

Cork Institute of Technology  
Bachelor of Science in Cell and Molecular Biology - Award

(NFQ Level 7)

Autumn 2006

**Bioanalytical Science**

(Time: 3 Hours)

Answer a total of FIVE questions.  
Answer TWO questions from Section A  
Answer THREE questions from Section B

Examiners: Prof. R. Fitzgerald  
Ms. C. Devaney  
Dr. L. Goold  
Ms. A. Ward

**Section A**

Q1. (a) State the possible physical states ( i.e. solid, liquid or gas) for both mobile and stationary phase in (i) partition chromatography and (ii) adsorption chromatography. Briefly explain the concept of resolution,  $R$ , in a chromatographic process. How is resolution determined from a chromatogram? Mention two experimental procedural changes that can be used to change resolution in a chromatographic process.

(8 marks)

(b) Describe the (i) head space analysis and (ii) stream splitting techniques of sample introduction in gas chromatographic analysis. In each case mention the main advantage associated with each technique.

(12 marks)

Q2. (a) Construct a labelled block diagram of a High Performance Liquid Chromatography (HPLC) instrument and use it to **briefly** describe the function of each component.

(8 marks)

(b) Describe reverse phase HPLC analysis under the following headings:- (i) polarity of mobile phase and stationary phase giving typical examples of each. (ii) order of elution of components of varying polarity and (iii) polarity changes to mobile phase required to increase elution power.

(6 marks)

**PTO**

Q2. (cont.)

- (c) Briefly explain the processes of isocratic and gradient elution modes of HPLC analysis. Indicate a type of sample mixture that would require the gradient mode for successful separation. Explain your answer.

(6 marks)

- Q3. The analysis of vanillin in vanilla essence was performed by gas chromatographic analysis using the internal standard method of quantitation. The internal standard chosen was p-hydroxybenzaldehyde. A series of standard solutions of vanillin were prepared in methanol solvent. Each of these solutions contained p-hydroxybenzaldehyde at a concentration of 200 ppm. The vanilla essence sample used for analysis was prepared by pipetting 200  $\mu$ l of the original sample of essence into a vial followed by 200  $\mu$ l of a 1000 ppm solution of p-hydroxybenzaldehyde and 600  $\mu$ l of methanol solvent. All the solutions were analysed by gas chromatography under similar conditions. The following peak area data was obtained from the analyses:-

SOLUTION ANALYSED	PEAK AREA OF VANILLIN	PEAK AREA OF P-HYDROXYBENZALDEHYDE
<b>Vanillin Standard (100ppm)</b>	5745 counts	4352 counts
<b>Vanillin Standard (200ppm)</b>	13044 counts	4857 counts
<b>Vanillin Standard (300ppm)</b>	18400 counts	4600 counts
<b>Vanillin Standard (400ppm)</b>	20540 counts	3920 counts
<b>Vanillin Standard (500ppm)</b>	27720 counts	4200 counts
<b>Vanilla Essence Sample</b>	14300 counts	4400 counts

- (a) Briefly explain why the internal standard method of quantitation is particularly suited to gas chromatographic analysis. Identify 4 criteria that have to be satisfied when choosing a particular chemical as an internal standard for gas chromatographic analysis.

(6 marks)

- (b) Use an appropriate plot to determine the ppm concentration of vanillin in the original vanilla essence sample as accurately as possible.

(14 marks)

## Section B

- Q4. (a) Outline the principle of a competitive OR a non-competitive ELISA. Illustrate your answer with a diagram of the assay format and calibration curve for the system. (12 marks)
- (b) Write a brief account of the optimisation parameters for this ELISA (8 marks)
- Q5. Write an account of the assessment of the precision and accuracy of an analytical method. Comment on the importance of an internal quality control system to an analytical laboratory. (20 marks)
- Q6 (a) Describe using a diagram for illustration, any immuno-analytical technique which uses an immuno-precipitation reaction. (15 marks)
- (b) Define each of the following;
- (i) Monoclonal antibody
  - (ii) Polyclonal antibody (5 marks)
- Q7. (a) (i) “A nucleus with an excess of neutrons will undergo neutron to proton transition. Conversely, nuclei which contain an excess of protons undergo proton to neutron transition”. Illustrate the above statements by reference to the decay of  $^{14}_6\text{C}$  ( carbon 14) AND  $^{125}_{53}\text{I}$  (iodine 125). (6 marks)
- (ii) Describe, with the aid of a diagram, the operation of EITHER a Geiger Counter OR a Scintillation Counter. (8 marks)
- (b) A centrifuge is a device used to increase sedimentation rates. Write a brief note on this device, mentioning in your answer the principle upon which it works and two precautions taken when using the device. (6 marks)