



W1-2-60-1-6

**JOMO KENYATTA UNIVERSITY  
OF  
AGRICULTURE AND TECHNOLOGY  
UNIVERSITY EXAMINATIONS 2024/2025**

**END OF SEMESTER EXAMINATION FOR THE DEGREE OF MASTER OF  
SCIENCE IN MEDICINAL PHYTOCHEMISTRY**

**TPS 3107: CHROMATOGRAPHIC METHODS OF ANALYSIS**

**DATE: DECEMBER 2024**

**TIME: 3 HOURS**

**INSTRUCTIONS: ANSWER ANY FOUR QUESTIONS**

**QUESTION ONE (25 MARKS)**

- a) Explain the principle of chromatography 4 Marks
- b) Classify chromatography based on the physical state of the mobile phase 6 Marks
- c) Summarize the common modes of chromatography 5 marks
- d) Discuss the role of chromatography in phytochemistry 10 marks

**QUESTION TWO (25 MARKS)**

- a) Discuss the principles, procedure, advantages, and limitations of thin-layer chromatography (TLC) as a separation technique. Include its applications in phytochemistry 10 marks
- b) Using a sketch and appropriate examples describe the interpretation of a TLC chromatogram 6 marks
- c) You performed a thin-layer chromatography experiment and obtained the following measurements: 5 marks
- The distance traveled by the solvent front (mobile phase) is 8.0 cm.
  - The distance traveled by Compound A is 6.4 cm.
  - The distance traveled by Compound B is 4.0 cm.

Calculate the retention factor ( $R_f$ ) values for both Compound A and Compound B. Then, interpret the  $R_f$  values in terms of polarity.

- d) Describe the specific role of TLC in phytochemical research **4 marks**

**QUESTION THREE (25 MARKS)**

- a) Using a diagram, explain the theory of column chromatography **6 marks**

- b) A researcher is analyzing a mixture of phytochemicals using a high-performance liquid chromatography (HPLC) system. The following data were obtained during the experiment: **5 marks**

- The width of the peak for a specific phytochemical is 0.5 minutes.
- The retention time for the same phytochemical is 5.0 minutes.
- The length of the column used for the separation is 25 cm.
- The number of theoretical plates ( $N$ ) calculated for the peak is 400.

- c) Highlight how the following factors affect column efficiency **4 marks**
- i. Column temperature
  - ii. Particle size of solid stationary phase
  - iii. Solvent
  - iv. Concentration of solutes

d) A researcher is tasked with isolating and purifying specific phytochemicals from a plant extract using column chromatography. The following details are provided:

- The plant extract contains a mixture of flavonoids, alkaloids, and terpenoids.
- The stationary phase used is silica gel, and the mobile phase is a gradient of hexane and ethyl acetate.
- The researcher plans to collect fractions and analyze them using TLC to monitor the separation of compounds.

- i. Describe the steps the researcher would take to set up and conduct the column chromatography process **4 marks**
- ii. How would the researcher determine the optimal mobile phase composition for effective separation of the desired phytochemical **3 marks**
- iii. If the researcher collected 10 fractions and found that fractions 4 and 7 contained distinct spots on the TLC plate corresponding to the target flavonoids, what additional steps should be taken to confirm the identity of these compounds **3 marks**

#### QUESTION FOUR (25 MARKS)

- a) Describe the principle operation of high performance liquid chromatography (HPLC) **5 marks**
- b) Discuss the different types of high-performance liquid chromatography (HPLC) techniques, including their principles, applications, and advantages **10 marks**

- c) Discuss the role of high-performance liquid chromatography (HPLC) in phytochemistry research. Include its applications in the identification and quantification of phytochemicals, and describe how HPLC can be utilized to analyze complex plant extracts. Provide examples to illustrate its importance in this field. **10 marks**

**QUESTION FIVE (25 MARKS)**

- a) State two factors that affect the order of elution in gas chromatography **2 marks**
- b) With an aid of a well labeled sketch, explain the principles and operational procedures of gas chromatography (GC) **7 marks**
- c) Discuss its applications in various fields such as environmental analysis, food safety, and pharmaceuticals. **4 marks**
- d) Analyze the impact of gas chromatography (GC) in phytochemistry research **4 marks**
- e) Describe how GC is utilized to analyze volatile and semi-volatile phytochemicals in plant extracts. Include specific examples of its applications in identifying essential oils, terpenes, or other bioactive compounds **6 marks**
- f) Highlight any two advantages of using GC in Phytochemistry **2 marks**

**QUESTION SIX (25 MARKS)**

- a) Outline the significance of ion chromatography in phytochemistry studies **10 marks**
- b) Describe the principle of operation of electrophoresis. **10 marks**
- c) Discuss the application of electrophoresis in the study of phytochemicals **5 marks**