

**CORK INSTITUTE OF TECHNOLOGY**  
**INSTITIÚID TEICNEOLAÍOCHTA CHORCAÍ**

**August Examinations 2013**

**Module Title:      ADVANCED CHROMATOGRAPHY**

**Module Code:          BIOT 8003**

**School:                  Biological Sciences**

**Programme Title:**    B.Sc. in Applied Biosciences and Biotechnology  
                              B.Sc. (Honours) in Pharmaceutical biotechnology

**Programme Code:**    SBIBI\_7\_Y3  
                              SPHBI\_8\_Y3

**External Examiner(s): Dr. Jerry Bird**

**Internal Examiner(s): Germain Levieille**

**Instructions:**        **Answer any 4 of the 5 questions asked. Each question carries a equal mark weighing.**  
                              **Please state clearly the questions addressed in your paper.**

**Duration:               2 Hours**

**Sitting:                  August 2013**

**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.

- Q1. a) Discuss the Plate Theory as a model of prediction of analytes behaviour in chromatography. (10 marks)
- b) Highlight the key factors affecting the efficiency of a chromatographic method based on the Plate theory rational. (5 marks)
- c) Discuss the Rate Theory, give its principles, mathematical components and equation. (10 marks)
- Q2. Two substances A and B are separated by column chromatography. Their retention times are A: 6.5 min and B: 11 min, on a 20 cm column. The widths of the peak bases were 0.35 and 0.68 min respectively.
- Calculate:
- (a.) the resolution of these peaks; (5 marks)
- (b.) the average number of plates in the column; (7 marks)
- (c.) the average plate height; (7 marks)
- (d.) calculate the minimum length of column to achieve a resolution of 1. (6 marks)
- Q3. a) Give a description of the method of solid phase microextraction used for headspace analysis. Include diagram and general description of the equipment used for this method. (15 marks)
- b) Discuss the applications of this technique? (10 marks)
- Q4. You have a 2-Liters aqueous solution containing 200.0 mM of compound C. This solution is extracted with 500.0 mL of diethyl ether and the aqueous phase is assayed and it is found that the concentration of compound C that remains is now at 40.0 mM.
- a) What is the equilibrium constant for this extraction system? (10 marks)
- b) How much compound C will remain in aqueous solution after three extractions? (10 marks)
- c) What will be the percentage of recovery of the compound C after three extractions (5 marks)
- Q5. a) Describe the general principles of HPLC. (8 marks)
- b) Describe the instrumentation used for HPLC. (5 marks)
- c) Indicate the factors to consider to optimise an HPLC method (5 marks)
- d) How does HPLC compare with other chromatographic methods, particularly Gas Chromatography? (7 marks)