

UNIVERSITI SAINS MALAYSIA

Second Semester Examination
2011/2012 Academic Session

June 2012

KAA 505 – Separation Techniques
[Kaedah Pemisahan]

Duration : 3 hours
[Masa : 3 jam]

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

Instructions:

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

Answer each question on a new page.

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

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

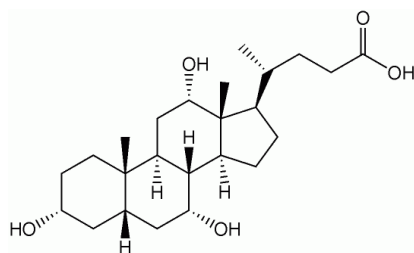
In the event on any discrepancies, the English version shall be used.

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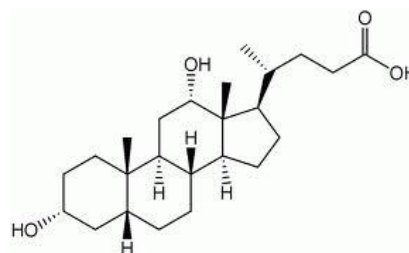
- 2 -

1. (a) An ultra-performance liquid chromatography-evaporative light scattering detection (UPLC-ELSD) method based on a Acquity UPLC HSS T3 column (2.1 × 50 mm, 1.8 μm) with gradient elution of 0.2% aqueous formic acid and acetonitrile for the simultaneous determination of four bile acids including cholic acid (CA), deoxycholic acid (DCA), chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) in a Chinese traditional medicine material is described. Under optimum conditions, the four target compounds were baseline separated within 10 min. Reversed phase HPLC with refractive index detector had previously been used for the determination of these acids.
- List the main parameters that need to be optimised for the ELSD detector.
 - What are the advantages that can be derived with the UPLC-ELSD technique?
 - What is the role of formic acid in the mobile phase?
 - What are some limitations of the use of the previously reported HPLC-refractive index method?

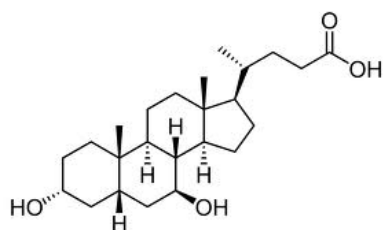
(15 marks)



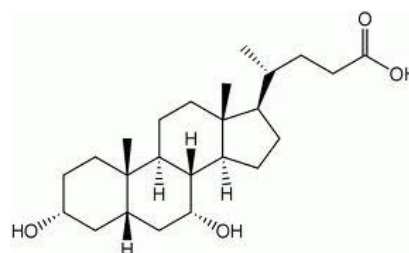
CA



DCA



CDCA



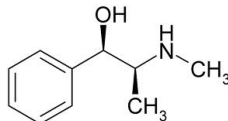
UDCA

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- (b) Describe the composition of the background electrolyte that will be suitable for the separation of the enantiomers of ephedrine in capillary electrophoresis (CE).

(5 marks)



Ephedrine

2. (a) *p*-amino benzoic acid ($pK_a = 4.19$) from a fermentation extract was separated using the following HPLC conditions:

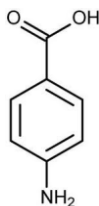
Mobile phase :	Methanol:hexane: 10 mM phosphate buffer, pH 5.0 (50:40:10 v/v%)
Detector :	254 nm
Column :	15 cm x 0.46 cm Zorbax Eclipse XDB-C18, 3 micron particles
Flow rate :	1 mL min ⁻¹
Oven temp :	25 deg C

Predict the retention time of the analyte when the:

- Mobile phase was changed to ethanol:hexane: 10 mM phosphate buffer, pH 5.0 (30:60:10 v/v%).
- pH of the buffer was changed to 3.0.
- pH of the buffer was changed to 8.0.
- The column was changed to a monolithic silica column.

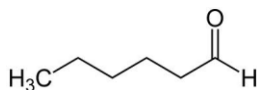
Provide justifications for your answers.

(14 marks)

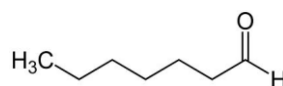
*p*-amino benzoic acid

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- (b) Discuss strategies how selectivity can be enhanced in solid phase extractions. (6 marks)
3. (a) Discuss the advantages and disadvantages of the direct and indirect gas chromatographic (GC) separation of chiral molecules. (10 marks)
- (b) Hexanal and heptanal are endogenous aldehydes that are formed from membranes and lipid oxidation. They have been found to be higher in cancer patients than in healthy subjects, thus suggesting as lung tumor biomarkers. Describe a liquid chromatographic analytical procedure, excluding sample preparation, for the determination of trace hexanal and heptanal in urine. (10 marks)



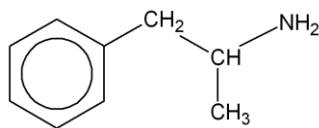
Hexanal



Heptanal

4. (a) Discuss how excessive peak tailings and long analysis times can be reduced in capillary gas chromatography. (6 marks)
- (b) Discuss the challenges in the analysis of microorganisms using capillary electrophoresis. (8 marks)
- (c) The analysis of acidic drugs in capillary electrophoresis is normally conducted using basic buffers. Why? (6 marks)
5. (a) Discuss the main features of the two dimensional GC technique. Compare its performance to that of the fast GC technique. (10 marks)
- (b) With the assistance of a schematic diagram, provide the necessary experimental conditions for the selective extraction of amphetamines and related compounds in urine sample using the liquid phase microextraction technique. (10 marks)

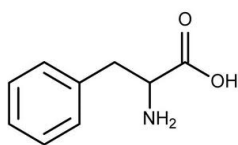
- 5 -



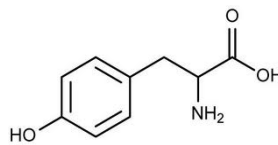
Amphetamine

6. (a) The micellar electrokinetic capillary electrophoresis method was used to separate the amino acids phenylalanine, tyrosine and tryptophan.
- (i) Suggest a suitable background electrolyte, with justifications, for the separation.
- (ii) What is the elution order of the amino acids? Give justifications.

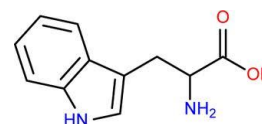
(7 marks)



Phenylalanine



Tyrosine



Tryptophan

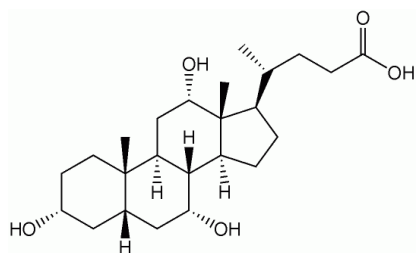
- (b) Describe with the assistance of a schematic diagram how you will automate the solid phase extraction of short chain fatty acids in an environmental sample to be analysed using GC.
- (c) Explain the working principles of the capacitively coupled contactless conductivity detector. Under what circumstances is this detector useful?

(7 marks)

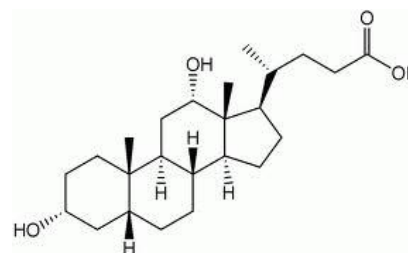
(6 marks)

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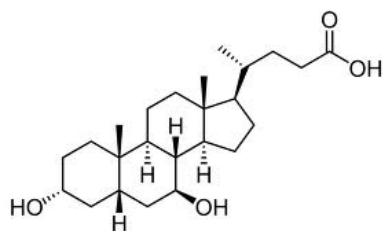
1. (a) Suatu kaedah ultra-prestasi kromatografi cecair-pengesanan pengwapan cahaya terserak (UPLC-ELSD) berasaskan turus Acquity UPLC HSS T3 (2.1×50 mm, $1.8 \mu\text{m}$) dengan elusi kecerunan 0.2% asid formik akueus dan asetonitril bagi penentuan serentak empat asid bil termasuklah asid kolic (CA), asid deoksikolik (DCA), asid senodeoksikolik (CDCA) and asid ursodeoksikolik (UDCA) di dalam suatu bahan ubat tradisional Cina telah diterangkan. Di dalam keadaan optimum, keempat sebatian diminati telah dipisahkan di dalam 10 min. HPLC fasa terbalik bersama pengesanan indeks biasan telah digunakan sebelum ini bagi penentuan asid ini.
- Senaraikan parameter utama yang perlu dioptimumkan bagi pengesanan ELSD.
 - Apakah kebaikan yang boleh diperolehi dengan kaedah UPLC-ELSD?
 - Apakah peranan asid formik di dalam fasa cecair ini?
 - Apakah beberapa penghadan penggunaan kaedah HPLC-indeks biasan yang telah di laporkan sebelum ini?



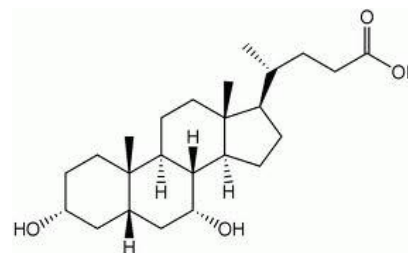
CA



DCA



CDCA



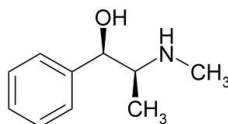
UDCA

(15 markah)

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- (b) Terangkan komposisi elektrolit latarbelakang yang sesuai bagi pemisahan enantiomer efedrina di dalam elektroforesis rerambut (CE).

(5 markah)



Efedrina

2. (a) Asid *p*-amino benzoik ($pK_a = 4.19$) daripada suatu ekstrak penapaian telah dipisahkan menggunakan keadaan HPLC berikut:

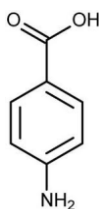
Fasa gerak	:	Metanol:heksana: 10 mM tampan fosfat, pH 5.0 (50:40:10 v/v%)
Pengesan	:	254 nm
Turus	:	15 cm x 0.46 cm Zorbax Eclipse XDB-C18, zarah 3 micron
Kadar alir	:	1 mL min ⁻¹
Suhu Ketuhar	:	25 deg C

Ramalkan masa penahanan apabila:

- Fasa gerak telah ditukarkan menjadi metanol:heksana: 10 mM tampan fosfat, pH 5.0 (30:60:10 v/v%).
- pH tampan ditukar menjadi 3.0.
- pH tampan ditukar menjadi 8.0.
- Turus ditukar kepada turus silika monolitik.

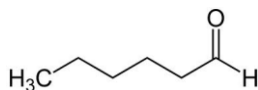
Beri alasan untuk jawapan anda.

(14 markah)

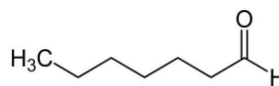
Asid benzoik *p*-amino

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- (b) Bincangkan strategi bagaimana kepilihan boleh ditingkatkan bagi pengekstrakan fasa pepejal. (6 markah)
3. (a) Bincangkan kebaikan dan keburukan pemisahan molekul kiral menggunakan kromatografi gas (GC) secara terus dan bukan terus. (10 markah)
- (b) Heksanal and heptanal adalah aldehid semula jadi yang terbentuk dari membran dan pengoksidaan lipid. Ianya telah didapati tinggi di dalam pesakit kanser jika dibandingkan dengan orang sihat, oleh itu mencadangkan sebagai penanda bio paru-paru. Terangkan suatu tatacara analisis kromatografi cecair, tanpa penyediaan sampel, bagi penentuan surihan heksanal and heptanal di dalam urin. (10 markah)



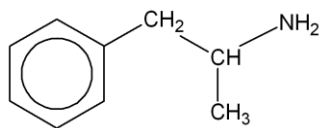
Heksanal



Heptanal

4. (a) Bincangkan bagaimana ekor puncak berlebihan dan masa analisis panjang boleh dikurangkan bagi kromatografi gas rerambut. (6 marks)
- (b) Bincangkan cabaran bagi analisis mikroorganisma menggunakan elektroforesis rerambut. (8 markah)
- (c) Analisis drug berasid di dalam elektroforesis rerambut biasanya dilakukan menggunakan tampan bebas. Mengapa? (6 markah)
5. (a) Bandingkan sifat utama kaedah GC dua dimensi. Bandingkan keupayaannya berbanding kaedah GC pantas. (10 markah)
- (b) Dengan dibantukan dengan satu gambarajah skema, berikan keadaan eksperimen yang perlu bagi pengekstrakan memilih amfetamina dan sebatian berkaitan di dalam sampel urin menggunakan kaedah pengekstrakan mikro fasa cecair. (10 markah)

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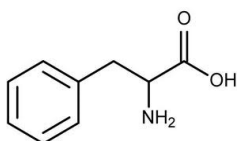
Amfetamina

6. (a) Kaedah elektroforesis rerambut elektrokinetik misel telah digunakan untuk memisahkan asid amino fenilalanina, tirosina and triptofan.

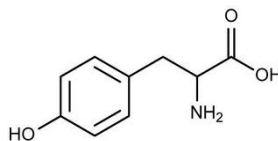
(i) Cadangkan satu elektrolit latarbelakang yang sesuai, berserta alasan, bagi pemisahan.

(ii) Apakah tertib elusi asid amino? Beri alasan.

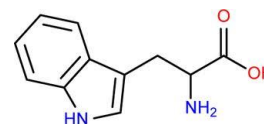
(7 markah)



Fenilalanina



Tirosina



Triptofan

(b) Terangkan, dengan bantuan satu gambarajah skema, bagaimana anda akan mengautomatiskan pengekstrakan fasa pepejal asid lemak rantai pendek di dalam suatu sampel persekitaran untuk di tentukan menggunakan GC.

(7 markah)

(c) Terangkan prinsip kerja pengesanan kekonduksian kupel teraruh. Di dalam keadaan bagaimana pengesanan ini berguna?

(6 markah)

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