Pharmaceutical and Biomedical Analysis

22CMC004

Semester 1 2022/23 In-Person Exam paper

This examination is to take place in-person at a central University venue under exam conditions. The standard length of time for this paper is **2 hours**.

You will not be able to leave the exam hall for the first 30 or final 15 minutes of your exam. Your invigilator will collect your exam paper when you have finished.

Help during the exam

Invigilators are not able to answer queries about the content of your exam paper. Instead, please make a note of your query in your answer script to be considered during the marking process.

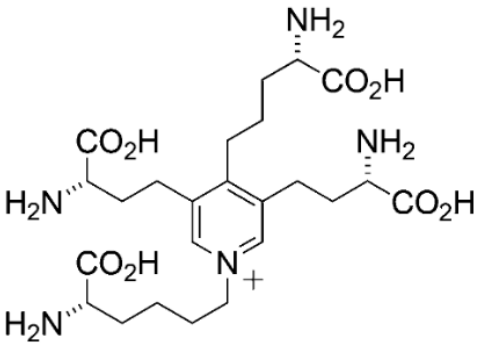
If you feel unwell, please raise your hand so that an invigilator can assist you.

You may use a calculator for this exam. It must comply with the University's Calculator Policy for In-Person exams, in particular that it must not be able to transmit or receive information (e.g. mobile devices and smart watches are **not** allowed).

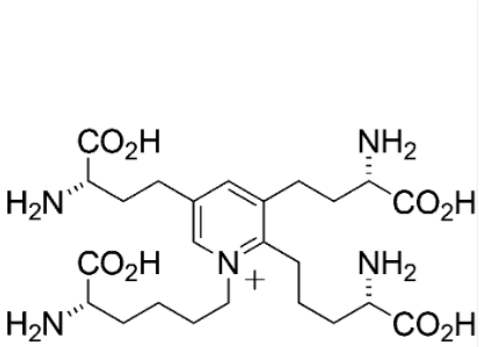
Answer **ALL** questions. Use a **SEPARATE ANSWER BOOK** for **EACH QUESTION**.

1. Answer **ALL** parts

Desmosine (DES) in human urine is a known biomarker of lung dysfunction which originates from the breakdown of the protein elastin. Analysing DES in urine is complicated by the presence of the isobaric interference isodesmosine (IDES) which can interfere with the separation obtained. IDES has the same mass as DES so cannot be resolved easily by using a mass spectrometer.



desmosine (**1**)



isodesmosine (**2**)

Analysing individual standards of DES and IDES by LC-MS, with an isocratic mobile phase of 20:80 methanol:water+0.1% formic acid at pH 2.5 on a 10 cm C-18 column gave the following chromatographic data. The unretained component t_m eluted at 1.15 minutes.

Table 1 Summary of retention times and peak widths for the two metabolites.

Metabolite	Retention time (t_r/min)	Peak Width ($W_{1/2}$/min)
DES	1.36	0.05
IDES	1.44	0.06

(a) Use the Purnell equation to calculate the resolution of this separation, making sure to show all your working, and comment on how well resolved the two species are.

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k'_{AB}}{1 + k'_{AB}}\right)$$

Equation 2 - The Purnell Equation.

[10 marks]

continued…

(b) Based on your answer to part a) justify which parameter should be optimised first to improve the resolution of the isobaric metabolites

[3 marks]

(c) Outline what are the different options available to improve the chromatographic resolution of DES and IDES, explain why each of the changes you propose could improve the separation of DES and IDES?

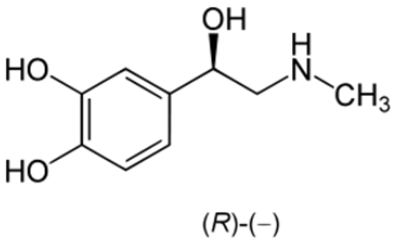
[12 marks]

2. Answer **ALL** parts

(a) With the aid of a diagram, describe how an enantiomer can be purified from a racemic mixture of compounds using a donor acceptor (Pirkle) type chiral stationary phase. What are the limitations of this approach?

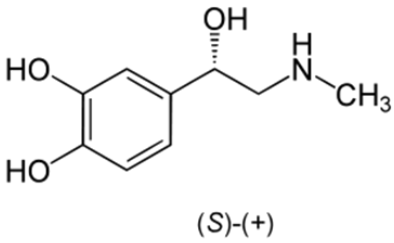
[6 marks]

(b) Develop a protocol for determining the enantiomeric purity of R-epinephrine using liquid chromatography. How would you accomplish the chiral separation and what would be the fastest and most cost-effective method for drug batch testing.



(*R*)-(−)

R-epinephrine (1)



(*S*)-(+)

S-epinephrine (2)

[6 marks]

(c) When designing a sampling procedure deciding how the samples will be taken is an important consideration. Describe the difference between a **Composite** and a **Replicate** sample and described the advantages and disadvantages of taking a composite sample.

[11 marks]

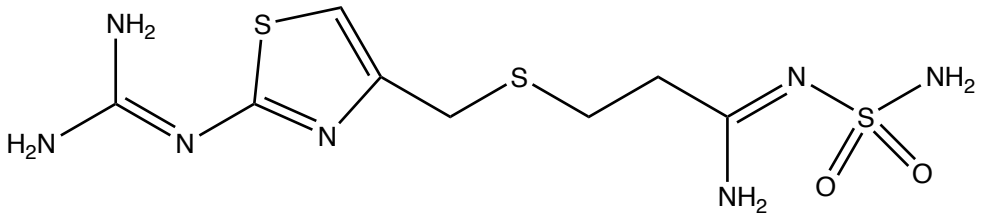
continued…

(d) Define the term Representative Sample

[2 marks]

3. Answer **ALL** parts

(a) Famotidine is a competitive histamine-2 (H_2) receptor antagonist that works to inhibit gastric acid secretion. It has a low first pass metabolism and is expected to be 15 – 25% protein bound in the blood. Using the structure and chemical information below propose a preparation, clean up and analysis method for a metabolism study of famotidine in urine.



Formula = $C_8H_{15}N_7O_2S_3$
Molar Mass = 337.44 $g.mol^{-1}$
pKa = 8.4

[20 marks]

(b) Valid Analytical Measure or VAM has six key principles. State VAM principle 2 and briefly discuss how this principle can be implemented in an analytical laboratory.

[5 marks]

4. Answer **ALL** parts

(a) With the aid of a diagram, illustrate a resistive pulse/s and describe what information can be gained from the signal

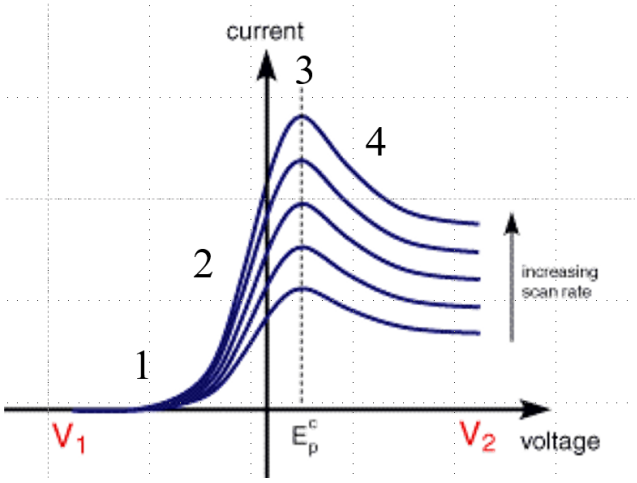
[8 marks]

(b) In a typical voltammetry experiment describe the three main modes of mass transport that occur.

[6 marks]

continued…

(c) Below is an image of a typical Linear sweep voltammogram, making reference to the concentration of the reactants at the electrodes surface and the diffusion field thickness describe what is happening at points 1-4 on the diagram.



Linear sweep Voltammogram

[8 marks]

(d) Name one example of a method used to create and control flow in an electrochemical setup and describe the relationship between your method and the current.

[3 marks]

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