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UNIVERSITI SAINS MALAYSIA

Second Semester Examination  
2010/2011 Academic Session

**KAA 505 – Separation Techniques**  
**[Kaedah Pemisahan]**

Duration : 3 hours  
*[Masa : 3 jam]*

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Please check that this examination paper consists of **TEN** printed pages before you begin.

**Instructions:**

Answer **FIVE** (5) questions only.

Begin your answer on a new page.

You may answer either in Bahasa Malaysia or in English.

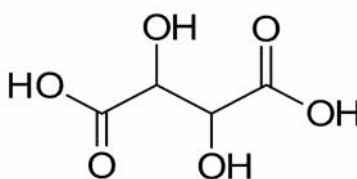
If a candidate answers more than five questions, only the first five answers will be graded.

In the event on any discrepancies, the English version shall be used.

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1. (a) (i) Discuss column technologies in capillary electrochromatography.
- (ii) Of the various technologies, which one do you think will dominate in the near future. Give your justifications.
- (12 marks)
- (b) Describe a direct (no derivatization needed) high performance liquid chromatographic (HPLC) method for the enantioseparation of tartaric acid (**I**). In your discussion, pay particular attention to the choice of mobile phase, type of column and detector used.

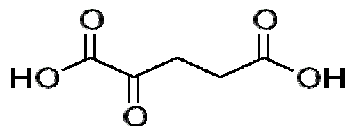


(8 marks)

2. (a) Alpha-ketoglutaric acid (**II**) and 5-hydroxymethylfurfural (**III**) were investigated as cancer cell damaging agents. An analytical method for the simultaneous determination of these analytes in human plasma was described in the literature as follows:  
 Plasma samples were directly treated with 0-(2,3,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PHH) to form the corresponding oximes, thus facilitating subsequent liquid-liquid extraction. After formation of the trimethylsilyl ethers, samples were analysed by gas chromatography-mass spectrometry (GC-MS). GC conditions: column, BPX5 (polar); 15 m x 0.25 mm internal diameter, 0.25 μm film thickness; carrier gas, helium; oven temperature, 300 C.
- (i) What is the role of PHH?
- (ii) Describe a suitable solventless extraction method that can be used for the isolation of the analytes prior to the GC-MS analysis.
- (iii) Comment of the quality of the chromatogram obtained.

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- (iv) What will be the prospect of the analysis of these analytes using capillary electrophoresis (CE) with capacitively coupled contactless conductivity detection instead? What precautions on the type of background electrolyte that need to be exercised if this option is chosen? .



(II)



(III)

(20 marks)

3. (a) Describe the various on-column preconcentration strategies that can be adopted to enhance sensitivity in capillary electrophoresis (CE).

(7 marks)

- (b) The development of the ultra pressure liquid chromatography (uPLC) is considered a significant innovation in liquid chromatography. Discuss the salient features of this technique.

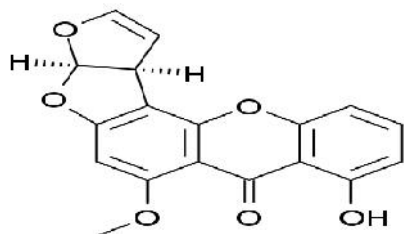
(8 marks)

- (c) Explain how you will analyse ppb levels of bromate anions in an underground water sample using CE.

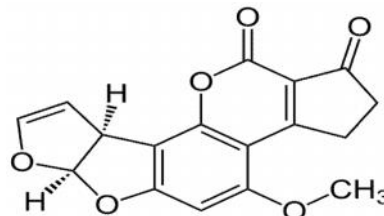
(5 marks)

4. (a) (i) Discuss the analytical challenges in the analysis of  $\text{ng kg}^{-1}$  levels of sterigmatocystin (VI) and aflatoxin B1 (V) in a moldy cheese sample.

- (ii) What strategies can be adopted to overcome these challenges?



(VI)



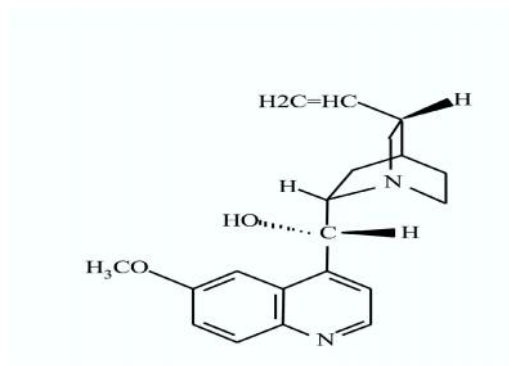
(V)

(12 marks)

- (b) Provide justifications for the following:
- (i) Theoretical plates in the order of hundreds of thousands are possible in CE.
  - (ii) Separations of enantiomers in GC and HPLC are normally conducted using the direct approach.
- (8 marks)
5. (a) Describe how the QuEChERS method can be used for the preparation of trace antioxidants in honey samples.
- (10 marks)
- (b) Describe strategies to improve **selectivity** in solid phase extraction.
- (10 marks)
6. (a) CE separation of the antimalarial drug quinine (VI) ( $pK_{a1}$ , 4.11,  $pK_{a2}$ , 8.00) in a pharmaceutical tablet was carried out using background electrolytes that had been adjusted to pH 4.5, and later changed to pH 6.0.
- (i) Comment on the migration times under the different pH conditions.
  - (ii) How can the enantiomers of quinine be separated?
  - (iii) Briefly discuss the migration mechanism.

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(iv) How is heat dissipated from the system?



(VI)

(15 marks)

(b) Discuss the practicality of molecular imprinted polymers for the routine analysis of analytes in body fluids.

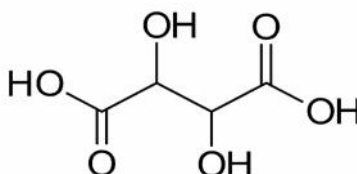
(5 marks)

- 7 -

1. (a) (i) Bincangkan teknologi turus untuk kromatografielektro rerambut.
- (ii) Diantara pelbagai teknologi, yang mana satu pada pendapat anda akan menonjol di dalam masa terdekat. Berikan justifikasi anda.

(12 markah)

- (b) Terangkan satu kaedah cecair prestasi tinggi (HPLC) terus (tanpa memerlukan penerbitan) bagi pemisahan enantio asid tartaric (I). Di dalam perbincangan anda, beri tumpuan terhadap pemilihan fasa gerak, jenis turus dan pengesan yang akan digunakan.



(I)

(8 markah)

2. (a) Asid  $\alpha$ -ketoglutarik (II) dan 5-hidroksimetilfurfural (III) telah diselidiki sebagai agen pemusnah sel kanser. Satu kaedah analisis bagi penentuan serentak analit ini di dalam plasma manusia telah diterangkan di dalam literatur:

Sampel plasma telah dioleh secara terus dengan

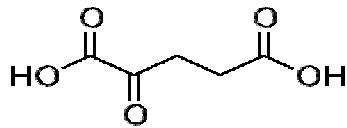
0-(2,3,5,6-pentafluorobenzil)hidroksilamina hidroklorida (PHH) untuk membentuk oxim sepadan, dengan itu membolehkan seterusnya pengekstrakan cecair-cecair. Setelah pembentukan eter trimetilsilil, sampel telah ditentukan menggunakan kromatografi gas-spektrometer jisim (GC-MS). Keadaan GC: turus, BPX5 (berkutub); 15 m x 0.25 mm diameter dalaman, 0.25  $\mu$ m lapisan filem; gas pembawa, helium; suhu ketuhar, 300 C.

- (i) Apakah peranan PHH?
- (ii) Terangkan satu kaedah pengekstrakan tanpa pelarut yang boleh digunakan bagi pemencilan analit sebelum penentuan GC-MS.
- (iii) Komen tentang kualiti kromatogram yang diperolehi.

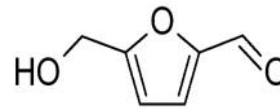
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- (iv) Apakah prospek penentuan analit ini menggunakan elektroforesis rerambut (CE) dengan pengesanan kekonduksian tanpa sentuh kupel teraruh? Apakah langkah tentang jenis elektrolit latarbelakang yang harus dititikberatkan jika pilihan ini telah digunakan? .



(II)

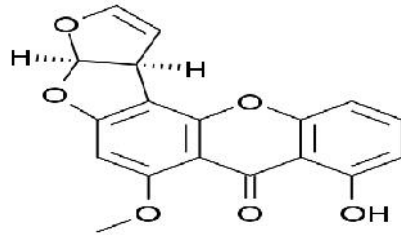


(III)

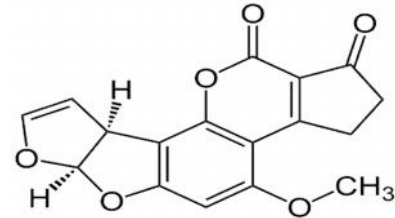
(20 markah)

3. (a) Terangkan pelbagai strategi prakepekatan dalam turus yang boleh dilakukan untuk meningkatkan kepekaan di dalam elektroforesis rerambut (CE).  
(7 markah)
- (b) Perkembangan kromatografi cecair tekanan ultra (uPLC) telah disifatkan sebagai inovasi ketara di dalam kromatografi cecair. Bincangkan sifat utama kaedah ini.  
(8 markah)
- (c) Terangkan bagaimana anda boleh menentukan paras ppb anion bromat di dalam air bawah tanah menggunakan CE.  
(5 markah)
4. (a) (i) Bincangkan cabaran analisis bagi penentuan paras  $\text{ng kg}^{-1}$  l sterigmatosistin (VI) and aflatoksin B1 (V) di dalam suatu sampel keju berkulat.

- (ii) Apakah strategi yang boleh di gunakan untuk mengatasi cabaran ini?



(VI)



(V)

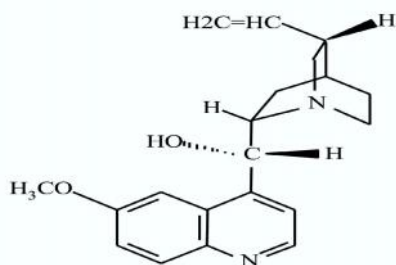
(12 markah)

- (b) Berikan justifikasi bagi berikut:
- (i) Plat teoretis ratusan ribu adalah mungkin bagi CE.
- (ii) Pemisahan enantiomer bagi GC dan HPLC biasanya dilakukan menggunakan pendekatan terus.
- (8 markah)
5. (a) Terangkan bagaimana kaedah QuEChERS boleh digunakan untuk penyediaan surihan antioksidan di dalam sampel madu. (10 markah)
- (b) Terangkan strategi untuk memperbaiki kepilhan di dalam pengekstrakan fasa pepejal. (10 markah)
6. (a) Pemisahan CE untuk drug antimalaria kuanina (VI) ( $pK_{a1}$ , 4.11,  $pK_{a2}$ , 8.00) di dalam tablet farmaseutik telah dilakukan menggunakan elektrolit latarbelakang yang pHnya telah dijadikan pH 4.5, dan seterusnya diubah menjadi pH 6.0
- (i) Komen tentang masa penghijrahan bagi pH berbeza.
- (ii) Bagaimanakah enantiomer kuinina boleh dipisahkan?
- (iii) Bincangkan dengan ringkas mekanisma penghijrahan.



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(iv) Bagaimanakah haba disebarikan daripada sistem?



(VI)

(15 markah)

(b) Bincang penggunaan polimer “imprinted” molekul bagi analisis rutin analit di dalam cecair badan.

(5 markah)

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