WI-2-60-1-6

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

UNIVERSITY EXAMINATIONS 2025/2026

EXAMINATION FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL CHEMISTRY

TPS 3103: INSTRUMENTAL METHODS OF ANALYSIS

DATE: DECEMBER 2025 **TIME: 3 HOURS**

QUESTION ONE (25 MARKS)

a) Explain the key differences between classical "wet chemistry" methods and instrumental methods of analysis in pharmaceutical sciences, providing examples of each. **3 Marks**

b) A pharmaceutical company synthesized a new compound with molecular formula $C_{16}H_{18}N_2O_4$. Describe how NMR, MS, and HPLC can be used together to confirm the structure, molecular weight, and purity of the compound. **3 Marks**

c) During quality control of paracetamol tablets, UV spectrophotometry gave inconsistent assay results (91–108% of label claim). Explain why HPLC provided more reliable results and why adherence to pharmacopeial methods is important. **3 marks**

d) A batch of atenolol tablets showed a degradation peak after accelerated stability testing. Explain why HPLC is preferred over UV spectroscopy for stability studies and describe corrective measures to ensure product stability. **3 marks**

e) In a bioequivalence study of ciprofloxacin, plasma concentrations are very low (ng–µg/mL). Explain why LC–MS/MS is the method of choice and describe the role of internal standards. **3 marks**

f) Describe the four basic components of an analytical instrument and explain their roles in pharmaceutical analysis. Using a UV–Vis spectrophotometer as an example, briefly explain how each component functions in measuring drug concentration. **5 marks**

g) Explain why transducers are essential in analytical instruments. Could we directly record optical or thermal signals? Provide one pharmaceutical example for each of the following types of transducers: optical, thermal, electrochemical, and mechanical. **5 marks**

QUESTION TWO (25 MARKS)

a) Explain why λmax is used in UV–Vis spectroscopy for drug analysis and provide an example from pharmaceutical applications. **5 marks**

b) A patient's plasma sample shows an absorbance of 0.450 at 277 nm. If the molar absorptivity ε = 12,000 $L \cdot mol^{-1} \cdot cm^{-1}$ and path length l = 1 cm, calculate the drug concentration in $mol \cdot L^{-1}$. **5 marks**

c) Discuss the applications of UV–Vis spectroscopy in pharmaceutical analysis and provide examples from case studies. **5 marks**

d) A suspected counterfeit paracetamol tablet is analyzed using FTIR. The spectrum lacks the sharp C=O band at 1650 cm^{-1} but shows strong carbonate peaks around 1400 cm^{-1}. Explain the significance of these observations. **5 marks**

e) An FTIR spectrum of ibuprofen mixed with lactose shows a shift of the C=O stretch from 1720 cm^{-1} (pure ibuprofen) to 1705 cm^{-1}. Explain what this shift indicates and its pharmaceutical implications. **5 marks**

QUESTION THREE (25 MARKS)

a) Explain the principles, key components, and limitations of Atomic Absorption Spectroscopy (AAS), and describe one example of its application in quality control of pharmaceutical products. **5 marks**

b) A ferrous sulfate tablet (expected 60 mg Fe/tablet) is analyzed using flame AAS. After digestion and dilution, the sample absorbance is 0.286. Calibration slope = 0.022 A/ppm. Dilution factor = 20. Determine the iron content per tablet and assess if it meets pharmacopeial specifications (±10%). **5 marks**

c) A herbal syrup sample is analyzed for lead (Pb) content using AAS. The sample absorbance is 0.248, and the calibration slope is 0.031 A/ppm. The WHO permissible limit for Pb is 10 ppm. Interpret the results and explain the pharmaceutical significance. **5 marks**

d) Define chromatography and explain its basic principle in the context of pharmaceutical analysis. Illustrate your answer with one example from herbal medicine or drug **5 marks**

purity testing.

e) Explain why in silica gel column chromatography of a mixture of aspirin and caffeine, caffeine elutes before aspirin. Include in your answer the roles of stationary and mobile phases, and discuss how this principle applies to herbal alkaloid extraction. **5 marks**

QUESTION FOUR (25 MARKS)

a) Define HPLC and GC. Explain the principle of operation of each, highlighting mobile and stationary phases. Give one pharmaceutical application for each. **5 marks**

b) Paracetamol tablet batch: RP-HPLC standard peak area = 10,000, sample = 9,600. GC–FID methanol: standard 12,000 (3000 ppm), sample 10,000. **5 marks**

(a) Calculate % assay of paracetamol. **2 Marks**

(b) Decide if batch meets pharmacopeial limits. **1 mark**

(c) Explain why HPLC and GC are suitable. **2 marks**

c) Discuss major components of HPLC and GC systems, explaining function and giving an example detector. **5 marks**

d (i) Give two pharmaceutical applications of ion-selective electrodes. **1 mark**

(ii) Fluoride ISE measures toothpaste slurry: E = 160 mV; standard 1 ppm → E = 150 mV; Nernst slope = 59 mV/decade. Calculate fluoride concentration. **2marks**

(iii) Conductometric titration: 50 mL HCl titrated with 0.1 M NaOH; equivalence at 48 mL. Calculate HCl concentration. **2 marks**

e (i) Explain why voltammetry is suitable for bioanalysis of drugs in plasma. **1 mark**

(ii) DPV shows dopamine = 0.8 µM (therapeutic range = 1.5–2.5 µM). Interpret the result. **1 mark**

(iii) How can voltammetry data guide therapy? **1 mark**

(iv) WFI batch shows conductivity = 2.0 µS/cm (USP limit ≤ 1.3 µS/cm). Is it compliant? **1 mark**

(v) What corrective action should be taken for non-compliant WFI? **1 mark**

QUESTION FIVE (25 MARKS)

a) (i) Define potentiometry. **1 mark**

(ii) Explain the main principle behind ion-selective electrodes in pharmaceutical analysis. **1 mark**

(iii) A batch of intravenous ciprofloxacin shows pH = 5.2 (BP specification 3.5–4.6). Does the batch comply with pharmacopeial standards? **1 mark**

(iv) What QC actions should be taken for non-compliant pH? **2 marks**

b) (i) Explain why voltammetry is suitable for bioanalysis of drugs in plasma. **1 mark**

(ii) DPV shows dopamine = 0.8 μM (therapeutic range = 1.5–2.5 μM). Interpret the result. **1 mark**

(iii) How can voltammetry data guide therapy? **1 mark**

(iv) WFI batch shows conductivity = 2.0 μS/cm (USP limit ≤ 1.3 μS/cm). Is it compliant? **1 mark**

(v) What corrective action should be taken for non-compliant WFI? **1 mark**

c) (i) Give two pharmaceutical applications of ion-selective electrodes. **1 mark**

(ii) Fluoride ISE measures toothpaste slurry: E = 160 mV; standard 1 ppm → E = 150 mV; Nernst slope = 59 mV/decade. Calculate fluoride concentration. **2 marks**

(iii) Conductometric titration: 50 mL HCl titrated with 0.1 M NaOH; equivalence at 48 mL. Calculate HCl concentration. **2 marks**

d) (i) Briefly explain the principle of TGA. **1 mark**

(ii) A 5 mg aspirin sample is heated from 25–400°C. Observed weight losses: 25–100°C → 2%, 140–150°C → 5%, 200–300°C → 90%. Assign the likely cause of each weight loss. **3 marks**

(iii) Comment on the thermal stability of aspirin based on this data. **1 mark**

e) (i) Briefly explain the principle of DSC. **1 mark**

(ii) DSC of paracetamol shows: Sample A → sharp endothermic peak at 169°C; Sample B → endothermic peak at 158°C followed by 169°C. Identify Form I and Form II and explain which is preferred for tablet compression. **2 mark**

(iii) DSC of ibuprofen + lactose mixture shows a broad melting peak at 70°C, compared to pure ibuprofen melting at 76°C. What does this suggest and what alternative excipient would you recommend? **2 marks**

QUESTION SIX (25 MARKS)

a) During stress testing of metformin hydrochloride, a degradation peak at m/z 73 is observed, corresponding to dimethylamine (DMA). The impurity level is 0.12%, while ICH Q3A/B guidelines require identification of impurities above 0.10%. **5 marks**

b) A sample of aspirin stored under stress conditions gives the following MS data: Parent drug m/z 180 ($[M+H]^+$) and degradation peak m/z 137. **5 marks**

c) **Question:** A hospital laboratory is tasked with monitoring plasma levels of fluoxetine in patients undergoing antidepressant therapy. The plasma samples contain proteins, salts, and endogenous metabolites that interfere with HPLC analysis. Which sample preparation technique would you recommend to ensure accurate HPLC analysis? Describe how this technique is performed and explain the outcomes and clinical significance of using it. **5 marks**

d) A pharmaceutical manufacturer suspects trace metal contamination in intravenous iron sucrose. The sample is viscous and contains complex organic matter, making direct ICP-MS analysis unsuitable. Which sample preparation technique should be used to analyze elemental impurities accurately? Outline the procedure and discuss the expected outcomes and regulatory implications. **5 marks**

e During stability testing of atenolol tablets, impurity Y was monitored using HPLC. The calibration regression equation obtained was $y = 1130x + 2$, $R^2 = 0.999$, where y = peak area and x = concentration in µg/mL. The standard deviation of the intercept is $\sigma = 2$.

(a) Calculate the Limit of Detection (LOD) and Limit of Quantification (LOQ). **3 Marks**

(b) A tablet sample gave a peak area of 565. Calculate the concentration of impurity Y and interpret whether it is quantifiable. **2 Marks**