



**DEVELOPMENT OF COMPREHENSIVE ANALYTICAL
METHODS FOR THE ASSESSMENT OF TOXIC
ELEMENTS IN SELECTED MALAYSIAN MEDICINAL
PLANTS AND THEIR FORMULATION**

BY

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ABSTRACT

Analysis of arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) in herbal medicinal products (HMP) encounter significant analytical challenges. This study aimed to develop, optimize and validate an analytical method for the determination of As, Hg, Cd and Pb in HMP using atomic absorption spectrometer (AAS). To overcome the limitations of hydride generation (HGAAS) and cold-vapor (CVAAS) techniques graphite furnace (GFAAS) was developed for the detection of As and Hg. The technique showed consistent levels of accuracy and precision in spiked HMP samples for As and Hg. The recoveries of arsenic measured by HGAAS – GFAAS were ranged between 81.3-91.5 % and 82.7-93% respectively. Mercury recoveries measured by CVAAS-GFAAS were ranged between 81.7-86.6 % and 80-86.7%. Sample preparation methods were developed by comparing three methods namely WD1, WD2 and WD3 which represented acid digestion with mixture of nitric-hydrochloric acids $\text{HNO}_3 - \text{HCl}$ in a ratio of 1:3 using conventional open vessel heating program, mixture of nitric acids - hydrochloric $\text{HNO}_3 - \text{HCl}$ in a ratio of 1:3 and a mixture of $\text{HNO}_3\text{-H}_2\text{O}_2$ in a ratio of 4:1 using close vessel microwave digestion respectively. The comparison was conducted on HMP spiked with various concentrations of As, Hg, Cd and Pb standard solutions. Recoveries of As were 78.1-82.3 % , 90.5-92.8% , 91.4-93.3%; Hg 74.2-82.1, 87.1-91.9, 86.2-91.4; Cd 81-85,88.2-93.4,87.7-93.5 and Pb 78.1-82.2, 88.1-90.9, 88.7-92.6 for WD1,WD2 and WD3 respectively. The statistical analysis indicated good recoveries by microwave digestion methods WD2 and WD3 compared to method WD1 as they had given a significant high recoveries ($p < 0.05$) compared to method WD1. In further study to evaluated the effect of ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) and palladium nitrate $\text{Pd}(\text{NO}_3)_2$ on the recovery values of As and Hg by comparing three digestion methods namely M1, M2 and M3 that represent microwave digestion without stabilizers addition, with addition of $\text{NH}_4\text{H}_2\text{PO}_4$ and with addition $\text{Pd}(\text{NO}_3)_2$ respectively. Recoveries of As were ranged between 88.5-90.8%, 90-93.7%, 96.1-104% and Hg 88.3-91.8% ,87.4-93.5%, 95.5-100.1% for M1,M2 and M3 respectively. Statistical analysis showed significant high recoveries ($p < 0.05$) for M3 compared to M1 for As and Hg analysis. Optimization of three digestion factors namely A, B and C time of digestion, reagent volume and temperature at high and low levels was performed by design of experiments (DOE) statistical approach. The optimum recoveries for As, Hg, Cd and Pb where found when factor A and C were at high level and factor B was low level. The developed method was validated by the analysis of As, Hg, Cd and Pb in apple leaves 1515 standard reference material (SRM) and applied for the analysis of some commercial HMP samples available in Malaysia. Excellent recoveries were obtained ranged between 98.2-102% for SRM. As was detected in 35%; Hg was found in 60%; Cd was found in 55% and Pb was found at 65% of the total number of the samples.

خلاصة البحث

الزرنِيخ و الزئبق و الكاديوم والرصاص تعتبر من العناصر الخطرة التي تتواجد في مستويات تركيز مختلفة في المنتجات العشبية الطبية. قياس هذه المعادن في مصفوفات معقدة يشكل صعوبات تحليلية كبيرة. لهذا فان هذه الدراسة صممت لغرض تطوير طريقة تحليلية موثوق بها مع تقنية إعداد العينات المرتبطة بها. هدفت هذه الدراسة إلى تطوير وتحسين والتحقق من صحة الطريقة التحليلية لتحديد الزرنِيخ و الزئبق و الكاديوم و الرصاص في المنتجات العشبية الطبية باستخدام مطياف الامتصاص الذري. للتغلب على القيود المفروضة على طريقة توليد الهيدريدات والبخار البارد تم تطوير تقنية الجرافيت الفرن كوسيلة بديلة للكشف عن الزرنِيخ و الزئبق. وأظهرت هذه التقنية مستويات مقبولة من الدقة في عينات من المنتجات العشبية الطبية وقد تراوحت استردادات الزرنِيخ في العينات التي تم قياسها من قبل تقنية توليد الهيدرات - وفرن الجرافيت بين 81.3-91.5% و 82.7-93% على التوالي. وقد تراوحت استردادات الزئبق التي تقاس بتقنية البخار البارد و فرن الكرافيت بين 81.7-86.6% و 80-86.7%. التقييم الإحصائي للعينات المستقلة دل على عدم وجود فروقات احصائية بين نتائج التقنيات المستخدمة. الجزء الثاني من الدراسة تم فيه تطوير طريقة اعداد العينات حيث تمت المقارنة بين ثلاثة طرق وهي الهضم بمزيج من حامض النيتريك و الهيدروكلوريك باستخدام طريقة التسخين التقليدية و كذلك باستخدام طريقة التسخين بالميكرويف و الهضم بمزيج حامض النيتريك و محلول بيروكسيد الهيدروجين باستخدام المايكرويف ايضا. وقد تراوحت استردادات الزرنِيخ و الزئبق والكاديوم و الرصاص ما بين 78.1-81.3%، 90.5-92.8%، 91.4-93.3%، 74.2-82.1%، 87.1-91.9%، 86.2-91.4% و 81-85% ، 88.2-93.4%، 87.7-93.5% و 78.1-82.2%، 88.1-90.9%، 88.7-92.6%. التقييم الاحصائي للعينات المستقلة دل على وجود فروقات احصائية هامة بين طريقتي الهضم بالميكرويف و طريقة الهضم باستخدام التسخين التقليدي. في هذا البحث تم ايضا دراسة تأثير المثبتات الغير عضوية على تثبت العناصر المتطايرة خلال عملية الهضم و هي فوسفات ثنائي الهيدروجين امونيوم و نترات البلاديوم. اثبتت الدراسة بالدلائل الحصائية ان اضافة نترات البلاديو كان لها تأثير ايجابي على استردادات الزرنِيخ. و الزئبق قمنا في هذا البحث ايضا بدراسة العوامل المؤثرة على عملية الهضم بالميكرويف و تحديدا الوقت المحدد للتفاعل و حجم الحامض المستعمل في عملية الهضم و درجة حرارة التفاعل و ذلك باستخدام النظام الاحصائي المسمى بتصميم التجارب. الاسترداد الامثل لجميع العادن وجد عندما كان عامل درجة الحرارة و الوقت في المستوى العالي.

APPROVAL PAGE

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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I would like to dedicate this study to my family especially to Isra my beloved daughter and my best friend, you have been the source of encouragement and spiritual support.

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TABLE OF CONTENTS

Abstract	ii
Abstract in Arabic	iii
Approval Page.....	iv
Declaration	v
Copyright	vi
Dedication	vii
Acknowledgements	viii
Table of contents	ix
List of Tables	xiii
List of Figures	xvi
List of Abbreviations	xviii
CHAPTER ONE: INTRODUCTION	1
1.1 Background Of The Study	1
1.2 Quality and Safety of Herbal Medicinal Products	3
1.2.1 Contamination of HMP with Heavy Metals	3
1.3 Elemental Analysis	5
1.3.1 Analytical Methods for Elements Analysis in HMP.....	6
1.3.2 Method Development for Elements Analysis in HMP	6
1.4 Problem Statement.....	8
1.5 Research Objectives.....	10
1.5.1 General Objectives	10
CHAPTER TWO: LITERATURE REVIEW.....	11
2.1 Herbal Medicinal Products an Overview.....	11
2.1.1 Importance and Uses of Medicinal Plants/herbal products.....	12
2.1.2 Quality and Safety Aspects of HMP	15
2.2 International Health Organizations.....	17
2.3 Effect of the Accumulation of Some Heavy Metals on Human Health	19
2.3.1 Arsenic	20
2.3.2 Mercury	21
2.3.3 Cadmium (Cd).....	22
2.3.4 Lead (Pb).....	23
2.4 Heavy Metals in Herbal Medicinal Products.....	24
2.5 Sample Preparation for Element Analysis.....	33
2.5.1 Quality Control (QC) of Sample Preparation Technique.....	34
2.5.2 Sample Preparation Procedures.....	35
2.5.2.1 Liquid Samples	35
2.5.2.2 Solid Samples	37
2.5.2.2.1 Dry Ashing.....	38
2.5.2.2.2 Wet Digestion	39
2.5.2.2.3 Conventional Open Vessel Digesting System	41
2.5.2.2.4 Close Vessel Microwave Heating System	45
2.5.2.2.5 Close Vessels Digestion System.....	46

2.5.2.2.6	Ultrasound-assisted extraction	51
2.5.2.2.7	Slurry Sample Preparation	54
2.6	Main Analytical Techniques Used for Elemental Analysis in Various Matrices	56
2.6.1	Analytical Methods	57
2.6.1.1	Atomic Absorption Spectroscopy (AAS)	57
2.6.1.1.1	Flame atomic absorption spectrometer (FAAS)	59
2.6.1.1.2	Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)	60
2.6.1.1.3	Hydride Generation Atomic Absorption Spectroscopy (HGAAS)	63
2.6.1.1.4	Cold Vapor Atomic Absorption Spectrometer (CVAAS)	64
2.6.1.1.5	Atomic Emission and Fluorescence Spectroscopy (AES) and (AFS)	66
2.6.1.1.6	Inductively Coupled Plasma Mass spectrometry (ICP-MS)	68
2.6.1.1.7	Inductively Coupled Plasma/Optical Emission Spectrometry (ICP-OES)	69
2.6.1.2	X-ray fluorescence (XRF)	72
2.6.1.3	Neutron activation analysis (NAA)	73
2.6.1.4	Anodic Stripping Voltammetry (ASV)	74
CHAPTER THREE:	METHODOLOGY	76
3.1	Chemicals and Reagents	77
3.2	Glassware	78
3.2.1	Treatment of glass wares	78
3.3	Equipment and Instrument	78
3.3.1	Microwave Digester	78
3.3.2	Atomic Absorption Spectrometer (AAS)	79
3.3.2.1	Graphite Furnace Atomic Absorption Spectrometer (GFAAS)	79
3.3.2.1.1	Heating Sequences Steps of GFAAS	80
3.3.2.2	Flow Injection atomic absorption spectrometer (FIAS)	81
3.3.2.2.1	Hydride Generation Atomic Absorption Spectrometer (HGAAS)	81
3.3.2.2.2	Cold Vapor Atomic Absorption Spectrometer (CVAAS)	81
3.4	Statistical Analysis	82
3.5	Standards and Reagents Preparation	83
3.5.1	Standards Preparation for GFAAS	83
3.5.2	Reagents and Standards Preparation for HGAAS	83
3.5.2.1	Preparation of Reducing Reagents	83
3.5.2.2	Carrier Solution	84
3.5.2.3	Arsenic Standards Preparations for HGAAS	84
3.5.3	Reagents and Standards for CVAAS	84
3.5.3.1	Reducing Solutions	84
3.5.3.2	Mercury stabilizer	84
3.5.3.3	Carrier Solution	85

3.5.3.4 Mercury Standards Preparations for CVAAS	85
3.6 Development and optimization of analytical method for the determination of As, Hg, Ca and Pb different techniques of AAS.....	85
3.6.1 Limit of detection (LOD) and limit of quantification (LOQ)	85
3.6.2 Linearity	86
3.6.3 Accuracy	86
3.6.4 Precision.....	87
3.6.5 Evaluation of GFAAS as an alternative method for the determination of As and Hg in HMP.....	87
3.6.6 Effect of Different Digestion Techniques on Elements Recoveries for Spectroscopic Analysis in HMP.....	88
3.6.6.1 Method WD1	89
3.6.6.2 Method WD2	89
3.6.6.3 Method WD3	90
3.6.7 Evaluation to the effect of inorganic stabilizers addition on As and Hg recoveries in HMP samples	90
3.6.7.1 M1: Microwave digestion without addition stabilizer.....	91
3.6.7.2 M2: Microwave digestion with NH ₄ H ₂ PO ₄ addition.....	91
3.6.7.3 M3: Microwave digestion with Pd (NO ₃) ₂ addition	91
3.6.8 Optimization of Digestion Factors using DOE	92
3.6.8.1 Sample Preparation for Optimization of Digestion Factors	92
3.7 Method Validation	93
3.7.1 Analysis of Standard Reference Material	93
3.8 Analysis of Commercial Herbal Medicinal Products	93

CHAPTER FOUR: RESULTS AND DISCUSSION95

4.1 Evaluation of GFAAS as an Alternative Instrumental Method for Determining arsenic and mercury in Herbal Medicinal Products	95
4.1.1 Arsenic Analysis in HMP	96
4.1.1.1 Analytical Merit for HGAAS and GFAAS Techniques for Arsenic Analysis.....	97
4.1.1.2 Optimization of the instrumental conditions for arsenic analysis by GFAAS	98
4.1.1.3 Accuracy and Precision of HGAAS and GFAAS for As analysis in Standard QC Samples.....	100
4.1.1.4 Comparison of HGAAS and GFAAS for As Analysis Using Matrix Spike Experiments	101
4.1.2 Mercury Analysis in HMP	108
4.1.2.1 Analytical Merit for CVAAS and GFAAS for Mercury Analysis.....	110
4.1.2.2 Optimization of the instrumental conditions for mercury analysis by GFAAS.....	111
4.1.2.3 Accuracy and Precision of CVAAS and GFAAS for Hg analysis in QC Standard Samples	113
4.1.2.4 Comparison of CVAAS and GFAAS for Hg Analysis Using Spike Matrix Experiments	114
4.2 Analytical parameters for cadmium and lead measurements using GFAAS	120

4.2.1 Linearity, LOD and LOQ for Cadmium and Lead Analysis by GFAAS Technique	120
4.2.2 Accuracy and Precision of GFAAS for Cd and Pb analysis in QC Standard Samples	120
4.2.2.1 Cadmium (Cd)	120
4.2.2.2 Lead (Pb)	121
4.3 Effect of Different Digestion Techniques on Elements Recoveries for Spectroscopic Analysis in Herbal Medicinal products	123
4.3.1 Comparison of digestion methods.....	125
4.3.1.1 Arsenic (As).....	125
4.3.1.2 Mercury (Hg).....	129
4.3.1.3 Cadmium (Cd).....	133
4.3.1.4 Lead (Pb)	137
4.4 Influence of Inorganic Stabilizers on Releasing Volatile As and Hg metal ions from Complex Matrices of H in Spectroscopic Elemental Analysis	147
4.4.1 Arsenic (As)	148
4.4.2 Mercury (Hg)	152
4.5 Optimisation of Various Digestion Parameters for the Determination of arsenic, MERCURY, CADMIUM and lead	158
4.5.1 Arsenic (As)	161
4.5.2 Lead (Pb).....	167
4.5.3 Mercury (Hg)	174
4.5.3.1 Cadmium (Cd).....	182
4.5.4 Analysis of As, Hg, Cd and Pb in Standard Reference Material (SRM).....	193
4.5.5 Analysis of As, Hg, Cd and Pb in Commercial Herbal Medicinal Products	193
CHAPTER FIVE: CONCLUSION	198
REFERENCES.....	203
OUTCOME OF THE STUDY	221
APPENDIX I: METALS STANDARD CURVES	223
APPENDIX II: OPTIMIZATION OF Pd (NO₃)₂ VOLUME.....	225
APPENDIX III:	226

LIST OF TABLES

<u>Table No.</u>		<u>Page No.</u>
Table 2.1	Permissible limits of major toxic metals in finished herbal product in different countries ($\mu\text{g/g}$).	18
Table 2.2	Mineral acids and oxidizing agents used for wet digestion	40
Table 2.3	Summary of AAS and ICP analytical techniques	71
Table 3.1	Metals concentration in SRM 1515	77
Table 3.2	Instrumental parameters (GFAAS) for the determination of As, Hg, Cd and Pb	80
Table 3.3	Instrumental parameters of HGAAS and CVAAS techniques for As and Hg analysis.	82
Table 3.4	Prepared concentrations QC for As, Hg, Cd and Pb	86
Table 3.5	Concentrations As and Hg spike HMP samples	88
Table 3.6	Concentration of As, Hg, Cd and Pb in spike samples	89
Table 4.1	Analytical parameters for HGAAS, GFAAS for As analysis	97
Table 4.2	Arsenic concentrations in QC standard solutions and respective accuracy and precision (RSD) of HGAAS and GFAAS	100
Table 4.3	Measured concentrations of As in spiked samples with $0.7 \mu\text{g/g}$ As standard accuracy and precision by HGAAS and GFAAS	102
Table 4.4	Measured concentrations of As in spiked samples with $0.9 \mu\text{g/g}$ As standard precision measured by HGAAS and GFAAS	102
Table 4.5	Analytical for CVAAS & GFAAS techniques for Hg analysis	110
Table 4.6	Mercury concentrations of three QC samples, recovery precision RSD measured by CVAAS, GFAAS techniques.	113
Table 4.7	Concentrations of Hg spiked samples $2 \mu\text{g/g}$ As standard accuracy and precision measured by CVAAS and GFAAS	114
Table 4.8	Concentrations of Hg spiked samples with $4 \mu\text{g/g}$ As standard accuracy and precision measured with CVAAS and GFAAS	115
Table 4.9	Linear ranges, R^2 LOD and LOQ values for Cd and Pb analysis by GFAAS	120

Table 4.10	Cadmium concentrations in QC standard solutions and their accuracy and precision (RSD) measured by GFAAS	121
Table 4.11	Lead concentrations in QC standard solutions respective accuracy precision (RSD) measured by GFAAS	121
Table 4.12	Linear ranges, R ² coefficient correlation, standard concentration absorbance for As, Hg, Cd and Pb measured by GFAAS	122
Table 4.13	Measured concentrations, and precision of As in spiked samples with 1 µg/g of As standard and digested by WD1, WD2 and WD3	126
Table 4.14	Measured concentrations, recoveries of As in spiked samples with 2 µg/g of As standard digested by WD1, WD2 and WD3	126
Table 4.15	Measured concentrations, precision of Hg in spiked samples with 6 µg/g of Hg standard digested by WD1,WD2, WD3	130
Table 4.16	Measured concentrations, precision of Hg in spiked samples with 9 µg/g of Hg standard digested by WD1,WD2, WD3	130
Table 4.17	Measured concentrations,precision of Cd in spiked sample 0.2 µg/g of Cd standard digested by WD1, WD2 and WD3	134
Table 4.18	Measured concentrations, precision of Cd in spiked samples 0.4 µg/g of Cd standard digested by WD1, WD2 and WD3	134
Table 4.19	Measured concentrations, precision of Pb in spiked samples with 5 µg/g of Pb standard digested by WD1,WD2 and WD3	138
Table 4.20	Measured concentrations, precision of Pb in spiked samples with 7 µg/g of Pb standard digested by WD1, WD2 and WD3	138
Table 4.21	Measured concentrations, precision of As in spiked samples 1 µg/g of As standard and digested following M1, M2 and M3	148
Table 4.22	Measured concentrations µg/g and precisions for HMP spiked with 2 µg/g of As standard and digested M1, M2 and M3	149
Table 4.23	Measured concentrations of Hg in spiked HMP samples with 6 µg/g of Hg standard and digested using M1, M2 and M3	152
Table 4.24	Measured concentrations, precision of Hg in spiked HMP samples with 9 µg/g of Hg digested using M1, M2 and M3	153
Table 4.25	Factors A,B and C with their levels and response for As, Hg, Cd and Pb	160
Table 4.26	Studied effects and their contribution percentages to the digestion processes	162

Table 4.27	ANOVA test for arsenic analysis in HMP	162
Table 4.28	Three factors with the actual and predicted response	164
Table 4.29	Studied effects and their contribution percentages to the digestion processes of Pb in HMP	167
Table 4.30	ANOVA test for lead analysis in HMP	169
Table 4.31	Three factors with actual and predicted response and residual values of Pb	170
Table 4.32	Studied effects and their contribution percentages to digestion processes of Hg in HMP	175
Table 4.33	ANOVA test for mercury analysis in HMP	177
Table 4.34	Three factors with actual, predicted and residual recovery values for Hg	178
Table 4.35	Studied effects and their contribution percentages to digestion processes of Cd in HMP	182
Table 4.36	ANOVA test for cadmium analysis in HMP	184
Table 4.37	Three factors, actual and predicted response with the residual values for Cd	185
Table 4.38	Concentrations of As, Hg, Cd and Pb in SRM and the recovery percentages	193
Table 4.39	Concentration of As, Hg, Cd and Pb $\mu\text{g/g}$ (\pm SD) in commercial HMP	194

LIST OF FIGURES

<u>Figure No.</u>		<u>Page No.</u>
Figure 2.1	Schematic heat transfer to samples by (A) conventional heating (B) microwave heating.	45
Figure 2.2	Basic atomic absorption spectrometer	58
Figure 2.3	Basic ICP-MS	68
Figure 2.4	Basic ICP-OES	70
Figure 3.1	Typical Graphite Tube Atomizer of AAS Analyst 800	80
Figure 4.1	Pyrolysis temperature curve of 15 ppb As standard solution	99
Figure 4.2	Atomization temperature curves of 15 ppb As standard solution	99
Figure 4.3	Comparison of HGAAS and GFAAS for the determination of As in three samples of HMP spiked with 0.7 $\mu\text{g/g}$	103
Figure 4.4	Comparison of HGAAS and GFAAS for the determination of As in three samples of HMP spiked with 0.9 $\mu\text{g/g}$	104
Figure 4.5	Pyrolysis temperature curve of 40 ppb Hg standard solution	111
Figure 4.6	Atomization temperature curve of 40 ppb Hg standard solution	112
Figure 4.7	Comparison between CVAAS and GFAAS for determination of mercury in three samples of HMP spiked with 2 $\mu\text{g/g}$.	116
Figure 4.8	Comparison between CVAAS and GFAAS for determination of mercury in three samples of HMP spiked with 4 $\mu\text{g/g}$	116
Figure 4.9	Comparison of arsenic concentration in three samples of HMP spiked with 1 $\mu\text{g/g}$ and digested with WD1, WD2, WD3.	127
Figure 4.10	Comparison of As concentrations in three samples of HMP spiked with 2 $\mu\text{g/g}$ and digested with WD1, WD2 and WD3.	128
Figure 4.11	Comparison of Hg concentrations in three samples of HMP spiked with 6 $\mu\text{g/g}$ and digested with methods A, B and C.	131
Figure 4.12	Comparison of Hg concentrations in three samples of HMP spiked with 9 $\mu\text{g/g}$ and digested with WD1, WD3 and WD3	132
Figure 4.13	Comparison of Cd concentrations in three samples of HMP spiked 0.2 $\mu\text{g/g}$ and digested with methods WD1, WD2, WD3.	135

Figure 4.14	Comparison of Cd concentrations in three samples of HMP spiked 0.4 $\mu\text{g/g}$ and digested with methods WD1, WD2, WD3.	136
Figure 4.15	Comparison of Pb concentrations in three samples of HMP spiked 5 $\mu\text{g/g}$ and digested with methods WD1, WD2, WD3	139
Figure 4.16	Comparison of Pb concentrations in three samples of HMP spiked 7 $\mu\text{g/g}$ and digested with methods WD1, WD2, WD3	140
Figure 4.17	Comparison of As concentrations in three samples of HMP spiked with 1 $\mu\text{g/g}$ and digested with methods M1, M2, M3.	150
Figure 4.18	Comparison of As concentrations in three samples of HMP spiked with 2 $\mu\text{g/g}$ and digested with methods M1, M2, M3.	150
Figure 4.19:	Comparison of Hg concentrations in three samples of HMP spiked with 6 $\mu\text{g/g}$ of Hg standard and digested M1, M2, M3.	154
Figure 4.20:	Comparison of Hg concentrations in three samples of HMP spiked with 9 $\mu\text{g/g}$ of Hg standard and digested M1, M2, M3.	154
Figure 4.21	Arsenic half normal plot	161
Figure 4.22	Response surface of BC interaction as significant factors	165
Figure 4.23	Cube plot for factors A, and C for As analysis in HMP	166
Figure 4.24	Lead half normal plot graph	168
Figure 4.25	Response surface for AB interaction.	171
Figure 4.26	Response surface of AC interaction for Pb recovery in HMP.	172
Figure 4.27	Response surface of BC interaction of Pb recovery in HMP.	173
Figure 4.28	Cube plot for ABC interaction of Pb recovery in HMP	174
Figure 4.29	Mercury half normal plot graph	176
Figure 4.30	Response surface for AC interaction for Hg recoveries in HMP	179
Figure 4.31	Response surface for BC interaction for Hg recoveries in HMP	180
Figure 4.32	Cube plot for ABC interaction for Hg analysis in HMP	181
Figure 4.33	Cadmium half normal plot graph	183
Figure 4.34	Response surface for AB interaction	186
Figure 4.35	Response surface of BC interaction	186
Figure 4.36	Cube plot for ABC interaction of Cd recovery in HMP.	187

LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectrometer
Al	Aluminum
AES	Atomic Emission Spectrometry
ANOVA	Analysis of Variance
ASV	Anodic Stripping Voltammetry
As	Arsenic
AFS	Atomic Fluorescence Spectroscopy
As ₂ O ₃	Arsenic Trioxide
Ba	Barium
B	Boron
AsO ₄ ³⁻	Arsenates
Ca	Calcium
Cd	Cadmium
CNS	Central Nervous System (CNS)
Co	Cobalt
Cr	Chromium
Cu	Copper
CVAAS	Cold Vapor Atomic Absorption Spectrometer
DRC	Dynamic Reaction Cell
DCA	Drug Control Authority
DOE	Design of Experiment
DTA	Direct Temperature Control
DPC	Direct Pressure Control
DPP	Differential Pulse Polarography
EDL	Electrodeless Discharge Lamp
FAAS	Flame Atomic Absorption Spectrometer
FDA	Food and Drug Administration
Fe	Iron
FIAS	Flow Injection Atomic Absorption Spectroscopy
G	Gram

GAP	Good Agricultural Practices
GC	Gas Chromatography
GFAAS	Graphite Furnace Atomic Absorption Spectrometer
GMP	Good Manufacturing Practices
GSP	Good Storage Practice
KMnO ₄	Potassium Permanganate
H ₂ O ₂	Hydrogen Peroxide
HCL	Hollow-Cathode Lamp
HCl	Hydrochloric Acid
HClO ₄	Perchloric Acid
Hg	Mercury
Hg ₂ ⁺⁺	Mercurous
Hg ²⁺	Mercuric
HGAAS	Hydride Generation Atomic Absorption
HGAFS	Hydride Generation Atomic Fluorescence Spectrometry
HNO ₃	Nitric Acid
HPLC	High Performance Liquid Chromatography
HF	Hydrofluoric acid
H ₂ SO ₄	Sulfuric acid
HMP	Herbal Medicinal Products
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively coupled plasma-mass spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
INAA	Instrumental Neutron Activation Analysis
Kg	Kilogram
KI	Potassium Iodide
L	Liter
LOD	Limit of Detection
LOQ	Limit of Quantification
LVMWD	Low Volume Microwave Digestion
Li	Lithium

MHS	Mercury Hydride System
Mg (NO ₃) ₂	Magnesium Nitrate
Mg	Magnesium
Mg	Milligram
µg	Microgram
Mn	Manganese
Mo	Molybdenum
NaBH ₄	Sodium-Borohydride
NaOH	Sodium Hydroxide
Ng	Nano-gram
NH ₄ H ₂ PO ₄	Ammonium Dihydrogen Phosphate
Ni	Nickel
NIST	National Institute of Standards and Technology
NAA	Neutron Activation Analysis
NPRA	National Pharmaceutical Regularity Agents
OCL	Oral Component Limit
OTC	Over The Counter
Pb	Lead
H ₂ PO ₄	Phosphoric acid
HClO ₄	Perchloric acid
PTFE	Polytetrafluorethylene
PL	Permissible Limit
KMnO ₄	Potassium Permanganate
Ppb	Part Per Billion
P	Phosphor
Ppm	Part Per Million
Ppt	Part Per Trillion
PTWI	The Provisional Tolerable Weekly Intake
ROS	Reactive Oxygen Species
RSD	Relative Standard Deviation
SD	Standard Deviation
S	Sulfur
SPSS	Statistical Package for the Social Sciences

SRM	Standard Reference Material
S	Sulfur
TCM	Traditional Chinese Medicine
TTM	Traditional Tibetan Medicine
Ti	Titanium
V	Vanadium
WHO	World Health Organization
XRF	X-Ray Fluorescence
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Medicinal plants have been used over the course of thousands of years for curatives and palliatives purposes. General knowledge of plants remedies in old cultures developed via trial and error over many centuries and the most important therapies were verbally passed through generations (Khan, 2014).

Currently medicinal plants and their preparation are still in high demand in many nations all over the world. The research with the aid of advanced technical approaches had well identified the most important plant species used around the world as medicinal and/ or herbal preparation furthermore they had successfully screened many of their chemical constitutes and reviewed their pharmacological activities to evaluate their therapeutic effectiveness. Therefore, medicinal plants and herbal medicinal products (HMP) continued to be accepted and supported by the global communities as an important practice of the entire healthcare system (Mosihuzzaman and Choudhary, 2008).

The importance of herbal medicinal products is indicated by the fact that more than 70% of the world's population rely on HMP for their prime healthcare needs. The growing importance of such products is referred not only to their claimed therapeutic characteristics but to the consumer's preference for HMP due to the growing concerns over the side effects of the modern medicines in addition to the abundance and the affordability of these products. Such factors have gained global economic importance to HMP which will drive the global market of these products to \$107 billion by the end of 2017 as estimated by Global Industry Analysis (Joshi and Shankar, 2016).

Owing to the rapid growing of HMP global market size, international health institutes such as World Health Organization (WHO) had offered guideline regarding quality control, safety and efficacy issues of HMP. Such guideline generally imposed the application of good manufacturing practices (GMP) worldwide and specifically recommended regular monitoring for heavy metal content in finished herbal products and raw medicinal plants materials (WHO 2007).

In Malaysia, versatile medical options are practiced in addition to the conventional medical system like reflexology and aromatherapy as well as various types of herbal preparation. Significant proportion of the population relies on HMP for their health care needs (Akram et al., 2015).

Malaysian Ministry of Health considered HMP as an essential part of the national healthcare structure. Therefore, initiation of quality assurance systems is of importance to promote rational use of such products (Zhang et al., 2012).

The Malaysian herbal industry has been recognized as a new growing sector in the national economy. Malaysian Government has identified herbal medicine industry as one of the key strategic sectors that will support the growth of the Malaysian economy. Therefore, this industry should possess the highest standard of quality, safety and efficacy to meet the international health certification standard and to achieve long-term success (Ahmed and Othman, 2013).

The registration criteria established by the Drug Control Authority (DCA) placed several regulations to confirm the safety and efficacy of herbal medicinal products before marketing process. Limits for heavy metals was an important factor listed in the registration criteria followed by further quality issues like microbial contamination, absence of adulterants and herbs with known adverse effects as well as

agreement with good manufacturing practice (GMP) and good storage practice (GSP) (Jayaraj, 2010).

1.2 QUALITY AND SAFETY OF HERBAL MEDICINAL PRODUCTS

Despite the health and economic importance of the HMP at global and local level there are many concerns exist to their usage. The quality and safety of HMP are major issues facing the manufacturing and marketing processes other than being critical issues for competent health authorities.

1.2.1 Contamination of HMP with Heavy Metals

In general, heavy metals are major environmental contaminants. Their presence in various samples such as soil, water and plants is mainly initiated from industrial wastes, mining activities and the use of pesticides and chemical fertilizers (Chan, 2003). Medicinal plants and their finished products are also vulnerable to metal contamination as stated by number of published researches worldwide (Saper et al., 2004; Martena et al., 2010; Harriet et al., 2012; Rehman et al., 2013).

The contaminants are either originated from the raw materials due to the capability of medicinal plants to accumulate pollutants especially heavy metals or they might develop during the manufacturing courses. Heavy metals accumulation in HMP poses health hazards that are subsequently provoke safety concerns among consumers (Manan et al., 2015).

The toxicity of some heavy metals such as arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) has been recognized to be a major health hazard. They do not have any