

TEST 2

NAME: _____

STUDENT NO.: _____

Please answer 8 questions of your choice. Only the eight first answered questions will be marked.

QUESTION 1:

Given the four following compounds: Acetone, acetaldehyde, ethane, ethanol.

a) What would be their elution order in gas chromatography (GC)? Justify.

b) What kind(s) of GC detector(s) would be appropriate for these compounds? Justify.

QUESTION 2:

Comment on the use of dansyl chloride reagent for high performance liquid chromatography.

a) What kind of compounds is it useful for and how does it enhance detection?

b) In terms of quantitative analysis by HPLC, what is the main advantage obtained from the use of dansyl chloride?

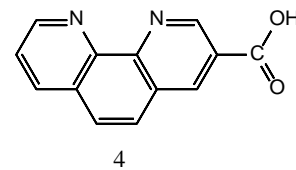
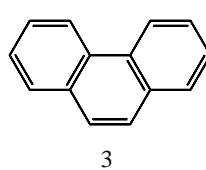
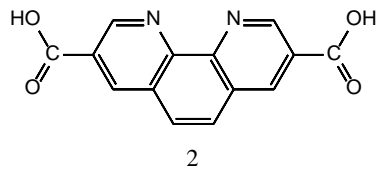
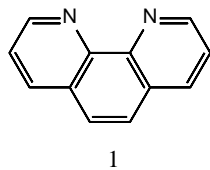
QUESTION 3:

a) What is the difference between the total ion current (TIC) and a selected ion current (SIC) in gas chromatography-mass spectrometry (GC/MS)?

b) In which ways are they both useful?

QUESTION 4:

Given the four compounds shown here:



Which mode of HPLC would you prefer to use for their complete separation, and what would be their elution order? Justify and mention the types of mobile and stationary phases used.

QUESTION 5:

How is a molecular UV absorption spectrum different from an excitation spectrum obtained using a spectrofluorimeter?

QUESTION 6:

The electrospray spectrum of a protein shows two ion series:

Series 1: m/z 772, 901, 1081, 1351, 1801

Series 2: m/z 794, 923, 1103, 1373, 1823

a) Calculate the molecular weight of this protein from Series 1, then from Series 2.

b) Knowing that Series 1 ions yield the correct molecular weight, suggest a reason why Series 2 would be observed, while leading to wrong mass results.

QUESTION 7:

Two amino acids, serine ($pI = 5.68$) and tryptophan ($pI = 5.89$), are to be separated by cation exchange chromatography on beads coated with sulfonic acid groups ($pK_a = -2$).

a) If the mobile phase is at pH 4, which amino acid forms the stronger interaction with the beads (and will elute second)? Justify.

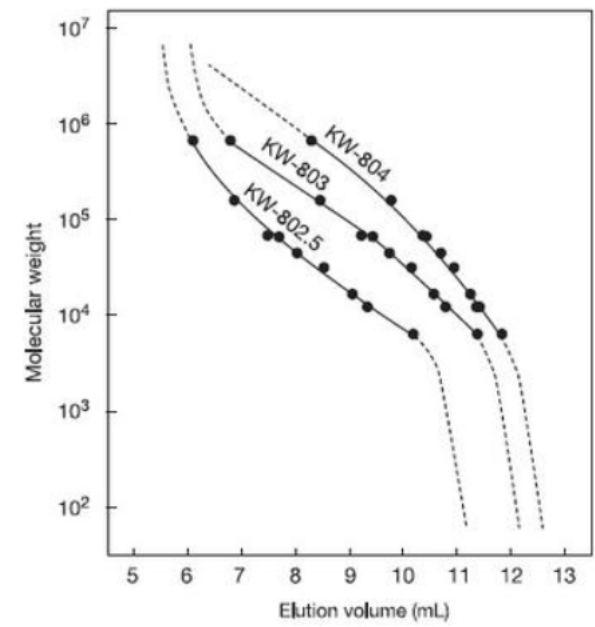
b) What would happen with a mobile phase of pH 8?

QUESTION 8:

A sample of dry KW-802.5 beads sold for size exclusion chromatography occupies 0.8 mL when dried and 1.2 mL once mixed with solvent.

a) Calculate V_m for a full 50 mL column when it is ready to use.

b) From the diagram below, determine V_0 and V_s for the same KW-802.5 commercial gel.



QUESTION 9:

What is K_d and how is it possible to determine this value during an affinity chromatographic experiment?

QUESTION 10:

a) Why is fluorescence detection more sensitive and selective than UV-absorbance for HPLC experiments?

b) Name two major disadvantages of fluorescence detection vs. UV-absorption.