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UNIVERSITY OF RUHUNA – FACULTY OF ALLIED HEALTH SCIENCES
DEPARTMENT OF PHARMACY
FOURTH BPHARM PART I EXAMINATION – MARCH/APRIL 2026
PH 4123 PHARMACEUTICAL ANALYSIS – SEQ

TIME: TWO HOURS

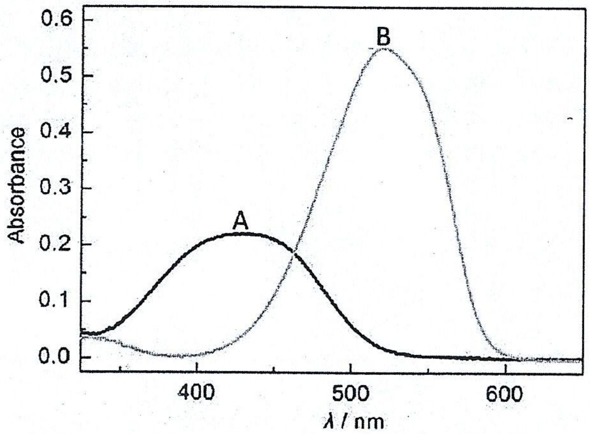
INSTRUCTIONS

- There are **four** questions in parts **A, B,** and **C** of this paper.
- Answer all questions.
- No paper should be removed from the examination hall.
- Do not use any correction fluid.
- Use illustrations where necessary.

PART A

01.

1.1 The methyl red indicator is red in acidic solutions (pH < 4.4) and yellow in alkaline solutions (pH > 6.2). The visible spectra of alkaline (**A**) and acidic (**B**) forms of methyl red (10 μM) are shown below.

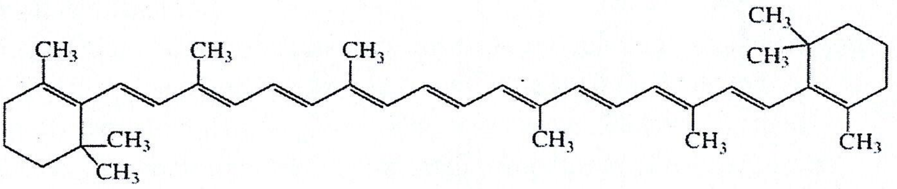


1.1.1 Estimate the λ_{max} of the two forms. *(**10 marks**)*

1.1.2 Calculate the % transmittance (%T) of the peak B at its λ_{max}. *(**05 marks**)*

1.1.3 Calculate the molar absorptivity of both forms at their λ_{max}. *(**10 marks**)*

1.2 β–carotene is a natural organic pigment present in carrots. Find the maximum absorption wavelength using Woodward-Fieser rules and show that these rules are unsatisfactory for predicting λ_{max} of polyenes beyond 4-double bonds. Experimentally λ_{max} of β–carotene is observed at 450 nm. (**25** ***marks***)



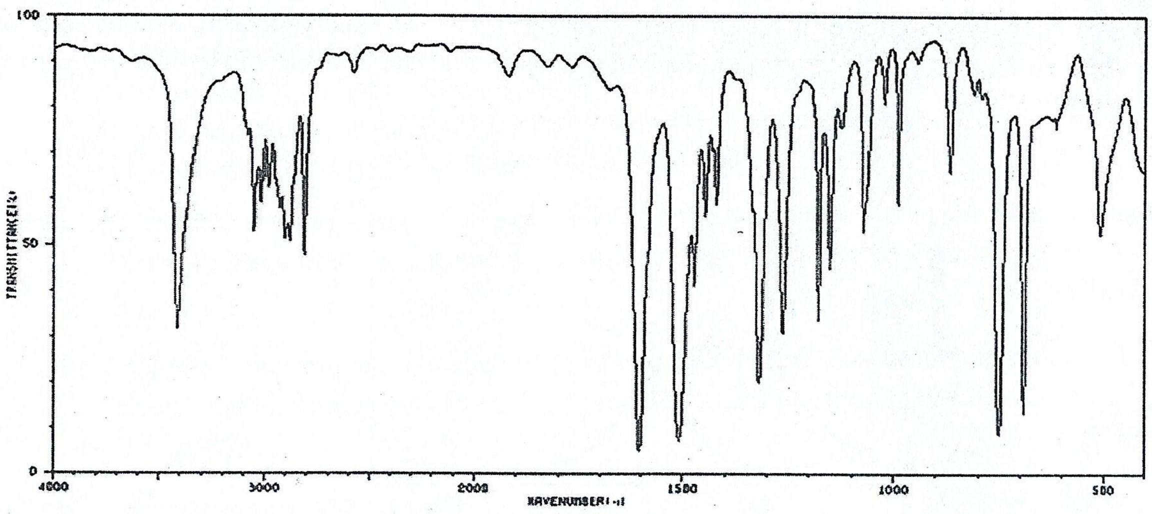
Note: *Base values for acyclic conjugated diene, homoannular conjugated diene, heteroannular conjugated diene are 217, 253, and 214 nm, respectively.*

1.3 Draw a Jablonski diagram, which illustrates the electronic states and the vibrational levels of a molecule, and explain the fates of molecules in the excited state. *(25 marks)*

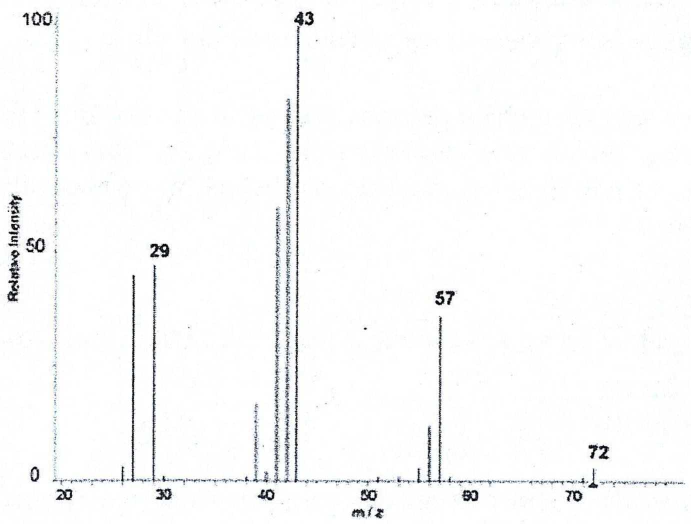
1.4 What are the primary species produced from a sample when it is exposed to a flame or furnace in the **Atomic Absorption Spectroscopy (AAS)** and **Flame Emission Spectroscopy (FES)** in a flame or furnace?
Give general sequence of reactions leading to the formation of these species. *(25 marks)*

02.

2.1 A compound exhibits the following infrared spectrum and has a molecular formula **C_7H_9N**. Its ^1H NMR spectrum shows a multiplet near 7 ppm with two other peaks. Deduce a likely structure for this compound with a brief explanation. *(25 marks)*



2.2 The following figure shows the mass spectrum of a hydrocarbon.



2.2.1 Propose a molecular formula for this compound assuming that the molecular ion can be detected. *(05 marks)*

2.2.2 By considering the relative intensities of the labelled peaks and giving reasons, suggest the most plausible structure for this hydrocarbon. (*10 marks*)

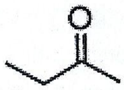
2.2.3 Draw the structural formulas of three possible isomers of this hydrocarbon. (*10 marks*)

2.3

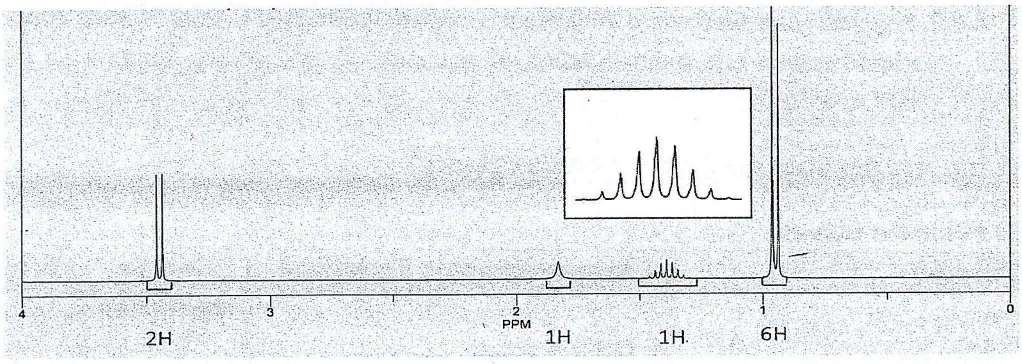
2.3.1 Briefly explain why ^{12}C is not important in NMR spectroscopy for the structure elucidation of organic compounds while ^{13}C will provide considerable information. (*10 marks*)

2.3.2 Draw the stick diagrams to show AX_2 and AMX spin systems' pattern of coupling in ^1H NMR spectra, in which one gives a *triplet* and the other gives a *doublet of doublets*. (*No extensive explanations needed, indicating the relationship of the coupling constant(s) in each spin system is sufficient*). (*10 marks*)

2.3.3 Giving reasons, briefly explain how you would use ^1H NMR spectroscopy to distinguish the following two compounds. (*10 marks*)



2.3.4 The ^1H NMR spectrum of the compound **X** ($C_4H_{10}O$) is shown below. The number of hydrogens represented by each peak are indicated underneath the spectrum. The peak at 1.40 ppm appears as a *septet of triplets*. Giving reasons, suggest a plausible chemical structure for the compound **X**. (*Note that the signal at 1.8 ppm is absent in D_2O).* (*20 marks*)



PART B

03.

3.1

3.1.1 What is diffusion? (*10 marks*)

3.1.2 Differentiate between steady state diffusion and unsteady state diffusion. (*20 marks*)

3.2 Polarimetry is a type of qualitative and quantitative analytical method.

3.2.1 Briefly explain the principle of polarimetry as an analytical method. (*20 marks*)

3.2.2 Sketch a diagram representing the main components of a polarimeter. (*15 marks*)

3.3 Potentiometry is an electroanalytical method.

3.3.1 Briefly describe the fundamental principle underlying pH determination by potentiometric method. (*20 marks*)

3.3.2 State the applications of potentiometry. (*15 marks*)

PART C

04.

4.1 A liquid herbal supplement was analyzed for contamination by pesticide **X** using a reversed-phase HPLC method with a C18 column and 20% acetonitrile in water as the mobile phase. An aliquot of 1.00 mL of the herbal supplement was diluted to 10.00 mL with distilled water. The diluted sample was injected and gave a peak at 15.5 min with an area of 375. A standard solution of pesticide **X** was prepared by dissolving 5.0 mg of **X** in 100.0 mL of distilled water. This standard solution was injected three times, giving peak areas of 1240, 1255, and 1250 at a retention time of 15.5 min. All injections were performed under identical chromatographic conditions using the same injection volume. Detection was carried out using a UV detector.

However, method validation data indicated that:

- the extraction recovery of pesticide **X** from the herbal matrix was 90% (i.e., only 90% of the actual amount present is recovered and detected during analysis).
- a blank matrix sample (herbal supplement without pesticide **X**) produced a peak at the same retention time (15.5 min) with an area of 25.

4.1.1 Calculate the concentration of pesticide **X** in the original herbal supplement, correcting for both blank contribution and extraction recovery, and express your answer as % w/v. *(**40 marks**)*

4.1.2 Suggest one analytical technique coupled with HPLC that would improve both the identification and quantification of pesticide **X**, and briefly explain its advantage over HPLC-UV. *(**10 marks**)*

4.2 List five detectors used in gas chromatography. *(**10 marks**)*

4.3 Fill in the blanks. *(**40 marks**)*

Purpose	**Chromatographic Technique**	**Principle behind the separation**
Detection of a volatile pesticide in a food sample	4.3.1	4.3.2
Monitoring the progress of an organic reaction	4.3.3	4.3.4
Rapid assessment of purity of fractions obtained from column chromatography	4.3.5	4.3.6
Purification of a protein such as an antibody	4.3.7	4.3.8
Determination of molecular weight distribution of a polymer sample	4.3.9	4.3.10

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