PROFILING OF ILLICIT CANNABIS SAMPLES USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY AND MULTIVARIATE ANALYSIS

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A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science (Forensic Science)

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Please be informed that the above mentioned project report be classified as **RESTRICTED** for a period of **five (5) years** from the date of this letter. The reasons for this classification are:

- The project involved the analysis of illicit cannabis seized from various parts of Malaysia.
- The developed method has not been rigorously tested.
- The study was part of an initial investigation of the profiling of seized drugs, carried out in collaboration with Jabatan Kimia Malaysia.

Thank you.

Sincerely yours,

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Special dedication towards these special people:

~Father~

~Mother~

~Friends~

~Family~

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ABSTRACT

Drug profiling is an important aspect of drug analysis. Physical and chemical properties of a seized drug samples can be accumulated, and provides intelligence information to combat drug trafficking and abuse. The purpose of this study was to profile drugs, especially illicit cannabis samples using high performance liquid chromatography (HPLC) and multivariate analysis. Herbal cannabis samples were extracted using methanol-chloroform mixture in 9:1 ratio. HPLC separation employed Onyx Monolithic C18 column. Mobile phase consisting of methanol-water (75:25) was used as eluent at a flow rate of 0.8 mL/min and analytes detected at 220 nm. Three major cannabinoids: cannabinoid (CBD), cannabinol (CBN) and tetrahydrocannbinol (THC) were separated within twenty five minutes. Peak area of the three cannabinoids obtained from HPLC analysis were then further analyzed using three types of multivariate analyses, which are Principal Component Analysis (PCA), Cluster analysis and Soft Independent Modeling Class Analogy (SIMCA) analysis. All multivariate analyses were performed using Unscrambler X 10.0. Results from these three multivariate analyses were compared to find which is more suited for profiling of illicit cannabis samples. HPLC profiling produced four groupings among fifty six cannabis extracts, these four groups were further sub-grouped by PCA into fifteen groups. Out of these fifteen groups, four main clusters were observed. Cluster analysis was also performed to confirm findings of PCA; results from Cluster analysis also revealed four clusters among the cannabis extracts. SIMCA was unsuccessful in profiling cannabis compared with PCA and Cluster analysis, since it produced different classes among the cannabis extracts. Hence, illicit cannabis samples were successfully profiled using PCA and cluster analysis to reveal the samples originating from different origins.

ABSTRAK

Pemprofilan dadah adalah satu aspek penting dalam analisis dadah. Maklumat fizikal dan kimia sampel dadah yang dirampas boleh dikumpulkan, profil sesuatu dadah menyediakan maklumat perisikan yang boleh membantu agensi-agensi penguatkuasaan undang-undang membanteras pengedaran dan penyalahgunaan dadah. Tujuan kajian ini adalah untuk menjalankan analisis pemprofilan sampel kanabis haram dengan menggunakan kaedah kromatografi cecair berprestasi tinggi (HPLC) dan analisis berbilang variat. Sampel kanabis diekstrak dengan larutan campuran metanol-kloroform dalam nisbah 9:1. Analisis HPLC dijalankan dengan menggunakan turus Monolithic Onyx C18. Metanol-air (75:25) dengan kadar alir 0.8 mL/min digunakan sebagai pengelusi dan analit dikesan pada 220 nm. Tiga kannabinoid utama iaitu kanabidiol (CBD), kanabinol (CBN) dan Δ^9-tetrahidrokanabinol (Δ^9-THC) telah dipisah dalam tempoh dua puluh lima minit. Luas puncak ketiga-tga kanabinoid daripada analisis HPLC dianalisis dengan menggunakan tiga jenis analisis berbilang variat iaitu Analisis Komponen Utama (PCA), Analisis Klaster dan *Soft Independent Modeling Class Analogy* (SIMCA). Perisian komputer Unscrambler X 10.0 digunakan untuk menjalankan analisis berbilang variat. Perbandingan antara tiga keputusan analisis berbilang variat adalah untuk mencari kaedah yang lebih sesuai bagi tujuan pemprofilan. Profil HPLC menunjukkan empat kumpulan daripada lima puluh enam ekstrak kanabis. PCA telah menghasilkan lima belas kumpulan kecil daripada empat kumpulan ini. Daripada lima belas kumpulan ini, terdapat empat kelompok utama. Analisis Klaster juga menghasilkan empat kelompok dan sekaligus mengesahkan keputusan PCA. Keputusan Analisis SIMCA tidak berjaya memprofilkan kanabis jika dibandingkan dengan PCA dan Analisis Klaster kerana SIMCA menghasilkan kelas yang berbeza. Sampel kanabis berjaya diprofil dengan menggunakan PCA dan Analisis Klaster yang mendedahkan bahawa sampel kanabis mempunyai asal-usul yang berbeza.

TABLE OF CONTENTS

CHAPTER	TOPIC		PAGE
	DECLARATION		ii
	DEDICATION		iii
	ACKNOWLEDGEMENT		iv
	ABSTRACT		v
	ABSTRAK		vi
	TABLE OF CONTENTS		vii
	LIST OF TABLES		xi
	LIST OF FIGURES		xiii
	LIST OF ABBREVIATIONS		xiv
	LIST OF APPENDICES		xv
1	**INRODUCTION**		
	1.1	Background of Study	1
	1.2	Statement of Problem	3
	1.3	Objectives of Study	3
	1.4	Scope of Study	4
	1.5	Significance of Study	4

1.6 Hypothesis of Study 4

2 LITERATURE REVIEW

2.1 Classification of Drugs 5

2.2 Trends of Drug Abuse in Malaysia 8

2.3 Cannabis 12

2.3.1 Cannabis Origins, Taxonomy & Nomenclature 12

2.3.2 Cannabis as Natural Medicinal Plant 14

2.3.3 Chemical Components of Cannabis 15

2.3.4 Forms of Cannabis Drugs 18

2.4 Drug Analysis 19

2.4.1 Identification of Drugs of Abuse 19

2.4.2 Quantitative Analysis of Drugs of Abuse 23

2.4.3 Forensic Toxicological Analysis of Cannabis 25

2.5 Monolithic Column 26

2.6 Drug Profiling 28

2.6.1 Multivariate Statistical Analysis 30

2.6.2 Principal Component Analysis (PCA) 31

2.6.3 PCA for Drug Analysis 33

2.6.4 Cluster and SIMCA Analysis 33

3 METHODOLOGY

3.1 Introduction 36

3.2 Chemicals & Reagents 36

3.3 Illicit Cannabis Samples 37

3.4 Apparatus 37

3.5 HPLC Instrument 38

3.6 Procedure 38

3.6.1 Extraction of Cannabis Samples 38

3.6.2 Physical Properties of Cannabis Samples 39

3.6.3 Microscopic Analysis of Cannabis Samples 39

3.6.4 High Performance Liquid Chromatography 39

3.6.5 Principal Component Analysis 40

3.6.6 Cluster and SIMCA Analysis 40

4 RESULTS AND DISCUSSION

4.1 Introduction 42

4.2 Illicit Cannabis Samples 42

4.3 Visual Inspection Microscopic Analysis of Illicit Cannabis Samples 44

4.4 HPLC Separation of Cannabis 46

4.4.1 HPLC separation of Cannabis Extracts 46

4.4.2 HPLC separation of Cannabis Standards 48

4.4.3 Chromatographic Profile of Illicit Cannabis 49

4.5 Principal Component Analysis using Unscrambler X 10.0 52

4.5.1 Loading Plot 54

4.5.2 Correlations Loading Plot 54

4.5.3 Score Plot 55

4.5.4 Comparison of HPLC and PCA for Profiling of Illicit Cannabis Extracts 59

4.6 Cluster Analysis 61

4.7 SIMCA Analysis 63

5 CONCLUSIONS AND SUGGESTIONS

5.1 Conclusions 65

5.2 Suggestions 66

REFERENCES 68

APPENDICES 75

LIST OF TABLES

TABLE NO.	TITLE	PAGE
1.1	Number of cases and samples received by Department of Chemistry	2
1.2	Major types of drugs handled by Department of Chemistry from 2005 to 2007	2
2.1	Classification of drugs & their potential medicinal values	7
2.2	Cannabis arrest in South East Asia from 2004 – 2008	9
2.3	Latest reported rank and trend of drug abuse in Malaysia	10
2.4	Drug related arrests in Malaysia	10
2.5	Morphology of cannabis plant of different species	13
2.6	Chemical structure and physical properties of CBD, CBN and Δ^9-THC	17
2.7	Different solvent systems used to analyze illicit cannabis samples And R_f ×100 values for each cannabinoids	23
2.8	Comparison of traditional particulate column and Monolithic Column	27
3.1	Sample code corresponding to area of seizure for twenty five Illicit cannabis samples	37
4.1	Sample codes of cannabis analyzed	43
4.2	Variation of retention time and peak area for day-to-day analysis of three cannabinoids	46

4.3	Variation of retention time and peak area for within-day analysis of three cannabinoids	47
4.4	Groupings of fifty six cannabis extracts based on observation of chromatogram and peak area size	50
4.5	Color, shape & numbering codes to differentiate cannabis extracts from different area of seizures	53
4.6	Groupings of illicit cannabis samples using principal component analysis	55
4.7	Groupings of cannabis extracts based on HPLC Profile and PCA Profile	60

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Schematic diagram of stages involved in drug profiling	20
2.2	Microscopic examination of cannabis samples	21
2.3	Electron microscope image of silica skeleton and macropore of a silica based monolithic column	28
4.1	Cystolithic hairs observed on cannabis leaves	44
4.2	Head of grandular trichomes observed on cannabis leaves	45
4.3	Seed of cannabis plant observed under stereomicroscope	45
4.4	HPLC separation of three cannabinoid standards	48
4.5	HPLC separation of cannabis samples	51
4.6	Score plot of illicit cannabis extracts	56
4.7	Loading plot for illicit cannabis extracts	57
4.8	Correlations loading plot for illicit cannabis extracts	58
4.9	Dendrogram of illicit cannabis extracts	62
4.10	Si vs. Hi Plot for illicit cannabis extracts	64

LIST OF ABBREVIATIONS

CBD	-	Cannabinoid
CBN	-	Cannabinol
THC	-	Tetrahydrocannabinol
PCA	-	Principal Component Analysis
SIMCA	-	Soft Independent Modeling Class Analogy
RSD	-	Relative Standard Deviation
HPLC	-	High Performance Liquid Chromatography
GC	-	Gas Chromatography
PC	-	Principal Component
Rpm	-	Revolution per Minute
UNODC	-	United Nations Office on Drugs and Crime
UNAIDS	-	United Nations Join Programme on HIV/AIDS
UNDCP	-	United Nations International Drugs Control Programme
DAINAP	-	Drug Abuse Information Network for Asia and the Pacific

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
1	Mathematical equation involved in PCA, Cluster and SIMCA analysis	75
2	Unscrambler X 10.0 interface for importing Microsoft Excel data spreadsheet	76
3	Chromatographic data (peak area of cannabinoids) used to perform PCA	77
4	Unscrambler X 10.0 interface to perform PCA	78
5	Unscrambler X 10.0 interface to perform Cluster Analysis	79

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Drug usage is not a new occurrence, the usage of drugs dates back to 10, 000 years ago, where there are archaeological evidences showing the usage of psychoactive substances (Mechoulam, 1973). In Malaysia, drug abuse is reaching an alarming rate and has caused a stir to the country's economy, whereby it has been identified as one of the major social illness among youths in Malaysia. Over the past decade, the main problematic drugs in Malaysian context have been heroin and morphine with total estimate of drug abusers to be within 350,000 to 500,000 (Devaney *et al.*, 2005). However, for the past five years cannabis, heroin, and morphine were the most dominant drugs in Malaysian law enforcement statistics (UNODC, 2010). According to statistics from Department of Chemistry of Malaysia, the number of cases and samples analyzed for illicit cannabis samples are substantial. **Table 1.1** provides details on number of cases and samples of illicit cannabis analyzed by Department of Chemistry from the year 2007 till 2009.

Table 1.1 : Number of cases and samples of cannabis received by Department of Chemistry, Malaysia.

Year	Number of Cases	Number of Samples
2005	2,644	9,263
2006	3,344	21,738
2007	3,226	15,411
2008	2,403	11,075
2009	3,066	13,690

Though the figures are not showing a constant increase or decrease, but the quantity of cannabis samples received for analysis are indeed significant. This shows that cannabis is one of the most abused illicit drugs in Malaysia. Furthermore, cannabis is also one of the most highly trafficked drugs in Malaysian scenario (**Table 1.2)**.

Table 1.2 : Major types of drugs handled by Department of Chemistry, Malaysia from 2005 to 2007.

Type of Drug	2005		2006		2007	
	Cases	**Sample**	**Cases**	**Sample**	**Cases**	**Sample**
ATS	4,641	26,345	4,851	134,362	241	13,356
Cannabis	**2,644**	**9,263**	**3,344**	**21,738**	**3,199**	**15,411**
Cocaine	-	-	-	-	7	125
Codeine	490	1,995	364	2,694	318	4,162
Ecstasy	1,109	48,757	1,483	27,902	385	6,826
Heroin	9,348	49,017	6,908	33,481	8,699	42,136
Ketamine	61	345	192	1,247	428	4,926
Ketum (mitragynine)	205	814	599	4,933	819	10,715
Metamphetamine	-	-	-	-	5,779	70,876
Opium (Candu)	11	47	7	37	6	23
Tablets (poisons)	-	-	-	-	1,524	46,819
Others	416	2,439	201	1,975	189	2,654
TOTAL	**18,925**	**139,022**	**17,949**	**228,369**	**21,594**	**218,029**

From **Table 1.2** cannabis is found to be one of the top five major drugs trafficked. As **Table 1.1** and **Table 1.2** revealed the abuse of cannabis drug in Malaysia, stringent measures are needed to fight crime on trafficking of illicit cannabis samples. This is where the role of drug profiling comes in. The alarming number of seized cannabis samples submitted to forensic laboratories warrants a

need for drug profiling in Malaysia. Drug profiling is the extraction of drug sample's chemical and/ or physical profile based on properties that they portray (Esseiva *et al.*, 2006).

1.2 Statement of Problem

The core of this research is to profile illicit cannabis samples by assessing chemical properties and also by using a type of multivariate analysis known as Principal Component Analysis (PCA). Drug abuse and trafficking in Malaysia has reached an alarming rate. A call for draconian measures to curb drug-related crimes is required in order to preserve our future younger generations. Although drug profiling is not a new phenomenon for overseas countries like United States, Australia and United Kingdom, this branch of forensic study is a new field to be tackled in Malaysia. Extensive research is required in the field of drug profiling in Malaysia to engage in combating against drug abuse and trafficking. These are the problem statements which have initiated research on this area of study.

1.3 Objectives of Study

The objectives of this study are:

i. To develop drug profiling based on chemical properties of the cannabis using high performance liquid chromatography.
ii. To employ Principal Component Analysis as statistical tool to profile cannabis.

1.4 Scope of Study

The scope of research encompasses profiling of illicit cannabis samples using chemical properties and Principle Component Analysis.

1.5 Significance of Study

Characterizing properties of illicit drugs by means of forensic analysis is known as drug profile. Drug profiling can provide intelligence information by linking different samples to a common source. Besides, profiling also helps in retrieving valuable information about the operation of illicit drug supply networks. Adequate intelligence information can facilitate police officers and custom enforcers to narrow down illegal drug trafficking.

1.6 Hypothesis of Study

Illicit cannabis samples can be profiled based on their chemical properties using Principal Component Analysis (PCA). Grouping among illicit cannabis samples can be established using PCA to identify common origin.

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