

**CORK INSTITUTE OF TECHNOLOGY  
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ**

**Autumn Examinations 2010/11**

**Module Title:      Bioanalytical Science V**

**Module Code:        BIOT7002**

**School:                Biological Science**

**Programme Title:    Bachelor of Science in Applied Biosciences and Biotechnology – Year 3**

**Programme Code:    SBIBI\_7\_Y3**

**External Examiner(s):      Dr A. Nelson**

**Internal Examiner(s):      Dr L. Goold, Ms A. Ward**

**Instructions:            Attempt 2 questions from Section A and 2 questions from Section B**

**Duration:            2 Hours**

**Sitting:                Autumn 2011**

**Requirements for this examination:**

**Note to Candidates:** Please check the Programme Title and the Module Title to ensure that you have received the correct examination paper.  
If in doubt please contact an Invigilator.

## 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) A chromatography separation yielded the following data for a given set of experimental conditions:-

**Column Length:** 20 cm. **Phase Ratio** ( $V_s/V_m$ ): 0.25

**Retention Times:-** non retained component = **30 seconds**

Component A = **1.5 minutes**

Component B = **2.0 minutes**

**Peak Base Widths:** Component A = **10 seconds**

Component B = **15 seconds**

Calculate the following:-

(i) The capacity factor,  $k^1$ , for Component A.

(ii) The partition co-efficient,  $K_d$ , for component B.

(iii) The resolution between,  $R$ , between A and B

(iv) The length of column required to give a resolution value of 1.50

(10 Marks)

(20 Marks)

Q2. (a) Construct a labelled block diagram of a gas chromatographic instrument and use the diagram to give a brief description of how a sample solution containing a mixture of volatile compounds is separated and analysed by this instrument.

(8 marks)

(b) Compare the processes of isothermal and temperature programmed gas chromatographic analysis.

(4 marks)

(c) An alcoholic beverage sample was analysed by gas chromatography for its ethanol content using the internal standard method of quantitation. Isopropanol was chosen as the internal standard and was added to the sample and standards of ethanol so that all solutions had a constant concentration of isopropanol.

The following data were obtained for all solutions analysed:-

| <b>%(v/v) Ethanol</b> | <b>Area of Ethanol Peak<br/>(integration counts)</b> | <b>Area of Isopropanol Peak<br/>(integration counts)</b> |
|-----------------------|--|--|
| 2                     | 660947   | 1812442  |
| 4                     | 1269649  | 1800540  |
| 6                     | 1861078  | 1799212  |
| 8                     | 1709135  | 1220811  |
| 10                    | 3081370  | 1803780  |
| sample                | 1185944  | 1701250  |

Use a graphical method to accurately determine the %(v/v) of ethanol in the sample.

(8 marks)

(20 Marks)

Q3. (a) Draw a labelled block diagram of a HPLC instrument and briefly describe the function of each component labelled. (8 marks)

(b) Discuss the importance of mobile phase selection for successful separation in HPLC analysis. (Your discussion should include reference to normal and reverse modes of HPLC analysis, how polarity affects order of elution of components in both modes and how eluting strength of mobile phase can be changed in both modes.)

(12 marks)

(20 Marks)

## SECTION B

- Q4. (a) Outline the important experimental considerations in the design of a Polyacrylamide Gel Electrophoresis system (10 marks)
- (b) Write a brief overview of the analysis of Polyacrylamide gels post electrophoresis. (10 marks)
- (20 Marks)
- Q5. (a) Write an overview of immunoassay validation. In your answer, outline the key parameters required to perform validation experiments for a newly developed immunoassay system. (10 marks)
- (b) Write short notes on the use of non-isotopic labels used in modern immunoassay systems. (10 marks)
- (20 Marks)
- Q6. (a) Describe, with the aid of a diagram the principle of a heterogeneous non-competitive Enzyme Linked Immunosorbent Assay (ELISA) (12 marks)
- (b) Outline the main optimisation parameters required for a non-competitive ELISA. (8 marks)
- (20 Marks)