UNIVERSITY OF JOHANNESBURG

DEPARTMENT OF BIOCHEMISTRY

FACULTY	: Science	
DEPARTMENT	: Biochemistry	
CAMPUS	: APK	
MODULE	: BIC8X01	ADVANCED ANALYTICAL TECHNIQUES
SEMESTER	: First	
EXAM	: June 2019	

DATE	03 June 2019	**SESSION**	08:30 - 11:30
ASSESSOR(S)	MR. M. MHLONGO DR. T. HORNE DR. F. TUGIZIMANA DR. Z. ENGELBRECHT		
MODERATOR	DR E. MADALA		
DURATION	3 HOURS	**MARKS**	112

NUMBER OF PAGES: 7 PAGES

INSTRUCTIONS:

1. Answer ALL THE QUESTIONS.
2. Number your answers clearly

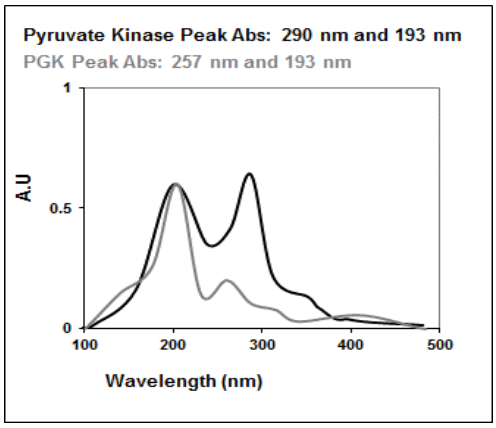
This paper comprises THREE sections. Answer ALL questions in each section. Use relevant equations and diagrams to add to your answers where applicable.

SECTION A - (DR. T HORNE) [22]

QUESTION 1 [10]

Pyruvate kinase is an enzyme that converts PEP into pyruvate via K^+ and Mg^{2+} ion cofactors and dephosphorylation generating ATP.

You isolate some pyruvate kinase in order to perform studies on it. When you compare its absorption spectra in water to the enzyme phosphoglycerate kinase (PGK) you observe a different profile despite the two enzymes having similar functions:



a) What does this comparison tell you about the composition of pyruvate kinase Versus other similar kinase enzymes? **(2)**

b) The spectra above is an example of an absolute spectra comparison. What spectrophotometric method could you use to determine the similarity between these two enzymes (Hint: isobestic points) **(1)**

c) What is the corresponding molar extinction coefficient of pyruvate kinase if you obtained this spectrum (A_{290nm} = 0.589) using a concentration of 165 mol / dm^3? (Assume a standard light path length) **(2)**

d) You would like to study the binding relationship between pyruvate kinase and PEP so you couple a fluorophore to PEP which quenches its signal upon binding to pyruvate kinase. However, low PEP fluorescence readings are observed before binding with pyruvate kinase. What could be the possible **technical** causes of this? **(2)**

e) Describe how you could use luminescence to study pyruvate kinase reaction efficiency rates **(3)**

QUESTION 2 **[6]**

You discover a new type of Low Density Lipoprotein which you name LDL1. You wish to study its interactions with cells in order to determine if it behaves like other LDLs.

a) You discover that LDL1 donates a new type of fatty acid of unknown function to the cells. What two critical pieces of information could you obtain about this fatty acid by using the following spectrofluoremetric methods **(NB* include a brief description of how you would use the method to obtain that information)**:

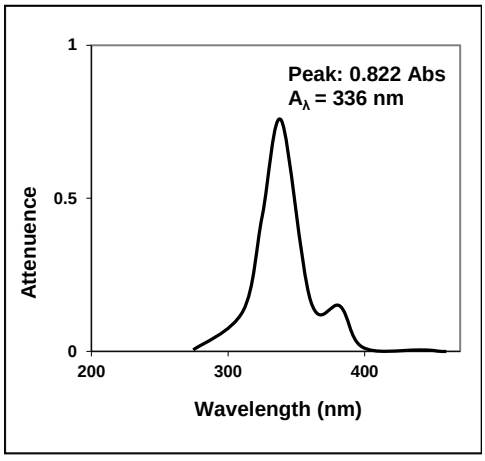
1) Fluorescence microscopy **(3)**

2) Quenching **(3)**

b) You discover that this fatty acid supplies the energy needed for synthesis of a large structural protein tetramer. Name one **spectrophotometric** and one **spectrofluorometric** application you could use to study the structure and/or orientation of this protein tetramer **(2)**

QUESTION 3 **[4]**

You isolate lysosomes and golgi bodies from other cell components and obtain an 'apparent' absorbance reading in order to compare with a standard for yield determination.



a) What is this application called and why would you need to employ it for this sample in particular? **(2)**

b) If you could further separate the lysosomes from the golgi bodies, how would you make use of the application in (a) to determine the molecular mass of your organelles? **(2)**

SECTION B - (DR. F TUGIZIMANA) **[20]**

QUESTION 1 **[5]**

a) Describe (3 lines max) Raman scattering (Raman effect) **(1)**

b) The shape of a Raman peak is important, not just its position. Use a typical Raman peak diagram to indicate 4 possible information that can be observed from a Raman peak. **(4)**

QUESTION 2 **[5]**

a) Name two possible information that can be provided by an FT-IR spectrometer configured to use a specific sampling device (e.g. transmission or attenuated total reflection) **(2)**

b) Write briefly about bending vibrations in IR (5 lines max). **(3)**

QUESTION 3 **[5]**

NMR spectroscopy is widely used in biochemical studies and medical applications. The resonance condition in NMR is satisfied in an external magnetic field. Describe/define the following:

a) The acquisition time **(1)**

b) The saturation **(1)**

c) The chemical shift **(1)**

d) Write down the equation for energy difference between two adjacent energy levels of magnetic dipole in an external magnetic field. Draw a diagram **(2)**

QUESTION 4 **[5]**

Signals recorded on a 1D ^{1}H NMR spectrum are chemical shifts representing frequencies from all NMR-visible nuclei in a sample. Thus, in 1D ^{1}H NMR metabolite profiling of plant extract samples, the generated spectrum is the result of the superposition of the NMR spectra of all NMR-visible single compounds present in the extracts.

a) To extract information from such analyses, data processing is required prior to statistical analyses. Explain (very briefly, 5 lines max) spectral 'bucketing or binning'. **(3)**

b) Due to the complexity of such samples, multidimensional NMR methods are often employed. Give two examples (full name) of such methods. **(2)**

SECTION C - (DR. Z ENGELBRECHT) **[20]**

QUESTION 1 **[5]**

a) What are the applications of analytical ultracentrifugation? **(5)**

QUESTION 2 **[5]**

Primary skeletal muscle tissues (from mouse) is sent to your lab for analysis. The client wants you to isolate the proteins and organelles from the tissue specimens for further analysis.

a) What technique will you use to ensure a successful separation? Mention the major steps in the correct order. **(4)**

b) How can you ensure that the membrane fractions are not contaminated with myosin? **(1)**

QUESTION 3 **[2]**

a) What is electroendosmosis and how can this phenomenon be resolved? **(4 x ½ = 2)**

QUESTION 4 **[8]**

You obtain two kidney specimens. One is from a healthy patient and the other from a patient that has chronic kidney failure. A proteomic approach is required to compare the two states (healthy vs disease) with each other.

a) What electrophoretic technique will you use to identify differentially expressed proteins in the damaged kidney? **(1)**

b) Explain the principle of the technique mentioned in (a). **(10×½=5)**

c) You identify two proteins (using the method in (a)) that are absent in the healthy kidney extract but expressed in the damaged kidney extract.

1) How will you isolate the proteins and with what other technique will you be able to identify them? **(4×½=2)**

SECTION D - (MR. M MHLONGO) **[50]**

QUESTION 1 **[10]**

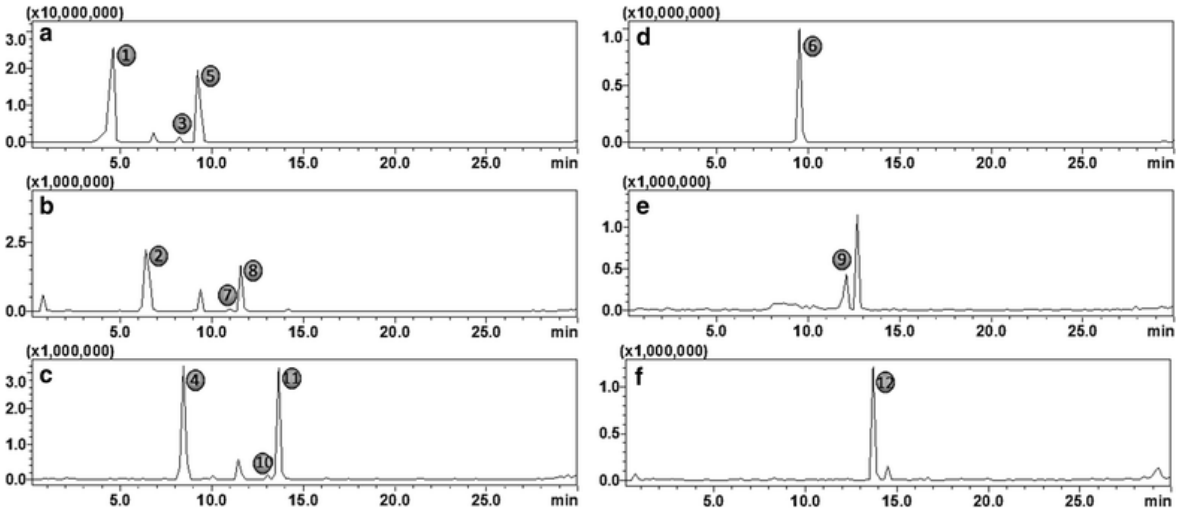
a) What is the selectivity factor of a chromatographic system and what influences this factor? **(2)**

b) Chromatographic columns are often compared with regards to the number of 'theoretical plates'. Explain what this is and why two well-separated analytes will have different values for the number of theoretical plates. **(4)**

c) Calculation of the resolution (Rs) between two adjacent peaks on an HPLC system with gradient elution gives a value of 0.85. Using the same column and mobile phase solvent(s), name (with explanation) four practical approaches that can be implemented in order to improve the resolution between the two metabolites. **(4)**

QUESTION 2 **[10]**

The following figure shows an elution order of two isobaric molecules (precursor: *m/z* 353) namley hydroxycinnamoyl-quinic acids (a-c) and hydroxycinnamoyl-isocitric acids (c-f) on C_{18} column using. A binary solvent mixture of MilliQ water containing 0.1% formic acid (eluent A) and methanol containing 0.1% formic acid (eluent B) was used with a constant flow rate of 0.4 mL/min.

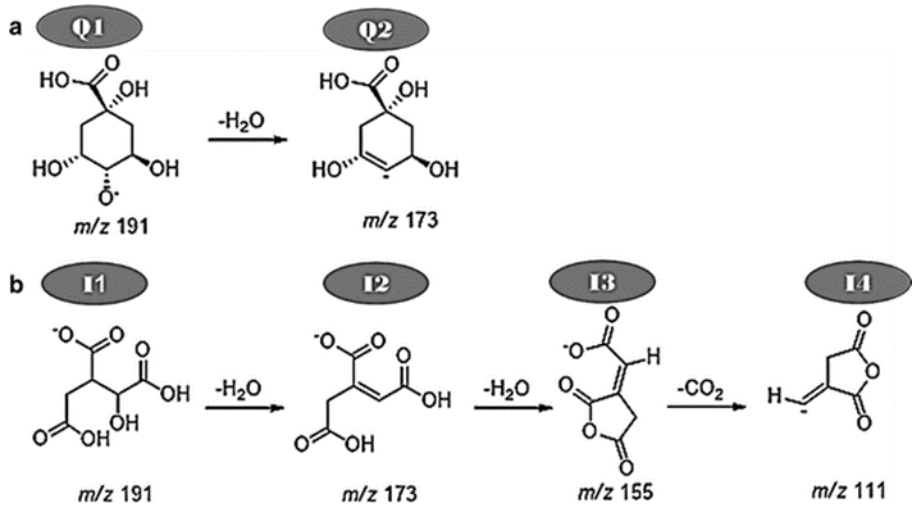


Key: *a* caffeoyl-quinic acids, *b* p-coumaroyl-quinic acid and *c* feruloyl-quinic acid
d caffeoyl-isocitric acid, *e* p-coumaroyl-isocitric acid and *f* feruloyl-isocitric acid

a) What type of chromatography was used to separate the above-mentioned molecules? Explain why the hydroxycinnamoyl-isocitric acids **(d-f)** elutes later than their counterparts **(a-c)** **(2)**

b) Explain in details the principles of the chromatographic method mentioned in **a**. i.e. SP and MP, type of molecules. **(3)**

c) The following figure shows the fragmentation mechanism and structural re-arrangement for the $[M–H]^-$ ion of quinic acid **(a)** and isocitric acid **(b)** in negative ionization. Draw spectra of the molecules (**a** and **b**) obtain by tandem MS performed in space. **NB!** Also, show the precursor ion. **(5)**



QUESTION 3 **[10]**

a) Tandem MS (MS/MS) was developed for structural elucidation of biomolecules. Describe the principles of a triple quadrupole (3Q) system for determination of the primary structure of peptides following tryptic digestion. **(4+4=8)**

b) Name two advantages that MS-based sequencing has above Edman sequencing **(2)**

QUESTION 4 **[10]**

a) Give an overview of how MS analysis is used in quantitative proteomics with reference to the Isobaric Tags for Relative and Absolute Quantitation (ITRAQ) technique. **(10)**

QUESTION 5 **[10]**

a) Define the following terms used in quantitative analysis:

1) Robustness **(1)**

2) Analytical specificity **(1)**

b) Quantitative measurements can be accepted with a degree of uncertainty. Distinguish between the two kinds of experimental errors and explain how you would identify them. **(4)**

c) When performing quantitative analysis is important to include an internal standard.

1) What is an ideal internal standard? **(2)**

2) If the analytical procedure involves preliminary sampling procedures, such as solid phase extraction, why is it important to add a known concentration of an internal standard at an early stage? **(2)**