TECHNOLOGICAL UNIVERSITY DUBLIN
TALLAGHT CAMPUS

Higher Certificate in Science
Bachelor of Science

Applied Biology
Bioanalysis

Full Time

Semester Three : January 2023

Chromatography Techniques & Measurement Systems

Internal Examiners
Dr Sinead Currivan-Macdonald
Dr Eugene Hickey

External Examiners
Dr David Page

Day **Saturday**
Date **7th January 2023**
Time **15:30-17:30**

Instructions to Candidates

Answer question one (compulsory) in Section A and two questions in Section B.

Total questions to be answered is three.

All questions are worth equal marks (100).

Total marks = 300

SECTION A- E. HICKEY

Question 1 (compulsory)

Answer any eight from twelve parts. Each part is worth 12.5 marks.

a) Distinguish between digital and analogue sensors and give an illustrative example of each.

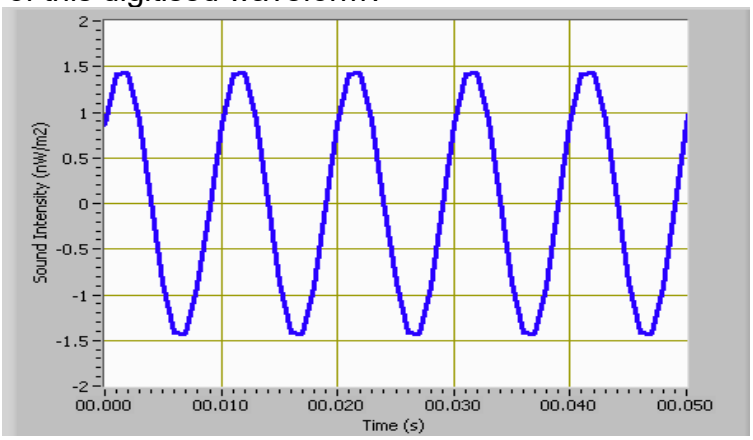
b) What process turns a signal into data?

c) The detector in the Scanning Electron Microscope produces a signal of size 15μV. It has an electrical resistance of 200kΩ and a bandwidth of Δf = 50MHz. Calculate the Shot Noise from the detector using $V_n = (2eVR\Delta f)^{½}$. Note the charge on the electron is $1.6x10^{-19}$C.

d) What types of filter would be appropriate to reduce the noise in the following signals:
- White noise where the signal of interest has frequencies between 100hz and 20kz
- 1/f noise which dominates the signal below 100hz
- A signal polluted by noise at 50hz and harmonics

e) Give four advantages for converting a signal into digital format as opposed to keeping it in analogue format.

f) Calculate the frequency in hertz of the signal shown below. This signal was digitised with a sample taken every 0.005s. Do a rough sketch in your answer copy (no graph paper needed) of the input signal, the samples taken, and the digitised waveform. What is the measured frequency of this digitised waveform?



g) A signal contains frequencies up to 150khz. What is the minimum sampling rate needed to capture this signal correctly?

h) What is meant by "Aliasing" of a signal when it is sampled?

i) An ADC has a range of -10V to 10V and digitises to 8 bits (256 levels). What is the digital resolution of this ADC?

j) A signal is digitised capturing 15s seconds of data with a sampling rate of 10khz and a sampling depth of 12 bits. What is the size in Bytes of the raw data file of this signal?

k) What is meant by the ***IP*** (internet protocol) address of a computer? Give an example of what an IP address would look like.

l) Distinguish between lossless and lossy compression using digital image formats as examples.

(8x12.5 = 100 marks)

Section B - S. Currivan-Macdonald

Answer any two questions

Question 2

Answer all parts (10 marks each)

a) What are the factors that contribute to resolution?

b) What are the components of a HPLC system?

c) Note two detectors that can be used with a modular HPLC system.

d) From a chromatogram the following values were obtained:

   i. t_R 3.67 min

   ii. $W_{1/2}$ 0.12 min

   Using the above values calculate efficiency.

e) List the three terms that contribute to band broadening as defined by the van Deemter equation.

f) What is a gradient in HPLC?

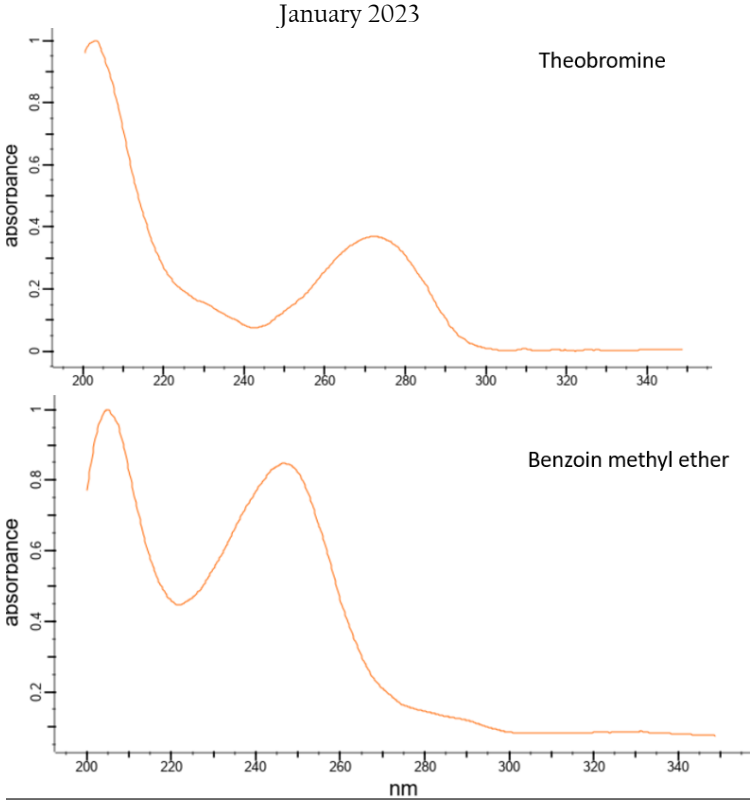
g) What factor us used to control retention in GC?

h) Calculate the resolution between the critical pairs (peak 6 and 7) in the table below:

	Peak 1	Peak 2	Peak 6	Peak 7
t_R	0.891	1.9	8.453	9.01
W_b	0.08	0.10	0.09	0.12
$W_{1/2}$	0.05	0.043	0.05	0.056

i) What is an internal standard and when is it used?

j) From the UV spectra below, what is the best wavelength of detection that should be set on the UV detector in your LC separation?


Theobromine
absorbance
0
0.2
0.4
0.6
0.8
1
200
220
240
260
280
300
320
340
Benzoin methyl ether
absorbance
0.2
0.4
0.6
0.8
1
200
220
240
260
280
300
320
340
nm

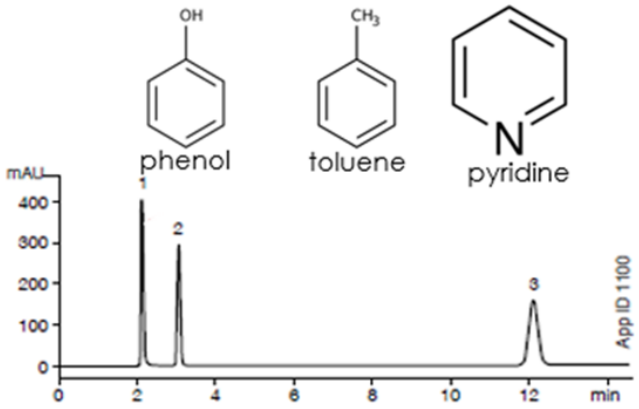
Question 3

100 marks

a) Describe two chromatographic methods usually encountered in HPLC. Within your answer briefly describe the stationary phase, mobile phase, and the typical analyte chemistry. (30 marks)

b) Briefly describe the factors influencing the A term of the van Deemter equation, the effects of the term, and suggest how this may be overcome. (30 marks)

c) In RPLC, two analytes are separated on a C18 column, with a mobile phase composition of 50 % methanol. Based on the following data, which compound will elute first? Within your answer justify your decision. (40 marks)



Analyte	Log P	Molecular weight (g/mol)
Phenol	1.5	94.11
Toluene	2.7	92.14
Pyridine	0.7	79.10

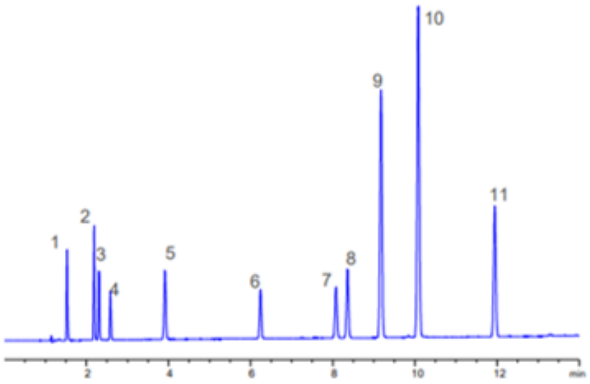
Question 3

100 marks

a) Draw a schematic of a GC system and note two detector types commonly found. Describe how one of those choices operates. (30 marks)

b) What are the advantages and disadvantages of reducing internal diameter of a GC column? (20 marks)

c) Based on the chromatogram below show your working for the following;



	Peak 1	Peak 2	Peak 7	Peak 8	Peak 11
t_R	1.891	2.20	8.100	8.453	9.01
W_b	0.08	0.10	0.10	0.09	0.12
$W_{1/2}$	0.05	0.043	0.05	0.05	0.056

i) Calculate the k of peaks 1 and 11 given that t_0 is 1.6 min

ii) Calculate the N of the separation

iii) The Rs between peaks 7 and 8 must be greater than 1.5 to pass a system suitability test, does this system passed based on your calculation? (50 marks)

Some useful equations:

$R_S = \frac{t_{R2} - t_{R1}}{\frac{1}{2}(W_1 + W_2)}$	$N = 5.54\left(\frac{t_R}{W_{0.5}}\right)^2$	$k = \frac{t_R - t_0}{t_0}$
$\alpha = \frac{k_2}{k_1}$	$t'_R = t_R - t_0$	$H = \frac{L}{N}$