

**CORK INSTITUTE OF TECHNOLOGY  
INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ**

**Semester 1 Examinations 2010/11**

**Bioanalytical Science V**

**Module Code: BIOT7002**

**School: Science**

**Programme Title: BSc in Applied Biosciences and Biotechnology**

**Programme Code: SBIBI\_7\_Y3**

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

**Internal Examiner(s): Dr. L. Goold**

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

**Duration: 2 hours**

**Sitting: Winter 2010**

**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. Write an account of the 4 main classes of chromatography i.e adsorption, partition, ion exchange and size exclusion. In the case of each class, indicate the nature of the stationary phase, the possible physical states of the mobile phase and explain the basis for separation of a mixture of components based on their interaction with the stationary phase

(20 marks)

- Q2. The analysis of vanillin in vanilla essence was performed by gas chromatographic analysis using the internal standard method of quantitation and flame ionisation detection. The internal standard chosen was p-hydroxybenzaldehyde, A series of standard solutions of vanillin were prepared in methanol solvent. The vanilla essence sample used for analysis was prepared by pipetting 200  $\mu\text{l}$  of the original sample of essence into a vial and diluting to 1.0  $\text{cm}^3$  (i.e.1000 $\mu\text{l}$ ) with methanol solvent. All the solutions (which also contained the internal standard at a fixed concentration) 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

PTO

(a) Construct a simple labelled block diagram of the instrument and use it to briefly describe the function of each component labelled. (6 marks)

(b) Describe the principles of operation of a flame ionisation detector (6 marks)

(c) Use an appropriate plot to determine the ppm concentration of vanillin in the original vanilla essence sample as accurately as possible. (8 marks)

Q3. (a) Draw a labelled block diagram of a HPLC instrument and briefly describe the function of each component labelled. (6 marks)

(b) Discuss the importance of the nature and composition of the mobile phase in relation to separation development in both normal and reverse modes of HPLC analysis. Briefly compare the gradient and isocratic elution modes of mobile phase operation and indicate the type of sample mixture that would require the gradient mode for successful separation. (8 marks)

(c) Describe in detail how a uv absorbance HPLC detector operates. Explain the process and usefulness of wavelength programming analysis associated with this type of detector. (6 marks)

**PTO**

## SECTION B

Q4. (a) Write an overview of Internal Quality Control (IQC) in immunoassays under the following headings:

(i) Basic IQC statistics (4 marks)

(ii) Quality Control Charts (4 marks)

(b) Briefly describe the principles & operation of an External Quality Assessment Scheme (EQAS) (6 marks)

(c) Define each of the following:

(i) International Standard (3 marks)

(ii) Laboratory Standard (3 marks)

Q5. (a) Describe the principle of **ONE** of the following immunoassay systems:

(i) Heterogeneous reagent excess ELISA

(ii) Homogeneous EMIT immunoassay

(Illustrate your answer with diagrams) (10 marks)

(b) List the main classes of immunoassay (4 marks)

(c) Write a brief overview of non-isotopic labels currently in use in modern immunoassay systems (6 marks)

Q6. (a) Write a brief 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) Describe the important experimental considerations in designing a Polyacrylamide Gel Electrophoresis (PAGE) system for protein separation. (10 marks)