UNIVERSITI TEKNOLOGI MALAYSIA

BORANG PENGESAHAN
LAPORAN AKHIR PENYELIDIKAN

TAJUK PROJEK : **DEVELOPMENT AND APPLICATION OF HIGH TEMPERATURE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF PESTICIDES AND PERSISTANT ORGANIC POLLUTANTS**

Saya **MOHD MARSIN SANAGI**
(HURUF BESAR)

Mengaku membenarkan **Laporan Akhir Penyelidikan** ini disimpan di Perpustakaan Universiti Teknologi Malaysia dengan syarat-syarat kegunaan seperti berikut :

1. Laporan Akhir Penyelidikan ini adalah hakmilik Universiti Teknologi Malaysia.
2. Perpustakaan Universiti Teknologi Malaysia dibenarkan membuat salinan untuk tujuan rujukan sahaja.
3. Perpustakaan dibenarkan membuat penjualan salinan Laporan Akhir Penyelidikan ini bagi kategori TIDAK TERHAD.
4. * Sila tandakan (/)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau Kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972).
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh Organisasi/badan di mana penyelidikan dijalankan).
☑	TIDAK TERHAD	

TANDATANGAN KETUA PENYELIDIK

MOHD MARSIN SANAGI

Nama & Cop Ketua Penyelidik

Tarikh : 11 April 2008

CATATAN : ***** *Jika Laporan Akhir Penyelidikan ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh laporan ini perlu dikelaskan sebagai SULIT dan TERHAD.*

End of Project Report Guidelines

A. Purpose

The purpose of the End of Project is to allow the IRPA Panels and their supporting group of experts to assess the results of research projects and the technology transfer actions to be taken.

B. Information Required

The following Information is required in the End of Project Report :

- Project summary for the Annual MPKSN Report;
- Extent of achievement of the original project objectives;
- Technology transfer and commercialisation approach;
- Benefits of the project, particularly project outputs and organisational outcomes; and
- Assessment of the project team, research approach, project schedule and project costs.

C. Responsibility

The End of Project Report should be completed by the Project Leader of the IRPA-funded project.

D. Timing

The End of Project Report should be submitted within three months of the completion of the research project.

E. Submission Procedure

One copy of the End of Project is to be mailed to :

IRPA Secretariat
Ministry of Science, Technology and the Environment
14th Floor, Wisma Sime Darby
Jalan Raja Laut
55662 Kuala Lumpur

End of Project Report

A. **Project number :** 09-02-06-0145 EA 001

Project title : Development and Application of High Temperature high performance liquid chromatography to the Analysis of Pesticides and Persistant Organic Pollutants

Project leader : Prof. Dr. Mohd Marsin Sanagi

Tel: +607-5536060 **Fax:** +607-5536080

B. **Summary for the MPKSN Report (**for publication in the Annual MPKSN Report, please summarise the project objectives, significant results achieved, research approach and team strucure)

The research project have successfully developed high-temperature high performance liquid chromatography (HT-HPLC) system, optimize separation of test compounds, and applied the developed method to the analysis of pesticides. The home-made micro-HPLC system utilized a micro-HPLC pump and a microflow-cell as the ultraviolet (UV) detection sytem.

Combined pressurized liquid extraction (PLE) and HT-HPLC method was successfully applied to the determination of selected persistent organopesticides (POPs) in soil samples. The pesticides include napthalene, fluorene, phenanthrene and fluoranthene. Extraction of analytes in the sample preparation step involves conventional Soxhlet extraction and PLE.

The PLE method was also applied to the extraction of natural vitamin E isomers from palm pressed fiber. The method also includes normal phase liquid chromatography for the separation of individual vitamin E isomers such as alpha-tocopherols, alpha-tocotrienols, gamma-tocotrienols and delta- tocotrienols.

The research findings lead to the development of liquid phase microextraction and dynamic liquid phase microextraction to the analysis of pesticides in different sample matrices.

Liquid membrane extraction (LME) method has been investigated and applied to the analysis of pesticides using micro-HPLC. Optimized conditions for the extraction enabled enhanced detection limits of the analytes.

C. Objectives achievement

- **Original project objectives** (Please state the specific project objectives as described in Section ll of the Application Form)

  TO DEVELOP HT-HPLC
  TO OPTIMIZE SEPARATION OF TEST COMPOUNDS
  TO EXPLORE THE APPLICATIONS OF HT-HPLC

- **Objectives Achieved** (Please state the extent to which the project objectives were achieved)

  All objectives were achieved.

- **Objectives not achieved** (Please identify the objectives that were not achieved and give reasons)

  Nil

D. Technology Transfer/Commercialisation Approach (Please describe the approach planned to transfer/commercialise the results of the project)

- Patent search has been conducted.
- Patent filing is in progress: PLEX: Pressurized Liquid Extraction For the Monitoring of Residue Oil Content and Natural Vitamin E in Palm Pressed Fiber

E. Benefits of the Project (Please identify the actual benefits arising from the project as defined in Section lll of the Application Form. For examples of outputs, organisational outcomes and sectoral/national impacts, please refer to Section lll of the Guidelines for the Application of R&D Funding under IRPA)

- **Outputs of the project and potential beneficiaries** (Please describe as specifically as possible the outputs achieved and provide an assessment of their significance to users)

  - High-temperature High Performance Liquid Chromatographic method for the analysis of Organophosphorus Pesticides.
    Beneficiaries: Chemical Laboratories
  - Pressurized Liquid Extraction For the Monitoring of Residue Oil Content and Natural Vitamin E in Palm Pressed Fiber.
    Beneficiaries: Palm oil mills laboratories

- **Organisational Outcomes** (Please describe as specifically as possible the organisational benefits arising from the project and provide an assessment of their significance)

  - Skilled researchers.
  - Skilled and trained scientists. (2 MSc Students, 1 Research Officer)
  - Articles in journals
  - Papers in conferences

- **National Impacts** (If known at this point in time, please describes specifically as possible the potential sectoral/national benefits arising from the project and provide an assessment of their significance)

  - 1 article in International Journal, 1 article in National Journal, 6 papers in national and international conferences
  - 1 Gold Medal award in SIIF 2006, Korea, and 1 Silver Medal award in ITEX 2006, Malaysia
  - 1 MSc Thesis, 1 BSc Project Report

F. Assessment of project structure

- **Project Team** (Please provide an assessment of how the project team performed and highlight any significant departures from plan in either structure or actual man-days utilised)

The project team is as planned.

- **Collaborations** (Please describe the nature of collaborations with other research organisations and/or industry)

The project was able to be condu.cted with the collaboration with the palm oil industry and agriculture industry

G. Assessment of Research Approach (Please highlight the main steps actually performed and indicate any major departure from the planned approach or any major difficulty encountered)

The project research approach was as planned.

H. Assessment of the Project Schedule (Please make any relevant comment regarding the actual duration of the project and highlight any significant variation from plan)

The project schedule was almost as planned.

I. Assessment of Project Costs (Please comment on the appropriateness of the original budget and highlight any major departure from the planned budget)

The financial performance is in line with plan.

J. Additional Project Funding Obtained (In case of involvement of other funding sources, please indicate the source and total funding provided)

Nil

K. Other Remarks (Please include any other comment which you feel is relevant for the evaluation of this project)

The researchers wish to thank Universiti Teknologi Malaysia and the Ministry of Science, Technology and Innovation Malaysia for facilities and financial support.

Date : 11 April 2008 **Signature :**

Benefits Report Guidelines

A. Purpose

The purpose of the Benefits Report is to allow the IRPA Panels and their supporting experts to assess the benefits derived from IRPA-funded research projects.

B. Information Required

The Project Leader is required to provide information on the results of the research project, specifically in the following areas:

- Direct outputs of the project;
- Organisational outcomes of the project; and
- Sectoral/national impacts of the project.

C. Responsibility

The Benefits Report should be completed by the Project Leader of the IRPA-funded project.

D. Timing

The Benefits Report is to be completed within three months of notification by the IRPA Secretariat. Only IRPA-funded projects identified by MPKSN are subject to this review. Generally, the Secretariat will notify Project Leaders of selected projects within 18 months of project completion.

E. Submissin Procedure

One copy of this report is to be mailed to :

IRPA Secretariat
Ministry of Science, Technology and the Environment
14th, Floor, Wisma Sime Darby
Jalan Raja Laut
55662 Kuala Lumpur

Benefit Report

I. Description of the Project

A. Project identification

1. **Project number :** 09-02-06-0145 EA 001

Project title : Development and Application of High Temperature high performance liquid chromatography to the Analysis of Pesticides and Persistant Organic Pollutants

2. **Project leader :** Prof. Dr. Mohd Marsin Sanagi

B. Type of research

Indicate the type of research of the project (Please see definitions in the Guidelines for completing the Application Form)

☐ Scientific research (fundamental research)

☑ Technology development (applied research)

☐ Product/process development (design and engineering)

☐ Social/policy research

C. Objectives of the project

1. Socio-economic objectives

Which socio-economic objectives are adressed by the project? (Please indentify the sector, SEO Category and SEO Group under which the project falls. Refer to the Malaysian R&D Classification System brochure for the SEO Group code)

Sector : Science and Engineering

SEO Category : Natural Science, Technology and Engineering

SEO Group and Code : Chemical Sciences (S5010300)

2. Fields of research

Which are the two main FOR Categories, FOR Groups, and FOR Areas of your project? (Please refer to the Malaysia R&D Classification System brochure for the FOR Group Code)

a. Primary field of research

FOR Category : Chemical Sciences

FOR Group and Code : Analytical Chemistry (F1030400)

FOR Area : Chemical and Physical Methods (F1030403)

b. Secondary field of research

FOR Category : Chemical Sciences

FOR Group and Code : Analytical Chemistry (F1030400)

FOR Area : Instrumentation Methods (F1030408)

D. Project duration

What was the duration of the project ?

36 Months

E. Project manpower

How many man-months did the project involve?

55.5 Man-months

F. Project costs

What were the total project expenses of the project?

RM 175,000.00

G. Project funding

Which were the funding sources for the project?

Funding sources	Total Allocation (RM)
IRPA	175,000.00

ll. Direct Outputs of the Project

A. Technical contribution of the project

1. **What was the achieved direct output of the project :**

For scientific (fundamental) research projects?

- ☐ Algorithm
- ☐ Structure
- ☐ Data
- ☐ Other, please specify : _____

For technology development (applied research) projects :

- ☑ Method/technique
- ☐ Demonstrator/prototype
- ☐ Other, please specify : _____

For product/process development (design and engineering) projects:

- ☐ Product/component
- ☐ Process
- ☐ Software
- ☐ Other, please specify : _____

2. How would you characterise the quality of this output?

- ☐ Significant breakthrough
- ☑ Major improvement
- ☐ Minor improvement

B. Contribution of the project to knowledge

1. How has the output of the project been documented?

- [✓] Detailed project report
- [ ] Product/process specification documents
- [ ] Other, please specify : _____

2. Did the project create an intellectual property stock?

- [ ] Patent obtained
- [ ] Patent pending
- [✓] Patent application will be filed
- [ ] Copyright

3. What publications are available?

- [✓] Articles (s) in scientific publications — How Many: 2
- [✓] Papers(s) delivered at conferences/seminars — How Many: 6
- [ ] Book
- [ ] Other, please specify : _____

4. How significant are citations of the results?

- [✓] Citations in national publications — How Many: ~1
- [✓] Citations in international publications — How Many: ~1
- [ ] None yet
- [ ] Not known

lll. Organisational Outcomes of the Project

A. Contribution of the project to expertise development

1. How did the project contribute to expertise?

- ☐ PhD degrees — How Many: _____
- ☑ MSc degrees — How Many: 2
- ☑ Research staff with new specialty — How Many: 1
- ☐ Other, please specify: _____

2. How significant is this expertise?

- ☑ One of the key areas of priority for Malaysia
- ☐ An important area, but not a priority one

B. Economic contribution of the project?

1. How has the economic contribution of the project materialised?

- ☐ Sales of manufactured product/equipment
- ☐ Royalties from licensing
- ☑ Cost savings
- ☑ Time savings
- ☐ Other, please specify : _____

2. How important is this economic contribution ?

- ☐ High economic contribution — Value: RM_____
- ☑ Medium economic contribution — Value: RM_____
- ☐ Low economic contribution — Value: RM_____

3. When has this economic contribution materialised?

- [ ] Already materialised
- [ ] Within months of project completion
- [ ] Within three years of project completion
- [ ] Expected in three years or more
- [x] Unknown

C Infrastructural contribution of the project

1. What infrastructural contribution has the project had?

- [ ] New equipment — Value: RM 69,977.00
- [x] New/improved facility — Investment : RM _____
- [ ] New information networks
- [ ] Other, please specify: _____

2. How significant is this infrastructural contribution for the organisation?

- [ ] Not significant/does not leverage other projects
- [x] Moderately significant
- [ ] Very significant/significantly leverages other projects

D. Contribution of the project to the organisation's reputation

1. How has the project contributed to increasing the reputation of the organisation

- [ ] Recognition as a Centre of Excellence
- [x] National award
- [x] International award
- [ ] Demand for advisory services
- [ ] Invitations to give speeches on conferences
- [ ] Visits from other organisations
- [ ] Other, please specify: _____

2. How important is the project's contribution to the organisation's reputation ?

☐ Not significant

☑ Moderately significant

☐ Very significant

1V. National Impacts of the Project

A. Contribution of the project to organisational linkages

1. Which kinds of linkages did the project create?

- [✓] Domestic industry linkages
- [ ] International industry linkages
- [ ] Linkages with domestic research institutions, universities
- [ ] Linkages with international research institutions, universities

2. What is the nature of the linkages?

- [ ] Staff exchanges
- [ ] Inter-organisational project team
- [ ] Research contract with a commercial client
- [✓] Informal consultation
- [✓] Other, please specify: Collaboration in Experimental Research

B. Social-economic contribution of the project

1. Who are the direct customer/beneficiaries of the project output?

Customers/beneficiaries:	Number:
Analytical Laboratories	Estimated >200

2. How has/will the socio-economic contribution of the project materialised ?

- [ ] Improvements in health
- [ ] Improvements in safety
- [✓] Improvements in the environment
- [ ] Improvements in energy consumption/supply
- [ ] Improvements in international relations
- [ ] Other, please specify: _____

3. How important is this socio-economic contribution?

☐ High social contribution

☑ Medium social contribution

☐ Low social contribution

4. When has/will this social contribution materialised?

☐ Already materialised

☐ Within three years of project completion

☑ Expected in three years or more

☐ Unknown

Date: 11 April 2008 **Signature:**

UNIVERSITI TEKNOLOGI MALAYSIA
Research Management Centre

PRELIMINARY IP SCREENING & TECHNOLOGY ASSESSMENT FORM

(To be completed by Project Leader submission of Final Report to RMC or whenever IP protection arrangement is required)

1. **PROJECT TITLE IDENTIFICATION** :

Project number : 09-02-06-0145 EA 001

Project Title: Development and Application of High Temperature high performance liquid chromatography To the Analysis of Pesticides and Persistant Organic Pollutants

Vote No: 74255

2. **PROJECT LEADER** :

Name : Prof. Dr. Mohd Marsin Sanagi

Address :

Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia,

81310 UTM Skudai, Johor

Tel : +607-5536060 Fax : +6-7-5536080 e-mail : marsin@kimia.fs.utm.my

3. **DIRECT OUTPUT OF PROJECT** *(Please tick where applicable)*

Scientific Research	Applied Research	Product/Process Development
☐ Algorithm	☑ Method/Technique	☐ Product / Component
☐ Structure	☐ Demonstration / Prototype	☐ Process
☐ Data		☐ Software
☐ Other, please specify	☐ Other, please specify	☐ Other, please specify
_____	_____	_____
_____	_____	_____
_____	_____	_____

4. INTELLECTUAL PROPERTY *(Please tick where applicable)*

☐ Not patentable	☐ Technology protected by patents
☐ Patent search required	☑ Patent pending
☐ Patent search completed and clean	☐ Monograph available
☐ Invention remains confidential	☐ Inventor technology champion
☐ No publications pending	☐ Inventor team player
☐ No prior claims to the technology	☐ Industrial partner identified

5. LIST OF EQUIPMENT BOUGHT USING THIS VOT

Intel Extreme Multimedia PC Package Intel Pentium D 2.8 GHz (RM 2450.00)

Accurel pp Capillary Membrane (RM 4850.00)

Micro straight-flowcell unit (RM 4,819.00)

Rheodyne 7520 micro-scale injector (RM 3,760.00); GC capillary column (RM 1,584.00);

SPME Fiver Holder and SPME Fiber Kit (RM 3,500.00)

Mcro-LC Pump (RM 48,034.00)

6. STATEMENT OF ACCOUNT

a) APPROVED FUNDING RM : 175,000.00

b) TOTAL SPENDING RM : 173,031.69

c) BALANCE RM : 1,968.31

7. TECHNICAL DESCRIPTION AND PERSPECTIVE

Please tick an executive summary of the new technology product, process, etc., describing how it works. Include brief analysis that compares it with competitive technology and signals the one that it may replace. Identify potential technology user group and the strategic means for exploitation.

a) Technology Description

- Pressurised Liquid Extraction (PLE) system is an extraction procedure that combines elevated temperature and high pressure with liquid solvents.
- The use of these physical parameters can result in rapid and efficient extraction of residue oil from palm pressed fiber (PPF) during routine monitoring process in palm oil mills.

b) Market Potential

- Monitoring of screw-press process by observing the total oil loss percentages in palm pressed fiber is crucial in affecting the overall crude palm oil (CPO) daily production.
- By introducing a fast and efficient extraction system, the oil loss percentages in PPF can be monitored in short while and leads to increased production yields.

c) Commercialisation Strategies

- Patent Search has been carried out.
- Patent Filing is in progress.

8. RESEARCH PERFORMANCE EVALUATION

a) FACULTY RESEARCH COORDINATOR

Research Status	( )	( )	( )	( )	( )	( )
Spending	( )	( )	( )	( )	( )	( )
Overall Status	( )	( )	( )	( )	( )	( )
	Excellent	Very Good	Good	Satisfactory	Fair	Weak

Comment/Recommendations :

……. Name : …….

Signature and stamp of JKPP Chairman

Date : …….

b) RMC EVALUATION

Research Status	( )	( )	( )	( )	( )	( )
Spending	( )	( )	( )	( )	( )	( )
Overall Status	( )	( )	( )	( )	( )	( )
	Excellent	Very Good	Good	Satisfactory	Fair	Weak

Comments :-

_____

_____

_____

_____

_____

_____

Recommendations :

☐ Needs further research

☐ Patent application recommended

☐ Market without patent

☐ No tangible product. Report to be filed as reference

…….. Name : …….

Signature and Stamp of Dean / Deputy Dean
Research Management Centre

Date : …….

Jurnal / Buku
Journal / Book -- PLEASE SEE ATTACHMENT --

No. Vot : 74255
Vote No.

Tajuk Kertas / Buku : -- PLEASE SEE ATTACHMENT --
Title

Penulis :
Authors

Bil. *No.*	Nama *Name*	No. K/P / Pasport *I.C Number / Passport*
1		
2		
3		
4		
5		

* Nama Jurnal :
Name of Journal

* Taraf Kertas : ☐ Antarabangsa *(International)*
Status

☐ Kebangsaan *(Local)*

*Volume : *No. :

** ISBN :

Penerbit / Tempat :
Publisher / Place

Tarikh Terbit :
Date

* Jurnal sahaja
** Buku sahaja

APPENDIX:
VOT 74255 PUBLICATIONS

1 article in International Journal, 1 article in National Journal, 6 papers in national and international conferences, 1 Gold Medal award in SIIF 2006, Korea and 1 Silver Medal award in ITEX 2006, Malaysia, 1 MSc Thesis, 1 BSc Project Report

Articles in Journals and Papers in Conferences

M. Marsin Sanagi, H. H. See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Determination of Carotene, Tocopherols and Tocotrienols in Residual Oil from Palm Pressed Fiber Using Pressurized Liquid Extraction – Normal Phase Liquid Chromatography" *Analytica Chimica Acta*, 538, 71-76 (2005)

M. Marsin Sanagi, Noorashikin Md Salleh, Hong Heng See, Wan Aini Wan Ibrahim and Ahmedy Naim, "Elevated Temperature Reversed-Phase High Performance liquid Chromatography of Polycyclic Aromatic Hydrocarbons on ODS-Silica Phase". *Buletin Kimia,* 21 (2005) 19-26.

Hong Heng See, M. Marsin Sanagi, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Determination of Carotene, Tocopherols and Tocotrienols in Residue Oil from Palm Pressed-Fiber using Pressurized Liquid Extraction – Normal Phase Liquid Chromatography" (G1-1). Presented at *Asian Chemical Congress* 2005, Seoul, Korea, 24-26 August 2005.

Hong Heng See, M. Marsin Sanagi, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Novel Applications of High Temperature Liquid Chromatography using Hydro-Organic and Superheated Water Eluent" (G1-8). Presented at *Asian Chemical Congress* 2005, Seoul, Korea, 24-26 August 2005.

Mohd Marsin Sanagi, Noorashikin Md Saleh, Hong Heng See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Application of High Temperature Liquid Chromatography to the Analysis of Polycyclic Aromatic Hydrocarbons in Soil", Paper presented at the *Fifth IMTGT UNINET Biannual Conference and International Seminar,* Tiara Convention Center, Medan North Sumatra, Indonesia, 22-23 June, 2006. (Organized by University of North Sumatra, Medan)

M. Marsin Sanagi*, Noorashikin Md Saleh, Hong Heng See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Elevated Temperature Reversed-Phase High Performance Liquid Chromatography of Polycyclic Aromatic Hydrocarbons on ODS-Silica Phase", Paper presented at the *Annual Fundamental Science Seminar 2005* (AFSS 2005), 4-5 July 2005, Institut Ibnu Sina, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia

M. Marsin Sanagi,* H. H. See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Enhanced Separation of Tocopherols and Tocotrienols Using Elevated Temperature High Performance Liquid Chromatography" Presented at Oils and Fats International Congress 2004, PWTC, 29 September 2004 – 2 October 2004.

M. Marsin Sanagi,* H. H. See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim "Determination of Carotene, Tocopherols and Tocotrienols in Palm Pressed Fiber Using Pressurized Extraction coupled to Elevated Temperature Liquid Chromatography" Presented at Oils and Fats International Congress 2004, PWTC, 29 September 2004 – 2 October 2004.

Thesis

"High-Temperature Liquid Chromatography and Pressurized Liquid Extraction of Polycyclic Aromatic Hydrocarbons" MSc Thesis. Ms Noorashikin Bt Md Saleh (2004 – 2006)

"Extraction of Total Residue Oil from Palm Pressed Fiber using Pressurized Liquid Extraction Method", Undergraduate Project Report. Shahrul Kamal bin Mat Akhir (July 2006 – May 2007)

Awards

Gold Medal at the Seoul International Invention Fair 2006 organized by Korea Invention Promotion Association in Seoul, Korea, 7-11 December 2006. Invention: "PLEX: Pressurized Liquid Extraction for the Monitoring of Residue Oil Content and Natural Vitamin E in Palm Pressed Fiber". Inventors: **Mohd Marsin Sanagi**, See Hong Heng, Wan Aini Wan Ibrahim, Ahmedy Abu Naim.

Silver Medal at the 17th International Invention, Innovation, Industrial Design & Technology Exhibition 2006 (ITEX 2006), Kuala Lumpur Convention Centre, 19-21 May 2006. Name of invention: "PLEX: Method for Pressurized Liquid Extraction (for the monitoring of residue oil content) and Separation of Natural Vitamin E in Palm Pressed Fiber", Inventors: **Mohd Marsin Sanagi**, See Hong Heng, Wan Aini Wan Ibrahim, Ahmedy Abu Naim.

DEVELOPMENT AND APPLICATION OF HIGH TEMPERATURE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF PESTICIDES AND PERSISTANT ORGANIC POLLUTANTS

MOHD MARSIN BIN SANAGI
WAN AINI WAN IBRAHIM
AHMEDY ABU NAIM
NOORASHIKIN MD SALEH
NORFAIRULZUKRY

PROJECT NUMBER : 09-02-06-0145 EA 001
RESEARCH VOTE NO: 74255

Jabatan Kimia
Fakulti Sains
Universiti Teknologi Malaysia

2008

UNIVERSITI TEKNOLOGI MALAYSIA

BORANG PENGESAHAN
LAPORAN AKHIR PENYELIDIKAN

TAJUK PROJEK : **DEVELOPMENT AND APPLICATION OF HIGH TEMPERATURE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF PESTICIDES AND PERSISTANT ORGANIC POLLUTANTS**

Saya **MOHD MARSIN SANAGI**
(HURUF BESAR)

Mengaku membenarkan **Laporan Akhir Penyelidikan** ini disimpan di Perpustakaan Universiti Teknologi Malaysia dengan syarat-syarat kegunaan seperti berikut :

1. Laporan Akhir Penyelidikan ini adalah hakmilik Universiti Teknologi Malaysia.
2. Perpustakaan Universiti Teknologi Malaysia dibenarkan membuat salinan untuk tujuan rujukan sahaja.
3. Perpustakaan dibenarkan membuat penjualan salinan Laporan Akhir Penyelidikan ini bagi kategori TIDAK TERHAD.
4. * Sila tandakan (/)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau Kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972).
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh Organisasi/badan di mana penyelidikan dijalankan).
☑	TIDAK TERHAD	

TANDATANGAN KETUA PENYELIDIK

MOHD MARSIN SANAGI

Nama & Cop Ketua Penyelidik

Tarikh : **11 April 2008**

CATATAN : ***** *Jika Laporan Akhir Penyelidikan ini SULIT atau TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh laporan ini perlu dikelaskan sebagai SULIT dan TERHAD.*

DEVELOPMENT AND APPLICATION OF HIGH TEMPERATURE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TO THE ANALYSIS OF PESTICIDES AND PERSISTANT ORGANIC POLLUTANTS

MOHD MARSIN BIN SANAGI
WAN AINI WAN IBRAHIM
AHMEDY ABU NAIM
NOORASHIKIN MD SALEH
NORFAIRULZUKRY

PROJECT NUMBER : 09-02-06-0145 EA 001
RESEARCH VOTE NO: 74255

Jabatan Kimia
Fakulti Sains
Universiti Teknologi Malaysia

2008

ACKNOWLEDGEMENT

We would like to thank Universiti Teknologi Malaysia (UTM) and Ministry of Science and Innovation (MOSTI) for scholarship and financial supports through the grant vote number 72455. We also would like to express our sincere appreciation to all researchers in the Separation Science Research Group (SSRG), Department of Chemistry, Faculty of Science, UTM, in particular Mr. Dadan Hermawan who have given their great support to this research.

ABSTRACT

A high temperature reversed-phase liquid chromatography (RP-LC) method has been developed to separate polycyclic aromatic hydrocarbons (PAHs) on ODS-silica stationary phase using various proportions of organic modifier in the mobile phase. The selectivity and retention patterns of four PAHs (naphthalene, fluorene, phenanthrene and fluoranthene) were investigated on a Hypersil ODS-silica column (4.6 mm ID × 100 mm, 5 µm) with ultraviolet detection at 254 nm. Excellent separations for the PAHs were achieved with acetonitrile-water 40:60 (v/v) as the mobile phase at a flow rate of 2.5 mL/min at column temperatures ranging from 25°C to 70°C. Pressurized liquid extraction (PLE) method was also developed to determine the quantity of the PAHs in soil samples. It was found that PLE could reduce sample preparation time and reduce organic solvent consumption to one-fifth of that required by Soxhlet extraction (United States Environmental Protection Agency, (US EPA) Method 3545). With dichloromethane-acetone 50:50 (v/v) as the extraction solvent, the optimum conditions were found to be 180°C at a pressure of 250 bar. For the extraction of spiked PAHs, it was found that PLE gave results that are comparable to or better than those obtained by Soxhlet extraction with highest recoveries of fluoranthene 94.4% for PLE and 73.6% for Soxhlet extraction. The developed high-temperature RP-LC method gave limits of detection (LOD) for the PAHs in the range of 0.60 to 1.08 ppm.

ABSTRAK

Kaedah kromatografi cecair fasa terbalik (RP-LC) telah dibangunkan bagi pemisahan hidrokarbon aromatik polisiklik (PAH) menggunakan turus ODS-silika dengan komposisi pelarut organik di dalam fasa bergerak yang berlainan. Pola kepilihan dan penahanan bagi empat PAH (naftalena, fluorena, fenantrena dan fluorantena) telah dikaji menggunakan turus Hypersil ODS-silika, 5 μm (4.6 mm × 100 mm) dengan pengesanan ultra lembayung pada 254 nm. Pemisahan sempurna bagi semua sebatian PAH telah diperolehi menggunakan fasa bergerak asetonitril-air 40:60 (v/v) pada kadar alir 2.5 mL/min dan julat suhu turus dari 25°C hingga 70°C. Kaedah pengekstrakan cecair tekanan tinggi (PLE) telah juga dibangunkan untuk menentukan kuantiti PAH di dalam sampel tanah. PLE didapati boleh menjimatkan masa penyediaan sampel dan mengurangkan penggunaan pelarut organik kepada satu per lima berbanding jumlah yang diperlukan bagi pengekstrakan Soxhlet (kaedah United States Environmental Protection Agency, (US EPA) 3545). Dengan menggunakan diklorometana-aseton 50:50 (v/v) sebagai pelarut pengekstrak, keadaan optimum yang diperolehi ialah suhu 180°C dan tekanan 150 bar. Bagi pengekstrakan PAH pakuan, PLE didapati menghasilkan peratus perolehan yang setanding atau lebih tinggi berbanding dengan menggunakan pengekstrakan Soxhlet dengan nilai perolehan tertinggi 94.4% bagi PLE dan 73.6% bagi pengekstrakan Soxhlet. Kaedah RP-LC suhu tinggi yang dibangunkan menghasilkan had pengesanan (LOD) bagi PAH dalam julat 0.60 hingga 1.08 ppm.

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CHAPTER 1

INTRODUCTION

1.1 High Performance Liquid Chromatography

Chromatography is a separation technique of two or more components in a mixture due to the differentiation in interaction (chemically or physically) or adsorption between mobile phase and stationary phase. In early, 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressurized liquid chromatography began to be used to increase flow through time, thus reducing purification times of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure was debated [1].

High Performance Liquid Chromatography (HPLC) was developed in the mid 1970's and quickly improved with the development of column packing materials and the additional convenience of on-line detectors. In the late 1970's, new methods including reversed phase liquid chromatography allowed for improved separation between very similar compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques [2]. Computers and automation were added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made.

1.2 Reversed Phase High Performance Liquid Chromatography

In many analytical laboratories, HPLC has become an indispensable technique for the analysis of samples, the determination of physical constants and the isolation of purified components from complex mixtures. Now, HPLC has found broad acceptance as the analytical technique of choice in many scientific and application-oriented areas such as life sciences, food, synthetic polymers and environmental chemistry. In addition, in order to meet legal requirements in application areas such as pharmaceutical and clinical chemistry, HPLC analysis protocols are standardized and validated [3].

Among the different available HPLC separation modes, e.g. normal phase (NPLC) or size exclusion chromatography (SEC), definitely reversed-phase liquid chromatography (RPLC) has taken and still takes a dominant position. It is estimated that presently about 80-90% of HPLC separations are performed using RPLC [4]. As already mentioned, the availability of many high quality RP columns together with the large number of tools to control and optimize separations substantially contribute to the great popularity of RPLC.

Although the development of RPLC already started about four decades ago, the technique is still very popular and of great and growing interest. Therefore, RPLC is a continuous subject of substantial research efforts in universities and with manufacturers as well. In turn, this results in a constant appearance of RPLC-oriented books, and scientific and technical papers [3-5]. In addition, new and also special equipment and columns for RPLC are continuously developed and marketed.

In general, a reversed phase system consists of a non-polar stationary phase, typically an alkyl ligand bonded to a silica surface, and a polar mobile phase. The mobile phase is generally a mixture of water and an organic modifier such as methanol or acetonitrile. Ideally, pure water could be used as a reversed-phase mobile phase. However, at room temperature, water is too weak of an eluent for all but the most polar of the solutes. For this reason, organic modifiers are used to increase the elution strength of the mobile phase [6].

The most popular column inner diameter chosen is 4.6 mm due to its earlier commercialization and widespread use. However, smaller diameter columns increase sensitivity if sample amount is limited (Table 1.1). Microbore and capillary columns enhanced sensitivity, and is the choice for sample-limited or trace analyses. Short 5 cm microbore column is ideal for rapid analysis for drug quality control (QC) at a flow rate of 100 to 200 μL/min using 5 cm × 1 mm i.d. microbore columns. Capillary columns and nanocapillary columns are ideal for electrospray and nanospray mass spectrometry (MS) due to the lower flow rates and enhanced MS sensitivity [7-8].

For column length, 15 cm is appropriate for most purposes. Shorter column lengths down to 5 cm are ideal for high speed drug QC applications. Smaller lengths are appropriate for screening once the appropriate phase with high selectivity has been identified. Longer column lengths have increased resolution but require longer analysis time and higher system pressures [9].

Table 1.1: General characteristics of the HPLC columns

Type	Column i.d.(mm)	Flow rate (μL/min)	Loading (μg)	Sensitivity
Analytical	4.6	500-2000	2000-10 000	1
Narrowbore	2.1	200-400	500-2000	5
Microbore	1.0	50-300	100-500	20
Capillary	0.15-0.75	1-30	50-100	40-1000
Nano	0.15	<1	<10	1000-8500

1.3 High Temperature Operations in Reversed-phased Liquid Chromatography

High-temperature liquid chromatography (HTLC) had limited use in the laboratory due to instrument and column limitations. New instrumentation is available that allows operation at temperatures of up to 200ºC with mobile phase preheating to eliminate thermal mismatch (see Section 1.3.1) [10-14]. This has generated increased interest in utilizing high-temperatures in separation work on a

more routine basis. Column selection, however, is still rather limited; no stationary phases other than those based on zirconia have been used at this extreme temperature for routine work. Stationary phases based on graphitic carbon, rigid polystyrene-divinylbenzene polymeric particles and polydentate silica phases should be stable at much higher temperatures than the traditional limits of 50 or 60ºC. One of the most intriguing aspects of HTLC is the ability to perform temperature programming [15-16].

The advantages of utilizing elevated temperatures in HPLC analysis are well documented in the literature. HTLC offers several distinct advantages to the separation scientist. Back pressure is reduced as the temperature is increased, allowing the use of stationary phases with smaller particle sizes for increased efficiency. The analyst can also operate at higher flow rates because of lower back pressure. The Van Deemter curve "flatten out" as a result of increased diffusion rates within the stationary phase and the mobile phase as the temperature is increased, allowing operations at flow rates that are many times the optimal velocity without the sacrifice in efficiency that is found at ambient temperature. The net result is faster and more efficient separations [17-20].

1.3.1 Instruments and Columns for High Temperature HPLC

The influence and important of the role of the temperature in HPLC has been neglected or underestimated for a long time. This is surprising because in HPLC temperature influences many relevant physical parameters such as viscosity, diffusivity of the analytes in mobile and stationary phases and also sample solubility. Working under controlled, fixed temperatures or temperature programming conditions is a strong tool to adjust and to improve retention and selectivity. In addition, higher temperatures also decrease eluent viscosity allowing significantly higher linear eluent velocities. As a result, retention times can be drastically reduced. Concomitantly at higher temperatures also the diffusivity of analytes in the mobile and stationary phases increases, resulting in much better efficiencies and improved peak shapes. Combining these latter effects, higher temperatures in RP-HPLC may provide better resolution and faster analysis.

In addition, at higher analysis temperatures also less amounts of organic modifiers in eluent are needed to achieve the same separation. In turn, this contributes to the reduction in the use of toxic organic solvents and hence contributes to the appearance of "greener" laboratories. Also opposite to the change in the amount of organic modifiers in eluent, which needs a certain equilibrium time, temperature is more flexible and easily adjustable parameter to optimize chromatographic separation. A recent review outlines the use of temperature programming with capillary and microbore columns. Temperature programming with microbore columns was reported in the literature as early as 1983 [21].

Instrument limitations have prevented the use of temperature programming with standard 4.6 mm i.d. column. One aspect critical to successful HTLC analysis with 4.6 mm i.d. column is adequate preheating of the mobile phase. Heat from a forced-air oven alone is sufficient to warm the mobile phase to the same temperature as the column because the columns used are lower in mass and operated at lower flow rates. If the mobile phase is not preheated, the cool mobile phase entering the heated column will warm up faster along the walls of the column than in the center. The warmer mobile phase in this region will flow faster than that in the column center and lead to band broadening. This 'thermal mismatch" band broadening is eliminated if the mobile phase is preheated [22-26]. Thermal mismatch band broadening can occur at temperatures as low as 80ºC with 4.6 mm i.d. columns.

New instrumentation is now commercially available to perform temperature-programmed HPLC at temperatures of up to 200ºC with 4.6 mm i.d. columns [15-16]. This technique allows the user to perform a temperature program to alter retention and selectivity in place of a solvent gradient. This is possible because hydrogen bonding effects in water are reduced as the temperature is increased, making it less polar so that water behaves like a moderately polar organic solvent like methanol or acetonitrile during the separation process. This means that many separations requiring a binary solvent gradient can be separated isocratically using a temperature program [27-29].

Although a number of column heaters have been available for several years that are capable of operation at temperatures of up to 100ºC, traditional silica based

column packings were only stable to about 60ºC when used with aprotic solvents. It was not until the creation of zirconia based stationary phases that high-temperature liquid chromatography was seriously investigated as a routine laboratory technique. Although these zirconia stationary phases are most often the only ones that come to mind for high-temperature use, there are other commercially available columns that can be used at temperatures of up to 200ºC.

A number of papers [12-15] have reported the evaluation a number of different stationary phases under HTLC conditions using temperature programming. The columns were ZirChom, PBD, CARB, DiamondBond column, C_8 polydentate silica column, PRP-1 polymeric column and HyperCarb column consisting of a graphitic carbon stationary phase. The C_8 and PRP-1 columns had an essentially flat baseline. The HyperCarb column exhibited a slight rise in the baseline starting at about 150°C continuing to 200°C. The zirconia based columns exhibited excessive column bleed under temperature-programmed high temperature HPLC conditions. The ZirChom PBD column had a steep rise in the baseline with a maximum absorbance at 220 nm. The ZirChom DiamondBond column had the largest baseline rise with a maximum absorbance of almost 1.5 AU at 220 nm. This large baseline rise was not observed with the C_8, PRP-1 or HyperCarb columns or when the columns were replaced with a stainless steel union in the instrument. This suggests that the observed baseline rise was caused by some material leaching from the packing of the zirconia columns during the temperature-programmed run. This material “bleeding” from the column absorbs in the UV at 254 nm and 220 nm.

The variation in retention time and efficiency was ±10% in most cases for each set of conditions. No lost of efficiency or retention was observed after exposure to high-temperatures even when combined with pH extremes. Stationary phase collapse due to extreme temperature or temperature-programming should have resulted in a loss of retention and efficiency. No significant change in back pressure was observed during the course of the evaluation with any of the columns during analysis [30]. Zirconia based stationary phase can be regarded as one of the latest high thermal stability stationary phases introduced and is able to withstand extended exposure to column temperature as high as 150ºC. Zirconia has very rich surface

chemistry and able to operate at a wider pH range of 1-14 [15]. In contrast, for conventional alkyl silane bonded phases, high temperature will directly accelerate the dissolution of silica in aqueous solution.

The advantages of zirconia as a stationary phase were more apparent with the development of polybutadiene-coated zirconia and carbon-clad zirconia stationary phase. Zirconia columns coated with polybutadiene (PBD) have been widely used as a reversed-phase HPLC stationary phase because it is a more durable substrate compared with conventional silica-based phases, while not imparting the high retentive characteristics of the aromatic polymer-based column. Carr and Li established a reversed phase/cation-exchange mixed-mode chromatographic system on PBD phases [31]. The existence of hydroxyl groups on the surface of zirconia control the surface chemistry and reflected in its cation and anion exchange properties.

1.4 The Improvement and Development of Silica as Reversed-Phase Stationary Phase

By far, silica is still the most popular substrate to manufacture RP stationary phases. Silica has a high mechanical strength that enables its use under the high pressure conditions encountered in HPLC. Furthermore, this substrate does not swell or shrink when exposed to organic solvents. Finally, its production and bonding chemistry is well understood and can be performed in much different morphology and also is reproducible. For this reason, silica seems the perfect starting material for the manufacturing of (bonded) phases for HPLC. During the last decades, many workers have suggested several different approaches for the synthesis of silica-based RP stationary phases and also to improve their chemical and thermal stability. Recently, the preparation and properties of different types of RP stationary phases were extensively discussed and reviewed by Nawrocki *et al*. [32].

However, depending on the physico-chemical properties of the stationary phase, the eluent composition and other experimental parameters silica and silica-based RP stationary phase are vulnerable to deterioration effects. This in turn may

result in early column failure. Silica dissolves slightly in the pH range 2-7. The saturation concentration is about 100 ppm and the value is somewhat dependent on the pH. Above approximately pH 7, however, silica dissolution is substantially accelerated. This in turn causes the impairment of the silica backbone of an RP stationary phase. This process generally results in reduced plate numbers and finally in column clogging. At acidic pH values of the eluent, another process is mainly responsible for the deterioration of RPLC stationary phases. The organic ligands of most of the presently manufactured RP phases are covalently bonded to the silica surface by mono- or polyfunctional siloxane bonds [4, 5, 33, 34].

Silica dissolution is greatly influenced by the nature of the starting substrate material. In principle, silica can be manufactured by a so-called silgel or a solgel process [35]. Apart from other differences solgel silicas have significantly thicker walls in their morphological structures compared to silgel-based silica types. Kirkland *et al*. [36] showed that bonded phases prepared from solgel silicas are significantly more stable towards aggressive alkaline eluents compared to silgel-based RP phases.

Another approach to improve the chemical stability of silica substrates and thus also of the resulting RP stationary phases is described by Neue *et al*. [34] called hybrid organic-inorganic technology process. Furthermore, Collins *et al*. [37] described that titanium grafting or zirconization of silica substrates may also substantially improve the chemical stability under neutral and high pH conditions [27].

In a later study, Kirkland *et al*. [36] investigated a number of RP C-18 phases under non-recycling conditions using freshly prepared eluents at pH 10. Neue *et al*. [34] have discussed another interesting concept for the preparation of alkyl-modified silicas of substantially improved chemical stability. However, silica may slowly dissolve during operation under some mobile phase conditions. If any degradation of the silica occurs there is, in essence, no loss of the stationary phase. Retention remains constant as only the silica surface remains. Eventually, however, the silica may dissolve enough for the column to no longer be of use. When this occurs it

should be a quick loss in performance not a slow change or gradual loss in retention [36].

1.5 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are a class of diverse organic compounds containing two or more fused aromatic rings of the carbon and hydrocarbon atoms. They are ubiquitous pollutants formed from the combustion of fossil fuels and are always found as a mixture of individual compounds. PAHs as one of the typical persistent organic compounds (POPs) featured in regional and global cycling. PAHs are emitted mainly into the atmosphere and have been detected at long distances from their source. Because of their low vapor pressures, compounds with five or more aromatic rings exist mainly adsorbed to airborne particulate matter, such as fly ash and soot [38].

PAHs are reported to have mutagenic and/or carcinogenic effects. The ability of polycyclic aromatic hydrocarbons (PAHs) to induce cancer has been documented by epidemiological studies of worker in coal tar, creosote, coal gas, coke, and cutting oil industries. The European Union (EU) has therefore developed a directive for controlling six PAHs in drinking water. Some analogues of these compounds, such as polycyclic aromatic sulfur heterocycles (PASHs), are also potentially mutagenic and carcinogenic. But, although they have a high bioaccumulation and have been found in some water and sediment samples, they have not been studied as extensively as PAHs [39].

PAHs are adsorbed strongly to the organic fraction of sediments and soils. Therefore, it can be concluded that sediments and soils are usually considered as the main sinks for PAHs in the environment and PAHs with four or more aromatic rings are persistent in the environment. In order to minimize or prevent adverse effects of POPs, many studies illustrate the fate of POPs in natural environments. In the past 20 years, numerous important researches focused on transport of POPs in multimedia environment, such as between soil/water, etc.

The World Health Organization has issued a guide value maximum of 10 mg/mL for the single PAH benzo[a]pyrene. Because certain PAH isomers exist and because many of these PAHs are toxic or even carcinogenic/mutagenic, the development of an accurate and sensitive separation method is needed. Recommended analytical procedures are documented or proposed in several USA and European guidelines including the US Environmental Protection Agency (EPA) method. International Standard Organization (ISO) methods, German Standard (DIN) method and Dutch Standard (NEN) method. The EPA methods comprises a list of 16 PAH priority pollutants; the German and Dutch standard methods only specify 6 and 10 PAHs, respectively. Because more and more PAHs are identified and investigated on their toxicity it can be expected that the list will be extended in the near future [40].

Some PAHs have been demonstrated to cause cancer in human. For over 200 years, it has been known that prolonged exposure in occupational settings to very high levels of coal tar, the principal toxic ingredient of which is benzo[a]pyrene, leads to cancer in humans [38]. In 1775, the occurrence of scrotal cancer in chimney sweeps was associated with the soot lodged in the crevices of their skin. Modern workers in coke oven and gas production plants likewise experience increased levels of lung and kidney cancer due to this PAH.

The main cause of lung cancer is the inhalation of cigarette smoke, which contains many carcinogenic compounds in addition to PAHs. It is difficult to deduce from health statistics the much smaller influences of pollutants such as PAHs from other sources. Research has been established that the PAH molecules themselves are not carcinogenic agents, rather they must be transformed by several metabolic reactions in the body before the actual cancer-causing species is produced. A number of PAHs have been found to cause tumors in laboratory animals that were exposed to PAHs through their food, from breathing contaminated air and when they were applied on their skin. When pregnant mice ate high doses of a PAH (benzo(a)pyrene) they experienced reproductive problems. In addition, the offspring of the pregnant mice showed birth defects and a decrease in their body weight. Other effects include damage to skin, body fluids and the immune system which helps the body fight disease. Animal studies showed that mice exposed to 308 parts per million (ppm) of

PAHs (specifically benzo(a)pyrene) in food for 10 days (short term exposure) had offspring with birth defects. Mice exposed to 923 ppm of benzo(a)pyrene in food for months developed problems in the liver and blood [40].

Phenanthrene is a tricycle aromatic hydrocarbon that is found in high concentrations in polycyclic aromatic hydrocarbon (PAH)-contaminated sediments, surface soils and waste sites. These hydrophobic contaminants are widely distributed in the environment, occurring as natural constituents of fossil fuels and their anthropogenic pyrolysis products. Unlike the higher-molecular-weight PAHs, phenanthrene does not pose a risk to human health, since it exhibit no genotoxic or carcinogenic effects. However, they have been shown to be toxic to fish and algae [41].

Phenanthrene is considered prototypic PAHs and serve as signature compound to detect PAH contamination, since their chemical structures is found in carcinogenic PAHs, such as benzo[a]pyrene. It has also been used as model PAH to determine factors that effect the bioavailability, biodegradation potential, and rate of microbial degradation of PAHs in the environment. A variety of bacterial species have been isolated that have the ability to utilize phenanthrene as the sole source of carbon and energy [41].

Fluorene is a solid colourless to white or pale yellow-green PAHs. Like most PAHs, fluorene is used to make dyes, plastics and pesticides. One of the most common ways fluorene can enter the human body is through breathing contaminated air. It can get into the lungs when the person breathes it. If a man works in a hazardous waste site where PAHs are disposed, he is likely to breathe fluorene or other PAHs. If a person eats or drinks food and water that are contaminated with PAHs, he or she could be exposed to PAHs. Once in the body, the PAHs can spread and target fat tissues. Target organs include kidneys, liver and fat [41].

1.6 Pressurized Liquid Extraction

Pressurized liquid extraction (PLE) is a relatively simple sample preparation technique for automated extraction of analytes in solid materials. At present it is competing with other techniques like microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) for the extraction of organic contaminants from various solid matrices. There are several reasons why these methodologies have evolved, and according to Wan and Wong [42] one of the major driving forces is the increasing demands from authorities to reduce the large volumes of organic solvents consumed by classic extraction methods such as Soxhlet.

The first reports on PLE appeared in 1995, presenting the basic experimental setup as well as extraction results for spiked pesticides and herbicides in soils and PAHs in urban dust. The recoveries obtained were in good agreement with Soxhlet data, demonstrating the great potential of PLE in terms of speed and reduced organic solvent consumption. The success of PLE, with a matrix independent quantitative recovery of a number of compounds after only a few minutes of static extractions, has been explained by the enhanced solubilization and desorption of analytes from the matrix occurring at elevated temperatures (50-200ºC) and pressures (7-20 MPa) [41].

Since PLE was demonstrated to be very efficient, the technique was rapidly accepted by the US Environmental Protection Agency (EPA) as a method for evaluation of solid wastes [40]. Another contributing factor for the rapid acceptance of PLE is that the method development is rather straight-forward. Often the organic solvent or combination of solvents utilized in existing Soxhlet method can simply be adopted by the PLE method. Consequently, the year after the first publications dealing with persistent organic pollutants (POPs) in soils and sediments were presented ever since, the number of publications dealing with PLE of POPs has increased, where the main focused has been on PAHs [41]. Surprisingly, one of the most well known POPs world-wide, namely polychlorinated biphenyls (PCBs), have been paid relatively little attention and the number of papers presented until today is limited.

1.7 Aim, Objectives and Scope of Research

1.7.1 Aim of Study

The aim of this study is to develop an environmentally friendly, efficient and a rapid separation method based on high temperature pressurized liquid extraction and HPLC for the analysis of polycyclic aromatic hydrocarbons.

1.7.2 Objectives of Study

The objectives of this study are:

a) To study the RP-HPLC separation of PAHs at ambient and high temperatures using various compositions of organic modifier in the mobile phase.
b) To develop pressurized liquid extraction method for PAHs in soil.
c) To compare the developed PLE method with Soxhlet extraction to soil samples.

1.7.3 Scope of Study

The separation of PAHs at ambient temperature was performed using mobile phase compositions acetonitrile-water (v/v) at 70:30, 60:40, 50:50 and 40:60. While the separation of PAHs at elevated temperature (50ºC, 60ºC, 70ºC) was carried out using mobile phase compositions acetonitrile-water (v/v) at 70:30, 60:40, 50:50, 40:60 and 30:70. The recoveries of PAHs using pressurized liquid extraction employed pressures ranging from 50 to 250 bar and temperatures ranging from 60°C to 250°C. While the recoveries of PAHs using soxhlet extraction was carried out with solvent extractor dichloromethane: acetone, hexane: acetone, dichloromethane and acetone.

CHAPTER II

EXPERIMENTAL

2.1 Instrumentation and Apparatus

2.11 High Temperature Reversed Phase Liquid Chromatography

The high temperature HPLC system consisted of a conventional HPLC system coupled with a column oven of a Shimadzu GC-8A Gas Chromatography (Shimadzu Kyoto, Japan)(Figure 2.1). HPLC separations were carried out using JASCO PU-980 HPLC pump for mobile phase delivery. Samples were injected into the system using a 25 μL loop for sample introduction. Analyte peaks were detected using a Shimadzu SPD-6A UV detector (Kyoto, Japan) and were recorded on a Waters 746 Data Module integrator (Mildford, USA).

A 30 cm length of stainless-steel tubing was placed in the oven between the injection valve and the column as pre-heating coil. The column used in this research was a packed 5μm Hypersil ODS-silica, 4.6 mm i.d. × 100 mm, (Agilent Technologies, USA). The column and the preheating coil were placed together in the oven. A thermometer (Zecol, England) with a temperature range of 20ºC-360ºC was placed inside the oven to measure the exact oven temperature.

For high temperature operations, column effluent was cooled by using ice water before it reached the detector. This procedure is important in stabilizing the baseline reading and avoids possible damage to the detector. In order to keep the

mobile phase from boiling when passing through the detector, aluminium tubing (0.1 mm i.d.) was placed at the outlet of the detector to serve as a restriction tubing to apply a back pressure in the detector cell.

Mobile phase used in this research was degassed using a vacuum-ultrasonic degassing procedure. Mobile phase was degassed in the ultrasonic bath (NEY 300 Ultrasonic, USA).

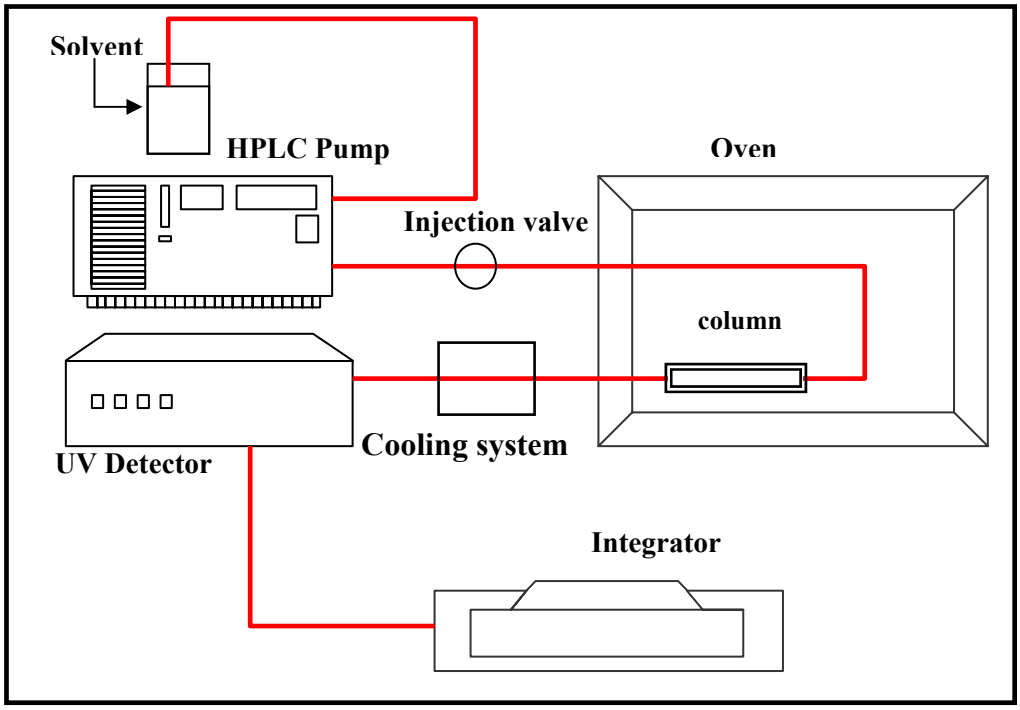


Figure 2.1: Diagrams of high temperature liquid chromatography system

2.1.2 Soxhlet Extraction

The Soxhlet extraction method was based on EPA method 3540. In summary, 30 g of soil were mixed with 30 g anhydrous sodium sulfate to adsorb moisture. The spiked standards of four PAHs was added in a soil sample and transfer into a cellulose extraction thimble and inserted into a Soxhlet assembly fitted with a 250 mL flask (Figure 2.2). The solvents used were (i) dichloromethane-acetone, (ii) hexane-acetone, (iii) dichloromethane, and (iv) acetone was heated for 20 hours. One third of the extract was concentrated to 10 mL on a rotary evaporator and evaporated under a gentle flow of nitrogen gas. Subsequently, 1 mL of solvent extract and 50 µL

of internal standard were added to the sample and aliquots of 2 μL of the extracts were injected into HPLC for analysis.

Figure 2.2. Soxhlet extraction apparatus

2.1.3 Pressurized Liquid Extraction

Extraction were done using JASCO PU 980 HPLC pump. Samples (7 g) were accurately weighed into a 11 mL cell. The sample cell were then closed, to finger tightness and placed in a Shimadzu GC 8A oven. Figure 2.3 shows a schematic diagram of pressurized liquid extraction system. Extractions were carried out using dichloromethane-acetone. Extractions were performed using preheated method. In this procedure, the sample cell were heated to the extraction temperature (the range of 60°C to 250°C was tested). When a gas chromatography oven was used, the cell was allowed to equilibrate for 10-15 min after the oven reached the set point temperature. The static valve was opened during the preheat step.

The pump valve was opened after the heat-up time and solvent was introduced into the cell at 2.0 mL/min until about 1 mL had accumulated in the collection vial. At that point the static valve was closed and the cell continued to

pressurize to the set point (50-250 bar). The static period for these studies was 5 min, because longer periods did not show improvement in recoveries. After the static period, the static valve was reopened, fresh solvent was introduced to flush the lines and cell, and the extract was collected in the vial. During this solvent flush step, 50-100% of the extraction cell volume of solvent was pumped into the cell.

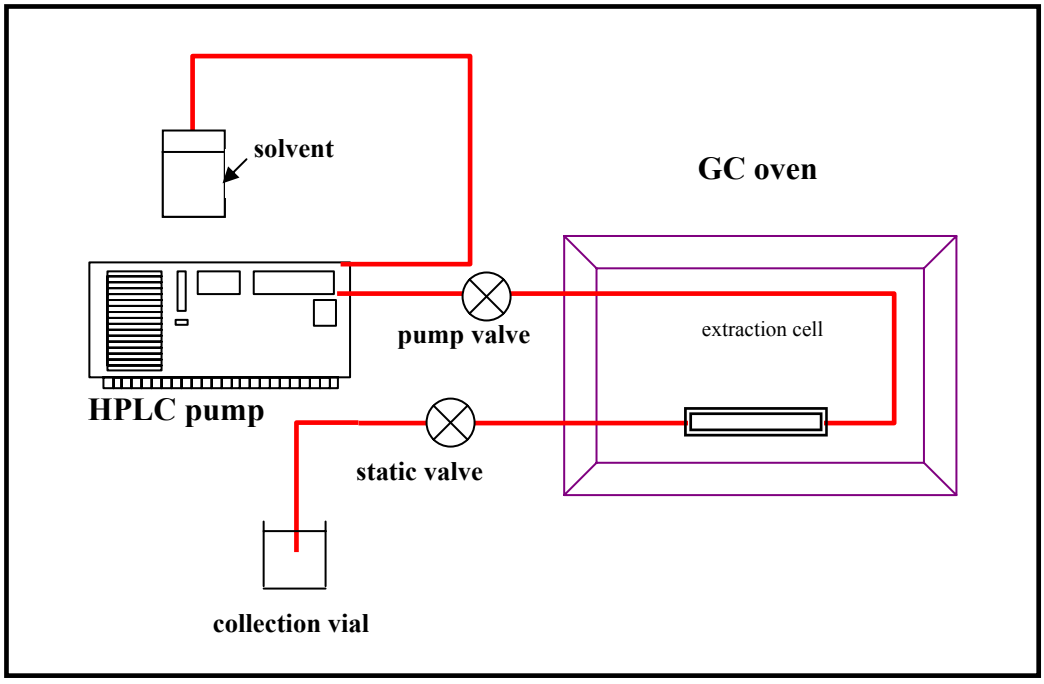


Figure 2.3. Schematic diagram of pressurized liquid extraction system

2.2 Soil Sample Preparation

The land soils was sampled from a known site (FELDA palm oil plantation, Serting, Negeri Sembilan) and transported to the laboratory where it was air-dried for 24 h and then sieved through <7 mm sieve. The fine powdered soils were then stored in air-tight containers for analysis.

2.3 Reagents and Chemicals

HPLC grade solvent acetonitrile was obtained from Scharlau (Barcelona, Spain). Methanol solvent was obtained from HyperSolv (BDH Laboratory, U.K.). The

polycyclic aromatic hydrocarbons (napthalene (70211), fluorene (46880), phenanthrene (77470), fluoranthene (46530) and internal standard (pyrene (82648) were obtained from Fluka Chemika, Sigma-Aldrich Chemic, Steinheim, (Switzerland). Double-distilled deionized water of at least 18 MΩ was purified by Nano ultra pure water system (Barnstead, USA).

2.4 Methods

2.4.1 High Temperature RPLC of PAHs on Silica column

Samples of polycyclic aromatic hydrocarbons (PAHs) dissolved in acetonitrile were injected in triplicate onto the column. Separation was carried out using acetonitrile-water: 70:30, 60:40, 50:50, 40:60 and 30:70 at high temperatures (40-70ºC) on silica column. The detection wavelength was set up at 254 nm and system was eluted at a flow rate of 1.0 mL/min and 2.5 mL/min. Then, flushed with fresh eluent for at least 30 min to stabilize the system. When a change was made to the mobile phase composition, the system was flushed overnight at a flow rate of 0.1-0.2 mL/min to make sure the column was equilibrated. At least 30 min was used to stabilize the column temperature and equilibrium of the whole system.

All the stock solutions for each analyte (napthalene, fluorene, phenanthrene and fluoranthene) were prepared at 1000 μg/mL by dissolving weighed amount of analytes in acetonitrile. Internal mixture of the analytes was prepared by mixing the specific quantities of analytes from respective stock solutions. The mixture solution was kept in the freezer to avoid sample degradation. Approximately 2 μL of sample mixture was injected into the HPLC system.

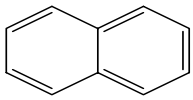
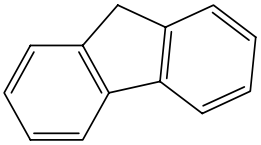
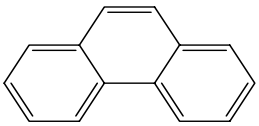
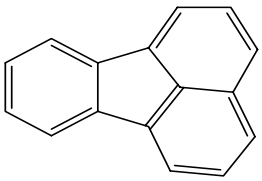
Retention factor, k; separation factor, α; theoretical number of separation plates per column length, N/m; plate height, H and the resolution, R_s were analysed and calculated. The column efficiency and retention characteristic for high temperature RP-HPLC system were observed and examined.

2.4.2 RPLC of PAHs on Silica column at Ambient Temperature

Samples of four PAHs (napthalene, fluorine, phenanthrene and fluoranthene) were dissolved in the acetonitrile and were kept in the freezer. Mixture solution was prepared by mixing the specific quantities of analytes from each stock solution. The prepared mixture solution was injected in triplicate onto the column.

Separations was carried out using different compositions (acetonitrile-water: 70-30; 60-40; 50-50; 40-60, v/v). The eluent flow rate was 1.0 mL/min and sample injection volume was 1 μL. UV detection of analytes for the comparison study was at 254 nm.

Table 2.1: Properties of four polycyclic aromatic hydrocarbons (PAHs)

Compound	Molecular Structure	Molecular Weight
Napthalene $C_{10}H_8$		128
Fluorene $C_{13}H_{10}$		166
Phenanthrene $C_{14}H_{10}$		178
Fluoranthene $C_{16}H_{10}$		202

2.5 Chromatographic Data

Retention time was calculated using:

$$t'_R = t_R - t_0$$

where, t_R is retention time and t_0 is column dead volume or column void volume.

The column capacity factor is given by:

$$k = \frac{t_R - t_0}{t_0}$$

where t_R is retention time and t_0 is column dead volume or column void volume.

Separation factor was calculated using:

$$\alpha = \frac{k_B}{k_A}$$

where k_B is capacity factor for peak B and k_A is capacity factor for peak A.

Resolution peak was calculated using:

$$R_s = \frac{2(t_{R2} - t_{R1})}{w1 + w2}$$

where t_{R2} is adjusted retention time for peak 2, t_{R1} is adjusted retention time for peak 1, w_1 is width of peak 1 and w_2 is width of peak 2.

Theoretical plate number is given by:

$$n = \left(\frac{t_R}{w}\right)^2$$

where t_R is adjusted retention time and w width of peak.

Peak height was obtained by using:

$$H = \frac{L}{n}$$

where L is column length and n is theoretical plate number.

2.6 Determining the Limit of Detection and Percent Recovery

Calibration graphs obtained were used to determine the limit of detection (LOD). The LOD was calculated using this formula:

$$\mathrm{LOD} = \frac{3\mathrm{x}\left(\dfrac{\sum\left(y_i - \bar{y}_i\right)^2}{x-1}\right)^{1/2}}{\text{slope}}$$

where y_i is peak area of analyte, $\bar{y}$ is a mean of peak area and x is quantity of samples.

CHAPTER III

HIGH TEMPERATURE LIQUID CHROMATOGRAPHY OF POLYCYCLIC AROMATIC HYDROCARBONS

In liquid chromatography, several mobile phases can be used to influence the strength of the interaction between the sample and stationary phase. The greater the elution strength of the mobile phase, the earlier is the component of the sample eluted. However, the use of ambient temperature operating in conventional RP-HPLC system with higher flow-rates to reduce analysis time is not recommended because of higher back pressure effects as well as increased operating incurred. In this work, the performance of ODS-silica stationary phase was studied to investigate the influence of elevated temperature to column efficiency and total analysis time and the importance of organic modifier in the mobile phase.

3.1 Separation of PAHs at Ambient Temperature

The PAHs were injected into the HPLC system in triplicates. From the chromatograms obtained (Figure 3.1), it was noted that the elution order for the four PAHs on ODS-silica column were in order of increasing molecular weight. Naphthalene with the lowest molecular weight was first eluted across the column followed by fluorene, phenanthrene and fluoranthene.

Figure 3.1: Separation of PAHs on ODS-silica at 24ºC (ambient temperature) with different mobile phase compositions. Separation conditions: ODS-silica column (100 mm × 4.6 mm I.D.) mobile phase: acetonitrile-water (a) 70:30; (b) 60:40; (c) 50:50; (d) 40:60 (v/v); flow rate: 1.0 mL/min; detection: UV absorbance at 254 nm. Peaks: (1) naphthalene; (2) fluorene; (3) phenanthrene; (4) fluoranthene.

The retention factor, *k*, separation factor, α, resolution, $R_{s,}$ and plate numbers per column length, *N*/m, are summarized in Table 3.1. It was observed that the retention factor for the PAHs decreased significantly with increasing percentage of organic modifier (acetonitrile) used in the mobile phase ranging from 40 to 70 (v/v). The test compounds were eluted within 60 min except for mobile phase composition of acetonitrile-water 40:60 (v/v). A time of approximately 20 min was required to separate all the compounds using acetonitrile-water 50:50 (v/v), 10 min using

acetonitrile-water 60:40 (v/v) and a mere 5 min using acetonitrile-water 70:30 (v/v) as the mobile phase.

Table 3.1: Performance characteristics of ODS-silica column in separating PAHs at ambient temperature. Separation conditions as in Fig. 3.1

PAHs	Mobile Phase Composition: Acetonitrile-Water (v/v) 70:30	60:40	50:50	40:60
	Retention time, t_R			
Naphthalene	2.77	4.33	6.68	22.30
Fluorene	3.80	6.74	11.68	-
Phenanthrene	4.20	7.69	13.64	-
Fluoranthene	5.32	10.40	19.62	-
	Retention factor, k			
Naphthalene	2.75	4.50	8.41	45.57
Fluorene	4.14	7.55	15.45	-
Phenanthrene	4.69	8.76	18.21	-
Fluoranthene	6.21	12.19	26.64	-
	Plate numbers per column length, N/m × 10^4			
Naphthalene	4.25	10.90	12.40	-
Fluorene	4.20	13.30	59.20	-
Phenanthrene	3.82	12.80	84.20	-
Fluoranthene	3.70	14.10	17.30	-
	Resolution, R_s			
Fluorene-Naphthalene	5.06	11.19	23.14	-
Phenanthrene-Fluorene	1.61	3.76	10.31	-
Fluoranthene-Phenanthrene	3.6	8.7	15.25	-
	Selectivity factor, α			
Fluorene-Naphthalene	1.50	1.68	1.84	-
Phenanthrene-Fluorene	1.13	1.16	1.18	-
Fluoranthene-Phenanthrene	1.32	1.39	1.46	-

[-] = Not eluted within 60 min

From the resolution data gathered in this study, all the compositions of mobile phases gave good resolutions based on the theoretical aspects; resolution of R_s = 1.0 represents an overlap of 2% (or 98% separation) and a resolution of R_s = 1.25 represents 99.4% or almost complete separation [12]. The results showed that

the resolution for each pair of components were greater than 1.25 and hence, sufficient for accurate quantification. It can be observed that the R_s values for all compound combinations were inversely proportional to the percentage of organic modifier used in the mobile phase. Similarly, the retention factor values increased consistently with the decrease of organic modifier percentage used in the mobile phase. For example, with acetonitrile-water 70:30 (v/v) the retention factor was 2.75 and increased to 45.57 with acetonitrile-water 40:60. Results of plate numbers per column length, *N*/m indicates that column efficiency of ODS-silica was in the good range (> 10 000/m). The results show that no significant relationship between the mobile phase composition with plate numbers per column length, *N*/m. The results obtained that selectivity factor was inversely proportional to the percentages of organic modifier used in the mobile phase. For example, selectivity factor for fluorene/naphthalene was 1.50 with acetonitrile-water 70:30 and increased to 1.84 with acetonitrile-water 50:50.

The variation of retention factors with different proportions of organic modifier in the mobile phase for PAHs compounds can be seen more clearly by creating two-dimensional plots (Figure 3.2). The results suggest that organic modifiers always play a very important role in activating the surface area of the stationary phase, increasing the solubility of the solutes in mobile phase, increasing the analytes transfer rates and reducing the total analysis time.

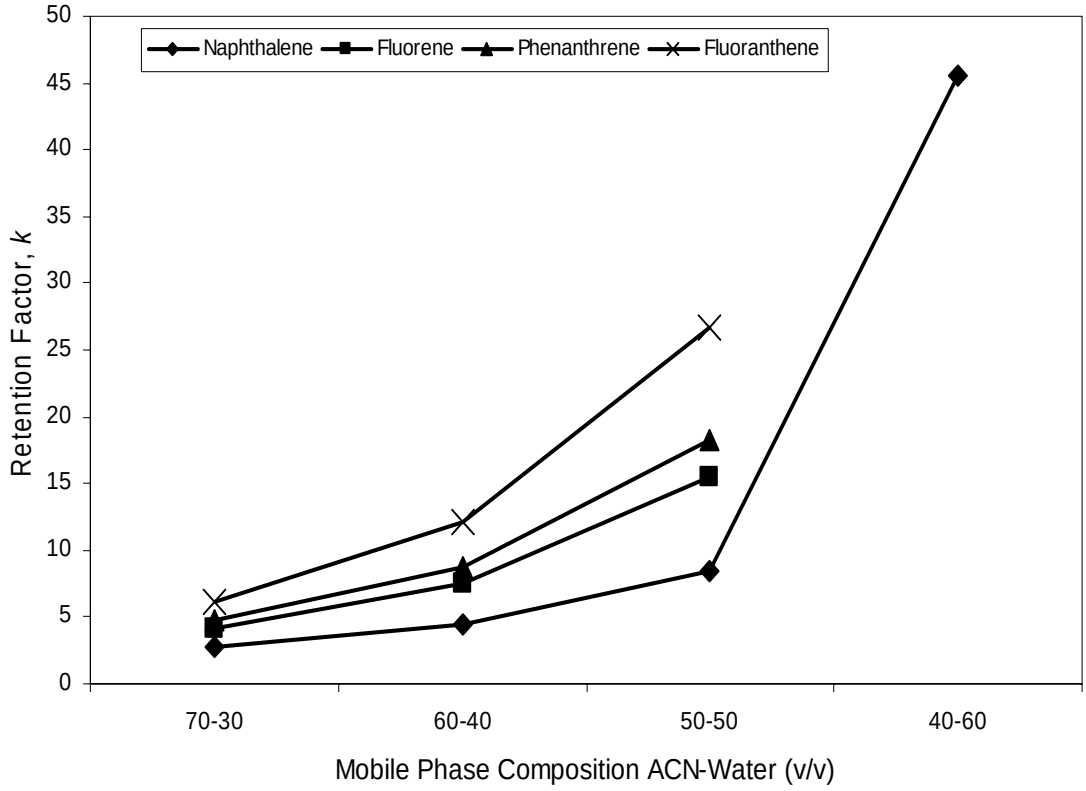


Figure 3.2: Variation of retention factor (*k*) with different proportions of organic modifier in the mobile phase for PAHs at 22ºC (ambient temperature). Chromatographic conditions as in Fig. 3.1

3.2 Separation of PAHs at Elevated Temperature

Experiments were carried out to determine the effect of temperature on the RP-HPLC separations of PAHs. Figure 3.3-3.5 show RP-HPLC separations of the PAHs on ODS-silica column at different column temperatures in the range of 40ºC to 70ºC with different acetonitrile-water compositions as the mobile phase with a flow rate of 1.0 mL/min. Good peak shapes were observed and the separations improved considerably as the temperature was increased. For each compound studied, there was marked decrease in the retention factor with increasing temperature from 40ºC to 70ºC with 10ºC increments using different proportions of acetonitrile (Table 3.2). For example, the retention factor of fluorene was 5.58 at 40ºC and it decreased to 3.75 at 70ºC. It was observed that a 10% decrease in concentration of acetonitrile in the mobile phase produced increasing retention factor at each column temperature, without significant loss in resolution and column efficiency. For example, at 40ºC,

the retention factor of naphthalene was 1.60 with acetonitrile-water 70:30 (v/v) and 21.37 with acetonitrile-water 40:60 (v/v).

Based on the retention factors, it was found that a 1% increase in acetonitrile concentration has approximately the same effect as a 4ºC increase in column temperature in controlling solute retention. For example, at 40ºC, 1% increase in the percentage of acetonitrile in the mobile phase composition of 60:40 (v/v) shows an increase of about 0.11 in the retention factor of naphthalene whereas a 4ºC increase in column temperature shows a decrease of about 0.100 in the retention factor of naphthalene. Bowermaster and McNair [48] observed similar results where a 1% increase in methanol concentration had approximately the same effect as a temperature increase of 4ºC. With acetonitrile-water 50:50 (v/v) eluent at 40ºC, the PAHs were eluted within 14 min, while at 70ºC the analysis time for the test compounds were reduced to about 5 min. It was observed that the retention factors for all PAHs studied were inversely proportional to temperature.

The variations of resolution and separation factors with temperature on RP-HPLC system were also studied in this work. For each compound studied, there was a systematic decrease in resolution with increasing temperature from 40ºC to 70ºC (Table 3.3). For example, the resolution and separation factor values for fluorene/naphthalene combination at 40ºC using acetonitrile-water 70:30 (v/v) were 7.62 and 1.53, respectively. Further increase in temperature to 70ºC decreased the resolution and separation factor values to 4.52 and 1.37, respectively.

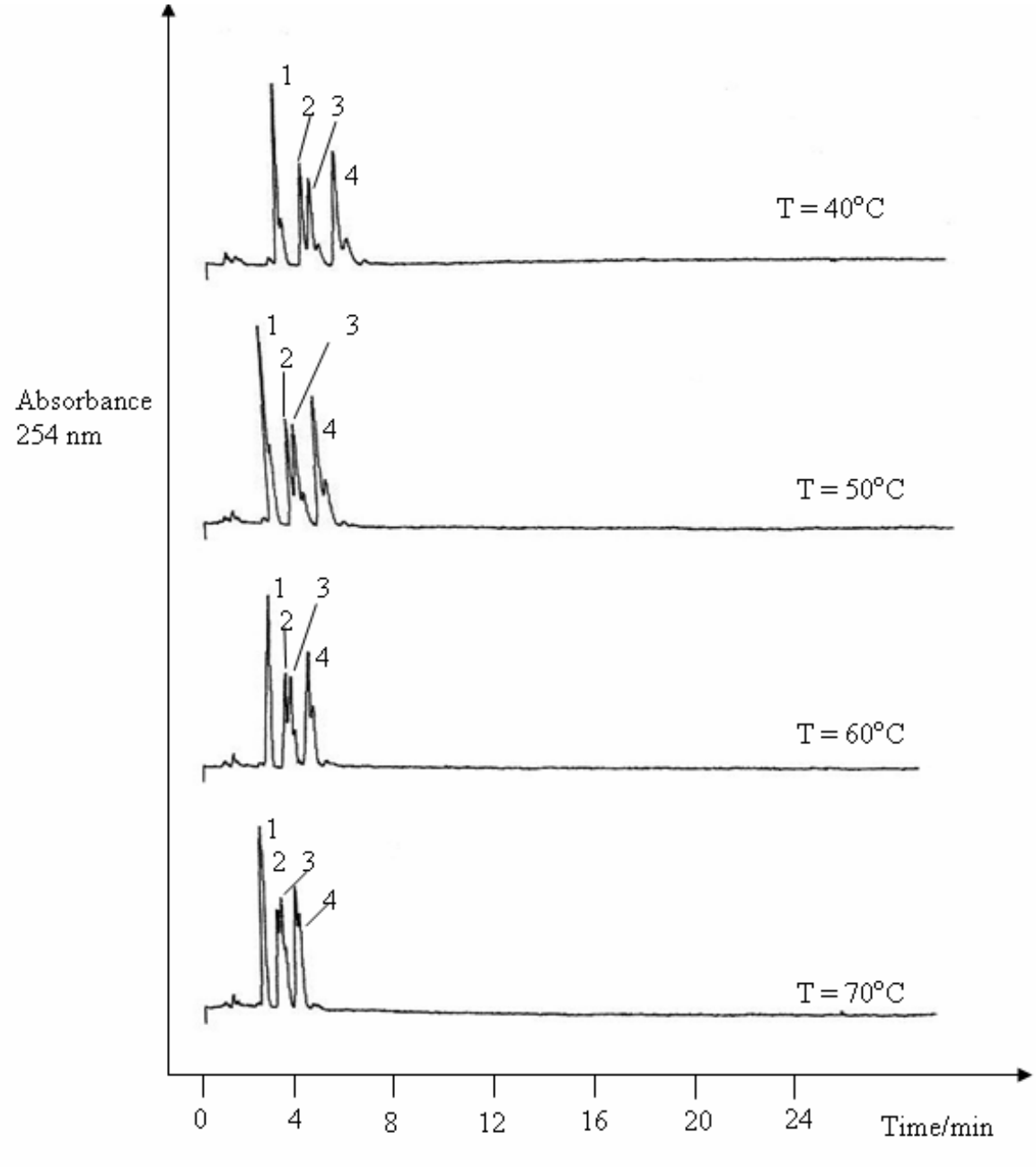


Figure 3.3: HPLC separations of PAHs using acetonitrile-water 70:30 (v/v) as eluent at different column temperatures. The separation condition: ODS-silica column (100 mm × 4.6 mm I.D.); flow rate: 1.0 mL/min; UV absorbance at 254 nm. Peaks: (1) naphthalene; (2) fluorene; (3) phenanthrene; (4) fluoranthene

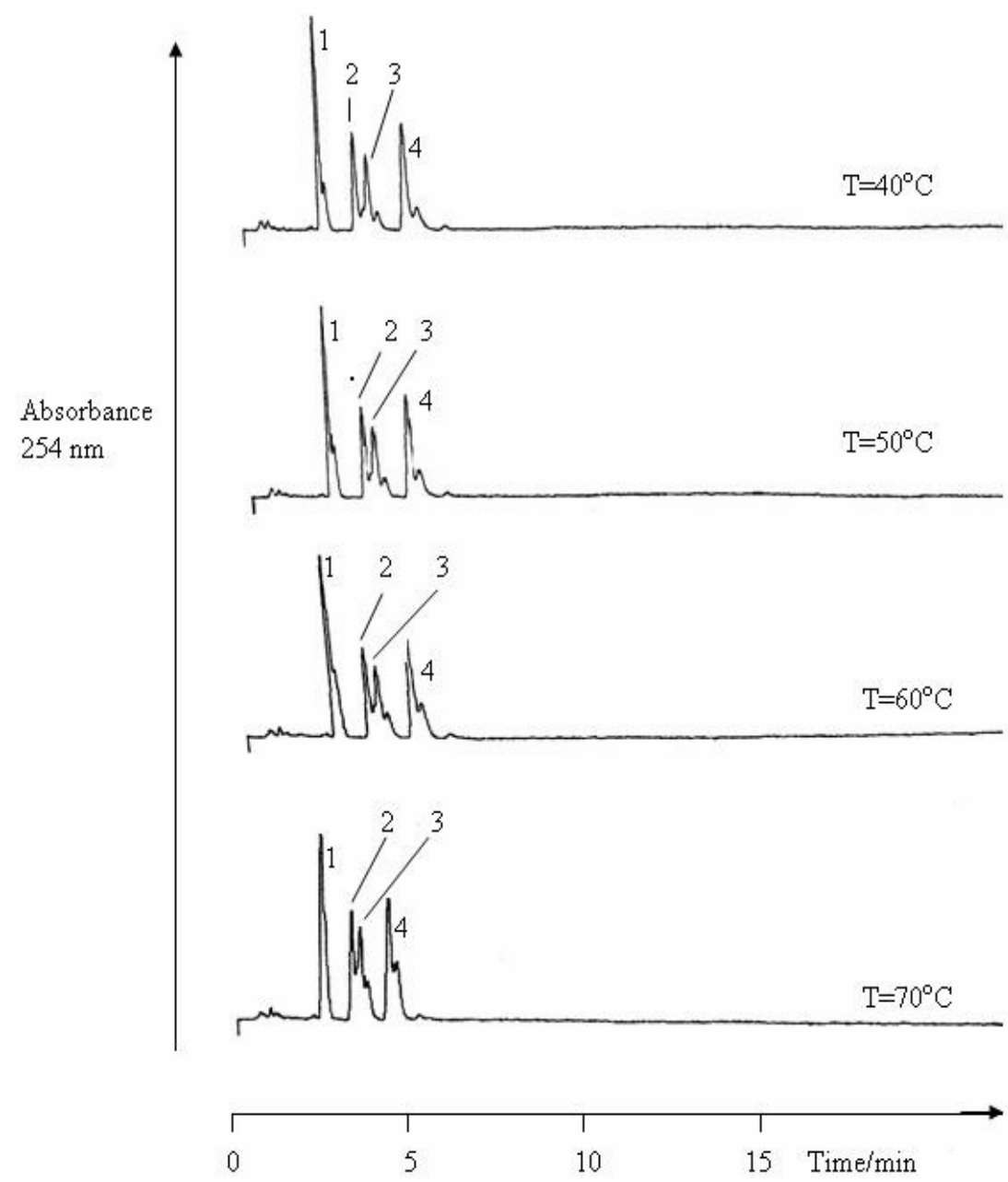


Figure 3.4: HPLC separations of PAHs using acetonitrile-water 60:40 (v/v) as eluent at different column temperatures. Separation conditions: ODS-silica column (100 mm × 4.6 mm I.D.); flow rate: 1.0 mL/min; detection: UV absorbance at 254 nm. Peaks: (1) naphthalene, (2) fluorene; (3) phenanthrene; (4) fluoranthene

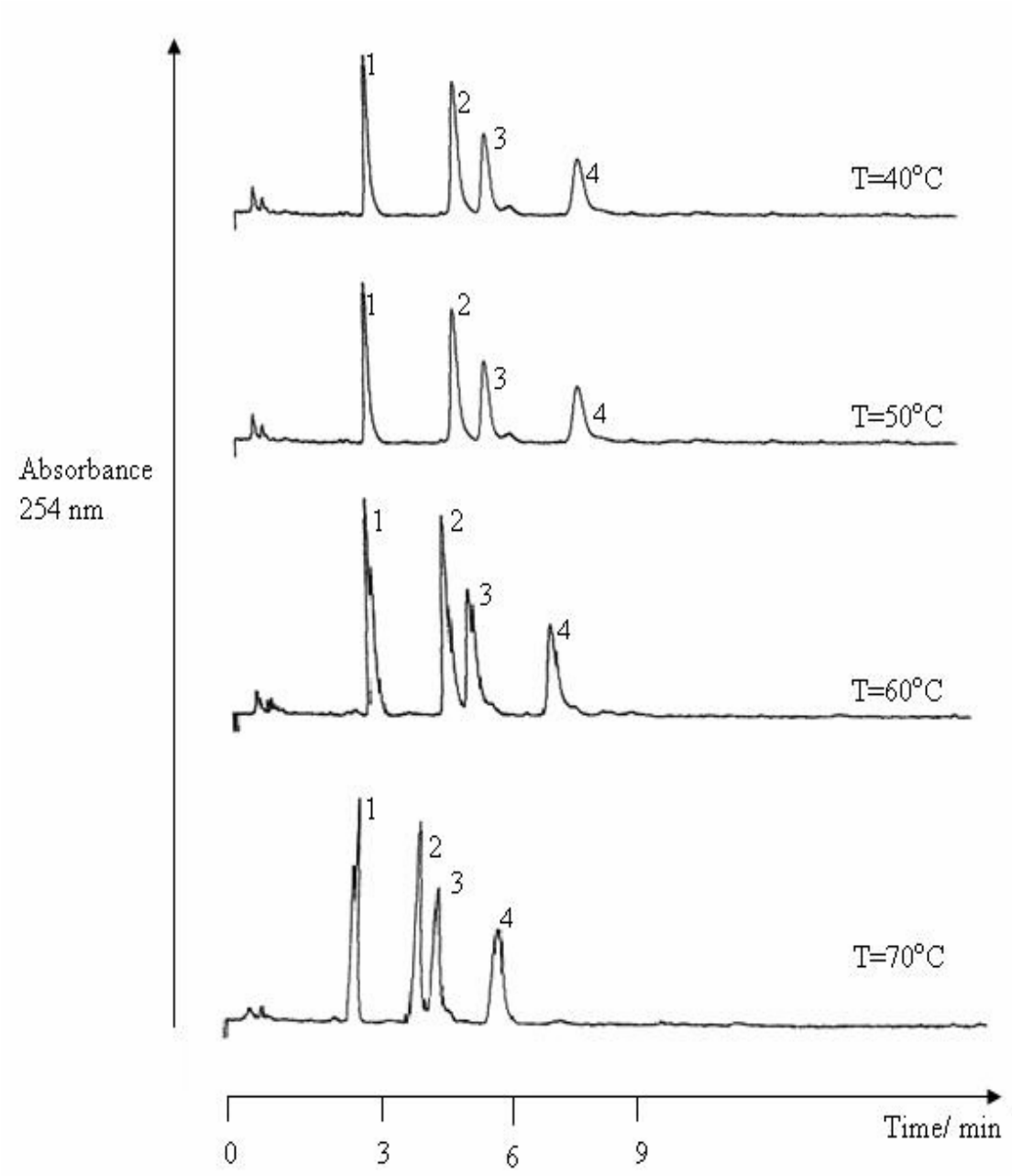


Figure 3.5: HPLC separations of PAHs using acetonitrile-water 50:50 (v/v) as eluent at different column temperatures. Separation conditions: ODS-silica column (100 mm × 4.6 mm I.D.); flow rate: 1.0 ml/min; detection: UV absorbance at 254 nm. Peaks: (1) naphthalene; (2) fluorene; (3) phenanthrene; (4) fluoranthene

Table 3.2: Retention time and retention factors of PAHs separated on ODS-silica at different column temperature using different proportions of organic modifier in the mobile phase with a flow rate of 1.0 mL/min. Separation condition as in Figures 3.1, 3.3-3.5

Compound	Mobile Phase Composition acetonitrile-water (v/v)	Retention Time, t_R Column Temperature (°C)				Retention Factor, k Column Temperature (°C)			
		40	50	60	70	40	50	60	70
Naphthalene	70:30	2.66	2.48	2.32	2.19	2.41(2.55)	1.60(0.00)	1.40(0.00)	1.29(2.27)
	60:40	3.46	3.25	2.98	2.74	3.50(0.00)	3.25(0.00)	3.00(2.56)	2.50(3.82)
	50:50	5.15	4.63	4.22	3.81	6.36(1.01)	5.68(1.48)	5.14(0.40)	2.56(0.60)
	40:60	13.53	11.52	10.17	8.86	21.37(3.49)	15.2(2.63)	7.98(1.42)	11.27(2.83)
Fluorene	70:30	3.58	3.27	2.98	2.75	3.68(1.51)	2.43(0.00)	2.09(0.00)	1.76(2.20)
	60:40	5.06	4.64	4.15	3.72	5.58(0.13)	5.07(0.14)	4.57(1.56)	3.75(1.10)
	50:50	8.60	7.51	6.54	5.75	11.28(1.46)	9.84(0.50)	8.52(0.30)	4.36(0.00)
	40:60	28.90	23.8	20.31	17.10	46.75(2.63)	32.48(5.96)	16.96(4.32)	22.64(2.50)
Phenanthrene	70:30	3.91	3.53	3.18	2.90	4.10(0.86)	2.70(0.26)	2.30(0.31)	1.92(2.28)
	60:40	5.63	5.11	4.52	4.00	6.32(0.00)	5.68(0.12)	5.10(3.87)	4.18(0.36)
	50:50	9.81	8.50	7.31	6.40	13.01(1.65)	11.26(1.42)	9.64(0.57)	4.94(0.00)
	40:60	35.19	28.63	24.12	20.10	57.17(1.62)	39.27(1.16)	20.29(3.93)	26.83(0.40)
Fluoranthene	70:30	4.87	4.32	3.82	3.43	5.36(0.56)	3.52(0.20)	2.95(0.48)	2.46(0.41)
	60:40	7.33	6.49	5.65	4.92	8.52(0.08)	7.48(0.28)	6.58(0.84)	5.28(0.40)
	50:50	13.42	11.49	9.77	8.24	18.17(1.30)	15.54(0.36)	13.22(1.86)	6.70(0.00)
	40:60	-	-	-	-	-	-	-	-

(R.S.D. %) was based on triplicate injections. [-] = Not eluted within 60 min

The resolution data demonstrated that the best separation was achieved at column temperature of 70ºC. The results show significant decrease in resolution values on going from 40ºC to 70ºC for all analytes studied. For example, with acetonitrile-water 50:50 (v/v), it was observed that when the column temperature was increased from 40ºC to 70ºC, the resolution values for fluoranthene/phenanthrene pair decreased from 13.51 to 6.42. Therefore, in order to achieve good resolution, optimal separation conditions with column temperature of 70ºC were used in subsequent experiments.

The overall column efficiency (*N*/m) observed for the silica column was reasonable (>10 000 plates/m) (Table 3.4). The results obtained indicated that the plate number, *N*, was proportional to the percentage of organic modifier used in the mobile phase. For example, at 40ºC, the plate number based on fluorene was 12.50×10^{4} m^{-1} with acetonitrile-water 70:30 (v/v) and 6.10×10^{4} m^{-1} with acetonitrile-water 40:60 (v/v). The apparent plate numbers show significant improvement on increasing the temperature from 40ºC to 70ºC. For example at 40ºC with acetonitrile-water 70:30 (v/v), the plate numbers per meter based on phenanthrene was 8.27×10^{4} m^{-1} and the value increased to 13.67×10^{4} m^{-1} at 70ºC. This was probably due to the increase in the diffusion coefficients of the mobile phase and the analytes with the increase in column temperature. The variation of column efficiency with different column temperature for PAHs can be observed clearly from Figure 3.6. From the figure, it is evident that the column efficiency was directly proportional to column temperature. Recent reviews by Yang *et al*. [11] have extensively discussed the temperature effects on column efficiency by increasing the temperature from 100ºC to 120ºC, the diffusivity is further increased and even better mass transfer results. However, the better mass transfer also causes a greater axial molecular diffusion and the lower column efficiency. This is the reason why the number of plates decreases when the temperature is raised higher.

Table 3.3: Resolution and separation factor values as a function of temperature ranging from 40ºC to 70ºC using different mobile phase compositions with a flow rate of 1.0mL/min. Separation conditions as in Figures 3.1, 3.3-3.5

Compounds	**Mobile Phase Composition acetonitrile-water (v/v)**	**Resolution, R_s (R.S.D%)**				**Separation factor, α (R.S.D. %)**			
		Column Temperature (°C)				**Column Temperature (°C)**			
		40	**50**	**60**	**70**	**40**	**50**	**60**	**70**
Fluorene Naphthalene	70:30	7.62(1.46)	6.91(1.01)	5.15(1.11)	4.52(0.45)	1.53(0.46)	1.52(0.47)	1.49(0.00)	1.37(2.97)
	60:40	10.49(1.57)	9.63(2.92)	8.88(1.29)	6.64(2.63)	1.13(0.44)	1.12(0.88)	1.11(0.46)	1.16(2.40)
	50:50	13.36(1.26)	11.79(0.54)	10.44(2.70)	9.77(1.09)	1.77(0.88)	1.73(0.50))	1.66(0.85)	1.71(1.30)
	40:60	32.54(3.04)	19.58(3.50)	19.41(1.70)	19.29(2.55)	2.18(0.23)	2.14(0.71)	2.12(1.19)	2.01(1.13)
Phenanthrene Fluorene	70:30	2.12(1.67)	1.74(0.40)	1.49(0.95)	1.20(1.60)	1.12(0.63)	1.11(0.00)	1.1(0.00)	1.09(0.65)
	60:40	6.00(3.28)	2.34(0.85)	2.23(3.3)	1.68(0.42)	1.13(0.00)	1.12(0.56)	1.11(1.89)	1.12(0.65)
	50:50	3.76(3.09)	3.21(2.58)	2.87(1.46)	2.53(0.52)	1.15(0.75)	1.14(0.45)	1.13(0.45)	1.13(0.43)
	40:60	12.30(4.87)	4.81(3.41)	3.76(1.83)	4.17(1.77)	1.22(0.43)	1.21(0.55)	1.20(0.59)	1.19(1.73)
Fluoranthene Phenanthrene	70:30	5.41(2.66)	4.73(0.44)	4.33(0.32)	3.90(5.50)	1.31(0.54)	1.31(0.58)	1.29(0.75)	1.28(1.68)
	60:40	7.35(2.27)	5.93(4.35)	5.80(3.88)	4.83(1.31)	1.35(0.48)	1.32(0.96)	1.30(1.65)	1.26(0.55)
	50:50	13.51(3.10)	8.65(1.14)	7.87(2.47)	6.42(0.64)	1.40(0.88)	1.38(1.02)	1.37(1.04)	1.35(0.98)
	40:60	-	-	-	-	-	-	-	-

(R.S.D. %) was based on triplicate injections ; [-] = not eluted within 60 min

Table 3.4: Plate numbers per column length, *N*/m, calculated based on the PAHs as a function of temperature (between 40ºC to 70ºC) using different mobile phase composition. Separation conditions as in Figures 3.1 3.3-3.5

Compound	Mobile Phase Composition (ACN-Water, v/v)	Plates Numbers per Meter × 10^4 (m^{-1}) (R.S.D. %) Column Temperature (ºC) 40ºC	50ºC	60ºC	70ºC
Naphthalene	70:30	12.09(1.10)	15.50(1.43)	15.30(1.50)	17.81(0.76)
	60:40	11.20(1.80)	12.80(1.30)	13.40(1.42)	15.76(1.03)
	50:50	10.70(0.47)	11.60(1.47)	12.68(1.25)	14.69(1.25)
	40:60	9.81(1.29)	9.28(1.32)	10.04(1.62)	12.32(0.90)
Fluorene	70:30	12.50(1.60)	12.80(1.58)	13.90(0.78)	19.10(0.38)
	60:40	11.84(1.24)	11.60(0.85)	11.30(1.35)	12.37(1.60)
	50:50	11.60(1.75)	10.9(0.91)	12.81(0.10)	14.30(0.43)
	40:60	6.10(1.17)	6.90(1.30)	8.68(0.30)	11.74(0.50)
Phenanthrene	70:30	13.90(0.39)	14.10(1.62)	15.00(0.64)	20.47(0.52)
	60:40	12.40(1.30)	13.40(1.80)	17.40(0.87)	18.75(0.40)
	50:50	10.10(0.77)	11.35(0.11)	13.34(0.16)	16.09(0.50)
	40:60	8.27(0.19)	9.90(0.22)	10.82(0.15)	13.67(0.27)
Fluoranthene	70:30	13.90(0.21)	14.80(0.33)	17.40(1.58)	19.20(0.00)
	60:40	12.40(0.40)	12.90(1.60)	14.70(0.78)	16.80(1.40)
	50:50	11.30(0.90)	12.40(0.87)	13.90(0.83)	14.30(0.75)
	40:60	-	-	-	-

(R.S.D. %) was based on triplicate injections; [-] = not eluted within 60 min

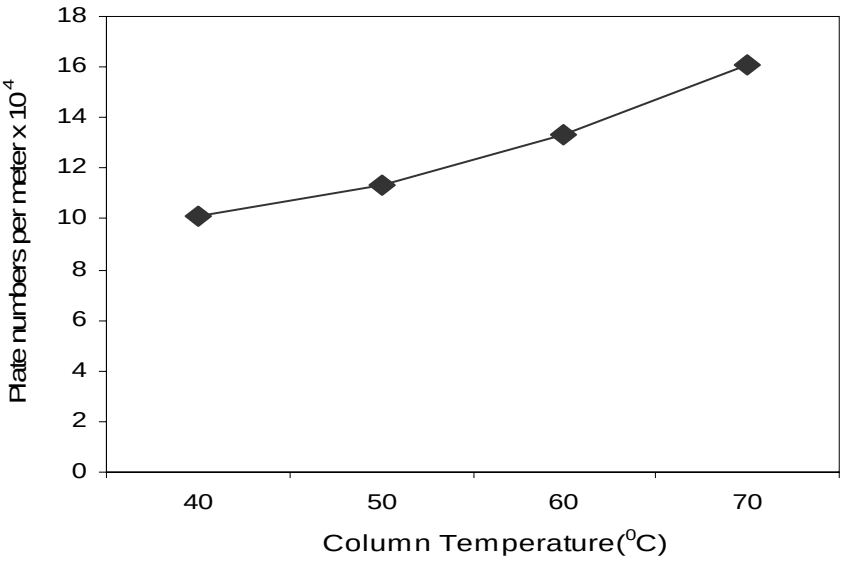


Figure 3.6: Variation of column efficiency calculated based on phenanthrene at different column temperatures with acetonitrile-water 50:50 (v/v) as the mobile phase

In order to expedite the advantages of high temperature operations, RP-HPLC separations of PAHs were performed using an elevated flow rate of 2.5 mL/min 40°C to 70°C with different mobile phase compositions ranging from 30:70 to 50:50 (v/v) (Figure 3.7-3.9). For a fixed mobile phase composition, it was observed that significantly improved peak shapes and faster analysis times were obtained at higher temperatures. With acetonitrile-water 30:70 (v/v) as the mobile phase, phenanthrene was eluted in about 50 min while fluoranthene, was not eluted within reasonable time (> 60 min) (Figure 3.7). However, at 70°C, all the compounds were eluted within acceptable time of approximately 43 min. The total analysis time at 70°C, was 19 min with acetonitrile-water 40:60 (v/v) as mobile phase (Figure 3.8) and further reduced to within 6 min with acetonitrile-water 50:50 (v/v) as mobile phase (Figure 3.9). These results suggest that marked decrease in the total analysis time is achievable with combined elevated temperature and increased organic proportions in the mobile phase. The results obtained are in good agreement with study reported by McCalley [49]. The author reported that working under controlled, temperature programming conditions is a strong tool to adjust and to improve retention time and selectivity. In addition, higher temperatures also decrease eluent viscosity allowing significantly higher linear eluent velocities. Combining these latter effects, higher temperatures in RP-HPLC may provide better resolution and faster analysis.

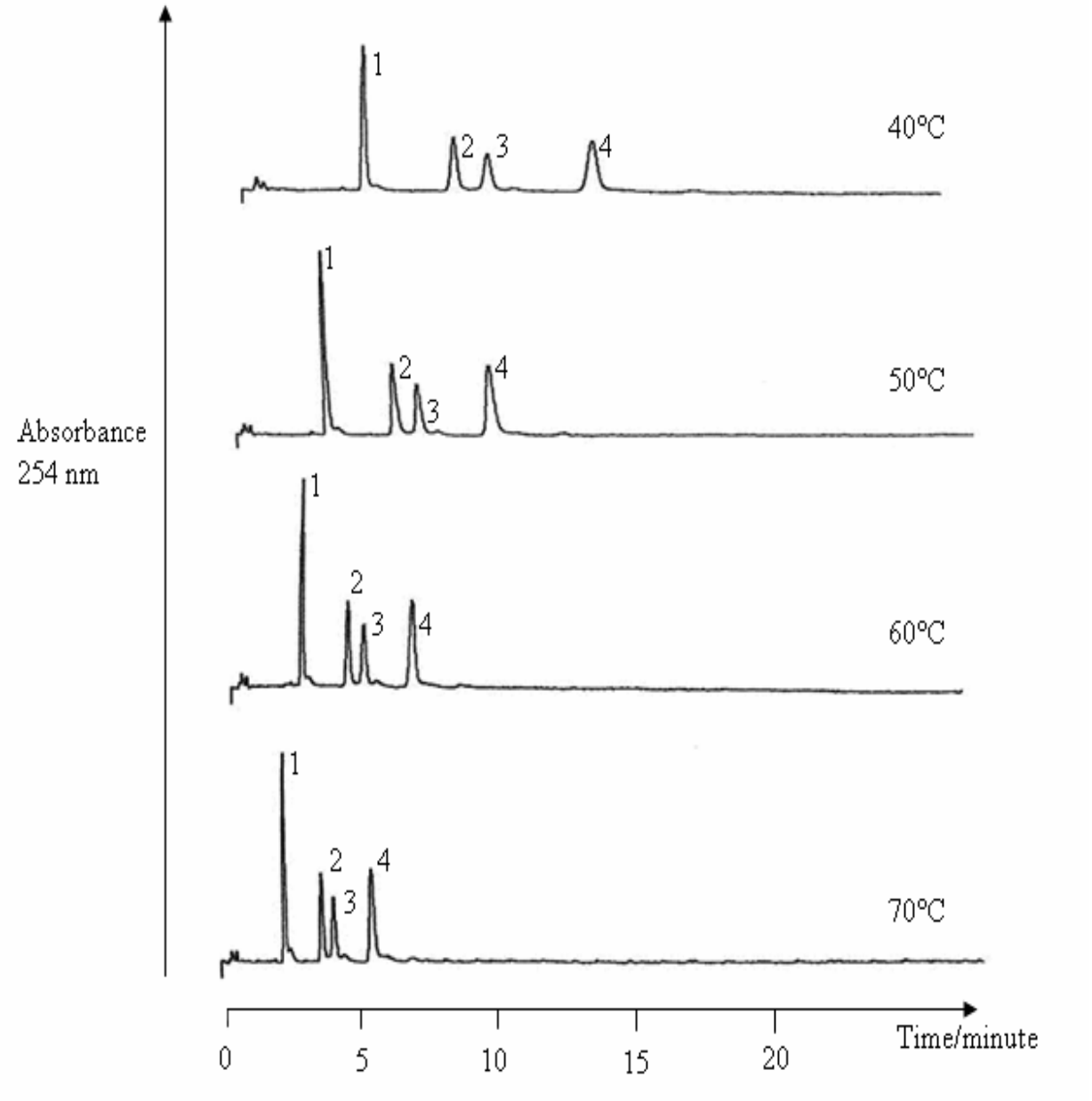


Figure 3.7: HPLC separations of PAHs compounds using acetonitrile-water 50:50 (v/v) as eluent at different column temperatures. Separation condition: ODS-silica column (100 mm × 4.6 mm I.D.), flow rate: 2.5 mL/min; detection: UV absorbance at 254 nm. Peaks: (1) naphthalene; (2) fluorene; (3) phenanthrene; (4) fluoranthene

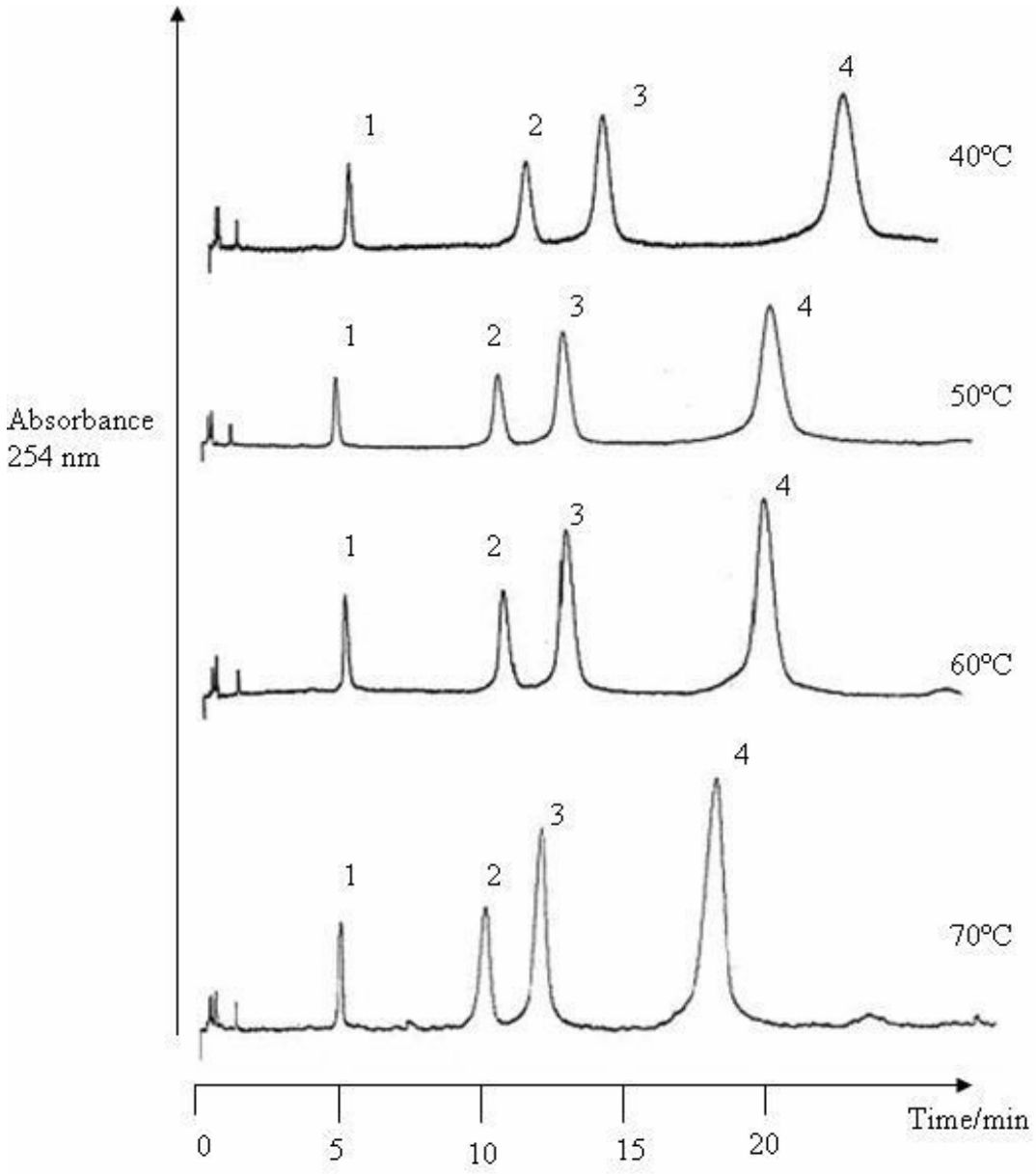


Figure 3.8: HPLC separations of PAHs using acetonitrile-water 40:60 (v/v) as eluent at different column temperatures. Separation condition: ODS-silica column (100 mm × 4.6 mm I.D.); flow rate: 2.5 mL/min; detection: UV absorbance at 254 nm. Peaks: (1) naphthalene; (2) fluorene; (3) phenanthrene; (4) fluoranthene

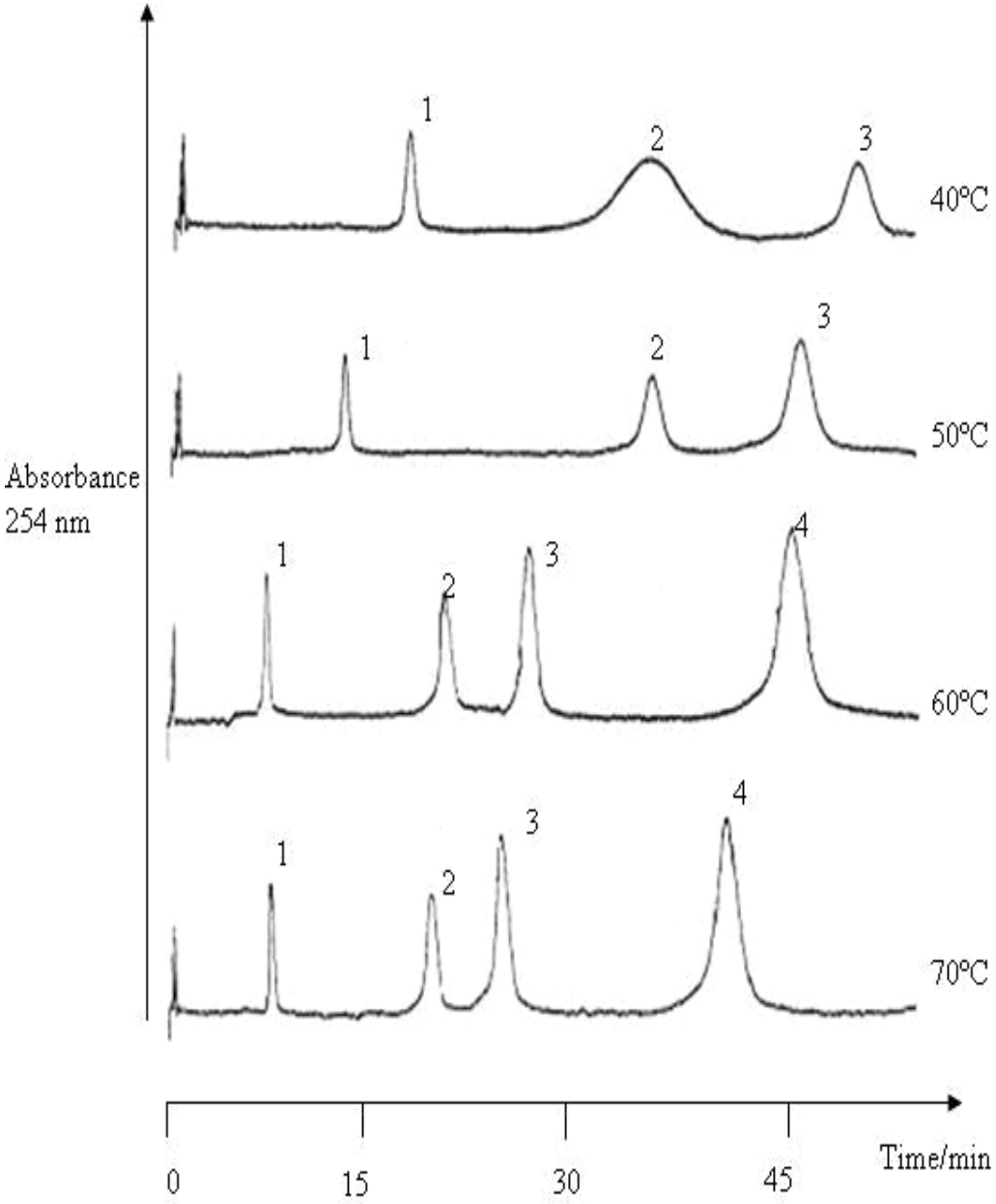


Figure 3.9: HPLC separations of PAHs using acetonitrile-water 30:70 (v/v) as eluent at different column temperatures. Separation conditions: ODS-silica column (100 mm × 4.6 mm I.D.), flow rate: 2.5 mL/min; detection: UV absorbance at 254 nm. Peaks: (1) naphthalene; (2) fluorene; (3) phenanthrene; (4) fluoranthene

The retention times and retention factors of the test compounds at different temperatures ranging from 40ºC to 70ºC with 10ºC increments using different proportions of organic modifiers were calculated (Table 3.5). There was marked decrease in retention factors for each analyte studied on going from 40ºC to 70ºC. For example, with acetonitrile-water 50:50 (v/v), the retention times of phenanthrene was 17.01 at 40ºC and 9.77 at 70ºC. At elevated flow rates coupled with elevated temperature and acetonitrile-water 30:70 (v/v) as the mobile phase, the PAHs were separated successfully in less than 45 min. This is due to the decrease in the eluent viscosity that allows increased eluent velocities at higher temperatures. The variation of separation factor (*k*) with different proportions of organic modifier in the mobile phase for PAHs can be seen more clearly by creating two-dimensional plots (Figure 3.10). The retention factor, *k*, shows the highest value at mobile phase composition acetonitrile-water 30:70 (v/v). These results suggest that organic modifiers play a very important role in activating the surface area of the stationary phase, increasing the solubility of the solutes in mobile phase and reducing total analysis time.

The retention times and retention factors were calculated and summarized in Table 3.5. The results suggest that temperature does effect the retention in RP-HPLC. Temperature is an important tool to control and optimize retention and selectivity in RP-HPLC. Similar study has been reported by Greibrokk and Anderson [47] who has studied the effects of temperature programming in the temperature range of 25-125ºC in miniaturized RP columns. In this study, the authors discussed the great and positive influence of temperature elevation on the reduction of analysis time, the improvement of peak shapes and the achievement of higher efficiencies.

Table 3.5: Retention times and retention factors of PAHs at different temperatures using different proportions of organic modifier with a flow rate of 2.5 mL/min. Separations condition as in Figures 3.7-3.9

Compounds	Mobile Phase Composition acetonitrile-water (v/v)	Retention time, t_r				Retention Factor, k			
		Column Temperature (°C)				Column Temperature (°C)			
		40	50	60	70	40	50	60	70
Naphthalene	50:50	4.32	3.25	2.53	2.25	7.86 (5.07)	7.47 (5.70)	5.50 (0.13)	5.00 (1.44)
	40:60	9.50	7.12	6.01	5.07	20.88 (1.41)	17.74 (0.23)	14.77 (0.90)	12.51 (2.98)
	30:70	19.41	14.15	10.41	8.50	59.10 (0.10)	48.65 (0.12)	40.47 (0.30)	23.08 (0.70)
Fluorene	50:50	7.59	5.67	4.17	3.59	14.61 (4.91)	13.76 (4.85)	9.72 (0.25)	8.560 (1.55)
	40:60	21.52	15.64	12.69	10.4	48.59 (1.72)	40.16 (0.52)	32.29 (0.14)	26.73 (4.31)
	30:70	35.65	37.09	26.04	20.47	109.372(0.10)	129.14(0.15)	102.73(0.10)	56.99 (0.25)
Phenanthrene	50:50	8.75	6.52	4.73	4.03	17.01 (4.72)	15.97 (4.77)	11.18 (0.10)	9.77 (0.12)
	40:60	26.71	19.19	15.34	12.47	60.55 (1.64)	49.50 (0.56)	39.26 (0.13)	32.24 (3.89)
	30:70	53.85	48.27	33.29	25.67	165.71 (0.43)	168.36 (0.00)	131.63(0.33)	71.71 (0.60)
Fluoranthene	50:50	12.46	9.09	6.45	5.39	24.64 (4.77)	22.68 (4.55)	15.6 (0.36)	13.39 (1.49)
	40:60	43.08	30.15	23.71	18.77	98.25 (1.38)	78.34 (0.17)	61.22 (0.22)	49.05 (0.57)
	30:70	-	-	56.79	42.23	-	-	225.26(0.11)	118.62(0.21)

R.S.D. %) was based on triplicate injections. [-] = not eluted within 60 min

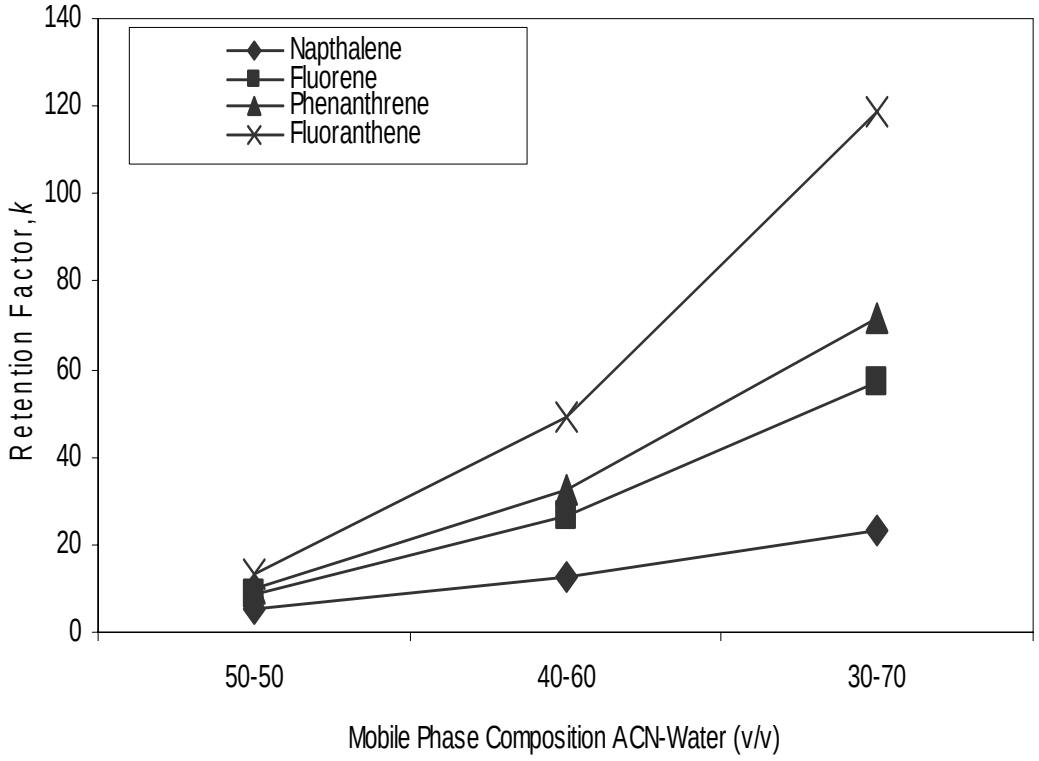


Figure 3.10: Variation of retention factor (k) for PAHs with mobile phase compositions. Separation conditions as in Figure 3.7-3.9

The variations of resolutions and separation factors of the PAHs with temperature in RP-HPLC were also studied (Table 3.6). For each test compound, there was a systematic decrease in resolution and separation factors with increasing temperature. For example, the R_s and α factor values for phenanthrene/fluorene combination at 40ºC using acetonitrile-water 40:60 (v/v) were 26.03 and 1.25, respectively. Further increase in temperature of up to 70ºC decreased the resolution and separation factor values to 6.99 and 1.21, respectively. The resolutions for each combination of components were almost always greater than 1.0, hence, sufficient for accurate quantification. In general, the R_s and α value were inversely proportional to the percentage of organic modifier used in the mobile phase at all temperatures studied.

Table 3.6: Resolutions and separation factors of the test compounds at different temperatures ranging from 40ºC to 70ºC using different mobile phase compositions with a flow rate of 2.5 mL/min. Separation conditions as in Figures 3.7-3.9

Compounds	**Mobile Phase Composition acetonitrile-water (v/v)**	**Resolution, R_s (R.S.D %) Column Temperature (°C)**				**Separation factor, α (R.S.D. %) Column Temperature (°C)**			
		40	**50**	**60**	**70**	**40**	**50**	**60**	**70**
Fluorene Naphthalene	50:50	15.48(4.05)	14.91(3.03)	14.26(0.57)	13.33(0.46)	1.85(0.80)	1.84(2.58)	1.77(0.12)	1.72(3.22)
	40:60	45.04(4.79)	23.31(0.35)	30.48(3.12)	31.46(0.19)	2.33(0.30)	2.19(2.17)	2.19(0.25)	2.14(1.51)
	30:70	46.22(0.31)	87.08 (1.23)	72.02(0.93)	75.51(0.31)	1.85(0.28)	2.66(0.50)	2.54(1.10)	2.47(1.70)
Phenanthrene Fluorene	50:50	4.60(0.23)	3.94(2.78)	3.85(1.07)	3.33(4.24)	1.17(0.54)	1.16(0.24)	1.15(0.18)	1.14(0.50)
	40:60	26.03(5.81)	18.38(0.29)	10.96(4.57)	6.99(0.35)	1.25(0.60)	1.22(0.92)	1.22(0.25)	1.21(0.35)
	30:70	80.07(0.76)	61.48(0.14)	49.96(1.98)	32.89(0.46)	1.52(1.90)	1.30(1.08)	1.28(0.44)	1.26(0.56)
Fluoranthene Phenanthrene	50:50	11.20(3.59)	9.79(3.63)	9.38(0.58)	8.42(3.74)	1.14(1.64)	1.42(0.65)	1.40(0.35)	1.37(1.13)
	40:60	74.72(7.10)	42.08(0.15)	23.04(0.21)	11.16(0.10)	1.62(0.26)	1.56(0.27)	1.56(0.27)	1.52(0.19)
	30:70	-	-	89.36(1.40)	66.26(0.69)	-	-	1.71(0.82)	1.65(1.70)

(R.S.D. %) was based on triplicate injections; [-] = not eluted within 60 min

Results obtain in this work has clearly shown that working under controlled temperature programming conditions is a strong tool to adjust and to improve retention and selectivity. In addition, higher temperatures also decrease eluent viscosity allowing significantly higher linear velocities. As a result, retention time can be drastically reduced. Concomitantly, at higher temperatures, the diffusivity of analytes in the mobile and stationary phases increases, resulting in much better efficiencies and improved peak shapes. Combining these latter effects, higher temperatures in RP-HPLC offer better resolution and faster analysis. In addition, at higher analysis temperatures, fewer amounts of organic modifiers in the eluent are needed to achieve the same separation. In turn, this contributes to reduce the use of organic solvent and hence contribute to the appearance of "greener" laboratories.

CHAPTER IV

PRESSURIZED LIQUID EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM SOIL SAMPLES

Pressurized liquid extraction (PLE) is a sample preparation technique for the extraction of analytes from solid materials. According to Wan and Wong [42] one of the major driving forces is the increasing demand from authorities to reduce the large volumes of organic solvents consumed by classical extraction methods such as Soxhlet. Another contributing factor for the rapid acceptance of PLE is that the method development is rather straight-forward. In this study, a PLE method was developed by using conventional HPLC instrument and the performance of the PLE system was tested using environmental soil samples.

4.1 Calibration of Standard

A calibration graph was constructed for each PAH studied. Standard mixtures of PAHs with different concentrations were prepared and pyrene as internal standard was added into each of the solution prepared. Linear calibration curves of the relative peak area and concentration were plotted and it was found that the correlation coefficients ranged from 0.9932 to 0.9952 (Figure 4.1). The calibration curves for naphthalene, fluorene, phenanthrene and fluoranthene generally show good linearity in the concentration range studied (1 to 10 ppm).

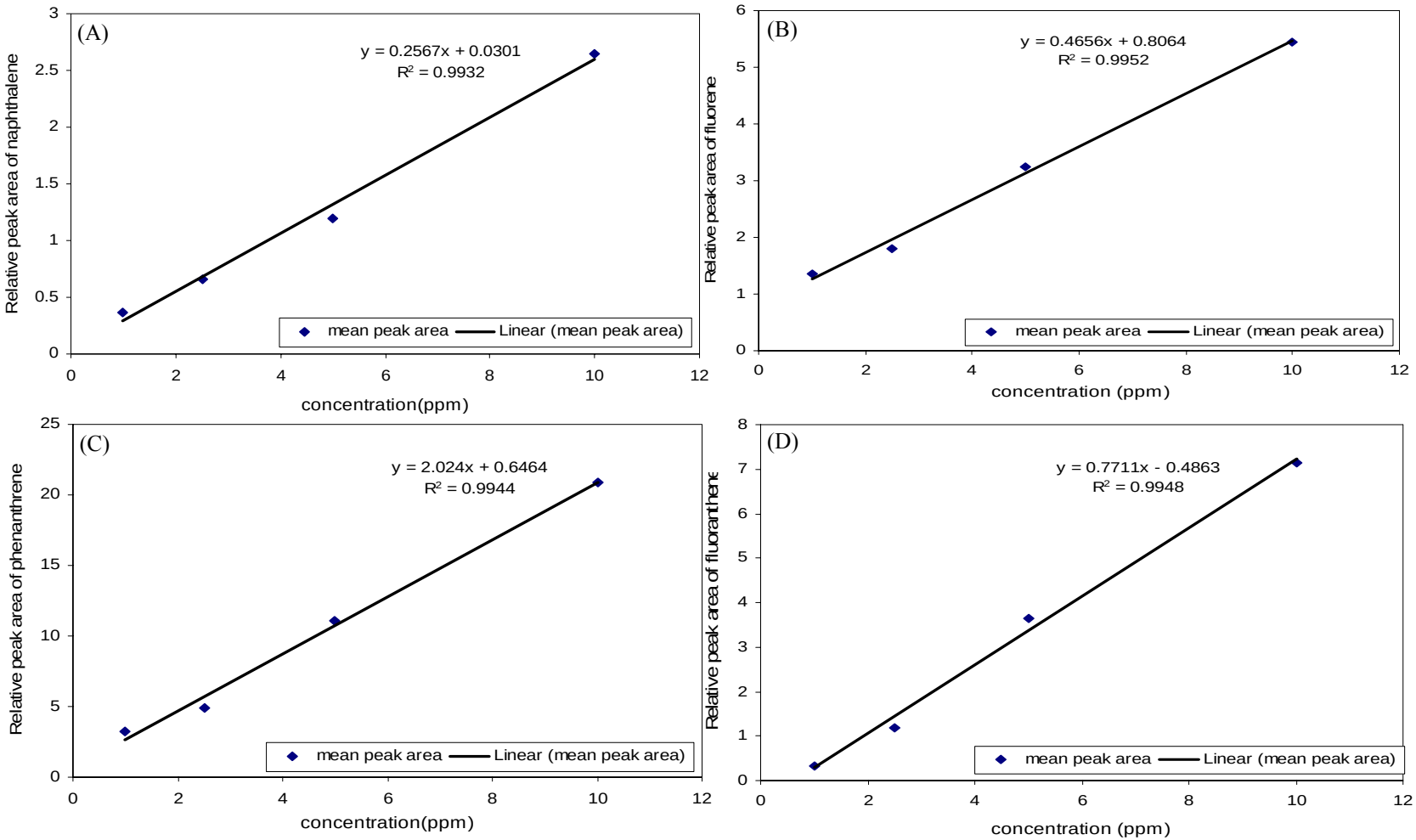


Figure 4.1: Calibration graphs (peak area of compound/peak area of internal standard vs. concentration of compound) of four PAHs studied using HT-RPLC: (A) naphthalene, (B) fluorene, (C) phenanthrene, (D) fluoranthene. Chromatographic conditions: mobile phase acetonitrile-water 40:60 (v/v); flow rate 2.5 mL/min; temperature 70°C; UV absorbance 254 nm; injection volume 5µL

4.2 Limit of Detection and Limit of Quantitation of PAHs Studied

The limit of detection (LOD) is defined as the instrument signal three times of the background signal while the limit of quantification (LOQ) is defined as the instrument signal ten times of the background signal. The LOD, LOQ, regression equation and correlation coefficient for each PAH studied are shown in Table 4.1. It was found that the LOD's of PAHs were in the range of 0.58 to 1.08 ppm whereas the LOQ's of PAHs were in the range of 1.93 to 3.6 ppm. Fluorene shows the lowest LOD (0.58 ppm). The low LOD and LOQ values obtained indicate good sensitivity of the UV detector towards PAHs.

Table 4.1: Limit of detection (LOD), limit of quantification (LOQ), regression equation and correlation coefficient of PAHs studied using HPLC

PAHs	Regression Equation	Correlation Coefficient, r^2	LOD (ppm)	LOQ (ppm)
Naphthalene	Y= 0.2567x + 0.0301	0.9932	1.08	3.33
Fluorene	Y= 0.4656x + 0.8064	0.9952	0.58	1.93
Phenanthrene	Y= 2.024x + 0.6464	0.9944	0.71	2.36
Fluoranthene	Y= 0.7711x – 0.4863	0.9948	0.92	3.07

4.3 Optimization of Pressurized Liquid Extraction (PLE) Conditions

It has been widely accepted that the main assumption for good accuracy of generated data is fine-tuning of the extraction process. In this study, optimized PLE conditions were investigated to be applied for the extraction of PAHs from soil samples. The extraction solvents, extraction pressure and extraction temperature were optimized and the reproducibility of method was also investigated.

4.3.1 Extraction Solvent

Due to their chemical-physical properties, PAHs, especially the higher molecular weight PAHs, are hardly degradable and tend to accumulate in the different environmental compartments. Their analysis usually involves extraction with different organic solvent, cleanup procedure and instrumental determination. In this work, the optimization of extraction solvent was carried out with the PAHs (0.5 mL of 50 ppm standard PAHs) spiked into soil samples (7 g). The soil samples were dried thermally in an oven at 105°C for 24 h prior to PLE. As Soxhlet extraction is often considered to be the benchmark technique, its inclusion in this work was considered essential for comparison with the PLE method developed in our laboratory.

In the PLE, the soil is mixed with anhydrous sodium sulfate. The advantages of this method are water is trapped through binding to sodium sulfate, soil exposure to laboratory air is minimal, the microbial activity is stopped due to the high salt concentration and sample treated in this way can be stored for a long period of time.

Liquid chromatographic analysis of extracts obtained from PLE of spiked soil samples were carried out (Appendix 1). It was found that different extraction solvent gives different percentage recoveries (Table 4.2). The recovery of PAHs ranged from 79.6% to 100.5% for dichloromethane-acetone, 69.5% to 94.1% for hexane-acetone and 20.7% to 112.5% for dichloromethane as extractant. The highest recovery was observed for phenanthrene (112.5%) and fluorene (87.8%) using dichloromethane as the extraction solvent. Low recoveries were obtained for naphthalene with dichloromethane as single solvent extractant indicating that the solvent was not suitable for naphthalene compared to other analytes. The similar results has been reported by Burkhardt *et al.* [38] who obtained a mean recovery for naphthalene of 67% compared with phenanthrene (102.2%) and fluoranthene (110.8%) using water-isopropanol as extraction solvents. Fisher *et al*. [50] found that spiking in more polar solvent resulted in higher recoveries. Similar results were also obtained by Eschenbach *et al.* [51] who explained that acetone showed greater efficiency than toluene or dichloromethane. All relative standard deviations for spiked standard PAHs were acceptable (R.S.D. 0.3-9.3).

Table 4.2: Percentage recovery of spiked PAHs in 7 g of soil sample

Solvent extraction	**% Recovery (R.S.D.)**			
	Naphthalene	**Fluorene**	**Phenanthrene**	**Fluoranthene**
Dichloromethane-acetone 50:50(v/v)	81.4 (0.3)	79.6 (0.5)	100.5 (3.1)	86.4 (2.3)
Hexane-acetone 50:50(v/v)	79.3 (8.9)	69.5 (9.3)	94.1 (3.1)	82.0 (0.5)
Dichloromethane 100 (v/v)	20.7 (2.1)	87.8 (6.2)	112.5 (3.2)	86.0 (5.0)

In order to observe the effect of extraction solvent on analyte recovery, the variation of extraction recovery was carried out. It was found that all percentage recoveries of each solvent extraction are more than 60% except for naphthalene with 20.7%. However, the results indicate that dichloromethane-acetone was suitable as extraction solvent for the extraction of PAHs from soils (Figure 4.2). All of the PAHs have been successfully extracted with this solvent with percentage recoveries of more than 60%. A previous report [41] suggested that recovery of PAHs depends on the molecular size of the PAH where PAHs with high-molecular weights tend to have stronger adsorption and formation of non-extractable residues. The results obtained by Eschenbach e*t al*. [51] show that acetone had greater efficiency than toluene or dichloromethane and soil moisture had no evident effect on the efficiency of PAHs extraction. These also show that solvent mixtures are more effective in extraction of PAHs from moist samples than a single solvent. In these studies, mixture of dichloromethane-acetone was applied for all subsequent extractions.

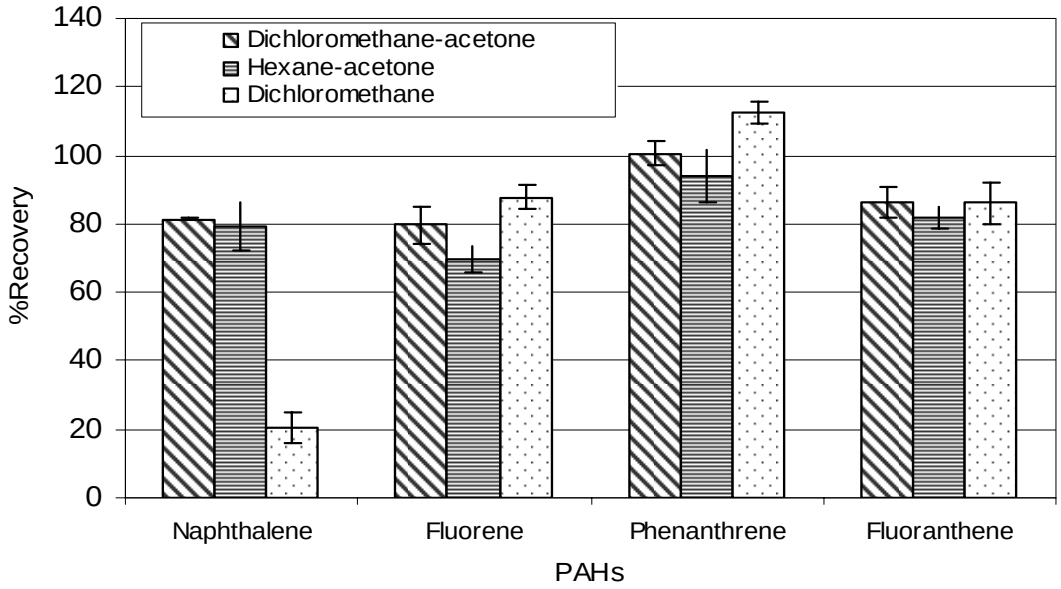


Figure 4.2: Average percentage recoveries of spiked 50 ppm PAHs in soil samples using different extraction solvents

4.3.2 Extraction Temperature

Temperature has a significant influence on the diffusion coefficient of solvents; hence the kinetic of extraction process and its overall efficiency is strongly dependent on this parameter. In this work, recoveries of PAHs from spiked samples were determined using different extraction temperatures in the range of 100°C-180°C with mixture of dichloromethane-acetone 50:50 (v/v) as the extraction solvent. Examples of chromatograms for the PAH determination are given in Appendix 2.

In general, higher recoveries of all PAHs studied were obtained at higher temperatures (Table 4.3). For example, at 100°C and 180°C, the recoveries of phenanthrene were 83.4% and 88.5%, respectively. A high temperature of 180°C gave highest recoveries for fluoranthene (94.4%) followed by fluorene (92.3%), phenanthrene (88.5%), and naphthalene (81.5%). The higher recoveries for the more hydrophobic compounds (phenanthrene and fluoranthene) can be explained because these compounds are generally more thermally stable. PAHs with high-molecular weight may have

stronger adsorption and formation of non-extractable residues. Hence, fluoranthene with the highest molecular weight show the highest recovery and naphthalene with the lowest molecular weight shows the lowest recovery.

The high recoveries at 180°C for the PAHs studied can be explained by the fact that at higher temperatures, there is a decrease in the viscosity of liquid solvents, thus allowing better penetration of matrix particles and enhancing extraction. Increased temperature can disrupt the strong solute-matrix interactions caused by van der Waals forces, hydrogen bonding, and dipole interactions of the solute molecules and active sites on the matrix. In addition, increased temperatures also decrease the surface tension of the solvent that facilitates better contact of the solvent with the analytes and enhance extraction [45].

Results obtained are in good agreement with those reported by Jaska *et al*. [52] who suggested that in order to enhance the recovery efficiency, extractions need to be performed at elevated temperature within the range of 50-180°C, depending on the boiling point of the analytes studied. In PLE, the samples are extracted at temperatures well above the boiling point of the extractant used. The kinetics of mass transfer can be greatly improved at elevated temperatures. Therefore, it is very well possible that a poor extraction solvent in a conventional extraction method such as Soxhlet extraction can be a good solvent in PLE at high temperatures.

Table 4.3: Percentage recoveries of spiked standard PAHs at 20 ppm in 7 g of soil samples at different extraction temperatures

Compound	**%Recovery (R.S.D.)**		
	Temperature		
	100°C	**150°C**	**180°C**
Naphthalene	42.2(2.5)	67.6(1.0)	81.5(1.0)
Fluorene	86.6(1.3)	91.6(1.4)	92.3(1.1)
Phenanthrene	83.4(1.7)	88.1(7.7)	88.5(1.8)
Fluoranthene	86.5(1.1)	87.3(1.5)	94.4(0.2)

(R.S.D. %) was based on triplicate injections

4.3.3 Amount of Spiked Standards

As PAHs exist in the environment in various concentrations, it was therefore of interest to investigate the effect of analytes concentration on the percentage recovery. Experiments were carried out to determine recovery of spiked analytes ranging from 20 ppm to lower concentration of 2.5 ppm. The results obtained for spiked PAHs (20 ppm, 5 ppm and 2.5 ppm) at extraction temperature of 180°C are shown in Table 4.4. Highest percentage recoveries were observed for spiked PAHs at 20 ppm. Marked decrease in recovery of naphthalene was observed on going from from 20 ppm to 2.5 ppm. For example, at 20 ppm gave a recovery of 81.5% and while 2.5 ppm gave a recovery of 33.9%. This was because naphthalene has the lowest boiling point 218°C compared to other analytes with average boiling point above 300°C and thus more easily lost during PLE.

Recoveries of naphthalene were low which were consistent with the reported data of Auer and Malissa and Marten *et al*. [53, 54]. Higher volatility and water solubility of naphthalene may account for the low recovery rate. On going from spiked concentration of 20 ppm to 2.5 ppm, phenanthrene and fluorene show about 8.2% and 9.2% decrease in the percentage recovery while fluoranthene show only a slight decrease in percentage recovery of about 3% respectively. Fisher *et al*. [50] reported that the recovery of PAHs depends on the molecular size of the PAHs. Additionally, the relative standard deviations are low (5%).

Table 4.4: Percentage recoveries of spiked standard PAHs at 20 ppm, 5 ppm and 2.5 ppm in 7 g of soil samples at 180°C

	%Recovery (extraction temperature 180°C)		
	Spiked PAHs concentration		
	20 ppm	5 ppm	2.5 ppm
Naphthalene	81.5(1.0)	56.4(0.1)	33.9(0.3)
Fluorene	92.3(1.1)	87.5(0.2)	83.1(0.2)
Phenanthrene	88.5(1.8)	82.9(0.8)	80.3(0.4)
Fluoranthene	87.3(1.5)	86.6(0.1)	84.3(0.2)

(R.S.D. %) was based on triplicate injections

4.3.4 Extraction Pressure

The use of pressure facilitates extractions of analytes from samples in which the analytes are trapped in matrix pores. Elevated pressure along with elevated temperature reduces the solvent surface tension that helps force the solvent into the pore to contact the analytes. In this study, the following pressures were used in order to determine the effects of pressure on the extraction efficiency: 50 bar, 100 bar and 150 bar. Higher pressures above 150 bar was not attempted in this study because only modest pressure in the range of 100-150 bar (10-15MPa) is usually needed to maintain the solvents as liquid in PLE [52]. The results show that the percentage recoveries of the analytes improve with pressure (Table 4.5). All the analytes studied showed the highest recovery at 150 bar. Fluorene show the highest percentage recovery (94.6%) followed by phenanthrene (94.3%), fluoranthene (86.9%) and naphthalene (52.2%). These results confirm the hypothesis that the increased pressures will allow analytes that are found in pores effectively blocked by solvent to be more rapidly extracted than is possible at room temperature and atmospheric pressure. Elevated pressure will also force the solvent into pores.

Table 4.5: Percent recovery of analyte studied at different extraction pressures

Compound	% Recovery (R.S.D.) Pressure (bar)		
	50	100	150
Naphthalene	44.9 (5.8)	49.0 (0.20)	52.2 (0.04)
Fluorene	82.5 (4.2)	87.8 (0.03)	94.6 (0.08)
Phenanthrene	78.5 (1.1)	83.1 (0.03)	94.3 (0.03)
Fluoranthene	77.3 (2.6)	81.5 (0.20)	86.9 (0.04)

Figure 4.3 gives a graphical summary of the results obtained for the average recovery of PAHs at different extraction pressures. At 150 bar, naphthalene shows about 7% improvement in percent recovery compared to lower pressure of 50 bar. Phenanthrene shows about 15% improvement in recovery from 78.5% to 94.3%. Meanwhile, phenanthrene shows the highest improvements from 78.5% to 94.3%. In addition to providing improved recoveries, operating at higher pressures does have a

practical aspect. Cell can be filled with solvent much more quickly at higher pressure more than 1500 psi (103 bar) especially when extracting samples having small particles such as soil and sediment [50]. Within the limit of the experiment, high pressure was found to have positive influence on the PLE studied and thus 150 bar was used in subsequent extractions.

A similar study has been reported by Richter *et al*. [45]. They studied the effect of pressure on recoveries of PAHs (fluorene, phenanthrene, anthracene, fluoranthene, benz[a,h]anthracene, etc.) by extracting silica loaded with PAHs at pressures ranging from 500 to 2500 psi (34.5 bar to 172.4 bar). They noted that the use of high pressures gave improved recoveries compared to the use of lower pressures. The improved recovery started with fluoranthene and most pronounced for benz[a,h]anthracene. The authors explained that certain amount of pressure is needed to maintain the solvents as liquids at or above their atmospheric boiling points. However these pressures need not be excessive.

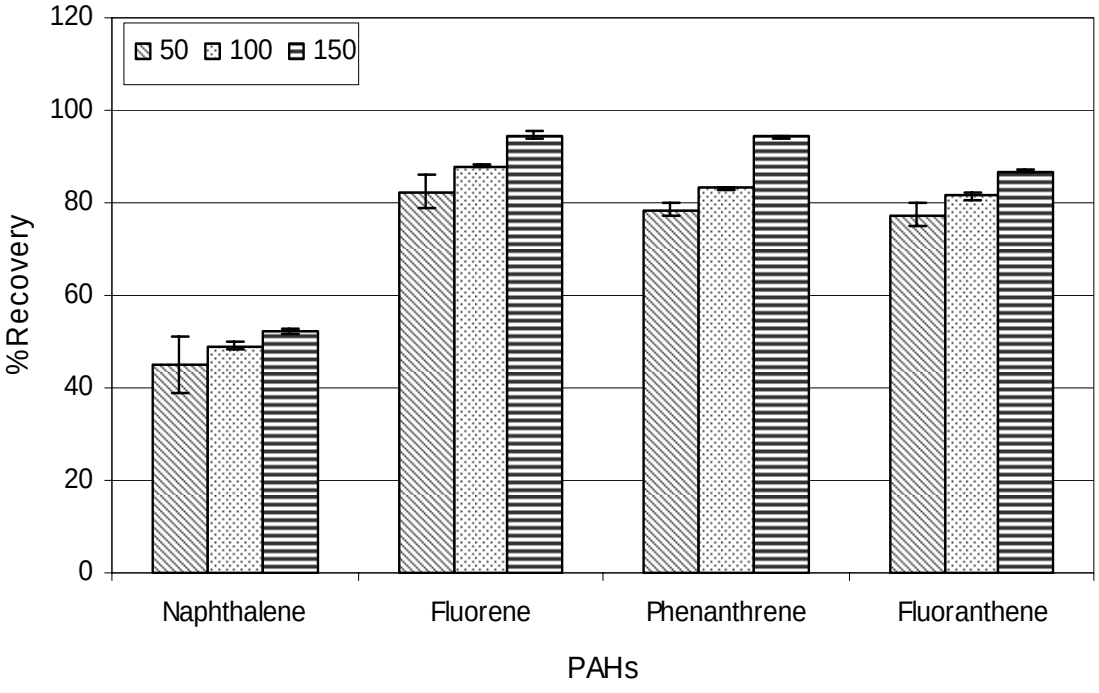


Figure 4.3: Average percentage recovery of PAHs studied at different extraction pressures

4.3.5 Reproducibility of PLE Method

Extraction temperature, extraction solvent and extraction pressure have significant influence on the diffusion coefficient of solvents; hence the kinetic of extraction process and its overall efficiency is strongly dependent on these parameters. It is thus of interest to determine the consistency in recovery of the developed method. Experiments were set out to study the reproducibility of the developed method on day 1 to day 3. The average value was calculated from three individual experiments, the percent recovery of the PAHs was performed under the optimized condition described above (section 4.2.3). The extraction by solvent mixture dichloromethane-acetone (50:50, v/v), extraction temperature 180°C and extraction pressure of 150 bar.

From the results obtained (Table 4.6), it is noted that there is no significant day-to-day differences in the efficiency of the developed method and the day-to-day R.S.D. values were lower. The highest value was obtained for fluorene (95.05%) at day 1 while the lowest recovery was naphthalene (63.38%) at day 2. The result showed that three-ring PAHs (fluorene, phenanthrene) appeared to be more efficiently extracted by PLE compared to the two-ring PAH (naphthalene), probably due to its higher volatility and thus more analyte loss during extraction.

Table 4.6: Relative percentage recoveries and standard deviation of analytes studied between day to day

	% Recovery			Mean %Recovery	% R.S.D.
Compound	**Day 1**	**Day 2**	**Day 3**		
Naphthalene	87.7	63.4	68.1	73.1	0.1_1
Fluorene	95.0	96.4	90.6	93.9	0.0_4
Phenanthrene	89.8	92.7	89.1	90.5	0.0_2
Fluoranthene	90.5	88.6	88.4	89.1	0.0_1

The results indicate that the developed method for PLE of PAHs has good reproducibility. The RSD's were good, ranging from 0.0_1 to 0.1_1. Therefore, the PLE

method proved to be readily applicable as a standard method for the extraction and analysis of PAHs. Currently available US EPA methods for the extraction of PAHs from contaminated soil include Method 3540 for Soxhlet extraction, Method 3541 for SFE and Method 3545 for PLE. Conte *et al*. [43] reported that PLE is advantageous because the parameters involved including ease of total automation that adds to better reproducibility, reduced time direct contact between operator and solvent vapour, reduced consumption of organic solvents, the subsequent storage and disposal.

4.4 Comparison of PLE with Soxhlet Extraction

In this study, PLE and Soxhlet extraction were carried out to determine whether the PLE method developed was in good agreement with conventional Soxhlet extraction method. The extraction efficiencies of PLE employing optimum extraction conditions were compared with the efficiencies of Soxhlet extraction for the extraction of PAHs from soil samples. PLE was completed in about 40 min for each extraction condition with 5 min for static extraction time whereas Soxhlet extraction was carried out for 20 h (follow EPA Method 3540). In our study, 5 min of static time was used because no difference in recovery was observed when longer static extraction time was used.

Temperature of organic solvent flowing out from the cell was measured and found to be less than 35°C, even with the extraction cell at 100°C. The fast loss of heat was due to the length of tubing from the cell to the collection vial. No cooling was used on the collection vials in our experiment. This was in agreement with conditions applied in an experiment described by Richter *et a*l. [45] who explained that cooling system was not necessary because no difference was seen with or without cooling on the collection vials in terms of recovery or precision when extraction the volatile compounds.

The quantities of representative PAHs extracted by each method tested at spiked 20 ppm PAHs in soil sample is shown in Table 4.7. The total PAH contents determined by each extraction method are in good agreement. PLE method showed good reproducibility for the majority of PAHs. Fluoranthene showed the highest recovery

(94.4%) followed by fluorene (92.3%), phenanthrene (88.5%) and naphthalene (81.5%). Based on data gathered, all PAHs are successfully extracted using PLE with minimum percentage recovery 81.47%. High temperature at 180°C along with polar solvent dichloromethane-acetone was found to be necessary to extract the PAHs quantitatively from soils.

Higher recoveries are shown for naphthalene using PLE with 81.5% compared with Soxhlet extraction 34.31%. It was probably because of increased solvent penetration from increased swelling of soil particles [38]. Similar result has been reported by Burkhardt *et al*. [38] who explained that extraction of PAHs compounds such as naphthalene and fluorene are more efficient by using PLE than Soxhlet extraction. Phenanthrene showed only slight difference in recoveries between PLE (88.5%) and Soxhlet extraction (90.1%) compared with other analytes which shows marked difference in recoveries (Figure 4.4). Similar study has been reported by Saim *et al.* [46] who explain where phenanthrene extracted using PLE gave similar concentration to that obtained by Soxhlet extraction whereas fluoranthene show the highest recovery using Soxhlet extraction. In comparison to establish methods such as sonication and Soxhlet, PLE offers the advantage of faster extraction times and lower solvent consumption.

Table 4.7: Comparison of extraction efficiencies of spiked 20 ppm PAHs in soil samples between Soxhlet and PLE

	% Recovery (R.S.D.)	
Compound	**Soxhlet**	**PLE (180C/150 bar)**
Naphthalene	34.31(8.3)	81.5(1.0)
Fluorene	82.2(2.7)	92.3(1.1)
Phenanthrene	90.1(6.1)	88.5(1.8)
Fluoranthene	73.6(1.2)	94.4(0.2)

*Soxhlet extraction conditions: solvent, 150 mL dichloromethane-acetone (50:50, v/v) heating 20 h

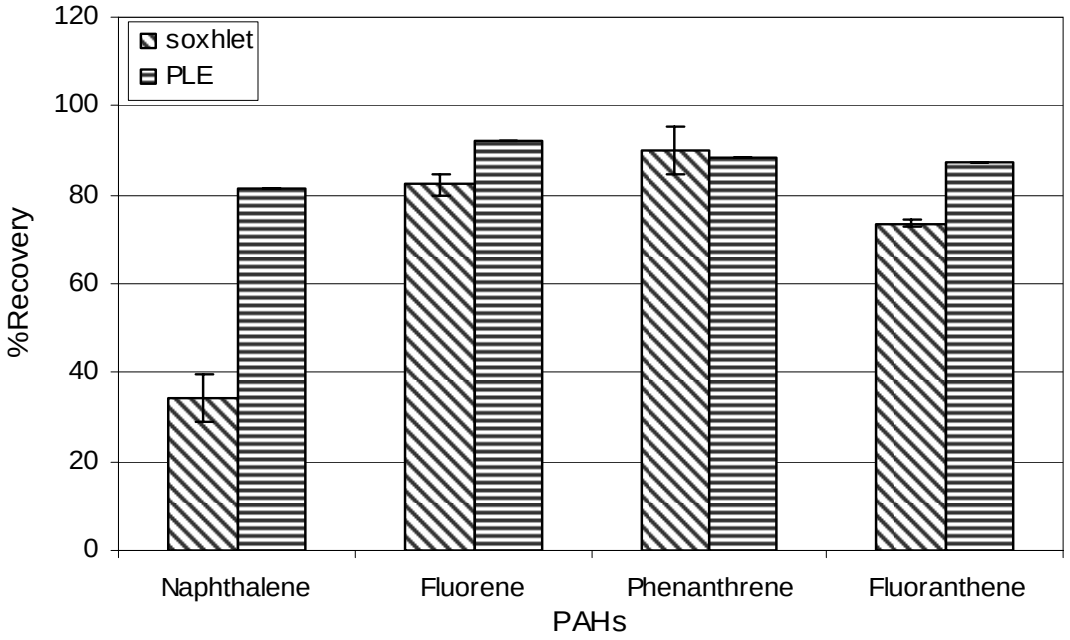


Figure 4.4: Average percentage recovery of spiked 20 ppm standard PAHs in soil sample

4.5 Application of the Developed PLE Method to the Analysis of Soil Samples

PLE of soil samples were performed at 180°C and pressure of 150 bar using dichloromethane-acetone 50:50 (v/v) with single 5 min extraction step. Five soil samples were used in this experiment were collected at different places from FELDA palm oil plantation at Serting, N.Sembilan. Sample 1-2 were collected near palm oil trees were black loamy sand and Sample 3-5 were obtained near a river were clay sand. All soils were sieved through a 2 mm sieve. Results of the HT-HPLC analysis are shown in Table 4.8. The PAHs detected in the samples are 2.2-60.7 μg/mL. Phenanthrene (4.1 to 49.4 μg/mL) fluorene (10.1 μg/mL and 11.6 μg/mL) fluoranthene (2.2-60.7 μg/mL). However naphthalene was not detected in the soil samples.

Table 4.8: PAHs concentrations in five soil samples using developed PLE method followed by HTLC determination

	Compound	Concentrations (ppm)	R.S.D.
Sample 1	Phenanthrene	8.9	0.66
	Fluoranthene	60.7	0.42
Sample 2	Phenanthrene	49.4	0.73
	Fluoranthene	23.7	0.60
Sample 3	Fluorene	11.6	0.81
	Fluoranthene	2.4	0.70
Sample 4	Fluorene	10.1	0.54
	Phenanthrene	4.1	1.00
	Fluoranthene	2.2	0.50
Sample 5	Phenanthrene	38.7	0.26
	Fluoranthene	42.8	0.60

Figure 4.5-4.6 shows HT-HPLC chromatograms of the PAHs extracted from five soil samples. Based on the chromatogram of HT-HPLC, phenanthrene and fluoranthene peaks were observed in Samples 1, 2, 4, 5. The amount of phenanthrene detected at higher concentrations in Sample 2 and Sample 5 are 49.4 μg/mL and 38.7 μg/mL, respectively. Fluoranthene were detected at higher concentrations in Sample 1 (60.7 μg/mL), followed by Sample 5 (42.8 μg/mL) and Sample 2 (23.7 μg/mL). Fluorene were detected in Sample 3 and 4 with concentrations of 11.6 μg/mL and 10.1 μg/mL, respectively. Lower concentrations were observed for fluoranthene compound in Sample 4 (2.2 μg/mL) and Sample 3 (2.4 μg/mL). All peaks were identified by retention time comparison against the standard PAHs. The results indicates that the differences in PAH content determined in different matrices of the soil samples depend closely on the interactions with the solid and liquid matrix components, interferences of the macromolecular compounds of the matrix and owing to their various volatility [55].

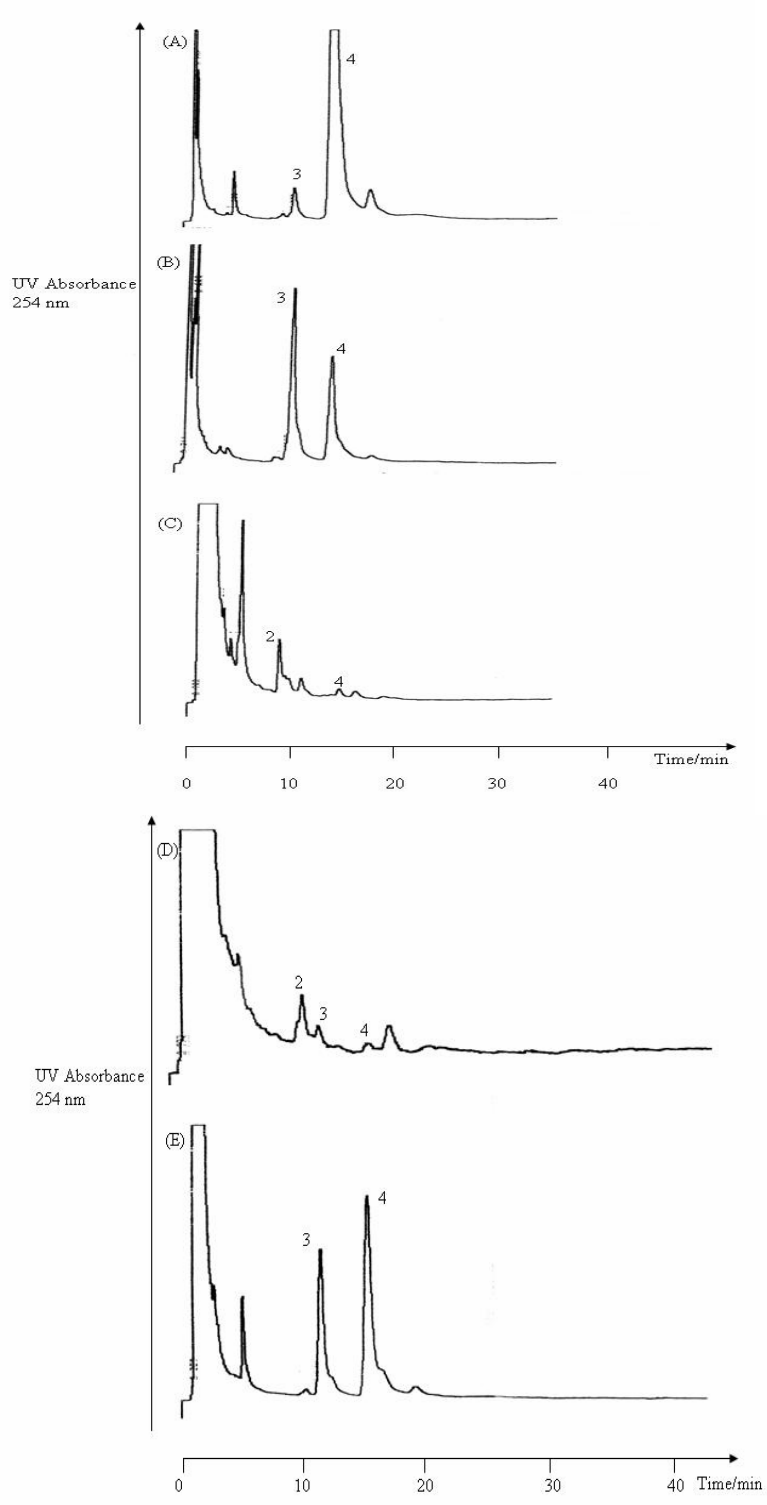


Figure 4.5: Chromatograms of PAHs extracted from soil samples using HTLC. (A) Sample 1; (B) Sample 2; (C) Sample 3; (D) Sample 4; (E) Sample 5. Chromatographic conditions: ODS-silica column (100 mm × 4.6 mm I.D.); mobile phase 40:60 (v/v); flow rate 2.5 mL/min; temperature 70°C; UV absorbance 254 nm; injection volume 5µL. Peaks: (2) fluorene; (3) phenanthrene; (4) fluoranthene.

The results obtained indicate that the developed method can be applied to the analysis of PAHs in soils. Popp *et al.* [44] reported a similar study explained that phenanthrene (581 ng/g) and fluoranthene (823 ng/g) were mostly detected in contaminated soil from river Mulde, German. Filipkowska *et al*. [55] show the average recoveries of PAHs from raw sediment samples was approximately 62% and phenanthrene and anthracene was observed in lower recovery, 47% and 21% respectively. All these are due to diverse a physical property which includes sorption, solubility and volatility of the compounds studied.

CHAPTER V

CONCLUSIONS AND SUGGESTIONS FOR FURTHER STUDY

5.1 Conclusions

The separations of polycyclic aromatic hydrocarbons (PAHs) by reversed phase liquid chromatography (RPLC) separation technique at elevated temperature have been successfully examined. The relationship between retention factor, k; separation factor, α; theoretical number of separation plates per column length, N/m and the resolution, R_s with different column temperatures were analyzed and demonstrated. It was observed that a better separation was achieved during the high operating temperature. The overall column efficiencies at high operating temperatures were much higher relative to those at ambient operating temperature conditions. High temperature operation in reversed phase liquid chromatography provides the opportunity to reduce the quantity of organic solvent used in mixed organic-water mobile phase, decreases total analysis time, and increases analyte mass transfer rates.

Four PAHs (naphthalene, fluorene, phenanthrene and fluoranthene) were successfully separated using RPLC at 70°C. Optimum separation conditions were selected for the separation of these compounds on ODS-silica column with a mobile phase acetonitrile-water 40:60 (v/v) at 70°C. It was found that by simply increasing the column temperature, excellent separation efficiencies were achieved without significant loss in resolution.

A method for the determination of PAHs has been developed using homemade pressurized liquid extraction (PLE) system and elevated temperature RPLC. PLE showed excellent efficiency in term of total extraction time, total solvent usage as well as the method reproducibility. PLE method demonstrated an outstanding performance with dichloromethane-acetone 50:50 (v/v) as solvent extraction, pressure 150 bar and extraction temperature 180°C. Successfully results were obtained when the method applied to the analysis of PAHs in soil samples. The PAHs detected in the samples are 2.2-60.7 µg/mL with Phenanthrene (4.1 to 49.4 µg/mL) fluorene (10.1 µg/mL and 11.6 µg/mL) fluoranthene (2.2-60.7 µg/mL). The results indicates that the differences in PAH content determined in different matrices of the soil samples depend closely on the interactions with the solid and liquid matrix components, interferences of the macromolecular compounds of the matrix and owing to their various volatility.

PLE have shown to be more powerful techniques compared to Soxhlet extraction for certain compounds. Saving in time, solvent usages are possible due to the high extraction efficiencies at high temperature /pressure conditions. An added advantage of method for PLE is the significantly shortened method development time because the same solvent used in existing extraction methods can be used, reducing the amount of time for method optimization. This PLE method of soils sample preparation has advantages over conventional Soxhlet extraction for sample automation, reduced extraction time and reduced solvent volume. Data showing the method R.S.D. and reproducibility prove method suitability for routine monitoring of the compounds of interest in samples of soil. Although we have not covered all classes of environmentally-important compounds, PLE of representative semi-volatile compounds in soils matrix as well as real samples indicate this technique is a viable technique among the new regime of rapid method for extraction. Recoveries using PLE were in all cases satisfactory, offering good agreement with established data reported.

5.2 Suggestions for Further Study

Although satisfactory results were obtained using high operating temperature RP-HPLC with silica column in this study, the percentage of organic modifier compositions should be considered. Therefore, further studies could be conducted using the modified silica such as titanium grafting or zirconization of silica substrates which highly stable stationary phase under neutral high pH conditions. Zirconia by itself has very rich surface chemistry and able to withstand extended exposure to column temperature as high as 200ºC.

Further studies could be conducted where temperature programming micro-column liquid chromatography separation technique can be utilized to improve the separation resolution when dealing with complex mixtures. The use of temperature programming requires fast heat transfer from the heating medium into a chromatographic system. Hence, micro bore column (I.D. < 1 min) or alternative highly stable capillary, which provides a very low heat capacity, can be utilized, as it is easily thermostated to a precise and constant temperature.

In order to minimize or prevent adverse effects of POPs, many studies illustrate the fate of POPs in natural environments. PAHs as one of the typical persistent organic compounds (POPs) featured in regional and global cycling. Further studies should be conduct using a different analytes which has been listing of 16 PAH priority pollutants comprises by US Environmental Protection Agency (EPA), German and Dutch standard methods only specify 6 and 10 PAHs, respectively. Because more and more PAHs are identified and investigated on their toxicity it can be expected that the list will be extended in the near future. PAHs are emitted mainly into the atmosphere and have been detected at long distances from their source. Extensively studies should be taken at environment samples such as vegetables, fish, marine sediment or other suspect places of distribution PAHs.

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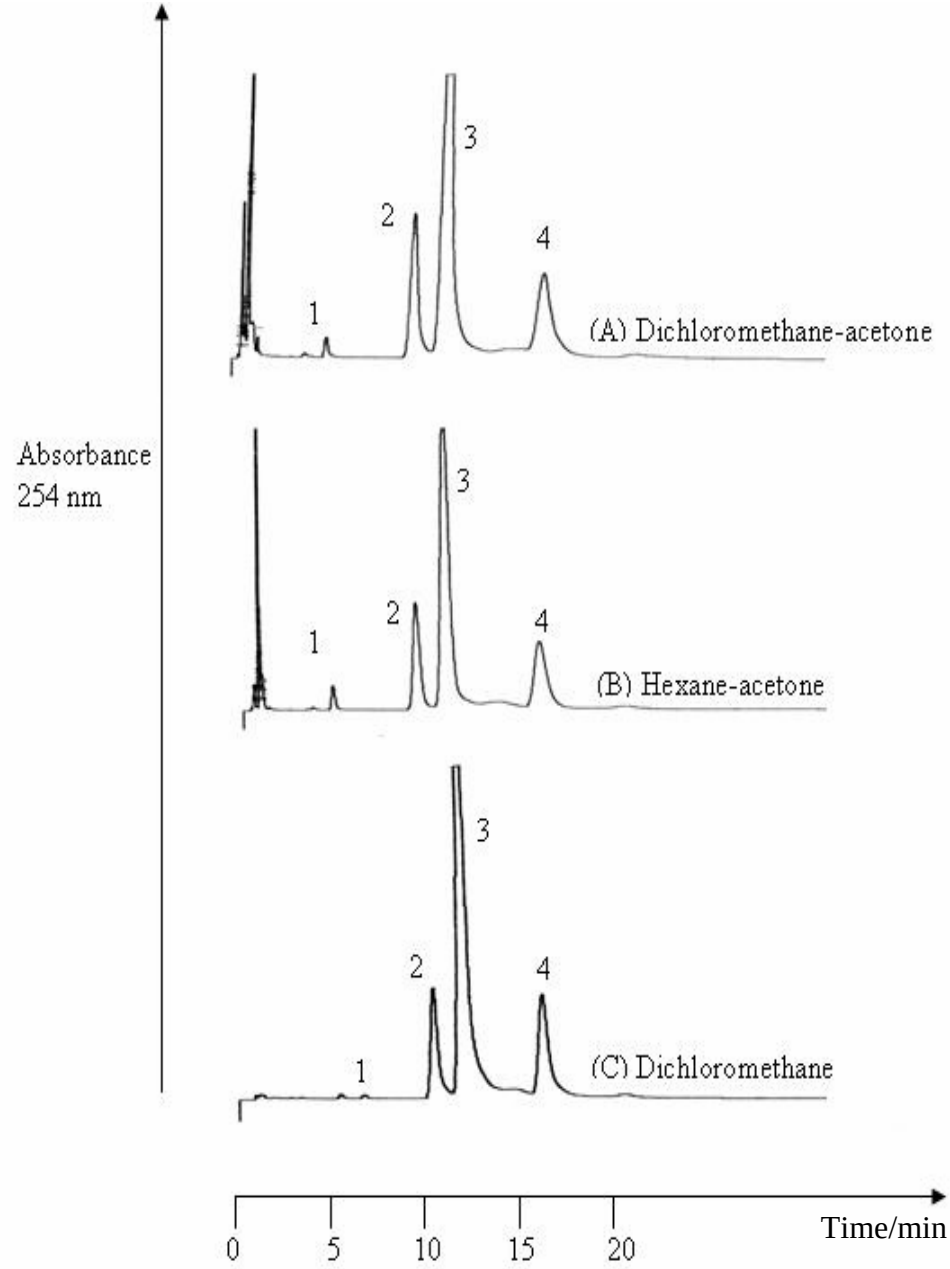
APPENDICES

Appendix 1



High temperature liquid chromatography of extracts from spiked PAHs (50ppm) in soils sample using PLE at different solvent extractions (A) Dichloromethane-acetone 50:50 (v/v); (B) hexane-acetone 50:50 (v/v); (C) Dichloromethane. Chromatographic condition: ODS-silica column (100mm × 4.6 mm I.D.); mobile phase acetonitrile-water 40:60 (v/v); flow rate 2.5 mL; temperature 70°C; UV absorbance 254 nm; injection volume 5µL. Peaks: (1) naphthalene; (2) fluorene; (3) phenanthrene; (4) fluoranthene.

Appendix 2

LIST OF PUBLICATIONS

Articles in Journals and Papers in Conferences

1. M. Marsin Sanagi, H. H. See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Determination of Carotene, Tocopherols and Tocotrienols in Residual Oil from Palm Pressed Fiber Using Pressurized Liquid Extraction – Normal Phase Liquid Chromatography" *Analytica Chimica Acta*, 538, 71-76 (2005)

2. M. Marsin Sanagi, Noorashikin Md Salleh, Hong Heng See, Wan Aini Wan Ibrahim and Ahmedy Naim, "Elevated Temperature Reversed-Phase High Performance liquid Chromatography of Polycyclic Aromatic Hydrocarbons on ODS-Silica Phase". *Buletin Kimia*, 21 (2005) 19-26.

3. Hong Heng See, M. Marsin Sanagi, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Determination of Carotene, Tocopherols and Tocotrienols in Residue Oil from Palm Pressed-Fiber using Pressurized Liquid Extraction – Normal Phase Liquid Chromatography" (G1-1). Presented at *Asian Chemical Congress* 2005, Seoul, Korea, 24-26 August 2005.

4. Hong Heng See, M. Marsin Sanagi, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Novel Applications of High Temperature Liquid Chromatography using Hydro-Organic and Superheated Water Eluent" (G1-8). Presented at *Asian Chemical Congress* 2005, Seoul, Korea, 24-26 August 2005.

5. Mohd Marsin Sanagi, Noorashikin Md Saleh, Hong Heng See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Application of High Temperature Liquid Chromatography to the Analysis of Polycyclic Aromatic Hydrocarbons in Soil", Paper presented at the *Fifth IMTGT UNINET Biannual Conference and International Seminar*, Tiara Convention Center, Medan North Sumatra, Indonesia, 22-23 June, 2006. (Organized by University of North Sumatra, Medan)

6. M. Marsin Sanagi*, Noorashikin Md Saleh, Hong Heng See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Elevated Temperature Reversed-Phase High Performance Liquid Chromatography of Polycyclic Aromatic Hydrocarbons on ODS-Silica Phase", Paper presented at the *Annual Fundamental Science Seminar 2005* (AFSS 2005), 4-5 July 2005, Institut Ibnu Sina, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia

7. M. Marsin Sanagi,* H. H. See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim, "Enhanced Separation of Tocopherols and Tocotrienols Using Elevated Temperature High Performance Liquid Chromatography" Presented at Oils and Fats International Congress 2004, PWTC, 29 September 2004 – 2 October 2004.

8. M. Marsin Sanagi,* H. H. See, Wan Aini Wan Ibrahim, Ahmedy Abu Naim "Determination of Carotene, Tocopherols and Tocotrienols in Palm Pressed Fiber Using Pressurized Extraction coupled to Elevated Temperature Liquid Chromatography" Presented at Oils and Fats International Congress 2004, PWTC, 29 September 2004 – 2 October 2004.

Thesis

1. "High-Temperature Liquid Chromatography and Pressurized Liquid Extraction of Polycyclic Aromatic Hydrocarbons" MSc Thesis. Ms Noorashikin Bt Md Saleh (2004 – 2006)

2. "Extraction of Total Residue Oil from Palm Pressed Fiber using Pressurized Liquid Extraction Method", Undergraduate Project Report. Shahrul Kamal bin Mat Akhir (July 2006 – May 2007)

Appendix 3

LIST OF AWARDS

1. **Gold Medal** at the Seoul International Invention Fair 2006 organized by Korea Invention Promotion Association in Seoul, Korea, 7-11 December 2006. Invention: "PLEX: Pressurized Liquid Extraction for the Monitoring of Residue Oil Content and Natural Vitamin E in Palm Pressed Fiber". Inventors: **Mohd Marsin Sanagi**, See Hong Heng, Wan Aini Wan Ibrahim, Ahmedy Abu Naim.

2. **Silver Medal** at the 17th International Invention, Innovation, Industrial Design & Technology Exhibition 2006 (ITEX 2006), Kuala Lumpur Convention Centre, 19-21 May 2006. Name of invention: "PLEX: Method for Pressurized Liquid Extraction (for the monitoring of residue oil content) and Separation of Natural Vitamin E in Palm Pressed Fiber", Inventors: **Mohd Marsin Sanagi**, See Hong Heng, Wan Aini Wan Ibrahim, Ahmedy Abu Naim.