UNIVERSITI SAINS MALAYSIA

Second Semester Examination
2009/2010 Academic Session

KAA 505 – Separation Techniques
[Kaedah Pemisahan]

Duration : 3 hours
[Masa : 3 jam]

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

Instructions:

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

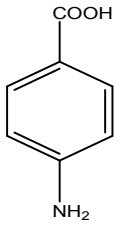
Begin your answer on a new page.

You may answer either in Bahasa Malaysia or in English.

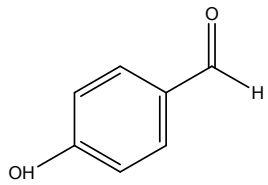
If a candidate answers more than five questions, only the first five answers will be graded.

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

1. (a) What are the optimum conditions for the liquid-liquid extraction of *p*-aminobenzoic acid (pK_a: 2.5 and 5.0) and *p*-hydroxybenzaldehyde (pK_a: 2.0 and 6.0) in a water sample. Draw the chemical structures of the analytes under the proposed conditions.



p-aminobenzoic acid



p-hydroxybenzaldehyde

(9 marks)

(b) Describe pressurised liquid extraction. What are its main advantages?

(6 marks)

(c) Discuss briefly the benefits of performing liquid chromatographic separations under elevated temperatures.

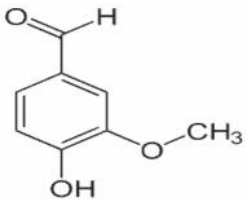
(5 marks)

2. (a) Trace levels of vanillin (pK_a: 7.38) were extracted using the two phase liquid phase microextraction technique. The pH of the water was acidified to 3.0.

(i) Comment on the efficiency of the extraction.

(ii) Draw a schematic diagram of the set-up.

(iii) What technique will be the most suitable for the quantitation of the extracted vanillin?



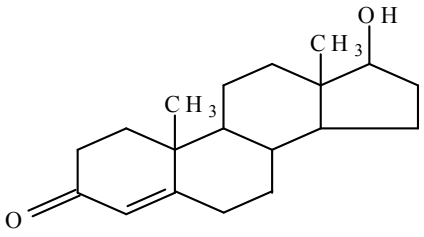
Vanillin

(10 marks)

(b) Discuss strategies how band broadening due to longitudinal diffusion and resistance to mass transfer can be reduced.

(10 marks)

3. (a) Describe a detailed gas chromatographic method for the analysis of testosterone in a biological fluid extract. Provide justifications for your chosen conditions.



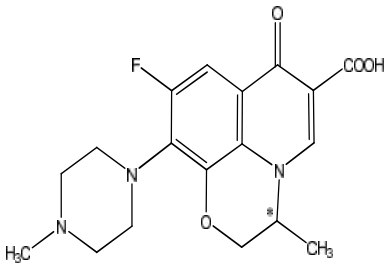
Testosterone

(10 marks)

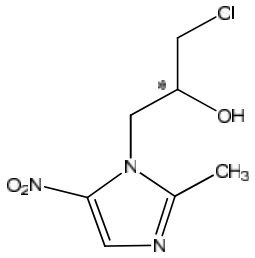
(b) Discuss the suitability of molecular imprinted polymers (MIPs) as sorbents in solid phase extraction compared to the more commonly used sorbent based on C18. Describe the major restrictions that prevents the widespread use of MIPs.

(10 marks)

4. A capillary electrophoretic method for the separation of the enantiomers of ofloxacin (pK_a: 6.00 and 8.00) and ornidazole (pK_a: 2.4) is described. Good chiral separation of the racemic mixtures was achieved in less than 16 min with resolution factors 5.45 and 6.28 for both ofloxacin and ornidazole enantiomers, respectively. Separation was conducted using a bare fused-silica capillary and a background electrolyte of 50 mM H_3PO_4 - 1 M tris solution; pH 1.85; containing 30 mg mL^{-1} of sulphated-β-cyclodextrin. The separation was carried out in reversed polarity mode at 25 °C, 18 kV and using hydrodynamic injection for 15 s.



Ofloxacin



Ornidazole

* chiral centre

(i) Describe the separation mechanism under the above conditions.

(ii) Explain the role of sulphated-β-cyclodextrin.

(iii) Comment on the migration time if β-cyclodextrin is used instead as a component in the background electrolyte.

(iii) What is meant by hydrodynamic introduction? Comment on the suitability of the method.

(iv) Suggest a suitable detector for the analysis.

(v) What other information is required for you to predict the elution order of the enantiomers?

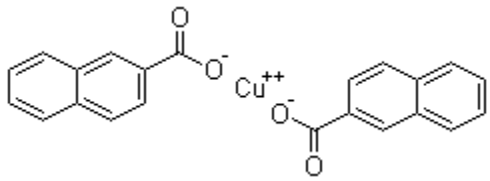
(20 marks)

5. (a) The following is an enquiry that was received by a testing lab from a client.

"Our company is a leading organisation that manufactures power cable and most of our power cables are installed underground. Our underground power cable are able to withstand termite attacks due to the termite repellent material (copper naphthenate, $Cu(C_{11}H_7O_2)_2$) used in the outer sheath jacket of the cable. We would like to send pieces of cable outer sheath samples for you to test the levels of the copper naphthenate in the sample. This is to counter check that a particular length of installed power cable complied to our standard"

(i) Describe a suitable sample preparation technique for the above task.

(ii) Suggest a suitable separation technique. Provide details of the proposed technique (e.g., type of column, mobile phase, background electrolyte, detector, etc.) and its justifications.



Copper naphthenate

(14 marks)

(b) Compare the main characteristics of liquid chromatographic separations using monolithic columns and ultra pressure liquid chromatography.

(6 marks)

6. (a) Aniline (pK_a: 4.6) in an extract was analysed using reversed-phase high performance liquid chromatography under the following conditions:

Column: 15 cm x 0.46 cm Zorbax Eclipse x DB-C18
Eluent: 90% aqueous/10% acetonitrile
Aqueous: 10 mM $Na_2HPO_4 \cdot 7H_2O$ + $xHClO_4$
Flow rate: 1 mL min^{-1}

(i) Sketch the plot of pH of mobile phase versus retention time over pH 2.0 -7.5.

(ii) What would be the main problems and how they can be overcome if the separation was conducted using mobile phase of pH 8.5.

(10 marks)

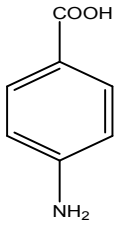
(b) Briefly discuss the following:

(i) Capabilities and limitations of the evaporative light scattering detector.

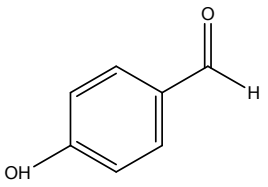
(ii) Viability of supercritical fluid extraction as the main extraction technique of the future.

(10 marks)

1. (a) Apakah keadaan optimum bagi pengekstrakan cecair-cecair asid *p*-aminobenzoik (pK_a: 2.5 dan 5.0) dan *p*-hidroksibenzaldehid (pK pK_a: 2.0 dan 6.0) di dalam suatu sampel air. Lukis struktur kimia analit di dalam keadaan yang dicadangkan.



Asid *p*-aminobenzoik



p-hidroksibenzaldehid

(9 markah)

(b) Terangkan pengekstrakan cecair termampat. Apakah kebaikan utamanya?

(6 markah)

(c) Bincangkan dengan ringkas kebaikan menjalankan pemisahan kromatografi cecair di bawah keadaan suhu tinggi.

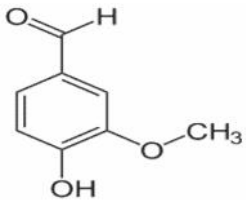
(5 markah)

2. (a) Paras surihan vanillin (pK_a: 7.38) telah diekstrak menggunakan kaedah dua fasa mikropengekstrakan fasa cecair. pH air telah diasidkan menjadi 3.0.

(i) Komen kecekapan pengekstrakan.

(ii) Lukis gambarajah radas.

(iii) Kaedah apakah yang paling sesuai bagi pengkuantitan vanillin terekstrak.



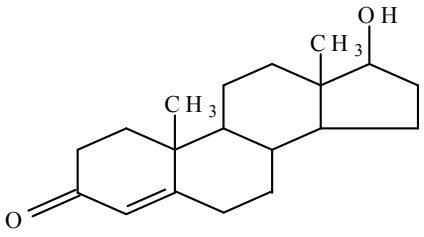
Vanillin

(10 markah)

(b) Bincang strategi bagaimana pelebaran jalur oleh pembauran membujur dan rintangan terhadap pemindahan jisim boleh dikurangkan.

(10 markah)

3. (a) Terangkan dengan terperinci kaedah kromatografi gas bagi analisis testosteron di dalam ekstrak cecair biologi. Berikan alasan bagi keadaan yang anda pilih.



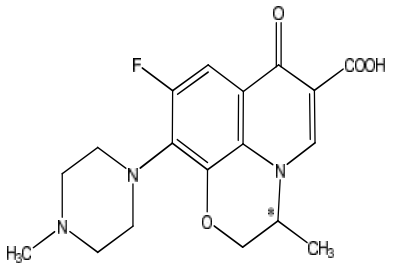
Testosteron

(10 markah)

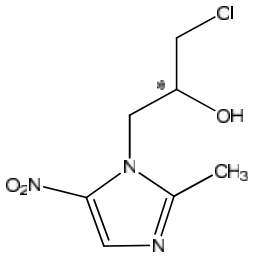
(b) Bincangkan kesesuaian polimer "imprinted" molekul (MIPs) sebagai penjerap di dalam pengekstrakan fasa pepejal berbanding dengan penjerap biasa yang berasaskan kepada C18. Terangkan penghadan utama yang menghalang penggunaan meluas MIPs.

(10 markah)

4. Suatu kaedah elektroferesis rerambut bagi pemisahan enantiomer ofloksasin (pK_a: 6.00 dan 8.00) dan ornidazol (pK_a: 2.4) telah dicadangkan. Pemisahan kiral yang baik bagi campuran rasemat telah dilakukan kurang dari 16 min dengan faktor resolusi 5.45 dan 6.28 bagi masing-masing enantiomer ofloksasin dan ornidazol. Pemisahan telah dilakukan menggunakan rerambut silika terlakur dan elektrolit latarbelakang 50 mM H_3PO_4 - 1 M tris; pH 1.85; mengandungi 30 mg mL^{-1} of β-siklodekstrin tersulfat. Pemisahan dilakukan dalam mod kekutuban terbalik pada $^{\circ}C$, 18 kV dan menggunakan penyuntikan hidrodinamik selama 15 s.



Ofloksasin



Ornidazol

* pusat kiral

(i) Terangkan mekanisma pemisahan di bawah keadaan di atas.

(ii) Terangkan peranan β-siklodekstrin tersulfat.

(iii) Komen tentang masa penghijrahan jika β-siklodekstrin telah digunakan sebagai komponen elektrolit latarbelakang.

(iv) Apakah yang dimaksudkan dengan pengenalan hidrodinamik? Komen kesesuaian kaedah ini.

(v) Cadangkan pengesan sesuai bagi analisis ini.

(vi) Apakah maklumat lain yang diperlukan bagi anda meramalkan tertib elusi enantiomer?

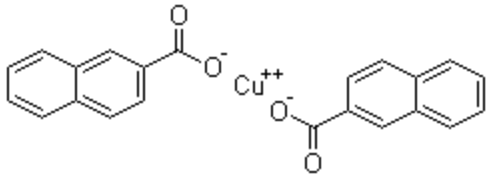
(20 markah)

5. (a) Di bawah merupakan satu pertanyaan yang telah diterima oleh sebuah makmal ujian daripada seorang pelanggan.

"Syarikat kami merupakan organisasi utama yang menghasilkan kabel kuasa dan kebanyakan kabel kuasa kami dipasang di bawah tanah. Kabel kuasa kami di bawah tanah boleh tahan dari diserang oleh anai-anai disebabkan oleh penggunaan bahan anti anai-anai (kuprum nafthenat, $Cu(C_{11}H_7O_2)_2$) diluar jaket kabel. Kami ingin menghantar beberapa sampel kabel untuk anda uji paras kuprum nafthenat di dalam sampel. Ini adalah untuk memastikan suatu bahagian kabel kuasa yang telah dipasang mematuhi piawai kami"

(i) Terangkan satu kaedah penyediaan sampel bagi tujuan di atas.

(ii) Cadangkan suatu kaedah pemisahan yang sesuai. Berikan maklumat lanjut kaedah yang dicadangkan (misalnya, jenis turus, fasa gerak, elektrolit latarbelakang, pengesan) dan justifikasinya.



Kuprum nafthenat

(14 markah)

(b) Bandingkan ciri utama pemisahan kromatografi cecair menggunakan turus monolitik dan kromatografi cecair prestasi ultra.

(6 markah)

6. (a) Anilina (pK_a: 4.6) di dalam suatu ekstrak telah dianalisis menggunakan kromatografi cecair prestasi tinggi fasa terbalik di bawah keadaan berikut:

Turus: 15 cm x 0.46 cm Zorbax Eclipse x DB-C18
Eluen: 90% akueus/10% asetonitril
Akueus: 10 mM $Na_2HPO_4 \bullet 7H_2O$ + x$HClO_4$
Kadar alir: 1 mL min^{-1}

(i) Lakarkan plot pH fasa gerak melawan masa penahanan dari pH 2.0 -7.5.

(ii) Apakah masalah utama dan bagaimana ia diatasi jika pemisahan telah dilakukan mengguna fasa gerak pH 8.5.

(10 markah)

(b) Bincang dengan ringkas perkara di bawah:

(i) Keupayaan dan penghadan pengesan penyerakan cahaya tersejat.

(ii) Keupayaan pengekstrakan cecair supergenting sebagai kaedah pengekstrakan utama di masa hadapan.

(10 markah)

oooOOooo