paint)
6	A: Where are you going? Are you going shopping?
-	
	B: Yes, some things for dinner (1 / buy)
7	B: Yes,some things for dinner. (I / buy) A: I don't know how to use the washing machine.
7	B: Yes, some things for dinner. (I / buy) A: I don't know how to use the washing machine. B: It's easy. you. (I / show)

8 A: What would you like to eat? B: \_\_\_\_\_\_a pizza, please. (I / have)