
UNIVERSITI SAINS MALAYSIA

Second Semester Examination
Academic Session 2004/2005

March 2005

KAA 505 – Separation Methods

Time : 3 hours

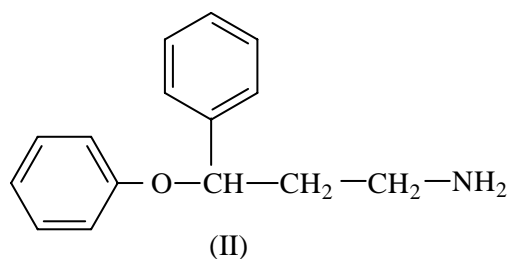
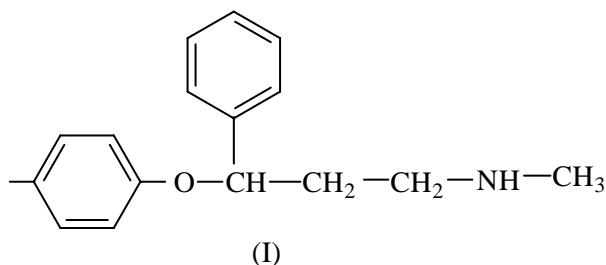
Please make sure this paper consists of SEVEN printed pages before answering the questions.

Answer FIVE questions. This paper contains SIX questions.

Only the first five questions answered by the candidate will be marked.

1. The determination of the antidepressant drug fluoxetine (I) and its metabolite norfluoxetine (II) in serum is an important part of monitoring its therapeutic use. A known amount of protriptyline is added to a serum sample as internal standard. A 0.5-mL aliquot of the serum is passed through a solid-phase extraction (SPE) cartridge containing silica particles with a bonded C₁₈ phase. After washing to remove interfering constituents from the sample matrix, the remaining constituents, including both analytes and the internal standard, are removed by washing the cartridge with 0.25 mL of a 25:75 v/v mixture of 0.1 M HClO₄ and acetonitrile. A 20-μL aliquot is injected onto a 15-cm x 4.6-mm HPLC column packed with a 5-μm C₈-bonded stationary phase. An isocratic mobile-phase mixture of 37.5:62.5 v/v acetonitrile and water (containing 1.5 g of tetramethylammonium perchlorate and 0.1 mL of 70% v/v HClO₄) is used.

- (i) What are the purposes of including the solid-phase extraction step?
- (ii) Why is an internal standard used in this analysis? What assumptions must be made about the internal standard?
- (iii) If the peaks for fluoxetine and protriptyline are not sufficiently resolved, how might you alter the chromatographic conditions to improve their separation?
- (iv) Why is tetramethylammonium perchlorate added to the mobile phase?
- (v) Suggest another alternative active site that can be used to replace C₁₈ for SPE. Explain the separation principles.



(20 marks)

2. (a) The concentration of polyaromatic hydrocarbons (PAHs) in soil can be determined by first extracting the PAHs with methylene chloride. The extract is then diluted, if necessary, and the PAHs are separated by HPLC with a fluorescence detector. In a typical analysis, a 2.013-g sample of dried soil is extracted with 20.00 mL of methylene chloride. After filtering to remove the soil, a 1-mL portion of the extract is taken and diluted to 10 mL with acetonitrile. Injecting 5 μL of the diluted extract into an HPLC unit gives a signal 0.217 (arbitrary units) for the PAH fluoranthene. When 5 μL of a 20.0-ppm fluoranthene standard is analyzed using the same conditions, a signal of 0.258 is measured. Report the concentration of fluoranthene in the soil in ppm.

(5 marks)

- (b) A capillary electrophoresis (CE) technique was applied to the separation of various organophosphorus pesticides (OPPs). Chemical structures of OPPs are shown in Figure 1, while the electrophoregram obtained and details of the experimental conditions are shown in Figure 2.
- What is the CE technique used?
 - Assign the peaks (A-C, Figure 2) to the appropriate OPP
 - Explain how the electroosmotic flow (EOF) can be measured.
 - What is the role of sodium dodecyl sulphate (SDS) in the running solution?
 - Suggest an alternative method to introduce samples.

Give brief explanations where necessary.

OPP	Log partition coefficient between octanol:water
Diazinon	3.02
Quinalphos	3.72
Chlorpyrifos	4.68

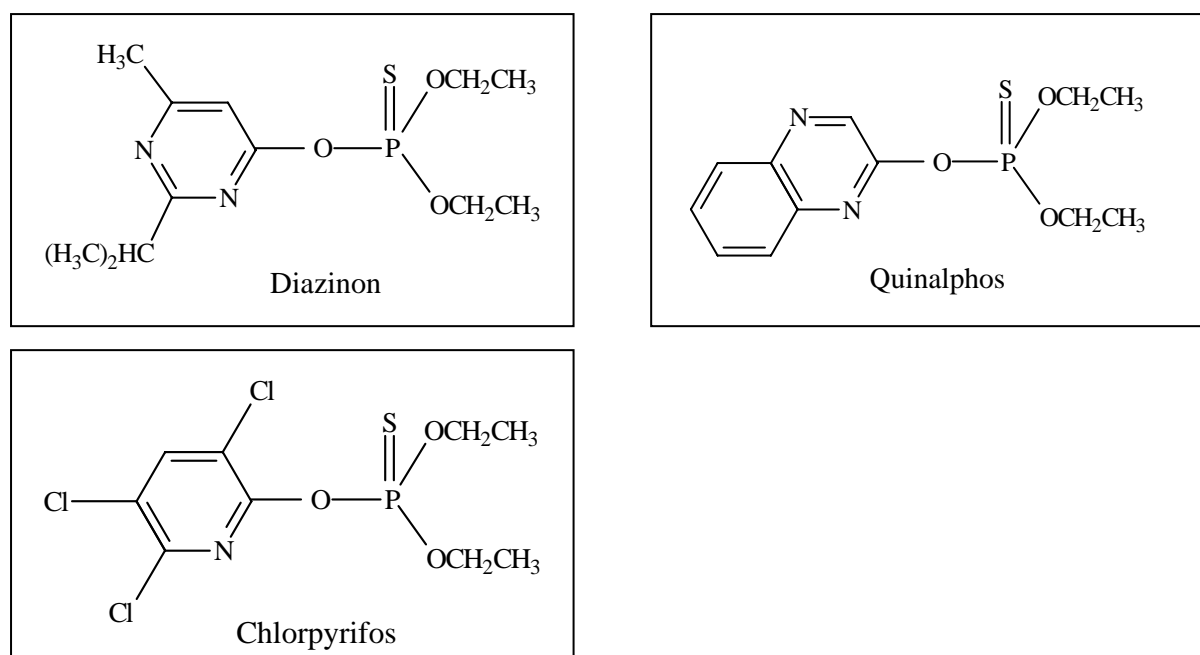


Figure 1. Chemical structures of OPPs

Figure 2: Electropherogram of the separation of hydrophobic OPPs in 5mM borate buffer (pH 9.3), 10 mM SDS and 5% v/v methanol. Conditions: applied potential 25 kV, sample injection for 10 s by hydrodynamically (2.8 kPa), samples are prepared in pure methanol, detection at 200 nm.

(15 marks)

...5/-

3. (a) What would be the effect of the following on the plate height of a GC column?
- (i) Increasing the weight of the stationary phase relative to the packing weight.
 - (ii) Decreasing the rate of sample injection.
 - (iii) Increasing the injection port temperature.
 - (iv) Increasing the flow rate of the mobile phase.
 - (v) Reducing the particle size of the packing.
 - (vi) Decreasing the column temperature.

Provide brief explanation to all answers.

(12 marks)

- (b) Discuss the optimization procedures that can be carried out in supercritical fluid chromatography.

(8 marks)

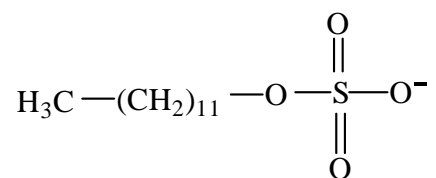
4. Discuss the following developments in separation science:

- (i) Monolithic materials as liquid chromatographic stationary phases.
- (ii) SPE sorbents based on molecularly imprinted polymers.

Highlight the current problems and explain how they were overcome.

(20 marks)

5. (a) Suggest two separation techniques (with justifications) that can be used for the determination of the surfactant shown below.



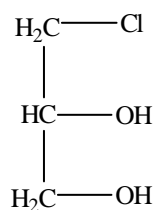
(7 marks)

- 6 -

- (b) Discuss the problems of interfacing a capillary electrophoresis unit to mass spectrometers. How have the major problems been overcome?

(7 marks)

- (c) The formation of 3-MCPD from vegetable proteins (such as soy sauce) has attracted a lot of attention due to its carcinogenic effects. What are the potential problems that can arise when 3-MCPD is analysed by using GC? Suggest how the problems can be solved.



(3-MCPD)

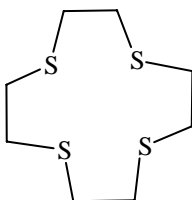
(6 marks)

6. (a) A liquid-liquid extraction procedure involving the thiacycrown ether ligand (12S4) and a mixture containing Ag^+ , Hg^{2+} and Cu^{2+} was reported. Picrate was used as counter ion. The dependence of the distribution coefficient (D) with variation in ligand (L) and picrate (Pic) concentrations are shown in Figures 3 and 4, respectively.

- (i) Propose the stoichiometries of the complex formed between the thiacycrown ether and the metal ions.
- (ii) Comment on the nature of the Hg^{2+} complex formed when the slope (s) is 0.3 (Figure 4).

(12 marks)

- (b) Based on the data obtained, describe an analytical application that uses the 12S4 thiacycrown ether, apart from liquid-liquid extraction.



(12S4)

(8 marks)

...7/-

- 7 -

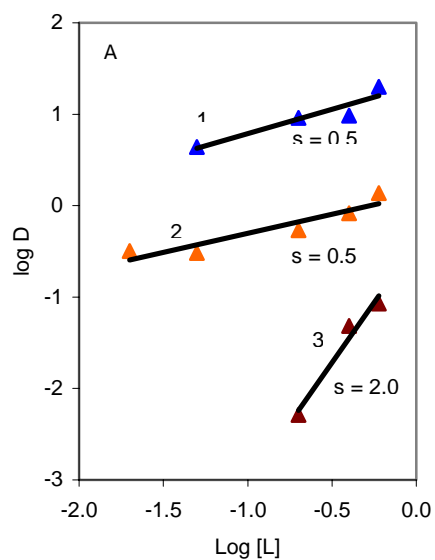


Figure 3: Effect of thiocrown ether (12S4) concentration on the distribution ratio of metal ions (1, Ag^+ ; 2, Hg^{2+} ; 3, Cu^{2+}) from picrate medium (1×10^{-3} M), pH = 5, s = slope.

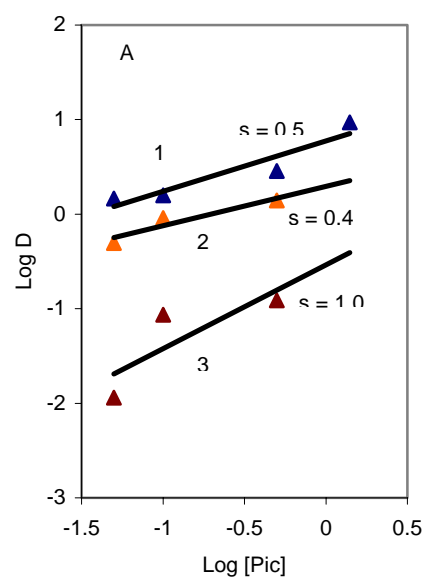


Figure 4: Effect of picric acid concentration on the distribution ratio of metal ions (1, Ag^+ ; 2, Hg^{2+} ; 3, Cu^{2+}) with (2×10^{-4} M), ligand, pH = 5, s = slope.

-000 O 000-