First Semester Examination
Academic Session 2020/2021

January 2021/February 2021

KAT344 – Separation Methods

Duration: 2 hours

Please check that this examination paper consists of **EIGHT (8)** pages of printed material before you begin the examination.

Instructions:

Answer **FOUR (4)** questions only.

SECTION A: Answer all the questions.

SECTION B: Answer **THREE (3)** questions only.

If a candidate answers more than four questions, only the first four questions in the answer sheet will be graded.

Answer each question on a new page.

You may answer the questions either in Bahasa Malaysia or in English.

SECTION A: ANSWER ALL THE QUESTIONS

1. (a) The concentrations of phenol distributed in hexane and water are 7.00 M and 5.00 M, respectively.

(i) Compute the distribution coefficient, K_D of phenol between hexane and water

(ii) After shaken process, the concentration of phenol remain in water is 0.415 M. Identify the concentration of phenol extracted in hexane.

(4 marks)

(b) The K_D of 1.00 g benzoic acid between water and chloroform is 49. Compute the weight of benzoic acid remain in 100 mL of water and extracted into 100 mL of chloroform.

(3 marks)

(c) The determination of additives, A (polar) and B (non-polar) in a preserved mango sample was done using solid phase microextraction (SPME) technique followed by gas chromatography-mass spectrometry (GC-MS). Given three different types of SPME fibres; polydimethylsiloxane (PDMS), polydimethylsiloxane-divinylbenzene (PDMS-DVB) and polyacrylate (PA), describe the most suitable fibre for the extraction of both compounds.

(2 marks)

(d) Hydrocarbons in an oil sample was extracted using microwave accelerated extraction (MAE) with hexane as the extraction solvent. However, none of hydrocarbons were extracted. Justify this observation.

(3 marks)

(e) The retention factor of sunset yellow dye separated using C18 column and mobile phase of acetonitrile/water (30/70) was 15. Describe how to manipulate the retention factor to the range between 1 and 10.

(4 marks)

(f) A sample mixture was analyzed using gas chromatography with flame ionization detector (GC-FID) on a 30-m capillary column. The following data were obtained:

Compound	Retention time (min)	Peak width (min)
A	8.70	1.06
B	10.90	0.78

Calculate the plate number and plate height for the column based on compound A.

(5 marks)

(g) In high performance liquid chromatography (HPLC) using packed column, justify the term in the van Deemter equation that has the least and the greatest influence to the efficiency of the separation.

(4 marks)

SECTION B: ANSWER THREE (3) QUESTIONS ONLY

2. (a) An analysis of vegetable sample that suspected to contain chlorinated hydrocarbons residue was done using headspace solid phase microextraction (HS-SPME) as sample preparation. The gas chromatography (GC) analysis was carried out using a bonded phase column (10 m × 0.2 mm) with maximum column temperature of 350 °C.

(i) Describe the importance of sample preparation in GC analysis.

(ii) In your opinion, suggest the suitable detector and injector type for the above analysis.

(iii) Justify your answer in (ii).

(iv) If the temperature of column was set at 400 °C, describe the effect to the column.

(10 marks)

(b) Figure 1 shows a chromatogram obtained in the analysis of a mixture of benzaldehyde, benzene, phenol and ethylbenzene using high performance liquid chromatography (HPLC) under the following conditions:

Column : Water Spherisorb C18 (250 mm × 4.6 mm × 5 µm)
Mobile phase : Methanol/water (50/50)
Flow rate : 1 mL/min
Detector : Ultraviolet (UV) at 254 nm

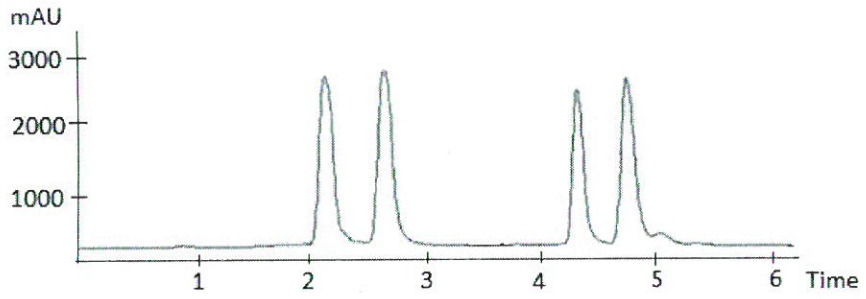

Figure 1

(i) Describe the elution order of the compounds.

(ii) Explain the effect to the separation of the compounds if you change the mobile phase composition to 20/80 (methanol/water). (Include the polarity of the stationary phase and analyte in the discussion).

(iii) Very low detection signals were obtained when the UV detector wavelength changed to 420 nm. Discuss this observation.

(15 marks)

3. (a) The separation of anionic and neutral molecules of dyes can be performed with capillary electrophoresis (CE). Explain approaches to obtain the efficient separation for these anionic and neutral molecules of dyes using the CE.

(10 marks)

(b) You are given a task to analyze sample mixture containing benzoic acid, hexanoic acid, 4-butylbenzoic acid, 2-nitrobenzyl alcohol and propanoic acid using HPLC. The separation was done using diol bonded column and hexane/ethyl acetate (75/25) as a mobile phase and ultraviolet (UV) detector at a wavelength of 254 nm. From the analysis, only three (3) peaks were observed.

(i) Discuss the reasons for the observation of only three (3) peaks.

(ii) Describe an approach to improve the separation of all compounds.

(8 marks)

(c) In ion analysis, discuss the rationale of each action taken in the following situations:

(i) Counter ion and buffer solution were added into the mobile phase prior to the separation of anions in water sample using reversed phase liquid chromatography.

(ii) Carboxylic acid cation exchanger was replaced by sulphonic acid cation exchanger for the analysis of sample below pH 4.

(7 marks)

4. (a) A method using gas chromatography (GC) was developed for the determination of essential oils (boiling point as in table below).

Essential oil	Boiling point (°C)
Limonene	176
Carvone	231
Lavender	204
Peru balsam	314

The essential oils were separated on a polyethylene glycol capillary column. Baseline separation for the essential oils were obtained using column temperature program as follows:

Initial temperature : 160 °C
Rate : 10 °C/min
Final temperature : 320 °C

(i) Explain the elution order of essential oils based on the principle of GC.

(ii) By changing the column to 50% phenyl-polydimethyl siloxane, all essential oils were eluted faster causing overlapping of peaks. Explain this observation.

(iii) Describe the expected peaks obtained if the analysis is done isothermally at 200 °C.

(13 marks)

(b) Size exclusion chromatography (SEC) was used in the separation of five (5) fatty acids, $CH_3(CH_2)_nCOOH$ where n is between 10 to 100. A polystyrene-based packing is used as the stationary phase with exclusion limit of 2.0×10^3 and permeation limit of 1.0×10^2. Explain if all the fatty acids will be separated into individual peak.

(6 marks)

(c) Discuss the solvent system and pressure required for an efficient supercritical fluid chromatography (SFC) analysis of volatile and non volatile polar compounds.

(6 marks)

5. (a) Capillary electrophoresis (CE) method was developed to separate five (5) components (similar masses) with buffer at pH 6.7. The components are A^+, B^{2+}, C^-, D (neutral species) and E (neutral species). In the analysis, the electroosmotic flow (EOF) was greater than the electrophoretic flow. The electrophoresis was set up with the injection end positive and the detection end negative.

(i) Explain the order of elution.

(ii) If the pH of buffer was decreased to 2.0 (assume that the charges of the components do not change), predict the effect to the separation.

(iii) If the silanols on the capillary wall are partially ionized, describe the effect to the separation.

(12 marks)

(b) The water soluble vitamins; niacinamide (neutral compound), riboflavin (neutral compound) and thiamine (cation) were separated by micellar electrokinetic capillary (MEKC) in 15 mM borate buffer (pH 8) with 50 mM sodium dodecyl sulphate (SDS). The migration times obtained were 8.1, 13.0 and 14.9 min for niacinamide, riboflavin and thiamine, respectively.

(i) Explain the reasons for both of the neutral vitamins, niacinamide and riboflavin obtained at different migration times.

(ii) If SDS is added in a very low concentration (< 50 mM) into the system, indicate the effect of the migration of the above vitamines.

(8 marks)

(c) The separation of biphenyl and terphenyl was done using high performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC). Using HPLC, the separation was achieved in 6 min with methanol/water as mobile phase compared to less than 2 min by SFC using carbon dioxide as mobile phase. Explain this observation.

(5 marks)

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