W1-2-60-1-6

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

UNIVERSITY EXAMINATION 2022/2023

MSC, MEDICINAL CHEMISTRY

SECOND SEMESTER SUPPLEMENTARY EXAMINATION

TPS 3103: INSTRUMENTAL METHODS OF ANALYSIS

DATE: DECEMBER 2023 **TIME: THREE HOURS**

Instructions : Answer any FOUR of the SIX questions

QUESTION ONE (25 MARKS)

a Differentiate between affinity and adsorption chromatography and state an example of each **4 marks**

b HPLC fitted with a UV detector is used in quantifying caffeine content in a diet cola sample containing caffeine, benzoic acid, and aspartame in the order of increasing polarity. Project the following: -

(i) the chromatogram for the diet cola sample (give reason) **5 marks**

(ii) the quantitative determination of caffeine **4 marks**

(iii) Explain the working of the detector during detection of caffeine **4 marks**

c Uncolored components can be visualized by three main ways in Thin layer chromatography. Name the three ways and explain the chemistry associated with any one of these **4 marks**

d Discuss how you would address resolution and retention time during method development in GLC **4 marks**

QUESTION TWO (25 MARKS)

a List the sections of a splitless injector port stating the function of each section. Hence describe the working of the device taking into consideration the precautions during the injections **5 marks**

b i. Explain the mechanism of separation in GC-MS **5 marks**

ii. Describe how GC-MS is used in identification of compounds from crude extract **5 marks**

c Explain separation by capillary zone electrophoresis for at least 3 arbitrary anions with different charges and concentration and present their layout in both the capillary tube and on the electropherogram **6 marks**

c Discuss the step of development in planar chromatography **4 marks**

QUESTION THREE (25 MARKS)

a Chromatography is the separation of sample components based on their distribution between two phases. Differentiate between the following as used/employed in chromatography: - **12 marks**

i. Sensitivity and selectivity

ii. TLC and column chromatography

iii. Retardation factor and resolution factor

iv. Split and splitless injector ports

v. Visualization by UV-light and by a locating reagent

vi. Adsorption and partition separation modes

b Study the data below generated following separation of four carotenoids U, V, W and X (polarity increasing in the order U,V,W,X) on a 35 cm normal phase HPLC column. The absorbance (K) of UV light was measured by a UV detector. Answer the question s that follow

Rt (min)	W_b	No. of peaks	Peak letter	Peak area	K
0		0	B	0	G
6	0.05	1	C	300	H
1		A			100
8	0.01			F	
5	1.24	2	D	50	1
9	0.02	1	E	400	30

i. How many peaks were detected after 1 min (letter A)? (1mark)

ii. Sketch the resultant chromatogram (2 marks)

iii. Assign the letters B-E to the carotenoids (3 marks)

iv. What is the column effluent at 8 mins (letter F)? Explain your answer (1 mark)

v. Provide suitable values for the absorbance (letter s G-I). Give reason for your values. (2 marks)

vi. Report the resolution for the two least retained carotenoids. (2 marks)

vii. Evaluate theoretical plate height for the most retained carotenoid (2 marks)

QUESTION FOUR (25 MARKS)

a Using the theory of column chromatography, explain how scientists evaluate column efficiency (use two analytes in the same sample) **7 marks**

b Discuss three methods used in improving efficiency in capillary zone electrophoresis **6 marks**

c Discuss the separation of say three analytes in HPLC and GLC **6 marks**

d Briefly explain the working of split and splitless injection port — 6 marks

QUESTION FIVE (25 MARKS)

a Consider the separation of sample components by capillary zone electrophoresis and explain their separation making reference to charge and size — 6 marks

b Discuss the working of the following detectors

i. Electron capture detector — 3 marks
ii. Ultra-Violet detector — 3marks

c Flow injection analysis is used in the pharmaceutical industry where absorbance of an analyte is employed. Discuss the determination of the concentration of analyte using FIA — 5 marks

d
i. Explain the working principle of supercritical fluid chromatography — 5 marks
ii. Why is supercritical fluid chromatography superior over gas chromatography — 3 marks

QUESTION SIX (25 MARKS)

a
i. Describe the different ionization techniques in mass spectrometry — 8 marks
ii. Explain data acquisition and interpretation in mass spectrometry — 10 marks

b Sketch a well labelled chromatogram showing poor resolution in thin layer chromatography and describe one factor that can be used to improve the resolution — 5 marks

c Explain the significance of the number of theoretical plates in column chromatography — 2 marks