

Cork Institute of Technology
Bachelor of Science in Applied Biosciences & Biotechnology
(SBIBI_7_Y3)
Autumn 2008
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. G. Walsh
Ms. C. Devaney
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Ms. A. Ward

Section A

- Q1. (a) Describe the nature of the stationary phase as well as the underlying principles of separation in (i) ion exchange chromatography and (ii) size exclusion chromatography.
(10 marks)
- (b) Indicate clearly how the following chromatographic data can be determined from a chromatogram.
- (i) Column Theoretical Plate Value, N
 - (ii) Resolution, R
 - (iii) Capacity Factor, k'
- (7 marks)
- (c) Describe the changes, if any, that are likely to occur to (i) N and (ii) retention time of a component when any one of the following chromatographic parameters are changed:-
(1) Flow rate of mobile phase, F (2) Particle size of stationary phase, u and (3) Length of column, L .
(3 marks)

PTO

- 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)
- (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 ul of the original sample of essence into a vial followed by 200 ul of a 1000 ppm solution of p-hydroxybenzaldehyde and 600 ul 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
Vanillin Standard (100ppm)	5745 counts	4352 counts
Vanillin Standard (200ppm)	13044 counts	4857 counts
Vanillin Standard (300ppm)	18400 counts	4600 counts
Vanillin Standard (400ppm)	20540 counts	3920 counts
Vanillin Standard (500ppm)	27720 counts	4200 counts
Vanilla Essence Sample	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) Define each of the following:
- (i) Heterogeneous immunoassay systems
 - (ii) Homogeneous immunoassay systems (10 marks)
- (b) Describe, using a labelled diagram, the principle of a heterogeneous non- competitive sandwich enzyme immunoassay. (10 marks)
- Q5. (a) Outline FOUR experimental considerations in the design of a Polyacrylamide Gel Electrophoresis (PAGE) system. (12 marks)
- (b) Describe TWO methods of analysis of gels post electrophoresis. (8 marks)
- Q6. (a) Outline the important assay optimisation parameters for a non-competitive sandwich ELISA. (10 marks)
- (b) Write a brief overview of immunoassay validation. (10 marks)
- Q7. Write an overview of centrifugation under the following headings:
- (i) Types of centrifugal separation (10 marks)
 - (ii) Rotor categories (5 marks)
 - (iii) General applications (5 marks)