
Contents

	Page
Abstract	i
Acknowledgement	ii
List of Figure	vi
List of Tables	ix
Abbreviation	xi
 Chapter 1 INTRODUCTION	 1
1.1 Coal	1
1.2 Batu Arang, Selangor	2
1.3 Coal Analysis	5
1.4 Characteristics of organic geochemistry: Biomarker	7
1.5 Objective of present study	12
 Chapter 2 METHODOLOGY	 14
2.1 Literature review	14
2.2 High-performance liquid chromatography (HPLC)	19
2.3 HPLC detectors	21
2.3.1 Refractive index	21
2.3.2 UV detector	23
2.4 HPLC Utilisation	25
2.4.1 Semi-preparative HPLC	25
2.4.2 Columns for HPLC	26
2.4.3 Normal- and reversed phase chromatography	26
2.4.4 Choice of mobile phases	27
2.5 Rock (coal) sample extraction using Soxhlet apparatus	28

Chapter 3	EXPERIMENTAL	30
3.1	Chemicals	31
3.2	High-performance liquid chromatography (HPLC)	33
3.3	Preparation of standard solutions	34
3.3.1	Preparation of stock solutions	34
3.3.2	Preparation of standard solutions of various concentrations.	34
3.4	Detection limit	35
3.5	Sampling	35
3.5.1	Sampling location	35
3.5.2	General procedure for sampling	35
3.5.3	Rock sample extraction	39
3.6	Sample Clean-up	40
3.7	Identification of polyaromatic hydrocarbons (PAHs) and aliphatic hydrocarbons in coal samples.	40
3.8	Determination of yield from Soxhlet extraction.	41
3.9	Preparative thin-layer chromatography (TLC)	43
3.10	Determination of TLC recovery of organic matter.	43
Chapter 4	RESULTS AND DISCUSSION	44
4.1	Sampling information	44
4.2	Development of Method	45
4.2.1	High Performance Liquid Chromatography (HPLC)	45
4.2.1 (a)	Choice of UV detector condition	45
4.2.1 (b)	RI detector	49
4.2.1 (c)	Choice of mobile phase and flow rate	51
4.2.2	Recovery	60
4.2.3	Detection limit	61
4.3	Sample Clean-up	62
4.4	Preliminary identification of PAHs and aliphatic hydrocarbons	63

	Page
4.5 Interferences	63
4.5.1 Method interferences	63
4.5.2 Matrix interferences	64
4.6 Evaluation of Analytical Data	64
4.6.1 Errors	64
4.7 Discussion	65
 CHAPTER 5 CONCLUSION	 71
 REFERENCES	 72
 Appendix A	 76
 Appendix B	 79
 Appendix C	 81
 Appendix D	 87

List of Figures.

Figure		Page
1.1	Locality map of study area.	3
1.2	Batu Arang coal mine and stratigraphic relationship between logged section	3
1.3 (a)	Photo taken of old mining pond in Batu Arang (near sampling station).	4
1.3 (b)	Photo taken of Sampling <u>Station A</u> shows the stratigraphic of different rock layers	4
1.4	An illustration of the varied structures which can be produced when the ubiquitous precursor hydrocarbon squalene is cyclised by different classes of organism.	11
2.1	Configuration of HPLC	15
2.2	Schematic diagram of HPLC	20
2.3	Refractive index detector (refractometer)	20
2.4	Chromatogram of <i>n</i> -alkane standard mixture	22
2.5	Flow cell for Ultra Violet (UV)/Visible absorbance detector	22
2.6	A variable UV/Vis detector light path	22
2.7	Chromatogram of PAHs standard mixture.	24
2.8	Chromatogram of <i>n</i> -alkane and PAHs standard mixtures.	24
2.9	Isolation of <i>n</i> -alkane and PAHs standard mixtures by Semi-preparative HPLC	25
2.10	HPLC analytical column	25
(a)	Jones Chromatography APEX® silica column	25
(b)	Jones Chromatography Genesis® silica column	25
2.11	Illustration of Soxhlet extraction in progress	29
3.1	Flow diagram of experiment procedure	30

Figure		Page
3.2	Structure of some reference Polyaromatic hydrocarbons (PAHs) standard	32
3.3 (a)	Photo taken shows stratigraphic layers of <i>BA1</i> , <i>BA2</i> and <i>BA3</i>	37
3.3 (b)	Photo taken shows stratigraphic layers of <i>BA2</i> , <i>BA3</i> and <i>BA4</i> .	37
3.3 (c)	Photo taken shows stratigraphic layers of <i>BA5</i> , <i>BA6</i> and <i>BA7</i> .	38
3.3 (d)	Photo taken shows stratigraphic layers of <i>BA8</i> , <i>BA9</i> and <i>BA10</i> .	38
3.4	Flow diagram of extraction procedure	39
4.1	HPLC analysis of standard Phenanthrene (Optimisation of UV detector)	46
(a)	$\lambda_{\max} = 280\text{nm}$	46
(b)	$\lambda_{\max} = 254\text{nm}$	46
4.2	HPLC analysis of standard Anthracene (Optimisation of UV detector)	47
(a)	$\lambda_{\max} = 280\text{nm}$	47
(b)	$\lambda_{\max} = 254\text{nm}$	47
4.3	HPLC analysis of hexane (Optimisation of UV detector)	48
(a)	Sensitivity = 0.030	48
(b)	Sensitivity = 0.035	48
(c)	Sensitivity = 0.040	48
4.4	HPLC analysis of standard Naphthalene (Optimisation of RI detector)	50
(a)	Sensitivity = 4	50
(b)	Sensitivity = 8	50
(c)	Sensitivity = 16	50
4.5 (a)	An illustration of normal phase short column chromatography	62
4.5 (b)	Separation (development) of coal extract on normal phase TLC plate.	62
Appd. B	HPLC analysis of standard utilising HPLC condition no.1	79

Figure	Page
Appd.B (a) Anthracene	79
(b) Naphthalene	79
(c) Hexane	80
(d) $n\text{-C}_{34}$	80
(e) mixture of $n\text{-C}_{30}$, $n\text{-C}_{34}$, $n\text{-C}_{36}$	80
Appd. C HPLC analysis of standard utilising HPLC condition no.3	81
(a) Naphthalene	81
(b) Flourene	82
(c) Phenanthrene	82
(d) Acenaphthene	83
(e) Benz(b)anthracene	83
(f) Flouranthene	83
(g) Chrysene	84
(h) Pyrene	84
(i) Benzo(e)pyrene	84
(j) Hexane	85
(k) $n\text{-C}_{34}$	85
(l) mixture of $n\text{-C}_{30}$, $n\text{-C}_{34}$, $n\text{-C}_{36}$	85
(m) Mixture of $n\text{-C}_{26}$, $n\text{-C}_{28}$, $n\text{-C}_{29}$, $n\text{-C}_{30}$, $n\text{-C}_{32}$	86
(n) Anthracene	86
(o) Mixture of $n\text{-C}_{12}$, $n\text{-C}_{14}$, $n\text{-C}_{16}$, $n\text{-C}_{18}$, $n\text{-C}_{20}$, $n\text{-C}_{22}$, $n\text{-C}_{24}$, $n\text{-C}_{26}$, $n\text{-C}_{28}$, $n\text{-C}_{30}$, $n\text{-C}_{32}$	86
D(a) Semipreparative HPLC analysis of hexane utilising HPLC condition.6	87
D(b) Semipreparative HPLC analysis of hexane utilising HPLC condition.9	88
D(c) Semipreparative HPLC analysis of coal extraxt utilising HPLC condition.6	89
D(d) Semipreparative HPLC analysis of coal extract utilising HPLC condition.9	90

List of Tables

Table		Page
1.1	Chemical aspects of the description of major functional groups in biomarkers.	8
1.2	Chemical structures of major biomarker families.	9-10
3.1	Physical description of sediments collected at <i>Station B</i> and sample <i>BA11</i> at <i>Station A</i> .	36
3.2	Data for the determination of yield from extraction of rock samples.	41
3.3	Percentage of TLC recovery of the organic matter (aliphatic and PAH) from Batu Arang coal rocks.	42
4.1	Numeric notation of PAHs and n-alkane standards.	44
4.2	HPLC retention time, t_r (RI detector) of standards from utilising hexane: propan-2-ol (95:5, V/V) mobile phase on APEX® silica column. (HPLC Condition 1) and injection volume of 5 μ L.	53
4.3	HPLC retention time, t_r (RI detector) of standards from utilising Hexane: Ethyl Acetate (95:5, V/V) mobile phase on APEX® silica column (HPLC Condition 3) and injection volume of 5 μ L.	54
4.4	HPLC retention time, t_r (RI detector) of standards from utilising Hexane: Ethyl acetate (98:2, V/V) mobile phase on APEX® silica column (HPLC Condition 4) and injection volume of 5 μ L.	55

Table

4.5	HPLC utilising Genesis column volume	retention time, t_r (RI detector) of standards from hexane: Ethyl Acetate (95:5, V/V) mobile phase on silica column (HPLC Condition 5) and injection μL .
4.6	HPLC utilising Water column	retention time, t_r (RI detector) of standards from hexane: ethyl acetate (95:5, V/V) mobile phase on Pak® Catridge Prep Nova-silica semi preparative LC Condition 6) and injection volume of 25 μL .
4.7	HPLC utilising Waters column	retention time, t_r (RI detector) of standards from hexane: Ethyl Acetate (97:3, V/V) mobile phase on Pak® Catridge Prep Nova-silica semi preparative LC Condition 7) and injection volume of 25 μL .
4.8	HPLC utilising Waters column	retention time, t_r (RI detector) of standards from hexane: Propan-2-ol (95:5, V/V) mobile phase on Pak® Catridge Prep Nova-silica semi preparative LC Condition 9) and injection volume of 25 μL .
4.9	The re- material	covery and percentage recovery of the organic in the Batu Arang coal rock samples
4.10	Detectio	limits ($\mu\text{g/mL}$) of PAHs by HPLC-UV analysis.
4.11	Polarity	index for a range of solvents

Abbreviation

Appd.	=	Appendix
C	=	Carbon
e.g.	=	example
Fig.	=	Figure
GC	=	Gas chromatography
GC-MS	=	Gas chromatography - mass spectrometry
H	=	Hydrogen
HC	=	Hydrocarbon
HPLC	=	High-performance liquid chromatography
ID	=	Internal diameter
LSC	=	Liquid-solid chromatography
min.	=	minute
<i>n</i> -	=	Normal-
PAHs	=	polyaromatic hydrocarbon
Prep.	=	Preparative
RI	=	Refractive index
r.i.u.	=	Refractive index units
SFC	=	supercritical fluid chromatography
TLC	=	Thin layer chromatography
tr or t_R	=	Retention time
UV	=	Ultraviolet
V. or Vol	=	Volume

$$V = \frac{V_0}{n} \frac{f}{t}$$