# 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 $HNO_3$ – HCl in a ratio of 1:3 using conventional open vessel heating program, mixture of nitric acids - hydrochloric $HNO_3$ – HCl in a ratio of 1:3 and a mixture of $HNO_3$-$H_2O_2$ 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 methodWD1. In further study to evaluated the effect of ammonium dihydrogen phosphate ($NH_4H_2PO_4$) and palladium nitrate Pd $(NO_3)_2$ 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 $NH_4H_2PO_4$ and with addition Pd $(NO_3)_2$ 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

الزرنيخ و الزئبق و الكادميوم والرصاص تعتبرمن العناصر الخطرة التي تتواجد في مستويات تركيز مختلفة في النتجات العشبية الطبية. قياس هذه المعادن في مصفوفات معقدة يشكل صعوبات تحليلية كبيرة. لهذا فان هذه الدراسة صممت لغرض تطوير طريقة تحليلة موثوق بها مع تقنية إعداد العينات المرتبطة بها. هدفت هذه الدراسة إلى تطوير وتحسين والتحقق من صحة الطريقة التحليلية لتحديد الزرنيخ و الزئبق و الكادميوم و الرصاص في النتجات 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 الزرنيخ و الزئبق. وأظهرت هذه التقنية مستويات مقبولة من الدقة في عينات من المنتجات العشبية الطبية وقد تراوحت استردادات الزرنيخ 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 النيترك و الهيدروكلويك باستخدام طريقة التسخين التقليدية و كذلك باستخدام طريقة التسخين بالميكرويف و الهضم بمزيج حامض النتيرك و محلول بيروكسيد الهيدوجين باستخدام المايكرويف ايضا. وقد تراوحت استردادات الزرنيخ و الزئبق والكادميوم و الرصاص ما بين 78.1-86.2,%91.9-87.1,%82.1-74.2و%93.3-91.4,%92.8-90.5,%81.3-91.4 و81-85% , 88.2-93.4%,87.7-93.5% و78.1-82.2%,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 تاثير المثبتات الغير عضوية على تثبت العناصر المتطايرة خلال عملية الهضم و هي فوسفات ثنائي الهيدروجين امونييوم و نترات البلاديوم. اثبتت الدراسة بالدلائ الحصائية ان اضافة نترات البلاديو كان لها تاثير ايجابي على استردادات الزرنيخ. والزئبق 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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(Reem Saadi Khalid)

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| AAS | Atomic Absorption Spectrometer |
| Al | Aluminum |
| AES | Atomic Emission Spectrometry |
| ANOVA | Analysis of Variance |
| ASV | Anodic Striping Voltammetry |
| As | Arsenic |
| AFS | Atomic Fluorescence Spectroscopy |
| $As_2O_3$ | 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 |
| $H_2O_2$ | 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 |
| $H_2SO_4$ | 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 |
| $NH_4H_2PO_4$ | 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 |
| $H_2PO_4$ | Phosphoric acid |
| $HClO_4$ | Perchloric acid |
| PTFE | Polytetrafluorethylene |
| PL | Permissible Limit |
| KMnO4 | 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 heath 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