ANGLIA RUSKIN UNIVERSITY

FACULTY OF SCIENCE AND ENGINEERING

SCHOOL OF LIFE SCIENCE

HERBAL MEDICINE USE IN EKITI STATE, NIGERIA: EPIDEMIOLOGICAL STUDY AND ANALYSIS OF TOXIC CONSTITUENTS

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A thesis in partial fulfilment of the requirements of Anglia Ruskin University for the degree of Doctor of Philosophy

Submitted: October 2018

ANGLIA RUSKIN UNIVERSITY

Acknowledgment

I thank the lord almighty for the gift of life and the resources to come this far.

I appreciate the sacrifice and support of my parents Dr. Kolawole and Mrs. Oluwayemisi Aina in ways so invaluable to me. I do not take for granted the blessing you are to me. Thank you a million times.

To my Wife Folasade, without whom none of this would have been possible; without whose self-denial of the valuable time away from home I should not have succeeded. You are a jewel of inestimable value and a solid rock. To my children thanks for their patience, I am coming home. To my siblings, thanks for your support and standing in the gap in my absence. I love you all.

I especially recognise and thank my supervisors, Dr. Lata Gautam and Dr. Sarah Hall. I really appreciate your selfless, thorough and considerate contributions and mentoring. It was a wonderful privilege that my path to have crossed those of such wonderful people like you. Thanks for believing in me even when I do not. To my external supervisor, Professor Padam Shimkhada, are due profound thanks for time devoted, invaluable contribution and warm, generous reception during our Liverpool meeting.

Thanks to laboratory technologists Kevin Bright and Joanne Hooson for their support and direction. It was fun working with you both. I also appreciate Analytix for offering to analyse some of my samples.

My sincere thanks and appreciation to every other person from my family, faculty staff, colleagues, research assistants and the larger society who have contributed in one way or another.

ABSTRACT

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Herbal medicines (HM) use and popularity as a form of complementary and alternative medicine (CAM) continue to increase globally. However, public health and safety concerns also continue to grow. Contamination of HM samples with chlorinated pesticides and heavy metals has been reported, likewise adulteration with prescription medication.

In this research, an interdisciplinary method drawn from public health and forensic chemistry was adopted. It examined the knowledge and use of HM in Ekiti state, Southwest Nigeria, with a population of over two million. A survey was used to explore public knowledge, use, and perception of HM effectiveness and safety. Textual analysis, inferential and descriptive statistics were used to analyse survey data. Hospital data from the state was also examined to determine HM related casualty and fatality figures over a period of 5 years (2010 to 2014) and the findings were compared with the survey findings. Ten commonly used HM identified in the survey were then analysed for the possible presence of prescription medicines and heavy metals using Gas Chromatography-Mass Spectrometry (GC-MS) and Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) respectively.

Findings of this research showed that 85.0% of the respondents have used HM in the last two years. Although 57.0% had concerns about the safety of uncertified HM, 37.3% used them anyway, while 31.9% used both uncertified and certified HMs. The use of HM (85.0%) was attributed to its effectiveness by 39.6% of users; while poor service delivery was the primary reason 45.2% of respondents did not use the orthodox health system. There was a significant association between HM use and the age, gender, level of education, religion, annual income and occupation of respondents, using the Chi-square analysis at a significance level of 0.05. Hospital records of patients (n=94,323) showed a small number of HM associated paediatric admissions (0.5%), adult admissions (0.06%), paediatric deaths (3.2%) and adult deaths (0.2%). Analysis of all the studied HMs showed that cadmium and copper were detected at above World Health Organization (WHO) permissible limits and one of the samples also had lead and zinc above the limit. However, none of the target pharmaceutical compounds was detected in the HM samples.

This research investigated the issues surrounding the use of HMs, their potential toxicity, casualties, and fatalities. The onset and progression of some medical problems as highlighted in this research may be connected with exposure to heavy metals when present above permissible limits. Additionally, even when metals are present below permissible limits, prolonged exposure may result in accumulative toxicity. Therefore this study highlights a major public health concern and a need to monitor and control HMs through appropriate legislative changes. This is the first multidisciplinary investigation of HMs in the study population and the wider public.

Key Words: Herbal medicine, use, and perception, casualty and fatality, drug analysis, heavy metals.

Table of Contents

List of Figures

List of Tables

List of Acronyms

1H NMR	Proton Nuclear Magnetic Resonance
AD	Anno Domini
ACT	Artemisinin-Based Combination Therapy
AFS	Atomic Fluorescence Spectrometry
AIDS	Acquired Immune Deficiency Syndrome
AMS	Ambient Mass Spectrometry
ANOVA	Analysis of Variance
ATM	African Traditional Medicine
ATR	Attenuated Total Reflectance
ATR-IR	Attenuated Total Reflectance Infrared Spectroscopy
ATSDR	Agency for Toxic Substances and Disease Registry
BC	Before Christ
BCE	Before Common Era
BBC	British Broadcasting Cooperation
CAM	Complementary and Alternative Medicine
CBN	Central Bank of Nigeria
CDC	Centre for Disease Control
CE	Common Era
CE-C4D	Capillary Electrophoresis Coupled with Contactless Conductivity Detection
CE-MS	Capillary Electrophoresis-Mass Spectrometry
CHM	Chinese Herbal Medicine
CHMP	Committee for Medicinal Products for Human Use
CI	Chemical Ionization
CNS	Central nervous system
CRM	Certified Reference Material
CVAAS	Cold Vapour Atomic Absorption Spectrometry
DART	Direct Analysis in Real Time
DCBI-MS	Desorption Corona Beam Ionisation-Mass Spectrometry
DNA	Deoxy Ribonucleic Acid
DSHE	Dietary Supplement Health and Education Act
DSPE	Dispersive Solid Phase Extraction
EDQM	European Directorate for the Quality of Medicines
E.G	Exempli Gratia
EIC	Extracted Ion Chromatogram
EKSG	Ekiti State Government
EKSHDP	Ekiti State Hospital Development Project
EMA	European Medicines Agency
EPA	Environmental Protection Agency
ESI	Electrospray Ionisation
ETAAS	Electro Thermal Atomic Absorption
EU	European Union
FAAS	Flame Atomic Absorption Spectroscopy
FAO	Food and Agriculture Organization
FDA	Food Drug Administration
FGN	Federal Government of Nigeria

FHB	Finished Herbal Product
FI	Flow Injection
FI-MS	Flow Injection-Mass Spectrometry
FMOH	Federal Ministry of Health
FREP	Faculty of Science and Technology Research Ethics Panel
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
GFR	Glomerular Filtration Rate
GMP	Good Manufacturing Practices
HDL	High-Density Lipoproteins
HIV	Human Immunodeficiency Virus
HM	Herbal Medicine
HMA	Herbal Material
HMB	Hospital Management Board
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin-Layer Chromatography
IBD	Irritable Bowel Disease
ICH	International Conference on Harmonisation
ICP-MS	Inductively Coupled Plasma Mass-Spectrometry
ICP-OES	Inductive Coupled Plasma-Optical Emission Spectrometer
ICT	Information Communication Technology
IFAD	International Fund for Agricultural Development
IHM	Indian Herbal Medicine
IS	Internal Standard
ISO	International Organization for Standardisation
IUGR	Intrauterine Growth Restriction
K-S	Kolmogorov-Smirnov
LC-MS-SPE	Liquid Chromatography-Mass Spectrometry-Solid Phase Extraction
HPLC-DAD	High Performance Liquid Chromatography-Photodiode-Array-Mass Spectrometry
LC-DAD	Liquid Chromatography with Diode Array Detector
LC-HRMS	Liquid Chromatography-High Resolution Mass Spectrometry
LC-MS	Liquid Chromatography-Mass Spectrometry
LC/TOF-MS	Liquid Chromatography with Time-of-Flight Mass Spectrometry
LC-MS-MS	Liquid Chromatography-Tandem Mass Spectrometry
LC-QTOF-MS	Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry
LDL	Low-Density Lipoproteins
LGA	Local Government Area
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MEKC	Micellar Electrokinetic Chromatography
MF	Matrix Factor
MHRA	Medicines and Healthcare Products Regulatory Agency
MPAR	Mean Peak Area Ratio
MW	Molecular Weight

NACA	National Agency for the Control of AIDS
NAFDAC	National Agency for Food and Drug Administration and Control
NBS	Nigerian Bureau of Statistics
NEEDS	National Economic Empowerment and Development Strategy
NGO	Non-Governmental Organizations
NIST	National Institute of Standards and Technology
NMR	Nuclear Magnetic Resonance
NPC	National Population Commission
NSAID	Non-Steroidal Anti-inflammatory Drugs
NSFDP	National Sanitation Foundation Draft Proposal
OECD	Organisation for Economic Co-operation and Development
ONS	Office for National Statistics
OR	Odds Ratio
PAR	Peak Area Ratio
PPB	Parts Per Billion
PPM	Parts Per Million
PRC	Pew Research Center
PSA	Primary Secondary Amine
Quechers	Quick, Easy, Cheap, Effective, Rugged, and Safe
RA	Research Assistant
Rc	Mean Relative Response Factor
Rcts	Randomised Controlled Trials
RHM	Raw Herbal Material
RRT	Relative Retention Time
RSD	Relative Standard Deviation
RT	Retention Time
SB	Still Birth
SCBD	Secretariat of the Convention on Biological Diversity
SD	Standard Deviation
SIM	Selected Ion Monitoring
SMOH	State Ministry of Health
SPE	Solid Phase Extraction
SPL	Spontaneous Preterm Labour
SPSS	Statistical Package for the Social Sciences
SRS	Simple Random Sampling
SVO	Subject-Verb-Object
S-W	Shapiro-Wilk
SWCTOX	Scientific Working Group for Forensic Toxicology
TB	Tuberculosis
TCAM	Traditional Complementary and Alternative Medicine
THR	Traditional Herbal Registration
TIC	Total Ion Chromatogram
TLC	Thin Layer Chromatography
TLC-SERS	Thin Layer Chromatography Combined with Surface-enhanced Raman Spectroscopy
TM	Traditional Medicine
TTM	Total Toxic Metals
UHPLC	Ultra-High Performance Liquid Chromatography

UIFD	Unexplained Intrapartum Foetal Death
UK	United Kingdom
UNESCO	United Nations Educational, Scientific and Cultural Organization
USA	United States of America
USD	United State Dollars
US-FDA	United States Food Drug Administration
USN-ICP-OES	Ultrasonic Nebulization Coupled to Inductively Coupled Plasma Optical Emission Spectrometry
WHO	World Health Organisation
XRFS	X-ray Fluorescence Spectrometry

List of Appendices

CHAPTER 1: INTRODUCTION

1.1 Herbal medicine use in ancient times

The dependence of humanity on nature for the provision of medicine through herbs has a long history (Barnes, Anderson and Phillipson, 2007). Plants have proved to be rich sources of medicine and highly beneficial to the management and treatment of diverse diseases (Bako et al., 2005). Herbal medicines (HM) have played important roles in the healing process as well as containing many pharmaceutically active compounds, which are now used in pharmaceutics and commercially produced (Principe, 1988; Pearce and Puroshothaman, 2002). Their use has been reported to be as far back as 60,000 years ago after a new cave was discovered in Iraq in 1960. The cave contained a grave of a Neanderthal man which was adorned with specific flowers known for their medicinal properties (Solecki, 1975). A Sumerian cuneiform clay tablet dating to about 2100 B.C also provides one of the first documented references to an HM instruction containing depictions of plant ingredients and the mixing instructions (Janick, 2003).

The 'Papyus Ebers' documentation which dates to about 1500 B.C describes an asthma remedy prepared by hot brick heating of a herbal mixture to produce fumes (Ebers Papyrus, 2011). The organised practice of Chinese HM is known to date back over 2000 years (Beinfield and Korngold, 1995). The Emperor's textbook of herbs that was put together around the first century B.C. was one of the earliest compilations on HM (Mabey, 1988). A huge HM pharmacopeia was also developed around 1000 A.D. by Muslim physicians and the knowledge was documented in "The Canon", a book that was used by many medical schools for centuries (Dubick 1986; Saunders, 1965). In Europe, women practised healing arts especially after the fall of the Roman Empire in the 6th century (Buchman, 1979), a period in which previously acquired experience was used to determine when and what herbal remedies were needed, including dosage. In recent times medicinal plants have still constituted an efficacious component of various traditional (African traditional, Ayurveda, Chinese, Homeopathic and Unani) and modern medicines. However, many factors ranging from economic to scientific have influenced the use of herbal remedies in various parts of the world. In particular, an increase in scientific knowledge through rigorous research in HM has helped to identify its potential dangers and benefits, as further discussed in Section 1.6.

1.2 Complementary and alternative medicine (CAM)

The term “traditional, complementary and alternative medicine” (TCAM or CAM) has been used to summarise various nonconventional medical practices. Complementary and alternative medicine is a general description of a group of varying healthcare systems, medical practices and associated products. These are not regarded as part of conventional (or orthodox) medical practice nor are they included in public healthcare systems or administered by a practitioner in an orthodox medical setting (Adams et al., 2009). Complementary medicine therapies such as aromatherapy, acupuncture, massage, visualisation yoga and herbal medicine are practices used alongside modern medicine. Alternative medicines, on the other hand, are complete non-orthodox methods of healthcare. The World Health Organization (WHO) defined CAM to include all forms of healthcare practice which are not within the official health sector (Eisenberg et al., 1993). This definition included in CAM some of the traditional systems of medicine such as Unani, Ayurvedic, osteopathy, naturopathy, homeopathy, chiropractic, traditional healing and medicine men. Some of these practices now lie within the official health sector, especially in countries where local legislation supports them. Thus what is referred to as CAM or dietary supplements in some Western countries is actually an official health product in other countries such as China or India (Warude and Patwardhan, 2005). To avoid ambiguity, some of the healthcare provisions or practices which are not within the official health sectors of some developed countries are licensed as dietary and regulated as such, especially in the United States of America (Lewith, Jonas and Walach, 2010).

Nonetheless, traditional medicine practices together with complementary and alternative medicine such as Ayurvedic and others around the world have herbal medicine as a component. Knowledge of plant ingredients is increasing, but information on their clinical pharmacology and their mode of action is still largely unavailable, reducing the potential for standardisation, evaluation and further use of HM (Ma et al., 2009). Herbal medicines are materials or preparations obtained from the parts of one or more plants either raw or processed, containing substances with therapeutic characteristics and other benefits to human health (WHO, 2008). The WHO fact sheet on traditional medicine (2008) explains the four HM types:

I. Herbs: Raw plant material such as fruit, stems, leaves, seed, flowers, bark rhizomes, wood, roots or other parts of the plant that can be powdered or fragmented.
II. Herbal materials: Plant materials such as resins, gum, oil (fixed or essential), herbs, dried powder and fresh juice. These materials are often produced by different local

preparations which include roasting, steaming or honey stir-baking, mixing with alcohol or other substitutes.

III. Herbal preparations: Powdered or comminuted herbal extracts or materials, fatty oils of herbal material. They are precursors of finished herbal products and are produced by fractionating, extracting, purifying and concentrating the herb. Heating of herbal materials in alcoholic drinks, honey or both, or in different media are among other preparation methods used.

IV. Finished herbal products: Herbal mixtures are produced from one or more herbs and often referred to as mixture herbal products when more than one is used. Mixture herbal products can contain excipients and active ingredients. But mixture or finished products are not considered herbal if there is an addition of any chemically active compound, including artificial compounds and/or constituents extracted and isolated from herbal materials.

The World Health Organization has identified the contributions of traditional medicine (a form of CAM) in the 21st century, especially in management and prevention of various diseases which include malaria, tuberculosis and human immunodeficiency virus infection. Therefore, to further advance its prospect the WHO published its first comprehensive traditional medicine strategy 14 years ago for 2002 to 2005 (WHO, 2002), and a 2014 to 2023 strategy published 3 years ago (WHO, 2013). The 2014 to 2023 strategy is centred on traditional complementary and alternative medicine (TCAM) which includes:

- Development of suitable national policies for management of TCAM to improve the knowledge base.
- Regulation of practices, products and practitioners for stronger quality assurance, safety, effectiveness and right use of TCAM.
- Integration of TCAM options into healthcare service delivery and self-health care to increase universal health coverage.

1.3 Examples of traditional medicine practice

As stated by the WHO, it defines “traditional medicine as including diverse health practices, approaches, knowledge and beliefs incorporating plant, animal, and/or mineral based medicines, spiritual therapies, manual techniques and exercises applied singularly or in combination to maintain well-being, as well as to treat, diagnose or prevent illness” (WHO, 2018). A number of selected traditional medical practices are discussed below.

1.3.1 Traditional Chinese Medical Practice

Traditional Chinese medicine (TCM) is made up of many distinct practices dating back over 5,000 years, centring on ancient Taoist philosophy and is practised alongside western medicine in many of China‘s healthcare facilities (Katti et al., 2014). Traditional Chinese medicine is deployed in other countries such as the USA and the continents of Europe and Africa. An estimated 3.1 million adults in the USA used acupuncture in 2006; according to the National Health Survey of 2007 (Barnes and Bloom 2008). This survey reported that about 17% of adults used various natural products including herbs, and HM was the most common traditional medical therapy. TCM practitioners use various therapies in health propagation and treatment of disease, e.g. in Chinese herbal medicine and acupuncture, which are discussed below.

1.3.1.1 Chinese herbal medicine

Chinese herbal medicine (CHM) is traditionally one of the most important components of TCM (Xu and Yang, 2009). It has an extensive and valuable treatment history first reported by Shen-Nong, who lived nearly 5,000 years ago (Lu et al., 2004). Shen-Nong discovered over 70 herbs with medicinal value, describing their properties after tasting hundreds of the herbs daily and determining their suitability as herbal therapies (Kuang, 2000). Several books and documents have been discovered describing various Chinese herbal formulations and proprieties. An example is the book “52 Bing-Fang” (recipes for 52 ailments) that was found in an ancient Chinese tomb and describes over 240 herbal medicines and 52 prescriptions (Wang, 1992). Other ancient Chinese scripts also give details of herbal use; e.g.Shan-Hai-Jing, Zhou-Li and Li-Ji’s book of rites, Shang-Shu and Shi-Jing’s book of songs, and the Zuo-Zhuan script. Shi-Jing described the medicinal effect of herbs, recording about 170 types of CHM, including 80 plant species with their planting and harvesting times (Shi-Jing, 2013). The Chinese book of geography, “Shan-Hai-Jing” recorded 45 species of plants of therapeutic value which include species for mood, general health, treatment of disease and also those that are poisonous to humans (Hu, 2008).

Trade in herbs between China and other countries in Southeast Asia is extensive, spanning over 2,000 years and includes herbs such as poria, cinnamon, taurine, ginseng, velvet, kelp from Korea, and others such as saffron, clove, senna, myrrh, benzoin and frankincense (Xiao, Liu and Xiao, 2001). The development of CHM has come a long way through Chinese civilisation over thousands of years to meet contemporary need and demand. Despite the primitive and crude technology used in the olden days of CHM processing, modern research has supported the underlying concept (Zhao et al., 2010). More than 40 CHM dosage forms are now available. These include tincture, powder, bolus/pills, paste, granules, decoction,

tablets and liquids (injection and oral). Also available are plasters, liniment, poultices and ointment forms of CHM for external application. Aerosol and nanomised forms of CHM have also become available (Tang and Ling, 2007; Zhang, 2006; Ding and Hong, 2007; Zhao et al., 2010).

1.3.2 Indian herbal medicine

The history of Indian herbal medicine (IHM), also known as Ayurvedic medicine, dates back to the Hindu period of about 6000 to 4000 B.C with the earliest reference found in the Rig Veda (Tipton, 2008). The Rig Veda is an old collection of Vedic Sanskrit hymns that includes the Atharvaveda, which is the fourth and final documentation in Hindu literature. However, Bhava Prakasha's Bhava-Mishra is the most important herbal/plant document, which is also well respected among Ayurvedic practitioners in modern day practice (Dev, 1988; Prasad, 2000; Prasad and Narayana, 2007).

The oldest text of Ayurvedic medicine was written by Nagarjunan Rasa Vaisesika, who was regarded as an important Buddhist philosopher following the death of Buddha (Stanford Encyclopedia of Philosophy, 2013). This text, written during the 5th century, describes different principles of drug action and their composition (Ayurvedic medicine, 2013). However, the Charaka Samhita was the first documentation of the principles and practice of Ayurveda with therapeutic emphasis (Bagde et al., 2013). It divided drugs of plant origin into 50 groups according to their pharmacologic and therapeutic characteristics. This text was translated into numerous languages including Tibetan and Chinese (300 CE), Greek (300 BCE), Persian and Arabic (700 CE), which shows how useful and respected it was (Devarkar, Mane and Aswale, 2012).

The World Health Organisation has already documented about 21,000 plants that are used for medicinal purposes globally. 2500 of the species originated from India and 150 of these are commercially very profitable products (Rajesham, Ravindernath and Bikshapathi, 2012). Often referred to as the world's botanical garden, India occupies the enviable position of being the largest producer of medicinal herbs globally (Seth and Sharma, 2004). About 20% of the world's plants species are indigenous to the Indian subcontinent and a large percentage of these are of medicinal value and still used in contemporary Ayurvedic practice (Singh, 2006). The medicinal plant species are mostly obtained from the wild (80%), with about 10% being cultivated for trade (*Ibid*).

About 50% of medicines documented in the British Pharmacopoeia are from western Himalayan medicinal plants (Dev, 1997). The western Himalaya provides about 80% of Ayurveda herbal medicines, 33% of orthodox and 46% of unani (Baragi, Patgiri and

Prajapati, 2008). Well over 7,500 plant species are used in contemporary IHM, and a larger number of plant species (about 25,000) are still being used in traditional medicine (Joy et al., 1998). Contemporary use includes antimalarial therapy, tonics, antipyretics, aphrodisiacs, hepatoprotectants, expectorants, diuretics and antirheumatics (Aggarwal et al., 2011; Mukherjee and Wahile, 2006) as well as therapy for certain central nervous system disorders (Kumar and Khanu, 2012; Kulkarni, Girish and Kumar, 2012). The use of these herbs and herbal preparations (including spices) for medicinal purpose has been well documented for the management of chronic disease in humans, e.g. in diabetes and asthma (Sharma et al., 2007).

1.3.3 African traditional medicine

African Traditional medicine (ATM) is a non-conventional system of disease management that employs various processes of consultation with herbalists, priests, media and diverse traditional deities together with herbal use (MacGaffey, 2016). The origin of HM use in Africa is difficult to ascertain due to poor documentation of ATM practice and development over time, which still continues to be a problem (Sowofora, 1993; Elujoba, Odeleye and Ogunyemi, 2005; Okigbo and Mmeka, 2006; Aniago, 2015; Masango and Nyasse, 2015). Historically knowledge of ATM was passed down orally from generation to generation and therefore not documented (Van Wyk, Van Oudtshoorn and Gericke, 1999). This has accordingly affected our understanding of the workings of the system and its practice.

The earliest recorded history of ATM is the “Circa”, of about 1770 B.C. in the code of Hammurabi Babylon and that of 1550 BC in the code of Egypt (Veileux and Steven, 2006). According to the document the history of herbs goes as far back as 5000 years and beyond to the Sumerians’ description of the therapeutic use of plants such as caraway, thyme and laurel. Also documented was the 1000 B.C. old Egyptian ancient medicine’s use of opium, garlic, wheat and barley for medicine. The practice of African traditional medicine has a spiritual and nonspiritual aspect, the latter defined as herbalism (Jolles and Jolles, 2000). The spiritual aspect of ATM entails healing, which is divine and anchored in religious belief which is supposed to allow for supernatural powers (*Ibid*). Conversely, in many cases there is no distinctness in the practice of a traditional healer as a divine or as a herbalist. Before the establishment of orthodox medicine, ATM was the major medical system at the disposal of millions of people in urban and rural communities of Africa, but the advent of Europeans was a noticeable landmark in the annals of this ancient culture and tradition (Romero-Daza, 2002; Abdullahi, 2011).

The traditional healer mainly diagnoses and treats disease in light of the underlying psychological concept and treats symptoms using medicinal plants (Gurib-Fakim, 2006 and Gurib-Fakim et al., 2010). This makes ATM holistic, ministering both to the body and the mind. African diversity is typified in its varying rich biological and cultural forms, characterised by regional contrast in healing practice (Gurib-Fakim, 2006). During the colonial regime, traditional medical practice was banned due to a general perception of its being magic and witchcraft. It was ruled illegal by the colonial powers, thereby generating conflict in areas where the native culture was regarded as witchcraft (Helwig, 2005). In this period efforts were also made to regulate the sale of HM as colonialism and Christianity spread through Africa. There was no investigation of the soundness of traditional medical practices because many foreigners were of the opinion that traditional medical practitioners were to be dismissed as superstitious (Onwuanibe, 1979). This opinion still persists because orthodox health practitioners, including doctors, have continually despised traditional practitioners and practice, despite its impact in meeting and securing the basic health of the population (Conserve Africa, 2002). Moreover, until recently HM in Africa was generally not thoroughly researched and only loosely regulated (Mills et al., 2005). But in recent times attempts to synergise both conventional and traditional medicine practice have yielded results. As observed from personal experience in clinical work in Nigeria, this collaboration is demonstrated by a series of training sessions for traditional birth attendants organised by conventional health professionals and the development of effective referral practice.

1.4 Use and belief in traditional medicine in Ekiti State

1.4.1 Traditional medicine belief in Nigeria

About 75% of Nigerians still consult traditional healers for a variety of healthcare provision (Adefolaju, 2011). This shows that most Nigerians have retained a deep belief in traditional medical practice and its practitioners, hence relying on them for their various health care needs. There are many components of traditional medicine: maternity care, spiritual therapy, herbal medicine, circumcision, traditional orthopaedics, psychiatric care, aromatherapy, homeopathy, massage, music therapy and a host of others (Adefolaju, 2011). Practitioners of traditional medicine gained knowledge of herbal medicine through apprenticeship or inheritance. Historically a lot of the practitioners carried out their traditional medical practice as community service without financial reward, thereby enhancing the sincerity and effectiveness of the practice (Owumi, 1993).

As a result, traditional medicine practitioners (TMP) were respected in their community and were entrusted to provide health care. This care is provided through the use of herbs,

mineral substances, animal products or a combination of these. The practice revolves around the cultural, social and religious background, knowledge, attitudes and beliefs that characterise the community with regard to causation of disease, disability, social, mental and physical wellbeing (WHO, 2005b). There are various kinds of traditional health care practitioner across the different ethnic groups in Nigeria (see section 1.4.2), aside from the provision of western health care system.

1.4.2 Traditional Medicine Practitioner and herbal medicine use in Nigeria

Yoruba, being one of the ethnic groups in Nigeria to which Ekiti State belongs, calls a traditional medical practitioner "Babalawo" (Olayiwola, 1987). Yoruba is also the local language spoken by this ethnic group. In Yoruba land, the Babalawo, a specialist in Ifa (Oracle) divination, plays an important role in traditional healing, while specialists in herbs are known as Onisegun (herbalist) (*Ibid*). The herbalist, unlike the Babalawo, is limited in his expertise of spiritual matters, but the use of HM is common to the practice both of Babalawo and Onisegun. Undoubtedly the development of a conventional health system especially in Yoruba land has been unimpressive; with over half of the populace lacking access to conventional health care. The unacceptable level of accessibility to conventional health facilities is noticed more in urban slums and rural settlements (Oluwatayo, 2015; Mafimisebi and Oguntade, 2011). This problem makes alternative medicine a convenient choice in disease control in such areas. However, with HM being an integral part of TM, various herbal medicines have been found to be therapeutic in Nigeria, e.g. *Rauwolfia vomitoria* (Afzel) applied in the treatment of insomnia, hypertension, convulsion and stroke (Amole, Yemitan and Oshikoya, 2009). Some further examples are included in Table 1.1.

Table1.1: Examples of herbal medicine/ herbs and their therapeutic use in Nigeria

Herbal medicines/ herbs	Therapeutic use	Reference
Ocimum gratissimum leaves	Diarrheal diseases	Ilori et al., 1996
Parasidi Macfad seeds	Urinary tract infections	Oyelami et al., 2005
Carica papaya L seeds	Intestinal parasitosis	Okeniyi et al., 2007
Garcinia kola Heckel leaves	Analgesic and anti-inflammatory	Adegbehingbe et al., 2008
Aloe vera gel	Scabies	Oyelami et al., 2009
Undisclosed herbal preparation	Management of HIV	Onifade et al., 2013

A study of the frequency of complementary and alternative medicine use among hypertensive patients attending hospital appointments at a Nigerian tertiary hospital reported lower use of CAM, of which herbal medicinal products were the most commonly used (Amira and Okubadejo, 2007). The reduced use of CAM among these hypertensive patients may be due to desirable results' being achieved by the use of orthodox antihypertensive medication. Hypertension has been known to be controlled with appropriate choice and dosage of medication, the efficacy of which can be directly observed by a blood pressure check (Spirk et al., 2018). However, complicated diseases such as cancer still have enormous scientific research on-going in determining more acceptable and effective measures in its prevention, cause and treatment. It is only natural for cancer patients to want to try alternative methods in the face of modern scientific efforts that have not been able to provide satisfactory treatment.

In Ekiti State, a study conducted in one of the local government areas reported 74.3% of the 521 respondents used HM in the treatment of malaria (Olorunniyi and Morenikeji, 2013). This is similar to a study in urban Lagos, in which 66.8% of the 388 respondents used HM for management of various ailments (Oreagba, Oshikoya and Amachree, 2011). Other studies have also reported high use of HM in Nigeria in recent times (Olorunniyi and Morenikeji, 2013; Awodele et al., 2014; Okoronkwo et al., 2014; Duru et al., 2016). The HMs used are either locally made, refined, imported, certified or uncertified. Vendors of various herbal remedies are now almost ubiquitous in Nigeria, selling in traffic gridlocks, on highways, at bus stops bus and stations, events and, surprisingly, in some orthodox health facilities. In addition studies have reported high use of uncertified HM among cancer patients in Nigeria (Ezeome and Anarado, 2007), among pregnant women for antenatal care (Fakeye, Adisa and Musa, 2009), in patients with epilepsy (Danesi and Adetunji, 1994), diabetes mellitus (Ogbera et al., 2010) and children suffering from chronic diseases such as asthma, epilepsy and sickle cell anaemia (Oshikoya et al., 2008).

Extemporaneous HM is not included in the class of HM that needs the certification of the National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria (NAFDAC, 2005). They are preparations made by a practitioner and given on a one-to-one basis to a patient in the vicinity of preparation (NAFDAC, 2005). However, some extemporaneous HM samples have been commercialised, adding to the burden of uncertified HM use. Hence a review of the criteria for HM certification has become very necessary in Nigeria.

1.4.3 Traditional and orthodox medicine practice in Ekiti State

The orthodox and traditional medical system provides health care services in Ekiti State, coordinated by the state ministry of health (Ekiti State Hospital Development Project, 2011). The increased use of HM has been attributed to challenges with the availability and affordability of orthodox medicine. These challenges, such as affordability of service cost, quality of service rendered, closeness to home, staff attitude, neatness of the environment, availability of required services and drugs have been reported to influence patient's patronage of orthodox medicine in Ekiti State (Omotosho, 2010). Furthermore, of the sixteen local government areas (LGA) in Ekiti State, only two LGAs (Irepodun/Ifelodun and Ekiti East) have a higher number of their population benefiting from the health facilities located in their LGA (Odeyemi, 2014). Therefore the use of HM in Ekiti State is a combination of factors. The choice is made based on the situation of individuals at the time of decision-making. A household poverty indicator by healthcare in Ekiti State reported 57.5% of respondents make use of HM for their health care, due to problems associated with the financial capacity to offset hospital cost and proximity of government hospitals (Oluwatayo, 2008). Despite the availability of modern health care facilities in some communities and no sociocultural belief against its use, the inability of residents to afford the services has increased the use of HM (Adesiji and Komolafe, 2013).

As an example, from an enquiry during a field study in Nigeria, an original artemisinin-based combination therapy (ACT) drug used in the treatment of malaria costs about 1 Naira, 500 (US $4.9) in Ekiti State. Conversely, HM therapy for malaria costs an average of 200 Naira (US $0.57) (using the Central Bank of Nigeria exchange rate as of September 2018), and could even be self-prepared at no cost, provided the appropriate herb and preparation methods are known. Hence the choice is predictable, considering the cost of both options with ACT being over 70% more expensive. The poor coverage of the National Health Insurance scheme means that patients have to "pay as you go" for healthcare, otherwise known as "out of pocket" expenditure on health, which has further contributed to the challenges of the use of orthodox medical practice in Nigeria. A study on healthcare financing in Nigeria reported that about 69% of Nigerians expend on health through "out of pocket financing" (Uzochukwu et al., 2015). Furthermore, the prevalence of drug-resistant strains of bacteria and malaria parasite in Nigeria may also explain the use of herbal medicine by patients in the treatment of infection and infestation, which to them have defied conventional solution (Nasir et al., 2015).

In addition, countries in Africa are reported to be the most affected by increasing world-wide tuberculosis (TB) and malaria-drug resistance (WHO, 2010). The counterfeiting and

adulteration of modern antibiotics and other orthodox medication have further added to the problem of drug resistance. This is evidenced, for example, by an analysis of over 3,000 orthodox anti-malarial medications bought in Enugu, Nigeria which showed 9.3% to be of low and substandard quality, containing suboptimal amounts of the active ingredient required (Kaur et al., 2015). In addition, it was reported that 1.2% of the samples were falsified and 1.3% degraded. These present the risk of patients receiving incorrect doses and subsequent development of resistance in the malarial parasites. Other contributory factors to drug resistance were highlighted in the worldwide country-situation analysis response to antimicrobial resistance report (WHO, 2010). They included poor laboratory capacity, infrastructure and data management, as factors which discourage productive surveillance. The sale of antibiotics and other anti-infective medicines without a prescription, lack of standard treatment guidelines and growing potential for antimicrobial medicine overuse were also highlighted as discouraging factors (*Ibid*). The effects of these highlighted problems are likely contributors to the increase in use of herbal medicine as an alternative. The contribution of these and other factors has not been explored in Ekiti State, hence the need for this research to address the gap in knowledge.

1.5 Use and trade of herbal medicine globally

1.5.1 Developmental and economic implication

The WHO has encouraged developing countries to develop and use local medication suiting individual circumstances, particularly for primary healthcare use (WHO, 2009). This call was important in other ways, to reduce the cost of drug importation by developing countries and also to look inwards in meeting their primary healthcare need. As a result, countries in far East Asia have achieved about 75% of their care need through traditional medicine practice, herbal medicine development and use (WHO, 2009). The Secretariat of the Convention on Biological Diversity (SCBD) reported worldwide use of herbal medicines has markedly increased, and estimated that sales of herbal products in U.S dollars globally reached $60 billion in 2000 (SCBD, 2014). In 2008, an estimated world market of HM worth $83 billion was reported (Nutraceuticals World, 2013). This is an estimated increase of $23 billion in 8 years. In 2017 the world market in herbal supplements and remedies was reported to be about $107 billion (Global Industry Analysts Inc, 2013). Obviously, this is not an economic trend contributed to by herbal product use in developing countries alone. In some countries with improved socioeconomic development and improved quality of health care, the use of herbal medicine has also increased. This is attested by the increased number of herbal retail outlets and medical facilities in these countries (Bloom and Standing, 2001).

In the United Kingdom (UK) an estimated £1.6 billion annual CAM "out-of-pocket" expenditure was reported (Ernst and White, 2000), herbal remedies being a well-known and widespread CAM form. The UK Medicines and Healthcare products Regulatory Agency (MHRA) conducted a study on the perception of HM and its use (MHRA, 2008). It was reported from the survey that 35% of adults (n=2,032) had used HM in the previous two years and 89% of these adults believed most HM were safe to use. It is crucial to emphasise the extent to which use and interest in HM are growing. In the last ten years about 40% of all healthcare delivery in China was HM related, while the use of HM at least once by population percentage in USA, France, Belgium, Australia and Canada is estimated at 42%, 75%, 38%, 48% and 70%, respectively as seen in Figure 1.1 (Foster et al., 2000; WHO, 2002).

The increased use of HM, despite advances and availability of orthodox medicine, especially in developed countries, poses some form of paradox. Use of alternative medicine should otherwise be more reduced with advances in orthodox medical care. However, the reasons for HM use may vary from personal, cultural, religious, economic and other perspective, as discussed in Section 1.4.3. But then poverty, lack of good medical facilities, a culture of self-care and self-medication have been reported to influence the use in developing countries (Van der Geest and Hardon, 1990; Oshikoya et al., 2008). Factors which influence the use in developed countries are different, as discussed further in Section 1.5.2.

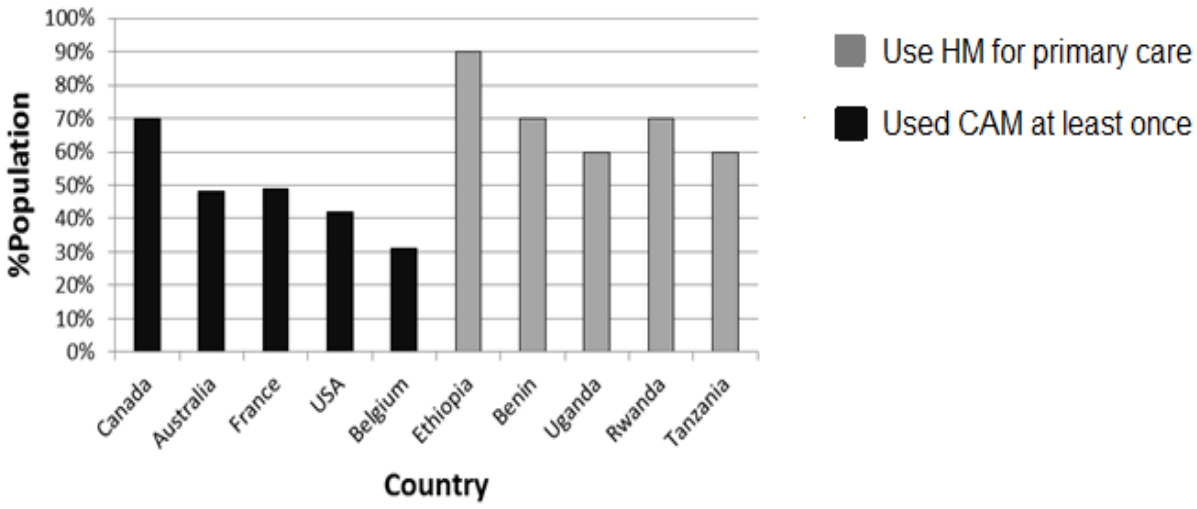


Figure1.1: Herbal medicine and CAM use in selected developing and developed countries (WHO, 2002)

1.5.2 Cultural and socioeconomic aspects of herbal medicine use

Social, cultural, socioeconomic and political factors have been found to influence the use of HM in otherwise industrialised or developed countries (Astin, 1998; Eskinazi, 2001; Ong and Bodeker, 2002). An example is the USA, where increases in the cost of prescription medicines and a renewed interest in natural products or organic alternative, has accounted for about 20% of the population's using HM (Bent, 2008). Also, as people migrate from different nations and cultures, cultural preservation may account for the use of HM in some ethnic minorities residing in developed countries (Ma 1999; Kronenberg et al., 2002).

As humans interact and develop relationships through friendship, marriage, workplace, educational placement, tourism and sport, it is plausible that people pass on ideas and beliefs to others. As a result, a part of the ethnic majority of developed countries may have used HM due to various forms of multicultural interaction. It has also been suggested that the increasing side-effect of pharmaceutical drugs, increasing drug-resistant illness and general dissatisfaction with orthodox medicine are other reasons for increased use of HM in developed countries (Chan, 1996). In addition, orthodox medical practitioners are viewed as not paying much attention to body mechanisms and often intervening to block them (Chan, 1996), unlike CAM practitioners, who support the body's natural capacity to regenerate and participate in the recovery of the patient. However, survival instinct is an inherent characteristic of humans, which often informs our choice including that of health care. Thus the use of HM is a complex combination of varying factors in individuals and localised population. Accordingly part of this research aims, through a survey, to understand the factors that influence the use of herbal medicine in Ekiti State, Nigeria.

1.6 Safety and efficacy of herbal medicine

1.6.1 Safety profile of herbal medicine

Herbal medicines contain various pharmacologically active compounds and the constituents behind the therapeutic effect are sometimes undefined (Schulz, Hansel and Tyler, 2001). Plants constituents such as tannins, polysaccharides and mucilage regulate the action of any "active principles" in the HM. Although these compounds occur naturally in the various plants used in the HM, adulterants and contaminants in HM can also produce significant pharmacological activity. The multi-active natural components of HM have made it difficult to test the efficacy and reproducibility of any positive therapeutic claim. Hence HM has to be viewed as a whole and characterised following clinical trials. Accurate characterisation will make reproducibility of analytical methods in HM research possible. Studies have revealed that the exact effect of the whole herb is not achievable or mimicked by administration of

purified and isolated components of the herb (Haq, 2004). Regardless, many HM randomised controlled trials (RCTs) have been reported with published meta-analyses and they include disease conditions such as early-stage breast and colon cancer, osteoarthritis of the knee and irritable bowel syndrome (Guo, Canter and Ernst, 2007; Li et al., 2013; Su et al., 2014; Hausenblas et al., 2015; Walton, Whitten and Hawrelak, 2016; Lee et al., 2016 and Izzo et al., 2016).

Research on HM poses several challenges, ethical, financial, study-design, product-standardisation, and regulatory before embarking on a new drug study in clinical trial. The WHO issued operational guidelines in 2005 on regulatory measures required to reinforce herbal products' clinical trial (WHO, 2005). However, the widespread acceptance and use of HM may be indicative of their efficacy and safety. The lack of efficacy and safety guarantee of HM, on the other hand, is largely due to inadequate pharmacological, pharmacokinetic and clinical data on many herbal medicinal products. In many cases, the evidence presented in some literature is marked by flawed methodology and quality, with a risk of bias which limits its findings. As a result claims of effectiveness seem to be based largely on poor-quality evidence from which firm conclusions cannot be drawn (Izzo et al., 2016).

HMs are, nevertheless, generally considered by some to be effective and a safe form of medication, which results in its yearly active patronage (Philomena, 2011). This belief in HM safety is understandable considering that a minimum of 100,000 people die in the USA annually as a result of pharmaceutical compound-related toxicity and about 8% of total admissions to hospital are as a result of adverse reactions to pharmaceutical drugs (Nasri and Shirzad, 2013). This fatality figure is about 3 times the number (of deaths) resulting from drunk drivers, meaning people are 3 times more likely to be killed by taking pharmaceutical medication than drunk driving (*ibid*). By contrast, death or hospitalisation due to HM is rare in the USA, as reported by the author. A detailed study of HM-related casualty also reported a significantly low incidence of adverse effect and a few severe clinical reactions (Posadzki et al., 2013; Di Lorenzo et al., 2015). However, as no similar data exist within the study population in this research, a study of HM-related casualty and fatality was carried out to ascertain the figures (Section 2.11).

1.6.2 Contamination and adulteration of herbal medicine

Regardless of the positive perception of the use of HM, its acclaimed therapeutic satisfaction and the disappointment with safety and effectiveness of orthodox medicines, the safety of herbal remedies continues to remain a dominant concern (Olorunniyi and Morenikeji, 2013; Awodele et al., 2014; Okoronkwo et al., 2014; Duru et al., 2016). Concerns have been repeatedly raised about the adulteration and contamination of HM (Ang and Lee, 2000; Ang,

2008; Ernst, 2002b; Snyman et al., 2005). Adulteration of HM is the substitution of HM in whole or in part with extraneous, impure, inferior or improper entities (Zhang et al., 2012). These improper entities include undeclared pharmaceutical compounds, which this research aims to investigate by analysing HM obtained from Ekiti State (see Chapter 4). According to a United States Food Drug Administration (US-FDA) study, undeclared pharmaceutical compounds were found in 572 dietary supplements between 2007 and 2014: sexual enhancers accounting for 238 of the entries, weight loss medication 228, and muscle builders 90 (Justa and Caldas, 2015). This further emphasises the seriousness of the issues at hand and a potential increase in trend if not promptly checked.

On the other hand, contamination of HM is the unwanted addition of impurities with microbiological or chemical characteristics, or of other foreign matter, in a finished herbal product, its starting or intermediate product or during sampling, production, packaging, storage or transport (WHO, 2012). Such contaminants may include heavy metals, which are very dangerous to human health as further discussed in Section 4.4.2 to 4.4.11. While adulteration is illegal, efforts to reduce contamination are provided for by various legal guidelines issued by different countries (Corrigan, 2003; European Medicines Agency, 2005; Kunle, Egharevba and Ahmadu, 2012) and particularly for heavy metals as summarised in Table 4.3 (Chapter 4). Reports of heavy metals in HM, in addition to adulteration with prescription medications (Garg et al., 2011; Posadzki, Watson and Ernst, 2013), has increased the toxic potential of HM, especially the uncertified forms (Chan, Chiu and Lau, 2003). Certification by regulatory bodies reduces the incidence of unpleasant effects from HM, through appropriate legislation and monitoring, although some HMs naturally contain harmful substances toxic to humans (Skoulidis, Alexander and Davies, 2005; Webb et al., 2005; Moreira et al., 2014).

Fung and Linn (2017) have shown that most HM adulteration is with prescription medications, which have therapeutic properties effective in treatment or management of medical conditions the HM is claimed to achieve. The detection of diuretic hydrochlorothiazide, sibutramine, bumetanide in sliming herbal medicine (Moreira et al., 2013; Khazan et al., 2014) and dexamethasone in herbal medicine used for the treatment of joint pain (Park et al., 2016) are a few examples of this worrying trend of adulteration. These adulterants are typically cheaper than standard brands due to profitability, hence raising suspicion of their illegal sourcing. The US-FDA took action against such counterfeit, adulterated and dangerous dietary supplements and HMs (Burton, 2013). One publication found such adulteration in Nigeria, with the detection of HIV drugs in HM used by patients for the management of HIV (Gini et al., 2016). Nonetheless, Gini (2016) focuses mainly on HIV treatment, unlike this research which examines the possibility of commonly abused

pharmaceutical compounds' being added to HM as an adulterant in Ekiti State. Such adulteration will likely target a larger population with greater potential economic benefit to the manufacturer. No publication has examined any HM in Nigeria or Ekiti State for commonly abused pharmaceutical compounds; hence this research aims to address this gap and the choice of target pharmaceutical compound in this research is outlined in Section 3.2.

Heavy metals such as selenium, thallium and arsenic have been detected in a few herbal products in Nigeria (Ajasa et al., 2004) and in other forms of HM around the world (Joob and Wiwanitkit, 2016). No publication has reported similar research in Ekiti State. Hence it has become essential to assess the safety of commonly used HM in Ekiti State by analysis for selected heavy metals as outlined in Section 3.3. Importantly, the toxic effects of heavy metals, particularly on human health, are not unknown. A study by Jin et al (2013) reported neural tube defect and anencephaly in babies and renal impairment in adults due to mercury, lead, cadmium and arsenic toxicity from various forms of environmental exposure. In south west Nigeria cases of acute renal failure associated with uncertified herbal medicine use and consequent fatalities were reported, though the particular component of the HM responsible for the damage was unknown (Kadiri, Arije and Salako, 1999). In addition, traditional HM-related liver fibrosis has been reported in Uganda (Auerbach et al., 2012); also the toxicity of various other herbal medicinal products (Ekor, 2014) and an 11-year retrospective study of aconite poisoning in mainland China (Li et al., 2016). Herbal medicine related fatalities were also reported in Ekiti State, after the deaths of two children (Olatunya et al., 2015). This unfortunate event has further reinforced the need for tighter HM control and evaluation. Therefore part of this research also aims to analyse HM used in Ekiti State for heavy metals and undeclared pharmaceutical compounds as a contribution to knowledge of HM safety.

Various analytical techniques have been used in the past for the detection and quantification of pharmaceutical compounds and heavy metals in HM, which is further discussed in Section 1.7.

1.7 Review of analytical methods for screening of pharmaceutical compounds and heavy metals in herbal medicine

The analysis of HM for pharmaceutical adulterants and heavy metals has been carried out using several techniques. These are examined in sections 1.7.1 and 1.7.3.

1.7.1 Analytical method for the screening of selected pharmaceutical adulterants in herbal medicine

Various analytical techniques exist for the screening of pharmaceutical adulterants in herbal products. These techniques include Liquid Chromatography (LC), Gas Chromatography (GC), Capillary Electrophoresis (CE), Flow Injection (FI), Ambient Mass Spectrometry (AMS), Attenuated Total Reflectance-Fourier Transform Infra-Red Spectroscopy (ATR-FTIR) and Thin Layer Chromatography (TLC). The use of any analytical technique is determined by the peculiarity of the analysis and the desired result. The volatility and thermal stability of a compound, for example, determine its analysis using Gas chromatography–mass spectrometry (GC-MS) (Rocha, Amaral and Oliveira, 2016). However, GC-MS enables a cost-effective analysis, with good quantitative results and high resolution, which makes its use beneficial (Sahil et al., 2011). Ambient mass spectrometry, on the other hand, has the advantage of providing data quickly and with little sample preparation (Zhou et al., 2011), while CE is fast, economical and requires a small sample size (Sombra et al., 2005). Hyphenated techniques improve accuracy; e.g. the Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) synchronised with Nuclear magnetic resonance (NMR), which can reliably identify and quantify adulterating compounds that may be present (Rundlöf et al., 2014). In addition, the technique does not require the use of a reference standard, as quantification may be carried out using an internal standard (Rundlöf et al., 2010; Holzgrabe and Malet-Martino, 2011). A technique such as Attenuated total reflectance infrared spectroscopy (ATR-IR) needs no sample preparation which is one of its advantages, thus permitting the screening of samples in either liquid or solid state (Deconinck et al., 2014).

TLC is reported to have high-throughput potential, inexpensive verification of analyte quality, simple operation and portability. However, this technique is only preliminary and non-confirmatory, requiring reference chemicals and a visualisation agent (Risha et al., 2008). A comparison of some of these techniques is presented in Table 1.2 and Table 1.3 summarises some of the analytical techniques that have been used in the screening of herbal products for pharmaceutical compounds.

Table1.2: Comparison of some analytical techniques used to analyse pharmaceutical adulterants in herbal products

Technique	Advantages	Disadvantages	Reference
LC-MS & HPLC	Analyses heavy, polar and organic compounds	Commercial database is not available	Vaclavik, Krynitsky and Rader 2014
GC & GC-MS	Fast, good accuracy, high sensitivity and resolution. Commercial database available	Only separates volatile compounds	Metz et al., 2007
^{1}H NMR	Detects specific structural components	Expensive, time-consuming.	Thet, 2018
CE & CE-MS	Small sample size required, low cost, rapid analysis and high separation efficiency.	Less accurate	Chatham and Blackband, 2001
TLC & HPTLC	Fast, simple, inexpensive, ability to use various solvents.	Short stationary phase, less sensitive, temperature and humidity may affect results	Labmate, 2018
ATR-IR	No sample preparation and the instruments can be compact and portable.	Less sensitive and not specific	Deconinck et al., 2014

LC-MS = Liquid chromatography-mass spectrometry; HPLC = High performance liquid chromatography; ^{1}H NMR = Proton nuclear magnetic resonance; CE-MS = Capillary electrophoresis-mass spectrometry; HPTLC = High-performance thin-layer chromatography.

Table1.3: Selected analytical methods used for the screening of some pharmaceutical adulterants in herbal products

Technique	Analyte/ target compound	Reference
	Mass spectrometry-based techniques	
LC-MS	Hypoglycaemic pharmaceutical (metformin)	Wu et al., 2012
GC-MS	Diphenoxylate and tramadol, 58 pharmaceuticals including (stimulants, weight reducers, NSAIDs, steroids), Sildenafil, vardenafil, tadalafil	Foroughi et al.,2017; AU et al., 2000 Liu, woo and Koo, 2001; Man et al., 2009
UHPLC	steroids, hypoglycaemic products, and antihypertensive agents, non-opioid analgesics and NSAIDs	Becue, Van Poucke and Van Peteghem, 2011; Kesting, Huang and Sorensen, 2010; Li et al., 2010
LC-QTOF-MS	Phosphodiesterase 5 inhibitors such as sildenafil	Aqai et al., 2013 and Aqai et al., 2013
FI-MS	Phosphodiesterase 5 inhibitors, weight-losing pharmaceutical	Song, El-Demerdash and Lee, 2012; Song et al., 2014
AMS and DART	oral hypoglycaemic pharmaceuticals	Zhou et al., 2011
DCBI-MS	weight-loss regimens fenfluramine, phenolphthalein, and sibutramine	Wang et al., 2012
	Capillary electrophoresis	
MEKC with ESI	NSAIDs, analgesics, antipyretic stimulants, and anxiolytics	Cheng et al., 2001
CE-MS	Anorexic drugs, antidepressants and anxiolytics	Almeida, Ribeiro and Polese, 2000; de Carvalho et al., 2010
CE-C^4D	Fluoxetine, flurazepam, fenproporex, sibutramine, sertraline, paroxetine, bupropion, and amfepramone	de Carvalho et al., 2010
	Hyphenated techniques	
LC-DAD and LC-MS	Sildenafil like compounds and analogue	Schramek, Wollein and Eisenreich, 2015
LC-QTOF-MS with NMR	Illegal drugs	Johansson et al., 2014
ATR-IR	Sibutramine	Deconinck et al., 2014
LC-HRMS and LC-MS-SPE/NMR	Valsartan, indapamide and amlodipine	William, 2012
	Thin-layered-based analytical techniques	
TLC-SERS	Antidiabetic drugs, Phosphodiesterase 5 inhibitors	Zhu et al., 2015; Lv et al., 2015

UHPLC = Ultra-High Performance Liquid Chromatography; FI-MS = Flow injection –mass spectrometry; DART = Direct analysis in real time; DCBI-MS Desorption corona beam ionisation - mass spectrometry; MEKC = Micellar electrokinetic chromatography; ESI = Electrospray ionization; CE-C^4D = capillary electrophoresis coupled with contactless conductivity detection; LC-DAD = Liquid chromatography with diode array detector; LC-HRMS = Liquid chromatography–high resolution mass spectrometry; LC MS SPE = Liquid chromatography–mass spectrometry -solid phase extraction; TLC-SERS = Thin layer chromatography combined with surface-enhanced Raman spectroscopy; LC- MS-SPE = High performance liquid chromatography -photodiode-array-mass spectrometry.

As HM adulteration tries to evade detection, more advanced and sensitive techniques have been developed, such as wooden-tip electrospray ionisation mass spectrometry (Hu et al., 2016), Core-shell Column Coupled to Tandem Mass Spectrometry (Al Lawati et al., 2017) among many others. In this research, GC-MS was used to screen selected HM for pharmaceutical adulterants, due its availability and the advantages highlighted in Table 1.2. The target analytes were non-volatile and thermally stable, making them suitable to be analysed with GC-MS.

1.7.2 Sample preparation for the extraction of pharmaceutical compounds in herbal products

The selection of a suitable sample preparation method for the extraction of pharmaceutical adulterants from herbal products depends on factors such as type and complexity of product matrix, product form (solid or liquid), analysis time and instrument sensitivity (Chen et al., 2009). For LC–MS, CE–MS and direct MS techniques, simple “dilute and shoot” sample preparation is generally used prior to instrumental analysis. This sample preparation method uses organic solvent such as methanol or acetonitrile or a mixture of both with water for extraction, and it is commonly used for solid samples such as powders, capsule contents, and homogenized tablets involving sonication or shacking (Vaclavik, Krynitsky and Rader, 2014). Liquid samples are usually just mixed with a solvent and the mixture is then centrifuged, filtered and diluted, requiring no clean-up of the sample (Chen et al., 2009). The “dilute and shoot” method typically takes about 20 minutes but may take up to 30 minutes with the GC-MS method, where derivatisation may be required (Van Thuyne and Delbeke, 2005).

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method of sample preparation uses a mixture of the sample with water and acetonitrile followed by separation of the aqueous and organic phases by addition of salts for the extraction of the sample (Vaclavik, Krynitsky and Rader, 2014). The majority of the polar constituents of the sample are retained in the aqueous phase, while the analytes are transferred to the acetonitrile component of the solution (Anastassiades et al., 2003). This sample preparation method has been used in the analysis of herbal products (Lacina et al., 2012), although for improved extraction soaking of the sample for about 30 minutes may be essential for plant toxins. However, this is not required for the extraction of pharmaceutical adulterants because they are an anthropogenic addition to the sample and are not a constituent of the sample matrix (Vaclavik , Krynitsky and Rader, 2014).

In some other studies, a further sample clean-up step is used to differentiate a co-extracted matrix of the sample. Some of these further clean-up steps include removal of lipophilic

components by nonpolar solvents (Vaclavik, Krynitsky and Rader, 2014), mixed-mode octylsilyl (C8)- cation exchange (Becue, Van Poucke and Van Peteghem, 2011), solid phase extraction (SPE) using cation exchange (Wu et al., 2012) cartridges, or dispersive SPE (dSPE) using octadecylsilyl (C18) (Vaclavik, Krynitsky and Rader, 2014) or primary secondary amine (PSA) sorbents (Stepan, Cuhra and Barsova, 2008 ; Wu et al., 2014).

1.7.3 Analytical method for the screening of heavy metals in herbal medicine

A number of analytical methods have been employed to screen herbal products for heavy metals. These include techniques such as Graphite furnace atomic absorption spectrometry (GFAAS), Flame atomic absorption spectroscopy (FAAS), Atomic fluorescence spectrometry (AFS), Inductively coupled plasma optical emission spectrometry (ICP-OES) or mass spectrometry (ICP-MS), Cold vapour atomic absorption spectrometry (CVAAS), X-ray fluorescence spectrometry (XRFS). The choice of analytical method, as discussed for the analysis of pharmaceutical compounds, is also dependent on the peculiarity of the analysis and the desired result.

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) can be used to measure most elements in the periodic table. Additionally, it is a spectroscopic technique with a wide concentration range, which can measure elements at both high and trace concentrations, unlike other techniques used in elemental analysis (Mohammed, 2014). It has a low detection limit with limited spectral and matrix interference. Inductively coupled plasma-mass spectrometry (ICP-MS) has similar advantages and is also useful in isotope analysis; however, matrix effect is a disadvantage of ICP-MS (Perkin Elmer, 2004). The capital and operating cost of ICP-MS and ICP-OES are higher than that of FAAS. However, problems such as a poor detection limit, matrix effects, capacity for limited element analyses and longer analysis time are among the disadvantages of FAAS, unlike ICP-OES and ICP-MS (Macfadden, 2004). Most of the analytical methods are, however, destructive and the type of sample that can be analysed varies from liquid or gaseous in ICP-OES to solid samples in XRFS. Solid samples can be digested and analysed in liquid form for metal analysis, though it may increase analysis time (Perkin Elmer, 2004). A summary of the comparison of the various analytical techniques used in metal analysis is presented in Table 1.4.

Table1.4: Comparison of selected elemental analysis techniques (Perkin Elmer, 2004)

Properties	Flame AAS	GFAAS	ICP-OES	ICP-MS	XRF
Limits of Detection	High ppm	Sub ppb*	Sub ppb	Sub ppb	Sub ppm**
Analytical potential	Single element	Single element	Simultaneous Multi-element analysis	Simultaneous multi-element analysis	Simultaneous multi-element analysis
Usability	Very easy	More difficult	Easy	More difficult	Easy
Development of Method	Easy	Easy	Fairly easy	More difficult	More difficult
Automated Operation	No	Yes	Yes	Yes	Yes
Procurement Cost	Low	Medium	High	Very high	Very high
Operating Costs	Low	High	Medium	High	Low

*ppb =parts per billion, **ppm = parts per million

To achieve desired results, in view of the potentially complex plant matrix of HM samples, the correct choice of an analytical method is essential. The use of several analytical techniques in the screening of heavy metals in herbal products is summarised in Table 1.5.

Table1.5: Selected analytical methods used to screen for heavy metals in herbal products

Technique	Analyte/ target compound	Reference
Flame atomic absorption spectrometry (FAAS)	Cu, Zn, Pb, Cd Mn, Mg and Si	Dong and Zhu, 2002; Wei et al., 2003; Qin et al., 2015; Gómez-Nieto et al., 2017
Graphite furnace atomic absorption spectrometry (GFAAS)	Pb, Cd, Cu, As, Hg, Cr and Ni	Nie *et al*., 2008; Yuan *et al*., 2009; Zhong, Ren and Zhao, 2016
Cold vapour atomic absorption spectrometry (CVAAS)	As and Hg	Yuan et al., 2009; Ang and Lee, 2006; Krishna and Karunasagar 2015
Atomic fluorescence spectrometry (AFS)	As	Lu et al., 2017
Inductively coupled plasma optical emission spectrometry (ICP-OES)	Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se and Zn	Santos Júnior et al., 2017; Sium et al., 2016; Cindric, 2013; Cindric et al., 2015
Inductively coupled plasma mass spectrometry (ICP-MS)	Cr, Zn, Mn, Se, Cu and V, Mg, Al, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Sr, Sb, Ba, As, Cd, Hg and Pb, Cd, Mo and Sn	Shao et al., 2007 Nookabkaew et al., 2006; van der Voet et al., 2008; Christopher and Thompson, 2013
USN-ICP-OES and ETAAS	Al, Cd, Pb, Fe and Vd	Gomez et al., 2007
X-ray fluorescence spectrometry (XRFS)	Pb, Hg, As Zn, Mn Fe and AU	Saper et al., 2004; Woolf et al., 2008; Wang et al., 2008; Sánchez–Pomales et al., 2013
Energy Dispersive X-ray Fluorescence Analysis (EDXRF) and Neutron Activation Analysis (NAA)	Al, Ba, Br, Ca, Co, Cr, Cu, Fe, Hf, K, La, Mn, Na Rb, Sm, Sc, Th, V and Zn	Gwarzo et al., 2017 Koch et al., 2007

USN-ICP-OES = Ultrasonic nebulisation coupled to inductively coupled plasma optical emission spectrometry; ETAAS = Electro-thermal atomic absorption

1.7.4 Sample preparation for the screening of metals in herbal products

Sample preparation of herbal products for the analysis of metals takes into account variations in the matrix compositions such as oils, fat, and proteins, to mention a few. Particular attention is, however, needed to prevent contamination from reagents and lab ware such as vessels, and mortar and pestle (Sastre et al. 2002). Optimal workings of the

atomic spectrometric and plasma-based methods need a nebulisation system for the introduction of the sample. Accordingly, the sample needs to be in liquid form and properly digested (Smichowski and Londonio, 2018). Digestion of the sample can be achieved e.g. by using microwave-assisted digestion, dry ashing or open-vessel digestion (Sium et al., 2016).

Microwave-assisted digestion is most used for the digestion of solid samples, although it is also useful for digestion of liquid samples which can be directly put into the polytetrafluoroethylene microwave oven vessels (Smichowski and Londonio, 2018). Microwave digestion reduces the risk of contamination from the environment and also ensures faster analysis. It uses several acids for its digestion, such as nitric acid (HNO_3), hydrogen peroxide (H_2O_2), perchloric acid ($HClO_4$), hydrochloric acid (HCl), sulphuric acid (H_2SO_4), phosphoric acid (H_3PO_4) and hydrofluoric acid (HF), which are often in mixture of various combinations and proportion, depending on the sample matrix (Konieczynski and Wesolowski, 2012, 2014; Pasias et al., 2010; Santos Júnior et al., 2017; Novotnik et al., 2015; Basgel and Erdemoglu, 2006).

Dry ashing is a very useful sample preparation method which uses less acid and aids complete digestion of the organic constituent. Nonetheless, dry ashing presents limitations such as volatilisation losses of some elements including As, Hg, Cd, Se and Pb from high ashing temperature. To avoid loss of volatile metals and boost digestion efficiency ashing aids, magnesium nitrate ($Mg(NO_3)_2$) have been used in previous research (May, 1982; Cervera et al., 1994; Welna et al., 2011), while other studies pre-digested the sample with nitric acid (Brumbaugh and Walther, 1989). Considering the sustainability issues with the use of fewer chemicals and recovery efficiency, $Mg(NO_3)_2$ was used in this study. Furthermore, a pyrolysis temperature setting not exceeding 350°C has been said to prevent loss of volatile metals such as cadmium in the absence of a matrix modifier (Jorhem, 2000; Welz and Sperling, 1998; Schlemmer and Radziuk, 1999). Hence in this study, the pyrolysis temperature did not exceed 350°C, as stated in Section 3.6.3.2.1. The dry ashing method has been used in various publications for sample preparation of HM and the ash dissolved in various acids such as HNO_3 and HCl (Santos Júnior et al., 2017; Pytlakowska et al., 2012) or a mixture of both acids (Malik et al., 2013).

Open-vessel wet digestion or conventional wet digestion on hot plates using glass ware (tubes or beakers) or Teflon have been carried out for a long time (Welna et al., 2011). It is especially useful for simple samples such as food and plant products including HM, but not commonly suitable for samples requiring prolonged digestion times of up to 24 hours *(ibid).* Open-vessel wet digestion provides results that are reliable and accurate (Abentroth Klaic et

al., 2011; Türkmen and Ciminli, 2007; Kira and Maihara, 2007). Generally, HNO_3, HCl, H_2SO_4, H_3PO_4, $HClO_4$, HF and H_2O_2 are used for digestion of organic samples such as rock, soil, alloy, silicate and mineral (Uddin et al., 2016). However, they have now been used for wet digestion of herbal products (Altinting, Altundag and Tuzen, 2014; Gomez et al., 2007; Sium et al., 2016). Some of these digestion reagents are highly toxic, requiring special training and handling. Concentrated HNO_3 is mostly used for digestion of organic materials, as a result of the higher solubility of its salt (nitrate salt) in water, accessibility, and affordability compared to other acid and their salts (Sastre et al., 2002). However, its low oxidation potential can cause incomplete digestion of substrates rich in organic constituents (Welna et al., 2011).

Hence the addition of another acid improves digestion. Aqua regia (HCl with HNO_3 (3:1) v/v), for example, is very useful for the digestion of complex matrices such as a combination of various plant and non-plant matrices used in this research (David, 2000). The addition of HCl is particularly safe in ICP-OES analysis and it is less viscous; therefore it prevents interference in the transport of solutions, unlike the addition of H_2SO_4 instead (Welna et al., 2011). Other acid combinations have also been reported for optimal sample digestion, examples of which include; HNO_3 and H_2O_2 (Santos Júnior et al., 2017), HNO_3, H_2SO_4, H_2O_2 and HCl (Sium et al., 2016), HNO_3, $HClO_4$ and HF (Gomez et al., 2007).

1.8 Quality control of herbal medicine

The quality of HM is influenced by many factors which range from medicinal herb cultivation to its end product. Hence standardisation is an important tool in quality control of HM. Standardisation of herbal medicines requires the setting of standard qualitative, consistent and quantitative values, reflecting quality assurance, safety, reproducibility and efficacy (Kunle, Egharevba and Ahmadu, 2012). Specific and precise standards are determined through research, leading to the development of a group of characteristics expected to be maintained by individual HM. As an example, electrophoretic, fluorescence and chromatographic data unique to different HMs and associated comparative chemometric analysis have been published (Mazina et al., 2015). However, challenges of HM quality control to a large extent still include factors such as variable quality and source of the raw material, varying chemical and natural components of plant materials, existence of chemo cultivars and chemo-varieties, inability to determine the active principle in many HMs and unavailability of analytical methods that are selective or commercially available reference standards (Kunle, Egharevba and Ahmadu, 2012). These issues are beyond the scope of this study. However, analysis for pharmaceutical compounds and heavy metals as performed in this research is essential for quality control measures in safeguarding public

health. A WHO report highlighted points of focus in the quality control of HM (WHO, 1998; 2007a; 2007b and 2011), two of which are relevant to this research:

1. Micro and macroscopic examination to identify safe variants and detection of possible adulterants
2. Toxicological studies to determine potentially toxic elements such as pesticide residues, harmful microorganisms and other studies to determine the lethal dose (LD50).

1.9 Regulatory framework of herbal medicine

1.9.1 Global perspective

There are various ways countries regard and define herbs, medicinal plants and their constituents. Countries have also developed different methods of manufacturing, licensing, trading and dispensing of herbal therapies to guarantee the quality, safety and efficacy (DSHE, 1994; Ameh et al., 2010; Human Medicine Regulation, 2012).

In the UK and EU, for example, if a herbal product is considered to be a medicine, it needs marketing authorisation (Product licence) or may be licensed employing a simplified Traditional Herbal Registration (THR), appropriate to use without medical supervision (Human Medicine Regulation, 2012). To attain THR for a product manufacturers or suppliers will have to show at least 30 years' history of traditional use of the herbal product (15 years of which should be in the EU), compliance with appropriate manufacturing standards, evidence of safety and availability of appropriate product information to users (Human Medicine Regulation, 2012). The THR framework is for non-severe, self-limiting conditions such as respiratory problems, infections (bacterial, viral, and fungal diseases), cold, skin and other less serious conditions. Conversely, unlicensed remedies may be produced and distributed by a practitioner in meeting individual patient needs after a one-to-one consultation without any legal restriction. These unlicensed products are not allowed to be marketed as HM until a THR or full marketing authorisation (product licence) has been obtained (*Ibid*).

In the USA HM is categorised as a dietary supplement and is not recognised as a medicine. According to the Dietary Supplement Health and Education Act (DSHE 1994), a dietary supplement includes any product (aside from tobacco) aimed at supplementing the diet with dietary ingredients such as amino acids, minerals, vitamins, herbs or other botanicals. Classification as a food supplement makes its regulation less strict but the dietary supplements' current Good Manufacturing Practices (GMPS or cGMPs) are, however, enforced for all manufacturers of HM. The food GMPs focus on all manufacturing aspects

including positive identification and purity assurance for whole herbs using macro-identification and organoleptic, use of techniques such as HPLC / TLC or microscopy for extracts and powders, identification of material source, detailed documentation, hygiene and personnel training (FDA, 2015).

In Asian countries such as India, Korea and China, traditional HM is accorded the same respect as modern pharmaceuticals with both included in the national health scheme (Ameh et al., 2010). The process of approving traditional HM includes approval for both clinical trial and marketing. The requirement for registration of HM includes pharmacological or toxicological data, pharmaceutical and general product data, and clinical data. Also required is the certification of manufacturers and marketers by the local drug regulatory agencies with an emphasis on good GMP and supply ethics (Ameh et al., 2010). However, HM regulatory frameworks are constantly evolving as more research is carried out on herbal products globally.

1.9.2 Regulatory framework of herbal medicine in Nigeria

The ability of regulatory bodies to monitor herbal products appropriately, coupled with the absence of appropriate legislation is a huge challenge in some countries like Nigeria and by extension Ekiti State. The National Agency for Food and Drug Administration and Control (NAFDAC), the Nigerian government regulatory agency's experience attests to this. A former head of the agency in a newspaper article highlighted lack of documentation, secrecy of real components, poor coordination of practitioners' activities and the challenges in determination of actual ingredients as among the difficulties faced in regulating HM in Nigeria (*The Nation newspaper*, 2008). This was attested by Akinleye (2008), who reported imprecise dosage, incorrect diagnosis, poor hygienic settings and secrecy of some healing methods and the lack of documentation on patients as among the challenges to safe HM practice in Nigeria.

According to the Director General of NAFDAC, compliance with manufacturing and regulatory standards by manufacturers of HM in Nigeria is still a herculean task, despite Nigeria's having a national policy on traditional medicine (Elujoba, 2013). Regulatory requirements were said to be perceived to create bureaucracies and discourage manufacturing. This perception still persists, due to a lack of local agenda for herbal medicine research and development by research institutions and the pharmaceutical industries in creating an indigenous phytomedicinal standardisation. This has made effective regulatory requirements difficult to achieve. As a result, current manufacturers' therapeutic claims and formulations are unverifiable and the regulators are also at a disadvantage. Conversely, guidelines are available for manufactured herbal products both imported and

local (WHO, 2003; NAFDAC, 2013). Herbal medicines have not yet been introduced in the essential health-care system in Nigeria. Regardless, a lot of people use HM in Nigeria.

NAFDAC is saddled with the responsibility of controlling and regulating the importation, manufacture, export, sale, advertising, distribution and use of medical devices, drugs, cosmetics, food, chemicals and packaged water (NAFDAC, 2005). According to NAFDAC (2013), regulations guiding the operation of HM practice in Nigeria are:

I. Herbal medicines and related products registration regulations, 2005
II. Herbal medicines and related products advertisement regulations, 2005
III. Herbal medicines and related products labelling regulations, 2005

According to the NAFDAC (2013), the requirement for registration of HM in Nigeria include

I. A detailed dossier on the source of each herbal product contained in the formulation
II. An acceptable quality and safety analysis certificate for the accredited laboratory
III. Factory certification for quality assurance
IV. A licence to practise and certification of the herbal medicine practitioner

Advertisement and labelling regulations also include various requirements to ensure safety and quality control of HM in Nigeria. Perhaps the most challenging problems with HM in Nigeria are the unavailability of safety regulations and inadequate standardisation (Ekeanyanwu, 2011). Standardisation of HMs may be particularly difficult because they contain hundreds of chemical constituents with little or no identification and no evidence of which constituent might be responsible for the claimed therapeutic effect (WHO, 2005). Nonetheless, advances in science have aimed to overcome these problems through quality control measures, as discussed in Section 1.8. Therefore the safety and control of HM needs to be reviewed in Nigeria; hence the need for this research as part of addressing the problems.

1.10. Prospect of herbal medicine

1.10.1 Global prospect of herbal medicine

About 75% of the therapeutic compounds of plant origin used worldwide were derived from traditional/folk medicine; with 25% of prescription medicines available worldwide obtained from plant sources (Wachtel-Galor and Benzie, 2011). Findings on traditional medicine and medicinal plants practices for 35 years (1970-2005) in Nigeria showed the results in herbal medicine research and development activities (Osemene, Elujoba and Ilori, 2011a). The

publication reported the degree of contribution of HM in various aspects of medicine, such as in anti-infective drugs or anti-microbial drugs (32.48%), gastro-intestinal tract drugs (9.69%), analgesics, antipyretics and non-steroidal anti-inflammatory agents (8.69%), cardiovascular agents (5.41%), toxicity studies (5.27%), hypoglycaemic agents (4.27%) and molluscicidal (3.85%). Other areas where research was moderately undertaken were in oxytocic (2.85%) and dermatological agents (2.71%), insecticides (2.42%), anaesthetics (2.28%), drugs affecting the blood (2.28%) and tranquillisers (2.14%) (*Ibid*). The use of herbs in the advancement of modern pharmaceuticals will continue, especially in the event of multi-resistant antibiotics and a need for more effective medication. Besides the use of herbs in pharmaceutical development, the growth of HM practice as an established system of medicine and efforts towards orthodox medicine standard is ongoing (Mukherjee and Wahile, 2006; Aggarwal et al., 2011; Kulkarni, Girish and Kumar, 2012).

Traditional medicine is believed to be a cultural health practice developed over time and within a particular belief system (WHO, 2000). But this is also true of orthodox medicine, which also evolved over time and became an established healthcare system through rigorous scientific support. The improvement of traditional medicine through research and rigorous scientific study is paramount and vital to its advancement. Improved and increased research focused on the efficacy, safety, mechanism of action, regulation of standards for practice, training of medical students and conventional medical practitioners will be highly beneficial to the advancement of traditional medicine (Bhattacharya, 2000 and Marcus, 2001).

1.10.2 Prospect of herbal medicine in Nigeria

The Federal Government of Nigeria (FGN) has taken steps to institutionalise traditional medicine in an attempt to expand healthcare coverage in the country. This necessitated the enunciation of the Traditional Medicine Policy for Nigeria (2007). The Nigerian Traditional Medicine Policy complies with the regional strategy implementation for the African region on promoting the role of traditional medicine in the health system (WHO, 2000). The objectives of the Nigerian medicine policy include (FGN, 2007):

I. Development and facilitation of traditional medicine use in the official healthcare system in Nigeria
II. Maximise the benefit and economic potential of traditional medicine practice to attain requirement of the National Economic Empowerment and Development Strategy (NEEDS).
III. Establishment of an institutional framework for traditional medicine which will be country-specific.

However, this policy has not made any progress in the nine years since its implementation. Use of herbal medicine in Nigeria still faces challenges from government officials who regard it with disrespect and disdain (Adefolaju, 2011), as discussed in Section 1.3.3. Colonisation has been reported to have harmed the development of HM in Nigeria (Matthias, Osayi and Opara, 2012), but then India was also under colonial rule but seems to have developed her traditional medical practice way ahead of Nigeria. Successive Nigerian administrations have remained reluctant to accord herbal medicine a position in their national health-care delivery system as is the case in China, Ghana and even India (Adefolaju, 2011). Reportedly, HM is practised in contemporary Nigeria without any enabling legislation to standardise and regulate its practice, as is the norm in other parts of the world (WHO, 2011). The many challenges facing standardised orthodox medical practice in Nigeria may have hindered the progression of traditional medical practice for healthcare delivery. This does not, however, mean nothing is being done. Despite the hurdles, HM research and collaboration with the orthodox health system is on-going. The future of HM practice in Nigeria lies in the effective coordination of the various research outputs on HM, industrial collaboration and the sincerity of the government in providing direction and an enabling environment with an emphasis on safety.

1.11. Research aim and objective

Public health and toxicological analysis of herbal medicine and its use in Ekiti State have become essential, as is apparent from the literature review in this chapter, especially as shown in Section 1.6.2. Accordingly, this research seeks to fill the gap in knowledge by exploring the sociological and public-health aspect of HM use. Commonly used HMs are identified and samples analysed in the laboratory. Hospital data was then examined on HM-related cases in the state. This is the first multifaceted study in Nigeria. The research process is presented in Figure 1.2.

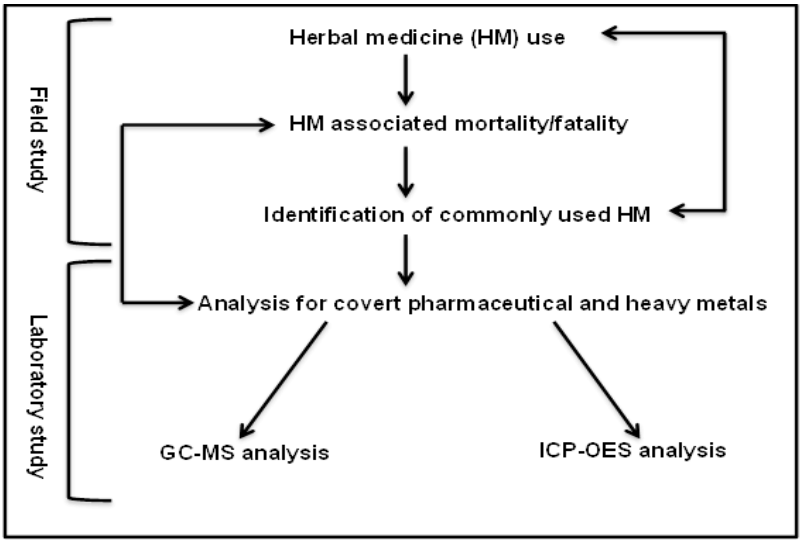


Figure1.2: Flow diagram of the research process

The findings from the public health survey, HM laboratory analysis and HM-associated hospital cases will provide a multifaceted understanding of HM use and its potential impact on the study population. Therefore the research aims to:

I. Study the pattern and use of herbal medicine in Ekiti State, and the effect of socio-economic factors using structured questionnaires.

II. Study reasons for non-use of orthodox health facilities in Ekiti State using structured questionnaires.

III. Determine the casualty and fatality figures associated with the use of HM using data obtained from 16 government hospitals in Ekiti State between the years 2010 and 2014.

IV. Identify and quantify heavy metals in uncertified and certified herbal medicine used in Ekiti State. Metals with known toxic profiles reported in the literature (arsenic, mercury, lead, cadmium, chromium, manganese, nickel, selenium, zinc and copper) will be analysed using ICP-OES.

V. Identify organic constituents such as analgesics (acetaminophen) non-steroidal anti-inflammatory drugs (ibuprofen, diclofenac), opiates (codeine, tramadol), steroid (dexamethasone), CNS stimulant (caffeine), antihistamine (chlorpheniramine), antidepressant/weight loss (fluoxetine) and benzodiazepine (diazepam) in uncertified and certified herbal medicine in Ekiti State using GC-MS

VI. Propose an effective public health measure in herbal medicine use and control in Ekiti State through the deployment of research findings for policy recommendation.

Research questions posed in Section 1.11.1 were used to achieve the aims and objectives.

1.11.1 Research questions

I. What are the pattern and use of herbal medicine in Ekiti State and the influence of socio-economic factors?

II. What are the reasons for non-use of orthodox health-care facilities in Ekiti State?

III. What are the casualty and fatality figures associated with herbal medicine use in Ekiti State between the years 2010 and 2014?

IV. What heavy metals and organic constituents are present in both uncertified and certified herbal medicine used in Ekiti State?

V. What policy recommendations may be proposed through the findings from this study?

CHAPTER 2: SURVEY OF THE PATTERN AND USE OF HERBAL MEDICINE IN EKITI STATE

2.1 Introduction

Research into the complex reasons associated with preference for herbal medicine reveals the interplay of many factors. The role of personal and cultural beliefs, philosophical mindset on health and life come to bear (Ernst and White, 2000). Respondents' comparison of the experience of orthodox healthcare and complementary medicine systems also plays a vital role (Astin, 1998). Therefore in this chapter, the sociological and public health aspects of HM are the first research component explored, examining its stance within the social awareness and its popularity as an alternative to orthodox medicine. This is achieved through the use of an interview-based questionnaire centred on determining the general public's knowledge of HM in Ekiti State. The use and perception of HM against a socioeconomic and educational background was explored in addressing the following two research questions;

- What is the pattern and use of herbal medicine in Ekiti State?
- How do socio-economic factors influence the use of HM in Ekiti State?

The analysed survey data offers a sociological framework which will be compared with analytical data of HM samples (see Chapter 4) obtained as part of this research. This is undertaken to see the correlation between peoples' perception and the reality in HM samples found in Ekiti State. In this study, HM refers to any material as defined in Section 1.2.

2.2 Research method

2.2.1 Justification of study method

A survey is an approach which may be used to gather information, to observe patterns of association between variables from particular studies (Sue and Ritter, 2012; Bryman, 2012). Surveys may employ qualitative methods, quantitative methods, or both mixed, and then data may be obtained either by use of structured interview, self-completion questionnaire, or a mixed-mode approach (De Vaus, 2001).

Qualitative research may be difficult to define appropriately due to its different, multifaceted paradigms (Hitchcock and Hughes, 2016, pg. 26). Some definitions of qualitative research have been criticised as too simplistic and neglecting other areas of the research design such as that which defines it as a form of research producing findings that are not reached by

statistical process or other quantification methods (Strauss and Corbin 1998, pg. 10-11). Another definition refers to qualitative research as a collection of comprehensive data on variables over a period, in a naturalistic environment, so as to gain awareness which may be impossible applying other research methods (Gay, Mills and Airasian, 2011, pg. 627). This definition's use of variables could be conflicting, as this epistemological postulation is based on complicated and interrelated social phenomena that sometimes cannot be defined by remote variables. Through condensation of a variety of literature, qualitative research may be defined as a developed method aimed at a descriptive interpretation of people's construct of meanings in relation to their experience of the world within their natural environment of phenomena or social occurrence through an inductive and interpretative approach (Patton, 2015, pg. 39–41; Denzin and Lincoln, 2017, pg. 3; Miles, Huberman and Saldana, 2013, pg. 6–7).

By contrast, quantitative research explains phenomena in terms of numerical data which are mathematically and statistically analysed. It may be defined as an empirical research method which examines social occurrences or human issues by testing proposed theories made up of variables that are measured using statistical analysis and numbers to determine how and to what extent the theory describes or forecasts phenomena that are of interest (Gay, Mills and Airasian, 2011; Creswell and Creswell, 2017). A quantitative approach requires that the researcher uses a preconstructed standardised tool or response categorisation that the respondents' diverse views and experience are expected to fit. There are a lot of distinguishing differences between qualitative and quantitative research. In this study, quantitative approaches were adopted but with the use of some open-ended questions.

Research methods are not limited only to qualitative and quantitative studies, but results are likely affected by the way they are used. The validity of the result of a study is more likely to be dependent on how right is the epistemology of the method. As long as the philosophical idea is the same in combined methods, their selection and application should not be problematic (Trochim, Donnelly and Arora, 2015). The aim, scope and the nature of inquiry are important in deciding the choice of research method, rather than a dichotomy between quantitative and qualitative methods (Siverman, 2015). The differences in an epistemological idea of some particular methods should not discourage a researcher employing qualitative method from using data collection methods typical of quantitative research, and vice versa, but instead use methods based on the rationale or justification of the methods to be used in the study (Howe, 1992; Johnson and Onwuegbuzie, 2004; Palinkas et al., 2011a, b, c).

Aside from other qualities of qualitative research, it is more in-depth, takes more time and requires a small sample size. The large sample size in this study (Section 2.4.1) does not

favour a qualitative study likewise other characteristics. Therefore the use of textual data in quantitative research was adopted. This is one of the ways of combining qualitative and quantitative research method characteristics which has been called “quantitizing” of qualitative data (Sandelowski, Voils and Knafl, 2009). Although it may be criticised as a non-integrative approach which seeks to quantify otherwise qualitative data, pragmatic justification of the usefulness of this method in analysis of HM use and associated issues is more important. Textual data were collected by the use of expansion, substitution and general open-ended questions (O'Cathain and Thomas, 2004).

Closed-ended questions are usually used in quantitative research; however, they may not be ideal for exploring complex and dynamic issues of HM use and health-seeking behaviour and unreported preference for HM in the population of interest in this study. Determination of various and prominent types of HM used by the sample population for subsequent laboratory analytical examination was better explored by open-ended questions. Other benefits of open-ended questions have been reported, such as their potential to increase response rate and identify new issues (Iversen, Bjertnæs and Skudal, 2014). A previous study reported that quantitative ratings were possibly more for positive than for the negative comments, and also observed qualitative comments helped validate quantitative ratings (Santuzzi, Brodnik and Rinehart-Thompson, 2009). Strength of open-ended responses is their potential to allow a direct view of a participant’s line of thought. For instance, it has been argued that open-ended questions explore views on the respondent’s mind as at the interview time which were presumably salient before the question was asked and afterward (RePass, 1971). Similarly, it has been noted that open-ended questions have the characteristic “nonreactivity” as an advantage, which does not prompt participants to think of specific causes or treatments, unlike close-ended question (Iyengar, 1996).

Despite these advantages, open-ended questions are not as often used due to lack of knowledge of the best ways to obtain and present respondents’ comments for intended uses especially in health research (Rijisk, Ammentorp and Kofoed, 2012). They have rarely been analysed as proper qualitative data and are also more rarely used in combination with quantitative results obtained from the same questionnaires (Stoneman, Sturgis and Allum, 2013). Additionally, there is a vast number of published works on quantitative data collection, but few have been published in scientific journals about collection procedure and handling of data from open-ended questions (Iversen, Bjertnæs and Skudal, 2014; Gilles et al., 2017). It has been recommended that open-ended questions should not be asked unless replies will be analysed (Boynton and Greenhalgh, 2004). This may be a waste of participants’ time and probably unethical. But resources and time required for data input and analysis may be a

major constraint (Mccoil et al., 2001). In addition, open-ended responses have conventionally been seen as more strenuous to analyse than the closed-ended question (Schuman and Presser, 1996), because human coding is almost always used. However, these factors should be factored in during the process of the study design, as was the case in this study. Open-ended responses have been categorised as qualitative (Stickler et al., 1992; Bankauskaite and Saarelma 2003), some argue they are not (Boulton, Fitzpatrick and Swinburn, 1996), and some argue they are semi-qualitative (Murphy et al., 1998). Its use however, cuts across both qualitative and quantitative research.

There has been debate on the usefulness of the open-ended questions with emphases on their advantages and disadvantages (Krosnick & Schuman, 1988; Schwarz & Hippler, 1991) and the debate is still ongoing. Experts in this field have also identified the merits of each method (Lazarsfeld, 1944 and Krosnick, 1999). The usability of open-ended methods in research continues to be reported; likewise its relevance (Roberts et al., 2014). It was, however, reported that the benefit of using open-ended in addition to standard closed-ended questions was found to be practically nil in mental health prediction (Friborg and Rosenvinge, 2013), though the same study showed that open-ended questions revealed more comprehensive information than closed questions. Therefore, the choice will ultimately depend on the target result by individual researcher. Hence the need for open-ended question in this research is premised on the desired result which is to understand the pattern and use of HM in respondents' own words, including their opinion which can be measured to determine and predict the phenomenon of interest.

The approach adopted in this study can be implemented by various means, e.g. either paper-based, online or telephone. Telephone response rates have drastically reduced in recent years and sample size coverage problems are increasing (Singer, 2006). The model selected for this research was the use of self-administered paper-based survey and face to face interview. A paper-based survey was used to prevent sample selection bias, considering the level of education, literacy and level of Information communication technology (ICT) access in the population sample. Ekiti State has only about 3.1% of her population with access to personal computers; about 7% of households have access to the internet and 2.2% of the population have access to internet (NBS, 2012). Although there was an improvement to about 27% of the estimated population having access to internet in 2016 (NBS, 2016), this is still not adequate for an online survey. Hence the need for a paper-based survey in this study. An online survey is cheaper and a more sustainable approach but loss of data and software error can be a real setback (Sue and Ritter, 2012). A paper-based survey can be more time consuming and tedious to carry out than an online survey.

For this study adequate time was given for survey participants to rest, refresh and continue. The paper-based survey allows for verification of participants' identity because they are physically present. A comparison of the outcome of paper-based and online surveys has shown similar results were obtained (Hallfors et al., 2000). However, this study was carried out in the United States of America, with 87.4 % internet penetration and 42.6 % internet use, compared with Africa's 28.6 % internet penetration and 9.8 % internet use (Internet world statistics, 2015). Therefore in this survey, a mixed mode method was used which entails one sample, one-time duration, one questionnaire but different modes for different respondents, as seen in Section 2.2.2.

2.2.2 Self-administered and face- to- face interview mix

A mixed-mode approach compensates for the deficiencies of each combined mode at an inexpensive cost and provides the best of both methods. The most economical method may not be adequate to a given study. However, a mixed-mode design explores an explicit adjustment between cost and error, especially measurement error, frame or coverage error (non-sampling error), and non-response error (Biemer and Lyberg, 2003; Palinkas et al., 2015). There are at least four cognitive demanding steps required in answering a questionnaire; understanding of the question, memory recall of information needed, assessment of recalled information and link to the question asked, followed by communication of the answer (Tourangeau,1984; Bajekal et al., 2004). Therefore different questionnaire methods place different cognitive demands on respondents, such as the demand for literacy in the self-administration method. Face-to-face interview structured questionnaires only require verbal and listening skills, provided the question is asked in the language the respondents understand. This method was used in this survey for respondents with poor literacy levels and the questions were asked in the language they understood (Yoruba). A main disadvantage of the face-to-face interview method is interviewer bias (Lonna et al., 2014). However, interviewers were trained and the needs to be satisfied by each question were discussed extensively to overcome this bias. The use of a structured questionnaire was also very important in overcoming the bias.

Thus this study adopted a quantitative approach, using some open-ended questions requiring textual analysis. The questionnaires were administered using the mixed mode approach (self-administered and face-to-face interview).

2.3 Research setting and sampling

2.3.1 Research location

This study was conducted in Ekiti State in Southwest Nigeria, one of the 36 states of the country. Ekiti State was created on the 1st of October 1996 out of Ondo State as a political structure, but the history and existence of Ekiti far outdates that political creation (Bello, 2009, EKSG, 2016). Ekiti lies in the tropics, between latitudes 70°151′ and 80°51′ north of the Equator and longitudes 40°51′ and 50°451′ east of the Greenwich meridian (EKSG, 2016). Figure 2.1 gives a pictorial view of the location of the study in the wider African landscape.

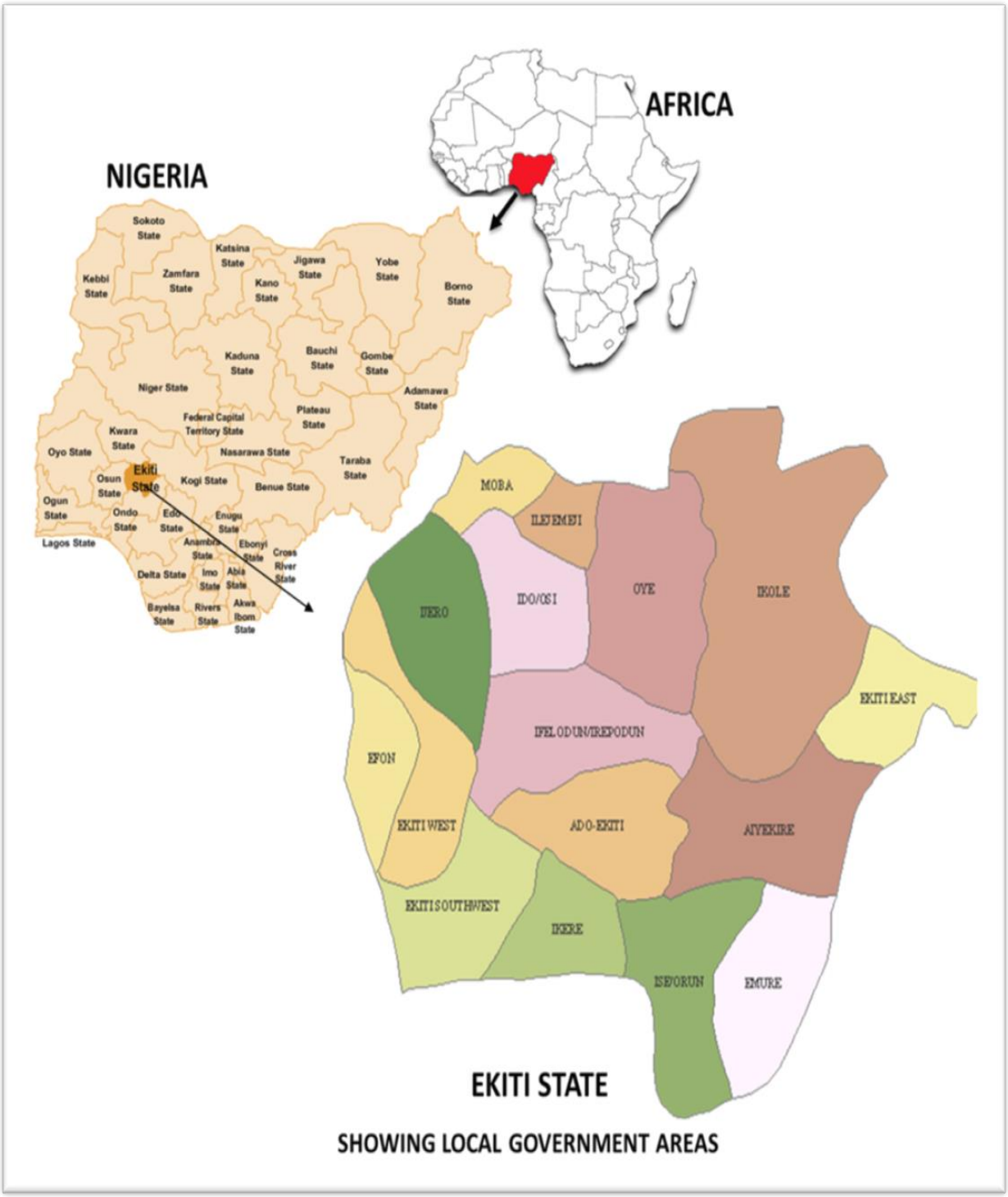


Figure 2.1: Map of Africa, Nigeria and Ekiti State (Malizumedia, 2018; Word press, 2013)

Ekiti State has a land area of 5887.890 sq km bordering Kwara and Kogi State to the South, Osun State to the East, Ondo State to the East and South (EKSG, 2016)

Ekiti State is made up of 16 Local Government Councils, as shown in (Table 2.1), with a population of 2,384,212, of whom 1,215,487 are male and 1,183,470 female (NPC, 2006).

Table 2.1: Local government areas in Ekiti State and their headquarters

SN	Local Government	Headquarters
1	Ado-Ekiti	Ado-Ekiti
2	Efon-Alaaye	Efon-Alaaye
3	Ekiti East	Omuo-Ekiti
4	Ekiti South West	Ilawe-Ekiti
5	Ekiti West	Aramoko
6	Emure	Emure-Ekiti
7	Gbonyin (Aiyekire)	Ode-Ekiti
8	Ise/Orun	Ise-Ekiti
9	Ido-Osi	Ido-Ekiti
10	Ijero	Ijero-Ekiti
11	Ikere	Ikere-Ekiti
12	Ikole	Ikole-Ekiti
13	Ilejemeje	Iye-Ekiti
14	Irepodun/Ifelodun	Igede-Ekiti
15	Moba	Otun-Ekiti
16	Oye	Oye-Ekiti

2.3.2 People and culture

Ekiti State people also known as the “Ekitis” are culturally homogeneous, form one of the biggest ethnic groups in Yorubaland and speak a form of Yoruba called Ekiti (Oguntomisin, 1981). Ekiti is one of the several subgroups of the Yoruba nation (Olagunju, 2012). The Yorubas dominate the South-western part of Nigeria and some have been reported to be located in contemporary Republics of Togo and Benin in West Africa, Cuba and some Caribbean countries (Abimbola, 2006). The Nigerian Yoruba population is about 30 million, constituting about 21% of the total population of Nigeria (Ogundele, 2007). Ekiti people are mainly Christians and Muslims while some are traditional religionists and worship local deities such as Sango, Ifa and Ogun.

According to EKSG (2016), Ekiti State is made up of over various large, small, ancient and modern towns totalling about 127. In ancient times Ekiti men were mainly farmers, while women engaged in trade. However, in recent times the majority of residents have been either civil servant employed by the state or engage in various business enterprises, while farming has been left to a few who are peasant farmers in rural communities (EKSG, 2016). The rulers of the towns are known as Obas traditionally, but there are Local Government Area (LGA) chairpersons who are political administrators of LGAs comprising various towns and villages.

2.3.3 Climate and vegetation

Ekiti State has a tropical climate with rainy and dry seasons. It has a tropical forest in the south and Guinea Savannah in the northern region (EKSG, 2016).

2.3.4 Health system in Ekiti State

The orthodox and traditional medical systems provide healthcare services in Ekiti State, coordinated by the state ministry of health (EKSHDP, 2011). At the Local Government Area (LGA) level, there are 283 primary healthcare facilities, made up of comprehensive health centres, basic health centres and maternity centres/dispensary centres. There are 17 general hospitals, 3 specialist hospitals and 2 tertiary health facilities, one owned by the state and the other by the federal government. In addition, there are 163 registered private hospitals and 7 faith-based mission health centres (EKSHDP, 2011). The coordination of health service, manpower development in the health sector and formulation of health policy are all the responsibility of the state Ministry of Health. Despite the number of health facilities available, only two LGAs (Irepodun/Ifelodun and Ekiti East LGAs) of the existing sixteen have a higher number of her population benefiting from domiciled health facilities (Odeyemi, 2014). This may not be unconnected with socio-economic factors such as age, sex, type of illness, perceived quality of service and access to services, which influence willingness to engage with certain medical options (Tipping and Segal, 1995; Akinnawo and Oguntimehin, 1997).

People have been reported to make choices between the orthodox and traditional health systems according to the type of illness (Orubuloye, 1999) and other factors as discussed in Section 1.4.3. These factors ultimately influence the choice between orthodox and the traditional health system. The challenges associated with the orthodox health system in Ekiti State, such as affordability of service cost, quality of service rendered, closeness to home, staff attitude, how neat is the environment, availability of required drugs and services have also informed patients' patronage (Omotosho, 2010).

Accessibility of healthcare facilities has also been reported to affect medical service patronage (Omotosho, 2009). While there are no available data for Ekiti State, the doctor-to-patient ratio in Nigeria is 1:2500 and nurse/midwives to people ratio is 1:1600, compared with the United Kingdom with a 1:357 doctor-to-people ratio and 1:114 nurse/midwife to people ratio (World Development Indicator, 2014). The ratio estimated by the WHO to provide adequate coverage for primary health care need is 1:400 for doctors, nurses/midwives to people ratio (World Health Report, 2006). Considering the poor coverage and exclusion of the Nigerian health insurance scheme, out-of-pocket expenditure on health is 71.1% in Nigeria, compared with 9.7% in the UK and 11.1% in the United States (World

Development Indicator, 2014). The poverty headcount revealed 46% of the rural population and 52.8% of the general population live below the poverty line (World Bank Indicator, 2010). An International Fund for Agricultural Development (IFAD) article reported that about 70% of Nigerians live on less than US$1.25 a day, and 80% of its rural population live below the poverty line (IFAD, 2015). This is no different in Ekiti State. The poverty incidence in Ekiti state is 41.9%; while 38.3% consider themselves to be poor or very poor, 58% consider themselves moderately and 2.3% consider themselves to be rich (NBS, 2012). The state has a poverty gap of 22.7%, an absolute poverty rate of 55.9% and a 12.1% unemployment rate (NBS, 2012). Hence the correlation of these statistics with the "out-of-pocket" expenditure on health gives a grim picture of effective healthcare delivery. Nevertheless, the five-year health strategic plan of Ekiti State sought to improve health care delivery (EKSHDP, 2011).

Africans have been reported initially to administer traditional before orthodox medicine and resort to it when orthodox efforts fail (Omololu, Ogunlade and Gopaldsani, 2008). A study in Ido/Osi LGA of Ekiti State reported a majority (74.3%) of the 521 respondents used herbal medicine to treat malaria (Olorunniyi and Morenikeji, 2013). These include respondents who used HM together with orthodox medicine, while 21.7% of respondents only used orthodox medicine. The use and practice of traditional medicine with HM as the major component in localised communities of Ekiti State has been documented in other studies (Kayode and Kayode 2008; Egbebi, 2011; Olanipekun, Kayode and Akomolafe, 2013). However, no study has covered the entire state, examining the factors which influence the use of HM in Ekiti State. This is part of the gap in knowledge this research aims to bridge.

2.3.5 Study population

The study population was made up of residents of Ekiti State, at the time of the study with a population of 2,384,212 (NPC, 2006).

2.3.5.1 Inclusion and exclusion criteria

Participants were of 18 years of age and above, resident in Ekiti State at the time of the study and had lived in the state for at least 2 years. The study excluded persons below the age of 18 years and anyone who had not resided in the state for at least 2 years. In addition, those who did not consent to participate in the study and those who met the inclusion criteria but for reasons such as significant distress during the interview or those unable to sit for the interview /self-administered questionnaire were also excluded.

2.4 Sampling procedures and sample size

2.4.1 Determination of Sample size

The formula for calculating a sample size based on a proportion of people who use HM was used (Cochran, 2007). The formula was developed for lager study population, which is the case in this survey.

Equation 2.1:

$$n = \frac{z^2 \times pq}{d^2}$$

Where n = required minimum sample size in the study
z = Z-score or Standard normal deviation
p = proportion of people who use HM in the study area
q = 1-p
d = the acceptable error level
The sample size was calculated based on an acceptable error level of 3% (0.03) and a confidence interval of 95% corresponding to a Z-score of 1.96. These are social science research recommended values for standard deviation and error levels (Bryman and Cramer, 1990).
The proportion of HM users in Ekiti State is unknown from previous research, so maximum heterogeneity was assumed for the study (i.e. a 50/50 split in users and non-users of HM) with
p = 0.5.
Therefore the sample size required for this study (n) =

$$\frac{1.96 \times 1.96 \times 0.5 \times 0.5}{0.03 \times 0.03} \qquad n = 1067.11$$

The minimum sample size required for this study was about 1067 participants, assuming 50% of the population use HM.

2.4.2 Sampling procedure

The method of selecting a proportion of the entire population as representative of the population in a study is known as sampling (Polite and Hunger, 1999). In this research, a multistage sampling technique was used, which entails sample selection in at least two stages. The initial stage involves selecting clusters of population units containing more units than are required for a final sample (Mason, 2002). The first stage involved in this study was stratifying Ekiti State into the existing 16 LGAs, as shown in Table 2.1. Stratified sampling is

a process of using stratification variables which may be geographical or non-geographical to increase the efficiency of a sample design (Patton, 2005). It involves the population stratification into homogeneous strata. A geographical stratification variable was used in this study, and the homogenous nature of Ekiti State made this sampling appropriate (EKSG, 2016). The strata which in this case are the LGAs that are non-overlapping, have precise borders, and collectively include everyone in the target population, with each person appearing in only one stratum i.e. an LGA. Stratified sampling enables application of various selection and estimation procedures to different strata; and a variety of information on the various strata can be obtained. It also permits analysis of different interests for different groups (Patton, 2005). The risk of forming a stratum where no information is available does arise; however, the use of HM is a state-wide phenomenon in the study population (Kayode and Kayode, 2008; Egbebi, 2011; Olanipekun, Kayode and Akomolafe, 2013). Therefore every stratum is sure to have information on the use and knowledge of HM in this study.

Simple Random Sampling (SRS) was used at the second stage of sampling in the study. It is a selection process based on probability, with each sample in the population given the probability of being selected equally (Mason, 2002). The names of towns in each LGA were obtained, a list was made and numbers allocated to each town. The allocated numbers were written out on separate pieces of paper. The small pieces of paper were rolled into tiny bits by a different person who was not a participant in the research and all bits mixed together in a basket. The researcher picked from the basket with eyes closed. This was repeated for each LGA and two bits were picked each time, making a total of 32 bits. These 32 bits made up the 32 towns used for the study (Table 2.2). The same method was used in the third stage. The list of the residential quarters in each of the selected towns was made and 5 residential quarters were picked as described above, making a total of 160. SRS is simple and easy to use but requires a complete list to be made of the entire unit in the study population (Patton, 2005). This can be particularly difficult for a large sample and can be expensive in a large geographical study such as this. However, the list of towns in each LGA was supplied by the respective LGA authorities, while the list of the residential quarters in each town was supplied by the traditional council of the towns. This helped considerably to reduce cost and time in this study. The list of town and villages chosen for the study is given in Table 2.2

Table 2.2: Selected location of study population

S/N	Local Government	Towns /villages
1	Ado-Ekiti	Odo
		Bolorunduro
2	Efon-Alaaye	Efon
		Igbo-olofin
3	Ekiti East	Ilasa
		Isinbode
4	Ekiti South West	Ilawe
		Ogotun
5	Ekiti West	Erijiyan
		Aramoko
6	Emure	Sasere
		Eporo
7	Gbonyin (Aiyekire)	Aisegba
		Ijan
8	Ise/Orun	Ise
		Orun
9	Ido-Osi	Ifaki
		Ayetoro
10	Ijero	Iloro
		Ikoro
11	Ikere	Onigemo
		Odolofin
12	Ikole	Ayedun
		Ipao
13	Ilejemeje	Iye
		Eda
14	Irepodun/Ifelodun	Esure
		Awo
15	Moba	Igogo Ekiti
		Irare
16	Oye	Osin
		Itapa

Systemic random sampling was used in the fourth stage, in selecting 10 households from the selected residential quarters and one person from each household made up the final sample population. The houses in each residential quarter were assigned a number due to the poor house numbering practice in Ekiti State. Each residential quarter had an average of 250 houses. The first local government studied had 236 households. A sampling factor was determined by dividing the population size (N) by the selected sample size (n) (Patton, 2005).

Equation 2.2: Sampling factor= N/n

Where N = population size

n= selected sample size

Therefore the sampling factor is calculated:

N = 236

n = 10

236/10=23.6 ≈24

Therefore 24 pieces of paper were numbered 1 to 24 and afterwards the start number “5” was picked using SRS as described earlier. This means the 5th household in the residential quarters was the starting point and the 24th household followed i.e. house numbers 5, 29, 53 77,101,125,149, 173,197 and 221 participated in the study. This process was repeated for all the earlier selected 160 residential quarters.

Systemic random sampling is simple to use and offers the chance of even sampling of the population at equal distance. However, one disadvantage of systemic random sampling is periodicity bias (Mason, 2002), but no periodicity was observed because samples were randomly distributed in each sampling cycle. One person from each household participated in the study to make the sample size. In households with more than one person who met the inclusion criteria and who was willing to participate, simple random sampling was used to choose one person. This was done by secretly rolling papers of a “yes” and many “no” into bits and members of such household were asked to pick one. The person who picked “yes” participated in the study. As a result, 50 people participated in the study from each of the 32 towns from the 16 local government areas of Ekiti State. Thus there was a total of 1600 participants representing 1600 households.

2.5 Research instruments: design and measurements

The data collection tool for this study was a questionnaire developed from previous studies on the use of HM (Ezeome et al., 2007; Okoronkwo et al., 2014; Kummet et al., 2015; Duru et al., 2016) as well as personal experience of the researcher. The questionnaire was focused on exploring the general public’s knowledge of HM, its use and perception. The questions asked were both open- and close-ended. The open-ended questions enabled participants to give multiple and flexible responses and were employed to identify HM used, reasons for HM preference and non-orthodox preference. It also included adverse effects experienced, how they were managed and how the safety of uncertified HM can be achieved.

The questionnaire was divided into three sections. The first recorded the bio-data of the participants, their age range, gender, level of education, religion, occupation and annual income. The second section elicited their knowledge and use of HM both certified and uncertified. It also investigated the type, name and frequency of HM used by participants. The third investigated the participant’s perception of HM effectiveness, its safety, reasons

that informed the use of HM, why hospital assistance was not sought, and regulatory framework. Any adverse effect associated with HM use was also reported in this section and how it was managed by participants. Various steps were taken in developing the questionnaire, as listed below:

I. Identification of key concepts and research question needs
II. Development of questions to test these concepts and meet research question needs
III. Input from other social science research in the questionnaire
IV. Pilot testing of the questionnaire (Section 2.6.4)
V. Discussion of field experience and effectiveness of the tool with the data collectors
VI. Discussion with a statistician on the validity of the questionnaire
VII. Corrections made and input added (Section 2.6.4)
VIII. Development of the final questionnaire.

The full questionnaire can be found in Appendix XVI

The questionnaires were either self-administered or interview-based. Literacy level and personal preference determined how they were administered. Respondents who were literate and willing to have the tool self-administered used self-administered questionnaires. Respondents who indicated that they were not literate or who were literate but preferred the interview method had the tool administered by an interview-based method.

2.6 Organisation of the study

2.6.1 Preparation for field work

The field work commenced in January 2016, after ethical approval, enlisting and training of research assistants and research tool pilot testing.

2.6.2 Ethical matters

Prior to commencement of the study ethical approvals were sought from the Faculty of Science and Technology research ethics panel (FREP), Ekiti State hospital management board and Ekiti State university teaching hospital (for access to hospital data) (Appendix XIX).

Candidate participants were requested to give their consent to participate in the study by signing the participant consent form (Appendix XV) after reading the participant information sheet. Participants who were not literate had the participant information explained to them in Yoruba and verbal consent was accepted.

2.6.3 Enlistment and training

Research assistants (RAs) were recruited for the study. They helped with data collection, especially coding to ensure intercoder reliability (Section 2.12.1). Recruitment was conducted by:

I. Placing an advertisement for research assistants in a variety of public places; with qualification outlined.
II. Received applications were examined and shortlisted applicants interviewed.
III. Successful applicants were enlisted and trained.

The study questionnaire review, piloting and finalisation were part of the training component. It also gave guidelines on the field protocols, duties and roles of the RAs. Training focused on administration of the questionnaire and a unified translation of the questions to Yoruba for those who did not understand the English language.

The study hired a total of 10 RAs for the survey. They had good knowledge and deep understanding of the culture of the indigenes, combined with English and Yoruba language proficiency. The RAs were aged between 22 and 34 years and helped in the administration of the questionnaires. Two of the RAs were later selected in light of good performance during the field work to help with the coding of the open-ended questions. The research assistants were trained using data from the pilot study in textual guidance so as to assign the same category to the same response in order to assess intercoder reliability and improve the coding process.

2.6.4 Pilot study

A pilot study is a fundamental aspect of a research process. It is the test of a method and process on a small scale before its use on a large (Porta, 2008). Thus the fundamental aim of undertaking a pilot study is to assess the feasibility of the research method before its application on a larger-scale study. This is essential to prevent possibly unexpected occurrences with grave consequence while undertaking a large-scale study which may eventually destroy all the research efforts and input. A pilot study can be employed in both qualitative and quantitative research (Lancaster, Dodd and Williamson, 2004). It helps in assessing the adequacy of the process (including unexpected answers, recruitment rate), resources (e.g. questionnaire completion time, training needs), management (e.g. questions and space needed for an answer, data variability) and research question (Van Teijlingen and Hundley, 2001). A pilot study cannot be used to test the research hypothesis since it requires a small sample and is correspondingly imprecise (*ibid*).

The pilot study was carried out to determine its feasibility on a large scale. The questionnaire intended for use in this study was piloted in two towns in two LGAs in the state. The questionnaires were pretested on the 12th of January, 2016. Each RA conducted at least 2 self-administered and 2 interview-based questionnaires. This served as a form of field training for the RAs.

Forty questionnaires were administered in each town, making a total of eighty obtained. The problems encountered during the fieldwork were discussed. It was observed that some of the questions on HM use were ambiguous and needed to be limited to a time frame. As a result the questions were rephrased to focus on the use of HM within the last two years: questions 9, 11 and 13 on the questionnaire (Appendix XVI). Also, some of the questions needed to be reworded to ask about HM in general rather than uncertified HM only, which gave a limited response. Afterwards, data collection started on 19th January 2016 and ended on 18th April 2016.

2.7 Data quality assurance mechanisms

Many actions were taken to guarantee quality assurance of the data, which makes the result reliable. The quality assurance mechanism required the constructs of dependability, credibility, transferability, trustworthiness and confirmability were enhanced (Flick, 2014). The coding of the data was performed by the researcher, who is experienced in data analysis and social research, and 2 research assistants who were trained. Meticulous transcription and analysis of the data were undertaken to ensure dependability of the result. The researcher was open to creation of new themes; and these were coded as they emerged to ensure confirmability (Flick, 2014). The initial data were analysed early to identify gaps and the guides were constantly reviewed as needed to bridge these gaps. This was carried out to maintain trustworthiness of the results produced. Data log sheets and questionnaire monitoring sheets were used to prevent double entry of data and entry errors. These steps helped to ensure the quality of the data.

2.8 Data collection, management and analysis

2.8.1 Data collection

Data were collected using a questionnaire as the instrument. Each self-administered questionnaire was completed in about 10 minutes, the interview-administered in about 20 minutes. Each RA together with the researcher administered about 10 questionnaires each day. The questionnaire was used to obtain both qualitative and quantitative data with open

and closed-ended questions. Completed questionnaires were checked for error and incomplete entries by the researcher. Identified mistakes or queries relating to the information were verified with the respondents or RAs.

The respondents were warm and eager to tell more than was asked about their use of HM. They were particularly happy with the research interest in HM, believing it will help to improve knowledge and secure greater scientific input.

2.8.2 Data management

Completed questionnaires were compiled for the data entry. Data were entered into Statistical Software for the Social Sciences (SPSS) for Windows (version 20.0) for analysis after they had been coded. Each questionnaire was numbered according to its serial number on the SPSS to ensure accuracy and then archived. After entry by the researcher, each data set was cross-checked after every 50th entry to prevent entry error. Where errors were identified the affected questionnaires were retrieved and the error was corrected.

2.8.3 Data analysis

Analysis of textual data from open-ended response can be performed manually or a computer automated system can be used and an instrumental or representational approach adopted. The latter was deployed in this study and manual coding of the response was employed. Manual coding is believed to be costly and time-consuming but so also is building a dictionary, although it may not be as expensive as manual coding. Manual coding is very helpful in combatting problems associated with ambiguity, such as recognizing homonyms (Roberts and Popping, 1996) and is also the most suitable for the representational approach adopted in this study. A vital point to consider in text analysis is the determination of the point of view at which the data is to be coded: that of the researcher's theory or respondents' point of view? The instrumental approach interprets texts according to the researcher's theory; the representational approach interprets text to understand author's point of view or meaning (Shapiro, 1997).

Instrumental text analysis method identifies individual and societal traits which members of the society are possibly unaware of, while representational method characterises texts in a manner intended by the sources. It has been argued that requirements for semantic validity are better achieved through representational than instrumental views, although some researchers prefer the instrumental view since the associated computer-assisted coding is faster, though not cheaper; with the need for a dictionary to be developed (Popping, 2012). Semantic validity requires coding in line with the research question and placing answers in

appropriate category fit to reflect the category's meaning with an agreement between persons familiar with the understudied text and language (Krippendorff, 2012). So the representational approach was adopted in this study to map out the meaning intended by the participants with an understanding of the social circumstances in which the response originated. This perspective will help to clarify both reasons for the choice of use of HM and also for non-hospital use peculiar to and within the study population.

2.8.3.1 Textual analysis

There are three methods by which text can be analysed in textual analysis in quantitative design: thematic, semantic and network (Roberts and Popping, 1996). In this study thematic text analysis was applied, requiring scanning for occurrence of themes in response to the question asked. While semantic text analysis employs the subject-verb-object (SVO) relationship seen in clauses or sentences and the explicit, implicit, inflected nature of the verb or related modifiers and the subject-object, the network text analysis organises the related SVO in a network (*Ibid*). Semantic and network analysis are usually employed in long responses.

In using representational thematic analysis parts of the text were selected and themes assigned manually by the researcher and research assistant. This means of coding ensure interpretation of the manifest response and also an assessment of latent meaning, making the method better than computer-assisted coding. Coding which is the process of systematic transformation and aggregation of raw data into units enabling accurate description of pertinent content features (Holsti, 1969) was performed by multiple coders (Researcher and 2 research assistants; (refer to section 2.6.3) to ensure intercoder reliability (Eccleston et al., 2001; Weston et al., 2001). The codes generated were not mutually exclusive; some respondents had more than one response to some of the questions which fell into two or more categories. These were entered as individual response in the separate categories. Thus some of the total responses to some of the questions exceed the total of participants. An example of the coding of some of the responses is contained in Table 2.4.

It has been asserted that intersubjectivity of the results obtained from computer-assisted text analysis remains a fundamental advantage (Heinrich, 1996). However, the major objective of inter-coder reliability is to minimise subjective bias. There are calculations available to assess agreement among coders using the percentage agreement, Cohen's kappa coefficient, and for agreement between more than one coder using Fleiss kappa coefficient or the Scott's pi (Scott, 1955; Cohen, 1960; Fleiss, 1971; Neuendorf, 2002). Krippendorff's alpha coefficient is also used which is applicable to any number of coders

(Krippendorff, 2004b). There is a host of other tests used, such as the Pearson r and the Spearman Rho, the contingency coefficient, the concordance correlation coefficient, the intra-class correlation coefficient. Krippendorff's alpha (α) is the most reliable because it allows more than two coders, presents no issues with missing data in the calculation of alpha and all levels of measurement can be tested (Hayes and Krippendorff, 2007).
Conversely, unlike other methods (with the exception of percentage agreement) which measure the agreement between the observed and expected outcome, Krippendorff's equation measures the disagreement between the observed and expected outcomes, which makes it conceptually and computationally difficult. Intercoder reliability was, however, carried out using the Krippendorff alpha method with the use of the online intercoder reliability calculator for three or more coders (Recal3) developed by Deen Freelon, which is suitable for the intended use in this study (Freelon, 2010). A $\alpha \geq 0.800$ is acceptable while $\alpha \geq 0.667$ is the lowest acceptable in case tentative conclusions are to be drawn (Krippendorff, 2004a, pg. 241).

Aside from intercoder reliability's being calculated during the training period with the result from the pilot study, this was also performed 5 times during the coding period (14 days coding period). It was done at about 2 days interval, in the morning before the start of the coding day by coding responses from 20 questionnaires that were duplicated and shared among the 3 coders. Considering both face-to-face and self- administered questionnaires were used, it was essential to assess areas of agreement and disagreement in data interpretation. This allowed for a constant feedback on any coding problem and clarifying the appropriate category. Although this reduced the target volume of work to be done on such days, it was a quality-control measure that was essential to achieving a credible result. This is also essential to minimise factors such as leniency/stringency error, perception difference, stereotyping or halo effect which may affect intercoder reliability (Stemler, 2004). The coding concluded, data were analysed as described in Section 2.8.5. Data input was performed by the researcher, enabling a quality control check on the coded and processed data.

2.8.3.2 Quantitative data analysis

Inferential and descriptive statistics were carried out on the quantitative aspect of the data using SPSS for Windows version 20.0. Comparison of socio-demographic factors and the use or non-use of HM was made using inferential analysis. A Pearson Chi-square test was used to examine the influence of various independent variables on various phenomena in the study and a P value ≤ 0.05 was considered to be statistically significant. The test is inferential non-parametric statistics, which may be administered on frequency data. It is

employed in testing the differences between the expected and observed frequencies occurring asides sample error, either chance or other factors (Mehta and Patel, 2012). A two-way chi-square test was used to examine the difference between the socioeconomic characteristics and the use of HM (Table 2.20) and on the perception of HM safety (Table 2.21).

A minimum count of 5 on SPSS in each cell of the chi-squared table confirms the validity of the chi-squared test. However, for counts below 5, some exact tests can be used for data testing, such as the Monte Carlo Exact or Fishers Exact, to give a valid chi-squared statistic (*Ibid).* The test was used to compare the influence of education, gender and annual income on the use of HM and perception of its safety. The analysis helped further comprehension of the interplay of several factors in the use of HM.

2.9 Data interpretation

The mixed method of data interpretation used in this study included reduction, display and transformation correlation, intertwining comparison and integration of data (Section 2.8.3). The reliability of result and conclusion are further strengthened using the findings from this approach (Onwuegbuzie and Teddlies, 2003; Palinkas et al., 2015). Ensuring the interaction of the data enabled the selection of the right model that optimally reflected reality in the study and its internal validity (Palinkas et al., 2015). Once analysis is completed, the results will be disseminated to the Ekiti State Ministry of Health, Ekiti State Hospital Management Board and other stakeholders in the Ekiti State health sector for the development of a forward approach in HM use and regulation in Ekiti State.

2.10 Role of the researcher

The researcher's direct involvement in this study was likely to introduce various ethical and personal issues due to his positionality and reflexivity in the study as described in Sections 2.10.1 and 2.10.2.

2.10.1 Positionality

Positionality is needed in exploring the phenomenon in a study without the researcher's influence on the outcome (Rose 1997; Bourke, 2014). Positionality is the way people are defined by their existing network of relationships, which may change and be analysed as the research progresses (Maher and Tetreault, 1994). It may include aspects of identity (class, gender, age) or research experience (such as involvement in previous projects, research training) which can affect the relationship between the researched and the researcher

(Hopkins, 2007). Understanding positionality and its effective role is vital to credible data collection and analysis because encounters, processes and outcomes of studies can be influenced by various identities of the researcher (Unluer, 2012).

The researcher's being a health professional and a stakeholder in the Ekiti State health system created multiple identities (those of physician, government official, researcher and Christian) which might influence the research process. Although it is required by the methodological perspective to the study that the researcher be an "outsider", he was an "insider" not least because of his familiarity with the study environment. Also, some of the participants referred to the researcher as an insider due to a common ethnic origin, shared local language and familiarity with the study environment. Some other participants, however, referred to the researcher as an outsider due to his socio-economic status, religion, education and profession. This mixed insider-outsider stance had its implication for the research. The researcher's ethnic similarity, local language proficiency and awareness of cultural expectations helped to develop a cordial and warm relationship with the participants (see also De Andrade, 2000; Harvey, 1996; Sheriff, 2001; Chavez, 2008; Throsby and Evans, 2013; Longhurst and Johnston, 2014).

The researcher's being an insider helped with the logistics and procurement of materials and documents needed in this research. Being an insider afforded insight in understanding the emotional, cognitive, economic, psychological and cultural workings of the study participants. However, the insider position can predispose to interview bias harmful to the data collection process (Bourke, 2014). Therefore the researcher needed to be continually reminded of his research role. Participants who were familiar with his professional background might be inclined to respond in a way believed to please the researcher or absolve them of potential scrutiny (Lloyd and Hopkins, 2015). Beginning the interview with a disclaimer of a non-judgemental stance encouraged participants to open up, especially about how they managed the adverse effects of HM use (DeLyser, 2001, Bourke, 2014). This disclaimer also helped promote discussion of the reasons for non-hospital use which the participants may want to say little about, believing the researcher knew what most of the problems were. In general, the insider-outsider identity presented methodological advantages and difficulties, especially on issues pertaining to research process positionality, the researcher's awareness of self and knowledge gained as a result of his position in the social strata (Chavez, 2008). The positionality of the researcher was properly managed in this study, drawing on his own diverse social identities (Unluer, 2012). It helped minimise biases in the research result while interacting with the study participants in order to carry out an ethical and reliable study (Hopkins, 2007).

2.10.2 Reflexivity

The beliefs, values, education and interests of the researcher could influence research outcome, hence reflexivity was important in this study. Reflexivity is a self-examination which may result in in-depth understanding of selves and others in relation to particular social environments, fostering deeper awareness of how social context influences peoples' personality and behaviour (Danielewicz, 2001 pg.155). Reflexivity means the researcher's self-appraisal that helps to identify and take responsibility for role in and influence on the study and how it affects people and study settings, data collection and interpretation. It also challenges the views of the resulting knowledge as objective and independent of the researcher (Berger, 2015). Reflexivity entails a consciousness of the contribution to the establishment of definition by the researcher all through the research procedure and accepting the impossibility of being 'outside of one's subject matter during the research. In this study, the researcher had the same cultural background as the study population, which could have influenced his reflection on the actions and behaviour of respondents concerning the use, choice and efficacy of HM in the study environment.

But as a result of his knowledge of the effects of such use, choice and perception, the researcher was careful not to influence the research process. Additionally, the use of a structured questionnaire in data collection reduced the involvement of the researcher in construction of meaning in the study. The use of theories necessitating data collection and analysis minimised researcher's bias especially with the provision of an option for self-administered questionnaire which prevented influence from the researcher's interest.

2.11 Herbal medicine associated casualty and fatality; method

The HM-related causality and fatality figures were studied in Ekiti state. This study was carried out on a 5-year retrospective record of patients admitted to female and male medical wards, children's emergency wards and records of obstetric delivery in the 16 Ekiti State-owned specialist and general hospitals and 1 tertiary hospital in Ekiti State (Ekiti State university teaching hospital). The period of study was from January 1, 2010 to December 31, 2014 (5 years). The population under study was all patients admitted to the three wards, medical ward, children's emergency and labour ward. Available records on the wards from nurses' report, case notes obtained from the hospital's department of medical records and records from the Hospital Management Board (HMB) were used in the study. Only deaths that were certified were included in the study. The study narrowed search criteria to admissions and deaths due to poison agents, poisoning and herbal medicine for medical and paediatric study. HM related causality and fatality was then narrowed down.

A review of obstetric records of patients with stillbirth (SB) was identified and used for the study. SB was determined as the delivery of a baby with a birth weight equal or greater than 1kg, of 28 weeks gestational age or more, of 35 cm or more in body length, who died prior to or during labour (WHO, 2004). Data were retrieved from SB case files of patients with maternal exposure to HM in pregnancy. The data obtained included maternal age, level of education, religion, occupation and possible cause of SB by Baird-Pattinson's classification which includes foetal abnormality, antepartum haemorrhage, unexplained intrapartum foetal death, intrapartum-related infection, hypertensive disorder, maternal disease, spontaneous preterm labour, and intrauterine growth restriction (Pattinson; De Jong and Theron, 1989).
Unknown among the probable causes of death, foetal abnormality, intrauterine growth restriction, spontaneous preterm labour and unexplained intrapartum foetal death were identified and examined for history of HM use in pregnancy. SPSS package version 20 was used to analyse the obtained data.

2.12 Results and discussion

2.12.1 Introduction

This section presents the analysis and results of the data collected from both the survey and hospital records. Analysis of the data is performed according to objectives I, II and III of this research (Section 1.11). The survey analysed the pattern and extent to which respondents use HM in Ekiti State and how socio-economic and educational factors influence this use. The study also explored their perception of safety, management of adverse effects and reasons for non-hospital use of HM. Cross-tabulation, frequencies, percentages and charts were used to present the results.

2.12.2 Response rate, categorisation and reliability

A total of 1600 respondents participated in the research, 984 of whom used self-administered questionnaires and 616 interview-administered. However, 1265 respondents constituted the sample for the study due to exclusion of 335 questionnaires (Table 2.3). The excluded entries were either incomplete or damaged beyond use. The self-administered questionnaire had 253 entries excluded for incompleteness and 10 for damage (total 263), while interview-administered questionnaires had 64 entries excluded for incompleteness and 8 for damage.

Table 2.3: Methods of questionnaire administration

	Total respondents (%)	Incomplete entry (%)	Damaged questionnaire (%)	Total excluded (%)	Actual sample size (%)
Self-administered questionnaire	984 (61.5%)	253 (25.7%)	10 (1.0%)	263 (26.7%)	721 (73.3%)
Face to Face Interview administered questionnaire	616 (38.5%)	64 (10.4%)	8 (1.3%)	72 (11.7%)	544 (88.3%)
Total	1600	317	18	335	1265

There was a higher nonresponse rate among the self-administered than interview-administered questionnaires, which is similar to findings from previous studies (Christensen et al., 2013; Ekholm et al., 2009). The exclusion of 64 participants due to incomplete entry from face-to-face interview-administered questionnaires (Table 2.3) could have been as a result of likely bias due to different RAs. However, without proper training of the RAs the number of respondents excluded through this data collection method could have been higher. Also, the highest number of incomplete entries arising in self-administered questionnaires might have been due to problems at any of the cognitive steps required in answering a questionnaire, as discussed in Section 2.2.2. However, RAs were present to assist where necessary, which likely reduced the potential number of excluded respondents. The responses of the participants were coded by placing them in various categories, as described in Section 2.8.3.1 and examples are shown in Table 2.4.

Table 2.4: Exemplar table of categorisation of participants' response

Question: Why have you not used HM in the last two years?	
Response	Category
I prefer to visit a quality Doctor in the hospital	Personal preference
It can be poisonous and can make me sick	Attendant health risk
I just don't like to take HM	Personal preference
Some of those HM you don't know their source and what they really contain	Lack of knowledge on available HM
Question: Why have you used HM in the last two years?	
It is easy to find and buy	Availability
It really works for me when I use it	Effectiveness
It is not expensive for me	Affordability
My money can buy it and more if I need it again and no need to be looking for where to buy drugs	Affordability and availability
Natural is the best no need to be taking artificial chemical into my system	Natural(ity)

The analysis of textual data was subjected to an inter-coder reliability test as described in Section 2.8.3.1. The inter-coder reliability test showed there was an excellent agreement between coders during the coding stage with the Krippendorff α >0.800 for all open-ended questions coded (see Table 2.5).

Table 2.5: Table showing inter-coder reliability at different periods of testing

Test period	Krippendorff α	Inter-coder reliability
Pilot stage (n=80)	0.823	yes
Day 1 (n=20)	0.845	yes
Day 3 (n=20)	0.896	yes
Day 6 (n=20)	1	yes
Day 10 (n=20)	0.949	yes
Day 12 (n=20)	1	yes

α≥0.800 is acceptable

When observed disagreement (Do) = 0 and α = 1, it is said to be perfect reliability. But when there is agreement as if results were obtained by chance, observed disagreement (Do) = expected disagreement (De) premised on an interpretation of chance and α = 0 which shows an absence of reliability (Krippendorff, 2011, pg. 1). Inter-coder reliability improved over time (as evidenced by increasing Krippendorff α in Table 2.5) which indicated better agreement

between coders and thus validates the reliability of the result from this study. Hence it can be used in answering research questions I and II (Section 1.11.1).

2.12.3 Socio-demographic indicators

Socio-demographic characteristics of the respondents, including age, sex, level of education, religion, occupation and annual income (Table 2.6).

Table 2.6: Socio-demographic background of respondents

Variable	Frequency (N=1265)	Percentage (%)
Age (years)		
18-29	322	25.5
30-49	547	43.2
50-69	358	28.3
70 and above	38	3.0
Gender		
Male	713	56.4
Female	552	43.6
Level of Education		
No formal education	191	15.1
Primary	245	19.4
Secondary	340	26.9
Tertiary	489	38.7
Religion		
Christianity	879	69.5
Islam	346	27.4
African traditional religion	40	3.2
Occupation		
Student	84	6.6
Civil servant	491	38.8
Farmer	76	6.0
Self Employed	501	39.6
Others	113	8.9
Annual Income(Naira)		
Low Income (≤ 600,000)	1037	82.0
Middle income(601,000 to 2.4M)	179	14.2
High Income (≥2.4M)	49	3.9

As outlined in Table 2.6, there were more male (56.4%) than female respondents (43.6%) in the study. This is not unusual when compared with the population distribution in the state: 51.0% male and 49.6% female (NPC, 2006). The ages of respondents ranged between 18 and over 70 years; 43.2% were aged 30-49, 3.0% above 70. The greater proportion were 30-49, which corroborates previous population statistics, with a higher proportion of youth and middle-aged in the state (NPC, 2006). This is further noted when the age range 18-29

years, which made up 25.5% of the study population, is added, making a total of 68.7% of the study population between the age range 18-49 years. The latest statistical publication reports higher youth and middle-aged residents in Ekiti State aged 15-44 years, accounting for 38.8%, 45-69 accounting for 37.6 % and over 70 accounting for 6.32% (NBS, 2012).

Formal education is the pursuit of knowledge, understanding and skill in the curriculum of an institutionalised educational environment (Livingstone, 1999). About 84.9% of the respondents had a form of formal education, while 15.1% were without (Table 2.6). Most had a tertiary level of education which included degrees from post-secondary institutions such as polytechnics, universities and colleges of education or agriculture. This group was followed by that with a secondary level secondary or grammar school qualification. Respondents with primary level of education were those who only completed elementary or primary school.

The youth (15-24 years) and adult (25 years and above) literacy rate in any language including the English is 98.6% and 79.5% in Ekiti State respectively (NBS, 2012). A recent publication reports 73% of the Ekiti population are literate (NPC, 2015). As compared with the 57% having successfully self-administered questionnaires used in this study, the state can be said to have more literate people. This fact reflects the official state and widely acknowledged ascription, “the fountain of Knowledge” (EKSG, 2016), the boast of higher educational qualification of state citizens as compared with those of other Nigerian states.

Christianity is the most practised religion in Ekiti State, with 69.5% of adherent respondents, 27.4% Muslim and 3.2% of African traditional religion, although before the introduction of Christianity to Ekiti in 1893 African traditional religion was the only religion practised (Bello, 2009). Published data showed that in 1963 there were 10.6% Muslims, 79.0% Christians and 10.4% others among the Ekitis (Ostien, 2012). When compared with this study result, there is shown a marked decrease in the proportion of Muslims in the state, although African traditional religion was not mentioned in the previous study. However, the majority of Ekiti people still seem to hold their African religious belief, which may have influenced their use of HM, regardless of religion (Table 2.20), although further evidence from this study does not support this hypothesis. The findings of this study regarding the significance of religion in the use of HM are further discussed in Section 2.12.3.

The majority of respondents were self-employed, with 39.6% engaged in various trades as their source of income. Most of these trade ventures are small scale businesses ranging from sales of mobile phone top-up cards, hairdressing and barber shops, to artisan workshops, small restaurants and food trade in the market. This group was followed by civil servants (38.8%), employed by the state and federal government as employees in various cadres. Students accounted for about 6.6% of the study population. The low student

representation may not be unconnected with most higher education institutions' being shut at the time of the study. This occurred following strike action embarked on by the staff of the institutions, due to non-payment of their salaries by the government.

Farmers constituted 6% of the study population. These were peasant farmers who resided mainly in the rural areas of the study environment. The other groups, such as retirees, priests and politicians constituted 8.9% of the study population and are categorised as "others" (occupation with a number of participants below 20). This reflects the non-industrialised nature of the state, where the majority are either employed by the government as cleaners, clerks, drivers, teachers, doctors, lectures or administrative officers or engaged in small-scale business. This affected the annual income of the respondents in this study (Table 2.6): most (82%) were in a low, a minority (3.9%) in a high-income class.

The gap between the income classes was very wide, especially with few respondents n a middle-income class (14.2%). The high and middle-income classes often consist of senior civil servants, owners of big business, politicians and professionals. A study in Enugu Nigeria showed a similar result, with a low-income class accounting for about 76% of respondents, 19% middle-income class and 4.2% high-income class (Okoronkwo et al., 2014). The findings from this study highlight the income inequality in the study population. Income inequality describes the extent of uneven distribution of income among a population. It can be measured in various ways such as the "Gini coefficient" coefficient, which is 0 when everyone has the same income accrued to them and 1 when an individual has the whole income. Zero signifies perfect equality and a coefficient of one signifies complete inequality (OECD, 2017).

The Gini coefficient for Nigeria is reported to be between 0.50 and 0.70, one of the most unequal (Bakare, 2012). The UK scores 0.358 and the US 0.394, making the US slightly more unequal. By contrast, Iceland, a much more equal society has a score of 0.244 (OECD, 2017). However, inequality and particularly income inequality is a global problem and it has been called a defining challenge of our time. A Pew Research Center (PRC) survey reported that over 60% of worldwide respondents consider the gap between the rich and the poor a major challenge (PRC, 2014). Income inequality has been reported to be due to people's and groups' differing productive potential, which in turn results in differing income and wage levels (Dabla-Norris et al., 2015). Income inequality and poverty have also theoretically been found to be inseparably connected with the presence of one usually implying that of the other (Bourguignon, 2004; Burtless and Smeeding, 2002).

About 70% of Nigerians live on less than US $1.25 daily, and 80% of its rural population lives below the poverty line (IFAD, 2015), which reduces life expectancy. A recent study reported that the life expectancy rate and unemployment create inequality (Ogbeide and Agu, 2015). While there is no correlation between unemployment and poverty in Nigeria, the report found a direct correlation between inequality and poverty and at the same time an indirect correlation between them via unemployment's creating inequality and inequality leading to poverty. The majority of respondents in this study (39.6%) are self-employed (Table 2.6). Although the majority can be said to be employed, a good number of them may be said to be underemployed with a consequent effect on their income. Underemployment can be defined as insufficient in maximising skills or potential and generating an income relative to a standard of living (Maynard and Feldman, 2011). Hence, in addition to the finding of Ogbeide and Agu (2015), the link between poverty and income inequality may be through underemployment, although a further study on the skills and type of employment engaged in by the population under study may be needed to confirm this view. How these socio-demographic characteristics statistically affect the use of HM and perception of its safety is discussed Section 2.12.10.

2.12.4 Knowledge of herbal medicine

Question 2.1: Do you know what HM is?

All the respondents in this study (100%, n=1265) have heard of and know what HM is.

Question 2.2: Do you know the difference between certified and uncertified HM?

Table 2.7: Respondents' knowledge of certification of herbal medicine

Response	Frequency	Percentage (%)
Yes	1139	90.0
No	126	10.0
Total	1265	100.0

All the respondents (100%) have heard about HM and know what it is. This is not unusual considering the long history of HM use not only in Ekiti State but in Yoruba land traditionally and historically (Borokini and Lawal, 2014), as discussed in Section 1.3.3. Orthodox medicine was introduced to Nigeria in the Yoruba land of Abeokuta in the 1860s by Roman Catholic missionaries (Metz, 1991). Before then HM was extensively practised and HM practitioners were an integral part of the Yoruba community, with various degrees of specialisation (Bello, 2009; Olagunju, 2012). This historical and cultural fact is reflected in the knowledge of HM reported by participants. However, with modernisation and advances

in almost all spheres of life, the need for regulation and standardisation of HM was inevitable. Some HMs are now being properly packaged, subjected to laboratory tests and approved for public use by NAFDAC (Oguntade and Oluwalana, 2009). These are the certified form of HM, while a lot of others are still not registered or regulated. According to Table 2.7 the majority of respondents (90%) knew the difference between government certified and uncertified HM. The high level of knowledge about certified and uncertified HM may be attributed to the various campaigns and public awareness of NAFDAC of fake and counterfeit drugs in Nigeria (Ofuani, Kuye and Ogundele, 2015). The national campaign was successful in urging people always to check for the NAFDAC number on any medication before it is consumed. This awareness is thus reflected in this study and is an indicator of the effectiveness of the government effort at informing the public.

2.12.5 Use of herbal medicine in Ekiti State

Question 2.3: Have you used HM in the last two years?

The reported use of HM was high in this study: 85.0% of the respondents had used HM in the previous two years (Table 2.8). The high percentage of HM users in this study (85.0%) is similar to a finding of a previous study carried out in a small part of Ekiti State where 74.3% of the 521 respondents had used herbal medicine in the treatment of malaria (Olorunniyi and Morenikeji, 2013).

Table 2.8: Respondents' use of herbal medicine in Ekiti State

Response	Frequency	Percentage (%)
Yes	1075	85.0
No	190	15.0
Total	1265	100.0

This is also similar to findings of studies in urban Lagos and the UK in which 66.8% of the 388 respondents used HM in Lagos and an average one-year prevalence of 64.2% HM use in the UK (Oreagba, Oshikoya and Amachree, 2011; Posadzki et al., 2013). Previous studies, as stated earlier, have focused on specific HMs or were carried out outside Ekiti State, which makes comparative analysis difficult. However, findings on HM use in this research are higher than those in Lagos and the UK, which could indicate that the use of HM is prominent in Ekiti State.

Question 2.4: Why have you not used herbal medicine in the last two years?

Although the reasons for HM use vary, respondents who have not used HM in the last 2 years in this study attributed their non-use to various factors. These include potential health risk (61.1%), personal preference (25.8%), and lack of knowledge of particular HMs (13.2%) (Table 2.9).

Table 2.9: Respondents' reasons for non-use of herbal medicine in Ekiti State

Response	Frequency	Percentage (%)
Risk to health	116	61.1
Personal preference	49	25.8
Lack of knowledge about specific HM	25	13.2
Total	190	100.0

A previous study reported that 26.4% of the 129 non-HM users attributed their reluctance to safety concerns (primary reason among respondents who gave an answer), although, 31% of the respondents gave no reason, while the remaining 43% gave different reasons (Oreagba, Oshikoya and Amachree, 2011). Findings from this study also showed more people (61.1%) cited safety concerns. These factors were influenced by personal experience or that of friends and family as expressed by the respondents. In addition, the effort of the Nigerian government through various print and audio-visual media to raise awareness of the health risks of consuming unregistered products may be responsible for the high "risk to health" response among non-users. This is because more people are aware of NAFDAC's warnings and sensitisation about consumption of unregistered medicinal and food products (Ofuani, Kuye and Ogundele, 2015). However, the results from this study still show high use of uncertified HM despite government efforts (Table 2.10). This further emphasises the need for this research in identifying underlining reasons for the trend.

Question 2.5: What type of HM have you used in the last two years?

Table 2.10: Class of herbal medicine used by respondents in Ekiti State

Response	Frequency	Percentage (%)
Uncertified	401	37.3
Both	343	31.9
Certified	331	30.8
Total	1075	100.0

According to Table 2.10, among the respondents who had used HM in the previous 2 years, uncertified HM was the most used (37.3%) followed by respondents who used both. This finding is novel because there are no published studies in this regard distinguishing certified and uncertified HM and comparing their use even in Nigeria. Despite its being a novel finding, it is of significant concern, especially to public health. Accordingly, the commonly consumed HMs are further analysed for their toxic potential in Chapter 4. The difference between the use of certified and uncertified HM may be small, probably due to public awareness, but the category of people who use both (31.9%) may make a difference to the actual result where respondents cite order of use. Table 2.12 better explains this use, giving a clearer assessment of the type of HM used by respondents.

Question 2.6: How many times have you used HM in the last two years?

Table 2.11: Frequency of herbal medicine use by respondents

Response	Frequency	Percentage (%)
Once- twice	142	13.2
3-10 times	363	33.8
Over 10 times	570	53.0
Total	1075	100

The frequency of HM use in this study varied from once to over 10 times in the previous 2 years, which is important in assessing health impact. The majority of respondents (53%) used HM over 10 times in the previous 2 years (Table 2.11). This highlights the high frequency of HM use in the study population. Similar studies reported frequent use of HM in Nigeria, especially among specific groups such as pregnant women and people living with chronic illness (Ezeome and Anarado, 2007; Fakeye et al., 2009; Osamor and Owumi, 2010). Publications on the frequency of use are limited for other countries, but a publication reported lifetime use of HM among 65.3 % (n=118) of Americans of Hmong ethnicity (Lor et al., 2016). This may allude to the cultural influence of HM use, as previously discussed in Section 1.5.2.

Question 2.7: What is the name of the HM you have used in the last two years?

The high use of uncertified HM (Table 2.10) is reflected in the name of the HM commonly used by the respondents.

Table 2.12: Types of herbal medicine used by respondents in Ekiti State

Response	Frequency	Percentage (%)	Type
M and T Capsule	104	6.8	certified
Original malaria/yellow fever and typhoid	76	4.9	uncertified
Aromalegun	100	6.5	uncertified
Body pain	93	6.0	certified
Supa A1	105	6.8	certified
Eroxy 5000	211	13.7	certified
Wadco total blood cure	110	7.1	uncertified
Male tonic	178	11.6	uncertified
YK original malaria	107	6.9	uncertified
Wadco pile and dysentry	89	5.8	uncertified
Local herbal mixture	358	23.2	uncertified
Others	10	0.6	
Total	1541		
Certified	513	33.3	
Uncertified	1018	66.1	

In total, uncertified and unregulated HM was the most commonly used type, accounting for 66.1%, while certified HM accounted for 33.3% (Table 2.12). This further alludes to the findings in Table 2.10, which showed uncertified HM as the most used form of HM. According to Table 2.12, 0.6% of the respondents used other types of HM which included different brands, some certified and some uncertified. The HMs identified by respondents (Table 2.12), with the exception of local herbal mixture, were analysed for heavy metals and the possible presence of pharmaceuticals as part of this research (see Chapter 4). This would help to identify potential toxic components in these HMs.

2.12.6 Preference for herbal medicine use and Hospital use

Question 2.8: Why do you prefer to use herbal medicine?

The reasons for HM preference varied among the respondents, 39.6% of whom cited its effectiveness, 31.9% its affordability and 21.5% its availability (Table 2.13).

Table 2.13: Reasons for preference of herbal medicine use

Response	Frequency	Percentage (%)
Effectiveness	538	39.6
Affordability	434	31.9
Availability	292	21.5
Naturality	96	7.1
Total	1360	100.0

Effectiveness of HM was a major factor in the choice of HM in this study. Its role as a major factor in HM preference has also been reported previously (Mafimisebi and Oguntade, 2011, Adesiji and Komolafe, 2013). Affordability was the second most important determinant, followed by availability. These reasons for HM use have been reported in the literature, where several factors were found to have contributed to the resurgence of public interest in HM, including claims of its effectiveness, a preference for natural therapies and alternative medicines, dissatisfaction with orthodox pharmaceutical outcomes, high cost and side effects of orthodox medicine, distrust of physicians' abilities and self-medication habit (Bandaranayake, 2006). Anecdotal information from friends, religious influence and level of spiritual consciousness has also been known to influence the use of HM (Astin, 1998; Zeil, 1999; Parle and Bansal, 2006). The high use of HM in developing countries was reported to be likely due to its acceptability, availability, accessibility and affordability by the majority of the populace (Elvin-Lewis, 2000). Some of these factors have also been identified in this study across age and gender (Figure 2.2 and 2.3).

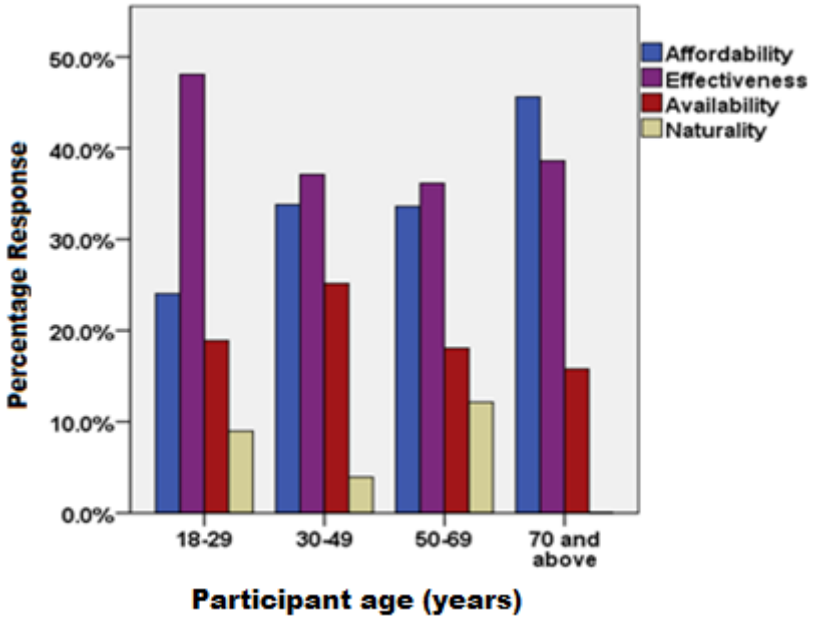


Figure 2.2: Reasons for HM use by age

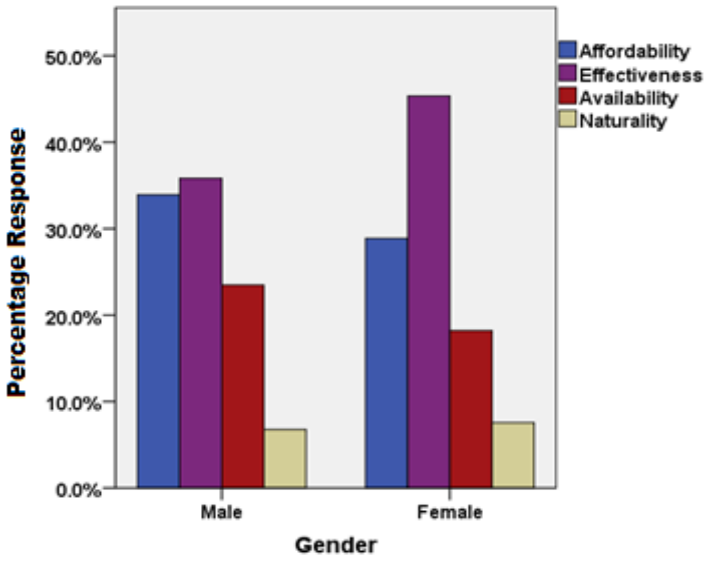


Figure 2.3: Reasons for HM use by gender

Although only 7.1% of the respondents in this study attributed their use of HM to its natural and organic properties, the misconception of the natural properties' being non-toxic and free of adverse effects is a common belief in both developed and developing nations (UNESCO, 2013). Perception of HM as natural and thus a reason for its use is not peculiar to the sample population in this study.

Belief in the effectiveness of HM as a major reason for its use cut across the economic and educational attributes of the respondents (Figures 2.4 and 2.5) including age and gender,

as seen in Figures 2.2 and 2.3. The effect of background characteristics on the use of HM and statistical correlation is further discussed in section 2.12.10.

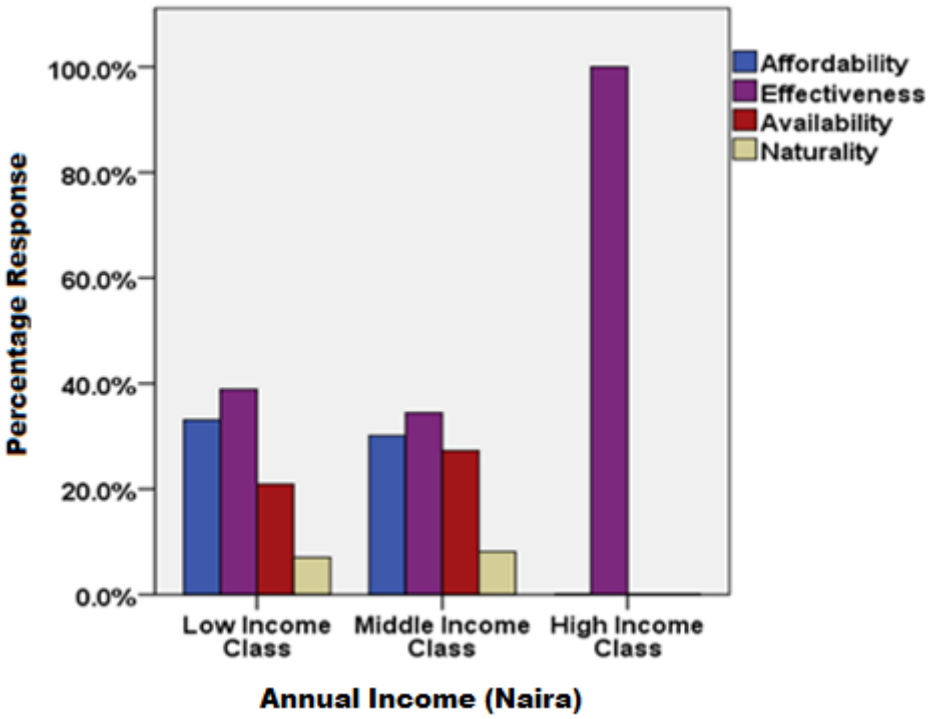


Figure 2.4: Reasons for HM use by economic background

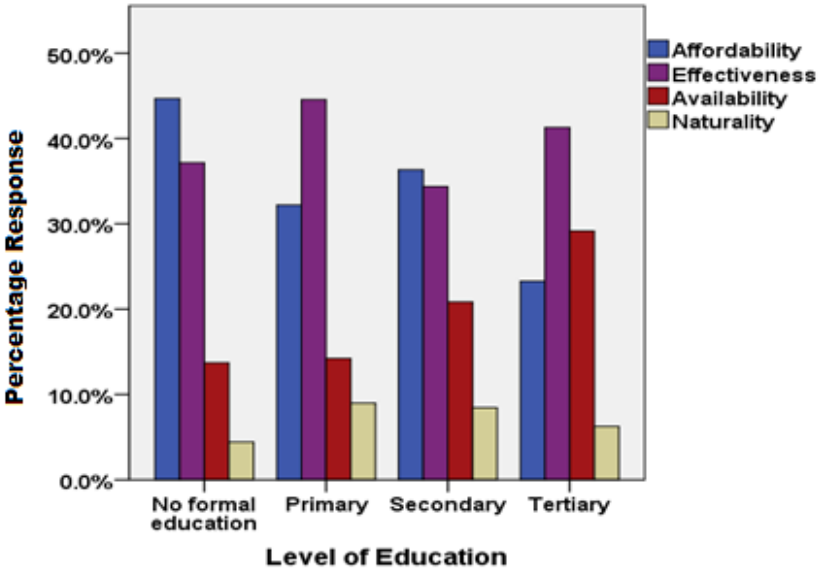


Figure 2.5: Reasons for HM use by educational background

In view of the findings on the reasons for preference of HM use it was appropriate to examine the effectiveness of orthodox medicine in Ekiti State and Nigeria as a whole for purposes of comparison.

The WHO currently estimates that about 10% of pharmaceuticals in circulation worldwide and 25% in developing countries are fake (Amadi and Amadi, 2014). An estimated 75% of these fake drugs are produced in India, 7% in Egypt and 6% in China (OECD, 2008). While Indian law prohibits the sale of fake drugs in the country, there is no prohibition of their export (Aminu et al., 2011). The problems of manufacture, importation and sales of fake drugs in Nigeria has markedly improved from 41% in 2002 to 10% in 2011 (Amadi and Amadi, 2014; Akunyili, 2007). However, a WHO study has shown that about 70% of pharmaceutical medication in circulation in Nigeria is either adulterated or fake (Wertheimer and Wang, 2012, WHO, 2015). This consequently affects the effectiveness of the medicines for their intended purpose. The near ubiquity of counterfeit medicine in hospital pharmacies and outside pharmacies together with poor service delivery in hospitals and health centres has possibly contributed more to the level of dissatisfaction with orthodox health care. Dissatisfaction might have indirectly increased HM use. But more important is the concern at possible adulteration of HMs as patronage increases and a desire to make it very effective. Therefore in this research HMs are analysed for possible pharmaceutical adulterants.

Question 2.9: Why do you not prefer to use conventional health facilities?

Dissatisfaction with orthodox health care was further highlighted in this study as the majority of respondents (45.2%) attributed poor service delivery in the hospital to non-use of hospitals (Table 2.14). The poor services experienced included long hospital waiting times, the hostile and rude attitude of health workers, poor infrastructure and lack of communication. A comparative assessment of herbal and orthodox medicine in Nigeria reported HM was rated higher than orthodox medicine in terms of efficacy, affordability, availability, safety and level of advertisement (Osemene, Elujoba and Ilori, 2011).

Table 2.14: Reasons for non-use of conventional health facilities in Ekiti State

Response	Frequency	Percentage (%)
Poor service delivery	534	45.2
High hospital cost	396	33.5
Unorthodox belief	141	11.9
Do visit the hospital	110	9.3
Total	1181	100.0

The role of quality service delivery in patient's choice of healthcare is vital, and this has been reported previously (Cheraghi-Sohi et al., 2008; Faber et al., 2009; Aikins, Ahmed and Adzimah, 2014). Poor service delivery, as pointed out in this study was identified across the educational levels of the respondents (Figure 2.6), although the majority of respondents with no formal education blamed prohibitively high hospital costs for failure to use hospitals.

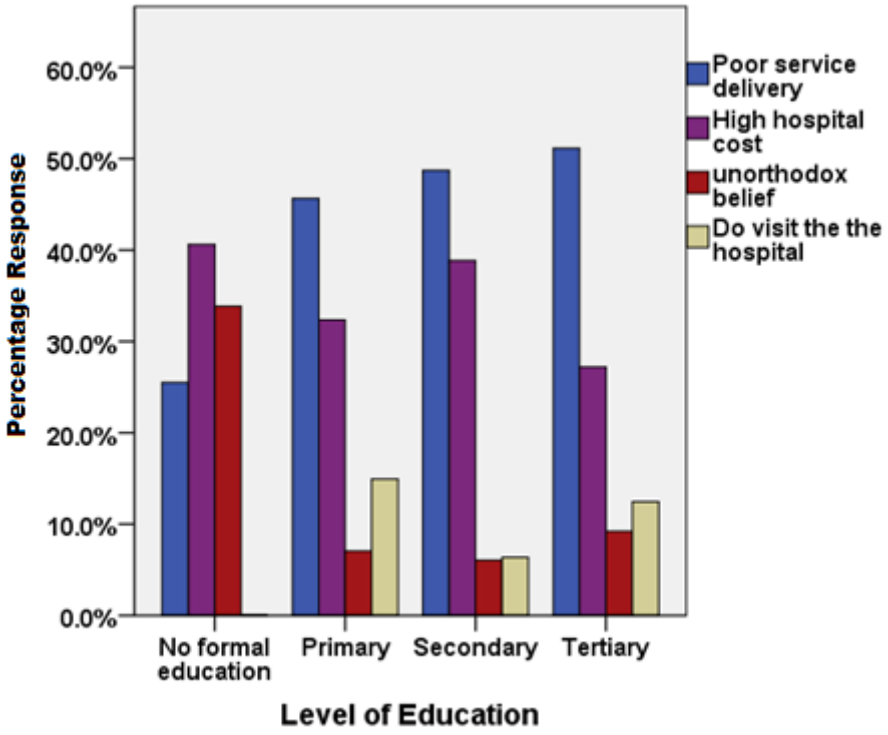


Figure 2.6: Reasons for not using hospitals by educational background

Tangibility, reliability, responsiveness, assurance and empathy are vital tools for quality healthcare service delivery (Aikins, Ahmed and Adzimah, 2014). While most orthodox health facilities in Ekiti State lack many of these, HM practice has been shown to be empathic, responsive and assuring, although its tangibility and reliability remain controversial.

The high cost of hospital service was also cited by 27% of respondents as a reason for avoiding use of hospitals. Using the Central Bank of Nigeria (CBN) currency exchange rate on the 27th September 2018 of one United State Dollar (1USD) to 305 Nigerian Naira (CBN, 2018) as discussed in Section 1.4.3, orthodox health care can be very expensive for an average Ekiti family, where the majority live on less than US $1,967 annually (Table 2.6). Hence, there is a plausible choice between the use and non-use of HM as further discussed in Section 2.12.10.

A household poverty indicator by healthcare in Ekiti State reported 57.5% of 240 respondents make use of HM in their health care, due to (problems associated with financial)

incapacity to meet hospital costs and proximity of government hospitals (Oluwatayo, 2008). This may be responsible for prohibitive hospital cost being the second most commonly cited reason for not using hospitals (33.5%) (Table 2.14) and affordability being the second most frequently cited reason for use of HM (31.9%) (Table 2.13). A comparative study has shown that social deprivation is strongly related to overall life-expectancy and mortality (Daniels, Kennedy and Kawachi, 2000). The middle-income class in relatively unequal societies like Nigeria have worse health than even poorer residents in a more equal society like the UK. The gap between the income class in this study is wide, with 82% of respondents in the low-income class, 3.9% in the high-income class and 14.2% in the middle-income class (Table 2.6) in a health system still largely financed by out-of-pocket expenditure (69% by patients in Nigeria), as shown in Figure 2.7, compared with 11% out-of-pocket expenditure in the UK (Wilson, 2017).

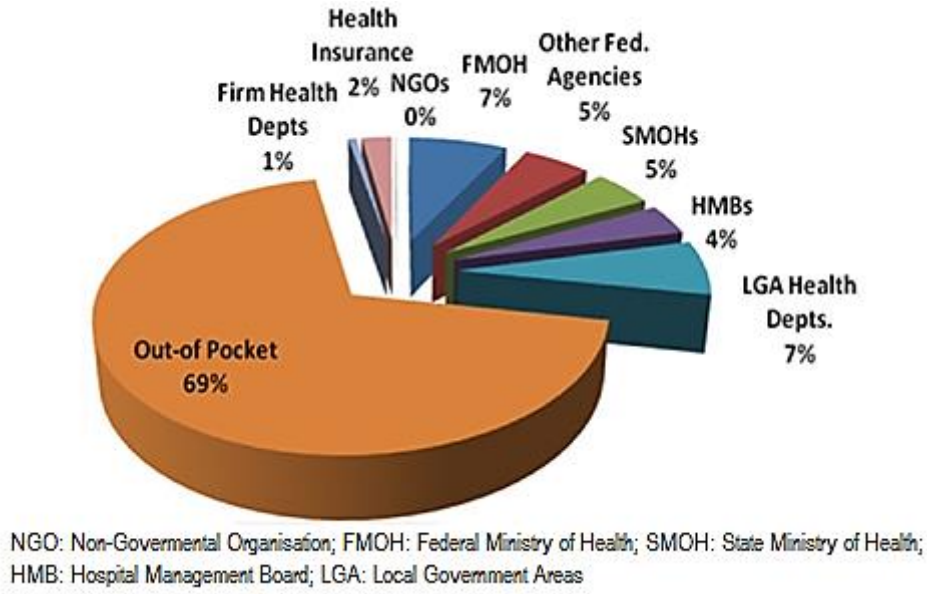


Figure 2.7: Sources of health-care financing in Nigeria (Uzochukwu et al., 2015).

This explains how income inequality can lead to health inequality. Health insurance helps in health care financing as everyone contributes in proportion to their capacity a percentage regardless of their income, with high-income earners contributing more. This makes for more equal access to healthcare and reduces health inequality.

Considering the study environment and despite the availability of modern health-care facilities in communities, the inability of residents to afford hospital services has likely contributed to the increased patronage of HM (Adesiji and Komolafe, 2013). However, the inability to afford hospital costs might not be attributed to poverty; as affordability and poverty cannot be said to be the same even though they are not mutually exclusive. Further statistical analysis sheds light on this issue in Section 2.12.10. A published report showed

that the average per-subject cost of treating headache in the UK is £39 and can go up to £42,000 for more complex cases (Fineberg et al., 2013). But for the health insurance policy in the UK, treatment would likely not be affordable for an average UK citizen who earns £528 average gross weekly on a full-time basis (ONS, 2015). Conversely, poverty seems to be used to mean affordability in many contexts. Although more respondents in the low-income class ascribed high hospital cost to non-use of hospitals, poor service delivery was still the reason most frequently cited (Figure 2.8).

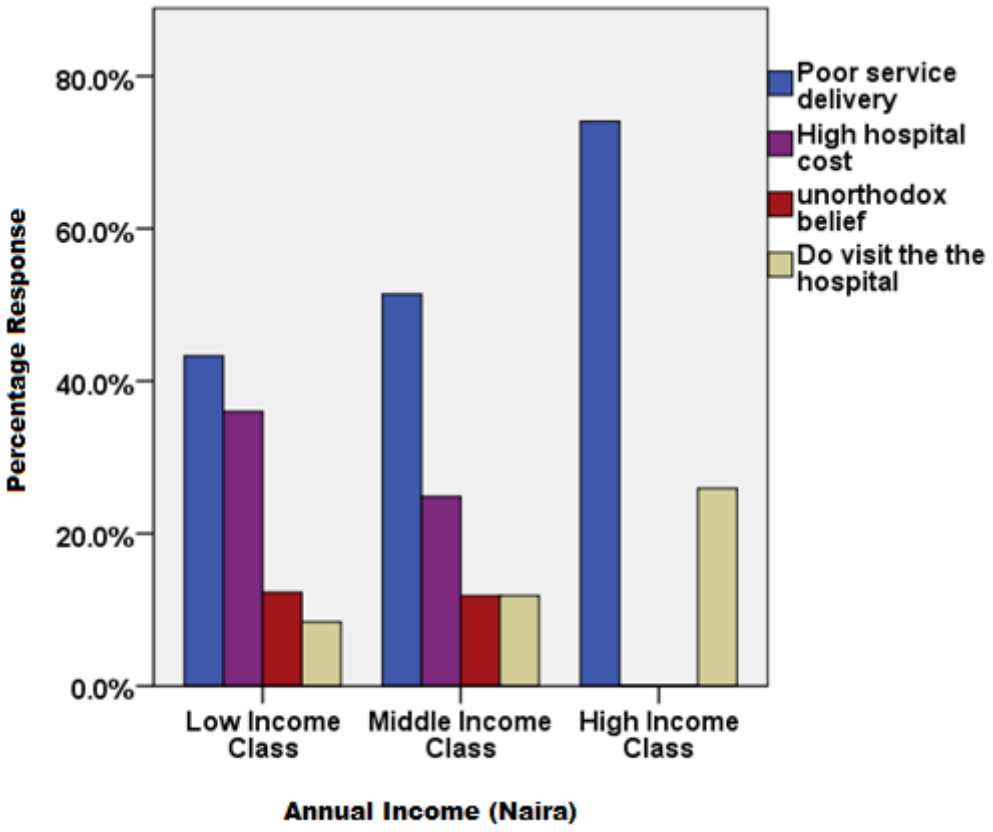


Figure 2.8: Reasons for not using hospitals by economic background

Figure 2.8 shows that, apart from the health-care financing issues in the study environment, poor service delivery by orthodox health facilities has become as serious a problem which requires serious intervention. But then poor service delivery in orthodox health-care facilities in this study cannot be viewed in isolation, without relating it to other factors in this study. It has been argued that societies with high-income inequality, as found in this study, are the most unlikely to invest in human capital resources and social services such as health care (Kaplan et al., 1996; Kawachi et al., 1997). The consequences are more serious for low-income and middle-income earners who may not be able to afford the luxury which health care has become, while high-income earners can seek help at higher cost. Suffice to say

that egalitarian societies have been found to achieve a better health outcome (Wilkinson, 1996).

About 11.9% of the respondents in this study cited unorthodox belief for not attending hospital, while 9.3% both attend hospital and use HM. The unorthodox belief of some respondents may, however, not be unconnected with dissatisfaction and disappointment with the orthodox medical system as discussed earlier in this section.

2.12.7 Perception of safety and effectiveness of herbal medicine

Question 2.10: Was the HM effective for its intended use?

This study showed the majority of respondents (83.6%) affirmed the effectiveness of the HM they had taken (Table 2.15). Various studies have also reported the effectiveness of herbal preparations on the various diseases and conditions studied (McClure, Flower and price, 2014). A study carried out in Nigeria reported the effectiveness of HM on HIV patients and also revealed instances where orthodox medicine produced worse results than those produced by HM (Onifade et al., 2013). A review of clinical studies of herbal therapy in irritable bowel disease (IBD), for example, showed much difference in the efficacy of HM than placebo (Ng et al., 2013).

Table 2.15: Perception of Effectiveness of herbal medicine

Response	Frequency	Percentage (%)
Yes	899	83.6
No	176	16.4
Total	1075	100.0

Other studies have also reported findings alluding to the perception of effectiveness of HM (Oh et al., 2010; Teng et al., 2010; Puataweepong et al., 2012; Gary and Rutledge, 2013; Ladas et al., 2014). Satisfaction with results produced by HM is evident from this study, although a comparative use of orthodox medicine was not possible and the types of aliment for which HM was used may provide more clues as to its effectiveness.

Question 2.11: Do you think it is safe to take uncertified HM?

This study showed that more than half (57.3%) of the respondents who took HM believed it is not safe to take uncertified HM, while 42.7% believed it was safe (Table 2.16). The perception of HM safety noted here is corroborated by previous findings in this study which showed that 61.1% of respondents who did not use HM cited its possible risk to health as a reason for its avoidance. (Table 2.9). Regardless, most respondents (37.3%) used

uncertified HM and 31.9% used both certified and uncertified concurrently (Table 2.10). Evaluation of the types of HM used by respondents (Table 2.12) also showed that 66.1% used HM that was uncertified.

Table 2.16: Perception of safety of herbal medicine

Response	Frequency	Percentage (%)
Yes	464	42.7
No	623	57.3
Total	1087	100.0

By contrast, a study of the use of HM in Ikorodu Lagos State Nigeria reported that the majority (82.4%) of 400 respondents believed HMs are safe for use and 9.7% believed they may not be safe; though the study did not distinguish between certified and uncertified HM (Awodele et al., 2014). The same study also reported that 12.6% of 400 respondents had experienced adverse effects, while 61.9% had not. These differ from the findings in this study, where 48.3% reported adverse effects ranging from nausea and vomiting to generalised bodily weakness, while 52.7% experienced no adverse effects (see Table 2.17).

HM-related adverse effects depend on the type, form and quantity of HM taken. Like orthodox medicine, HM also causes different adverse effects on the user. Those associated with various forms of HM have been reported in literature to include headache, abdominal discomfort and allergic reaction (Posadzki, Watson and Ernst 2013; Awodele et al., 2014; Awodele et al., 2013; Duru et al., 2016). It is clear from this study that adverse effect monitoring (pharmacovigilance) of HM must be adequate and discussion on monitoring of pharmacovigilance of HM is imperative**.** Regardless of adverse effect, 83.6% of the respondents said the HM they used was effective for the intended purpose, while 16.4% said it was not (Table 2.15). As a result of the increase in use of HM, as previously discussed in the literature review (Chapter1), the safety testing of herbal medicine has become essential.

Although there are perceptions of prejudice against the safety of herbal medicines (Zhang et al., 2015), several publications have raised safety concerns about HM and its practice. Studies have reported HMs as containing pharmaceutical adulterants and heavy metal contamination (Ajasa et al., 2004; Ernst and Pittler, 2002; Posadzki, Watson and Ernst 2013). Some HMs naturally contains harmful substances which have been reported to be toxic to humans (Moreira et al., 2014) and contamination of HM with microorganisms in Ekiti State has also been reported (Oluyege and Adelabu, 2010). Given the high rate of HM use found in this study (Table 2.8) and the high perception of uncertified HM's being unsafe

(Table 2.16), it is important to determine how safe the HMs used in Ekiti State are and Chapter 4 undertakes this.

2.12.8 Herbal medicine's adverse effects and their management

Question 2.12: What adverse effect did you experience with herbal medicine use?

As discussed in Section 2.12.17 above, over half of the respondents (52.7%) did not report any adverse effect (Table 2.17). Those who experienced an adverse effect reported severity ranging from mild to severe, which necessitated the type of intervention the respondents took, as shown in Table 2.18.

Table 2.17: Result showing adverse effect of HM experienced by respondents

Response / Effect	Frequency	Percentage (%)
None	566	52.7
Abdominal discomfort	150	14.0
Nausea and Vomiting	201	18.7
Headache	82	7.6
Stooling	62	5.8
Generalised bodily weakness	14	1.3
Total	1075	100.0

Question 2.13: How did you manage the adverse effect?

Among the respondents, 42.5% managed the adverse effect by resting, while effects wore off, 25% attended hospital, 21.6% took orthodox medicine (self-prescribed), and 10.9% took other types of HM to counteract the effect of the previous HM (Table 2.18).

Table 2.18: Result showing how adverse effects of HM were managed by respondents

Response	Frequency	Percentage (%)
Take other HM	56	10.9
Rest until it self-limits	219	42.5
Visit the hospital	129	25.0
Take orthodox medicine	111	21.6
Total	515	100.0

Apparently, mild effects were managed by rest, moderate effects by taking other HM or orthodox medicine and severe effects mostly by resort to the orthodox health system in the hospital. In developed countries, adverse effects of HM are mainly treated in orthodox ways (Posadzki, Watson and Ernst, 2013).

2.12.9 How can safety of herbal medicine be improved?

Question 2.14: How do you think the safety of HM can be achieved?

It is known that HM practice has a long cultural history in many societies including Yoruba. As a result, some practitioners may still be comfortable practising without regulation, relying on personal experience for treatment and hinging on tradition to legitimise practice. With advances in science, analysis and a regulatory framework for HM use are now more realisable. In this study, 46.5% of the respondents believed improved government monitoring would help to achieve safety of HM, while 40.1% believed public enlightenment would help. Also, 11.1% believed use of appropriate production and good hygienic preparation would help improve the safety of HM, while 2.3% said they do not know how its safety could be improved (Table 2.19). The government was believed to have a great responsibility for ensuring the safety of HM by the majority of participants. It can possibly be achieved through law on appropriate manufacturing standards, prevention of false claims and circulation of hazardous herbal products.

Table 2.19: How respondents think herbal medicine safety can be improved

Response	Frequency	Percentage (%)
Government monitoring	577	46.5
Use of appropriate production material and hygiene	138	11.1
Public enlightenment	498	40.1
Do not know	29	2.3
Total	1242	100.0

The guidelines of NAFDAC stipulate that no manufacturing, import, advertisement, sale or distribution of herbal medicinal and related products should be carried out in Nigeria until it has been registered in conformity with the necessary regulations (NAFDAC, 2005). The guidelines did not include extemporaneous preparations made by a practitioner and given to a patient on a one-to-one basis in the area in which it was prepared (NAFDAC, 2005). However, 23.3% of the respondents in this study used local herbal mixtures (Table 2.12), most of which can be classified as extemporaneous. Thus it is important that the safety of those who use these preparations be secured through the development of a thorough public enlightenment scheme, monitoring, screening, and legislation for extemporaneous HM. The publication and dissemination of these research findings will help to promote this course of action and also form a template for further policy formulation on HM use, especially in Ekiti State.

2.12.10 Effect of background characteristics on use of herbal medicine and perception of safety

The association between the use of herbal medicine and the socioeconomic background of respondents was evaluated using Chi-square (χ^2) analysis. The information thereby obtained will help to understand if there is a significant influence of any of the social, economic or demographic characteristics of the respondents on the use of HM. A level of significance $p \leq 0.05$ will mean a significant association between the observed factor and the use of HM, while a level of significance $p > 0.05$ will mean no significant association.

According to Table 2.20 a significant number of men in this study used HM (89.9%), more than women (78.6%) ($p < 0.001$, $\chi^2=31.008$ and $df =3$). This finding is not consistent with those of studies from developed countries, where women showed greater use than men; 12.7% of women and 1.1% of men (Harrison et al., 2004), 8.3 % women and 2.9% men (Raji et al., 2005) and the same in other studies (Gardiner et al., 2007; Bakhotmah and Alzahrani, 2010). A previous study in Nigeria, however, found no significant association between being female and use of HM (Onyeka et al., 2012).

Table 2.20: Significance of background characteristics on use of herbal medicine

Socio-demographic characteristics	Use of HM	Non-use of HM	Total	*p*-value
Age (years)				
18-29	274 (85.1%)	48 (14.9%)	322 (100%)	$p = 0.005$*
30-49	474 (86.7%)	73 (13.3%)	547 (100%)	$\chi^2 = 12.996$
50-69	289 (80.7%)	69 (19.3%)	358 (100%)	*df*= 3
70 and above	38 (100.0%)	0	38 (100%)	
Total	1075 (85.0%)	190 (15.0%)	1265 (100%)	
Gender				
Male	641 (89.9%)	72 (10.1%)	713 (100%)	$p < 0.001$*
Female	434 (78.6%)	118 (21.4%)	552 (100%)	$\chi^2 = 31.008$
Total	1075 (85.0%)	190 (15.0%)	1265 (100%)	*df* = 1
Level of Education				
No formal education	169 (88.5%)	22 (11.5%)	191 (100%)	$p = 0.001$*
Primary	224 (91.4%)	21 (8.6%)	245 (100%)	$\chi^2 = 16.670$
Secondary	287 (84.4%)	53 (15.6%)	340 (100%)	*df*= 3
Tertiary	395 (80.8%)	94 (19.2%)	489 (100%)	
Total	1075 (85.0%)	190 (15.0%)	1265 (100%)	
Religion				
Christianity	751 (85.4%)	128 (14.6%)	879 (100%)	$p = 0.009$*
Islam	284 (82.1%)	62 (17.9%)	346 (100%)	$\chi^2 = 9.493$
African traditional religion	40 (100.0%)	0	40 (100%)	*df*= 2
Total	1075 (85.0%)	190 (15.0%)	1265 (100%)	
Annual Income(Naira)				
Low Income(≤ 600,000)	890 (85.8%)	147 (14.2%)	1037 (100%)	
Middle income (601,000 to 2.4M)	158 (88.3%)	21 (11.7%)	179 (100%)	$p < 0.001$*
High Income (≥2.4M)	27 (55.1%)	22 (44.9%)	49 (100%)	$\chi^2 = 36.366$
Total	1075 (85.0%)	190 (15.0%)	1265 (100%)	*df*= 2
Occupation				
Student	69 (82.1%)	15 (17.9%)	84 (100%)	
Civil servant	395 (80.4%)	96 (19.6%)	491 (100%)	$p < 0.001$*
Farmer	76 (100.0%)	0	76 (100%)	$\chi^2 = 26.701$
Business	431 (86.0%)	70 (14.0%)	501 (100%)	*df*= 4
Other	104 (92.0%)	9 (8.0%)	113 (100%)	
Total	1075 (85.0%)	190 (15.0%)	1265 (100%)	

*Significant association; *df* = Degree of Freedom

It has been reported that women take fewer risks than men (Harris and Jerkins 2006 and Hoffman et al., 2013), and this may be true of the sample population of this study. A majority of female respondents cited risk to health and lack of knowledge of most of HM widely available as their chief reasons for avoiding HM. Their male counterparts, by contrast, cited personal preference mainly followed by risk to health (Figure 2.9). Findings of this study also show that more males took uncertified HM than did female respondents (Figure 2.10).

It was also observed from this study that a significant number of males believe it is safe to take uncertified HM, while a significant number of females believe it is not (Table 2.21). Studies have also reported that more women than men tend to seek orthodox health care (Green and pope, 1999; Bertakis et al., 2000; Chang et al., 2012).

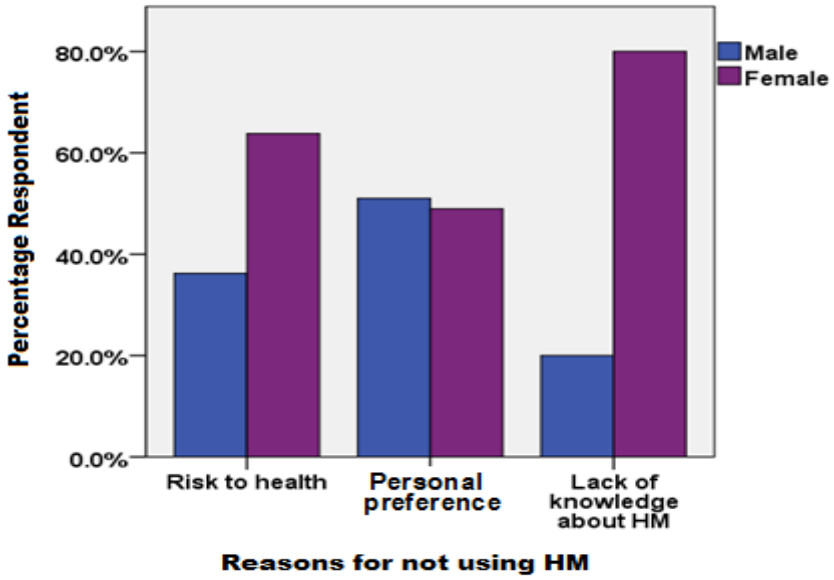


Figure 2.9: Reasons for not using herbal medicine by gender

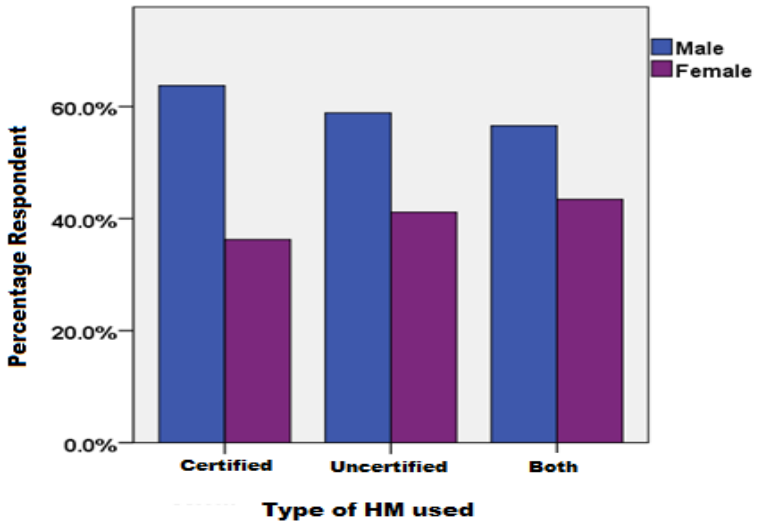


Figure 2.10: Type of herbal medicine used by gender

Other factors contributed to the use of HM, e.g. level of education. The use of HM was highest among respondents for whom the highest level was primary education (Table 2.20).

Though the use of HM was lowest among respondents with a tertiary education, still notable is the high use of HM among respondents with tertiary level of education (see Table 2.20). There was a significant relationship between respondents' level of education and use of HM

(p = 0.001, χ^2=16.670, df =3). There was a significant association between annual income and HM use (p < 0.001, χ^2=36.366, df =2); highest use among respondents of the middle-income class, while the least use was among the high annual income group. A previous study published that about 85% of Nigerians use HM for healthcare, psychological and social benefit due to dissatisfaction with orthodox medical care and poverty (Oshikoya et al., 2008). While dissatisfaction with orthodox medical care has been highlighted as the main reason for not using hospitals, it has also been observed that poverty was not the main reason respondents used HM in this study.

A higher level of education may be associated with increased ability to make informed choice; higher income makes these choices more realisable. Low level of education and income power have been reported to influence the choice of HM use (Gardiner et al., 2007; Barnes and Bloom, 2008; Okoronkwo et al., 2014). The studies associated low income with use of HM; which is different from what was found in this study, where the highest use was not among the low-income earners. Reinforcing this point, a Nigerian study reported a similar finding among pregnant women attending a tertiary health facility, where use of HM was higher among respondents who earned more (Duru et al., 2016). However, studies in developed countries have reported an insignificant relationship between income and HM use, but a significant relationship between higher level of education and HM use (Du et al., 2014; Kummet et al., 2015). Another study reported no significant relationship between levels of education and HM use (Oreagba, Oshikoya and Amachree, 2011). These variations may be due to factors such as respondents' socioeconomic characteristics or peculiarities of the study population, such as culture. Hence the findings in this study reflect the likely peculiarity of the people living in Ekiti State, where middle income was associated with more use of HM.

There was a significant association between the age of the respondents and use of HM in this study (p = 0.005, χ^2 =12.996, df =3). Respondents of 70 years and above have all (100%) used HM in the last 2 years, followed by the age group 30-49 years of whom 86.7% have also used HM during the period. A publication about the general population and HM use reported a significant relationship between age and use of HM (Gardiner et al., 2007), while another study reported no significant difference (Okoronkwo et al., 2015). This study showed that the use of HM increased with the age of the respondents, although the age group 50-69 years did not follow the trend. The occupation of the respondents was also significantly associated with HM use (p < 0.001, χ^2=75.504, df = 8) as more farmers (100%) used HM (Table 2.20) than did other occupation and this may relate to the annual income of the respondents, which had a significant association with HM use in this study (Table 2.20). The type of occupation largely determines accruable income.

There was 100% use of HM in the last 2 years among respondents who practised African traditional religion and 85.4% use among Christians. There was a significant relationship between religious affiliation of the respondents and HM use (p = 0.009, χ^2=9.493, df = 2) (Table 2.20). The use of HM as an integral part of African traditional religion is documented; this is in addition to the role of African traditional religionist as the custodian of African traditional medicine (White, 2015). Although some studies reported no significant association between religion and HM use (Hughes et al., 2013; Adesiji and Komolafe, 2013; Opara and Osayi, 2016), other studies have reported otherwise (Hulela and Thobega, 2013; Ethel and Amy, 2014). Furthermore, research has shown that the religious beliefs and spiritual practices of patients have a powerful influence on their decisions about treatment choice, coping with chronic disease and end of life care (Puchalski, 2001; McCormick et al., 2012). This study has shown the potential influence religion has on the use of HM, with 100% of respondents practising African traditional religion having used HM in the last two years. There is apparently a correlation between their religious belief and their choice of health care.

Table 2.21.Significance of background characteristics on the perception of safety of HM

Question 10.1: Do you think it is safe to take Uncertified HM				
Socio-demographic characteristics	Yes	No	Total	*p*-value
Participant age(years)				
18-29	107 (39.1%)	167 (60.9%)	274 (100%)	$p < 0.001$*
30-49	195 (40.8%)	283 (59.2%)	478 (100%)	χ^2=29.104
50-69	130 (43.8%)	167 (56.2%)	297 (100%)	*df*=3
70 and above	32 (84.2%)	6 (15.8%)	38 (100%)	
Gender				$p = 0.034$*
Male	294 (45.3%)	355 (54.7%)	649 (100%)	χ^2=4.499
Female	170 (38.8%)	268 (61.2%)	438 (100%)	*df*=1
Level of Education				
No formal education	111 (64.2%)	62 (35.8%)	173 (100%)	$p < 0.001$*
Primary	101 (45.1%)	123 (54.9%)	224 (100%)	χ^2=54.801
Secondary	126 (43.9%)	161 (56.1%)	287 (100%)	*df*= 3
Tertiary	126 (31.3%)	277 (68.7%)	403 (100%)	
Religion				
Christianity	316 (41.4%)	447 (58.3%)	763 (100%)	$p = 0.278$
Islam	127 (44.7%)	157 (55.3%)	284 (100%)	χ^2 =2.558
African traditional religion	21 (52.5%)	19 (47.5%)	40 (100%)	*df*=2
Occupation				
Student	33 (47.8%)	36 (52.2%)	69 (100%)	$p < 0.001$*
Civil servant	140 (34.7%)	263 (65.3%)	403 (100%)	χ^2 =36.688
Farmer	53 (69.7%)	23 (30.3%)	76 (100%)	*df*=4
Self Employed	199 (45.7%)	236 (54.3%)	435 (100%)	
Others	39 (37.5%)	65 (62.5%)	104 (100%)	
Annual Income(Naira)				
Low Income (≤600,000)	403 (45.1%)	491 (54.9%)	894 (100%)	$p < 0.001$*
Middle income (601,000 to 2.4M)	57 (36.1%)	101 (63.9%)	158 (100%)	χ^2 =18.891
High Income (≥2.4M)	4 (11.4%)	31 (88.6%)	35 (100%)	*df*=2

*Significant association; *df* = Degree of Freedom

The influence of demographic characteristics of the respondents on their perception of safety was also studied. This is to examine how the safety of HM is viewed across economic, educational, religious and other characteristics of the respondents. According to Table 2.21, there was a significant association between age, gender, annual income, occupation and perception of HM safety. The exception was religious belief, which had no significant association ($p = 0.278$, χ^2=2.558 and $df = 2$). The significant influence of education on the perception of safety of HM was reported in the literature (Fakeye, Adisa and Musa, 2009), and is consistent with the findings of this study. The non-significant association between religion and perception of safety of HM in this study may be attributed to the cross-cultural

belief in HM use in the sample population. This hypothesis may be further supported by the high number of respondents across religious divides that use HM (Table 2.20).

Therefore, much as there is a significant influence of religion on the use of HM among participants (Table 2.20), it had no significant influence on the perception of safety of HM (Table 2.21). A significant number of male respondents, of 70 years and above, having no formal education, farmers, in low annual income class believe it is safe to take uncertified HM. On the other hand, a significant number of youths between 18-29 years of age, females, and respondents with tertiary level of education, civil servants and high-income earners believe it is not safe to take uncertified HM. This is expected, considering that level of education and annual income affect the level of exposure which may have informed the views expressed by these groups of respondents.

The relationship between an exposure and an outcome is quantified using the odds ratio (OR), representing the odds of an outcome's occurring, provided a certain exposure to the odds of the outcome happening in the absence of the exposure being studied (Szumilas, 2010). While modelling of the data and a logistic regression would have helped predict future characteristics associated with the use of HM, not enough predictor variables were examined in this study to make such prediction reliable (Peduzzi et al., 1996). A future study may explore this subject employing more variables.

2.13 Herbal medicine related casualty and fatality in Ekiti State hospitals

A five-year retrospective study of casualties and fatalities related to HM between 2010 and 2014 from 17 government hospitals (see Section 2.11) examined 94,323 patient records. Findings showed that 0.5% of general paediatric admissions were related to herbal medicine use and 3.2% of the general paediatric deaths were related to herbal medicine use (Table 2.22).

Table 2.22: Herbal medicine related paediatric casualty and fatality

Total admission	Total Death	HM related Admission	HM related death
23363	1146	107(0.5%)	37(3.2%)

In a separate four-year retrospective study carried out in a tertiary hospital in Ekiti State 16 (0.3%) of the total paediatric admissions (5,256) were as a result of HM use. Although the total paediatric deaths were not stated, 2 of the 16 patients died (Olatuya et al., 2015). The

HM related paediatric casualties in this research (0.5%) are higher than published research (0.3%) *(Ibid*): although this study is a five-year retrospective analysis. However, in this research HM related casualties and fatalities in adult patients revealed lower figures (see Table 2.23). HM related medical admissions were only 0.06% of the total medical admissions over the study period and HM related death was 0.2% of the total deaths recorded (Table 2.23).

Table 2.23: Herbal medicine related medical casualty and fatality in adults

Total Admission	Total death	HM related admission	HM related death
52871	1964	34 (0.06%)	4 (0.2%)

Although about 25% of the respondents in the survey who experienced an adverse effect after taking HM (n=515) indicated that they attended hospital for treatment of the effects (Table 2.18), this is not corroborated in reality, with 0.06% of the medical admissions' (n=52871) being HM related. An explanation may be that some patients do not disclose their use of HM to doctors in the hospital (Shen et al., 2002; Downer et al., 1994; Ezeome and Anarado, 2007; Farooqui et al., 2016; Djuv, Nilsen and Steinsbekk, 2013). Doctors have also been reported to underestimate the use of HM by patients and so often do not ask about it when taking the clinical history from patients (Giveon, 2003). Conversely, there are a number of publications on HM related casualties and fatalities (Cosyns et al., 1999; Ernst, 2002; Assiri, 2012), and it is likely that the severity of the HM adverse effects often warranted a disclosure and probably made the doctor search more for possible causes to the point of exploring HM use.

Nonetheless, socio-demographic characteristics of the adult casualties and fatalities also obtained from the hospital records showed the highest casualties among the age group 30-49, male patients, patients with a primary level of education, Christians, and patients who were self-employed (Table 2.24). The fatalities were also highest among the age group 30-49, but then among patients with tertiary level of education, female patients, African traditional religion worshipers and civil servants.

Table 2.24: Socio-demographic characteristics of HM associated casualty and fatality of adult medical patients

Socio-demographic factors of patients	Outcome of effect		
	Survived % (n=30)	Fatality % (n=4)	Casualty % (n=34)
Age (years)			
18-29	4 (100.0%)	0	4 (11.8%)
30-49	16 (84.2%)	3 (15.8%)	19 (55.9%)
50-69	7 (87.5%)	1 (12.5%)	8 (23.5%)
70 and above	3 (100.0%)	0	3 (8.8%)
Total	30 (88.2%)	4 (11.8%)	34 (100.0%)
Gender			
Male	27 (90.0%)	3 (10.0%)	30 (88.2%)
Female	3 (75.0%)	1 (15.0%)	4 (11.6%)
Total	30 (88.2%)	4 (11.2%)	34 (100.0%)
Level of Education			
No formal education	0	0	0
Primary	13 (92.9%)	1 (7.1%)	14 (41.2%)
Secondary	10 (90.9%)	1 (9.1%)	11 (32.3%)
Tertiary	7 (77.8%)	2 (22.2%)	9 (26.5%)
Total	30 (88.2%)	4 (11.2%)	34 (100.0%)
Religion			
Christianity	25 (92.6%)	2 (7.4%)	27 (79.4%)
Islam	4 (80.0%)	1 (20.0%)	5 (14.7%)
African traditional religion	1 (50.0%)	1 (50.0%)	2 (5.9)
Total	30 (88.2%)	4 (11.2%)	34 (100.0%)
Occupation			
Student	2 (100.0%)	0	2 (5.9%)
Civil servant	0	2 (100.0%)	2 (5.9%)
Farmer	1 (100.0%)	0	1 (2.9%)
Business	25 (92.6%)	2 (7.4%)	27 (79.4%)
Others	2 (100.0%)	0	2 (5.9%)
Total	30 (88.2%)	4 (11.2%)	34 (100.0%)

Comparison of the socio-demographic characteristics of HM users in the survey study (Table 2.20) and that of adult HM related casualties from hospital records showed that the age group 30-49 years, the second highest users of HM, had the highest casualties and fatalities (Table 2.25). This finding mirrors the survey findings revealing how involved this age group is in HM use, with attendant health consequences. Hence this is worth signposting as a potential target group for future HM related policies and awareness programmes. Similarly, more males than females used HM (Table 2.20) and males also accounted for the highest casualty figures, although fatalities were higher in females (Table 2.25). People who practised African traditional religion used HM the most; they also had the highest fatalities of

the group admitted, although the lowest casualties. African traditional religion being the epicentre of HM practice (White, 2015), belief in HM is sacrosanct. This is also apparent from the survey data, where 100% of African traditional worshipers have used HM in the last two years (Table 2.20). As a result, they may likely use more HM and probably use other HMs to counteract undesirable effects, as observed in Table 2.18. The efficacy of such practice may be responsible for the low casualty rates recorded when it is successful and the cumulative damage when it fails may be responsible for the high fatalities recorded when they eventually attend hospital (Table 2.25). However, this needs further evaluation by proper history taking of HM use in hospital patients. A comparison of the results obtained from the survey study on the use of HM and the findings from the hospital record is shown in Table 2.25.

Table 2.25: Table comparing the use of HM with adult casualty and fatality figures

Socio-demographic factor	Use of HM (%) (n=1139)	HM related casualty (%) (n=34)	HM related fatality (%) (n=4)
Age(years)			
18-29	85.1	11.8	0
30-49	86.7	55.9	15.8
50-69	80.7	23.5	12.5
70 and above	100.0	8.8	0
Gender			
Male	89.9	88.2	10.0
Female	78.6	11.6	15.0
Level of Education			
No formal education	88.5	0	0
Primary	91.4	41.2	7.1
Secondary	84.4	32.3	9.1
Tertiary	80.8	26.5	22.2
Religion			
Christianity	85.4	79.4	7.4
Islam	82.1	14.7	20.0
African traditional religion	100.0	5.9	50.0
Occupation			
Student	82.1	5.9	0
Civil servant	80.4	5.9	100.0
Farmer	100.0	2.9	0
Self Employed	86.0	79.4	7.4
Others	92.0	5.9	0

The use of HM was highest among farmers in the survey, whereas casualties were highest among self-employed people and fatalities were highest among civil servants (Table 2.25).

This is not unusual considering that the self-employed constituted the largest percentage of the participants in this survey (39.6%), followed by civil servants (38.8%), while farmers were the least represented (6%) (Table 2.6). This may, therefore, be a reflection of the population distribution and hospital admissions along occupational lines in Ekiti State, which these fatality and casualty figures further highlight. The annual income of patients could not be obtained from the hospital data, therefore the economic correlation with the casualty and fatality figures could not be ascertained. But from Table 2.25, the level of education may be helpful, considering that the highest use of HM and casualties were among people with a primary level of education. Conversely, fatalities were highest among patients with a tertiary level of education. Therefore the impact of the undesirable effects of HM seems to be more serious in people with tertiary education, who likely form the bulk of respondents in the high-income class.

Obstetric fatalities in the form of stillbirth (SB) related to HM were 26 (3.9%) of the total 668 stillbirths. Herbal medicine related SBs were further divided according to the Baird-Pattinson aetiology (Section 2.11). Stillbirths with a history of HM use in pregnancy were only identified within four Baird-Pattinson classifications: spontaneous preterm labour (SPL) (42%), foetal abnormality (34%) intrauterine growth restriction (IUGR) (15.4%) and unexplained intrapartum foetal death (UIFD) (7.7%) (Table 2.26). The use of HM among pregnant women is common in Nigeria (Duru et al., 2016; Adisa, Agbom and Fakeye 2015; Fakeye, Adisa and Musa, 2009). However, people respond and react differently to different HM. A study has shown intrapartum use of HM in healthy babies (Louik et al., 2010), while other studies have reported foetal abnormalities (Chuang et al., 2006; Johnson and Sibeko, 2003; Jurgens, 2003; Low, 2009). In addition, HM-induced IUGR and foetal abnormalities in experimental animals have been reported (Wang et al., 2012; Li et al., 2012).

Table 2.26: Herbal medicine related obstetric fatalities

Total birth	Stillbirth	HM-related fatality	HM-related fatality (n=26)			
			SPL	Foetal abnormality	IUGR	UIFD
18089	668	26 (3.9%)	11 (42%)	9 (34%)	4 (15.4)%	2 (7.7%)

HM-associated stillbirth has also been reported in the literature (Choi, Han and Ahn, 2013; Neogi et al., 2016), and a 3-year retrospective study in Ghana reported HM to be associated with 5.7% of the total stillbirths (Alhassan et al., 2016), which is higher than the finding in this study of 3.9% (Table 2.26). Nevertheless, other studies have shown no significant

association between HM use and negative foetal outcome (Singh et al., 2015). The source of toxicity in some of the HM could be related to direct toxicity from the herbal medicines, misuse, contamination (with heavy metals, pesticide among others), adulteration (e.g with known pharmaceutical compounds) or uncontrolled confounding. Unlike the adult and paediatric HM- related casualties and fatalities where a diagnosis was made as regards HM culpability, this is not the case in an obstetric setting. Other factors could have been responsible for the obstetric outcome and most of the hospital records examined in this study only pointed to continuous use of HM by the patients, but no other definitive diagnosis was made. In addition, use of a control group would be useful in drawing a stronger inference. Nonetheless, more work needs to be done to ascertain the safety of HM, as later undertaken in Chapter 4.

2.14 Conclusion

This study has shown that a majority of the population have used HM in the last 2 years, cutting across economic class and with the highest use among the middle-income class. This does not suggest that poverty was a major factor in the use of HM, especially considering the income inequality in the population and the lack of universal health coverage. The use of HM was commonest among respondents with a primary level of education, of African traditional religion, the self-employed and respondents of 70 years of age and above. More males used HM than did females. The effectiveness of HM was a major and most frequently cited reason for respondents' use of it. The personal experience of the majority attested to the intended effectiveness of HM. Though the majority of respondents knew the difference between certified and uncertified HM, uncertified HM was most commonly used.

A majority of the study population believed it was not safe to take uncertified HM, although, more than half had not experienced any adverse effect associated with its use. Those who had suffered adverse effects reported different ways of ameliorating their condition, depending on its severity. A retrospective study of HM-related casualties and fatalities reinforced some of the survey findings, e.g. male predominance in the use of HM and casualty figures. These casualty and fatality figures could appear to be not too alarming and could be mitigated. This mitigation is essential in preserving lives and preventing waste of hospital resources.

Paradoxically, participants revert to orthodox medical care, especially when their illness gets out of control and/or HM adverse effects become severe. This further highlights the need for adequate investment in the health care system as it becomes the last resort in severe cases.

This is important, considering the findings of this study on reasons' for not using hospitals corroborating the health infrastructural / service deficit, which possibly contributed to the use of HM. However, the choice is limited to acclaimed effective HM and an orthodox health system's offering an unsatisfactory service. While affordability was also an important consideration for people who used HM in this study, high hospital cost was another reason for which they did not patronise the orthodox medical service. It is a choice between affordable HM and an expensive orthodox medical service, especially in the absence of adequate health insurance coverage. This study has shown that there is a significant association between annual income, education, gender, age, religion, occupation and the use of HM. There is also an impact of HM use reflected in the number of HM- related hospital admissions and deaths. Although there is a cultural history of HM use in the study population, socio-economic and socio-demographic factors were determinants of its use in light of prevalent income inequality and absence of adequate health provision.

2.15 Limitations of the study

The following three limitations to this study have been identified:

I. There were more incomplete entries in the self-administered questionnaires than on the interview-administered method, which reduced the number of eventual participants in the study.
II. Poor record keeping practice in most of the hospitals made it difficult to examine the casualty and fatality figures associated with HM use.
III. Most of the towns were visited during the day, at which time some of the farmer residents would usually be at work far away from the residential areas. This led to the exclusion of some of this group of people from the likelihood of participation in the study.

CHAPTER 3: GC-MS AND ICP-OES METHOD DEVELOPMENT AND VALIDATION FOR THE DETECTION OF PHARMACEUTICALS AND HEAVY METALS IN SELECTED HERBAL MEDICINE

3.1 Introduction to method validation

The aim of validating an analytical method is to present its suitability for a planned purpose (Ellison, Barwick and Farrant, 2009). After the development and validation of an analytical method, it can be deployed in analysing target compounds. Accordingly the adopted method for Gas Chromatography-Mass Spectrometry (GC-MS) and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) in this research was validated to make it suitable for the detection and quantification of target analytes (selected pharmaceutical compounds and heavy metal) in selected HM samples.

Guidelines and publication for method development and validation such as the International Conference on Harmonisation (ICH) guideline, Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method development, International Organization for Standardisation (ISO) and other guidance documents are widely available (ISO, 1990; ICH, 2005 and SWGTOX, 2013). The ICH guideline was used in this research for method validation, alongside the use of the ISO (1990) for the statistical assessment of the linear calibration function (ICH, 2005; ISO, 1990).

The validation studies conducted include specificity, accuracy, precision, linearity, linear range, limit of detection and limit of quantification. Although not all of the validation parameters are applicable for all types of test, these are typical validation parameters and are needed to determine the suitability of the method for the intended analytical use in GC-MS and ICP-OES. Autosampler stability was, however, added to the parameters required for the validation of GC-MS method.

3.1.1 Selectivity

Selectivity is defined as the capacity for unequivocal assessment of analyte in the presence of matrix which is likely present; such as impurities and degradation products (ICH, 2005).

3.1.2 Linearity and linear range

Linearity is defined by ICH (2005) as the ability of a method to produce results within a range, which correspond to the target analyte concentration in the sample. Linearity is examined over an extensive range of concentration from low to high. Determining the linear range, aside from the aim outlined by the definition, also helps in the assessment of the

sensitivity of the analytical instrument and method (Ellison, Barwick and Farrant, 2009). There are both instrument and method linearity. Instrumental linearity is defined as the capacity of the detector to produce effects that are directly, or by means of set mathematical variation, proportional to the standard concentration. Method linearity, however, starts from extraction of samples to instrumental analysis and is assessed at the same concentration range as the analyte concentration (*Ibid*). Instrument linearity was examined in this study as shown in Sections 3.7.5 and 3.8.2.

3.1.3 Limits of Detection (LOD) and Limits of Quantification (LOQ)

The LOD and LOQ are calculated at the lower limit of the linear range. LOD is described as the lowest concentration at which an analysed compound can be detected but not reliably quantified. Limit of quantification is the lowest concentration at which an analysed compound can be quantified within permissible precision and accuracy (Peters, Drummer and Mussholf, 2007). LOD can also be defined as the lowest concentration of analyte that can be validly differentiated from the blank (Thompson, Ellison and Wood, 2002). The LOD and LOQ were determined using the ICH method, based on the slope and the standard deviation of the response (ICH, 2005).

Limit of detection = $3.3\ \sigma / S$ (Equation 3.1)

Limit of quantitation = $10\ \sigma / S$ (Equation 3.2)

Where σ = the standard deviation of y (intercepts of the linear regression line)

S = the slope of the calibration curve

The standard deviation of the y-intercepts is obtained from the calibration curve of the triplicate run of analyte plotted in Microsoft Excel (see Figure 3.12), while the slope is obtained from the average linear regression equation. LOD and LOQ are statistically calculated using this method which accounts for both instrumental and sample preparation uncertainties. When the LOD is calculated from the standard deviation (SD) of replicates of several analyses of the same solution the uncertainty also accounts for the error obtained from the autosampler (Thompson, Ellison and Wood, 2002).

3.1.4 Precision and accuracy

According to ICH (2005) precision is defined as the degree of concurrence between repeated multiple sampling of a homogenous sample. This is often also reported as standard deviation or Percentage Relative Standard Deviation (%RSD). In this study, precision is examined for both the standards and sample. This method ascertains the total precision of

the method, adding the contributions of uncertainties from instrumental analysis and sample preparation. Precision is often measured as repeatability and reproducibility (Peters, Drummer and Musshoff, 2007). Repeatability is the precision of the analytical technique by replicate assessment within a limited period. Reproducibility refers to the precision between laboratories. Accuracy is the nearness of concurrence between the real and experimental values (Miller and Miller, 2010), often reported as the % recovery from the true concentration.

3.1.5 Autosampler stability

Stability assesses possible reduction in the concentration of analyte (due to decomposition) in both the sample and standard over a given time in storage ambience. This is assessed through analysis of a mixed standard at specified time intervals, using the previously validated analytical method (SWGTOX, 2013). The stability of the mixed pharmaceutical compounds on the autosampler for the entire duration of a typical run is examined in Section 3.7.4

3.1.6 Matrix effect

The phenomenon of matrix effect is the combined effect of all components, besides the analyte which co-elute with the compounds of interest. This was examined for the GC-MS analysis because these co-eluents interfere with the ionisation process in the mass spectrometer causing suppression or enhancement of the ionisation process that negatively affects quantification of the desired compounds (Kebarle and Tang, 1993). The assessment and management of matrix effects are essential when a method is validated because they can lead to inaccurate measurement of the selected analyte (Chambers et al., 2007; Chiu et al., 2010). For GC-MS matrix effect examination post-extraction spiked matrix comparison was used in this study (Matuszewski, Constanzer and Chavez-Eng, 2003) as shown in Section 3.6.4.5.

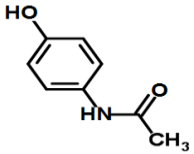
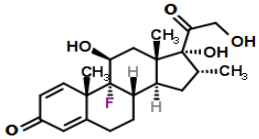
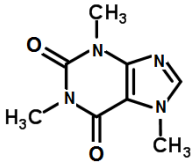
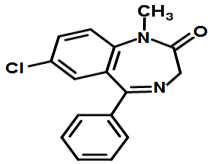
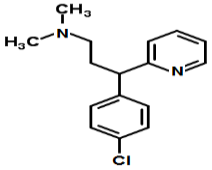
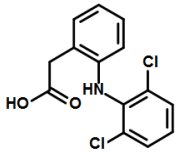
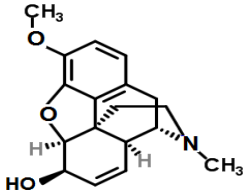
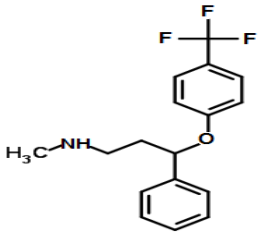
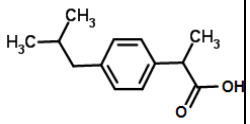
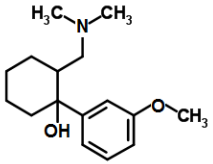
In ICP-OES analysis matrix effect may deter its ability in qualitative identification and quantitative measurement of target compounds by its effect on intensity and resolution of observed signals. The ionic-to-atomic line intensity ratio may be examined in order to detect plasma-related matrix, but then proper digestion of the complex sample matrix significantly reduces matrix effect (Todolí et al., 2002). Hence this parameter was tested in this research considering the complexity of the plant-based matrix in the herbal medicine samples and its potential effect on quantification of compounds of interest.

3.2 Introduction to target pharmaceutical compounds

As discussed in section 1.6.2, the increase in the adulteration of HM with undeclared pharmaceutical compounds has been reported extensively (Patel et al., 2012; Zhang et al., 2012). Bearing in mind the economic worth of HM global trade, as discussed in Section1.5, adulteration with pharmaceutical compounds to provide quicker effects for monetary gain have been reported (Wheatley and Spink, 2013). Hence this research seeks to explore possible adulteration of HM in Ekiti State as no publication exists on the subject.

The analytes of interest in this study include 9 commonly abused pharmaceutical compounds and one central nervous system (CNS) stimulant. The drugs of interest are an analgesic (acetaminophen), non-steroidal anti-inflammatory drugs (ibuprofen, diclofenac), opiates (codeine, tramadol), a steroid (dexamethasone), a CNS stimulant (caffeine), an antihistamine (chlorpheniramine), an antidepressant (fluoxetine) and a benzodiazepine (diazepam). These target compounds are substances that have been reported in publications to be present in some HM (Vaclavik, Krynitsky and Rader, 2014b) and are also known to be medications commonly abused in Ekiti State (Akindutire and Adegboyega, 2012; Atoyebi and Atoyebi, 2013). Further rationale for the choice of target pharmaceutical compounds has been previously discussed in Section 1.6.2. The chemical characteristics of the target compounds are summarised in Table 3.1.

Table 3.1: Properties of the pharmaceuticals of interest (U.S. Pharmacopeia, 2012)

Analyte name and structure	Class of compound	Molecular weight(g/mol)	pKa	Acidic/ Basic	Analyte name and structure	Class of compound	Molecular weight(g/mol)	pKa	Acidic/ Basic
Acetaminophen 	Analgesic	151.163	9.5	Acid	Dexamethasone 	Steroid	392.46	12.14	Neutral
Caffeine 	CNS Stimulant	194.191	14.0	Basic	Diazepam 	Anxiolytic	284.74	3.4	Basic
Chlorpheniramine 	Anti – histamine	274.789	9.47	Basic	Diclofenac 	NSAID	296.149	4.15	Acidic
Codeine 	Opiate analgesic	299.37	10.60	Basic	Fluoxetine 	Antidepressant /weight loss	309.32	9.8	Basic
Ibuprofen 	NSAID	206.281	4.91	Acidic	Tramadol 	Opioid analgesic	263.38	9.23	Basic

3.3 Introduction to target heavy metals

Agricultural sector practice (such as the use of pesticides and herbicides) and industrial sectors (such as inappropriate waste disposal) have contributed to an anthropogenic introduction of heavy metals into virtually all forms of the ecosystem (Lokhande, Singare and Pimple, 2011). These heavy metals find their way into plants, herbal medicine (HM), cosmetics and other products through the contamination of soil and the aquatic environment (Leung, Cai and Wong, 2006). Although some heavy metals are naturally present in the environment and required for optimal body functioning at trace levels, they may become very toxic at higher concentrations (Lane and Morel, 2000; Plum, Rink and Haase, 2010). Due to the potential toxicity of heavy metals to humans, it has become necessary to determine their concentration in products consumed by humans, one of which is herbal medicine.

The heavy metals in this study were selected on the basis of the European Medicines Agency's (EMA) Committee for Medicinal Products for Human use (CHMP) metals of safety concerns (EMA, 2008), the WHO publication of toxic metals found in herbal medicines (WHO, 2007) and from previous publications on metals detected in Nigerian herbal medicine (Ajasa et al., 2004 and Orisakwe et al., 2006). The British and European Pharmacopoeias (MHRA, 2013; EDQM, 2013) state the minimum metals that should be analysed in all herbal remedies to include cadmium, mercury and lead.

Hence the following metals were selected to be analysed in HM in this study; arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni), selenium (Se) and zinc (Zn). Scandium, a rare earth isotope, was used as an internal standard as recommended by EPA (EPA, 2013). Scandium has excellent recovery on ICP and no spectral interference with the selected metals in this study as reported in a previous publication (Chiweshe et al., 2016), therefore it meets the criteria for an internal standard.

3.4 Selection of GC-MS method

The identification and quantification of pharmaceutical compounds from HM samples were carried out using GC-MS. This is a well-used technique in HM analysis for pharmaceutical compounds and it possesses various advantages, as discussed in Section 1.7. The volatility and thermal stability of target analytes are the major determining factors for GC analysis (Rocha, Amaral and Oliveira, 2016), which is a major consideration in this study. In addition, the product analysis of GC-MS chemical reactions (such as products of hydrolysis) is found

to be useful and further helps to elucidate and identify structures (Patel et al., 2014; Vaclavik, Krynitsky and Rader, 2014a and 2014b).

3.4.1 Selection of internal standard

An internal standard (IS) is essential for GC-MS analysis. It is a reference compound used to establish the consistency of the standard instrumental efficiency of individual sample analysis and to confirm consistency and reproducibility of samples (Carlin and Dean, 2013). A good internal standard must possess certain qualities such as an ability to elute near peaks of the target compounds without overlapping them. In addition, a good IS should be stable and have similar chemical properties to those of the target compounds without any interference or reaction with their analysis (McNair and Miller, 2009). These characteristics were considered when deciding on a suitable internal standard for GC-MS analysis in this study (fluoxetine–d_5).

3.4.2 Selection of extraction technique for herbal medicine analysis

The isolation of pharmaceutical compounds from HM samples required a general extraction protocol that enabled the simultaneous detection of compounds with varying chemical characteristics (Table 3.1) and solubility.

The acidity or basicity of the pharmaceutical compound to be extracted is often considered, as shown in Table 3.1. Chloroform and ammonium hydroxide have been used in a previous study for the simultaneous extraction of basic pharmaceutical compounds from HM (Au et al., 2000). Conversely, a single extraction method with methanol and/or ethanol has been used for the extraction of both acidic and basic pharmaceutical compounds similar to those of this study in HM samples (Liu, Woo and Koh, 2001; Lau et al., 2003; Kim et al., 2014). However, methanol was used in this study following results from a prior solubility study of target pharmaceutical compounds (Section 3.7).

3.5 Selection of ICP-OES method

The identification and quantification of heavy metals from HM samples were carried out using ICP-OES due to its availability and suitability to the research, as discussed in Section 1.7.

3.5.1 Selection of digestion method for herbal medicine analysis

Dry ashing and open vessel digestion methods were used in this study due to their availability and suitability to the research. The sample preparation methods have been discussed previously in Section 1.7.

3.6 Materials and method

All the porcelain crucible, tongs, spatula and glassware was cleaned by soaking in aqua regia overnight for 12 hours and rinsed with ultrapure water before use for ICP-OES analysis.

3.6.1 Chemicals and reagents

Technical grade acetaminophen, caffeine, chlorpheniramine maleate, codeine, dexamethasone, diazepam, diclofenac sodium, ibuprofen sodium, tramadol hydrochloride (all powder), fluoxetine hydrochloride and fluoxetine- d_5 (liquid), and HPLC grade methanol were purchased from Sigma-Aldrich, Haverhill, UK. This is in addition to a 65% v/v (high-purity trace metal content) nitric acid and 37% v/v hydrochloric acid (trace metal grade) which were used for sample digestion and trace metals recovery. Ashing agent 99.99% magnesium nitrate hexahydrate (trace metals grade) was also purchased from Sigma-Aldrich, Haverhill, UK. Multi-elemental stock solutions of 100 ppm ICP standard As, Cd, Cr, Cu, Hg, Pb, Se, Mn, Ni and Zn were obtained from SPEXertificate® (Stanmore, Middlesex, United Kingdom). A certified reference material (CRM) IAEA-359 and 1000 ppm of scandium purchased from Sigma Aldrich UK was used for recovery studies and internal standard respectively. Ultrapure water (resistivity 18.0 MΩ) obtained by an Elga purelab option s7 system (Elga lab water, Wycombe United Kingdom) was used for all dilutions and infusions. Preparation of calibration standards was performed by dilution of reference multi-elemental standard solution in deionised water.

3.6.2 GC-MS method

The solubility of pure pharmaceutical standards at 1 mg/ml (0.8 mg/ml highest concentration used in this research) in various organic solvents (chloroform, ethyl acetate, acetone and methanol) and ammonium hydroxide was evaluated prior to further analysis.

3.6.2.1 Calibration standard preparation

Individual standards were analysed, followed by a mixed standard in the presence of the internal standard (fluoxetine-d_5) to obtain each compound's retention time and mass

spectrum for possible identification. To detect and quantify possible pharmaceuticals in samples of herbal medicine in this research a 14-point calibration was developed for the target pharmaceutical compounds, except for acetaminophen and dexamethasone, which had 10- point calibration. Standards ranging from 0.002 to 0.8 mg/ml (0.01 to 0.8 mg/ml for dexamethasone and acetaminophen) were prepared in methanol following solubility results in Section 3.7. The 10-point calibration for acetaminophen and dexamethasone was due to non-detection of peaks below 0.01 mg/ml, as compared with others at 0.002 mg/ml from preliminary studies. The calibration standards were made by addition of a known volume of prepared standard at 2 mg/ml starting concentration in methanol, an internal standard solution (fluoxetine–d_5) at 0.5 mg/ml starting concentration and made up to 1 ml for the final concentrations and subsequent analysis. The concentration of internal standard used was 0.25 mg/ml at each sample injection, which is within the sample concentration range.

3.6.2.2 Sample preparation

Extraction was performed by weighing out 200 mg of HM samples, thoroughly homogenised and extracted using 1 ml of methanol with internal standard (0.25 mg/ml fluoxetine-d_5). The mixture was vortexed for 1 minute and ultra-sonicated for 30 minutes. The mixture was then centrifuged at 4000 rpm for 5 minutes and the resultant supernatant was collected and filtered using 0.2 µm membrane filters. The solution was made up to 2 ml with addition of methanol and transferred into GC-MS glass vials for analysis. This step was repeated for all HM samples studied. All analysis was conducted in triplicate unless otherwise specified.

3.6.2.3 GC-MS parameters

A PerkinElmer Clarus 500 GC-MS was used for instrumental analysis. An Equity-5 column was used, which is made up of a cross-linked poly 5% diphenyl/95% dimethylsiloxane stationary phase, with 30 m length by 0.25 mm internal diameter and a 0.25 µm film thickness.

The GC-MS parameters used to analyse the pharmaceutical standard and HM samples are shown in Table 3.2. This instrumental method was adapted from two previous publications on the analytical screening of Chinese proprietary medicine and herbal weight loss supplements for undeclared therapeutic substances and pharmaceuticals (Liu, woo and Koo, 2001 and Khazan et al., 2014). The method was subsequently validated according to the ICH guidelines.

Table 3.2: Adapted GC-MS method

Parameters	Method used in this study
Instrument	PerkinElmer Clarus 500 GC-MS
Capillary Column	Equity-5ms ; 30 m Length, 0.25 mm diameter,0.25 µm film
Initial oven temperature	80°C
Initial temperature hold time	1 min
Ramp rate	15°C/ min
Final oven temperature	300°C
Final temperature hold time	5 mins
Total running time	26 mins
Injection port temperature	250°C
Transfer line temperature	300°C
Injection volume	1 µL
Split/Splitless mode	Splitless
Carrier gas	Helium
Carrier gas flow rate	1 mL/min
Mass spectrophotometer	
Ionization mode	Electron Impact (EI)
Ionization energy	70 eV
Solvent delay	2 mins
Scan range	40 – 350 amu

The samples were run in scan mode and also in selected ion monitoring (SIM Mode) using three ions shown in Table 3.4. Data were obtained using the compatible software TurboMassTM (PerkinElmer, 2006), and analysed using Microsoft excel package 2013.

3.6.3 ICP-OES method

3.6.3.1 Calibration standard preparation

The multi-element standard (As, Cd, Cr, Cu, Hg, Pb, Se, Mn, Ni and Zn) was prepared in 5% nitric acid at 100 ppm stock solution by the manufacturer. A working solution of 10 ppm was made and subsequent concentrations were made in deionised water. The internal standard was prepared in 5% nitric acid at 1000 ppm stock solution by the manufacturer. A working solution of 10 ppm was made in deionised water and a final concentration of 0.1 ppm was used throughout this study as the internal standard. To detect and quantify the chosen heavy metals in herbal medicine samples in this study a 12-point calibration was prepared for the target heavy metals ranging between 0.001and 5 ppm.

3.6.3.2 Sample preparation

Herbal medicine samples were thoroughly homogenised and subsequently analysed using dry ashing and wet digestion method, as described below.

3.6.3.2.1 Dry ashing

The modified method of Pytlakowska et al (2012) was adopted. Firstly a 1 mg/ml solution of Magnesium nitrate hexahydrate ($Mg(NO_3)_2$) ashing agent was prepared in deionised water, boiled and filtered (Narwal, Dhankhar and Sangwan, 2012). Afterwards, 1 g of the respective HM samples were weighed out into a porcelain crucible and 1.0 ml of $Mg(NO_3)_2$ was added. The mixture was swirled gently and evaporated on the hot plate at about 180^0 C. The crucible containing the sample was placed in the cold muffle furnace. The furnace temperature was gradually increased to 350 ^{0}C in an hour and then maintained over another 9 hours. The resulting ash was then dissolved in a 10 ml hot 20% HCl solution v/v and filtered through a Whatman filter paper. The solution was then brought to volume (25 ml) with deionised water. Blanks were prepared in the same way as the samples. Three replicates were prepared and analysed for each sample. The blank digests were similarly processed.

3.6.3.2.2 Open vessel wet digestion

According to a modified method of David (2000) and Orisakwe et al (2006), 1 g of each sample was carefully weighed and 15.0 ml of aqua-regia (1:3, HNO_3: HCl) was added to the sample for digestion in a beaker. The beaker was then heated on a hot plate at 150^oC in the fume cupboard for 2 hours. Afterwards the beaker was cooled then filtration and rinsing followed. The filtrate was then made up to 25 ml with deionised water for analysis. Samples were analysed in triplicate and blanks were prepared in the same way and analysed.

3.6.3.3 ICP-OES parameters

ICP-OES instrumental parameters are shown in Table 3.3, using an axial viewing Vista-MPX™ (Varian, UK). The analytical signal was measured by triplicate analysis and a two-point background correction. To prevent any possible instrumental memory effect wash time with deionised water was set at 120 seconds to ensure complete washout between samples analysis. High-purity argon (99.995%) (Supplied by BOC Cambridge, UK) was used as the carrier gas and to maintain the plasma.

Table 3.3: ICP-OES instrument parameters

ICP-OES instrument	
Instrument Name	Vista-MPX™
Company	Varian Analytical Instrument
Software	ICP-Expert
Optical system	Echelle Polychromator
Wavelength range	175-785 nm
RF generator	40 MHz
Detector	Megapixel charged-coupled device detector
Plasma view	Axial
Nebuliser type	Concentric
Sample uptake method	Peristaltic pump
Peristaltic pump tube and diameter	White/white 1.02 mm
Optical system resolving power	Normal
Plasma torch	Quartz, fixed, 3.0 mm injector tube
Parameters	Values
Rf power	1200 watts
Nebuliser gas flow rate	1 L/min
Auxiliary gas flow rate	1.5 L/min
Sample introduction rate	15 rpm

3.6.4 Method validation for GC-MS and ICP-OES

3.6.4.1 Selectivity

For GC-MS analysis selectivity of the method was assessed by monitoring the blanks for any interference and interpreting the mass spectra of the analysed standards in scan mode to distinguish between the co-eluting compounds, and then selected ion monitoring (SIM) mode to ensure a selective and sensitive method. During analysis methanol blanks were analysed before, during and at the end of each sample.

Assessment of selectivity for ICP-OES analysis was made in combination with an accuracy study (Section 3.8.4) to check for possible spectral interference using a multi-elemental mixed standard at a concentration of 0.2 ppm and matrix interference using a spiked CRM standard. To choose the optimal analytical wavelengths three wavelengths (nm) were measured for each target heavy metal. The three wavelengths were selected by visual examination based on non-overlapping peaks and non-spectral interference. The wavelengths that met these criteria and had the highest intensity were selected. The validity of the wavelength selection for non-spectral interference was further examined with the RSD% of the recovery (20% accepted criterion) (Peter, Drummer and Musshoff, 2007) at the

3 wavelengths. One wavelength was then selected for each target heavy meal for a further validation study by the RSD% values and visual examination of spectra.

3.6.4.2 GC-MS autosampler stability and repeatability study

Repeatability, precision and autosampler stability of the studied analyte were tested at three concentrations (low, middle and high concentrations) 0.008, 0.08 and 0.8 mg/ml, with the exception of acetaminophen and dexamethasone where the lowest concentration was 0.01 mg/ml. Each concentration was investigated in triplicate and repeated 11 times. The ratio of the analyte response to the internal standard response known as the Peak Area Ratio (PAR) and its standard deviation (n=33) were documented over a 50 hours period. The various RSDs were calculated and examined within the acceptable range to assess the stability of the analytes. A similar method was used to assess the auto-sampler stability of the internal standard.

3.6.4.3 Linearity and linear range

The linearity of the GC-MS method was determined by analysis of 14 concentrations of the mixed drug standard and 10 concentrations for dexamethasone and acetaminophen in triplicate injections, as stated in Section 3.6.2.1.

The linearity of the ICP-OES method was evaluated by preparing multi-element standard solutions (as described in Section 3.6.3.1) at 12 concentrations in the presence of a 0.1 ppm internal standard (scandium). A linear plot of the instrument response (ratio of intensity of analyte and that of the internal standard for ICP-OES and ratio of peak area of analyte and that of internal standard for GC-MS) against the concentration was obtained. A linear regression line of best fit was obtained from least squares and the linear regression equation was calculated (Ellison, Barwick and Farrant, 2009; SWGTOX, 2013).

SPSS was used to conduct a Shapiro-Wilk statistical test of normality of the residual and runs test for randomness, with a given *p*-value. For Shapiro-Wilk the null hypothesis is retained when the *p*-value is greater than the chosen 0.05 significant value, meaning a normally distributed residual. This is similar to runs test where a *p*-value greater than the chosen 0.05 significant level also retains the null hypothesis; indicating randomly distributed data (Peters, Drummer and Musshoff, 2007).

3.6.4.4 Limit of detection (LOD) and limit of quantification (LOQ)

As described in Section 3.1.3, using the calibration results (Sections 3.6.2.1 and 3.6.3.1), the standard deviation of the y-intercepts was calculated from the calibration graph of the 3

replicates data using Microsoft Excel, while the slope was also obtained from the calibration graph.

3.6.4.5 Matrix effect

Two sets of samples were prepared for determination of the matrix effect in GC-MS at concentrations used for calibration (see Section 3.6.3.1). The first samples were prepared using a neat mixed standard in methanol solution and the second set of samples was prepared by spiking an extracted HM sample (HM Sample 9 was used) with mixed standard at the same concentrations as the neat mixed standard solution in methanol. All samples were subsequently analysed using the GC-MS method (as shown in Table 3.2). The matrix effects which are expressed as matrix factor (MF) were calculated by comparison of the target analytes' mean peak area ratio (MPAR) in post-extraction spiked samples with the mean peak area ratio (MPAR) of the target analyte in the standard solution (Silvestro, Tarcomnicu and Savu, 2013) as seen in (Equation 3.3).

Equation 3.3:

Matrix Factor (MF) = MPAR in presence of matrix ions/MPAR in the neat solution

The result obtained is interpreted as:
MF=1 shows no matrix effect
MF<1 shows ion suppression
MF>1 shows ion enhancement
The matrix effect in ICP-OES is carried out along with recovery and accuracy studies using CRM.

3.6.4.6 Recovery and accuracy

Recovery studies were used to assess selectivity, accuracy, precision, repeatability and matrix effect. A pretested HM (HM6) sample which had none of the analyste was spiked with target compounds (Table 3.1) at three concentrations 0.008 mg/ml (0.01 mg/ml for acetaminophen and dexamethasone), 0.08 mg/ml and 0.8 mg/ml as for the stability study (see Section 3.6.4.2). The spiking is performed with a mixed standard, where the ratio of the standards' concentration relates to their unspiked sample's concentrations, as shown in Equation 3.4. Samples were then prepared using the method described in Section 3.6.2.2 and subsequently analysed. Analyses were performed within a 12-hour period and repeated three times for the intra-day repeatability study in GC-MS analysis. Spiked and unspiked levels are established with replicates (n = 9) for recovery study. The standard calibration was used to calculate the amount recovered from the initial concentration added. This was done

using the formula as expressed in Equation 3.4 (Matuszewski, Constanzer and Chavez-Eng, 2003).

Equation 3.4:

Recovery = $C_0 – (C_1/C_2)$ X 100

C_0= Concentration of target compound in unspiked sample

C_1= Concentration of recovered target compound from spiked sample

C_2= concentration of target compound added to spiked sample

The recovery study for ICP-OES was carried out using a CRM by spiking it prior to sample preparation with the multi-elemental standard at low, intermediate and high concentration (0.002, 0.2 and 2 ppm respectively). The spiked CRM was then prepared in the same way as the other samples, as described in Section 3.6.3.2. The concentration of metals in the CRM is shown in the certificate (Appendix XVIII). Results were calculated using Equation 3.4 and reported on a dry weight basis as mean ± standard deviation (SD) (see Table 3.19).

For both GC-MS and ICP-OES accuracy was assessed by percentage recovery and precision by the relative standard deviation (%RSD). According to the United States Pharmacopeia Convention (2012), a precision level of less than 20% is acceptable, while 70-150% recovery is acceptable for accuracy studies.

3.7 Result and discussion of GC-MS method validation

Prior solubility evaluation showed pharmaceutical standards were completely soluble in methanol at 1 mg/ml. Thus the extraction solvent used in this study was methanol. Several studies have also used methanol for the extraction of pharmaceutical compounds from herbal medicine (Pang et al., 2009; Bogusz et al., 2006; Balayssac et al., 2009).

3.7.1 Identification of compound

The authentication of the identity of a sample is examined in several ways through the use of a chromatogram and mass spectrum. A chromatogram is collected from a standard such as the chromatogram shown in Figure 3.1 and the peak retention time is compared with sample retention time or relative retention time. For mass spectrometry the fragmentation pattern and the relative abundance of selected ions are specific to individual molecules; hence it provides a valid identity for the compound. For unknown samples, library databases exist, such as NIST (NIST, 2006), which can be used for tentative identification. These databases compare mass spectra of known samples with the unknown sample to provide an initial identification. Confirmation can then be obtained using the presumed known standard.

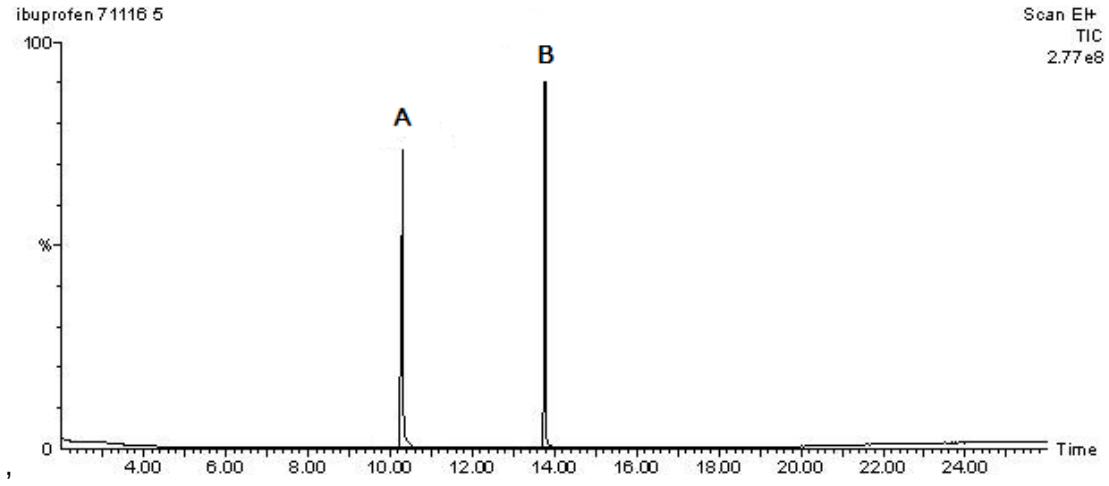


Figure 3.1: Chromatogram of ibuprofen (0.5 mg/ml) and internal standard fluoxetine-d_5 (0.25 mg/ml)

The chromatogram above (Figure 3.1) indicates two peaks; peak A which is of a known standard (ibuprofen) was documented at 10.26 minutes while peak B at 13.87 minutes was the internal standard fluoxetine-d_5.

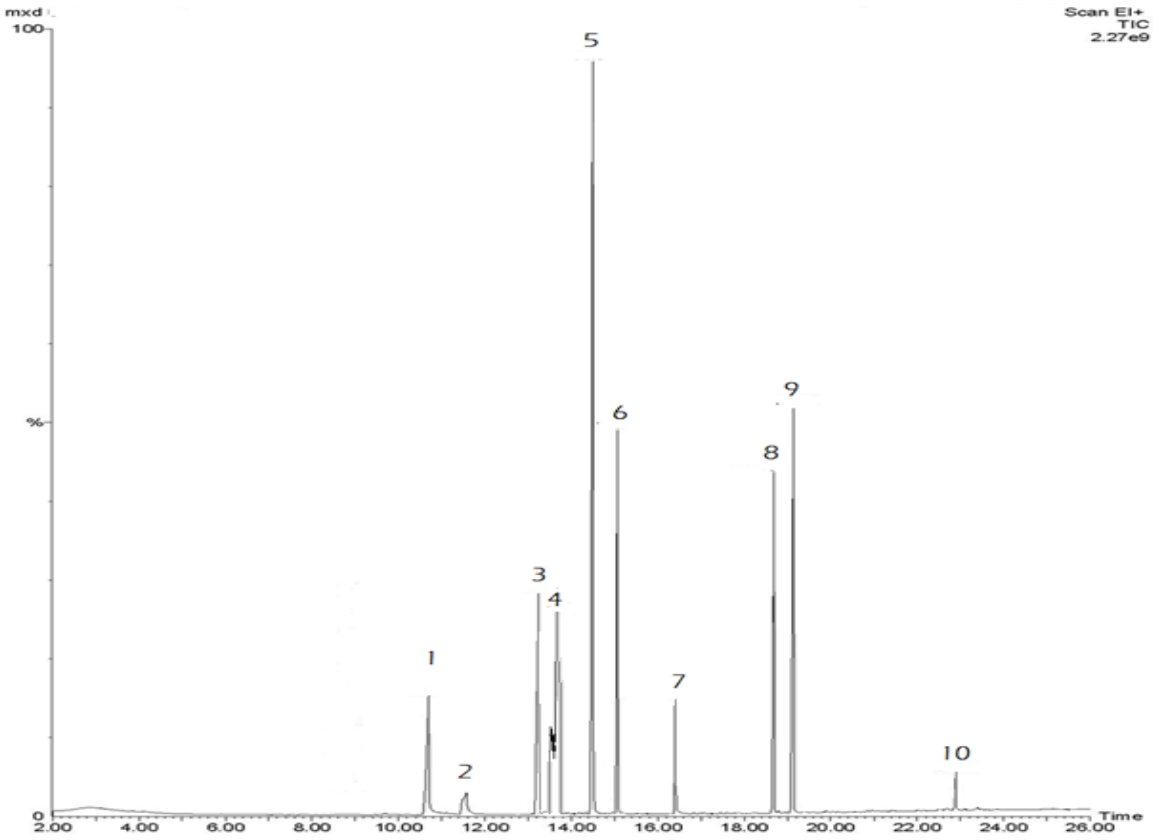


Figure 3.2: Chromatogram of mixed standard (0.5 mg/ml) (1) ibuprofen, (2) acetaminophen, (3) caffeine, (4) fluoxetine+fluoxetine-d_5, (5) tramadol, (6) chlorpheniramine, (7) diclofenac, (8) codeine, (9) diazepam, and (10) dexamethasone.

Figure 3.2 shows an example of a chromatogram where 10 analyte and an internal standard were detected simultaneously for a mixed drug standard. Standards were initially analysed individually to obtain retention time data with the internal standard, before the mixed standard analysis. Two sets of information on an analysed sample can be obtained from the chromatogram. The first is the retention time (RT), which is the first time the peaks were detected. The RT for compounds is obtained by running known standards of the compounds prior to attempts at identifying any unknown samples.

Retention times of all the compounds were recorded, as shown in Table 3.4, alongside the relative retention time (RRT). RRT is an expression of an analyte retention time relative to the internal standard's retention time and is considered a reliable measure for compound identification (EPA, 2017).

Calculation of the standard deviation of the retention time shows there is a little deviation between the RT ranging from ±0.026 to 0.005 minutes across all target compounds. The relative retention time (RRT) of a sample is expected to be within ±0.06 of the RRT of the standard for valid identification (EPA, 2017).

Table 3.4: Retention times, relative retention times and major ions of target compounds

Analyte	RT[a] (mins)	RRT[b]	Major ions m/z and % abundance				
			Q	C1		C2	
Acetaminophen	11.39 ± 0.026*	0.82	109	151	30%	43	24%
Caffeine	13.24 ± 0.007	0.96	194	109	74%	67	45%
Chlorpheniramine	15.15 ± 0.006	1.09	203	58	64%	274	10%
Codeine	18.81 ± 0.005	1.36	299	162	55%	115	32%
Dexamethasone	22.82 ± 0.006*	1.65	122	121	40%	392	5%
Diazepam	19.24 ± 0.005	1.39	256	283	95%	284	72%
Diclofenac	16.47 ± 0.006	1.19	214	242	46%	296	15%
Fluoxetine	13.64 ± 0.005	0.98	44	104	53%	309	10%
Ibuprofen	10.24 ± 0.005	0.74	161	91	75%	206	47%
Tramadol	14.65 ± 0.005	1.06	58	263	38%	59	36%
Fluoxetine-d_5	13.87 ± 0.007	-	43	109	35%	314	10%

[a] Retention Time (mean RT ± SD) (n=42); * (n=30); [b] Relative retention time; Q = quantifying ion (100%), C1 and C2 = confirmation ions

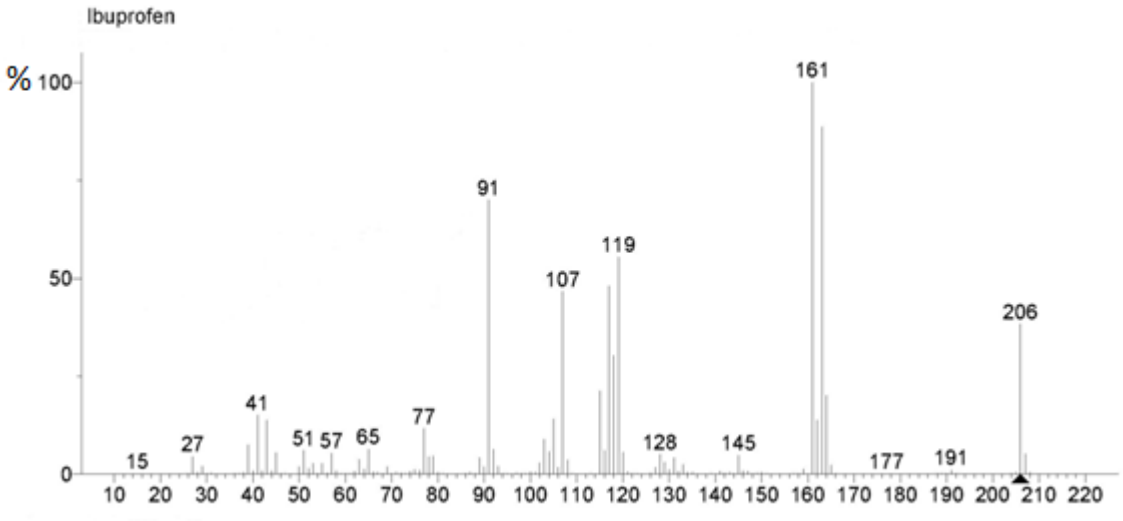


Figure 3.3: Exemplar mass spectrum of ibuprofen standard

The spectrum above (Figure 3.3) shows the obtained fragment ions for the ibuprofen standard in the mixed drug standard. The quantifying ion for ibuprofen was m/z 161 at 100% relative intensity, while the other ions recorded were m/z 91 (confirmation ion) at 74.69% relative intensity and second confirmation ion m/z 206 at 46.72% relative intensity (Table 3.4). In addition, compounds can be quantified by employing the mass spectrum; using the abundance ratio/peak area of the base ion of unknown sample to the internal standard against various standard concentrations (Fiehn, 2016).

3.7.2 Selectivity

During analysis of methanol blanks before, during and at the end of each analyte run no interference at the retention times of the analysed compounds was observed. Figure 3.4 shows a mid-run methanol blank.

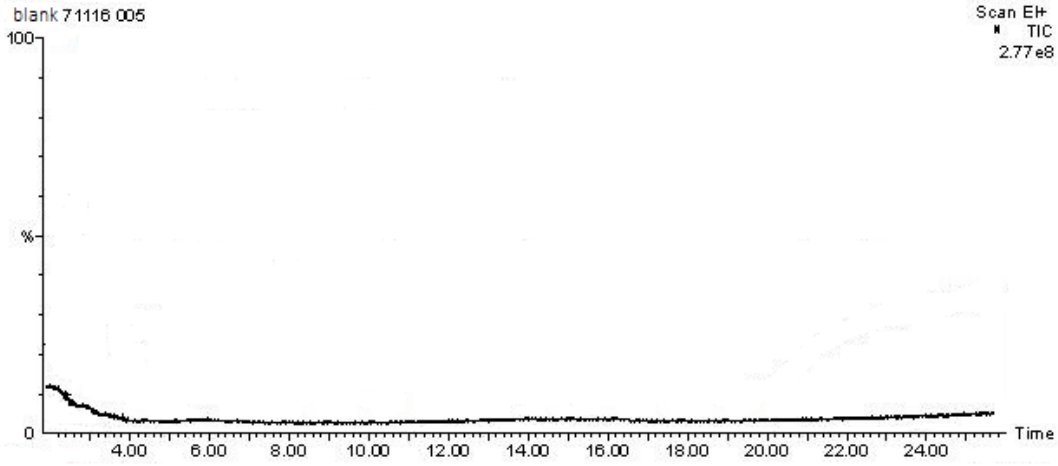


Figure 3.4: Mid-run methanol blank

Acetaminophen, caffeine, chlorpheniramine, codeine, dexamethasone, diazepam, diclofenac, ibuprofen, tramadol and fluoxetine-d_5 were all resolved in scan mode (see Figure 3.2) while fluoxetine and fluoxetine-d_5 exhibited co-elution at 13.70 ± 0.14 minutes, which is expected because the latter is the isotopically labelled form of fluoxetine culminating in structures that are chemically alike, with a 5 atomic mass units difference, thus matching retention times on the chromatogram. With different molecular and confirmation ions, extracted ion chromatogram (EIC) was employed for data analysis (see Figure 3.5) to distinguish the two compounds.

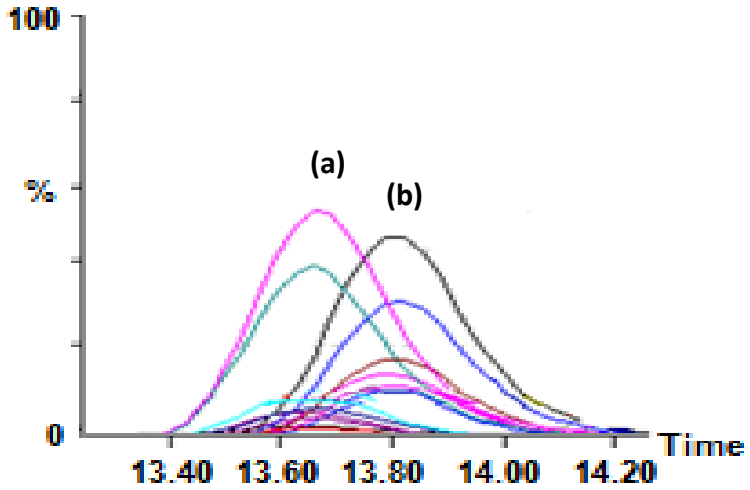


Figure 3.5: EIC of fluoxetine (a) and fluoxetine–d_5 (b) (0.25 mg/ml)

Scanning for target ions only allows SIM to differentiate between co-eluting compounds, which makes it more selective. By implementing the selected ions m/z 44, 104 and 309 for fluoxetine and m/z 43, 109 and 314 for fluoxetine-d_5 the compounds were separately identified. Hence, as a result of the selectivity of the analytical method in SIM and scan mode, method results were obtained from analysis using SIM.

3.7.3 Internal standard stability

The stability of the internal standard fluoxetine-d_5 (0.25 mg/ml) in a mixed standard over 50-hour duration was investigated using a simultaneous detection method in selected ion monitoring mode (selected ions in Table 3.4). Over the 50 hours the IS (0.25 mg/ml) was stable in the autosampler; with fluctuations less than 10% RSD in most hours, as indicated by the standard deviation of error bars at respective hour (see Figure 3.6). These deviations are within the recommended ±10% RSD for most of the studied time (Peters, Drummer and Musshoff, 2007) and those that are outside the recommended RSD (i.e 15, 30 and 40 hours)

are random throughout the runs. In Figure 3.6 the horizontal lines indicate the 15% RSD and the mean relative response factor (Rc).

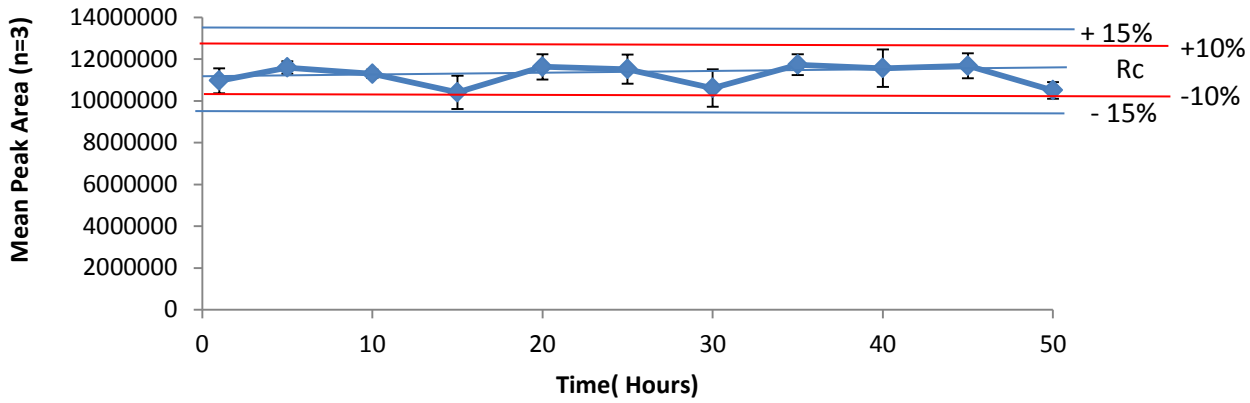
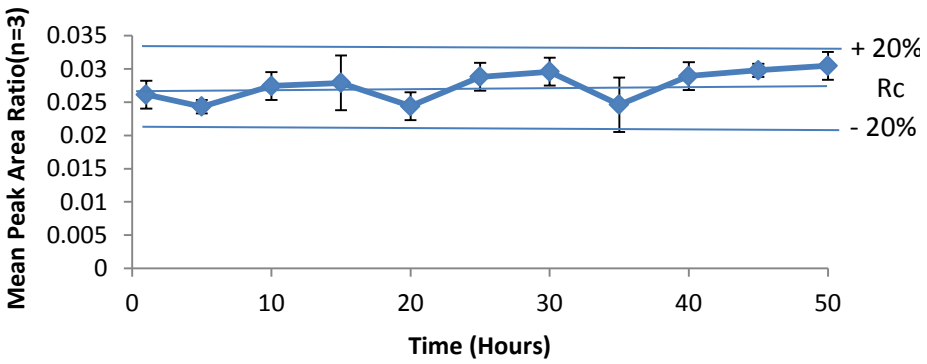


Figure 3.6: 50-hour stability results for internal standard fluoxetine-d_5 (mg/ml) mean peak area

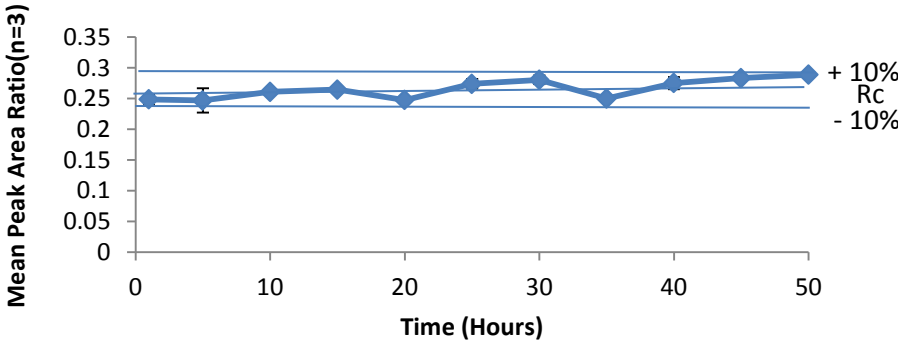
As shown in Figure 3.6, although responses at 15 and 30 hours seem to fall outside 10% but within 15% RSD, these may be a result of fluctuation in instrument reading, as the other nine points were within 10% RSD. The mean concentration at each level is expected to be within ±15% of the nominal concentration (EMA, 2012). Therefore the plot presented above in Figure 3.6 shows that the internal standard is stable across 50-hour storage (directly on the autosampler), which covers the analytical run used in this research. This result shows its suitability and selection, as discussed earlier in Section 3.4.1.

3.7.4 Autosampler stability of target compounds

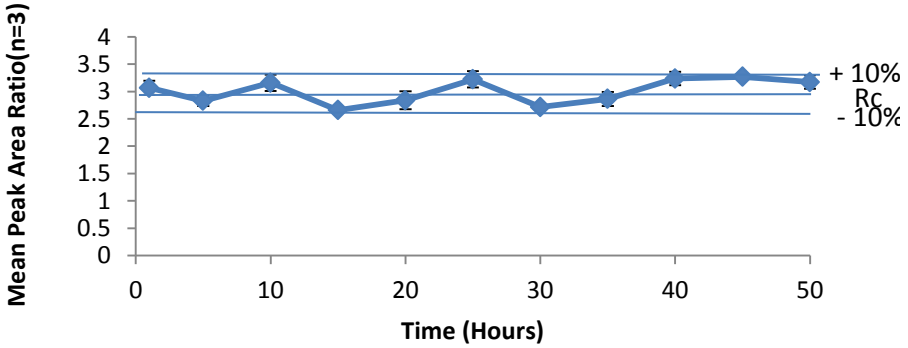
The 50 hours autosampler stability study for all the target compounds can be found in Appendices II, III and IV; however, an example is shown in Figure 3.7, which shows a graphical representation of the stability study of ibuprofen at 0.008, 0.08 and 0.8 mg/ml. As decreasing concentration is analysed, the peak areas and the resulting ratio also decrease, resulting in increased errors and larger variance, as seen in the stability study of the compounds (Saar et al., 2012).



(a) Stability study of ibuprofen at low concentration (0.008 mg/ml)



(b) Stability study of ibuprofen at mid concentration (0.08 mg/ml)



(c) Stability study of ibuprofen at high concentration (0.8 mg/ml)

Figure 3.7: Stability plot of mean PAR (n=3) over 50 hours for ibuprofen at different concentrations. Horizontal lines indicate the acceptance criteria of mean ±20% and ±10% respectively.

All compounds have points within the acceptable 10% RSD at the lowest concentration, with the exception of ibuprofen (Figure 3.7), caffeine, codeine, acetaminophen, tramadol and dexamethasone which were within ±20% RSD (Appendix II). An RSD of 20% is accepted, especially at the lowest concentration (Peters, Drummer and Musshoff, 2007). The higher deviation may be explained considering the LOD for ibuprofen is 0.007 mg/ml (Table 3.8) which is close to the minimum concentration (0.008 mg/ml) used in the stability study. The lowest concentration is, however, within the linear range (Table 3.8) for all analytes. Nonetheless, at the mid-range concentration (0.08 mg/ml) and high concentration (0.8 mg/ml) all RSD were within ±10% RSD, as shown for example in Figure 3.7 for ibuprofen. Overall all compounds are within the recommended RSD at various concentrations (Appendix II, III, and IV) and thus stable over the 50-hour period.

3.7.5 Linearity and linear range

As shown in Figure 3.8 for ibuprofen, other compounds have also resulted in a linear relationship with an increase in concentration resulting in an increase in analytical response (Appendix I). As expected it was, however, apparent that some compounds did not follow a linear trend over the whole concentration range (0.002 mg/ml to 0.8 mg/ml), as in the examples of caffeine, ibuprofen and acetaminophen (refer to Appendix I). There are points that will tail off and plateau expectedly at very high and very low concentration with non-linear instrumental response (Peters, Drummer and Musshoff, 2007). These concentration points are considered to be beyond the compound linear range.

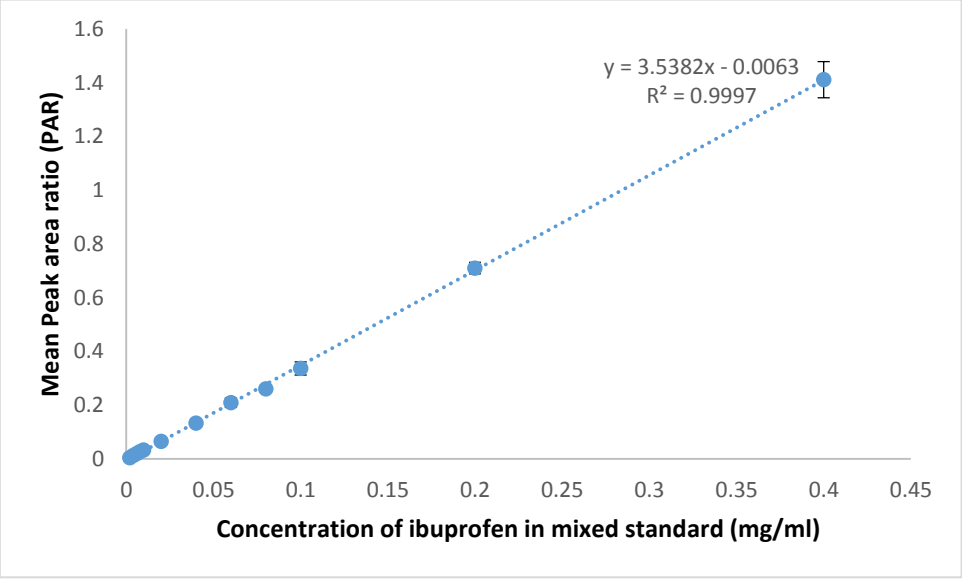


Figure 3.8: Linearity plot of ibuprofen; mean peak area ratio against concentration 0.006 mg/ml to 0.4 mg/ml (n=3)

Correlation coefficients (R^2) were obtained from linear plots (refer to Table 3.5). The correlation coefficient ranges from 0.0 to 1.0, measuring the extent of the linear relationship between x and y residuals. The larger the R^2 value, the closer the data points' correlation. An R^2 value of 1 represents a tentative perfect fit, which needs further assessment, as discussed subsequently in this section. Furthermore, an R^2 value of 0.99 would be needed for a 10% relative uncertainty in the linear range (Ellison, Barwick and Farrant, 2009). Compared with the R^2 data obtained from the linearity study, all conform to 10% relative uncertainties. It is, however, paramount to note in general that R^2 cannot be used as an evaluation of linearity. It is only used to show good linearity when data are evenly distributed and without anomaly (SWGTOX, 2013).

Table 3.5: Mean calibration equation and R^2 values at 0.002 mg/ml to 0.8 mg/ml

Analyte	Regression equation	R^2
Acetaminophen*	y = 1.0032x - 0.0088	0.9948
Caffeine	y = 3.0128x - 0.0234	0.9959
Chlorpheniramine	y = 6.6113x + 0.0031	0.9976
Codeine	y = 8.4886x + 0.0254	0.9978
Dexamethasone*	y = 3.7532x - 0.008	0.9993
Diazepam	y = 3.331x - 0.0168	0.9964
Diclofenac	y = 2.6065x - 0.0164	0.9960
Fluoxetine	y = 9.4254x - 0.0263	0.9975
Ibuprofen	y = 4.3082x - 0.0542	0.9893
Tramadol	y = 3.6051x - 0.0126	0.9971

*Concentration at 0.01 to 0.8 mg/ml

Evaluation of linearity data was further carried out by a graph of the mean peak area ratio of each compound divided by their corresponding concentration against the log of the concentrations (Huber, 2007). If acceptable linearity is attained, the ensuing plot is then expected to be within ±10% of the mean relative response factor. The mean relative response factor (Rc) in this case was the mean peak area ratio of the corresponding compound. There is a possibility of predicting the linear range as the method is considered linear untill the relative response drops outside the accepted 10% RSD. Figure 3.9 shows the plot for ibuprofen where data points outside the linear range were seen at the lowest and highest concentrations.

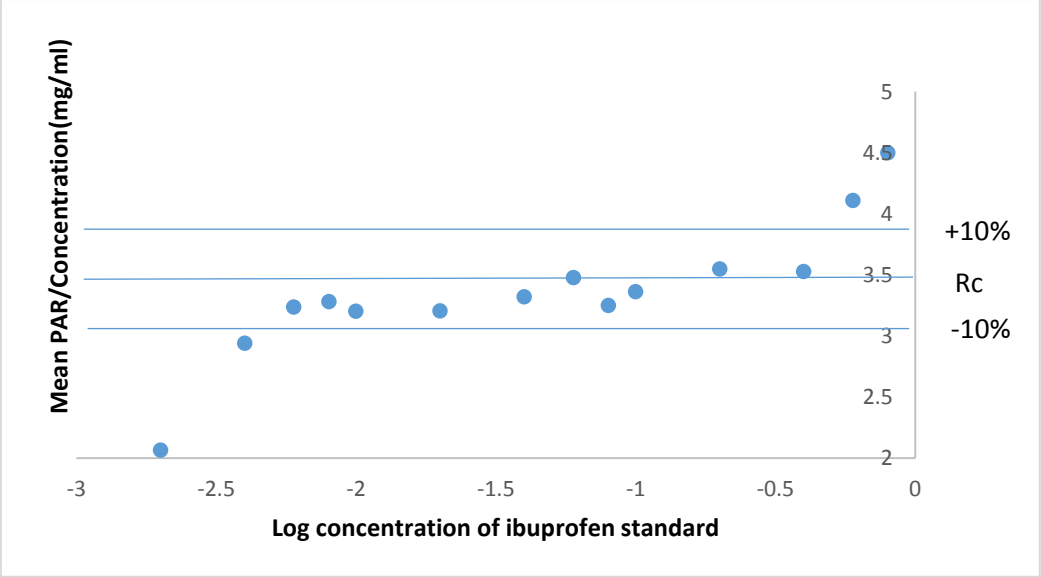


Figure 3.9: Peak area ratio of ibuprofen/concentration versus log concentration

These data points move the regression line, leading to a change in the intercept with an ensuing correlation coefficient farther away from the accepted 0.99, as was the case with other target compounds, as seen in Appendix V.

The data points for ibuprofen shown in Figure 3.9 are spread around the mean, while the non-linear data points at the higher end of the concentration range are outside expected parameters, thus showing a loss of linearity. Upon examination of the log graphs (Appendix V), linear graphs were plotted for a predicted linear range. Results that showed outliers and "tailing off" and "plateauing" responses at the higher and lower concentrations were removed until the data points fell within the accepted RSD limits. In the case of ibuprofen, the linear range was 0.006 to 0.4 mg/ml. As previously mentioned, log plots should not be used in isolation in making a judgment of linearity and linear range.

To check the predictions the residual can be useful in identifying problems through a poorly fitting curve (Ellison, Barwick and Farrant, 2009). For a good data fit the residuals are supposed to be scattered randomly around zero. A problem is suspected in the data when there is a deviation from this pattern of distribution. As an example, a curved pattern of distribution may point to the line of best fit's being forced through data points with a non-linear course (*ibid*).

On analysis of the residuals tailing and plateauing were apparent at lower and upper concentrations. This caused a non-random distribution of the residual around zero and thus pointed to a loss of linearity. As with the log graphs, on removing the non-linear data points,

residual plots were obtained. Figure 3.10 shows for ibuprofen the random distribution of residuals around zero in a linear range 0.006 to 0.4 mg/ml.

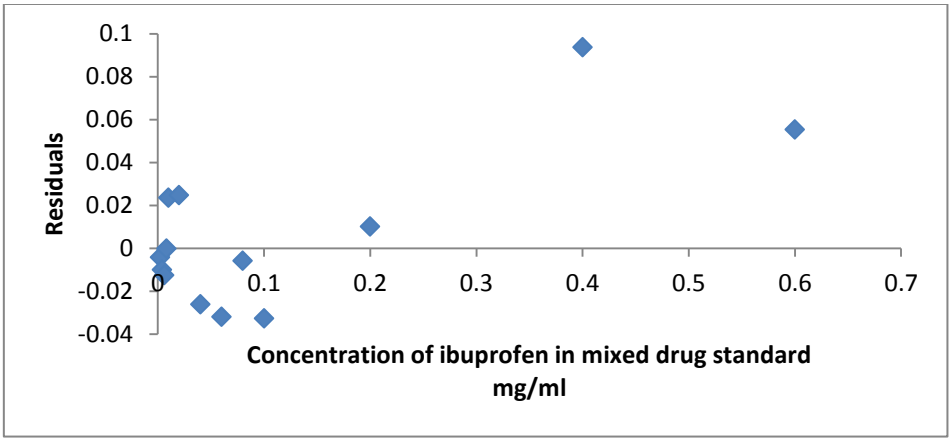


Figure 3.10: Residual plot of ibuprofen

On similar analysis all compounds and their corresponding residual analysis (Appendix VI), concentration ranges were evaluated and the linear range obtained is presented in Table 3.8. Correlation coefficients and associated standard deviations show a strong linear correlation between data.

Additional residual plots analysis was carried out to assess the normality of the results using the Shapiro-Wilk test (S-W test) from IBM SPSS Statistics. This analysis is built on the correlation between the data and their normal scores (Peat and Barton, 2005) and gives a capacity preferable to that of the Kolmogorov-Smirnov test (K-S test), regardless of Lilliefors correction (Steinskog, 2007). The Shapiro-Wilk test has been recommended as the foremost alternative for normality testing of data where the sample size is less than 50 (Thode, 2002; Elliott and Woodward, 2007).

The S-W test involves a comparison of predicted analytical response and analytical response, PAR, acquired through the residual analysis (linear range as per Table 3.8). The residuals are then obtained by calculating the difference between the two analytical responses (Miller and Miller, 2007). The statistical test retains a null hypothesis if there is a normal distribution of error. An average, together with the standard deviation and a p-value are generated. *p*-values denote the probability of an observed value greater than or equal to the critical value i.e. 0.05 if the null hypothesis is true, meaning a normal distribution. There are two summations of interpreting these values: if the *p*-value is lower than the significance level of 0.05, the null hypothesis must be rejected, which means a result that is significantly different from non-normal distribution; if the *p*-value is higher than the significance level of 0.05 the hypothesis is retained, as the results are of normal distribution (Bressolle, Bromet-

Petit and Audran, 1996). A 95% confidence limit has been used throughout the statistical analysis of the linearity study; hence the significance level $p \leq 0.05$ was used.

Normality is depicted by Gaussian distribution or the typical bell-shaped curve. Alongside the data produced by the SPSS statistical analysis, graphical presentations of the data analysed in the test are displayed on distribution histograms. The histogram is symmetrical around the mean, thus showing normal distribution.

Skewness, a measure of symmetry or its absence, and kurtosis, a measure of the data being peaked or flat as compared with a normal distribution (NIST, 2011), were recorded for all compounds (see Table 3.6). Caffeine, e.g., has a skewness value of 0.298, which would suggest that the data are skewed slightly to the right as it is a positive as opposed to a negative value that would indicate data are skewed to the left. It is also very close to 0, which indicates symmetry and normal distribution (*ibid*). In kurtosis, a higher value indicates more distinct peaks near the mean, which then decline rapidly resulting in heavy tails. A lower and negative value indicates a flat rather than a sharp peak near the mean. Similarly, value of 0 indicates normal distribution (*ibid)*.

Table 3.6: Results of Shapiro-Wilk test; null hypothesis and corresponding *p*-value

Analyte	Kurtosis	Skewness	*p*-value	Accept the null hypothesis
Acetaminophen	1.263	0.093	0.915	Yes
Caffeine	1.607	0.298	0.058	Yes
Chlorpheniramine	1.630	0.321	0.071	Yes
Codeine	0.612	0.191	0.420	Yes
Dexamethasone	1.451	-0.379	0.097	Yes
Diazepam	0.215	-0.361	0.231	Yes
Diclofenac	0.905	-0.263	0.209	Yes
Fluoxetine	2.447	-0.849	0.141	Yes
Ibuprofen	3.095	-0.736	0.070	Yes
Tramadol	3.204	0.855	0.059	Yes

Employing the S-W test for all compounds, the null hypothesis was retained throughout the linear range as determined by residual statistics, which indicates a normal distribution of error. All *p*-values were above the required 0.05 confidence limit and ranged from 0.058 to 0.915.

After the S-W test, it was important to evaluate the randomness of the errors with the Wald-Wolfowitz runs test (Wapole et al., 2012). A small positive or negative residual from the line of best fit is expected in linearity, which is to occur at random. But then, considering the earlier summation of the tailing off and plateauing at low and high concentrations, forcing the

line of best fit to pass through set of data points on a curve can lead to series of non-random positive and negative residuals, which are technically a series of “runs”. Using the same principle as of S-W test for Runs test, if the errors are normally distributed, the statistical test returns a null hypothesis, a mean, with corresponding standard deviation and a *p*-value. When the *p*-value is less than the significance level of 0.05, the null hypothesis is rejected, indicating a result significantly different from a random distribution. If the *p*-value is higher than the significance level of 0.05, the hypothesis is retained as the results are of random distribution (Epshtein, 2004).

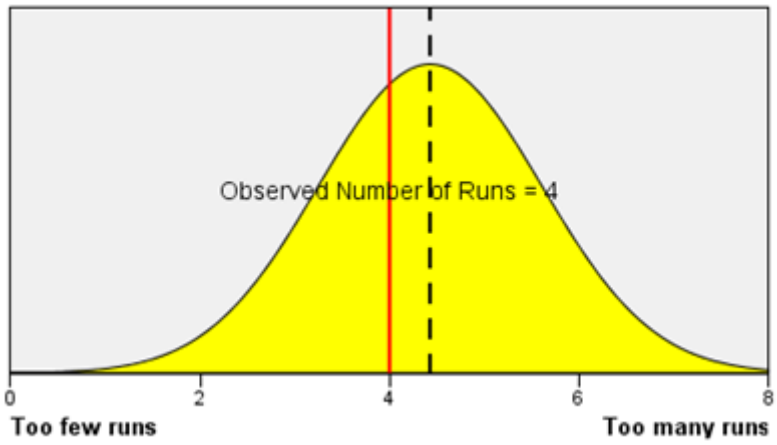


Figure 3.11: Runs Test showing Gaussian distribution curve for ibuprofen data based on residuals

The runs test checks the number of runs and uses the measure of how small it is either to accept or reject the null hypothesis of random distribution at 95% confidence limit (Epshtein, 2004). A sample of the graphical representation returned by the software SPSS statistical package for ibuprofen is shown in Figure 3.11. If the *p*-value is less than the 0.05 confidence interval, then the number of runs is within one of the two tails. The *p*-value will increase as the number of runs nears the centre of peak, thus indicating normal distribution and mutually independent errors. As illustrated in Figure 3.11 with the red line and description across it, the number of observed runs was 4, with a *p*-value of 0.164 (Table 3.7); this means the null hypothesis of normal distribution can be retained.

Table 3.7: Result of Wald-Wolfowitz runs test; null hypothesis and *p*-values

Analyte	*p*-value	Accept the null hypothesis
Acetaminophen	0.094	yes
Caffeine	0.120	yes
Chlorpheniramine	1.000	yes
Codeine	0.056	yes
Dexamethasone	0.429	yes
Diazepam	0.239	yes
Diclofenac	0.132	yes
Fluoxetine	0.131	yes
Ibuprofen	0.164	yes
Tramadol	0.520	yes

Therefore the null hypothesis was retained for all compounds with *p*-values ranging from 0.056 to 1.000 for peak area ratio values, indicating a random distribution of error(s) (Table.3.7).

Therefore the data obtained were linear, normally disturbed and randomly distributed error; hence their suitability for use in this research.

3.7.6 Limits of detection and quantification

To calculate the LOD and LOQ of pharmaceutical compounds which were in salt form (chlorpheniramine maleate, diclofenac sodium, fluoxetine hydrochloride, ibuprofen sodium and tramadol hydrochloride) the calculations below were used. All other analytes were already available as the free base form.

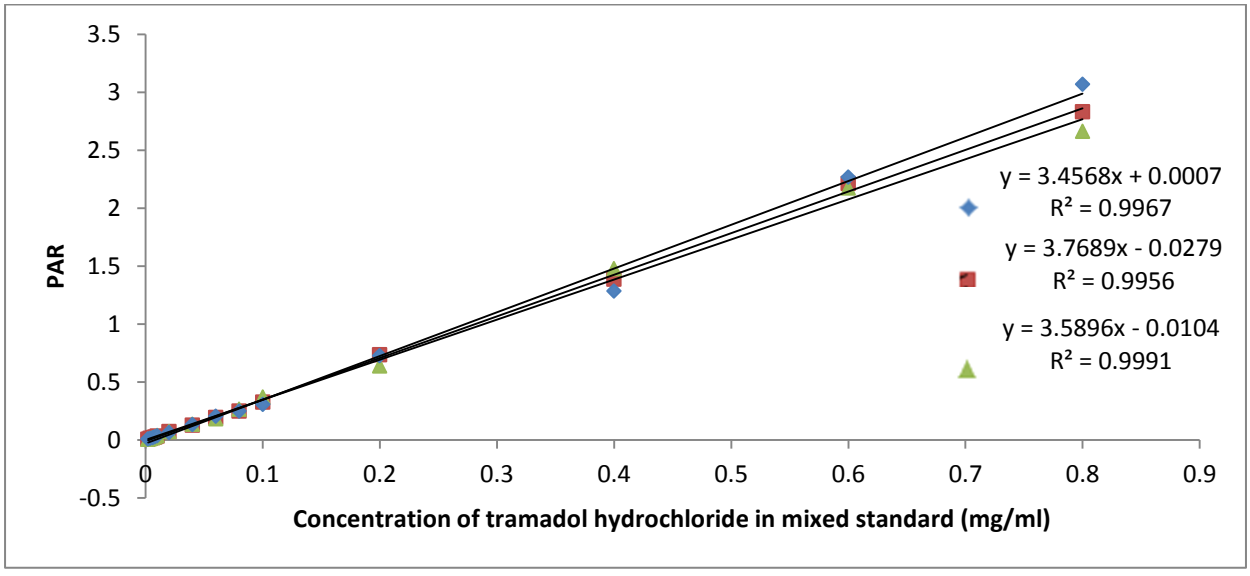


Figure 3.12: Calibration graph for triplicate analysis of tramadol hydrochloride

Calculation 1: According to Equation 3.1, using the standard deviation of the intercept (0.0138) and slope (3.6051) for tramadol hydrochloride in Figure 3.12;

LOD= (3.3 X 0.0138)/ 3.6051 = 0.0126 mg/ml

Calculation 2:- concentration converted to base form from salt form

Molecular weight (MW) of Tramadol = 263.4 (Table 3.1)

MW of Tramadol HCl. = 299.8

263.4 / 299.8 = 0.88

Therefore the free base concentration = LOD concentration x 0.88

0.0126 x 0.88 = 0.0111 mg/ml

Hence the LOQ and LOD of compounds in salt form have been adjusted accordingly, as presented in Table 3.8. All other LOD and LOQ were calculated in similar manner to tramadol hydrochloride.as exemplified in Figure 3.12 and presented in Table 3.8.

The LODs ranged from 0.003 mg/ml for chlorpheniramine to 0.012 mg/ml for codeine, the LOQs from 0.008 mg/ml for chlorpheniramine to 0.036 mg/ml for codeine. By contrast, the LODs in this research are lower than those reported in the literature for ibuprofen, acetaminophen, caffeine and dexamethasone. As shown in Table 3.8, LODs were calculated at 0.007, 0.010, 0.008 and 0.010 mg/ml respectively. These are remarkably lower than those reported at 0.070, 0.020, 0.028 and 0.116 mg/ml (Liu, woo and Koh, 2001; Belal, Awad and Clark, 2009). LODs obtained for chlorpheniramine and diazepam (0.003 and 0.009 mg/ml respectively) are slightly higher than those reported by Liu, Woo and Koh (2001); LODs of 0.002 mg/ml and 0.001 mg/ml respectively.

The LOD for diclofenac in this study (0.011 mg/ml) was higher than that reported in the literature (Liu, woo and Koh, 2001); though the literature employed the use of LC-MS, as was the case for ibuprofen, which had a lower LOD in this study than was reported in the publication. A comparative study of Liquid chromatography-tandem mass spectrometry (LC-MS-MS) versus GC-MS for the analysis and detection of some benzodiazepines from urine reported that LC-MS-MS had lower LOD and LOQ values than GC-MS (Perez et al., 2016). While this research alludes to the finding with the LOD of diclofenac being higher, on the other hand, there may be an indication of enhanced sensitivity of the GC-MS method used in this study for the detection of ibuprofen, considering its lower LOD (Table 3.8).

Table 3.8: LOD and LOQ table of target analyte compared with other GC-MS publications

Analyte	LOQ (mg/ml)	LOD (mg/ml)	Linear range (mg/ml)	LOD in Literature (mg/ml)
Acetaminophen	0.031	0.010	0.02 - 0.6	0.020[1]
Caffeine	0.024	0.008	0.006 - 0.4	0.028[2]
Chlorpheniramine	0.008	0.003	0.002 - 0.8	0.002[2]
Codeine	0.036	0.012	0.006 - 0.8	0.004[2]
Dexamethasone	0.032	0.010	0.01 - 0.8	0.116[2]
Diazepam	0.027	0.009	0.006 - 0.2	0.001[2]
Diclofenac	0.033	0.011	0.004 - 0.6	0.0004[2]
Ibuprofen	0.020	0.007	0.006 - 0.4	0.070[2]
Tramadol	0.034	0.011	0.004 - 0.8	0.00001,[3] 0.006[1]
Fluoxetine	0.012	0.004	0.004 - 0.8	0.0001[4]

[1]Belal, Awad and Clark, 2009; [2] Liu, Woo and Koh 2001; [3]Gambaro et al., 2003; [4]Crifasi , Le and Long, 1997

The LODs, with the exception of acetaminophen, were within the linear range shown in Table 3.8. This asserts the reliability of the calibration curve in quantifying pharmaceutical compounds around the LOD concentration. The LOD of acetaminophen (0.010 mg/ml) was, however, close to the lowest concentration within the linear range (0.02 mg/ml). Hence this may not be a major concern. In addition, it is important to consider that this method is used for the simultaneous detection of these compounds. For multiple compounds, one method may not be quite optimal for all. This is demonstrated in the high LODs for tramadol and fluoxetine compared with those in Table 3.8. This does not, however, affect the method being validated.

3.7.7 Matrix effect

Matrix effect can be expressed in two ways: ion enhancement or ion suppression (Panuwet et al., 2016). According to (Table 3.9), the matrix factors of the HM matrix on the entire target compound were less than one, suggesting ion suppression (see Section 3.6.4.5). This ion suppression of the target compound is likely caused by ions of naturally occurring compounds in the matrix. Unlike ion enhancement, where explanations are available as to why it occurs, the mechanism of ion suppression has not yet been explained. A hypothesis suggests that matrix constituents can compete with or suppress the ionisation potential of the analyte in the gas phase. It also competes with the binding of target ions through opposite electron affinity, causing loss of intensity in electron ionisation (EI) (Panuwet et al., 2016). However, suppression effects caused by co-eluting compounds can be seen in GC with either electron or chemical ionisation (CI). Co-eluting matrix constituents such as aliphatic acids, aldehydes, sterols, phthalates, alcohol and caffeine contribute to such effects

(Yu and Xu, 2012). Some of these compounds are naturally present in most plant products such as HM samples analysed in this research.

Table 3.9: Table showing matrix factor of target compound in spiked HM sample

Analyte	Matrix Factor
Acetaminophen	0.986
Caffeine	0.936
Chlorpheniramine	0.918
Codeine	0.931
Dexamethasone	0.916
Diazepam	0.927
Diclofenac	0.936
Fluoxetine	0.934
Ibuprofen	0.935
Tramadol	0.916

The degree of ion suppression may vary from compound to compound and from sample to sample, and can also be dependent on the sample preparation method (Bonfiglio et al., 1999). Methanol's being the solvent used for sample preparation (Section 3.6.2.2) had some of the naturally occurring compounds in the HM analysed soluble in it (see Section 4.3.1) and may thus have contributed to the ion suppression.

The concentration of the analyte monitored, which relates to the matrix/analyte ratio, may also be responsible for ion suppression (Van Hout et al., 2000). However, use of analyte concentrations which reflects those that will be encountered in real conditions helped to eliminate this possibility. While there are no acceptable levels of matrix effect yet available, the result of the recovery and accuracy study in (Table 3.10) shows that the matrix effect observed does not significantly affect the detection or quantification of the target analyte from HM samples. In addition, use of the isotopically labelled analogue of the target compound as an internal standard is the most efficient way to tackle matrix effects (Colby and McCaman, 1979; Berg and Strand, 2011). In theory, the same level of ion suppression or enhancement will be seen for the target compound and its isotopically labelled analogue. Therefore the ratio of the two signals should be unaffected, allowing for accurate quantification (*Ibid*). Thus use of an isotopically labelled analogue (fluoxetine-d_5) of one of the target compounds (fluoxetine) as the internal standard helped to evaluate the matrix effect.

3.7.8 Recovery and accuracy

The recovery study using spiked HM matrix was used to assess the method's precision and accuracy, as previously mentioned in Section 3.6.4.6. Figure 3.13 shows exemplar SIM chromatograms of a spiked HM sample (HM9) used for the recovery study scanning three ions as shown in Table 3.4

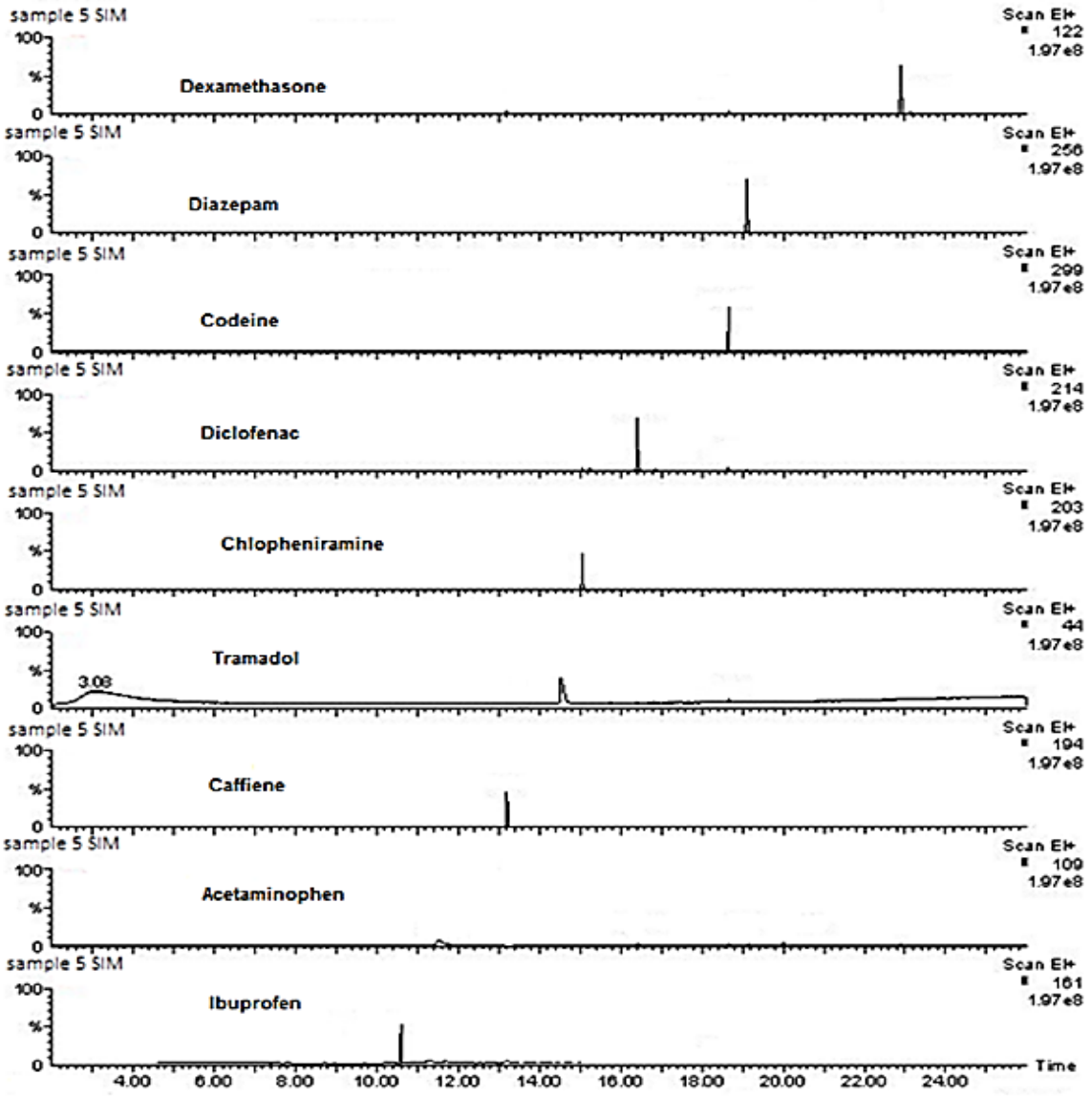


Figure 3.13: Exemplar SIM chromatogram of spiked HM sample at 0.8 mg/ml (HM9) showing the target compounds at retention times (bottom to top) of 10.23, 11.37, 13.25, 14.65, 15.16, 16.48, 18.80, 19.24, and 22.81 minutes respectively

Table 3.10 shows the recovery data calculated using Equation 3.4 in Section 3.6.4.6. For example, using HM9 containing no ibuprofen (C_0) spiked with ibuprofen at 0.8 mg/ml (C_2) and 0.795 mg/ml (C_1) mean concentration (n = 9, SD = 0.032) was recovered. Recovery is then calculated using:

Recovery = 0.8/0.795 X 100 = 99.38%

%RSD = 0.032/0.795 X 100 = 4.03

The recovery (%) and %RSD was also calculated for the other two concentrations of ibuprofen and the three concentrations of all the other analytes, as shown in Table 3.10

At all concentrations (low, middle and high), the recovery ranged from 70-103.75% and method precision was between 3.4 -11.4%. According to the United States Pharmacopeia Convention (2012), a precision level of not more than 20% is acceptable, while 70-150% recovery is acceptable for accuracy studies.

Table 3.10: Results obtained from recovery studies of target analytes in spiked HM sample

Analyte	Amount Added (mg/ml)	Amount recovered (mg/ml)±SD (n=9)	Recovery (%)	RSD (%)
Acetaminophen	0.01	0.007±0.0008	71.20	11.4
	0.08	0.078±0.004	78.00	5.1
	0.8	0.690±0.051	86.25	7.4
Caffeine	0.008	0.006±0.0004	76.25	6.6
	0.08	0.068±0.005	85.00	7.4
	0.8	0.830±0.030	103.75	3.6
Chlorpheniramine	0.008	0.007±0.0006	90.00	8.3
	0.08	0.077±0.005	96.25	6.5
	0.8	0.760±0.026	95.00	3.4
Codeine	0.008	0.006±0.0004	77.50	6.5
	0.08	0.066±0.005	82.50	7.8
	0.8	0.740±0.051	92.50	6.9
Tramadol	0.008	0.006±0.0005	71.25	8.8
	0.08	0.075±0.004	93.75	5.6
	0.8	0.780±0.053	97.50	6.7
Dexamethasone	0.01	0.007±0.0006	73.00	8.2
	0.08	0.076±0.0041	95.00	5.4
	0.8	0.820±0.041	102.5	5.0
Diazepam	0.008	0.006±0.0005	78.75	7.9
	0.08	0.078±0.004	97.50	5.4
	0.8	0.790±0.054	98.75	6.9
Diclofenac	0.008	0.006±0.0005	70.00	8.9
	0.08	0.068±0.004	85.00	6.0
	0.8	0.720±0.059	90.00	8.2
Ibuprofen	0.008	0.003±0.0002	70,00	7.1
	0.08	0.066±0.004	82.64	6.2
	0.8	0.795±0.032	99.38	4.0
Fluoxetine	0.008	0.007±0.0005	86.25	7.3
	0.08	0.074±0.004	92.50	5.3
	0.8	0.821±0.056	102.63	6.9
Fluoxetine-d_5 (internal standard)	0.25	0.192±0.013	76.80	6.6

Thus these results are within an acceptable range. The recovery studies show the potential of the selected method for the detection of the compounds of interest in this research using methanol as the extracting solvent with little interference from the matrix.

3.7.9 Precision study

Data from the stability study (Section 3.7.4) were used to assess instrument precision, while data from the recovery study were used to assess method precision (Section 3.7.8). Table 3.11 shows the data obtained for a mixed standard at three concentrations, with their corresponding PAR.

Table 3.11: Result of repeatability study of the target analyte over a 50-hour period

Analyte	Intra-assay PAR repeatability (n=33)[a]					
	0.008 mg/ml	RSD %	0.08 mg/ml	RSD %	0.8 mg/ml	RSD %
Caffeine	0.023±0.002	8.7	0.241±0.007	3.3	2.211±0.133	6.0
Chlorpheniramine	0.049±0.003	6.1	0.513±0.040	7.8	5.326±0.212	4.0
Codeine	0.076±0.004	5.3	0.712±0.024	3.4	6.676±0.301	4.5
Diazepam	0.023±0.002	9.1	0.264±0.009	3.4	2.346±0.123	5.2
Diclofenac	0.021±0.002	9.5	0.160±0.012	7.5	2.102±0.142	6.8
Ibuprofen	0.028±0.002	7.1	0.264±0.016	6.1	3.464±0.143	4.1
Tramadol	0.028±0.002	7.1	0.283±0.008	2.8	2.654±0.120	4.5
Fluoxetine	0.076±0.004	5.3	0.726±0.023	3.2	7.540±0.323	4.3
Acetaminophen	0.005±0.0004[b]	8.0	0.068±0.003	4.4	0.915±0.051	5.5
Dexamethasone	0.035±0.003[b]	8.6	0.270±0.015	5.6	3.141±0.180	5.0

[a] Triplicate analysis at 11 time points from stability study (Section 3.8.4); [b]Concentration at 0.01mg/ml; PAR: Peak area ratio.

All studied compounds had precision less than 10% RSD at low concentration (Table 3.11), although up to 15% RSD is acceptable (Peters, Drummer and Musshoff, 2007). Diclofenac had the highest RSD at 9.5%, followed by caffeine at 8.7% at the lowest concentration analysed but the values still fall within acceptable levels. Although these are below 10% RSD, the lowest concentration of analysed diclofenac is below the LOD (0.011 mg/ml), while caffeine is at the LOD concentration (0.008 mg/ml) (Table 3.8), which may be the reason for the increased %RSD.

The PAR of the lowest concentration for acetaminophen and dexamethasone (0.01 mg/ml) show higher fluctuations in %RSD than those recorded at 0.8 mg/ml. This is to be expected (Ellison, Barwick and Farrant, 2009) because at this concentration the analytes are closer to their LOD (Table 3.8), and fluctuations in the analytical response may be more predominant, making peak areas less precise. Despite this, some compounds of interest had a lower %RSD than those recorded at the mid-range concentrations, e.g. chlorpheniramine with 6.1% RSD at 0.008 mg/ml compared to 7.8% at 0.08 mg/ml. This eventually improved at 0.8

mg/ml with RSD of 4.0% and may be as a result of increased sensitivity at the highest concentration, as compared with the fluctuations at low and mid concentrations.

All studied analytes were within 10% RSD at a concentration of 0.08 mg/ml. At this mid concentration range, precision was improved in all the compounds except for chlorpheniramine. At the highest concentration (0.8 mg/ml) all compounds were within 10% RSD. The results for the compounds are more precise than those recorded at the lowest concentration. This may be as a result of an improved signal of the analytical instrument at higher concentrations than the LOQ.

In general, the results obtained show that all the compounds at the studied concentrations are repeatable within the 50-hour period (Section 3.7.4) and all are within 10-15% RSD. Furthermore, with increasing concentration, the method precision is improved.

3.8 Result and discussion of ICP-OES method validation

3.8.1 Selectivity

The selectivity of the ICP-OES method was carried out as described in Section 3.6.5.1 to assess spectral interference. The three wavelengths selected by visual examination based on non-overlapping peaks, non-spectral interference and highest intensity are shown in Table 3.12. This is with the exception of selenium, which had only one wavelength that met the criteria. Wavelengths 206.279 nm and 203.985 nm for selenium had spectral interference with zinc wavelengths at 206.200 nm and 202.548 nm. Although the two interfering wavelengths for zinc could have been removed instead, they had higher intensity and less spectra interference than selenium.

Table 3.12: Wavelength selection using multielemental mixed standard at 0.2 ppm

Element	Wavelength 1(nm)	Wavelength 2 (nm)	Wavelength 3 (nm)
Arsenic	188.980	193.696	234.984
Cadmium	214.439	228.802	226.502
Chromium	276.653	276.259	267.716
Copper	324.754	222.778	224.700
Lead	220.353	405.781	283.305
Manganese	257.610	259.372	260.568
Mercury	194.164	184.887	253.652
Nickel	222.486	221.648	231.604
Selenium	196.026	206.279*	203.985**
Zinc	206.200	213.857	202.548
Scandium (IS)	335.372	361.383	

*Interference with wavelength 1 of zinc ** Interference with wavelength 3 of zinc.
Both*and ** not used

In addition, the results of the recovery study as discussed in Section 3.8.4 showed the selected wavelengths were specific for all the elements, with recoveries within an acceptable range. This implies that the examined wavelengths were devoid of significant spectral and matrix interference using the multi-elemental mixed standard and the spiked CRM standard. In addition, analysis of the blank solution after measuring the 1 ppm calibration standard showed no memory effect and inspection of the spectrum at each wavelength chosen to detect each analyte found no signal. Hence one wavelength was selected from the selectivity study. The wavelengths that had the highest emission intensity and without spectral and matrix interference were selected, as shown in Table 3.13.

Table 3.13: Wavelength used in this study

Element	Wavelength (nm)
Arsenic	188.980
Cadmium	214.439
Chromium	276.653
Copper	324.754
Lead	220.353
Manganese	257.610
Mercury	194.164
Nickel	222.486
Selenium	196.026
Zinc	206.200
Scandium (IS)	335.372

This is with the exception of selenium, which had only one wavelength at 196.026nm with high emission intensity and no spectral interference. These selected wavelengths were used for subsequent analysis in this study and for method validation.

3.8.2 Linearity and linear range

The calibration graph for chromium, as an example, is shown in Figure 3.14 with a smaller graph within highlighting the linearity at lower concentrations (calibration graph for other metals is included in Appendix VII). The linear regression equation and the correlation coefficient (R^2) are shown in Table 3.14.

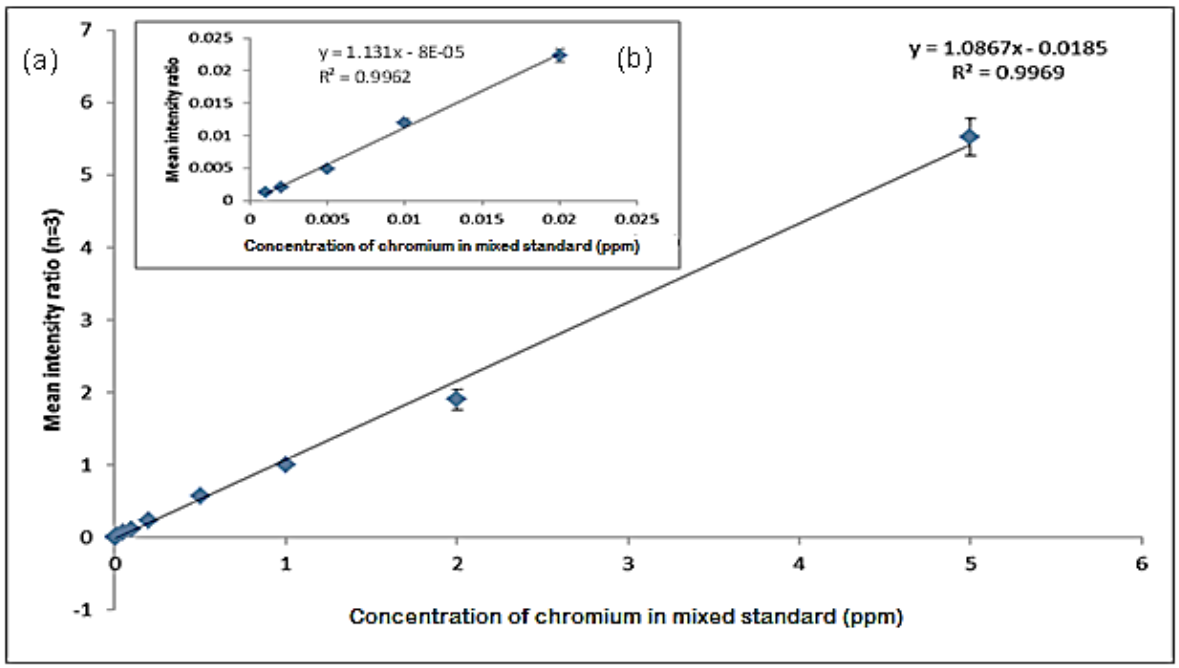


Figure 3.14: (a) Linearity plot of chromium with (b) linearity plot at lower concentration

At highest or lowest concentration range, concentrations showing non–linear behaviour were removed, giving a better R^2 value as closer to 1, as shown in (Table 3.14). These improved standard concentrations were then used as the working range. The linearity of the working range was subsequently assessed in this section using the same method, as described in Section 3.7.5 for GC-MS.

Table 3.14: Regression equation and R^2 values using the working range concentrations

Element	Regression equation	R^2
Arsenic	Y = 0.2848x + 0.0004	0.999
Cadmium	Y = 1.4458x - 0.005	0.999
Chromium	Y = 1.0867x - 0.0185	0.997
Copper	Y = 2.9884x + 0.0097	0.999
Lead	Y = 0.1113x + 0.005	0.999
Manganese	Y = 17.978x - 0.0418	0.998
Mercury	Y = 0.0508x + 0.001	0.998
Nickel	Y = 0.1927x + 0.0024	0.999
Selenium	Y = 0.0171x - 0.0005	0.999
Zinc	Y = 0.1799x - 0.0003	0.998

A plot of the ratio of peak intensity and concentration (Rc) against log of concentration, as described in Section 3.6.4.3, was used for further assessment of the linearity data. If ideal linearity is attained, then the ensuing plot should be within ±10% of the mean relative

response factor. Figure 3.15 is an example of this plot of chromium (in a mixed standard), showing the lowest concentration (0.001 ppm) is outside of 10% RSD. Therefore the linear range in Table 3.17 reflects this for chromium (0.002-5 ppm). The same approach was taken for the other metals analysed.

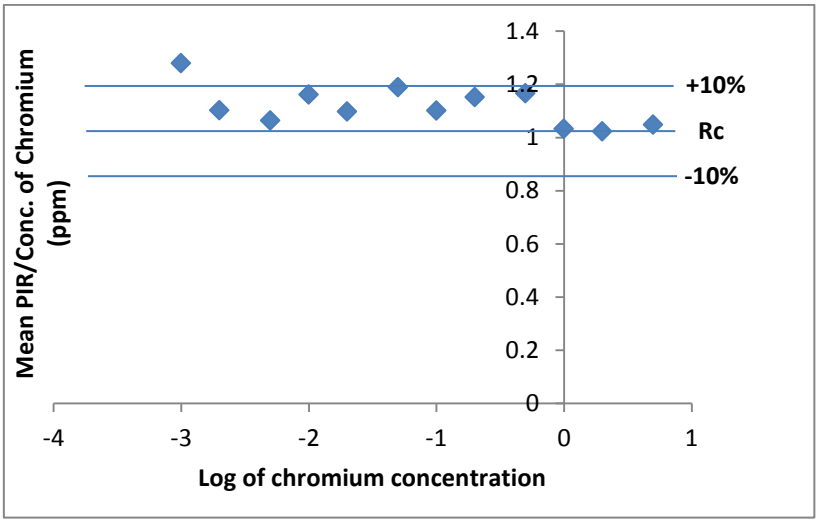


Figure 3.15: Log-linear plot of chromium

The data points shown in Figure 3.15 are spread around the mean, while the non-linear data point is at the lower level of the concentration range (0.001 ppm) thus showing a loss of linearity. The log-linear plot was run for other target metals (Appendix IX) to determine their linear range, as presented in Table 3.17.

Nonetheless, as also discussed in Section 3.7.5, a residual plot which allows checking for outliers, for example, those outside ±3 standard deviation (SWGTOX, 2013), corroborates earlier findings. The residual plot of the linear range showed that the calibration model was linear, as seen in Figure 3.16, for chromium and the rest of the selected metals, as shown in Appendix VIII. Although the residual plot for chromium in Figure 3.16 may appear to have a pattern, Shapiro-Wilk statistical analysis shows a normal distribution of the residuals (Table 3.15).

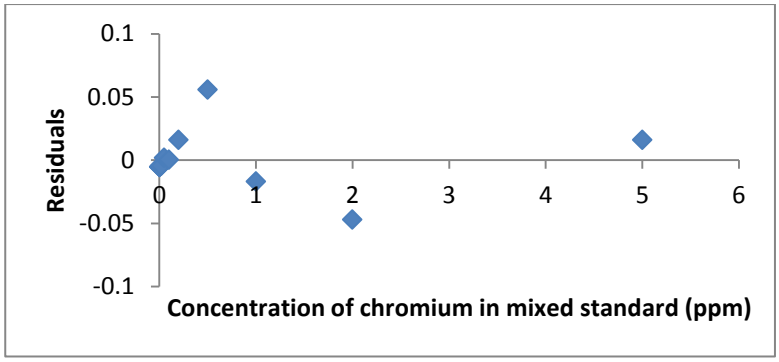


Figure 3.16: Residual plot of chromium

The Shapiro-Wilk statistical test performed for all the metals produced *p*-values ranging from 0.052 to 0.378 throughout the tested linear range residual (Table 3.15). These *p*-values were greater than the level of significance of 0.05; hence the null hypothesis was accepted, indicating normal distribution.

Table 3.15: Test for normality using Shapiro-Wilk test

Element	Kurtosis	Skewness	*p*-value	Accept the null hypothesis
Arsenic	2.338	0.999	0.090	yes
Cadmium	1.025	-0.790	0.139	yes
Chromium	3.172	0.572	0.073	yes
Copper	1.225	0.399	0.378	yes
Lead	3.275	0.339	0.055	yes
Manganese	2.637	-1.202	0.095	yes
Mercury	2.617	0.82	0.056	yes
Nickel	3.455	0.112	0.054	yes
Selenium	2.781	-0.845	0.091	yes
Zinc	3.964	-0.748	0.052	yes

As discussed in the GC-MS linearity study in Section 3.7.5, a normal distribution is not assumed in deriving the skewness and kurtosis tests. However, values close to symmetrical were obtained; e.g. chromium with kurtosis of 3.172 and skewness of 0.572.

It is also important to examine the randomness of the error using the Runs test. The Runs test produced *p*-values between 0.095 and 1.000 (Table 3.16), which were all greater than the 0.05 significance level. Hence the null hypothesis was accepted, which shows a random distribution of error.

Table 3.16: Values for Runs test

Elements	*p*-value	Accept the null hypothesis
Arsenic	0.565	yes
Cadmium	0.095	yes
Chromium	0.145	yes
Copper	0.835	yes
Lead	0.835	yes
Manganese	1.000	yes
Mercury	0.145	yes
Nickel	0.145	yes
Selenium	0.095	yes
Zinc	0.405	yes

3.8.3 Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Calculations of LOD and LOQ were carried out using the method outlined in Section 3.1.3

The values obtained are shown in Table 3.17, which showed LOD ranged between 0.010 and 0.035 ppm and LOQ ranged between 0.031 and 0.107 ppm for all the metals. The analysed LOD for arsenic and selenium are much lower than those reported in the literature at 0.017 ppm and 0.022 ppm in dry plant mass (Støving et al., 2013; Xiea et al., 2013; Senila et al., 2014; Ibrahim et al., 2011). Støving et al (2013) also reported similar LOD for copper (0.01 ppm) when analysing elemental impurities in tablets, according to US Pharmacopeia.

Other metals have varying LODs compared with those reported in other research, as shown in Table 3.17. This variation may not be unconnected with varying sample preparation methods, instrument calibration and properties of the metals in various mixed standard. While there are some volatile metals involved in this study, such as selenium and lead, the obtained LOD compared with those reported in the literature showed improved detection. Cadmium has a higher LOD (0.035 ppm) than reported in the literature. However, the result from the accuracy and precision study (Table 3.9) showed good recovery and %RSD of all the target metals including cadmium.

Table 3.17: LOD and LOQ values compared with other ICP-OES analysis of target element

Element	LOD (ppm)	LOQ (ppm)	Linear range (ppm)	LOD in literature (ppm)
Arsenic	0.017	0.052	0.005 - 5	0.05[1], 2.24[2], 0.045[3]
Cadmium	0.035	0.107	0.001 - 5	0.01[1,2 &4], 0.019[3],
Chromium	0.026	0.081	0.002 - 5	0.02[1], 0.1[2],0.075[3], 0.13[4]
Copper	0.010	0.031	0.001 - 5	0.01[1], 0.25[2], 0.12[3],0.2[4]
Lead	0.023	0.068	0.001 - 5	0.01[1&4], 0.12[2], 0.043[3]
Manganese	0.031	0.094	0.005 - 5	0.01[1], 0.05[2],0.030[3], 0.37[4]
Nickel	0.026	0.078	0.005 - 0.5	0.02[1], 0.75[2],0.13[3],0.01[4]
Selenium	0.022	0.068	0.005 - 0.2	0.45[2], 0.5[5]
Zinc	0.027	0.082	0.010 - 5	0.25[1], 0.10[2], 0.22[3],0.01[6]

[1]Støving et al., 2013 , [2]Xiea et al., 2013, [3] Senile et al., 2014, [4]Mihălţan et al., 2012,
[5] Ibrahim et al., 2011, [6]Milani et al., 2015

In addition, the LOQ values are lower than the published permissible level for various metals in herbal medicine (EMA, 2008). Thus the method is suitable for the quantification of metals in HM samples below permissible limits. The LOD values also fall within the linear range of the linearity data in this study. Thus this method is suitable for the detection and quantification of the target metals.

3.8.4 Recovery and accuracy

Following the method described in Section 3.6.4.6, the average recovery at three spiked concentrations across the three wavelengths (except selenium as discussed in section 3.8.1) were examined. They were within the recommended recovery range of 70-150%, according to the US Pharmacopeia and also with %RSD less than 10% across the selected wavelengths. Therefore one of the wavelengths was selected while considering other factors discussed in Section 3.8.1. The wavelengths used for method validation are thus selected in Table 3.12.

The detailed recovery study of the selected wavelength at the three levels of concentration analysed is shown in Table 3.18. The calculation for the recovery study is done as for GC-MS in Section 3.7.8. The recovery efficiency using the two digestion methods described in Section 3.6.3.2 was determined in order to select the best digestion method. It was observed from the recovery study that wet digestion was more efficient in the recovery of the target metals than dry ashing when percentage recovery from both methods was compared (Table 3.18). Similar studies have supported the efficiency of wet digestion as compared with dry ashing for most of the metals in this study (Yang et al., 2013; Zheng et al., 2013).

Table 3.18: Recovery studies from wet and dry ashing digestion method

		Wet Digestion			Dry Ashing		
Elements	Amount Added (ppm)	Amount recovered (ppm)±SD (n=3)	% Recovery	% RSD	Amount recovered (ppm)±SD (n=3)	% Recovery	% RSD
Arsenic	0.02	0.016±0.002	80.00	12.5	0.015±0.002	75.00	13.3
	0.2	0.180±0.013	90.00	7.2	0.163±0.016	81.50	9.8
	2	1.983±0.121	99.15	6.1	1.765±0.133	88.25	7.5
Cadmium	0.02	0.016±0.002	80.00	12.5	0.014±0.002	70.00	14.3
	0.2	0.178±0.014	89.00	7.9	0.157±0.015	78.50	9.6
	2	2.120±0.150	106.00	7.1	1.725±0.160	86.25	9.3
Chromium	0.02	0.017±0.002	85.00	11.8	0.014±0.001	70.00	7.1
	0.2	0.231±0.022	115.50	9.5	0.182±0.018	91.00	9.9
	2	2.070±0.160	103.50	7.7	1.919±0.180	95.95	9.4
Copper	0.02	0.015±0.001	75.00	6.7	0.014±0.001	70.00	7.1
	0.2	0.201±0.016	100.50	7.8	0.174±0.015	87.00	8.6
	2	1.982±0.113	99.10	5.7	1.858±0.101	92.90	5.4
Lead	0.02	0.020±0.002	100.00	10.0	0.018±0.002	90.00	11.1
	0.2	0.180±0.012	90.00	6.7	0.168±0.016	84.00	9.5
	2	2.315±0.155	115.75	6.7	1.874±0.138	93.70	7.4
Manganese	0.02	0.019±0.002	95.00	10.6	0.019±0.002	95.00	10.5
	0.2	0.198±0.017	99.00	8.6	0.188±0.014	94.00	7.5
	2	2.093±0.113	104.65	5.4	2.112±0.120	105.6	5.7
Mercury	0.02	0.018±0.002	90.00	11.1	0.018±0.001	90.00	5.6
	0.2	0.152±0.013	76.00	8.6	0.171±0.013	85.50	7.6
	2	1.887±0.141	94.35	7.5	1.899±0.129	94.95	6.8
Nickel	0.02	0.021±0.002	105.00	9.8	0.018±0.002	90.00	11.1
	0.2	0.218±0.012	109.00	5.5	0.176±0.015	88.00	8.5
	2	1.995±0.159	99.75	8.0	1.894±0.129	94.70	6.8
Selenium	0.02	0.018±0.002	90.00	11.0	0.014±0.002	70.00	14.3
	0.2	0.189±0.014	94.50	7.4	0.161±0.012	80.50	7.5
	2	1.927±0.121	96.35	6.3	1.667±0.104	83.35	6.2
Zinc	0.02	0.019±0.001	95.00	5.3	0.019±0.001	95.00	5.3
	0.2	0.215±0.019	107.50	8.9	0.196±0.016	98.00	8.2
	2	2.071±0.164	103.55	7.9	2.111±0.155	105.55	7.3

This is particularly so for the volatile metals in this study, such as arsenic, cadmium and zinc. Nonetheless, the recovery of metals using the dry ashing method had recovery within the acceptable range of between 70-150% even for volatile metals. This may be associated with the use of magnesium nitrate as the ashing aid, as discussed in Section 1.7.4. Thus both digestion methods seem efficient in the recovery of the target metals. Conversely, values from the open vessel wet digestion for selected HMs were subsequently presented and discussed in chapter 4 because of its higher recovery, as shown in Table 3.18. Other studies have also found wet digestion to be more efficient (Soylak et al., 2004).

3.8.5 Precision study

The same data shown in Table 3.18 were used to assess method precision. The precision studies showed that the %RSD were within the 15% acceptable range for all the metals at the chosen concentrations (0.02, 0.2 and 2 ppm). A 20% RSD is said to be acceptable for concentrations near the LOQ of the method (Perter, Drummer and Musshoff, 2007). Nonetheless, all the values were below 20% RSD for all analysed metals at all concentrations. Arsenic and cadmium had the highest RSD at 12.5% at a concentration of 0.02 ppm by wet digestion; this may be as a result of their potential volatility, as discussed in Section 1.7.4; although this is also expected at low concentrations and an allowance of up to 20% is permissible (Peter, Drummer and Musshoff, 2007). The high %RSD may be as a result of intensity inconsistency at the near LOQ concentration (0.052 ppm) for arsenic, for example (Table 3.17), as compared with higher concentrations (Ellison, Barwick and farrant, 2009). However, this is within the acceptable limit, as discussed earlier in this study, and therefore shows reproducibility. The same explanation applies to chromium, manganese and selenium which also had RSDs above 10% at 11.8%, 10.6% and 11.0% respectively. These RSDs improved at higher concentrations, as shown in Table 3.18. At 2 ppm the %RSD for all the metals were within 10%. Generally, these results suggest that the metals at the three concentration studied were precise, repeatable and within the acceptable range for accuracy and 15% RSD.

3.9 Summary

The results of method validation showed that the method to be used for the detection and quantification of the selected metals and pharmaceutical compounds in the selected HM is specific, linear and with a linear range that is suitable for the intended purpose. The LOQ concentrations in ICP-OES analysis are below the permissible levels of metals recommended by WHO (Table 4.3). The LOQ concentrations for target pharmaceuticals from GC-MS analysis were also below the usual therapeutic dose expected to be found in HM samples. The LOD for both ICP-OES and GC-MS were lower than those obtained from similar studies for most of the metals and pharmaceutical compounds. The results of the accuracy and precision studies show the reliability of the quantification that will be carried out on studied pharmaceutical compounds and metals.

CHAPTER 4: ANALYSIS OF PHARMACEUTICALS AND HEAVY METALS IN SELECTED HERBAL MEDICINE USING GC-MS AND ICP-OES

4.1 Introduction

The HMs analysed in this study were obtained as described in Section 4.2.1 to detect and quantify any possible pharmaceutical compounds and heavy metals using the method validated in Chapter 3. The variability of target compound within samples and location were also assessed. All HM samples were in powder form and prepared for GC-MS and ICP-OES analysis as described in Section 3.6.2.2 and 3.6.3.2 respectively.

4.2 Materials and method

The materials and method used for the analysis of HM for pharmaceutical adulterants and heavy metals are as shown in Section 3.6. The samples were thoroughly mixed; weighed and extracted, digested samples were made up to relevant concentration for GC-MS analysis and ICP-OES analysis.

4.2.1 Selection and sampling of herbal medicines

The HM samples selected were the 10 types most commonly used in Ekiti State. These were identified from the result the of survey study presented in Chapter 2 (Table 2.12). The details of the selected HM are shown in Table 4.1.

Analysed HM samples were obtained in replicate from five locations of Ekiti State, as shown and labelled 1 to 5 in Figure 4.1. This was essential to assess variability between locations.

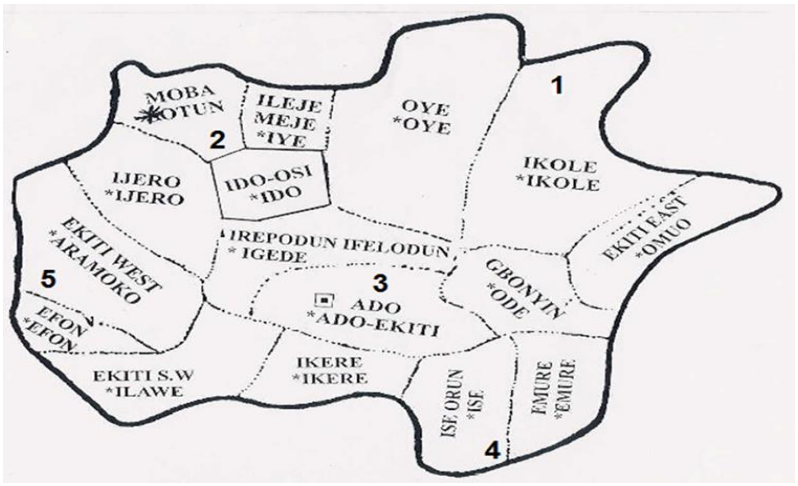


Figure 4.1: Map of Ekiti State showing various locations from which HM samples were obtained

Some HM samples were packaged in capsules, while others were powders wrapped in newspaper sheets or transparent plastic bags (see Appendix XVII). The HMs were all made up of various constituents, as declared, although some did not declare any (see Table 4.1). Sampling of the HM for analysis was carried out as follows:

I. Encapsulated HM samples in blister packs (HM3, HM6 and HM9 were samples of this type); two capsules were picked at random from 2 blister packs in each sample package. This was repeated for the four other replicates obtained from other locations, making a total of 20 capsules for each HM type. A total of 60 capsules were sampled and analysed for all blister pack HM.

II. Encapsulated HM samples not in a blister pack (HM1); four capsules were picked at random from their plastic bottles. This was done for the four other plastic bottles, making a total of 20 capsules.

III. Powdered samples packaged in paper sheets (HM2, HM4, HM5, HM7, HM8 and HM10); each was divided into two equal parts and samples weighed out from each bit. Four other replicates were weighed out using this method, allowing for 10 samples to be taken for each HM type to ensure representative sampling. A total of 60 were sampled and analysed for this type of HM. A summary of the sampling method is shown in Figure 4.2.

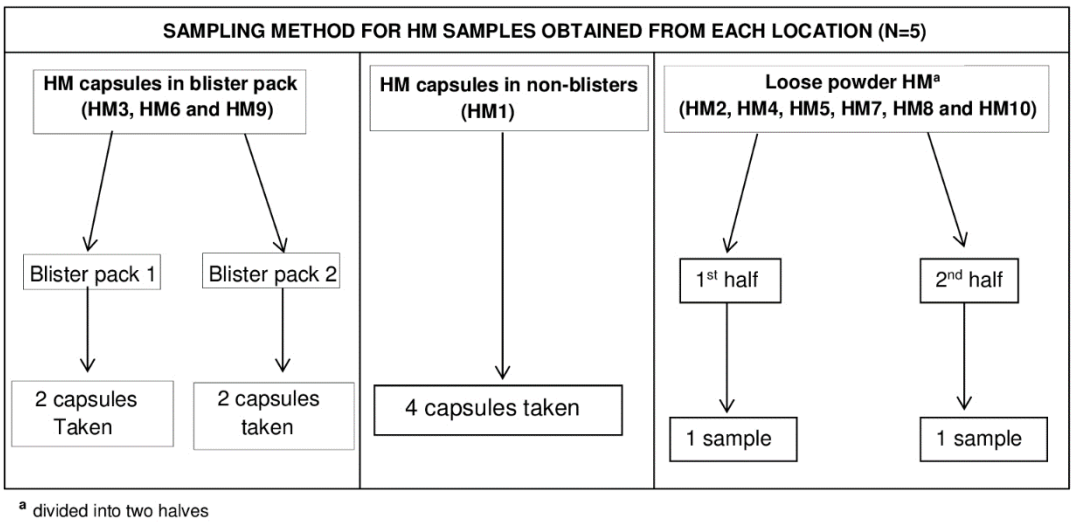


Figure 4.2: Herbal medicine sampling method for analytical studies

Table 4.1: Table of information on selected HM samples

HM No	*Product name*	*NAFDAC number*	*Manufacturer*	*Indication(S)*	*Dose and duration*	*Content*
HM1	Body pain	None[a]	Yemkem international	Pain	3 capsules twice daily	40% horse bone 30% bitter cola 30% potash
HM2	Male tonic	None[a]	Non specified	Low sperm count and increased sexual power	None	None declared
HM3	Supa A1	A7-0374L	Yemkem international	Immune booster, male and female infertility, jaundice and sickle cell disease.	2 capsules twice daily	15% cinchona succirubora and others[b]
HM4	Wadco total blood cure	None[a]	Wadco health foundation	Pain, yellow fever, typhoid, rheumatism	One spoon twice daily	None declared
HM5	Wadco pile & dysentery	None[a]	Wadco health foundation	Pain, dysentery, pile, infertility	Daily for two days	None declared
HM6	M&T Capsule	A7-0294L	Yemkem international	Malaria, typhoid	3 capsules twice daily	10% piper nigrum and others[c]
HM7	YK original malaria	None[a]	Dr. Abdul Kareem Yakub	Malaria, pains, fever and body weakness	Twice daily	None declared
HM8	Aromalegun	None[a]	Non specified	Pains, sleeplessness	Twice daily over two days	None declared
HM9	Eroxy 5000	A7-0303L	Yemkem international	Pile, pain, haemorrhoid, indigestion, energy and libido	3 capsules twice daily	25% garlic and others[d]
HM10	Original malaria/ yellow fever and typhoid	None[a]	Non specified	Malaria, typhoid, pain, sleeplessness and fever	Twice over two days	None declared

[a] uncertified HM ; [b]70% Markhamia tomantoas 20% Alstonia congensis [c]20% khaya ivorensis,50% colocasle antiguorum, 15% theobroma cacao [d]2% Eugqnia caryo phylatta, 2% piper nigrum, 16% zinigiba officianale, 15% Garcinia cola, 10% each of (Plumbayo zeylanica, Lantana,Eosinium and Entandrophrapma utile)

4.3 Results and discussion of analysis of herbal medicine using GC-MS

4.3.1 Primary Identification of pharmaceutical compounds in herbal medicine

Prior to analysis of HM samples for identification of pharmaceutical compounds using GC-MS known analytical standards of the pharmaceuticals of interest were run to acquire their retention times and mass spectra, as shown in Section 3.7.1. This analysis focused on samples that might contain the target compounds shown in Table 3.1; hence, where analytes of interest were identified and confirmed, samples were then taken forward to the quantification stage.

However, none of the HM samples was observed to contain any of the analytes of interest using the method of identification outlined above in Section 3.7.1, or if present were below the limit of detection of this study.

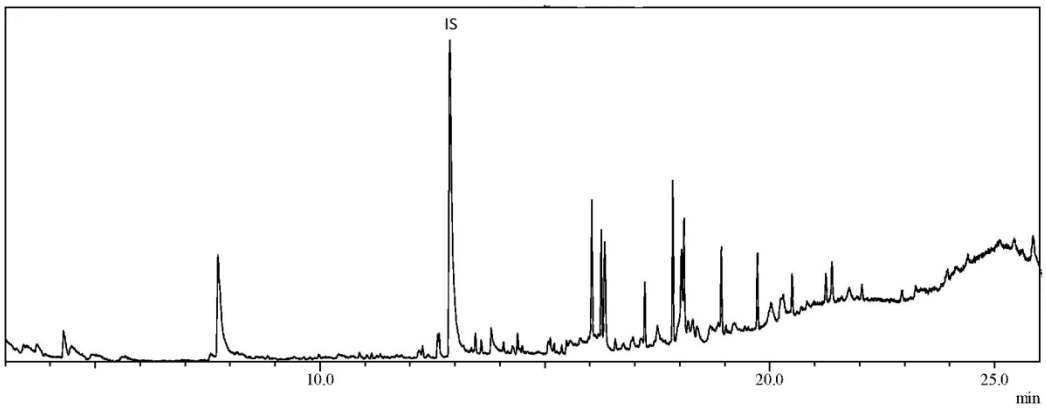


Figure 4.3: Chromatogram (TIC) of HM sample 3 showing internal standard (IS) fluoxetine-d_5 (0.25 mg/ml).

A sample TIC chromatogram of the HM sample (Figure 4.3, others in Appendix X) showed the presence of various tentatively identified organic constituents such as phytic acid, anabasine (plant alkaloids) and farneso (plant terpenes) and the internal standard (IS). They were plant matrices which were expected to be present. Hence such detection may attest to the suitability of the analytical method for the detection of constituent compounds in selected HM samples in this research. Regardless, the extraction of target analytes from spiked HM samples has been previously performed in this study (Section 3.6.4.6), including matrix effect and recovery study (Section 3.7.7 and 3.7.8). Therefore detection of the HM organic constituents may not have interfered with the detection of target analyte. However, despite the suitability of the TIC chromatogram of the mixed standard (Figure 3.2), a direct comparison of both chromatograms for the purpose of identification will be inaccurate because of the detected multiple organic constituents. Therefore EIC was used with the ions contained in Table 3.4 for each target analyte in the analysed HM samples. As none of the

target analytes was present in the analysed HM samples, further to confirm this, these samples were analysed in SIM using three ions at the retention times as shown in Table 3.4 at + and -1 minute. Results also showed none of the target compounds was present.

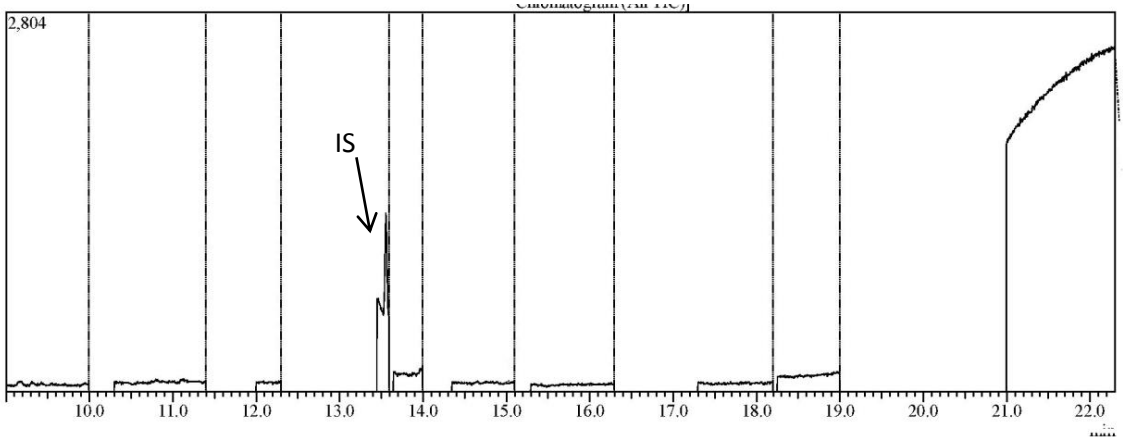


Figure 4.4: SIM chromatogram of HM sample 3 showing only the IS peak (m/z: 43, 109 and 314)

As seen in (Figure 4.4), no peaks were detected (except the internal standard) at the retention time of the studied analytes using selected ion monitoring. This was the same for all the HM samples analysed, providing further confirmation of the absence of the target analyte in the HM samples. The peak of the IS was distinct, as can be seen in (Figure 4.5), which also asserts the suitability of the method.

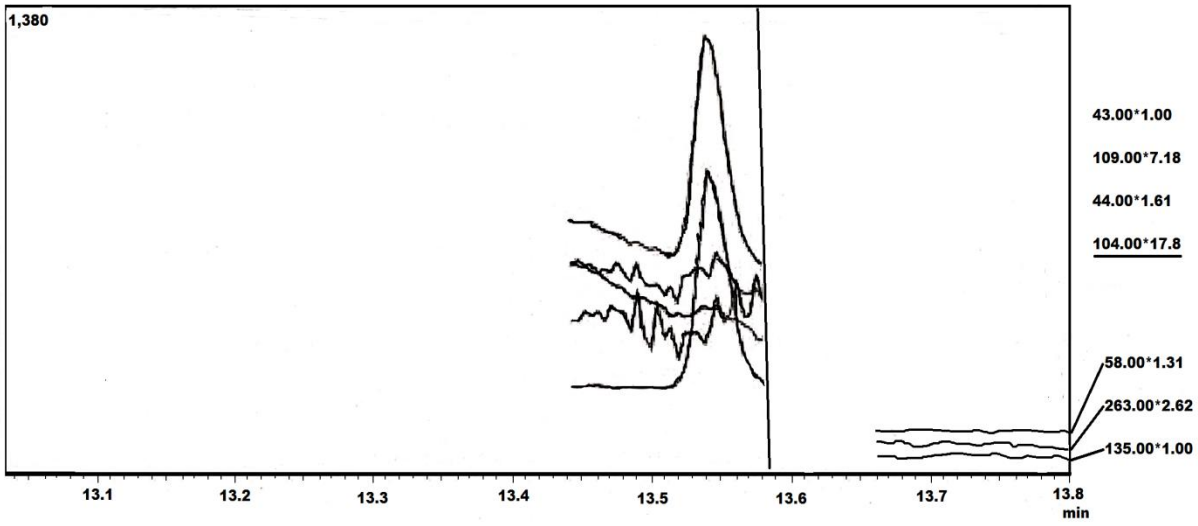


Figure 4.5: SIM chromatogram showing IS peak in HM sample 3 (m/z: 43, 109 and 314)

Absence of the target analytes was further ascertained by analysis of HM samples using another GC-MS (Shimadzu QP 2010) with the same parameters as those previously used with the Clarus GC-MS (Table 3.2) also failing to detect any of the target analytes. Therefore no quantification was carried out as none of the target analytes was detected.

The findings of this analysis differ from those of HM obtained from other countries where pharmaceutical compounds have been detected (Garg et al., 2011; Posadzki, Watson and Ernst, 2013; Justa, Neves and Caldas, 2015). The LOD of some analytes in this study methods was lower than the detected concentrations of the same analytes in other publications (Table 3.8). Hence these analytes would have been more likely to be detected if present using the method validated in this research.

However, further analysis of the HM samples for possible heavy metals was carried out, as discussed in Section 4.4.

4.4 Result and discussion of analysis of herbal medicine using ICP-OES

4.4.1 Elemental detection and quantification in herbal medicine samples

Following selected sample preparation via wet digestion (Section 3.8.4) and validation of the ICP-OES method (Section 3.8) all studied HM samples (Table 4.1) were analysed for selected heavy metals (Section 3.3). For all HM samples, of the 10 metals 9 were detected (i.e., As, Cd, Cr, Cu, Pb, Mn, Ni, Se and Zn) while Hg was not detected. The nine metals were detected above LOD and LOQ (Table 3.17).

To quantify the heavy metals detected in the HM samples the regression equation as shown in Table 3.14 was used. For example, according to the regression equation for chromium

$y= 1.0867x – 0.0185$

Because the mean intensity ratio (y) is known, the actual concentration of the sample can be calculated from the equation:

$x = (y+0.0185) / 1.0867$

So for HM1 as an example, the value of y which is the mean intensity ratio when analysed for chromium was 0.3564

Therefore x is calculated as shown below where y= 0.3564

$x = (0.3564+0.0185) / 1.0867=0.345$ ppm (µg/ml)

This shows that 0.345 µg/ml of chromium is contained in HM1. But then this is inconsistent with the original concentration calculated from the weight of the sample dissolved (1g of HM sample in 25 ml deionised water) (Section 3.6.3.2.2) with a concentration of 0.04 g/ml. Therefore the actual concentration of chromium is calculated by dividing the initially obtained concentration of chromium by the sample concentration:

0.345 µg/ml / 0.04 g/ml = 8.625 µg/g (ppm)

Hence HM1 contains 8.625 µg/g of chromium

These calculations were repeated for the other HM samples for the target metals detected. The results are presented in Table 4.2.

The permissible level of heavy metals varies from country to country, as seen in Table 4.3. Unfortunately, Nigeria has no guideline yet on the permissible level of heavy metals in herbal products consumed in the country. Therefore WHO recommended values (National Sanitation Foundation draft proposal values) are adopted in this study as permissible limits (WHO, 2007) as shown in Table 4.3.

Table 4.2: Table showing the average concentration of heavy metals detected in HM samples

Permissible limits*	2 ppm	0.3 ppm	2 ppm	3 ppm	10 ppm	0.1 ppm	44.6 to 339 ppm	1.6 ppm	25 ppm	100 ppm
HM samples (n=12)	Arsenic (ppm)	Cadmium (ppm)	Chromium (ppm)	Copper (ppm)	Lead (ppm)	Mercury (ppm)	Manganese (ppm)	Nickel (ppm)	Selenium (ppm)	Zinc (ppm)
HM1	0.233±0.018	0.669±0.048	8.625±0.615	15.134±0.898	3.972±0.264	ND	54.841±3.376	5.295±0.309	7.950±0.423	83.864±6.003
HM2	0.607±0.117	0.558±0.045	2.779±0.255	31.953±4.424	33.374±3.720	ND	91.469±2.813	5.148±0.910	7.965±0.435	131.428±6.662
HM3	0.473±0.032	0.531±0.032	1.774±0.091	14.753±0.909	1.396±0.052	ND	193.544±7.813	2.883±0.114	2.281±0.114	24.712±1.846
HM4	0.292±0.030	0.597±0.055	1.686±0.278	13.565±1.323	3.057±0.671	ND	85.431±2.846	1.936±0.387	5.342±0.943	25.585±5.763
HM5	0.390±0.035	0.705±0.122	4.712±0.561	57.175±4.806	5.907±0.322	ND	134.822±3.348	2.948±0.152	3.225±0.632	86.289±6.161
HM6	0.250±0.037	0.465±0.060	0.738±0.042	11.343±0.339	2.727±0.226	ND	88.774±9.885	1.406±0.299	9.023±0.789	19.286±1.269
HM7	0.302±0.037	0.473±0.029	0.538±0.055	4.912±0.669	1.612±0.207	ND	38.644±3.305	1.384±0.148	5.208±0.203	5.557±0.748
HM8	0.350±0.062	0.491±0.028	3.536±0.424	38.367±1.134	3.122±0.291	ND	30.578±3.292	8.234±0.352	3.016±0.669	93.070±3.898
HM9	0.255±0.039	0.458±0.047	3.908±0.372	58.888±6.604	1.772±0.117	ND	91.067±4.840	5.121±0.568	2.524±0.463	31.776±3.518
HM10	0.527±0.084	0.497±0.044	3.299±1.035	13.311±0.619	2.900±0.909	ND	63.151±1.341	1.872±0.481	4.050±0.789	14.891±1.665

Values in red are above the permissible limit. ND: Not detected * WHO, 2007

Table 4.3: Table of permissible levels of heavy metals in various countries (WHO, 2007)

Countries	Product type	As	Pb	Cd	Cr	Hg	Cu	TTM as Pb
For herbal medicines								
Canada	RHM	5 ppm	10 ppm	0.3 ppm	2 ppm	0.2 ppm	-	-
	FHP	0.01 mg/day	0.02 mg/day	0.006 mg/day	0.02 mg/day	0.02 mg/day	-	-
China	HM	2 ppm	10 ppm	1 ppm	-	0.5 ppm	-	20 ppm
Malaysia	FHP	5 mg/kg	10 mg/kg	-	-	0.5 mg/kg	-	-
Republic of Korea	HMA	-	-	-	-	-	-	30 ppm
Singapore	FHP	5 ppm	20 ppm	-	-	0.5 ppm	150 ppm	-
Thailand	HMA, FHB	4 ppm	10 ppm	0.3 ppm	-	-	-	-
WHO recommendations		10 mg/kg	0.3 mg/kg	-	-	-	-	-
For other herbal products								
NSFDP (Raw Dietary Supplement)		5 ppm	10 ppm	0.3 ppm	2 ppm	-	-	-
NSFDP (Finished Dietary Supplement)		0.01 mg/day	0.02 mg/day	0.06 mg/day	0.02 mg/day	0.02 mg/day	-	-

TTM = Total toxic metals; RHM = Raw herbal material; FHB = Finished herbal product; HMA = Herbal material; HM = herbal medicine; NSFDP = National sanitation foundation draft proposal

The average batch element concentrations of target metals in each HM sample are discussed according to individual metals from Sections 4.4.2 to 4.4.11.

4.4.2 Arsenic

The concentration of arsenic in all the HMs was below the permissible limits of 2 ppm (WHO, 2007). HM2 and HM10 appear to have a large standard deviation in the average concentration of arsenic (Figure 4.6). This may be a result of variation of arsenic across the five locations sampled, as discussed in Section 4.4.13

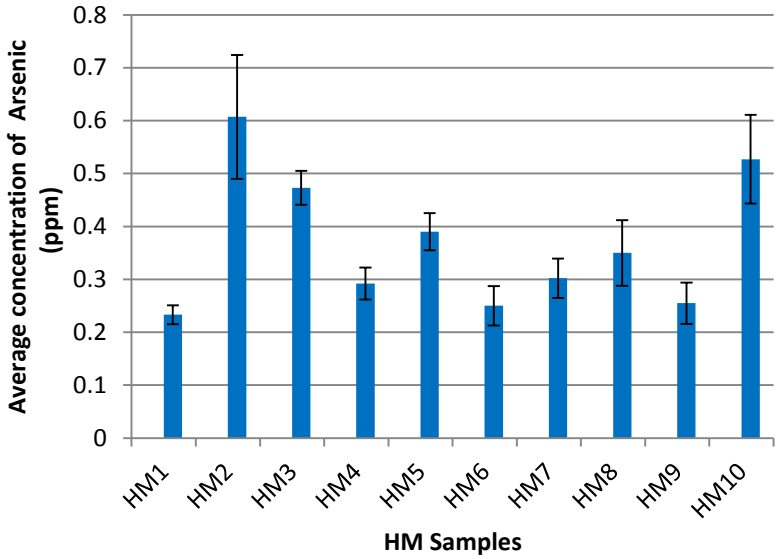


Figure 4.6: Average concentration of arsenic detected in HM samples

The results from Figure 4.6 show that the concentration of arsenic in HM samples ranged between 0.233±0.018 ppm for HM1 and 0.607±0.117 ppm for HM2. These values are above the LOQ in this research (Table 3.17) and are similar to concentrations of arsenic obtained from a previous study (0.301-1.108 ppm) which analysed HM samples from other parts of Nigeria (Adepoju-Bello et al., 2012).

However, arsenic is a naturally occurring constituent of the earth's crust and is largely abundant in the environment, in the air, land and water. This element can dissolve in water, so it can contaminate rivers, lakes or underground water by dissolving in rain, snow, or through improperly disposed industrial waste, used as insecticide and herbicide or preservative for wood (Fergusson, 1990). As a result, groundwater contamination with arsenic is a serious public health threat worldwide. This is because of people's exposure to e.g. high levels of inorganic arsenic from contaminated water used for irrigation of agricultural plant and various industrial processes (WHO, 2016).

Arsenic has also been reported in seafood, especially in the oil-producing states of Nigeria (Chinyere et al., 2012; Kingsley and Reginia, 2016). Hence its accumulation in plants supplied by the same river may not be unusual. In Ekiti State high concentrations of arsenic above permissible limit has been found in farmland (Isinkaye, 2012). This may be due to continual applications of pesticide and fertiliser (Atafar et al., 2010). Conversely, the effect of chronic arsenic exposure and its bioaccumulation from ingested arsenic-contaminated plant products has been investigated in various countries and found to be associated with detrimental health effects such as cancer and vascular disease (Gibb et al., 2011; Argos et al., 2010). Oral exposure, as in the case of HM, accounts for the majority of exposure routes in the general population (ATSDR, 2014).

4.4.3 Cadmium

All HM samples had cadmium levels above the permissible limits of 0.3 ppm (WHO, 2007). As seen in Figure 4.7, the highest concentration was in HM5 (0.705±0.122 ppm), an uncertified HM (Table 4.1), followed by HM1 (0.669±0.048 ppm), a certified HM sample. Cadmium levels were above the LOQ (Table 3.17) in all the HM samples.

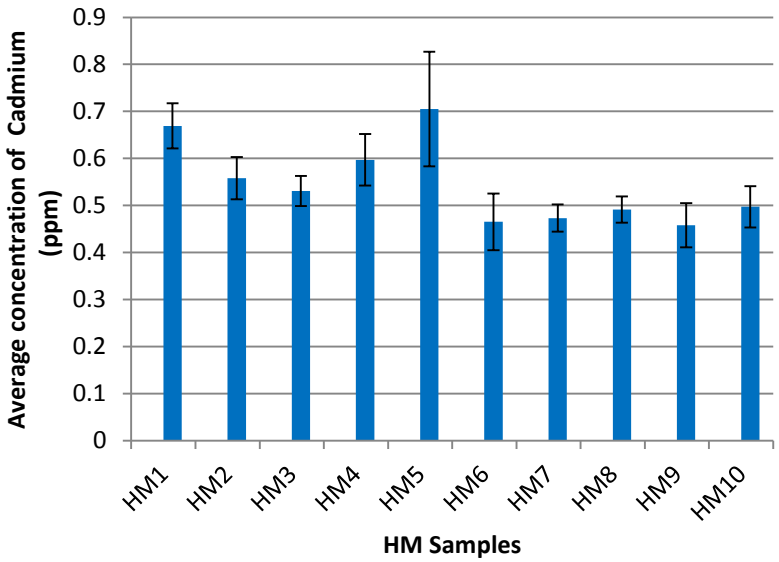


Figure 4.7: Average concentration of cadmium in HM samples

The concentration of cadmium in the HMs ranged between 0.705±0.122 ppm for HM5 and 0.458±0.047 ppm for HM9. This is low compared with values reported in HMs previously analysed in Nigeria (0.261 to 40.288 ppm) (Adepoju-Bello et al., 2012). The difference in the findings of this research may be associated with the variation in the location where samples were obtained, as per batch variation, which is further discussed in Section 4.4.13.

Cadmium is found in the earth's crust at about 0.1 ppm and often as an impurity in lead or zinc deposits (Wedepohl, 1995). Cadmium is used in commerce, e.g. the production of paint pigments, batteries, steel galvanisation, lasers, cosmetics and television screens (U.S. Geological Survey, 2012). The management of electronic waste is still problematic in Nigeria and dumpsites are a mixture of varying types of waste ranging from electronic to biological. The presence of high amounts of cadmium at dumpsites in Ekiti State and the bioaccumulation of the metal in plants, especially common edible vegetables, has previously been reported (Awokunmi, Adefemi and Asaolu, 2015). Additionally, in Nigeria and most developing nations, the marked increase in population and a need for mostly western used personal cars (known as "tokunbo") alongside an increase in automobile repair/workshops have contributed to increased environmental pollution. Suffice to say a marked increase in cadmium concentration in roadside vegetation and auto mechanic workshops in Nigeria has been reported (Fakayode and Olu-Owolabi, 2003; Ololade, 2014). These human activities may have contributed to the presence of Cd above the permissible limit in all the HM samples analysed; as it seems the source of environmental pollution especially from cars and their related products are a common contributing factor regardless of location. Noteworthy also is the publication of results of soil analysis in some areas of Ekiti State, which reported moderate cadmium pollution especially around sawmills (Ajibulu et al., 2013).

It has been reported that contamination of drugs and dietary supplements (designation for HM in the USA) may also be responsible for overexposure to cadmium (Abernethy et al., 2010). Ingestion of cadmium is one of the main routes of human exposure and intestinal absorption is higher in people who suffer calcium, zinc or iron deficiency (Nordberg, 2007). High cadmium exposure can produce long-term health effects. Cadmium toxicity mainly affects the kidney in humans primarily at the proximal tubule where cadmium is deposited causing Fanconi syndrome from cadmium-induced oxidative damage and also chronic glomerulonephritis (Thévenod, 2003; Fujiwara, Lee, and Tokumoto, 2012; Kim et al., 2015; Sabath and Robles-Osorio, 2012). The survey results of HM use reported in this research showed that 85% of the population (n=1265) used HM in Ekiti State (Table 2.8), of which the most commonly used types analysed in this research are now found to contain cadmium above permissible limits. More worrying are the findings on the challenges of renal care in an Ekiti State renal centre, which reported that chronic glomerulonephritis was the most prevalent (45.3%) kidney injury and one of the leading causes of end-stage renal failure in the facility (one of only two such facilities in the state) (Oluyombo et al., 2014). Therefore there may be a correlation between the renal findings and the findings of this research.
Cadmium has no physiological benefit to humans even at trace level (ATSDR, 2008). It affects the cardiovascular system in several ways such as its role in inducing hypertension

(Gallagher and Meliker, 2010), diabetes (Edwards and Prozialeck, 2009), peripheral arterial disease (Navas-Acien et al., 2005), sudden cardiac death (Menke et al., 2009) and myocardial infarction (Everett and Frithsen, 2008). Besides its acute toxicity, cadmium bioaccumulation in the renal cortex and liver is one of its chronic toxic effects, as a result of its long biological half-life (Hammer and Hammer, 2004; Adepoju-Bello and Alabi, 2005). Hence, with the results in this study showing the presence of cadmium above the permissible limit in all the HM samples analysed, it has become a serious public health concern, which this research intends to highlight for prompt action. Toxicity at low chronic exposure is also a cause for concern, as discussed in Section 4.4.12.

4.4.4 Chromium

Some of the HM samples had concentrations of chromium higher than the permissible level of 2 ppm. Samples HM1, HM2, HM5, HM8, HM9 and HM10 had levels of chromium higher than permissible limits, while HM3, HM4, HM6 and HM7 had concentrations of chromium below the permissible level (Table 4.2).

The concentrations of chromium in Government certified HM1 (8.630±0.615 ppm) and HM9 (3.908±0.372) were above the permissible limit (2 ppm) and HM1 contained the highest concentration of chromium. This points to the need for a more comprehensive testing protocol for HM certification in Nigeria.

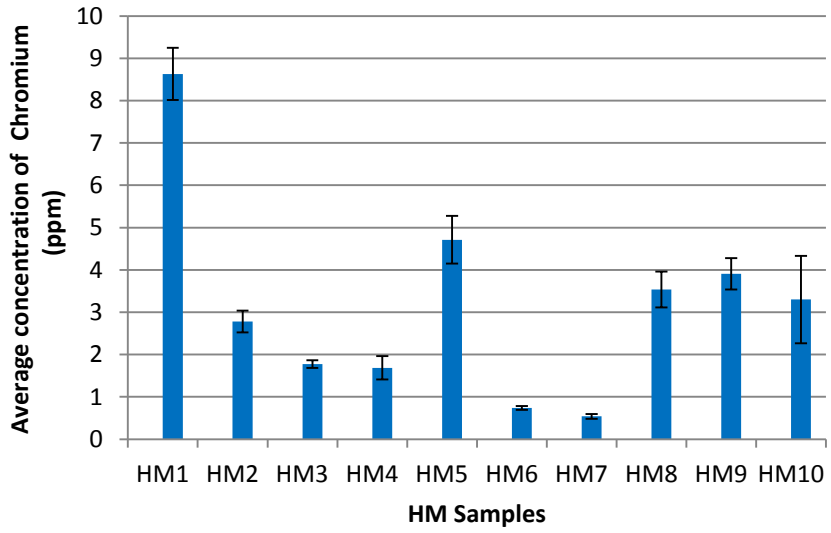


Figure 4.8: Average concentration of chromium in HM samples

The range of chromium concentration was between 8.630±0.615 ppm and 0.538±0.055 ppm with HM7 an uncertified HM containing the lowest concentration of chromium (Figure 4.8). Various publications on HM samples obtained from the Niger Delta area of Nigeria and Lagos State reported no chromium was detected (Ekeanyanwu et al., 2013; Idu, Oghale and

Jimoh, 2015). Nonetheless, another study on HMs obtained in the same Niger Delta area detected chromium levels similar to those in this study at 4.16% above WHO permissible limits (Igweze, Orisakwe and Obianime, 2012) . While the disparities may be as a result of various human activities in these regions, Chromium concentration or its detection may also vary according to the type and source of HM analysed.

Chromium occurs naturally in plants, animals, soil and rocks with oxidation states in a range of chromium (-II) to chromium (VI). Hexavalent chromium (Cr(VI) is toxic and carcinogenic, unlike chromium metal and Cr(III) ions (EPA 1984a, 2000; Salnikow and Zhitkovich, 2008). No speciation was performed in this research, hence total chromium was analysed. Natural processes and industries such as textile, leather, chemical and steel manufacture are responsible for chromium entering the environment. Various activities from chromate mining, such as seepage and improper disposal of mining and manufacturing tools have also been reported to contribute to groundwater contamination (Rom, 2007).

Impact evaluation of small-scale mining activities on groundwater quality and vegetation in some part of Ekiti State reported no significant impact (Talabi et al., 2015). On the other hand, a report from neighbouring Osun State (See Section 2.3.1) showed heavy metals geoaccumulation at a level up to extreme contamination at an illegal gold mining site (Olujimi et al., 2015). Also, the study of some auto-mechanic workshop soil in Ondo State (another neighbouring state; see Section 2.3.1) reported chromium concentration within permissible limit (Ololade, 2014). Other studies have reported statistically significant differences in chromium concentration between soil around petroleum product handling facilities and control samples in Lagos (Adeniyi and Afolabi, 2002). This is because heavy metals are associated with petroleum products (Albers, 2003) and thus activities of petroleum handling facilities can harm vegetation. Another study on soil samples from sawmill areas in Ekiti State reported concentrations of chromium suggesting natural origin with a little anthropogenic contribution (Ajibulu, 2013). All these sources potentially contribute to the presence of chromium in HM samples.

Chromium (III) is a nutrient essential to humans that is involved in the metabolism of protein, fat and glucose by potentiation of insulin activities. But exposure to high levels through its ingestion can result in adverse health effects such as increased cancer risk, liver damage and intestinal bleeding (ASTDR, 2013). The toxicity of chromium in humans through HM exposure may not be acute, but there is a preferential accumulation of chromium in the kidney leading to tubular damage as a result of chronic exposure (Wedeen and Qian, 1991). This associated kidney injury is as discussed for cadmium in Section 4.4.3 and the oral

exposure limit is further discussed in Section 4.4.12. The mechanism of kidney injury is also via oxidative stress (Zendehdel et al., 2015) as with cadmium.

4.4.5 Copper

All the HM samples had a concentration of copper above the permissible limit (3 ppm). HM9 (a certified HM) had the highest concentration of copper (58.888±6.604 ppm), followed by HM5, an uncertified HM (57.175±4.806 ppm) (Figure 4.9). The concentration of copper in the HM samples ranged between 4.912±0.669 ppm and 58.888±6.604 ppm**.**

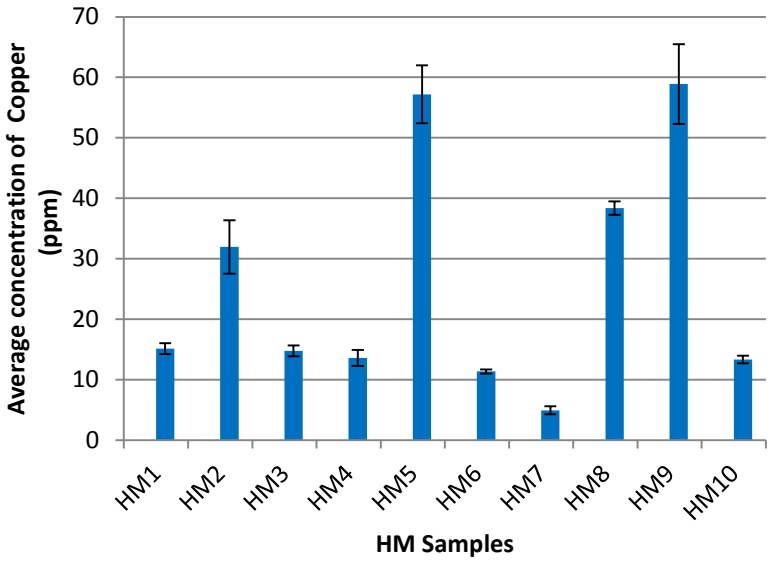


Figure 4.9: Average concentration of copper in HM samples

This concentration range is higher than that reported in HM samples obtained in Lagos, and particularly HM9 which was analysed by another study and reported to contain 0.20 ppm of copper (Onwordi ,Agbo and Ogunwande, 2015) compared with 58.888±6.604 ppm obtained in this study. The HM9 sample analysed in the study mentioned above was in liquid form, not the powdered form analysed in this study. This difference may be responsible for the wide disparity in concentration due to differences in the sample preparation method used for both types of sample (Gauglitz and Moore, 2014). Another study has also reported a higher concentration of copper and other heavy metals in powder samples than in their liquid form (Idu, Oghale and Jimoh, 2015).

HM6 in this study had 11.343±0.339 ppm of copper compared to 2.35 ppm of the same sample analysed in a previous study (Onwordi, Agbo and Ogunwande, 2015). The varying concentration of copper may be a result of a difference in the source of raw materials or

batch manufacturing process, although, as discussed in Section 4.4.13, there was no significant variation between the various batch concentrations of copper in HM samples analysed in this research (Appendix XIII). This insignificant variation in copper concentration found in this study may weaken the postulation as to the difference between concentration reported from Onwordi, Agbo and Ogunwande, (2015). But then the distance between the locations of the two studies may further assert variations as a result of a difference in the source of the raw materials used, or in the batch manufacturing process. Nevertheless, the high concentration of copper in the analysed samples is a serious safety concern.

Drinking water, food and copper supplements are the main sources of copper exposure from the environment. The copper content in plants varies according to the quality of the soil, source of water, geography, season and use of fertilisers which influences the concentration in plants (McLaughlin et al., 2000). Although studies of some selected farmland in Ekiti reported copper concentration in soil below the permissible limit (Adeyeye, 2005; Isinkaye, 2012), on the other hand a study of the soil and plants on dumpsites in Ekiti State revealed the concentration of copper above the WHO permissible limit in the soil sample and its high bioaccumulation in plant found at the dump site (Ayeni, Ajayi and Odeyemi, 2017). This is similar to findings from other parts of Nigeria (Ideriah et al., 2010; Idera and Oladele, 2015). Hence these may be responsible for the high concentration of copper in HMs found in Ekiti State (Table 4.2), although the source of the plants used for HM production cannot be established. Additionally, a significant concentration of copper was found in the soil of petroleum handling facilities above that of a control sample (Adeniyi and Afolabi, 2002) and auto workshop soil also showed a concentration above the WHO limit (Ololade, 2014). Thus there are indications that copper accumulation in soil and plant is directly linked to various human activities such as petroleum handling and waste disposal.

But then copper is a vital micronutrient and constituent of various proteins involved in a number of biological pathways essential to life (Rucker et al., 1998; Sánchez-Ferrer et al., 1995). At excess levels, copper can be toxic, with liver damage the most noticeable chronic effect (Zietz et al., 2003). At lower doses initial adverse reaction after acute exposure to copper is the vagal stimulation of the stomach, leading to a reflex response of nausea and vomiting (Araya et al., 2004). HM9 and HM5, for example, are being used by 13.69% and 5.78% of the sample population respectively (n=1018) (Table 2.12). These samples contain 58.888±6.604 ppm and 57.175±4.806 ppm of copper respectively (Table 4.2) which is 19 times higher than the permissible limit (3 ppm). This high concentration may be responsible for nausea and vomiting that was experienced by 18.7% of respondents (n=1075) in this research (Table 2.17).

While this may be an acute reaction, the effect of chronic exposure through prolonged use may lead to serious problems such as liver injury. Hence, inorganic constituents such as copper may contribute as much to HM-induced liver injury as those caused by the organic constituents reported in a previous publication (Ma, Peng and Hu, 2014). In addition, a study from a Nigerian tertiary hospital reported that 45.5% of the risk factors for liver disease in the hospital were due to the use of herbs (Nwokediuko et al., 2013). Although a similar report is not available for Ekiti State, findings from Nwokediuko et al (2013) did not specify which constituent was responsible for the liver damage (organic or inorganic). Hence the need for further study in this regard, as recommended in Section 5.2.

4.4.6 Lead

As shown in Figure 4.10, the highest concentration of lead (33.374±3.720 ppm) was found in HM2 (a non-certified HM), exceeding the permissible limit of 10 ppm and 3 times above. Conversely, all other HMs had lead concentrations below the permissible limit.

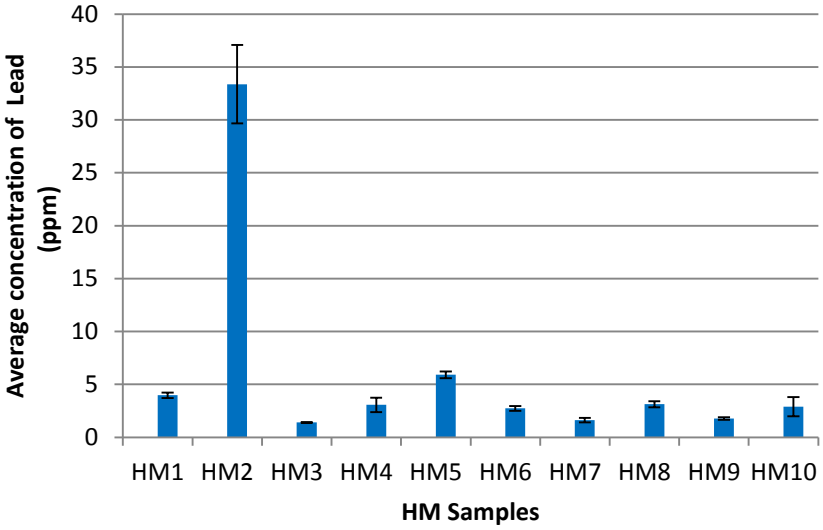


Figure 4.10: Average concentration of Lead in HM samples

The range of lead concentration was between (1.396±0.052 ppm and 33.374±3.720 ppm). This was similar to the range (1.54 to 33.80 ppm) reported in Nigeria (Onwordi, Agbo and Ogunwande, 2015) and those obtained from some Chinese HMs (Zheng et al., 2013). The concentration of lead (2.727±0.226 ppm) in HM6 (certified HM sample; M&T capsule) obtained from this study was similar to that reported for the same HM (2.850 ppm) obtained from a different location (Onwordi, Agbo and Ogunwande, 2015). This may suggest consistency in lead concentration in the HM sample from the manufacturer and also the robustness of the method used in this study. Nor was there any significant variation in the

average concentration of lead detected in each HM sample except for HM2, HM8 and HM 10 (Appendix XIII). There was also no significant variation in lead concentration between batches of HM sample, with the exception of HM8

Lead is a toxic metal which occurs naturally in the Earth's crust. Its extensive use has led to serious contamination of the environment, unsafe human exposure and public health issues in several more parts of the world (CDC, 2013c). Major sources of environmental contamination include industries such as smelting, mining, recycling and manufacturing and the persistent use of leaded products (paint, gasoline and aviation fuel) in some countries (Nigeria included) (Flora, Flora and Saxena, 2006). The initiative proposed to reduce the concentration of lead in gasoline to 0.15 g/L and finally to zero in Nigeria only exists on paper (Orisakwe, 2009). This is due to government negligence; predominance of old European used vehicles manufactured to run on lead gasoline and modern cars manufactured to use lead-free gasoline using leaded gasoline (Orisakwe, 2014). Lead is also used in various products such as ceramic glaze, paint, pigments, stained glass, lead crystal glassware, toys and jewellery and in some traditional medicines (Flora, Flora and Saxena, 2006). These human activities contaminate soil and water bodies with lead, which is eventually driven into the fauna and flora (Mclaughlin et al., 2000; Kingsley and Regina, 2016; Adewumi et al., 2017).

The elevated concentration of lead in soil samples above WHO permissible limit has been reported in petroleum handling areas in Nigeria (Adeniyi and Afolabi, 2002; Ololade, 2014; Nwaichi, Wegwu and Nwosu, 2014). This is not unusual considering the earlier discussion on the use of leaded gasoline. With 82.0% of respondents within the low-income group in this research (Table 2.6) and the high use of old vehicles designed to use leaded gasoline, it is inevitable that there will be a consequent environmental effect. Dumpsites and highway vegetation in Ekiti State have also been found to contain significant concentrations of lead (Awokumi et al., 2015) (Ajibulu et al., 2013), although other publications have reported lead concentration below the WHO permissible limit in other dump sites in Ekiti State (Shittu et al., 2015; Ayeni et al., 2017). Mining has been associated with contamination of soil with lead in China (Zhou et al., 2007), and in North West Nigeria (Tsuwang et al., 2014). Although cases of illegal mining have been reported in Ekiti State (The Nation, 2015), reports of heavy metal contamination as a result have not been documented. Conversely, analysis of farmland soil in Ekiti State reported a concentration of Pb below the WHO limit (Isinkaye, 2012) or not detected in some cases (Adeyeye, 2005). But then, only one of the analysed HMs (HM2) had a lead concentration above the permissible limit in this study.

Regardless, children are especially predisposed to the harmful consequences of lead and may suffer severe and permanent adverse health effects, inhibiting the development of the nervous system and brain (Cleveland et al., 2008). Exposure of pregnant women to high levels of lead can lead to stillbirth, minor malformation, miscarriage, premature birth and low birth weight (Flora, Pachauri and saxena, 2011). The effect of lead in HM may thus relate to the findings in Table 2.27 where there was 7.7% HM associated stillbirth, 42.0% HM associated spontaneous premature labour (SPL) and 34% HM associated foetal abnormality. Although there is no direct correlation, these findings will form the basis for further research, as suggested in Section 5.3. Accumulation of lead even at well below nontoxic levels, can have serious health effects (Lanphear et al., 2005), hence being below permissible levels may not suggest safety. Continuous exposure even at a low dose may be detrimental, so the need for estimation of daily oral limit and the availability of clearly written HM dosage are essential, as further discussed in Section 4.4.12.

Lead also causes long-term injury in adults, including kidney damage (Navas-Acien et al., 2007), as discussed in Section 4.4.3 for cadmium and risk of high blood pressure. The likelihood of HM-induced hypertension by heavy metal constituents such as lead, cadmium and arsenic (Gyamlani and Geraci, 2007) may be a vital contributor to the high prevalence of hypertension (55.5%) in Ekiti State (Olamoyegun et al., 2016), the more so considering that 85.0% of respondents used HM (Table 2.8) and heavy metals known to induce hypertension (lead, cadmium and arsenic) have been found in commonly used HM in this study. Therefore the need for stricter measures for certification of HM and monitoring has become essential.

4.4.7 Mercury

Mercury was not detected in any of the HMs using the validated method in this study, which may be due to non-use of hydride generation ICP-OES. However, some of the HMs were analysed externally at Analytix Limited using a Direct Mercury Analyser DMA-80 autosampler system (Appendix XIV) as part of its marketing offer. Considering mercury volatility, the analyser among other operating mechanisms traps mercury vapour for analysis; this is one of its advantages (Analytix, 2018).

The concentrations reported were HM1 (Sample 1) 7.65 ppb, HM2 (Sample 2) 15.80 ppb, HM4 (Sample 4) 0.203 ppb which were below the permissible limit of mercury (0.1 ppm) in herbal product (FAO/WHO, 1984). Other publications have reported the detection of mercury in HM samples (Adepoju-Bello et al., 2012) and some above permissible limits (Osadolor et al., 2015; Martena et al., 2009), although some others did not detect mercury (Idu, Oghale

and Jimoh, 2015). Thus mercury content of HM likely varies with location and detection in HM may depend on the method LOD, which should be lower than the permissible limit.

Mercury exists as elemental mercury mainly in nature or as sulphide and exists in the earth's crust at about 0.5 ppm. Anthropogenic mercury sources include mining (mercury and gold in particular) (Zeitz, Orr and Kaye, 2002). Human mercury exposure occurs mainly via ingestion of mercury attached to organic moieties (methyl, ethyl or dimethyl mercury) (WHO, 1991; Richardson, 1996). Mining has been associated with significantly high concentrations of mercury in the soil of the northwestern part of Nigeria (Tsuwang et al., 2014). The contribution of oil spillage in the Niger Delta area to high mercury concentration in soil and water above WHO permissible limit has been reported (Odunaike et al., 2013). High levels of mercury in the Ibadan southwest waterbody as a result of improper disposal of industrial effluent (into waterbody) have also been reported (Fakayode, 2005). A high concentration of mercury in the soil around automobile waste dump sites has been documented (Iwegbue, 2006b). All these human activities have an effect on the concentration of mercury in plant and by extension in HM.

Chronic exposure to elemental mercury at low-grade induces milder clinical findings and symptoms such as anorexia, gastrointestinal distress, weakness, fatigue and weight loss (Lucchini et al., 2002). At higher levels of exposure fine tremor with coarse shaking occurs and also gingivitis, erethism, excessive salivation and immune dysfunction (Meyer-Baron, schaeper and Seeber, 2002; Park et al., 2000). Concentrations of mercury in HMs analysed were below the permissible limit. However, this does not render redundant precautionary screening of HM for mercury content for public health safeguarding.

4.4.8 Manganese

The highest concentration of manganese (193.544±7.813 ppm) was found in HM3 (Figure 4.11), although this concentration and those obtained for other HM samples are all within the permissible limit of 339 ppm. The range of manganese concentration detected in the HMs was between 30.578±3.292 ppm and 193.544±7.813 ppm, which is within the permissible range of 44.6 ppm and 339 ppm (Table 4.2). Similar studies have reported much lower manganese concentrations in selected HM in Nigeria; 0.475 ppm and 1.446 ppm (Umar et al., 2016) and 0.012 ppm to 0.702 ppm (Ekeanyanwu et al., 2013). However, a higher concentration range as found in this study has been reported in selected HM from China (Zheng et al., 2013) and dry plant mass in Romania (Senila et al., 2014).

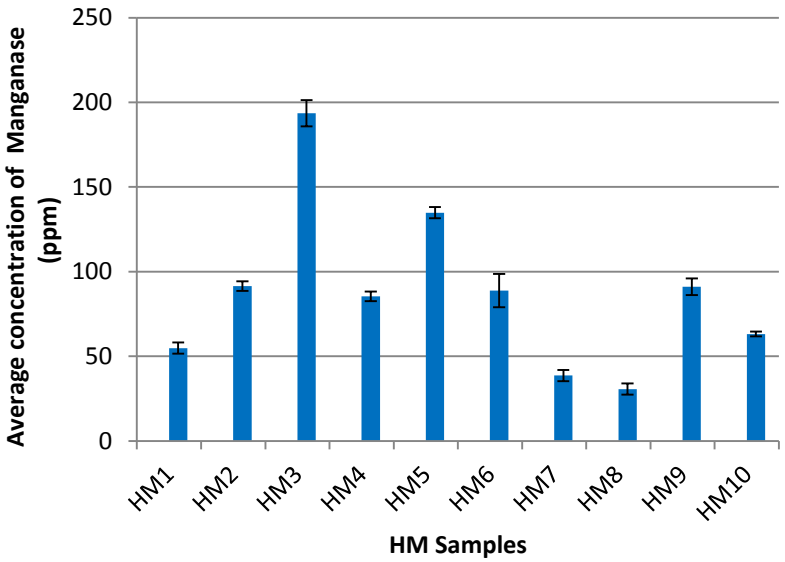


Figure 4.11: Average concentration of manganese in HM samples

Statistical analysis, however, shows only HM6 and HM8 had a significant variation in the concentration of manganese obtained from different locations (Appendix XIII).

Manganese is found in the environment mainly in its oxidised form either as Mn_3O_4 or MnO_2 (Nadaska, Lesny and Michalik, 2012). It is an element essential to human health as a co-factor in many enzymes and it is a requirement in normal growth, maintenance of immune cell activities and nerves, regulation of blood vitamins and sugar amid other functions (Guilarte, 2010; Aschner et al., 2007). Manganese is required at small levels through dietary intake, but exposure to food or water contaminated with high levels can portend toxic outcomes.

Industries such as mining of manganese dioxide, manufacturing of (dry-cell battery steel, aluminium cans, electronics,) smelting, welding, fungicides, pesticides and fertilisers have contributed to the increased manganese levels in the environment with eventual human toxicity (Keen and Lonnerdal, 1995; Ono, Komai and Yamada, 2002; Wang et al., 1998; Ferraz et al., 1988). Some of these activities also take place in Ekiti State, especially the use of pesticides, fungicides and fertilisers. However, the concentration of manganese in Ekiti farmland was found to be within acceptable limits (Isinkaye, 2012). Also, manganese uptake did not exceed the WHO limits for crops in test samples in an oil spill area in Nigeria (Nwaichi et al., 2014). In addition, the concentration of manganese in Ekiti dumpsite soils was reported to be generally lower than the permissible limits, but concentrations were higher than the permissible limit in plants (Shittu et al., 2015). This may indicate atmospheric deposition of the heavy metal in plants.

The major pathway for Mn absorption is oral via the gastrointestinal tract (Nadaska, Lesny and Michalik, 2012). Manganese toxicity is prominent in the central nervous system (Huang et al., 1993; Calne et al., 1994) although cardiovascular (Brurok et al., 1997) and liver (Goering, 2003), as well as reproductive and developmental toxicities (Sanchez et al., 1993), have also been noted. Hence the effect of manganese toxicity may be responsible for some of the HM-related obstetric outcomes and casualties highlighted in this study (Section 2.15). However, all analysed HM in this study had a manganese concentration within the permissible limit; but this does not render otiose further monitoring for quality control.

4.4.9 Nickel

From Figure 4.12 it can be observed that the concentration of nickel was highest in HM8, an (uncertified) sample (8.234±0.352 ppm), which is 5 times higher than the permissible limit at 1.6 ppm (WHO, 2007). Other HM samples above the permissible limit include certified samples HM1, HM2, HM9 and uncertified samples HM3, HM5 and HM10, while HM4, HM6 and HM7 had a concentration of nickel below the permissible limit (Table 4.2).

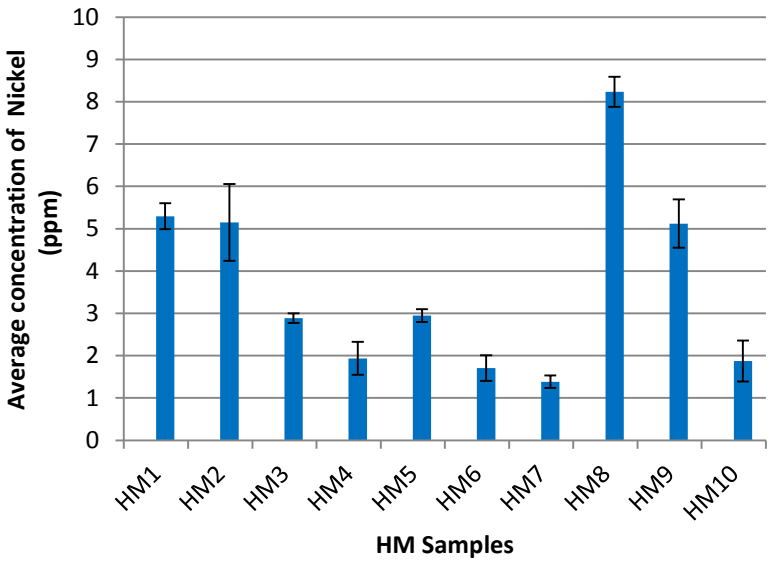


Figure 4.12: Average concentration of nickel in HM samples

The concentration of nickel ranged between (1.384±0.148 ppm and 8.234±0.352 ppm), which is lower compared with those found in selected HMs previously analysed in Nigeria (0.730 ppm to 54.000 ppm) (Onwordi, Agbo and Ogunwande, 2015) but close to those reported in China (0.4132 to 5.1210 ppm) (Zheng et al., 2013) and Turkey (0.650 to 8.690 ppm) (Soylak et al., 2004). Nonetheless, the concentration of nickel in HM6 (1.706±0.299 ppm) (Table 4.2) was close to that reported for the same HM (M&T capsule) in a previous

article at 2.480 ppm (Onwordi, Agbo and Ogunwande, 2015). The difference could be as a result of variation in the location, although findings from this study showed no significant variation in concentration of nickel between locations (Appendix XIII). The inter-batch variation in this study was an intra-state variation in location, while the sample obtained for the analysis in the publication mentioned was obtained in Lagos. This may suggest inter-state variation, probably as a result of a different manufacturing factory or source of the raw material, though both concentrations (cited and observed in this study) were above permissible limits.

Nickel is naturally occurring and essential trace metal for the nutritional needs of plants (Scott-Fordsmand, 1997; Barceloux, 1999; Klaassen, 1996). The major sources of trace metal pollution (especially As, Cr, Cu, Mn and Ni) in water bodies are non-ferrous metal smelters and domestic wastewater effluents with easy accumulation of these metals in aquatic plants (WHO, 1991; Barałkiewicz and Siepak, 1999). Nickel gets into the ambient air from activities such as combustion of fuel oil, diesel oil and coal, including the incineration of various wastes (Clayton and Clayton, 1994; Von-Burg, 1997) and may eventually find its way into flora.

Nickel is widely distributed evenly through the soil, but essentially accumulates on the surface of soil through deposition from various agricultural and industrial activities. A few foods may have acquired nickel through the manufacturing process, but in many cases, it is present naturally (WHO, 1991). The concentration of nickel in dumpsite soils was reported to be generally lower than the permissible limits but was higher than the permissible thresholds in the plants which grew on them (Shittu et al., 2015). There was report of a rapid increase in the level of Pb, Cd, Cu, Ni, and Zn with proximity to highways; with the metal concentration at a 50 m distance from the road nearing that of the natural background concentrations of the metals at the control sites. This may suggest metal deposits from automobile fuel combustion (Fakayode and Olu-Owolabi, 2003). Nonetheless, the concentration of nickel found in dumpsite soil in Ekiti State was below WHO limits; likewise, the nickel concentration found in the plants grown on them (Ayeni, Ajayi and Odeyemi, 2017). The effect of poor petroleum product handling on the environment has been reported, with a significant amount of nickel found in the soil around petroleum handling areas in Nigeria (Adeniyi and Afolabi, 2002) as also seen in other parts of the World (Davies,1997). Also, nickel concentration was above WHO limit in soil samples taken from auto workshops in southwest Nigeria (Fakayode and Olu-Owolabi, 2003; Ololade, 2014). These factors may also account for bioaccumulation of nickel in Ekiti State flora; which may eventually end up in the HMs studied.

Oral exposure is the main route of nickel toxicity which mainly targets the kidney and also affects the immune system, blood and the cardiovascular systems (Young, 1995; Nielsen et al., 1999). In addition, general population exposure to nickel is primarily through oral ingestion via contaminated water and food (Cempel and Nikel, 2006), of which HM is a part. Hence the focus of toxicity again targets the kidneys as with other metals such as arsenic, cadmium, lead and mercury. At present, there is no information on the chronic toxicity of nickel in humans.

4.4.10 Selenium

Selenium concentrations in all the HM samples were below the 25 ppm permissible limit (WHO, 2007). The highest concentration was found in HM6 at 9.023±0.789 ppm and the lowest concentration found in HM3 at 2.281±0.114 ppm (Figure 4.13).

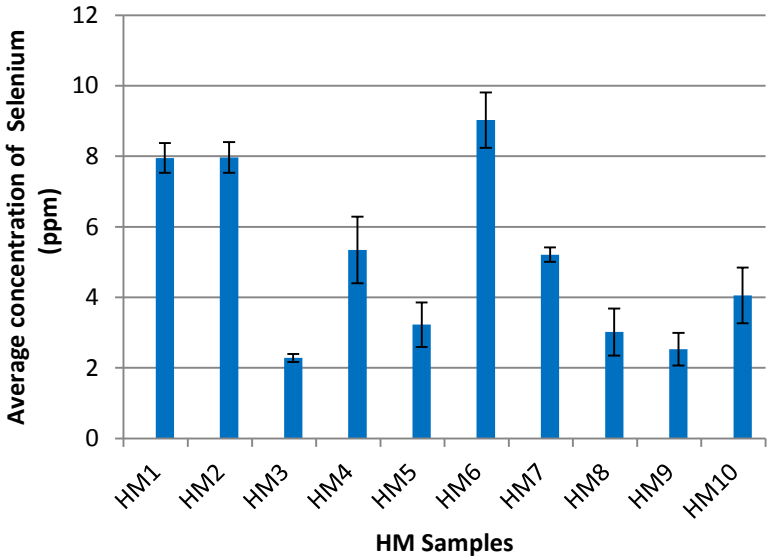


Figure 4.13: Average concentration of selenium in HM samples

The range of selenium concentration in this study is similar to that reported in HM from previous studies (0.232 to 6.460 ppm) in China (Zheng et al., 2013).

There was no significant variation in the concentration of selenium across the various locations for each HM sample (Appendix XIII). This is with the exception of HM7 (uncertified HM), which showed a significant variation in selenium concentration between different locations.

Selenium is a naturally occurring mineral required for good health and is abundant in a diverse diet from vegetables grown on seleniferous soils, grains, cereals and animal products such as fish, milk, eggs and meat (Lipiec et al., 2010; Fairweather-Tait et al., 2010;

Surai et al., 2009). The selenium dietary intake is influenced naturally by the selenium composition of soils on which the plant is grown (Thompson, 2004). Industries such as coal burning, mining and smelting of sulphide ores further contribute significantly to the pollution of the environment with selenium, including the soil (ATSDR, 2009). Previous analysis of farmland soil samples in Ekiti State did not detect selenium (Adeyeye, 2005). Analysis of soil in Kogi State showed one of the examined sites had selenium level above WHO limits (Oklo et al., 2013). Another study reported the concentration of selenium above the WHO limit in shallow groundwater in the residential and commercial areas of the metropolis of Ibadan, Nigeria (Effiong, 2017). These publications indicate that the bioavailability of selenium varies across locations, as reported by Thompson (2004), and its bioavailability in plants.

Selenium is essential in the regulation and maintenance of numerous cellular functions such as signal transduction, enzyme catalysis and at trace level for brain function (Rayman, 2000). However, it can result in acute or chronic toxicity at increased serum concentrations (Nuttall, 2006). At higher concentrations in soil selenium is taken up by plants leading to acute or chronic selenium toxicity in humans when consumed (Fan and Kizer, 1990; ATSDR, 2003). Its effect at increased levels is associated with several clinical findings and symptoms such as fatigue, nausea, memory loss, muscle and joint pain, musculoskeletal complaints, nail discoloration or brittleness, diarrhoea and vomiting (MacFarquhar, Broussard and Melstrom, 2010). Of all the metals, selenium has the smallest range between dietary deficiency and toxicity, deficient below 40 µg/day and toxic over 400 µg/day (Nuttal, 2006). As a result, selenium requires meticulous control of its human exposure. From the point of view of this study, this may not be a source of concern, considering concentrations were all below permissible limits. However, precautionary screening is essential as a requirement for certification of HMs in Ekiti State.

4.4.11 Zinc

Figure 4.14 shows HM2 (uncertified sample) had the highest concentration of zinc (131.428±6.662 ppm) and was the only one with a concentration above the permissible limit of 100 ppm (WHO, 2017). The range in zinc concentration was between 5.557±0.748 ppm and 131.428 ppm. This is higher than those reported previously in Nigerian HM, 8.500 and 38.900 ppm (Onwordi, Agbo and Ogunwande, 2015), 0.030 ppm and 0.420 ppm (Idu, Oghale and Jimoh, 2015), in Turkey 7.840 and 47.600 ppm (Soylak et al., 2004) and in Romania 18.800 and 64.400 ppm (Senila et al., 2014). But it is similar to the range detected in HM in China (6.574 and 162.410 ppm) (Zheng et al., 2013). The concentration of zinc in HM6 from this research (19.286±1.269 ppm) (Table 4.2) was higher than that previously

reported for the same sample (2.350 ppm) obtained from another state of Nigeria (Onwordi, Agbo and Ogunwande, 2015).

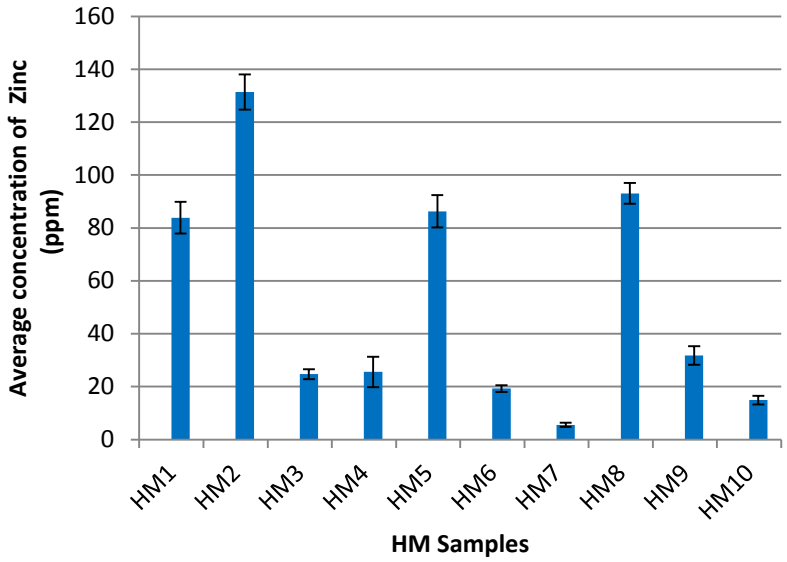


Figure 4.14: Average concentration of Zinc in HM samples

The explanation for this is similar to those with similar findings discussed earlier. Significant variation was observed in the concentration of zinc in HM6 samples obtained from different locations in the state (Appendix XIII). Likewise, there was no significant variation in the concentration of zinc across various locations in the state for the remaining HM samples, as further discussed in Section 4.4.13.

Human metabolism including the translation and replication of genetic material is driven by proteins and enzymes which contain zinc (Keith, Chih-chin and Carol, 2000). Zinc enters the environment through natural and anthropogenic activities. However, anthropogenic sources significantly account for more zinc in the environment than natural sources (Davies and Jones, 1988). The major sources of anthropogenic zinc in the environment (air, soil and water) are associated with metallurgic and mining activities (Wuana and Okieimen, 2011); in addition to the use of zinc in commercial products such as wood preservatives and fertilisers (Chaney, 1993).

In Ekiti State some of these activities, such as illegal mining, application of fertilisers and improper waste disposal are pursued and possibly contribute to anthropogenic zinc deposits. A study reported levels of zinc above the permissible limit in some agricultural farmlands (Adeyeye, 2005) in Ekiti State, although another study reported zinc levels in agricultural

farmlands below the permissible limit (Isinkaye, 2012). This may be a reflection of various activities going on in the various locations; and may be a result of the persistent application of zinc-containing fertiliser or pesticides on the latter (Jones and Jarvis, 2015). However, the concentration of zinc in an Ekiti State dumpsite soils was reported to be lower than the permissible limits (Shittu et al., 2015). Additionally, the uptake of zinc in plants found in oil-spill affected agricultural areas of Nigeria resulted in levels below the permissible limit for plants (Nwaichi et al., 2014). Another study reported Zn levels above the permissible limit in an auto workshop (Ololade, 2014). In addition, a published report showed an association between fuel combustion in cars with zinc deposits in plants (Fakayode and olu-owolabi, 2002) and contributions from an atmospheric source (Shittu et al., 2015). However, the results from this research showed all the HM samples had Zn levels below the WHO permissible limits, with the exception of HM2. So the presence of zinc in HM2 may reflect the activity around the source and the materials used in the manufacturing process. None of this can be ascertained because HM2 is not certified, with little or no information available on its content (see Table 4.1).

Absorption of toxic a concentration of zinc results in immediate symptoms such as nausea, abdominal pain, vomiting and later effects such as dizziness, lethargy and anaemia (Porea, Belmont and Mahoney, 2000). Chronic toxicity of zinc has been reported to be associated with copper deficiency (Ogiso et al., 1974). Copper deficiency presents with common symptoms which include leukopenia, anaemia, neutropenia, hypocupremia, impaired iron mobilisation, elevated plasma cholesterol and Low-Density Lipoproteins (LDL): High-Density Lipoproteins (HDL) cholesterol and abnormal cardiac activities (Sandstead, 1995; Fiske, McCoy and Kitchens, 1994). Hence HM2 with a zinc concentration above the permissible limit may be unhealthy, especially with prolonged use. The daily oral limit is discussed in section 4.4.12

4.4.12: Estimation of daily metal intake

With little or no information available, the amount of metal per dose cannot be estimated for some of the HM samples. This applies to all uncertified samples with no or an ambiguous prescription such as “daily for two days” (HM5) or “one spoon twice daily” (HM4) (Table 4.1). Nonetheless, certified HM samples had their prescription properly written out, which is a regulatory requirement. Hence it was possible to estimate the daily metal intake.

For example, Cd in HM1 has a concentration of 0.723 ppm (Table 4.2), which is the same as 0.723 µg/g

Each capsule’s (HM1) content weighed 0.366 g

Therefore each capsule contains 0.366 X 0.723 = 0.241 µg of Cd.

According to Table 4.1, the dosage for HM1 is 3 capsules twice daily. Thus a total of 6 capsules a day is recommended for HM1 according to the manufacturer.

Therefore daily intake of Cd from HM1 = 6 X 0.241 = 1.446 µg/day

Calculations for the other target metals that were above permissible limits in the HM samples that had a prescription are contained in Table 4.4 below.

Table 4.4: Daily oral exposure of metals in some HM sample

Metals	HM1 (0.366 g/capsule)		HM3 (0.322 g/capsule)		HM6 (0.277 g/capsule)		HM9 (0.342 g/capsule)	
	Amount in capsule (µg)	Daily intake (µg/day)	Amount in capsule (µg)	Daily intake (µg/day)	Amount in capsule (µg)	Daily intake (µg/day)	Amount in capsule (µg)	Daily intake (µg/day)
Cd	0.241	1.446	0.171	0.684	0.129	0.774	0.157	0.942
Cr	3.159	18.952	-	-	-	-	1.337	8.022
Cu	5.539	33.234	4.751	19.004	3.142	18.85	20.140	120.000
Ni	1.055	6.33	0.928	3.712	-	-	1.752	10.512

Cd = Cadmium; Cr = Chromium; Cu = Chromium; Ni = Nickel

Cadmium has a daily oral limit of 60 µg/day, chromium a daily limit of 20 µg/day (Table 4.3). The daily oral limit for copper and nickel are not stated. From these set limits the HM samples are within the daily oral limit of cadmium and chromium. This indicates that though the concentration of metals may be above the permissible limit in the HM, the quantity taken determines the amount of metals consumed by a user. While this may be true, the duration and frequency of use may have a significant impact on the amount of heavy metals which may gradually accumulate in the user even at levels below permissible limits. As an example, chronic low exposure to cadmium caused a decline in glomerular filtration rate (GFR) and damage to the renal proximal tubular in experimental animals (Thijssen et al., 2007).

But most of the HM samples did not include the duration of use, and it is important in discussing bioaccumulation of metals in humans. Therefore regulatory steps need to be taken to ensure prescriptions are properly written to ensure safety. In addition, sourcing of plant materials for HM is essential considering various activities affect their heavy metal composition, as discussed in Section 4.4.2 to 4.4.11. Accordingly, the intra-batch and inter-batch concentration of heavy metals in HM samples is analysed.

4.4.13: Intra-batch and Inter-batch concentration variability

The elemental variation between samples from each HM (Intra-batch variation) and the variation of the average elemental concentration in HMs between different geographical

locations (inter-batch variation) were examined and presented in Tables 4.5 and 4.6 for HM 1 (others in Appendix XIII). A two-way ANOVA statistical analysis was then carried out to determine how significant these variations were. The result of the statistical analysis is presented in Table 4.7 for HM1, the other results in Appendix XIII.

As earlier discussed for the individual metals analysed in Section 4.4.2 to 4.4.11, statistical analysis of intra-batch variability showed varying results. Analysis of target metals in HM 1, for example (Table 4.7), showed some had significant intra-batch variation, as seen with arsenic across all batches (others in Appendix XI). This may indicate inconsistency in the within-batch samples as it contains various constituents (Table 4.1). However, adequate steps were taken to ensure the homogeneity of the samples through their thorough mixing before analysis (Section 3.6.3.2). On the other hand, cadmium only had significant intra-batch variability across three batches. Furthermore, analysis of Cd from the 4^{th} batch (location 4) and 5^{th} batch (location 5) of HM1 showed no significant intra-batch variation, which may suggest consistency within the sample.

Table 4.5: Intra-batch concentration of metals in HM1

Element		Concentration (ppm)				
Arsenic		Sample 1 n=3	Sample 2 n=3	Sample 3 n=3	Sample 4 n=3	Average Conc
	Location 1	0.228±0.018	0.231±0.014	0.212±0.012	0.228±0.015	0.225±0.015
	Location 2	0.212±0.012	0.249±0.013	0.253±0.010	0.254±0.013	0.242±0.012
	Location 3	0.218±0.012	0.209±0.019	0.213±0.022	0.234±0.012	0.219±0.016
	Location 4	0.263±0.013	0.237±0.016	0.293±0.017	0.244±0.017	0.259±0.016
	Location 5	0.230±0.017	0.198±0.017	0.233±0.015	0.214±0.014	0.219±0.016
Cadmium	Location 1	0.991±0.041	0.919±0.034	0.882±0.032	0.881±0.042	0.918±0.037
	Location 2	0.741±0.042	0.729±0.024	0.672±0.039	0.641±0.033	0.696±0.036
	Location 3	0.731±0.042	0.719±0.036	0.682±0.038	0.626±0.037	0.690±0.036
	Location 4	0.714±0.043	0.702±0.028	0.665±0.022	0.599±0.047	0.676±0.037
	Location 5	0.674±0.038	0.662±0.028	0.625±0.029	0.559±0.041	0.637±0.034
Chromium	Location 1	8.333±0.760	8.226±0.865	8.139±0.509	9.034±0.367	8.437±0.629
	Location 2	7.881±0.652	7.796±0.343	8.693±0.588	7.990±0.685	8.089±0.565
	Location 3	9.097±0.733	9.791±0.336	8.892±0.470	8.985±0.838	9.189±0.596
	Location 4	8.119±0.756	8.022±0.674	8.225±0.931	8.921±0.531	8.327±0.726
	Location 5	9.016±0.768	8.812±0.503	9.714±0.363	8.903±0.586	9.111±0.558
Copper	Location 1	15.441±1.317	16.453±0.815	14.500±0.957	15.557±1.219	15.486±1.079
	Location 2	14.472±0.915	15.176±0.815	14.271±0.573	14.368±0.882	14.574±0.795
	Location 3	14.726±1.019	13.989±0.756	14.815±0.791	14.079±0.917	14.437±0.874
	Location 4	13.861±0.835	14.573±0.437	13.684±0.571	13.771±0.659	13.979±0.629
	Location 5	17.096±1.324	17.798±0.926	16.892±1.065	16.987±1.142	17.196±1.114
Lead	Location 1	4.805±0.445	4.675±0.187	4.283±0.150	4.474±0.593	4.560±0.344
	Location 2	4.180±0.263	4.357±0.145	3.838±0.176	3.987±0.235	4.091±0.205
	Location 3	2.560±0.420	2.672±0.095	2.276±0.055	2.367±0.309	2.468±0.219
	Location 4	4.397±0.595	4.903±0.068	4.075±0.261	4.238±0.354	4.403±0.320
	Location 5	4.386±0.471	4.722±0.145	4.063±0.103	4.183±0.200	4.339±0.230
Manganese	Location 1	61.709±3.203	59.874±1.413	53.658±1.788	51.965±2.497	56.801±2.225
	Location 2	57.855±4.069	60.020±2.675	49.803±2.963	53.110±3.743	55.197±3.362
	Location 3	58.141±3.731	61.305±2.667	52.089±2.767	51.396±3.620	55.733±3.196
	Location 4	56.813±4.556	59.318±3.028	52.490±2.623	47.653±4.143	54.068±3.587
	Location 5	55.452±5.530	56.788±3.954	50.129±4.044	47.249±4.509	52.404±4.509
Nickel	Location 1	6.188±0.369	5.853±0.190	5.137±0.228	5.444±0.299	5.655±0.271
	Location 2	4.476±0.294	4.540±0.155	3.724±0.184	4.031±0.262	4.193±0.224
	Location 3	6.170±0.374	6.335±0.268	5.518±0.278	5.425±0.363	5.862±0.321
	Location 4	4.936±0.391	5.441±0.338	4.613±0.298	4.657±0.450	4.912±0.369
	Location 5	6.069±0.386	6.405±0.329	5.747±0.338	5.190±0.384	5.853±0.359
Selenium	Location 1	9.402±0.702	9.067±0.523	8.351±0.561	8.658±0.632	8.869±0.604
	Location 2	9.078±0.347	9.143±0.207	8.327±0.236	8.633±0.314	8.795±0.276
	Location 3	6.685±0.310	7.594±0.215	6.778±0.225	7.430±0.321	7.122±0.268
	Location 4	8.613±0.370	9.441±0.410	9.256±0.463	8.257±0.522	8.892±0.441
	Location 5	6.287±0.566	6.623±0.509	5.965±0.518	5.408±0.564	6.071±0.539
Zinc	Location 1	89.709±7.725	95.374±4.935	80.658±3.310	81.964±5.879	86.926±5.462
	Location 2	86.643±8.979	90.708±5.584	79.892±4.281	80.199±8.319	84.360±6.791
	Location 3	87.800±5.941	89.964±2.190	80.148±4.290	82.055±6.143	84.992±4.641
	Location 4	85.239±5.742	82.425±5.690	71.597±5.649	73.241±5.801	78.126±5.721
	Location 5	90.635±9.518	88.970±5.764	81.312±6.515	78.755±7.811	84.918±7.402

In HM samples obtained across 5 locations, there was no significant inter-batch variability of all target elements in HMs 2, 3, 4, 5, 9 and 10 (Appendix XIII). There was a significant inter-batch variation of arsenic in HM1 (Table 4.7). Other HMs had significant inter-batch variation of Mn and Zn in HM6; Se in HM 7; Mn and Pb in HM 6. Herbal medicines 1 and 6 are manufactured by the same registered herbal medicine company in Nigeria. Hence, to meet commercial demand, it possibly sources its raw material from different places with likely anthropogenic contamination from e.g. petrochemicals and agrochemicals, as discussed earlier in Sections 4.4.2 to 4.4.11. This may be responsible for the significant inter-batch variation in arsenic for HM1 (P = 0.001) (Table 4.7), manganese and zinc in HM6 (P = 0.027 and 0.010 respectively) (Appendix XIII).

Table 4.6: Inter-batch concentration of metals in HM1

Element	Concentration (ppm)					
	*Location 1 n=12	*Location 2 n=12	*Location 3 n=12	*Location 4 n=12	*Location 5 n=12	Average conc
Arsenic	0.225±0.015	0.242±0.012	0.219±0.016	0.259±0.016	0.219±0.016	0.233±0.018
Cadmium	0.918±0.037	0.696±0.036	0.690±0.036	0.676±0.037	0.637±0.034	0.723±0.036
Chromium	8.437±0.629	8.089±0.565	9.189±0.596	8.327±0.726	9.111±0.558	8.630±0.615
Copper	15.486±1.079	14.574±0.795	14.437±0.874	13.979±0.629	17.196±1.114	15.134±0.898
Lead	4.560±0.344	4.091±0.205	2.468±0.219	4.403±0.320	4.339±0.230	3.972±0.264
Manganese	56.801±2.225	55.197±3.362	55.733±3.195	54.068±3.587	52.404±4.509	54.841±3.376
Nickel	5.655±0.271	4.193±0.224	5.862±0.321	4.912±0.369	5.853±0.359	5.295±0.309
Selenium	8.869±0.604	8.795±0.276	7.121±0.268	8.892±0.441	6.071±0.539	7.950±0.423
Zinc	86.926±5.462	84.360±6.791	84.992±4.641	78.126±5.721	84.918±7.402	83.864±6.003

*Locations where HM samples were obtained (refer to section 3.4.6 chapter 3).

The manufacturing process may also be a possible contributor to heavy metal contamination, e.g. from transport, the contact surface material of processing equipment, use of contaminated water, additives, preservatives and packaging (Morgan, 1999), which may vary across batches. The contribution of the manufacturing process to batch variability may be more prominent in small government-unregistered manufacturer which still employs a crude process in the manufacture of their products, as may be observed in HM 7 and 8 appearance and packaging (Appendix XVII). These crude manufacturing processes possibly keep production cost minimal and thus enhance profit.

Table 4.7: Intra-batch and inter-batch variability of heavy metals in HM1

Metals / Location	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	*P*-value	Sig.
Arsenic				
Location 1	0.003	Yes		
Location 2	0.003	Yes		
Location 3	0.018	Yes	0.001	Yes
Location 4	0.010	Yes		
Location 5	0.008	Yes		
Cadmium				
Location 1	0.010	Yes		
Location 2	0.004	Yes		
Location 3	0.029	Yes	0.111	No
Location 4	0.113	No		
Location 5	0.398	No		
Chromium				
Location 1	0.058	No		
Location 2	0.021	Yes		
Location 3	0.008	Yes	0.282	No
Location 4	0.314	No		
Location 5	0.397	No		
Copper				
Location 1	0.068	No		
Location 2	0.002	Yes		
Location 3	0.070	No	0.243	No
Location 4	0.003	Yes		
Location 5	0.040	Yes		
Lead				
Location 1	0.011	Yes		
Location 2	0.002	Yes		
Location 3	0.001	Yes	0.954	No
Location 4	0.016	Yes		
Location 5	0.094	No		
Manganese				
Location 1	0.728	No		
Location 2	0.034	Yes		
Location 3	0.081	No	0.399	No
Location 4	0.828	No		
Location 5	0.496	No		
Nickel				
Location 1	0.002	Yes		
Location 2	0.003	Yes		
Location 3	0.004	Yes	0.691	No
Location 4	0.218	No		
Location 5	0.013	Yes		
Selenium				
Location 1	0.001	Yes		
Location 2	0.119	No		
Location 3	0.879	No	0.398	No
Location 4	0.120	No		
Location 5	0.721	No		
Zinc				
Location 1	0.173	No		
Location 2	0.717	No		
Location 3	0.039	Yes	0.665	No
Location 4	0.082	No		
Location 5	0.665	No		

The effect of manufacturer's inter and intra-batch variability of excipients in pharmaceutical products on processability, quality, and performance of the finished drug product cannot be underestimated (Chatlapalli and Rohera, 2002), because it affects quality assurance. Likewise, intra and inter-batch variations of impurities of illicit drugs have been used to characterise the source and route of some illicit drugs (Kunalan et al., 2009). Variability in HM metal concentration may also be used to evaluate the impact of the activities going on in the areas where the raw materials are sourced (if declared), although other factors may also contribute to such variability. Regardless, detection and variability of heavy metals in HM still raises concerns with regard to their safety, as discussed earlier in Section 1.6.

In this study, there was inter-batch and intra-batch variation of target metals analysed in the various HM samples, as seen in Tables 4.5 and 4.6 respectively for HM1 (others in Appendix XI and XII). Challenges in HM quality control such as variable quality and source of the raw material together with the varying chemical and natural components of plant materials have been said to be possible contributors to inter-batch variation (Kunle et al., 2012). Since the source of the raw material possibly contributes to the presence of metals in HM samples, documentation of such by manufacturers has become imperative for detailed and correlation studies. However, most of the HM analysed in this research had no information on the source of the raw material.

4.4.14 Summary

In summary, all the HM samples both certified and uncertified, contain heavy metals and some above permissible limits. The presence of these metals in soil and water bodies with subsequent bioaccumulation in plants results from various human activities. Improper waste disposal, mining, poor handling of petrochemical products, by-products of car fuel combustion and other human activities that are peculiar to the study environment have been reported markedly to increase heavy metal deposits in plants (Section 4.4.2 to 4.4.11). This may also contribute to significant inter-batch and intra-batch variation, depending on where the plant products are sourced. Table 4.8 below gives a brief overview of the HM samples and their status with regard to the permissible limits. The green cells indicate metals present within the permissible limit; the red cells indicate those above permissible limits, while the purple cells indicate undetected metals.

Table 4.8: Table showing HM sample metals above and below the permissible limit

	Arsenic	Cadmium	Chromium	Copper	Lead	Mercury	Manganese	Nickel	Selenium	Zinc
HM1										
HM2										
HM3										
HM4										
HM5										
HM6										
HM7										
HM8										
HM9										
HM10										

Below permissible limit | Above permissible limit | Not detected

According to Table 4.8, HM2 had the most metals (6) above the permissible limit and was the only HM sample containing lead and zinc above such limit. Herbal medicine 2 is an uncertified herbal product, bearing no manufacturer's name, content or dosage (Table 4.1). However, its appearance is similar to that of the charred products of unknown substances (Appendix XVII). The charring, which is likely a production process, may have further contributed to the presence of metals.

While the estimation of oral intake was not possible for some of the HM samples, due to the absence of needed information, other HM samples provided this (Table 4.1). However, despite being able to estimate the daily oral intake of metals from some of the HM samples, lack of information on duration of use poses a further setback. This is because bioaccumulation of metals to toxic levels is possible from continuous exposure even below permissible limits, as discussed in sections 4.4.2 to 4.4.11. Noteworthy is the fact that 53% of the respondents in this study used HM more than 10 times in the preceding 2 years (Table 2.11).

CHAPTER 5: HERBAL MEDICINE USE IN EKITI STATE; GENERAL DISCUSSION OF EPIDEMIOLOGICAL AND ANALYTICAL FINDINGS

5.1 General discussion

The high awareness (100%) of HM in this study population (Section 2.12.4) may be a result of the many historical perspectives of HM use (Olayiwola, 1987). The introduction of orthodox medical care and its advances in Ekiti State provided a choice in health care decisions for the populace. Whilst orthodox medicine with the use of pharmaceutical compounds has developed through rigorous scientific procedure, the same cannot be said of African traditional medicine, which has HM at the heart of its practice. As a result, the safety and efficacy of HM remain problems, though in this study 85.0% of the respondents (n=1265) have used HM in the last 2 years (Table 2.8) and 53.0% have used it over 10 times in the same period. Conversely, deaths or hospitalisations due to HMs are rare (Nasri and Shirzad, 2013), but a lot of concerns have been expressed about the adulteration and contamination of HM and subsequent health consequences (Ang and Lee, 2000; Ang, 2008; Ernst, 2002a; Snyman et al., 2005).

In a country like Nigeria, HM samples and associated products are sold without any compulsory toxicological or safety assessment, due to lack of effective analytical instrumentation and weak regulations to guide the manufacturing process and quality control standards. In addition, these herbal products are persistently provided to consumers without a prescription in many instances (Bandaranayake, 2006), as highlighted in this study, especially in the case of uncertified HMs (Table 4.1). This is coupled with the fact that hazards associated with a substandard product are not fully understood by the consumer. Accordingly, a NAFDAC registration number (as seen in Table 4.1) often distinguishes certified HM from uncertified to ensure safety in Nigeria (Ofuani, Kuye and Ogundele, 2015), and this was recognised by 90.0% of the respondents in this study (Table 2.7). This makes consumers depend fully on the NAFDAC registration number printed on the product to show that the product has passed through thorough clinical or laboratory tests and is certified fit for consumption and distribution. However, 37.3% of the respondents (n=1075) still used uncertified HM, while 31.9% used both uncertified and certified (Table 2.10).

Lots of counterfeited products have been sold to consumers in Nigeria with fake NAFDAC numbers or without one, yet only NAFDAC agents know if a registration number is authentic or fake (Oladosu, Taiwo and Oyeleye, 2016). Though NAFDAC numbers can be verified online to ascertain the authenticity of any product (Hoax, 2012), this will add to the end-user cost of the product as a result of the financial implication of product registration and provision

of such service. It is notable that 31.9% of the sample population (n=1360) cited affordability as a strong reason for choosing HM (Table 2.13); the most common reason for use among people 70 years and above (Figure 2.2) and those with no formal education (Figure 2.5). Hence these groups are likely to be affected. High hospital costs was a common reason (33.5%) cited for avoiding hospital treatment (Table 2.14) (n=1181) and the most common reason given by those with no formal education (Figure 2.6). Thus it would appear to be in the interest of manufacturers of HM to keep the cost of HM as low as possible, keeping it more affordable than conventional medicine.

The majority (83.6%) of respondents (n=1075) believed HM to be effective (Table 2.15). Some publications have reported that patients view the use of HM in a positive light and are satisfied with its therapeutic effect, while also expressing their disappointment at the effectiveness and safety of orthodox medicine (Huxtable, 1990; Abbot and Ernst, 1997). Conversely, 57.0% of respondents regard HM as unsafe (Table 2.16). This may be interpreted to suggest that people use HM because it is effective and affordable, among other reasons, but are concerned about its safety. This concern is confirmed with the detection of heavy metals in HM and its potential bioaccumulation in the body with consequent harm to health (Section 4.4.2 to 4.4.11). Yet what are the choices available to respondents?

The increased use of HM has been attributed to limited availability and affordability of orthodox medicine (Omotosho, 2010). Poor service delivery and high hospital costs have, among other reasons, been identified in this study as discouraging patronage of the orthodox health system (Table 2.9). Also of note is the high disparity in income groups, especially considering that low-income groups were the majority (82.0%) in the study population (Table 2.6) and out-of-pocket expenditure accounts for about 69% of health care financing in Nigeria (Figure 2.7). On the other hand, however, the majority of low-income earners cited their disinclination to use hospitals to poor service delivery (Figure 2.9) and cited effectiveness as their main reason for HM use (Figure 2.4). Considering the aforementioned factors, the reality may be that orthodox medical care is not within the reach of most of the population. However, findings in this study suggest an alternate hypothesis: that, despite low income, the need for effective, efficient and dignified treatment is desirable. Being of low income should not imply deserving poor service delivery in the orthodox health care setting, or deserving of medication which may not be effective after all. A publication further supports this point in its dual population survey finding in Nigeria, reporting a vast majority believe it takes a longer time to see a doctor in an orthodox than in a traditional medicinal setting. It also reported that a vast majority of the respondents believe the caregiver devotes more time to the patient in the traditional than in the orthodox setting

(Nottidge, Akpanudo and Akinbami, 2011). It may appear that people try to avoid Orthodox health facilities as much as possible while trying to maximise a perceived effective, affordable and available alternative. Although it may not be safe, it is provided in a sympathetic environment with freedom to express fears and optimism without intimidation (Section 1.5.1). However, this does not downplay the fact that safety of HM is becoming a major problem as discussed in Section 1.6.2 and also from results obtained in this study about the potential toxicity of HM (Section 4.4).

Thus, the negative contributions of human activities such as mining, petrochemical handling, waste disposal, and illegal adulteration, especially in Ekiti State, may have contributed to HM toxicity through contamination with heavy metals and adulteration with pharmaceutical compounds. The GC-MS analysis of the 10 most commonly used HM samples in this study (Table 2.12) detected none of the target pharmaceutical compounds. This is a positive finding of public health importance. Also, the non-detection of pharmaceutical compounds in HM samples is consistent with findings from the survey aspect of this research. This is because adulteration of HM samples with an expensive orthodox medication will increase the cost of the HM; a consequence for its affordability. It is notable that affordability was the main reason (31.9%), after effectiveness, cited for use of HM (Table 2.13) and high hospital cost, after poor service delivery, was the main reason (33.5%) cited for the avoidance of orthodox health facilities (Table 2.14). Likewise, adulteration with a perceived ineffective orthodox medicine will be counterproductive. On the other hand, a publication reported the adulteration of HM with HIV drugs in Nigeria (Gini et al., 2016). But then HIV medication is free in Nigeria (NACA, 2017); therefore its use as an adulterant in a proclaimed HIV-curing HM may not be at an additional cost to its manufacturer.

The analyte of interest in this study included nine selected commonly abused pharmaceutical compounds and one central nervous system (CNS) stimulant (acetaminophen, caffeine, chlorpheniramine, codeine, dexamethasone, diazepam, diclofenac, fluoxetine, ibuprofen, tramadol) (Table 3.1). These substances have been reported to be present in some HMs (Vaclavik et al., 2014a and 2014b) and are also known to be medicines commonly misused in Ekiti State (Akindutire and Adegboyega, 2012; Atoyebi and Atoyebi, 2013). The epidemic of drug abuse, especially codeine, in Nigeria was brought to light in a recent British Broadcasting Cooperation (BBC) documentary (2018), resulting in the closure of three pharmaceutical companies implicated by NAFDAC (Vanguard newspaper, 2018). With the exception of HM2 and HM3, all the HMs offered pain relief as one of their many therapeutic benefits. Therefore it was thought likely that being the most commonly used types identified from the survey (Table2.12), they would contain some of the commonly abused analgesics or CNS stimulants that are taken by members of the

study population. That was not the case as none was detected in this study. Additionally, the commonest CNS stimulant (caffeine) and analgesics such as tramadol, which may enhance performance, was expected to be present in HM2 which claimed to increase sexual power (Table 4.1) but was not detected.

The non-detection of the pharmaceutical compounds may be due to their presence below the detection limit of the method used in this study, therefore requiring a more sensitive method. However, mass spectral changes when compared to the background reading especially during method validation studies (Section 3.7) would have highlighted such presence. Hence the non-detection of these pharmaceutical compounds may suggest a lack of adequate knowledge or technology to successfully implement such adulteration. It may also suggest a sincere commitment to herbal medicine development to compete with orthodox medicine or unfavourable socioeconomic parameters. The finding from this study supports the latter suggestion, considering 82.0% of the respondents (n=1265) are low-income earners (Table 2.6) whilst the two other suggestions about lack of adequate knowledge or technology and a sincere commitment to herbal medicine development cannot be substantiated from the findings in this study.

In as much as non-adulteration is a positive finding in this research, the same could not hold for heavy metal analysis. Though most of the contamination of HM may not be deliberate, it may also be a product of human activities on the environment (Lokhande, Singare and Pimple, 2011). All the HM samples contained a minimum of two heavy metals (Cd and Cu) above the WHO permissible limits (Table 4.2). The highest level of heavy metals above the permissible limits was in HM2. It is an uncertified HM used to cure low sperm count and increase sexual power (Table 4.1), and the only sample which contained lead and zinc above the permissible limit.

Masculinity and male sexuality in Nigeria are interrelated in such a way that men still dominate decisions on sexual matters. On the other hand, femininity and female sexuality are associated with being seductive, passive, cooperative and submissive (Oladeji, 2008; Adebayo and Olonisakin, 2014b). As a result, there is a high expectation of sexual performance in men, associated with their remaining in charge. This high expectation has possibly led to the high use of sexual performance enhancers in Nigeria and their potential danger has dominated public discussion for some time (Vanguard, 2011). However, the use of herbs to improve sexual performance has resulted in little or no therapeutic effect even after prolonged use (Osakinle and Omoniyi, 2010). The prolonged use of one such sexual enhancer, HM2, will probably do more harm to the consumer. HM2 was found to have a high concentration of lead and zinc (Table 4.2) which have been reported to decrease libido and

cause other reproductive problems at toxic levels (Pinon-Lataillade et al., 1995 and Telisman et al., 2000). This fact is consistent with the finding of Osakinle and Omoniyi (2010) on the uselessness of such HM and their toxic potential. Besides, 57.3% of respondents in this study who took HM (n=1087) also believe that uncertified HM (such as HM2) is not safe.

All the HM samples contain cadmium above the permissible limit. Though it is a heavy metal with exposure pervasive in humans, at toxic levels it affects male fertility (Akinloye et al., 2006 and Telisman et al., 2007) and women's reproductive health (Nagata et al., 2005). The toxicity of metals detected in this study has been further discussed in Section 4.4.2 to 4.4.11. The prevalence of secondary infertility was reported in 2015 to be 83.7% in Ekiti State (Peter and Temi, 2016), meaning that most men were previously fertile. The finding from this study could, therefore, provide a link between the high use of HM contaminated with heavy metals and the prevalence of male infertility in the state. This may provide the basis for further research to establish a direct correlation by analysis of specific HM samples used by patients who present in the urology clinic over a period of time for heavy metals. A study reported that of the 218 patients who attended the fertility clinic in a Nigerian tertiary hospital, 84% with an abnormal seminal fluid analysis result had a history of herbal use, while only 16% of the patients who had no history of herbal use had an abnormal result (Enuh et al., 2012). Besides, there are other medical conditions that may be directly or indirectly linked to the use of HM, as discussion of individual metals in Sections 4.4.2 to 4.4.11 has shown how they contribute to the development of various diseases (Navas-Acien et al., 2007; .Park et al., 2000; Calne et al., 1994; MacFarquhar, Porea, Belmont and Mahoney, 2000; Broussard and Melstrom, 2010). In the absence of this type of research which combines epidemiological and toxicological analysis of HM, there is a greater likelihood of misdiagnosis as most medical professionals have underestimated the potential toxicity of some HM (Giveon et al., 2003).

Lack of this knowledge may be responsible for the low HM-related casualty and fatality observed in this study (Section 2.13). In retrospect, many of the other medical conditions forming the bulk of casualty and fatality figures in the medical records examined might have been attributed to HM use through heavy metals exposure, although there may be other confounding conditions. The effect of HM seems to have been underestimated, considering other important causation of medical conditions; so doctors may not specifically ask patients about HM use. Conversely, the benefit of HM cannot be underestimated, as many reports have highlighted this as discussed in Sections 1.3 and 1.4. It remains a growing and popular alternative to orthodox medicine, but its safety needs to be improved. As suggested by the majority (46.5%) of respondents in this study (n=1242) (Table 2.19), the government needs

to improve its regulation and monitoring of HM use to make it safer for the health of the populace.

In summary, this study has successfully addressed the research questions (see Section 1.10.1) as extensively discussed in Chapters 2 and 4. It deployed analytical method which was considered suitable for both pharmaceutical and heavy metal analysis through its validation (Section 3.8). Heavy metal concentrations were detected and quantified in HM samples above the WHO recommended permissible limit as a result of calculated LOD and LOQ values which were lower than those permissible levels (Table 4.3). However, for the analysis of pharmaceutical compounds, the calculated LOD values were either lower or higher than those reported in previous publications (Table 3.8). Hence comparatively, the lower LOD values indicate an improved sensitivity of the validated method in this study for the analysis of some pharmaceutical compounds such as ibuprofen, dexamethasone, and caffeine while higher values indicate less sensitivity (Table 3.8). The use of other analytical techniques previously deployed for analysis of HM e.g. LC-MS and HPLC (Wu et al., 2012; Becue; Van Poucke and Van Peteghem, 2011) may improve the sensitivity of the latter such as reported for diclofenac in herbal product using Liquid Chromatography with time-of-flight mass spectrometry (LC/TOF-MS) (Savaliya et al., 2009). However this study ensured that the procedure leading to the non-detection of the target pharmaceutical compounds was scientifically valid and acceptable as shown in Section 4.3.1.

In the case of population-based survey, it adopted various methods and approaches (e.g. use of both self and interview-administered questionnaires, open and close-ended questions, multi-sampling method, and use of more than one independent coder) in other to reduce bias (e.g. sampling bias, recall bias, data collection and interpretive bias) and to achieve the inclusivity necessary to obtain a valid result (Section 2.1 - 2.9). Nevertheless, a survey of farm settlements which was not carried out as part of this study may have improved inclusivity of more farmers as an example (Table 2.6), who had gone to the farm when survey was carried out in their towns. This may be improved on in future studies.

In analysing hospital data, the findings provided an idea of the health burden associated with HM use within the study population (Section 2.13). However, proper documentation of HM use in patient medical records could have brought about a better result. Nonetheless, the limitations from the analysis of hospital data and the survey (Section 2.15) did not impact the results obtained in this research in any significant way.

Finally, herbal medicine and its toxicity served as a tool to assess health-seeking behaviour and related factors in Ekiti State, as an alternative to an orthodox medical system offering unsatisfactory service.

CHAPTER 6: CONCLUSION, RECOMMENDATIONS AND FUTURE WORK

6.1 Conclusion

This research has examined the use and epidemiology of herbal medicine in Ekiti State and analysed HM sample for toxic constituents. A multidisciplinary approach combining methods from public health and analytical chemistry was used. Conclusions are drawn in this chapter from the findings of this research and answer provided to each of the five research questions posed in Section 1.11.1

What are the pattern and use of herbal medicine in Ekiti State and the influence of socio-economic factors?

The result of this study indicates a high use of HM within the study population and a pattern that shows a dissociation between knowledge of the risk associated with HM use and their use by the respondents. The study showed that majority of the respondents used HM and also more frequently in the previous two years. However, despite knowing the difference between government-certified and uncertified HM, the uncertified type was the most commonly used. This is a serious concern which should warrant serious public health intervention. Furthermore, in answering part of the research question, findings showed that the socioeconomic factors may not have been the major influence determining HM use within the study population. Although there was a significant association found between the use of HM and the age, gender, level of education, religion, annual income and occupation of the respondents, HM use was found to be highest in the middle-income class.

Across all the income classes, the principal reason cited for HM use was its effectiveness amidst other reasons such as affordability, availability and natural properties. The effectiveness of HM was corroborated in this study, as the majority of respondents who used HM saying it was effective for the intended purpose. What then are the issues causing disinclination in the use of available orthodox health facilities in Ekiti State? These are answered by the second research question.

What are the reasons for not using orthodox healthcare facilities in Ekiti State?

The result from this research suggests that if service delivery was improved and the cost of orthodox health facilities were reduced there would likely be an increase in patronage. This is so because a few of the respondents did attend hospital, but poor service delivery in orthodox health-care facilities was the main reason given for their avoidance, regardless of respondents' income class. Other reasons cited by respondents were high hospital cost and unorthodox belief. Increased patronage of orthodox health facilities may reduce the high use

of HM and especially uncertified HM as highlighted in this study and may also reduce the mortality and morbidity in the study population. The effect of this HM use on the health of the population is examined by the third research question.

What are the casualty and fatality figures associated with herbal medicine use in Ekiti State between the years 2010 and 2014?

Analysis of hospital records showed low HM-associated casualty and fatality figures. This means that, compared with other disease conditions accounting for hospital admission and death in Ekiti State, HM's contribution was minimal. However with questions not always being asked by clinicians about the use of HMs in admissions, perhaps the information is also limited in patient's records and awareness needs to be improved. The findings from this study, for example, showed detection of heavy metals in most commonly used HM within the study population at concentrations above WHO permissible limits. Hence, it is likely that heavy metals from HM use could have contributed significantly to the pathophysiology of some of the disease conditions (e.g. hypertension, diabetes, and renal failure) found in the hospital record as pointed out in the discussions in Section 4.4.2 to 4.4.11. This highlights the importance of taking a history of HM use from patients by clinicians and the dissemination of this research finding, for example, will be helpful in raising the much needed awareness. The fourth research question contextualizes the issues by examining the potential toxicity of HM.

What heavy metals and organic constituents are present in both uncertified and certified herbal medicine used in Ekiti State?

Analysis of selected HM detected none of the target pharmaceuticals, but heavy metals were detected above the WHO permissible limit. Cadmium and copper were present in all analysed HM samples at levels above the WHO permissible limits, while some other HMs had chromium, lead, nickel and zinc above the limit. There was significant intra-batch and inter-batch variation of heavy metals in some of the HMs analysed. This may indicate the non-homogenous nature of some of the samples as steps to homogenise them were thoroughly carried out in this study during sample preparation. Hence, there may be variation in the manufacturing process or the source of the raw materials. These variations may impact the concentration of constituents in the final product significantly, thus a harmonisation of manufacturing standard and declaration of source of raw materials may be helpful as further discussed in Section 6.2.1.

Some of the HM samples analysed, such as HM1, HM3, HM6, and HM9, are certified and manufactured by big companies with likely influence on regulatory and enforcement

agencies. As a result, regulatory and enforcement efforts may be resisted by the manufacturers; as may be the dissemination of these research findings. Hence, enforcement of safety guidelines may be a herculean task, but this should not discourage improved efforts at ensuring the safety of HM production and use.

The non-detection of pharmaceutical compounds may be an indication that pharmaceutical adulteration of HM is not a problem in the study population, unlike other parts of the world. Perhaps, other sensitive analytical technique may be deployed in future analysis. However, detection of heavy metals increases concern about the safety of HM and the potential harm to people who use it, especially over a long period. Thus, a call for a more thorough analysis of HMs in Nigeria is needed as further discussed in Section 6.2.1. This need for action and intervention to safeguard the health of the public brings about the fifth research question.

What policy recommendations can be proposed in light of the findings from this study?

The various findings from this research call for quick and holistic intervention by all stakeholders. The points of intervention have been identified and recommendations made based on this research findings. They bother on areas of government, corporate and individual responsibilities which are discussed extensively in Section 6.2. The implementation of the recommendations by those concerned will mitigate the negative consequences highlighted in this study.

6.2 Recommendation

Findings of this research emphasise the need for multi-level recommendations because of the various players (state, manufacturers, and users) associated with HM regulation, production, marketing and use.

6.2.1 Recommendation to policymakers and regulatory agencies

A lot of human activities such as use of herbicides and pesticides, indiscriminate refuse dumping, and disregard of safety in petrochemical-related activities have been found to be likely contributors to the presence of heavy metals in plants used in HM. Therefore it is important to ensure best practice in the handling of petrochemical products and waste disposal. Likewise, the approval and use of any type of herbicide and pesticide should consider their impact on the environment, especially from their heavy metal content.

The variation in heavy metal concentration which was significant in some HMs analysed highlights the need to make HM manufacturers declare the source of plants used in their products by the appropriate government agency. This is essential to correlate the heavy

metal content of HM and human activities from the source environment to ensure safety. The likely contribution of various manufacturing processes to the presence of heavy metals and variation in HM means that there is a need to ensure manufacturing procedures and tools does not contribute to heavy metal constituents in the finished product. There is also a need to include heavy metal analysis as standard screening steps preliminary to certification of HM in areas where this has not been implemented.

The certification of HM helps to ensure the safety of the general populace. However, uncertified HM was the type acknowledged by respondents to be in greatest use. This means that a lot needs to be done to ensure improved certification of HM. A way of achieving this is to review the process of certifying HM and its cost implication. This is important, considering that affordability of HM was the second most frequently cited reason for the use of HM. Thus a certification process that will make HM very expensive may be counterproductive. There is also a need to improve regulation of the use of extemporaneous HM as it contributes to the number of uncertified HMs in circulation in the study population. The inclusion of HM in the state pharmacovigilance strategy is also much needed.

Public enlightenment on the use of uncertified HM is needed to reduce its incidence. However, that some certified HMs were found to contain heavy metals above the permissible limit also means that a lot needs to be done to ensure the safety of certified HMs as earlier recommended.

6.2.2 Recommendation to orthodox health-care management

Poor service delivery in orthodox care was highlighted as the main reason for avoidance of its use. This underscores the need for proper training and retraining of health professionals in effective service delivery, human relations, and respect for human dignity. The institution of an appropriate reward and sanction system in the health profession may help to achieve desirable results. Adequate monitoring of health facilities and professionals by the relevant authorities and prompt response to complaint and needs is also vital.

An evaluation of high hospital cost as one of the main reason for avoidance of orthodox health facilities along with the affordability of HM as the second reason for HM use points to the impact of cost in healthcare decision making. Accordingly, a reduction in out-of-pocket expenditure on health through improvement of universal health coverage and inclusion by the provision of health insurance are required. These will be beneficial to the vast majority of the study population who, according to this study, are in the low-income class.

Poor record-keeping practice in most hospitals was identified as one of the limitations to the evaluation of casualty and fatality figures associated with HM use. Therefore there needs to be an enforcement of proper record keeping of health data in all hospitals, which is essential to accurate health research, planning and intervention.

The reference to HM use casually in the medical records of patients was also observed in this study. Thus there is a need expressly to ask patients for details of any use of HM and maintain its detailed history. Such findings should also be properly documented in medical notes. This will likely discourage non-disclosure and underreporting of HM use.

6.2.3 Recommendation to HM users

The majority of respondents believe it is not safe to take uncertified HM but a majority still take it. It is important that users of HM consider safety above other factors in their healthcare decision making. In addition, where technology is available to verify NAFDAC numbers users should endeavor to utilise such.

6.2.4 Recommendation to HM manufactures

Manufacturers of HM should put in place protocols (e.g. testing mechanism for level of metals) which will ensure that their products are safe. They should also invest more in HM research and development, which will benefit them and the public.

6.3 Future work

As this research has taken place in Ekiti State using a multi-disciplinary approach, opportunities also exist for future study.

A nation-wide study of the pattern and use of HM may produce new findings and will also help the comparison of findings across different parts of Nigeria. This will require a bigger sample and financial commitment but it will be beneficial to both the government and the Nigerian nation.

Analysis of HM used by hospital patients for the presence of heavy metals and their correlation with medical conditions through analysis of biological samples e.g. of blood, hair, post-mortem liver and kidney for such heavy metals. This may be particularly useful in suspected HM with associated heavy metal poisoning.

As HM can be purchased from different countries, a transnational study of there content could also be carried out.

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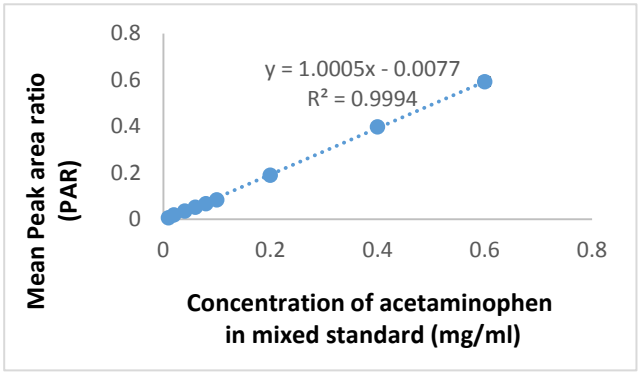
Appendix I: Linearity plot of pharmaceutical standard (mean peak area ratio (n=3) against concentration) (a) Acetaminophen, (b) Dexamethasone, (c) Tramadol, (d) Fluoxetine, (e) Codeine, (f) Chlorpheniramine, (g) Diclofenac, (h) Caffeine and (i) Diazepam.



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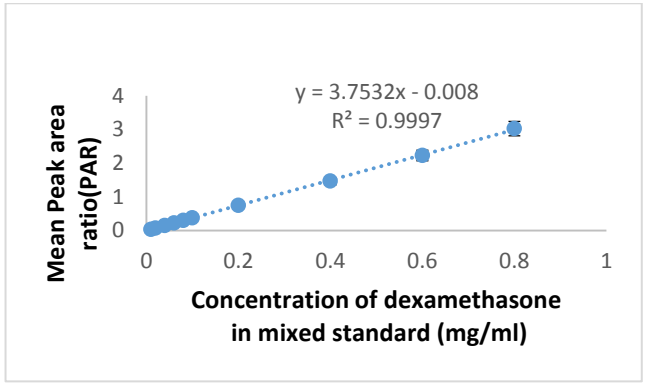

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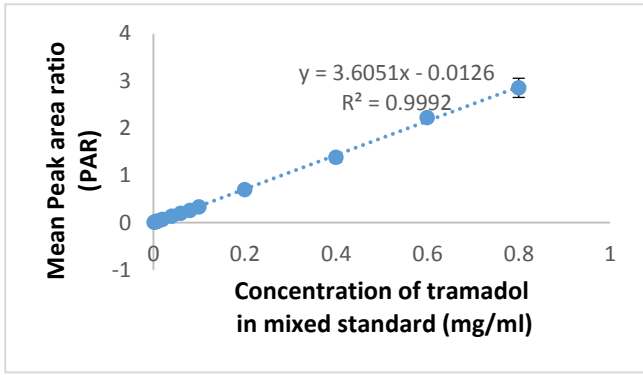

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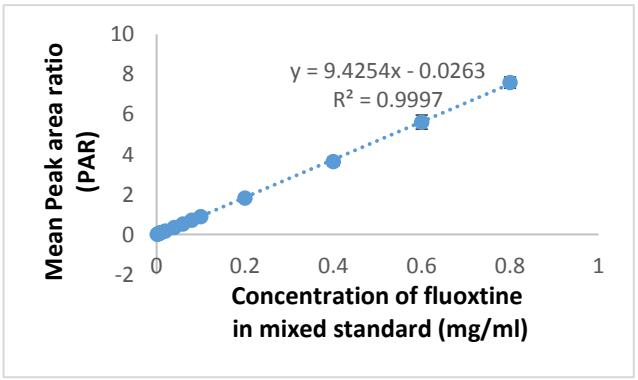

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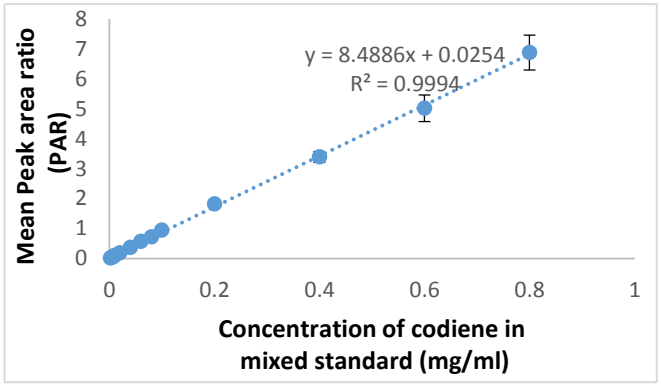

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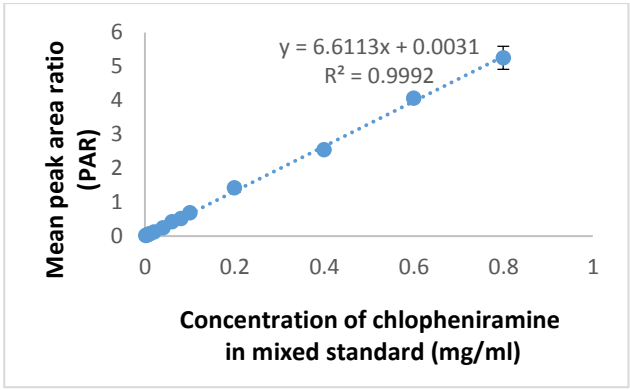

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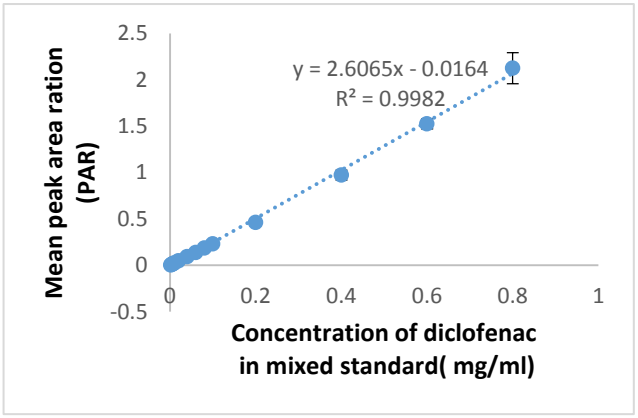

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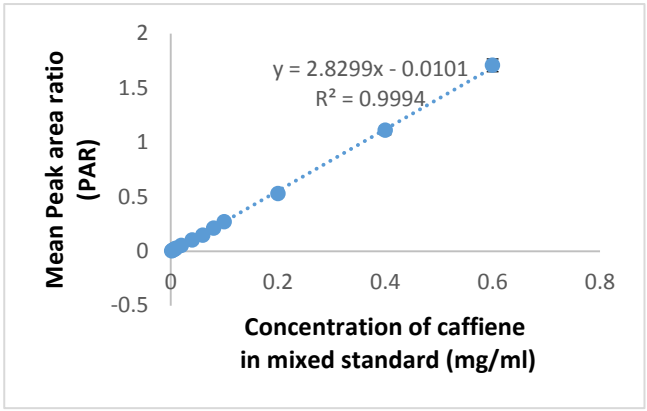

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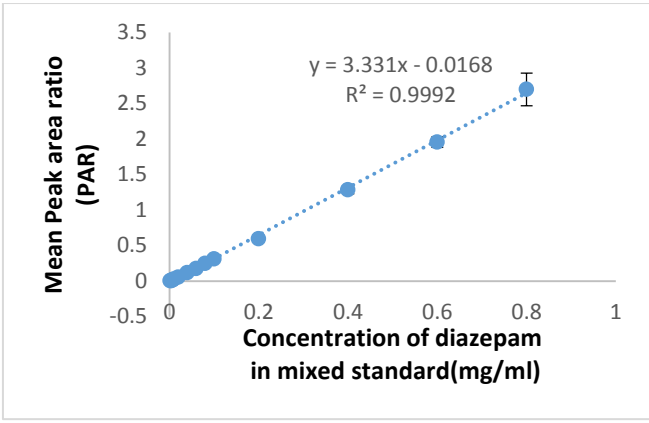
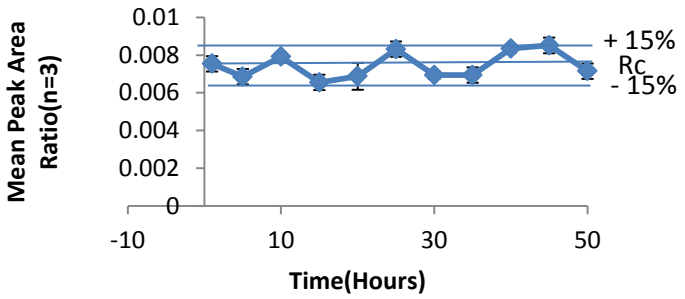
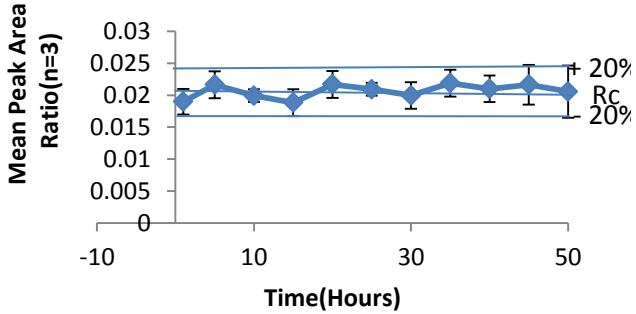


(i)

Appendix II: Graph of Mean Peak Area Ratio (n=3) over 50 hours stability trial, 0.008 mg/ml (a) Acetaminophen, (b) Caffeine, (c) Chlorpheniramine, (d) Codeine, (e) Dexamethasone, (f) Diazepam, (g) Diclofenac, (h) Fluoxetine and (i) Tramadol.



(a)



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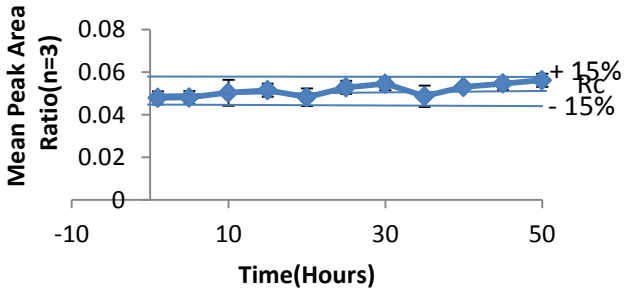

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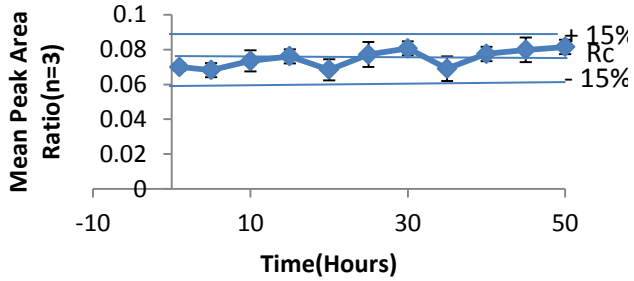

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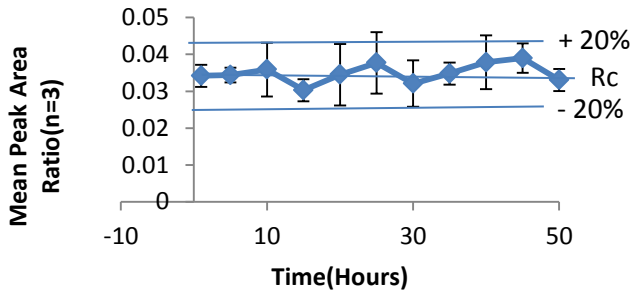

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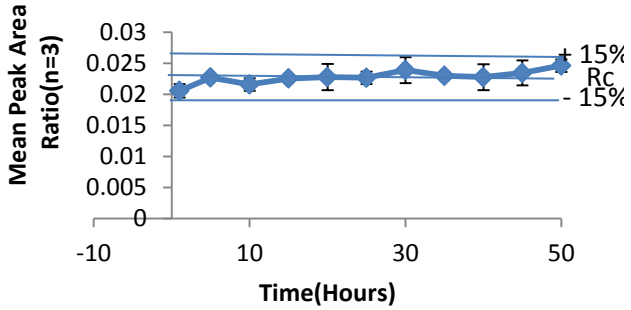
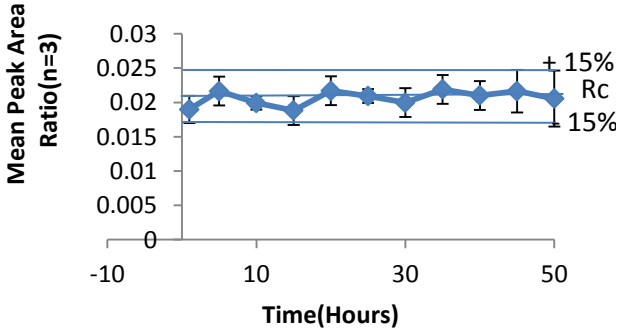
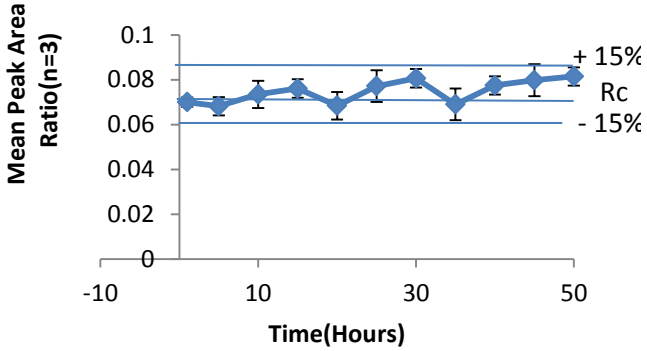


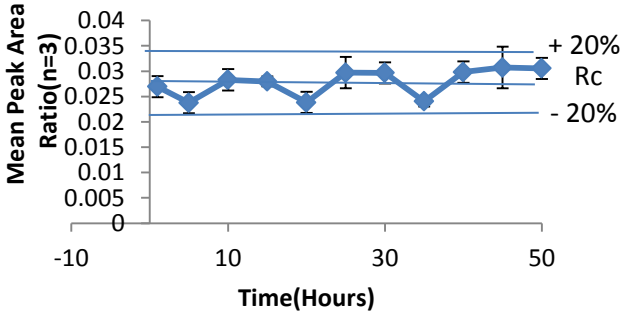
(f)



(g)

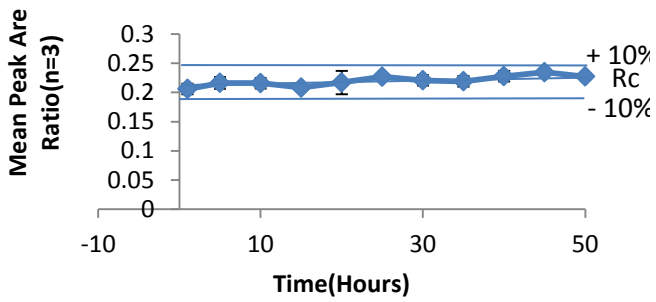


(h)

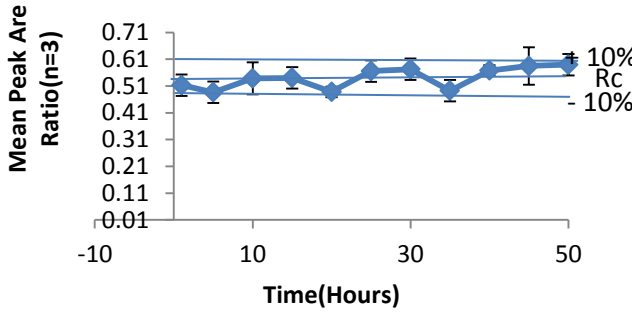


(i)

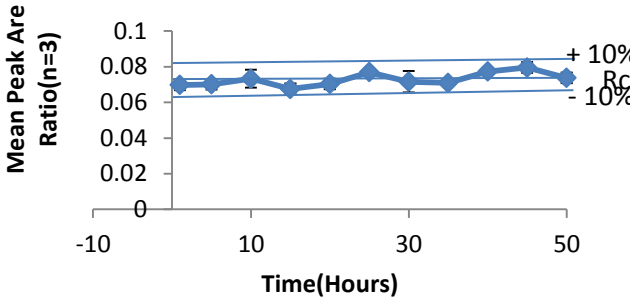
Appendix III: Graph of Mean Peak Area Ratio (n=3) over 50 hours stability trial, 0.08 mg/ml (a) Acetaminophen, (b) Caffeine, (c) Chlorpheniramine, (d) Codeine, (e) Dexamethasone, (f) Diazepam, (g) Diclofenac, (h) Fluoxetine and (i) Tramadol


Mean Peak Are Ratio(n=3)
0.3
0.25
0.2
0.15
0.1
0.05
0
-10
10
30
50
Time(Hours)
+ 10%
Rc
- 10%

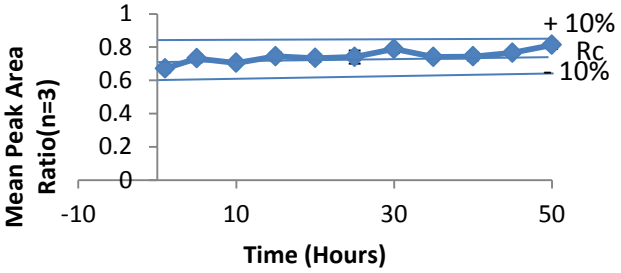
(a)


Mean Peak Are Ratio(n=3)
0.71
0.61
0.51
0.41
0.31
0.21
0.11
0.01
-10
10
30
50
Time(Hours)
+ 10%
Rc
- 10%

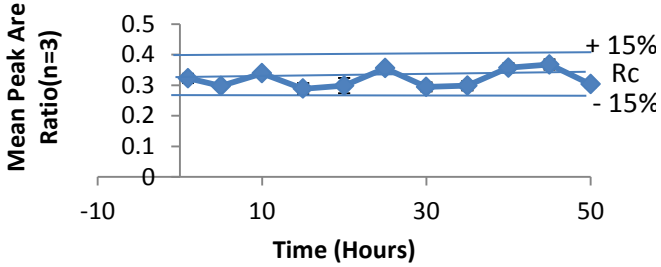
(b)


Mean Peak Are Ratio(n=3)
0.1
0.08
0.06
0.04
0.02
0
-10
10
30
50
Time(Hours)
+ 10%
Rc
- 10%

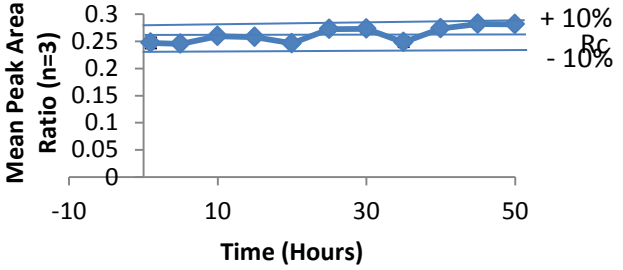
(c)


Mean Peak Area Ratio(n=3)
1
0.8
0.6
0.4
0.2
0
-10
10
30
50
Time (Hours)
+ 10%
Rc
- 10%

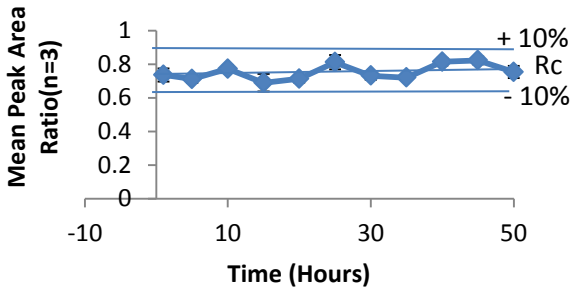
(d)


Mean Peak Are Ratio(n=3)
0.5
0.4
0.3
0.2
0.1
0
-10
10
30
50
Time (Hours)
+ 15%
Rc
- 15%

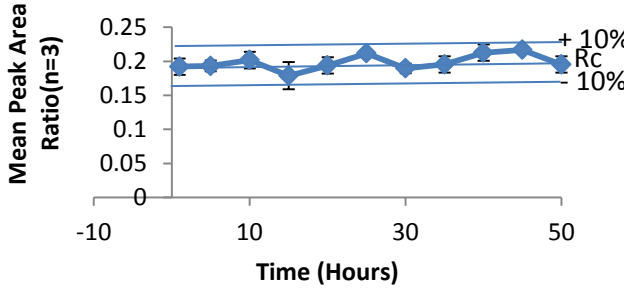
(e)


Mean Peak Area Ratio (n=3)
0.3
0.25
0.2
0.15
0.1
0.05
0
-10
10
30
50
Time (Hours)
+ 10%
Rc
- 10%

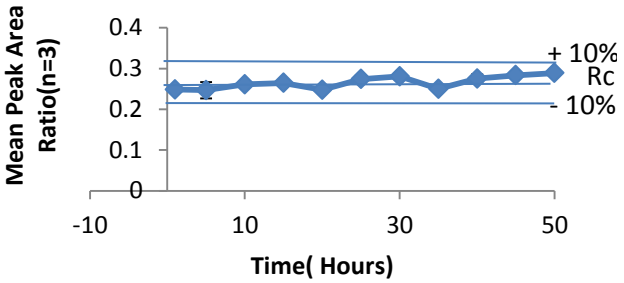
(f)



(g)

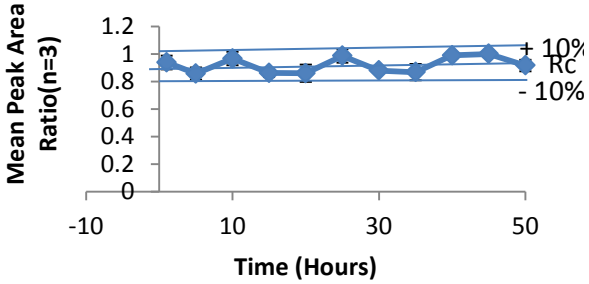


(h)

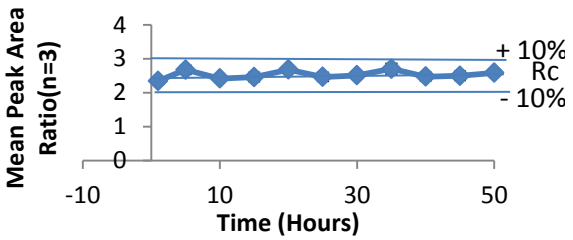


(i)

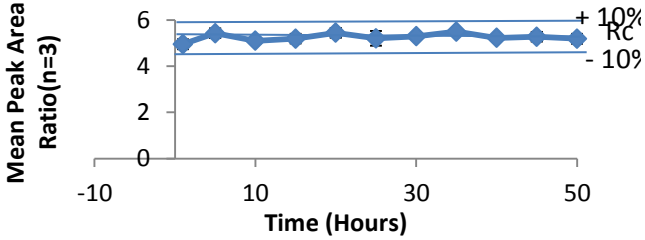
Appendix IV: Graph of Mean Peak Area Ratio (n=3) over 50 hours stability trial, 0.8 mg/ml (a) Acetaminophen, (b) Caffeine, (c) Chlorpheniramine, (d) Codeine, (e) Dexamethasone, (f) Diazepam, (g) Diclofenac, (h) Fluoxetine and (i) Tramadol .



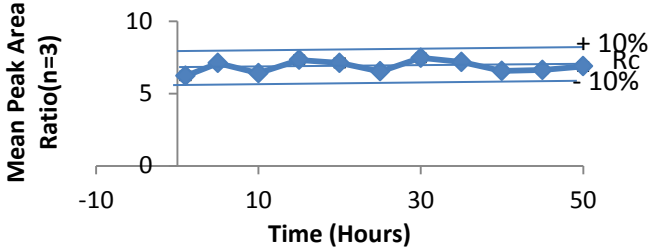
(a)



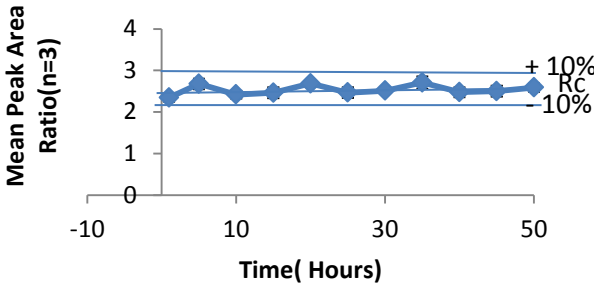
(b)



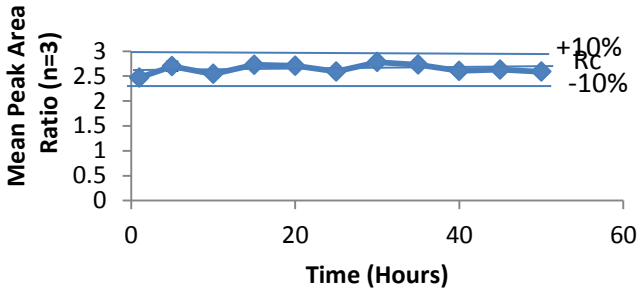
(c)



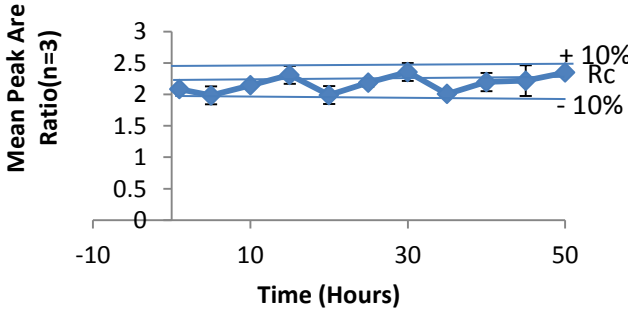
(d)



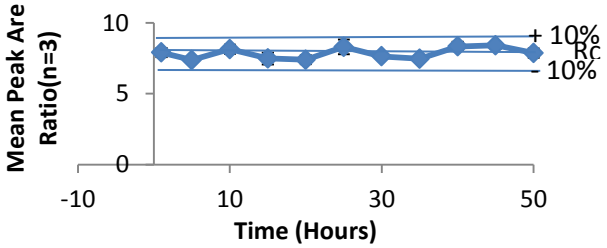
(e)



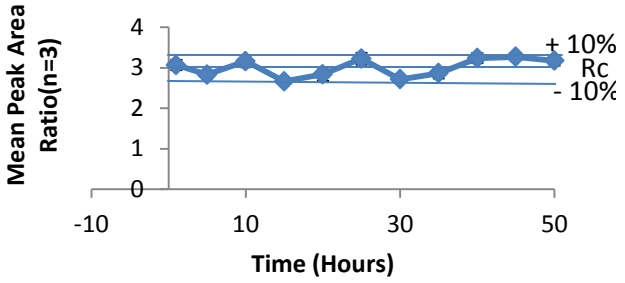
(f)



(g)

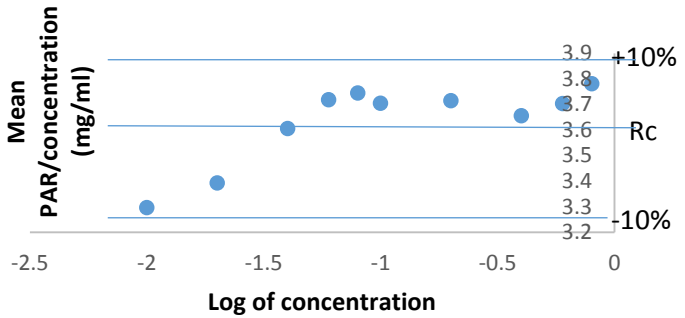


(h)

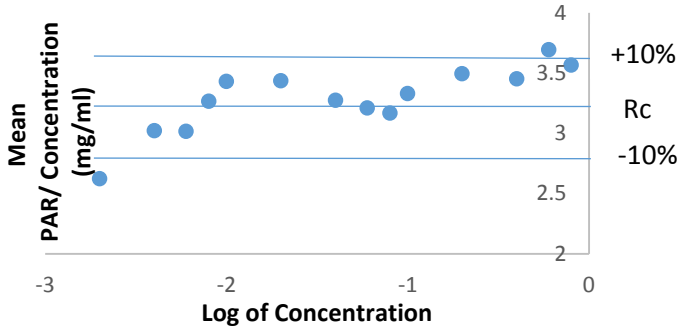


(i)

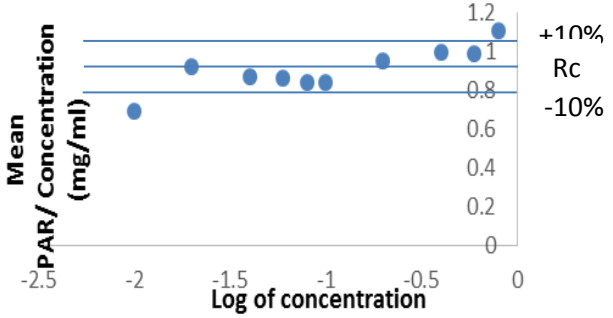
Appendix V: Log-linear plot of target analyte (a) Dexamethasone, (b) Tramadol, (c) Acetaminophen, (d) Fluoxetine, (e) Codeine, (f) chlorpheniramine, (g) Diclofenac, (h) caffeine, (i) Diazepam


Mean PAR/concentration (mg/ml)
+10%
Rc
-10%
Log of concentration

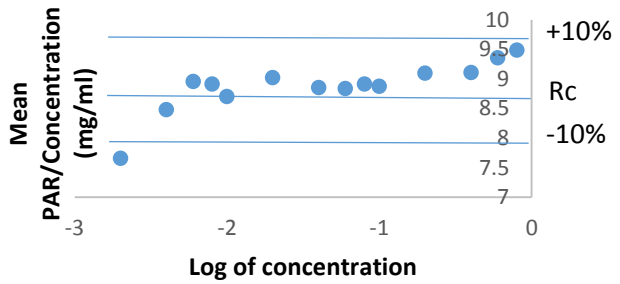
(a)


Mean PAR/ Concentration (mg/ml)
+10%
Rc
-10%
Log of Concentration

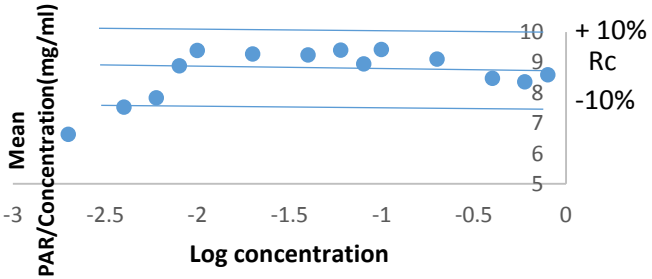
(b)


Mean PAR/ Concentration (mg/ml)
+10%
Rc
-10%
Log of concentration

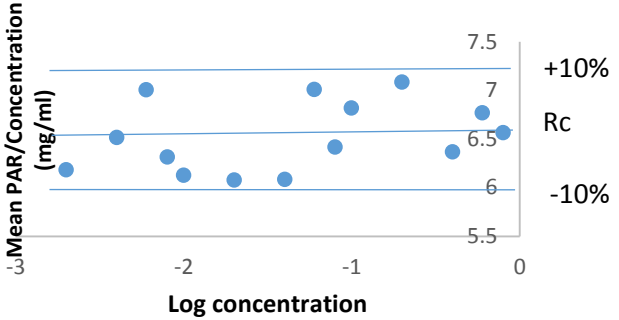
(c)


Mean PAR/Concentration (mg/ml)
+10%
Rc
-10%
Log of concentration

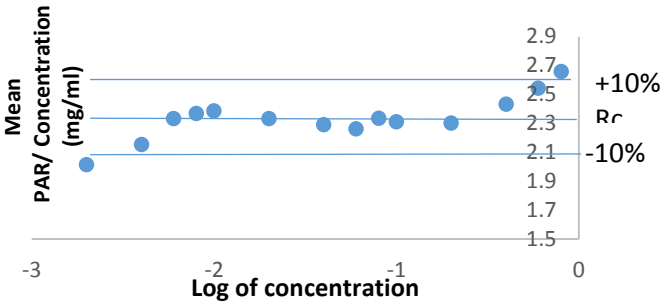
(d)


Mean PAR/Concentration(mg/ml)
+ 10%
Rc
-10%
Log concentration

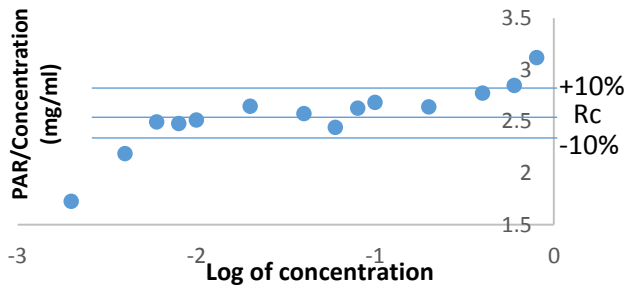
(e)


Mean PAR/Concentration (mg/ml)
+10%
Rc
-10%
Log concentration

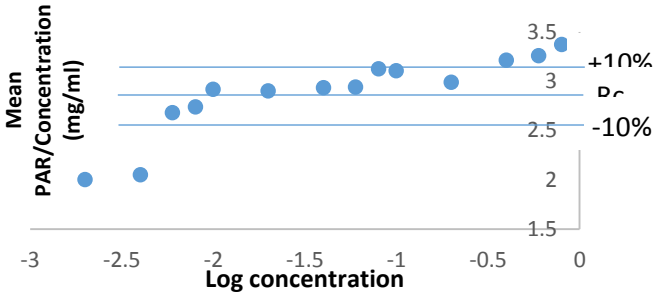
(f)



(g)

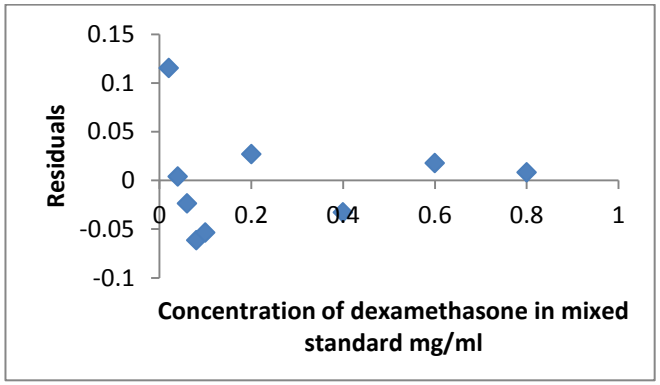


(h)

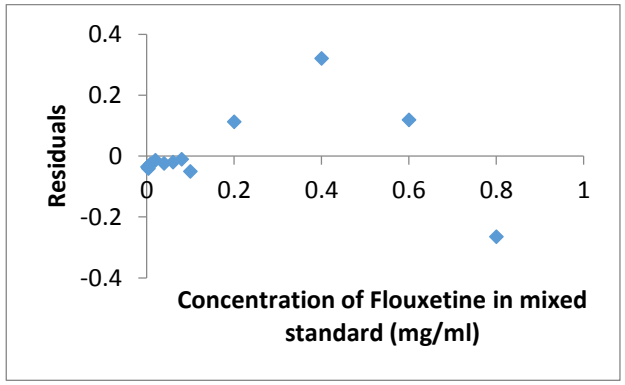


(i)

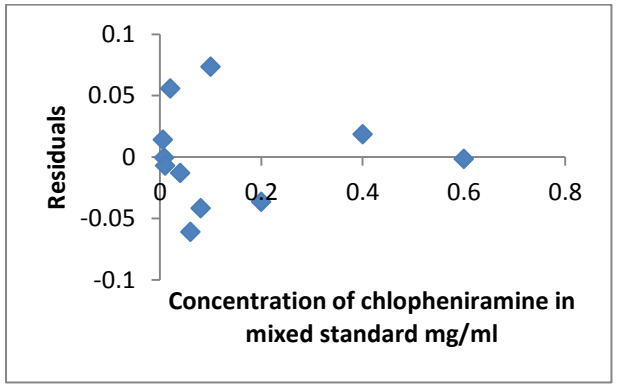
Appendix VI: Residual plot of target compounds (Peak area ratio of analyte/concentration versus log concentration) : (a) Dexamethasone, (b) Fluoxetine, (c) Chlorpheniramine, (d) Codeine, (e) Diclofenac, (f) Tramadol, (g) Acetaminophen, (h) Caffeine, (i) Diazepam.



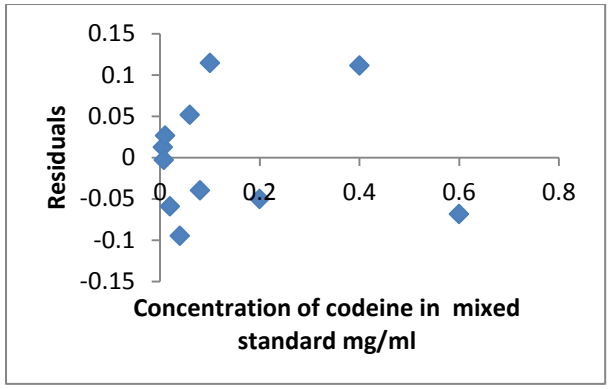
(a)



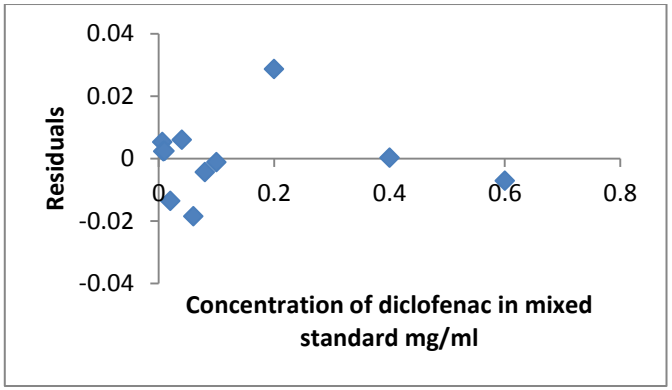
(b)



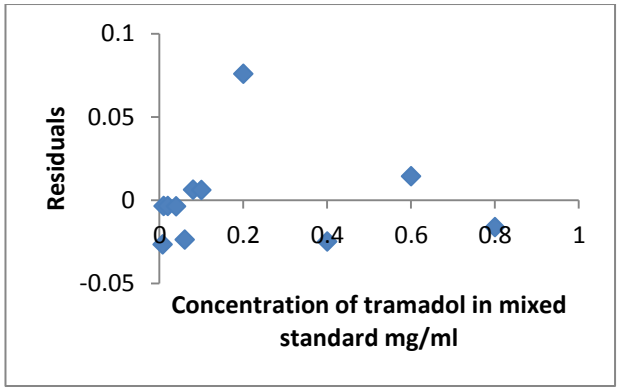
(c)



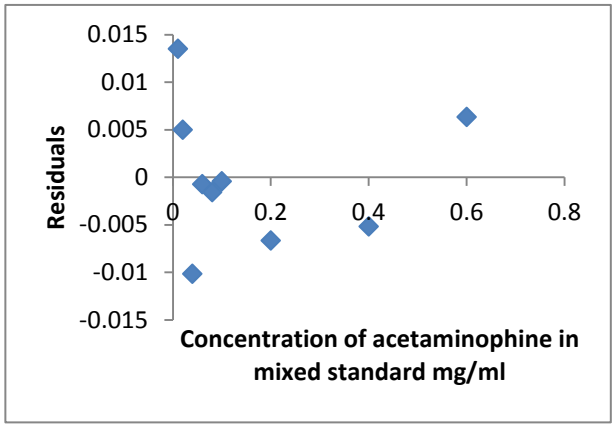
(d)



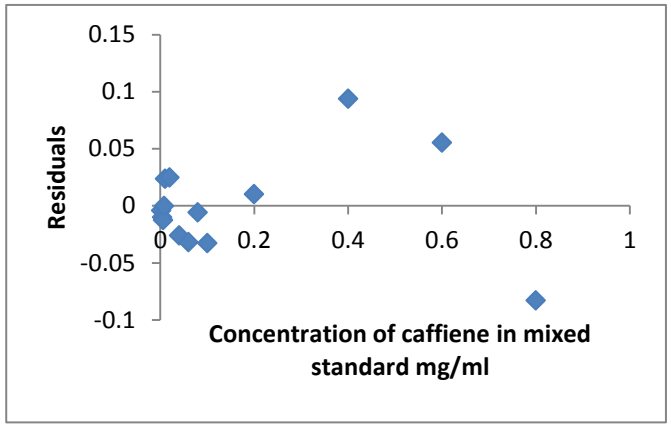
(e)



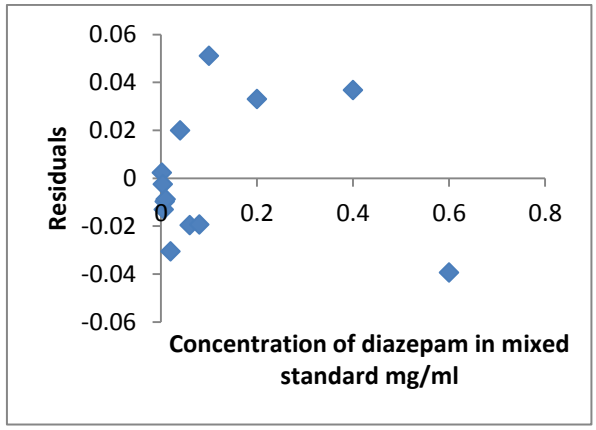
(f)



(g)

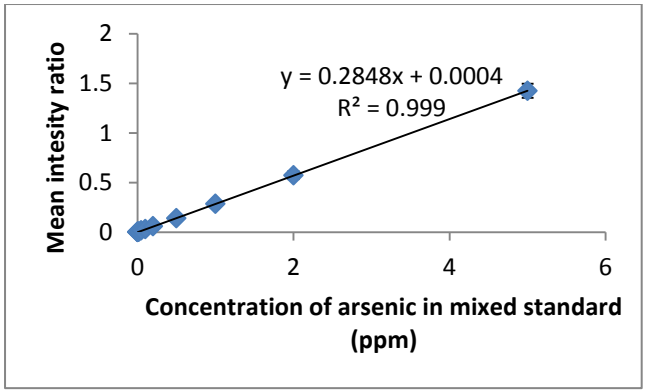


(h)

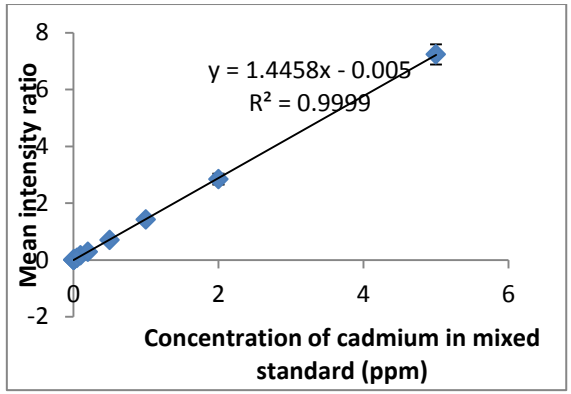


(i)

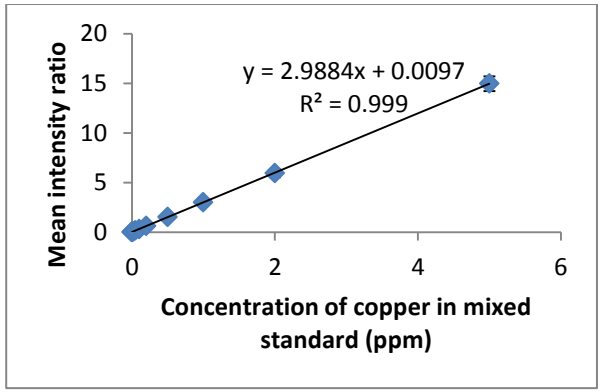
Appendix VII : Linearity plot of elemental standard (mean intensity ratio (n=3) against concentration : (a) Arsenic, (b) Cadmium, (c) Copper, (d) Lead, (e) Manganese, (f) Mercury, (g) Nickel, (h) Selenium and (i) Zinc


Mean intesity ratio
y = 0.2848x + 0.0004
R² = 0.999
Concentration of arsenic in mixed standard (ppm)

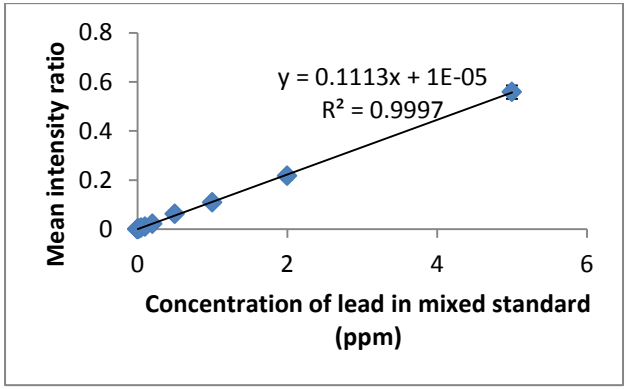
(a)


Mean intensity ratio
y = 1.4458x - 0.005
R² = 0.9999
Concentration of cadmium in mixed standard (ppm)

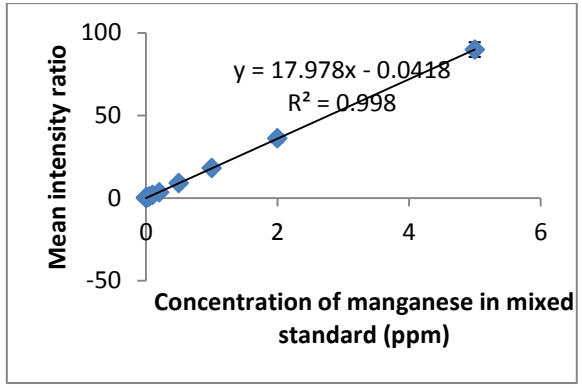
(b)


Mean intensity ratio
y = 2.9884x + 0.0097
R² = 0.999
Concentration of copper in mixed standard (ppm)

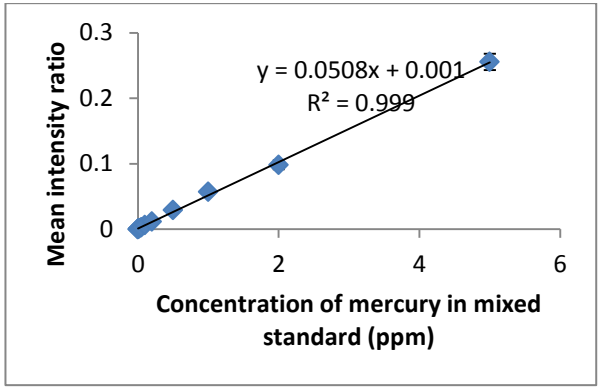
(c)


Mean intensity ratio
y = 0.1113x + 1E-05
R² = 0.9997
Concentration of lead in mixed standard (ppm)

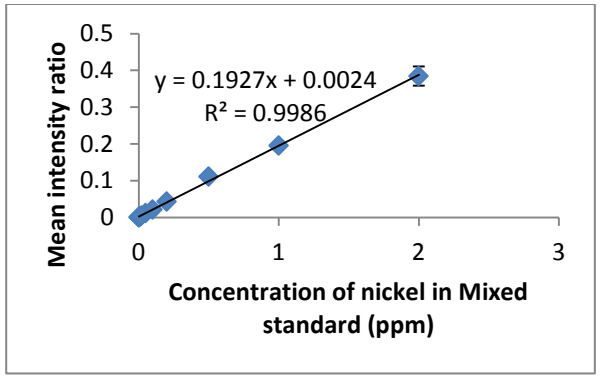
(d)


Mean intensity ratio
y = 17.978x - 0.0418
R² = 0.998
Concentration of manganese in mixed standard (ppm)

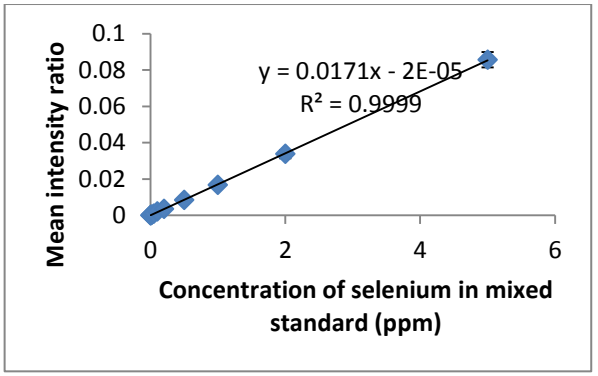
(e)


Mean intensity ratio
y = 0.0508x + 0.001
R² = 0.999
Concentration of mercury in mixed standard (ppm)

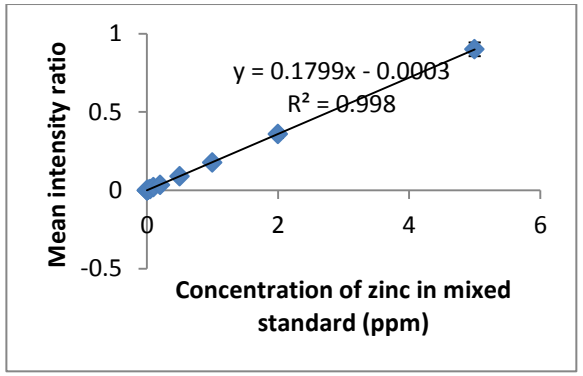
(f)



(g)

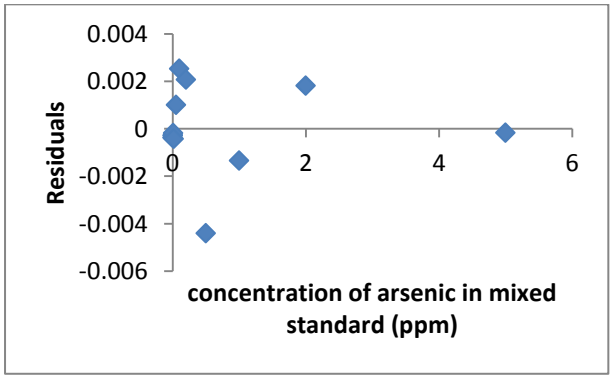


(h)

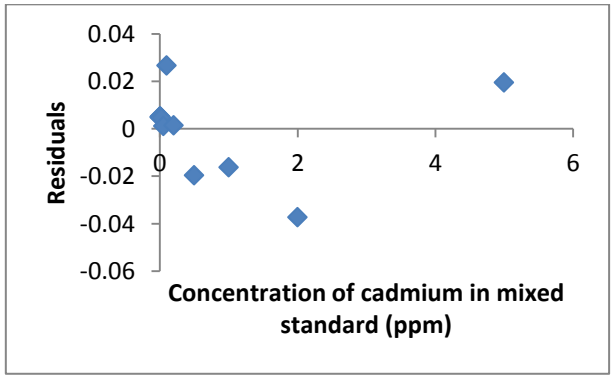


(i)

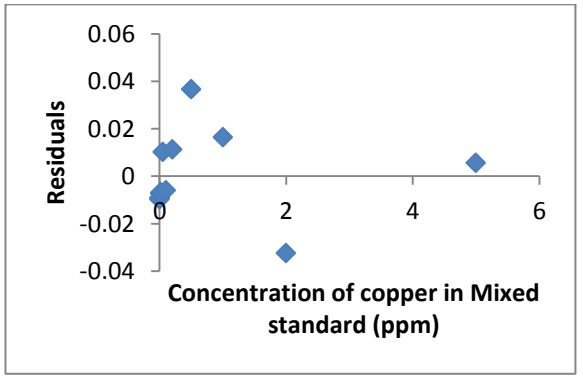
Appendix VIII : Residual plot of target element : (a) Arsenic, (b) Cadmium, (c) Copper, (d) Lead, (e) Manganese, (f) Mercury, (g) Nickel, (h) Selenium and (i) Zinc



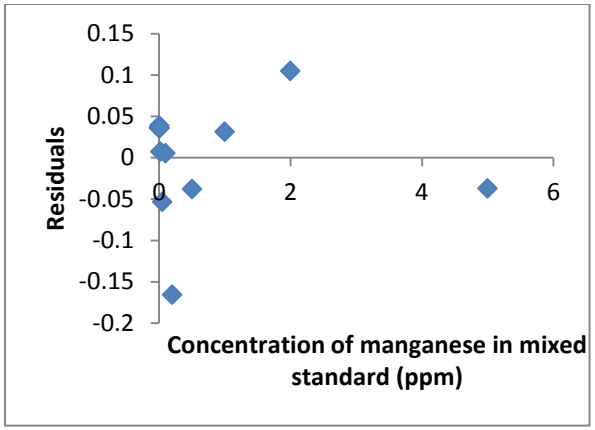
(a)



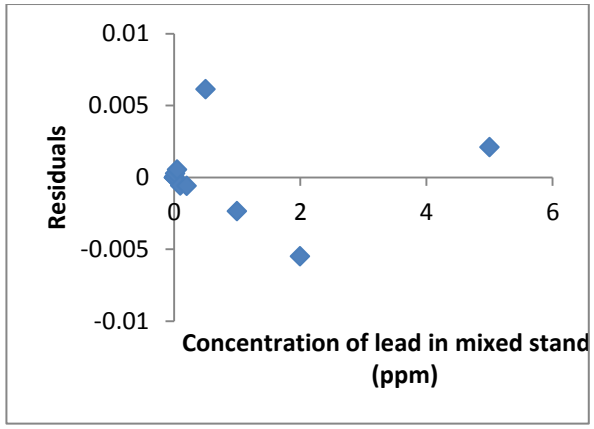
(b)



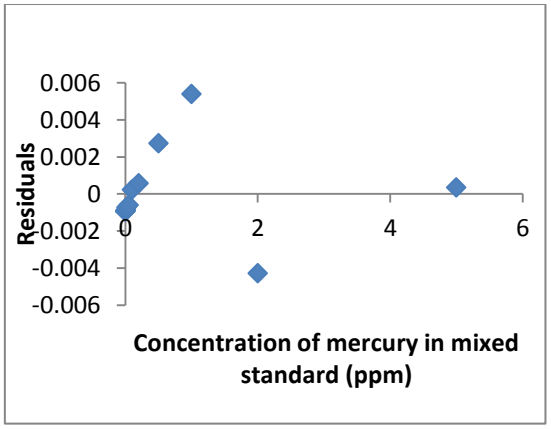
(c)



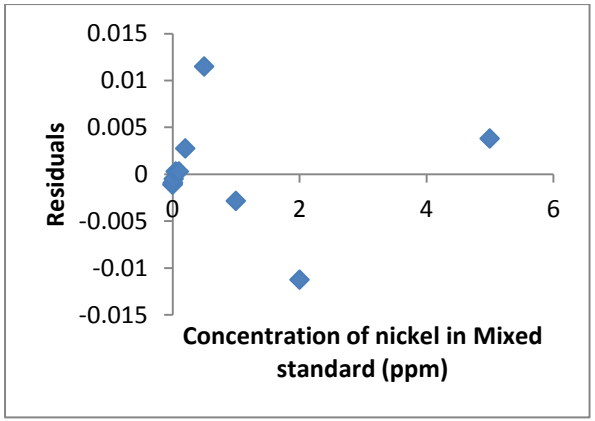
(d)



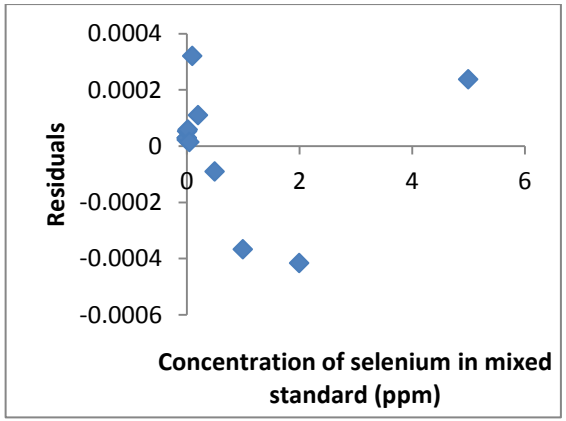
(e)



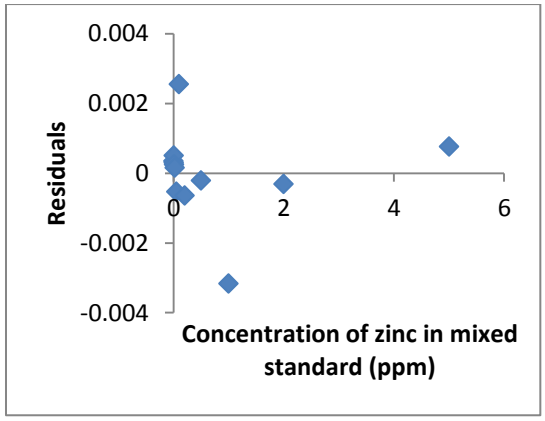
(f)



(g)

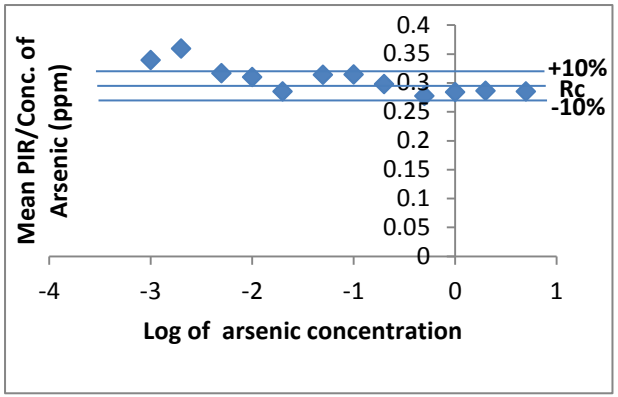


(h)

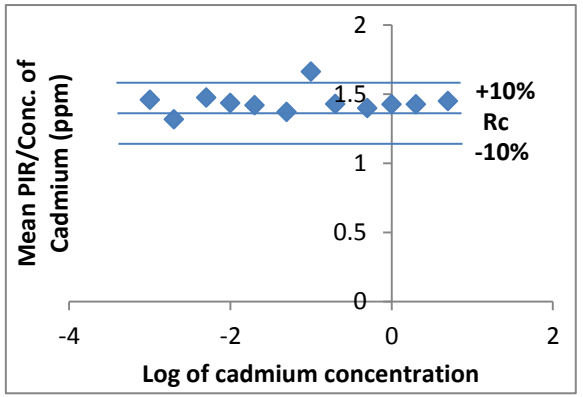


(i)

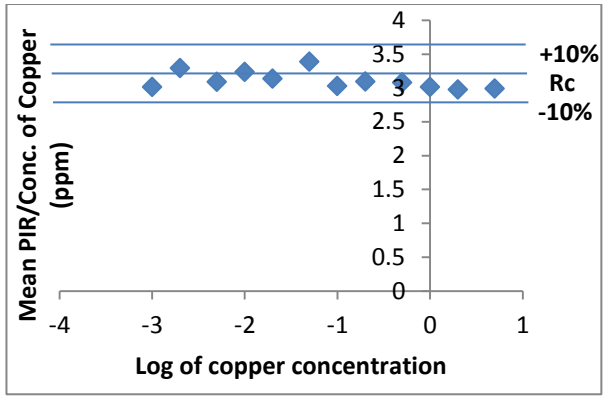
Appendix IX : Log-linear plot of target element: (a) Arsenic, (b) Cadmium, (c) Copper, (d) Lead, (e) Manganese, (f) Mercury, (g) Nickel, (h) Selenium and (i) Zinc


Mean PIR/Conc. of Arsenic (ppm)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
+10%
Rc
-10%
-4
-3
-2
-1
0
1
Log of arsenic concentration

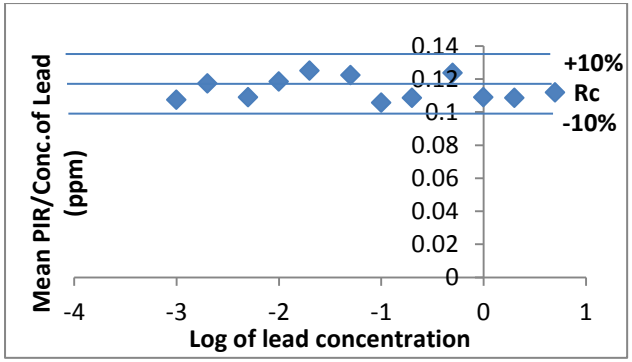
(a)


Mean PIR/Conc. of Cadmium (ppm)
2
1.5
1
0.5
0
+10%
Rc
-10%
-4
-2
0
2
Log of cadmium concentration

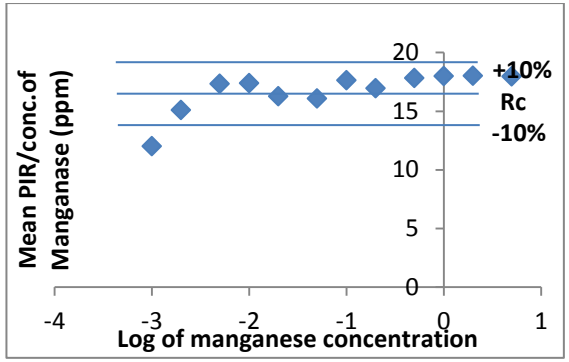
(b)


Mean PIR/Conc. of Copper (ppm)
4
3.5
3
2.5
2
1.5
1
0.5
0
+10%
Rc
-10%
-4
-3
-2
-1
0
1
Log of copper concentration

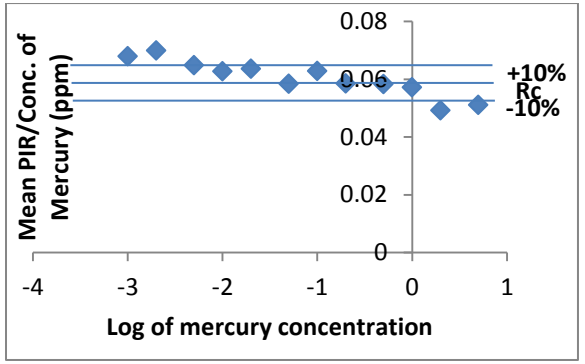
(c)


Mean PIR/Conc.of Lead (ppm)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
+10%
Rc
-10%
-4
-3
-2
-1
0
1
Log of lead concentration

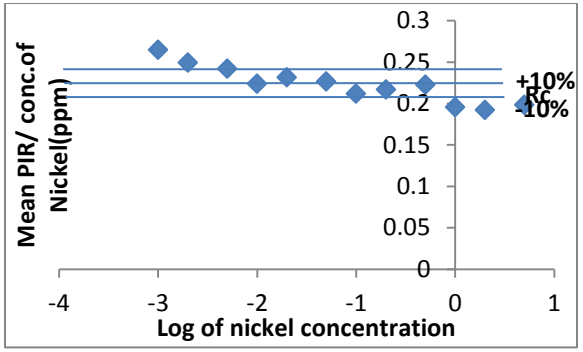
(d)


Mean PIR/conc.of Manganase (ppm)
20
15
10
5
0
+10%
Rc
-10%
-4
-3
-2
-1
0
1
Log of manganese concentration

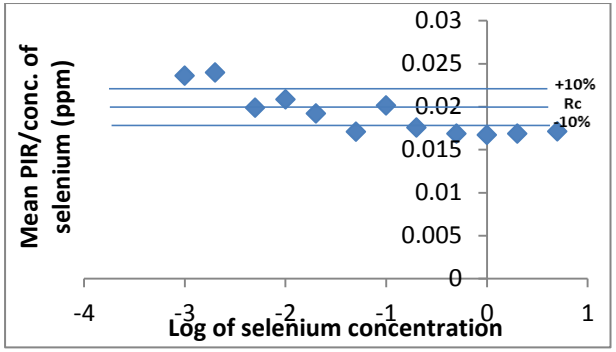
(e)


Mean PIR/Conc. of Mercury (ppm)
0.08
0.06
0.04
0.02
0
+10%
Rc
-10%
-4
-3
-2
-1
0
1
Log of mercury concentration

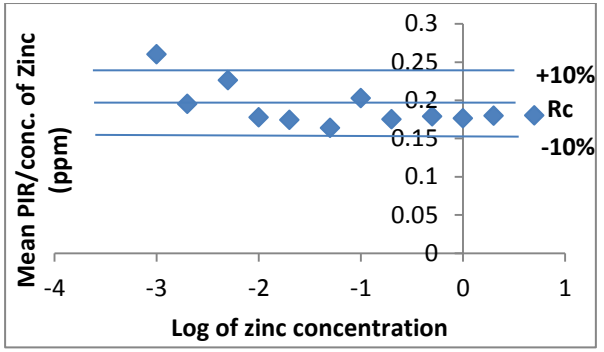
(f)


Mean PIR/ conc.of Nickel(ppm)
0.3
0.25
0.2
0.15
0.1
0.05
0
+10%
Rc
-10%
-4
-3
-2
-1
0
1
Log of nickel concentration

(g)


Mean PIR/conc. of selenium (ppm)
0.03
0.025
0.02
0.015
0.01
0.005
0
+10%
Rc
-10%
-4
-3
-2
-1
0
1
Log of selenium concentration

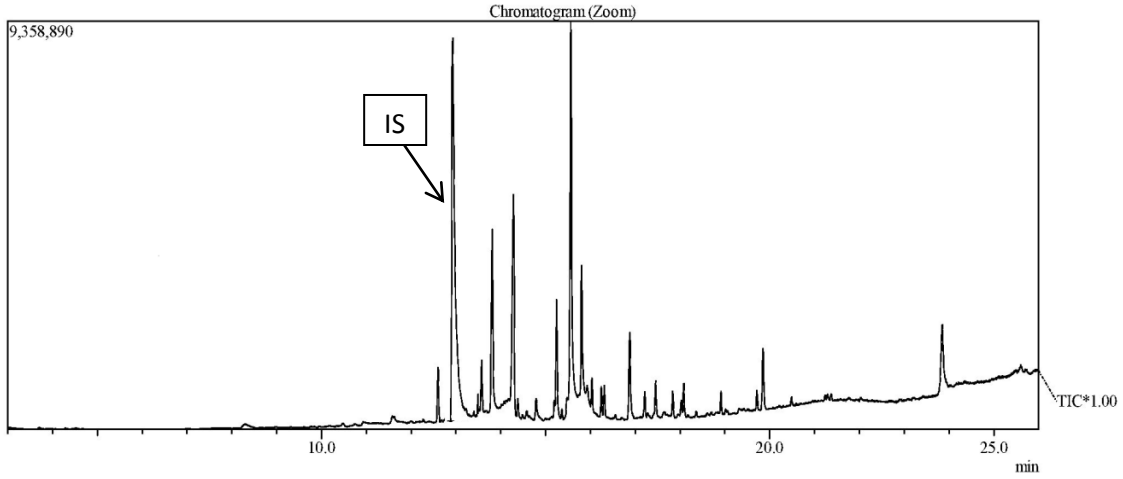
(h)


Mean PIR/conc. of Zinc (ppm)
0.3
0.25
0.2
0.15
0.1
0.05
0
+10%
Rc
-10%
-4
-3
-2
-1
0
1
Log of zinc concentration

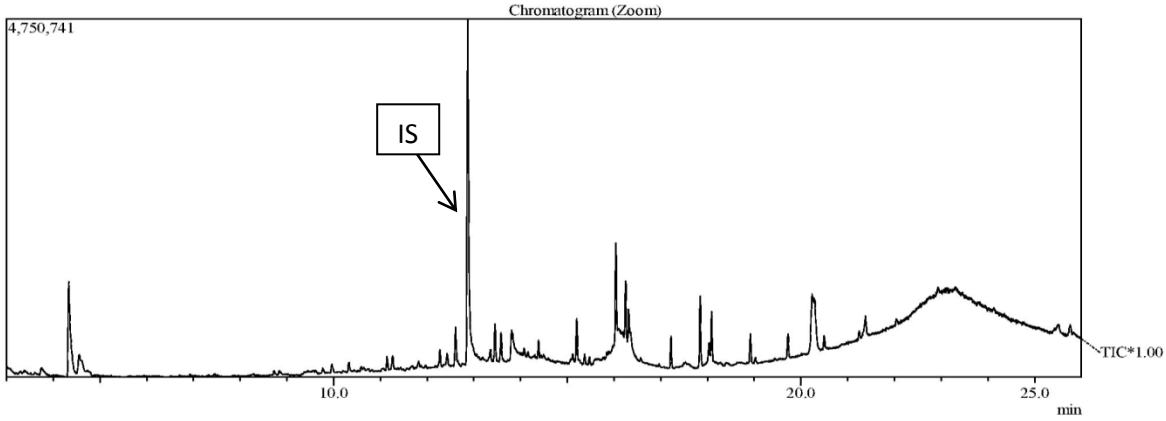
(i)

Appendix X: TIC chromatogram of HM samples 1 to 10 showing internal standard (IS)

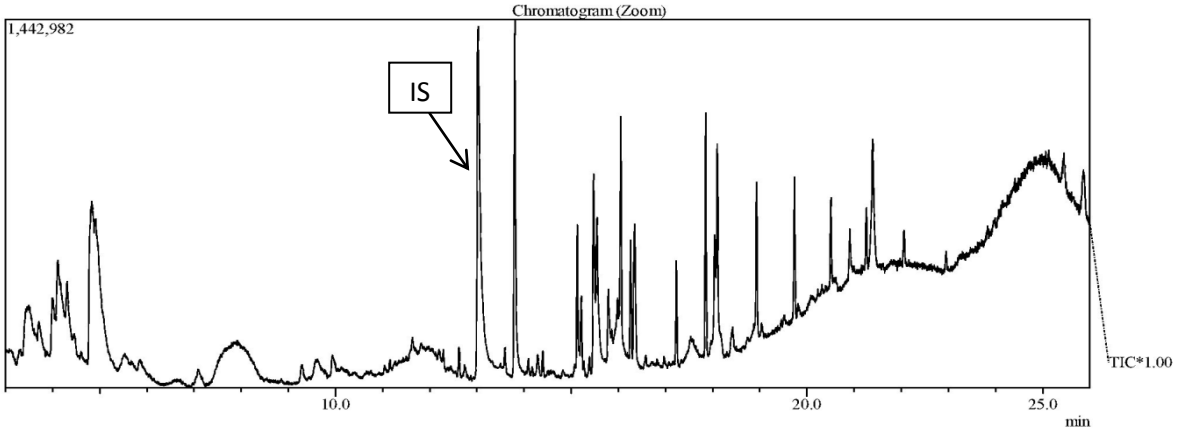
(a) HM Sample 1



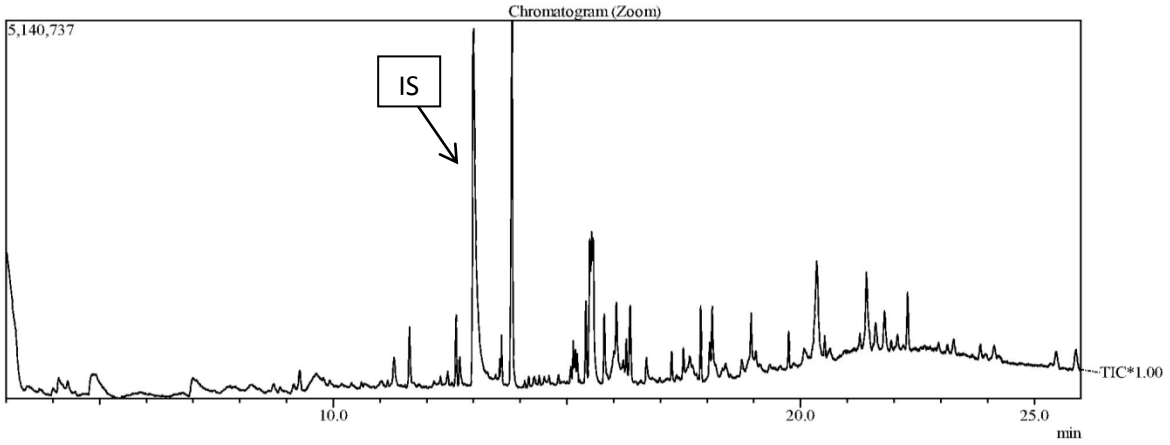
(b) HM Sample 2



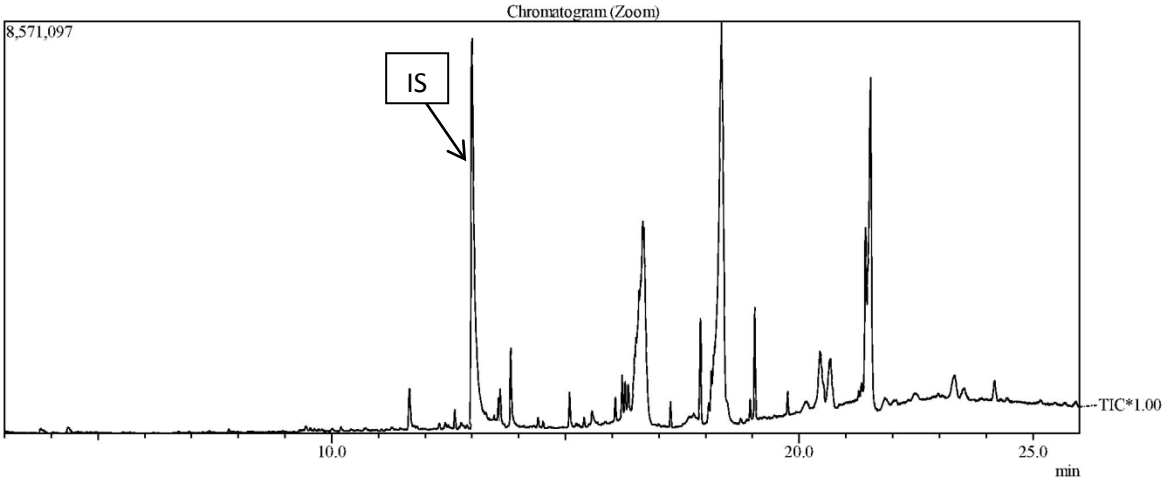
(c) HM Sample 4



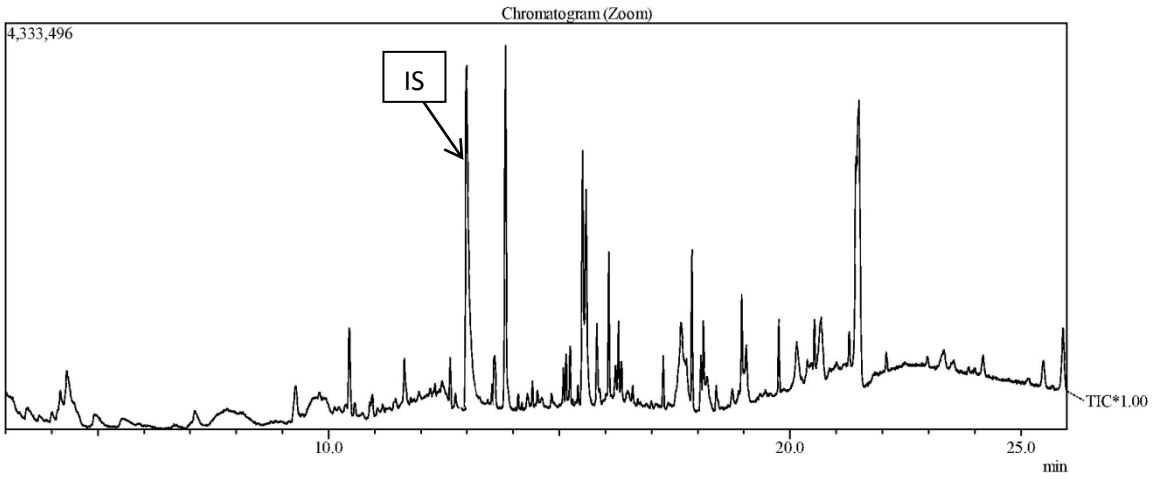
(d) HM Sample 5



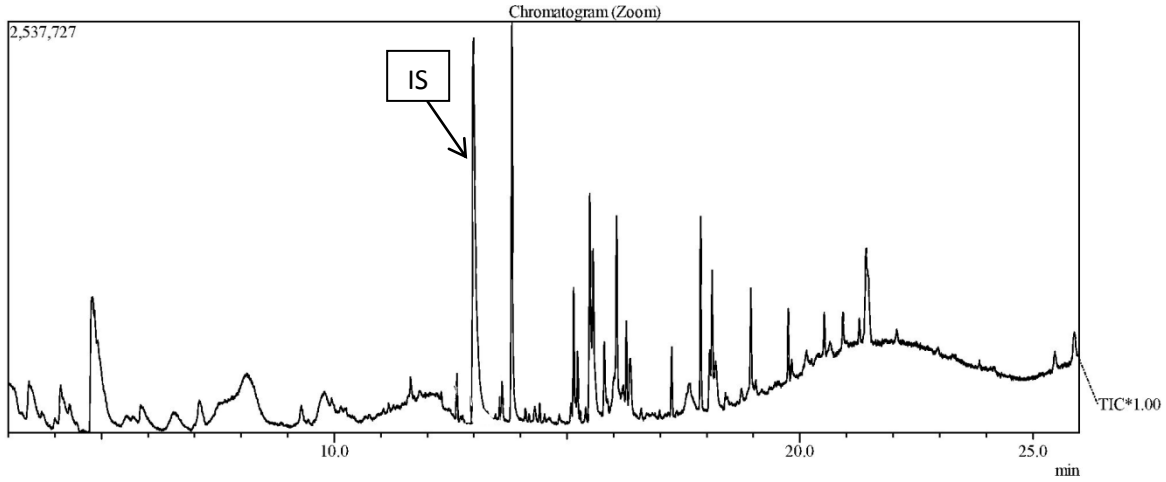
(e) HM Sample 6



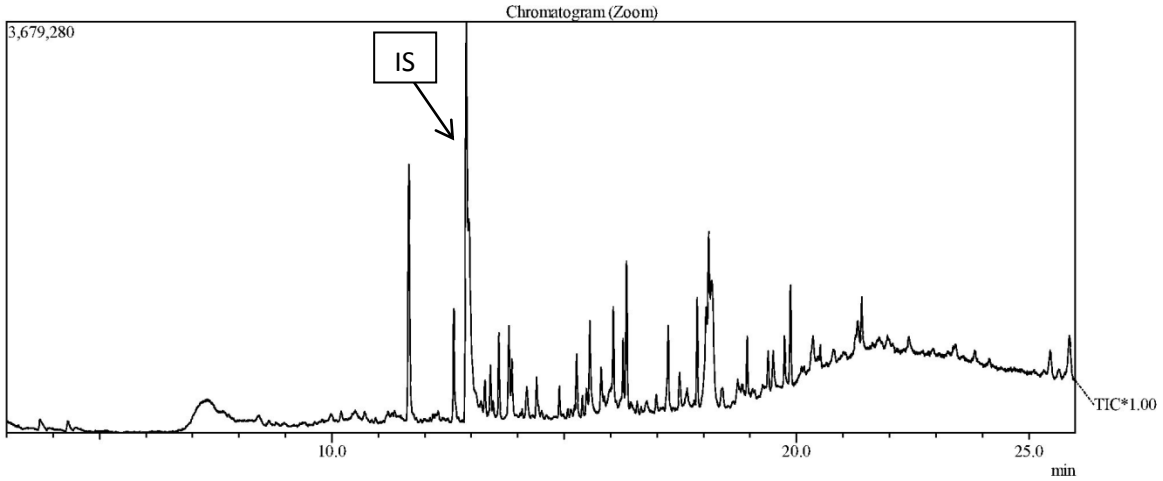
(f) HM Sample 7



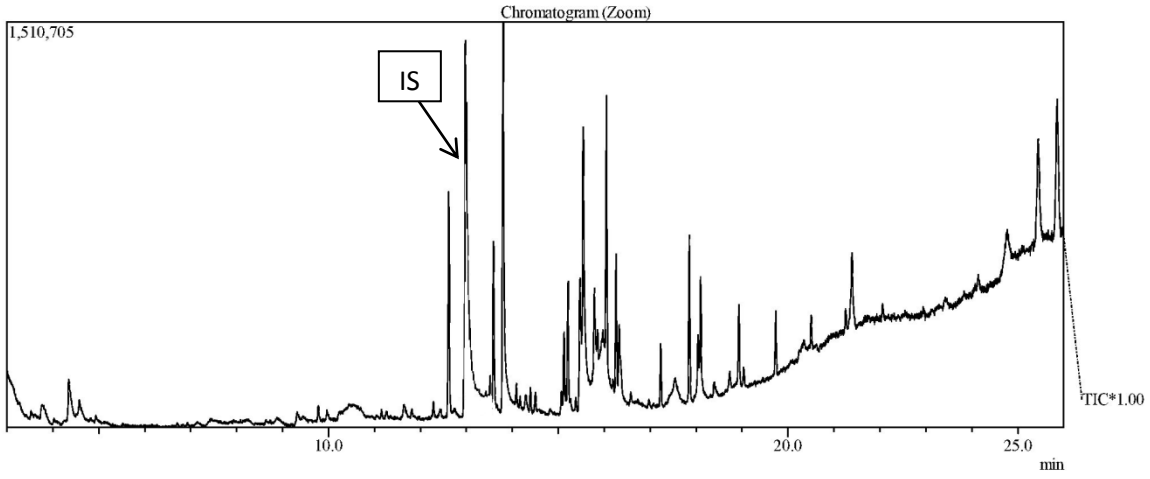
(g) HM Sample 8



(h) HM Sample 9



(i) HM Sample 10



Appendix XI: Table of intra-batch of metals in herbal medicine samples

(a) Intra-batch concentration of metals in HM2

Intra-batch concentration of metals in HM2				
Element	**Location**	**Sample 1**	**Sample 2**	**Average Conc**
Arsenic	Location 1	0.787±0.058	0.841±0.045	0.814±0.038
	Location 2	0.514±0.035	0.547±0.003	0.530±0.023
	Location 3	0.572±0.017	0.558±0.011	0.565±0.010
	Location 4	0.553±0.021	0.562±0.017	0.558±0.006
	Location 5	0.581±0.039	0.554±0.022	0.568±0.019
cadmium	Location 1	0.681±0.038	0.725±0.025	0.703±0.031
	Location 2	0.442±0.037	0.475±0.005	0.458±0.023
	Location 3	0.595±0.026	0.621±0.021	0.608±0.019
	Location 4	0.628±0.017	0.587±0.013	0.607±0.029
	Location 5	0.609±0.036	0.582±0.018	0.596±0.019
Chromium	Location 1	2.864±0.167	3.104±0.122	2.984±0.170
	Location 2	2.943±0.274	3.015±0.014	2.979±0.051
	Location 3	2.364±0.086	2.467±0.080	2.415±0.073
	Location 4	2.740±0.222	3.075±0.132	2.907±0.237
	Location 5	2.452±0.188	2.764±0.082	2.608±0.221
Copper	Location 1	37.158±3.645	38.323±1.855	37.741±0.824
	Location 2	33.011±2.647	29.266±3.321	31.138±2.648
	Location 3	36.922±2.380	33.177±4.269	35.050±3.324
	Location 4	30.490±2.500	27.330±2.787	28.910±2.234
	Location 5	28.528±2.437	25.325±1.417	26.927±2.265
Lead	Location 1	27.608±2.672	26.773±0.882	27.191±0.591
	Location 2	34.746±2.347	30.401±3.021	32.574±3.072
	Location 3	38.238±1.431	33.493±3.320	35.866±2.375
	Location 4	36.900±2.483	33.740±2.770	35.320±2.234
	Location 5	37.521±2.420	34.317±1.400	35.919±2.265
Manganese	Location 1	98.029±6.475	92.284±8.769	95.157±4.062
	Location 2	96.003±1.752	87.786±4.042	91.894±5.810
	Location 3	95.089±1.699	87.396±6.552	91.243±4.125
	Location 4	90.039±5.011	93.544±2.483	91.792±2.479
	Location 5	89.920±7.631	84.598±3.145	87.259±3.764
Nickel	Location 1	6.474±0.234	6.573±0.335	6.524±0.071
	Location 2	5.669±0.247	5.376±0.275	5.522±0.207
	Location 3	4.283±0.216	4.070±0.211	4.176±0.151
	Location 4	4.899±0.338	4.350±0.336	4.624±0.388
	Location 5	4.687±0.050	5.102±0.096	4.894±0.294
Selenium	Location 1	7.991±0.607	8.822±0.678	8.407±0.588
	Location 2	7.388±0.327	7.094±0.368	7.241±0.207
	Location 3	8.183±0.291	7.970±0.223	8.076±0.151
	Location 4	8.251±0.398	7.702±0.336	7.976±0.388
	Location 5	8.944±0.454	9.316±0.379	9.130±0.263
Zinc	Location 1	131.958±5.203	126.213±8.497	129.086±4.062
	Location 2	139.250±3.202	131.034±5.492	135.142±5.810
	Location 3	129.551±2.949	121.857±7.802	125.704±5.375
	Location 4	139.469±6.201	142.974±3.673	141.222±2.479
	Location 5	128.650±5.686	123.328±1.200	125.989±3.764

(b) Intra-batch concentration of metals in HM3

Intra-batch concentration of metals in HM3						
Element	Location	Sample 1	Sample 2	Sample 3	Sample 4	Average Conc
Arsenic	Location 1	0.385±0.018	0.328±0.013	0.331±0.007	0.369±0.010	0.353±0.012
	Location 2	0.386±0.031	0.448±0.025	0.461±0.058	0.417±0.037	0.428±0.038
	Location 3	0.454±0.033	0.467±0.039	0.393±0.038	0.402±0.033	0.429±0.036
	Location 4	0.669±0.099	0.788±0.012	0.769±0.040	0.705±0.011	0.733±0.040
	Location 5	0.458±0.042	0.431±0.025	0.387±0.030	0.409±0.035	0.421±0.032
cadmium	Location 1	0.618±0.041	0.632±0.033	0.564±0.030	0.561±0.036	0.594±0.035
	Location 2	0.608±0.046	0.594±0.014	0.533±0.020	0.563±0.025	0.574±0.026
	Location 3	0.715±0.031	0.640±0.030	0.650±0.026	0.701±0.026	0.676±0.028
	Location 4	0.538±0.039	0.468±0.108	0.474±0.019	0.546±0.020	0.506±0.047
	Location 5	0.387±0.030	0.409±0.035	0.431±0.025	0.458±0.042	0.421±0.033
Chromium	Location 1	2.561±0.081	2.321±0.125	2.080±0.119	2.245±0.136	2.303±0.115
	Location 2	1.858±0.316	1.891±0.056	1.783±0.048	1.582±0.068	1.778±0.122
	Location 3	1.672±0.077	1.775±0.018	1.459±0.072	1.597±0.076	1.626±0.061
	Location 4	1.740±0.134	1.748±0.045	1.547±0.037	1.670±0.132	1.676±0.087
	Location 5	1.472±0.149	1.646±0.043	1.401±0.032	1.423±0.053	1.486±0.069
Copper	Location 1	13.643±1.060	14.740±0.930	12.527±1.012	12.909±1.035	13.455±1.010
	Location 2	13.858±1.390	14.354±1.130	13.783±1.122	11.582±1.141	13.394±1.196
	Location 3	16.730±0.745	14.833±0.686	13.517±0.740	14.655±0.744	14.934±0.729
	Location 4	15.856±1.133	17.935±1.045	15.734±1.038	15.926±1.135	16.363±1.088
	Location 5	15.785±0.485	16.030±0.496	16.857±0.602	13.807±0.507	15.619±0.523
Lead	Location 1	1.270±0.034	1.250±0.022	1.029±0.026	1.199±0.083	1.187±0.041
	Location 2	1.435±0.284	1.437±0.024	1.165±0.035	1.366±0.016	1.351±0.090
	Location 3	1.298±0.022	1.511±0.049	1.614±0.025	1.436±0.032	1.465±0.032
	Location 4	1.558±0.015	1.356±0.007	1.549±0.104	1.479±0.102	1.486±0.057
	Location 5	1.407±0.003	1.652±0.014	1.479±0.120	1.429±0.024	1.492±0.040
Manganese	Location 1	202.923±9.411	193.179±8.705	187.872±7.996	210.088±2.621	198.515±7.183
	Location 2	197.855±10.276	200.020±3.881	185.803±9.170	180.110±11.950	190.947±8.819
	Location 3	197.411±8.780	200.575±7.716	183.359±7.816	190.666±8.669	193.003±8.245
	Location 4	198.873±7.132	196.378±5.604	180.550±3.199	184.713±6.719	190.128±5.663
	Location 5	199.675±10.175	201.011±8.599	188.352±8.689	191.472±9.154	195.128±9.154
Nickel	Location 1	2.640±0.154	2.951±0.098	2.470±0.137	2.711±0.143	2.693±0.133
	Location 2	3.058±0.330	3.091±0.070	2.983±0.062	2.782±0.081	2.978±0.136
	Location 3	3.167±0.085	3.270±0.026	2.954±0.080	3.092±0.084	3.121±0.069
	Location 4	2.806±0.174	3.042±0.084	2.614±0.077	2.737±0.172	2.800±0.127
	Location 5	3.056±0.073	2.518±0.062	2.883±0.179	2.833±0.108	2.823±0.105
Selenium	Location 1	2.192±0.137	2.432±0.091	1.951±0.130	2.121±0.147	2.174±0.126
	Location 2	2.036±0.334	2.068±0.074	1.961±0.066	1.759±0.085	1.956±0.140
	Location 3	3.296±0.086	3.474±0.028	3.158±0.082	3.371±0.087	3.325±0.071
	Location 4	1.943±0.060	2.371±0.067	2.136±0.157	2.066±0.155	2.129±0.109
	Location 5	1.833±0.198	2.006±0.092	1.668±0.081	1.783±0.127	1.823±0.124
Zinc	Location 1	27.035±3.2033	23.291±2.357	24.984±1.788	28.700±1.413	26.003±2.190
	Location 2	27.879±2.759	25.944±0.864	24.128±2.562	24.435±2.900	25.597±2.271
	Location 3	27.431±2.631	26.596±0.880	24.780±0.980	22.685±2.833	25.373±1.831
	Location 4	24.572±2.341	25.758±1.640	23.930±0.900	21.574±1.751	23.959±1.658
	Location 5	24.596±1.480	23.932±0.612	20.273±1.363	21.717±1.659	22.630±1.278

(c) Intra-batch concentration of metals in HM4

Intra-batch concentration of metals in HM4				
Element	Location	Sample 1	Sample 2	Average Conc
Arsenic	Location 1	0.279±0.021	0.313±0.008	0.296±0.024
	Location 2	0.284±0.037	0.317±0.005	0.300±0.023
	Location 3	0.251±0.013	0.238±0.007	0.244±0.010
	Location 4	0.307±0.015	0.346±0.011	0.327±0.028
	Location 5	0.305±0.030	0.277±0.012	0.291±0.019
Cadmium	Location 1	0.613±0.027	0.657±0.014	0.635±0.031
	Location 2	0.568±0.040	0.641±0.008	0.604±0.051
	Location 3	0.755±0.046	0.801±0.041	0.778±0.033
	Location 4	0.635±0.037	0.594±0.033	0.615±0.029
	Location 5	0.509±0.038	0.482±0.021	0.496±0.019
Chromium	Location 1	1.731±0.167	1.971±0.122	1.851±0.170
	Location 2	1.800±0.269	1.883±0.009	1.841±0.059
	Location 3	1.805±0.069	1.908±0.064	1.856±0.073
	Location 4	1.606±0.192	1.741±0.102	1.673±0.096
	Location 5	1.150±0.148	1.264±0.042	1.207±0.081
Copper	Location 1	13.605±1.282	14.770±0.327	14.188±0.824
	Location 2	13.810±1.060	12.065±1.028	12.937±1.234
	Location 3	13.785±0.843	12.340±0.832	13.063±0.838
	Location 4	12.689±0.500	11.529±0.787	12.109±0.820
	Location 5	16.628±1.437	14.425±1.417	15.527±1.558
Lead	Location 1	3.490±0.203	3.730±0.158	3.610±0.170
	Location 2	2.695±0.299	2.784±0.039	2.739±0.063
	Location 3	2.930±0.102	3.034±0.097	2.982±0.073
	Location 4	3.638±0.253	3.973±0.163	3.805±0.237
	Location 5	2.040±0.164	2.253±0.058	2.146±0.150
Manganese	Location 1	85.843±3.974	80.098±6.268	82.971±4.062
	Location 2	90.240±2.452	82.024±4.742	86.132±5.810
	Location 3	91.519±5.399	83.826±10.252	87.673±7.825
	Location 4	80.219±6.171	83.724±3.643	81.972±2.479
	Location 5	91.070±6.271	85.748±1.785	88.409±3.764
Nickel	Location 1	1.380±0.088	1.282±0.043	1.331±0.070
	Location 2	1.971±0.269	1.788±0.009	1.879±0.129
	Location 3	2.280±0.058	2.383±0.053	2.331±0.073
	Location 4	1.815±0.201	2.050±0.111	1.932±0.166
	Location 5	2.052±0.190	2.364±0.084	2.208±0.221
Selenium	Location 1	4.960±0.277	5.591±0.348	5.276±0.446
	Location 2	5.565±0.427	5.271±0.468	5.418±0.207
	Location 3	6.433±0.201	6.220±0.133	6.326±0.151
	Location 4	6.141±0.503	5.592±0.441	5.866±0.388
	Location 5	3.618±0.323	4.033±0.370	3.825±0.294
Zinc	Location 1	25.332±2.096	22.587±1.790	23.960±1.941
	Location 2	23.234±0.708	21.018±1.198	22.126±1.567
	Location 3	24.958±1.533	22.265±2.386	23.611±1.959
	Location 4	34.049±2.411	37.554±0.883	35.802±2.479
	Location 5	23.586±2.628	21.264±1.142	22.425±1.643

(d) Intra-batch concentration of metals in HM5

Intra-batch concentration of metals in HM5				
Element	Location	Sample 1	Sample 2	Average Conc
Arsenic	Location 1	0.400±0.028	0.434±0.014	0.417±0.024
	Location 2	0.407±0.050	0.440±0.018	0.423±0.023
	Location 3	0.395±0.013	0.382±0.007	0.388±0.010
	Location 4	0.316±0.034	0.355±0.030	0.336±0.028
	Location 5	0.400±0.043	0.372±0.025	0.386±0.019
cadmium	Location 1	0.854±0.072	0.898±0.059	0.876±0.031
	Location 2	0.792±0.060	0.825±0.028	0.808±0.023
	Location 3	0.701±0.045	0.727±0.040	0.714±0.019
	Location 4	0.765±0.020	0.724±0.016	0.744±0.029
	Location 5	0.589±0.032	0.562±0.015	0.576±0.019
Chromium	Location 1	4.364±0.319	4.695±0.390	4.530±0.234
	Location 2	4.228±0.319	3.934±0.361	4.081±0.207
	Location 3	4.898±0.190	4.685±0.121	4.791±0.151
	Location 4	5.879±0.434	5.330±0.372	5.605±0.388
	Location 5	4.348±0.323	4.763±0.370	4.555±0.294
Copper	Location 1	59.833±3.452	66.998±1.662	63.416±5.066
	Location 2	58.072±3.216	54.327±3.890	56.200±2.648
	Location 3	52.053±3.102	48.308±4.991	50.181±4.046
	Location 4	60.640±2.870	57.480±3.157	59.060±2.234
	Location 5	58.618±4.164	55.415±3.144	57.017±2.265
Lead	Location 1	6.040±0.515	6.439±0.616	6.240±0.283
	Location 2	6.099±0.347	5.806±0.375	5.952±0.207
	Location 3	5.683±0.424	5.470±0.419	5.576±0.151
	Location 4	5.849±0.438	5.300±0.436	5.574±0.388
	Location 5	5.987±0.385	6.402±0.432	6.194±0.294
Manganese	Location 1	136.256±6.657	130.511±9.951	133.383±4.062
	Location 2	141.475±4.709	133.259±6.999	137.367±5.810
	Location 3	136.958±1.797	129.264±6.650	133.111±4.223
	Location 4	127.799±4.631	134.304±2.103	131.052±4.600
	Location 5	141.860±5.171	136.538±0.685	139.199±3.764
Nickel	Location 1	3.025±0.215	3.265±0.170	3.145±0.170
	Location 2	2.901±0.306	3.098±0.047	2.999±0.139
	Location 3	2.810±0.286	3.014±0.280	2.912±0.144
	Location 4	2.790±0.242	3.125±0.152	2.957±0.237
	Location 5	2.571±0.222	2.883±0.116	2.727±0.221
Selenium	Location 1	3.021±0.206	3.261±0.161	3.141±0.170
	Location 2	2.301±0.319	2.390±0.059	2.345±0.063
	Location 3	2.910±0.194	3.213±0.189	3.061±0.215
	Location 4	3.898±0.283	4.233±0.193	4.065±0.237
	Location 5	3.405±0.258	3.617±0.152	3.511±0.150
Zinc	Location 1	92.019±6.484	86.274±8.778	89.147±4.062
	Location 2	92.380±3.839	84.164±6.129	88.272±5.810
	Location 3	86.281±5.041	78.587±9.894	82.434±7.468
	Location 4	91.430±6.528	95.935±4.000	93.682±3.186
	Location 5	80.570±7.061	75.248±2.575	77.909±3.764

(e) Intra-batch concentration of metals in HM 6

Intra-batch concentration of metals in HM 6						
Element	Location	Sample 1	Sample 2	Sample 3	Sample 4	Average Conc
Arsenic	Location 1	0.333±0.025	0.317±0.017	0.279±0.014	0.276±0.020	0.301±0.019
	Location 2	0.235±0.023	0.242±0.011	0.217±0.019	0.207±0.024	0.225±0.019
	Location 3	0.246±0.022	0.245±0.017	0.225±0.017	0.216±0.021	0.233±0.019
	Location 4	0.277±0.019	0.297±0.014	0.255±0.014	0.271±0.025	0.275±0.018
	Location 5	0.221±0.023	0.239±0.005	0.195±0.011	0.206±0.016	0.215±0.014
cadmium	Location 1	0.534±0.033	0.548±0.025	0.480±0.022	0.477±0.028	0.510±0.027
	Location 2	0.541±0.044	0.527±0.012	0.466±0.018	0.496±0.023	0.507±0.024
	Location 3	0.518±0.023	0.504±0.018	0.453±0.018	0.443±0.022	0.480±0.020
	Location 4	0.556±0.019	0.565±0.015	0.492±0.014	0.487±0.103	0.525±0.038
	Location 5	0.461±0.033	0.433±0.015	0.389±0.021	0.411±0.0257	0.424±0.024
Chromium	Location 1	0.768±0.053	0.711±0.048	0.641±0.042	0.792±0.045	0.728±0.047
	Location 2	0.736±0.021	0.798±0.015	0.811±0.048	0.767±0.026	0.778±0.027
	Location 3	0.769±0.033	0.755±0.027	0.704±0.027	0.694±0.032	0.730±0.030
	Location 4	0.712±0.048	0.731±0.020	0.648±0.019	0.612±0.107	0.676±0.048
	Location 5	0.813±0.043	0.785±0.026	0.742±0.031	0.763±0.036	0.776±0.034
Copper	Location 1	11.125±0.545	12.222±0.415	10.009±0.497	10.391±0.520	10.937±0.495
	Location 2	12.645±1.280	12.778±0.285	11.570±1.012	10.369±1.420	11.840±0.999
	Location 3	11.580±0.769	11.683±0.428	10.367±0.219	10.956±0.672	11.146±0.522
	Location 4	11.005±0.799	10.935±0.847	10.812±0.218	13.013±0.461	11.441±0.581
	Location 5	11.908±0.912	9.858±1.092	11.543±0.795	12.081±0.044	11.348±0.711
Lead	Location 1	2.931±0.199	3.171±0.154	2.690±0.193	2.860±0.210	2.913±0.189
	Location 2	2.680±0.301	2.713±0.041	2.605±0.033	2.404±0.052	2.601±0.107
	Location 3	2.441±0.051	2.544±0.043	2.228±0.046	2.366±0.050	2.395±0.035
	Location 4	2.807±0.186	3.043±0.096	2.615±0.089	2.738±0.184	2.801±0.138
	Location 5	2.934±0.162	3.107±0.056	2.7685±0.045	2.884±0.091	2.923±0.088
Manganese	Location 1	77.823±4.526	76.988±2.736	67.772±2.111	68.078±3.820	72.665±3.298
	Location 2	93.855±4.886	95.020±1.491	86.803±3.780	81.110±6.560	89.197±4.179
	Location 3	103.694±5.147	106.859±3.083	89.643±2.183	96.949±5.036	99.286±3.862
	Location 4	100.769±2.702	98.274±2.174	87.446±0.769	86.609±3.289	93.274±7.293
	Location 5	93.992±7.726	95.328±6.151	82.670±6.240	85.789±6.705	89.445±6.706
Nickel	Location 1	1.572±0.050	1.812±0.005	1.504±0.044	1.500±0.061	1.597±0.147
	Location 2	1.263±0.300	1.295±0.040	1.167±0.032	1.178±0.051	1.226±0.063
	Location 3	1.931±0.067	2.034±0.059	1.718±0.038	1.856±0.066	1.884±0.058
	Location 4	1.908±0.174	2.143±0.084	1.715±0.077	1.838±0.172	1.901±0.180
	Location 5	1.906±0.150	2.079±0.044	1.835±0.033	1.856±0.079	1.919±0.111
Selenium	Location 1	10.002±0.865	11.099±0.547	9.186±0.764	9.268±0.840	9.889±0.886
	Location 2	8.135±0.684	8.268±0.515	7.060±0.517	7.858±0.571	7.830±0.541
	Location 3	9.869±0.629	9.972±0.287	8.656±0.079	9.245±0.532	9.435±0.382
	Location 4	8.798±0.689	9.033±0.351	8.305±0.108	8.728±0.737	8.716±0.471
	Location 5	9.581±0.712	9.654±0.344	9.216±0.595	8.531±0.892	9.246±0.636
Zinc	Location 1	21.398±2.337	23.063±0.547	20.347±0.922	19.654±1.491	21.115±1.483
	Location 2	21.432±1.807	19.497±0.9124	17.681±1.610	17.988±1.948	19.149±1.717
	Location 3	20.431±2.231	21.596±0.480	19.780±0.580	17.687±2.433	19.873±1.640
	Location 4	19.132±1.941	19.818±1.240	18.490±0.500	16.134±1.351	18.394±1.601
	Location 5	19.113±1.180	18.449±0.312	17.791±1.063	16.234±1.359	17.897±1.233

(f) Intra-batch concentration of metals in HM7

Intra-batch concentration of metals in HM7				
Element	**Location**	**Sample 1**	**Sample 2**	**Average Conc**
Arsenic	Location 1	6.473±0.438	6.772±0.539	6.623±0.212
	Location 2	5.518±0.362	4.924±0.440	5.221±0.419
	Location 3	4.739±0.406	4.426±0.401	4.582±0.221
	Location 4	6.015±0.385	5.466±0.383	5.741±0.388
	Location 5	5.412±0.370	5.827±0.416	5.619±0.294
cadmium	Location 1	0.510±0.030	0.554±0.016	0.532±0.031
	Location 2	0.478±0.040	0.511±0.007	0.494±0.023
	Location 3	0.537±0.029	0.563±0.024	0.550±0.019
	Location 4	0.529±0.015	0.488±0.012	0.509±0.029
	Location 5	0.462±0.033	0.435±0.016	0.449±0.019
Chromium	Location 1	0.582±0.037	0.666±0.024	0.624±0.060
	Location 2	0.494±0.051	0.527±0.018	0.510±0.023
	Location 3	0.545±0.038	0.571±0.033	0.558±0.019
	Location 4	0.528±0.025	0.488±0.022	0.508±0.029
	Location 5	0.501±0.035	0.474±0.018	0.487±0.019
Copper	Location 1	5.145±0.178	5.776±0.249	5.460±0.446
	Location 2	5.030±0.369	4.737±0.410	4.884±0.207
	Location 3	5.033±0.230	4.820±0.162	4.926±0.151
	Location 4	5.741±0.466	5.192±0.404	5.466±0.388
	Location 5	3.618±0.323	4.033±0.370	3.825±0.294
Lead	Location 1	1.372±0.039	1.273±0.034	1.323±0.070
	Location 2	1.717±0.270	1.534±0.010	1.625±0.129
	Location 3	1.712±0.068	1.815±0.063	1.764±0.073
	Location 4	1.725±0.221	1.960±0.131	1.842±0.166
	Location 5	1.350±0.164	1.662±0.058	1.506±0.221
Manganese	Location 1	41.428±4.485	45.593±2.695	43.511±2.945
	Location 2	38.997±1.747	35.252±2.421	37.124±2.648
	Location 3	40.482±2.476	36.737±4.365	38.610±3.420
	Location 4	41.040±2.141	37.880±2.429	39.460±2.234
	Location 5	36.118±2.527	32.915±1.507	34.517±2.265
Nickel	Location 1	1.350±0.078	1.252±0.033	1.301±0.070
	Location 2	1.729±0.264	1.547±0.005	1.638±0.129
	Location 3	1.260±0.088	1.363±0.083	1.311±0.073
	Location 4	1.325±0.101	1.460±0.011	1.392±0.096
	Location 5	1.238±0.130	1.321±0.024	1.279±0.059
Selenium	Location 1	5.098±0.245	5.729±0.316	5.414±0.446
	Location 2	5.509±0.439	5.215±0.481	5.362±0.207
	Location 3	5.157±0.181	4.944±0.112	5.051±0.151
	Location 4	5.217±0.273	4.668±0.211	4.942±0.388
	Location 5	5.061±0.433	5.476±0.480	5.269±0.294
Zinc	Location 1	6.473±0.438	6.772±0.539	6.623±0.212
	Location 2	5.518±0.362	4.924±0.440	5.221±0.419
	Location 3	4.739±0.406	4.426±0.401	4.582±0.221
	Location 4	6.015±0.385	5.466±0.383	5.741±0.388
	Location 5	5.412±0.370	5.827±0.416	5.619±0.294

(g) Intra-batch concentration of metals in HM8

Intra-batch concentration of metals in HM8				
Element	Location	Sample 1	Sample 2	Average Conc
Arsenic	Location 1	0.403±0.029	0.457±0.016	0.430±0.038
	Location 2	0.314±0.038	0.347±0.005	0.330±0.023
	Location 3	0.380±0.016	0.366±0.010	0.373±0.010
	Location 4	0.256±0.015	0.265±0.012	0.261±0.006
	Location 5	0.370±0.032	0.342±0.015	0.356±0.019
cadmium	Location 1	0.541±0.024	0.585±0.011	0.563±0.031
	Location 2	0.542±0.037	0.615±0.005	0.578±0.051
	Location 3	0.365±0.041	0.411±0.035	0.388±0.033
	Location 4	0.562±0.035	0.521±0.031	0.542±0.029
	Location 5	0.489±0.033	0.462±0.016	0.476±0.019
Chromium	Location 1	2.862±0.125	3.102±0.080	2.982±0.170
	Location 2	3.948±0.278	3.737±0.019	3.842±0.149
	Location 3	3.154±0.091	3.257±0.085	3.205±0.073
	Location 4	3.803±0.272	4.138±0.182	3.970±0.237
	Location 5	3.522±0.188	3.834±0.082	3.678±0.221
Copper	Location 1	36.698±4.654	37.863±2.864	37.281±0.824
	Location 2	40.485±1.747	36.740±2.421	38.613±2.648
	Location 3	39.042±1.336	35.297±3.225	37.169±2.280
	Location 4	40.524±3.040	37.364±3.327	38.944±2.234
	Location 5	41.430±2.437	38.227±1.417	39.829±2.265
Lead	Location 1	2.790±0.153	3.030±0.108	2.910±0.170
	Location 2	2.985±0.315	3.074±0.055	3.029±0.063
	Location 3	3.194±0.102	3.297±0.097	3.245±0.073
	Location 4	2.688±0.233	3.023±0.143	2.855±0.237
	Location 5	3.462±0.168	3.674±0.062	3.568±0.150
Manganese	Location 1	29.102±3.225	28.267±1.435	28.685±0.591
	Location 2	33.087±2.057	29.742±2.731	31.415±2.365
	Location 3	29.683±2.141	26.938±3.030	28.311±2.585
	Location 4	37.589±1.791	34.429±2.078	36.009±2.234
	Location 5	30.074±2.544	26.870±1.523	28.472±2.265
Nickel	Location 1	7.643±0.723	8.474±0.794	8.059±0.588
	Location 2	8.318±0.327	8.024±0.408	8.171±0.207
	Location 3	9.035±0.291	8.343±0.223	8.689±0.489
	Location 4	8.739±0.461	8.190±0.399	8.464±0.388
	Location 5	7.578±0.259	7.993±0.306	7.785±0.294
Selenium	Location 1	2.844±0.232	3.084±0.187	2.964±0.170
	Location 2	2.368±0.273	2.441±0.013	2.404±0.051
	Location 3	2.405±0.069	2.508±0.064	2.456±0.073
	Location 4	3.040±0.272	3.375±0.182	3.207±0.237
	Location 5	3.890±0.195	4.203±0.089	4.046±0.221
Zinc	Location 1	92.565±8.811	98.310±5.517	95.438±4.062
	Location 2	94.327±1.768	88.111±4.058	91.219±4.396
	Location 3	90.958±3.767	83.264±8.620	87.111±6.193
	Location 4	93.735±2.139	99.533±4.667	96.634±4.100
	Location 5	97.608±5.686	92.286±1.200	94.947±3.764

(h) Intra-batch concentration of metals in HM9

Intra-batch concentration of metals in HM 9						
Election	Location	Sample 1	Sample 2	Sample 3	Sample 4	Average Conc
Arsenic	Location 1	0.243±0.019	0.237±0.011	0.209±0.008	0.205±0.014	0.224±0.019
	Location 2	0.250±0.019	0.257±0.007	0.232±0.015	0.222±0.020	0.240±0.016
	Location 3	0.319±0.024	0.318±0.018	0.298±0.018	0.288±0.023	0.306±0.015
	Location 4	0.289±0.021	0.310±0.016	0.267±0.015	0.283±0.027	0.287±0.018
	Location 5	0.227±0.020	0.244±0.003	0.200±0.008	0.212±0.013	0.221±0.019
Cadmium	Location 1	0.554±0.021	0.568±0.013	0.500±0.010	0.496±0.016	0.530±0.037
	Location 2	0.448±0.043	0.434±0.010	0.373±0.016	0.403±0.021	0.414±0.034
	Location 3	0.558±0.019	0.544±0.014	0.493±0.014	0.483±0.018	0.519±0.037
	Location 4	0.453±0.013	0.462±0.009	0.389±0.008	0.383±0.096	0.422±0.041
	Location 5	0.515±0.031	0.487±0.013	0.443±0.019	0.465±0.024	0.478±0.031
Chromium	Location 1	4.462±0.156	4.702±0.111	4.221±0.150	4.390±0.167	4.444±0.200
	Location 2	3.940±0.279	3.973±0.019	3.865±0.011	3.664±0.031	3.861±0.139
	Location 3	3.441±0.076	3.544±0.067	3.228±0.070	3.366±0.075	3.395±0.133
	Location 4	3.918±0.192	4.153±0.102	3.725±0.095	3.848±0.190	3.911±0.180
	Location 5	3.940±0.197	4.114±0.091	3.775±0.080	3.891±0.126	3.930±0.141
Copper	Location 1	57.159±5.474	58.324±3.684	51.108±3.059	51.414±4.768	54.501±3.774
	Location 2	56.953±3.526	58.118±0.131	49.901±2.420	49.208±4.200	53.545±4.641
	Location 3	72.342±5.147	71.507±3.083	65.291±2.183	65.597±7.036	68.684±4.362
	Location 4	57.930±4.150	59.435±3.622	51.607±2.217	50.770±4.737	54.935±4.383
	Location 5	65.819±6.731	67.155±5.156	60.497±5.245	57.616±5.711	62.772±4.482
Lead	Location 1	1.831±0.056	2.071±0.011	1.763±0.050	1.759±0.067	1.856±0.147
	Location 2	1.943±0.326	1.975±0.066	1.847±0.058	1.858±0.077	1.906±0.063
	Location 3	1.668±0.058	1.771±0.050	1.455±0.029	1.593±0.057	1.622±0.049
	Location 4	1.755±0.125	1.990±0.035	1.721±0.028	1.685±0.123	1.788±0.138
	Location 5	1.674±0.145	1.624±0.074	1.603±0.028	1.847±0.039	1.687±0.111
Manganese	Location 1	95.176±4.519	94.011±6.309	87.960±3.894	88.266±7.603	91.353±3.774
	Location 2	98.240±2.131	97.075±5.526	90.023±4.420	89.330±6.200	93.667±4.641
	Location 3	88.321±6.242	87.486±4.178	81.269±3.278	73.576±8.131	82.663±5.457
	Location 4	97.535±5.545	90.375±6.133	91.212±3.612	99.040±3.017	94.540±4.383
	Location 5	96.159±8.281	87.956±10.261	90.837±3.795	97.495±5.706	93.112±4.482
Nickel	Location 1	5.403±0.481	5.393±0.374	5.112±0.459	5.281±0.560	5.297±0.136
	Location 2	4.996±0.588	5.028±0.329	4.713±0.321	4.419±0.340	4.789±0.284
	Location 3	5.978±0.486	6.450±0.478	5.765±0.481	5.903±0.485	6.024±0.297
	Location 4	4.918±0.192	5.153±0.102	4.725±0.095	4.848±0.190	4.911±0.180
	Location 5	4.592±0.197	4.765±0.091	4.427±0.080	4.542±0.126	4.581±0.141
Selenium	Location 1	2.826±0.184	3.066±0.139	2.585±0.178	2.754±0.195	2.808±0.200
	Location 2	2.648±0.279	2.944±0.019	2.573±0.011	2.371±0.031	2.634±0.237
	Location 3	3.121±0.097	3.224±0.089	2.908±0.092	3.046±0.096	3.075±0.133
	Location 4	2.144±0.258	2.379±0.168	1.951±0.161	2.074±0.256	2.137±0.180
	Location 5	1.975±0.193	2.149±0.087	1.810±0.076	1.925±0.122	1.965±0.141
Zinc	Location 1	34.052±3.075	35.717±1.285	33.001±1.660	32.307±2.229	33.769±1.483
	Location 2	29.786±2.137	27.851±1.242	26.035±1.940	26.341±2.278	27.503±1.717
	Location 3	37.121±2.571	38.286±0.821	36.470±0.921	34.376±2.774	36.563±1.640
	Location 4	32.038±2.482	32.724±1.781	31.396±1.042	29.040±1.893	31.299±1.601
	Location 5	30.959±1.990	30.295±1.122	29.637±1.873	28.080±2.169	29.743±1.233

(i) Intra-batch concentration of metals in HM10

Intra-batch concentration of metals in HM10				
Element		**Sample 1**	**Sample 2**	**Average Conc**
Arsenic	Location 1	0.485±0.028	0.539±0.015	0.512±0.038
	Location 2	0.482±0.027	0.515±0.005	0.498±0.023
	Location 3	0.561±0.049	0.588±0.043	0.574±0.019
	Location 4	0.611±0.019	0.660±0.016	0.636±0.034
	Location 5	0.427±0.033	0.399±0.016	0.413±0.019
cadmium	Location 1	0.533±0.037	0.587±0.024	0.560±0.038
	Location 2	0.491±0.048	0.524±0.015	0.507±0.023
	Location 3	0.636±0.019	0.623±0.014	0.629±0.010
	Location 4	0.486±0.025	0.494±0.022	0.490±0.006
	Location 5	0.483±0.037	0.456±0.019	0.470±0.019
Chromium	Location 1	2.144±0.151	2.294±0.106	2.219±0.106
	Location 2	2.512±0.279	2.585±0.019	2.548±0.051
	Location 3	4.631±0.325	4.704±0.319	4.667±0.052
	Location 4	3.899±0.233	4.234±0.143	4.067±0.237
	Location 5	2.838±0.165	3.151±0.059	2.994±0.221
Copper	Location 1	13.138±1.662	14.303±0.707	13.721±0.824
	Location 2	13.075±0.653	12.330±0.621	12.703±0.527
	Location 3	14.154±0.913	12.709±0.902	13.432±0.908
	Location 4	14.629±0.533	13.469±0.820	14.049±0.820
	Location 5	13.751±1.490	11.547±1.470	12.649±1.558
Lead	Location 1	2.540±0.263	2.780±0.218	2.660±0.170
	Location 2	2.355±0.316	2.444±0.056	2.399±0.063
	Location 3	3.530±0.092	3.634±0.086	3.582±0.073
	Location 4	3.888±0.256	4.223±0.166	4.055±0.237
	Location 5	1.699±0.155	1.911±0.049	1.805±0.150
Manganese	Location 1	66.749±3.394	61.004±5.688	63.877±4.062
	Location 2	68.474±1.839	60.258±4.129	64.366±5.810
	Location 3	65.834±0.817	62.141±5.670	63.988±3.243
	Location 4	59.445±4.141	62.950±1.613	61.198±2.479
	Location 5	64.990±4.186	59.668±2.700	62.329±3.764
Nickel	Location 1	2.582±0.063	2.722±0.058	2.652±0.099
	Location 2	1.932±0.266	2.015±0.006	1.973±0.059
	Location 3	1.555±0.037	1.354±0.032	1.454±0.142
	Location 4	1.466±0.212	1.601±0.122	1.533±0.096
	Location 5	1.691±0.163	1.806±0.057	1.748±0.081
Selenium	Location 1	3.701±0.316	4.132±0.387	3.917±0.305
	Location 2	4.627±0.317	4.133±0.358	4.380±0.349
	Location 3	2.860±0.183	2.647±0.115	2.754±0.151
	Location 4	4.980±0.503	4.614±0.441	4.797±0.259
	Location 5	4.196±0.262	4.611±0.309	4.404±0.294
Zinc	Location 1	16.751±1.999	17.916±1.044	17.334±0.824
	Location 2	13.300±0.860	12.855±0.828	13.077±0.315
	Location 3	15.169±0.715	13.724±0.704	14.447±0.710
	Location 4	14.479±0.670	13.319±0.957	13.899±0.820
	Location 5	16.298±1.337	15.095±1.417	15.697±0.851

Appendix XII: Table of inter-batch concentration of metals in herbal medicine samples

(a) Inter-batch concentration of metals in HM2

Inter-batch concentration of metals in HM2						
	Location 1	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.814±0.051	0.530±0.019	0.565±0.014	0.558±0.019	0.568±0.030	0.607±0.117
cadmium	0.582±0.031	0.481±0.040	0.563±0.013	0.570±0.018	0.594±0.032	0.558±0.045
Chromium	2.984±0.145	2.979±0.144	2.415±0.083	2.907±0.177	2.608±0.135	2.779±0.255
Copper	37.741±2.750	31.138±2.984	35.050±3.324	28.910±2.643	26.927±1.927	31.953±4.424
Lead	27.191±1.777	32.574±2.684	35.866±2.375	35.320±2.626	35.919±1.910	33.374±3.720
Manganese	95.157±7.622	91.894±2.897	91.243±4.125	91.792±3.747	87.259±5.388	91.469±2.813
Nickel	6.524±0.284	5.522±0.261	4.176±0.213	4.624±0.337	4.894±0.073	5.148±0.910
Selenium	8.407±0.642	7.241±0.348	8.076±0.257	7.976±0.367	8.125±0.227	7.965±0.435
Zinc	129.086±6.850	135.142±4.347	125.704±5.375	141.222±4.937	125.989±3.443	131.428±6.662

(b) Inter-batch concentration of metals in HM3

Inter-batch concentration of metals in HM3						
Element	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.353±0.012	0.428±0.038	0.429±0.036	0.733±0.040	0.421±0.033	0.473±0.032
cadmium	0.548±0.041	0.654±0.045	0.626±0.026	0.505±0.026	0.321±0.020	0.531±0.032
Chromium	2.303±0.115	1.778±0.122	1.626±0.061	1.676±0.087	1.486±0.069	1.774±0.091
Copper	13.455±1.010	13.394±1.196	14.934±0.729	16.363±1.088	15.619±0.523	14.753±0.909
Lead	1.187±0.041	1.351±0.090	1.465±0.032	1.486±0.057	1.492±0.040	1.396±0.052
Manganese	198.515±7.183	190.947±8.819	193.003±8.245	190.128±5.663	195.128±9.154	193.544±7.813
Nickel	2.693±0.133	2.978±0.136	3.121±0.069	2.800±0.127	2.823±0.105	2.883±0.114
Selenium	2.174±0.126	1.956±0.140	3.325±0.071	2.129±0.109	1.823±0.124	2.281±0.114
Zinc	26.002±2.190	25.597±2.271	25.374±1.831	23.959±1.658	22.630±1.278	24.712±1.846

(c) Inter-batch concentration of metals in HM4

Inter-batch concentration of metals in HM4						
Element	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.296±0.014	0.300±0.021	0.244±0.010	0.327±0.013	0.291±0.021	0.292±0.030
cadmium	0.635±0.020	0.604±0.024	0.778±0.043	0.615±0.035	0.496±0.029	0.626±0.101
Chromium	1.592±0.077	1.600±0.078	1.624±0.100	1.502±0.104	1.328±0.100	1.529±0.122
Copper	14.188±0.805	12.937±1.044	13.063±0.838	12.109±0.643	15.527±1.427	13.565±1.323
Lead	3.610±0.181	2.739±0.169	2.982±0.099	3.805±0.208	2.146±0.111	3.057±0.671
Manganese	82.971±5.121	86.132±3.597	87.673±7.825	81.972±4.907	88.409±4.028	85.431±2.846
Nickel	1.331±0.066	1.879±0.139	2.331±0.055	1.932±0.156	2.208±0.137	1.936±0.387
Selenium	5.276±0.312	5.418±0.448	6.326±0.167	5.866±0.472	3.825±0.347	5.342±0.943
Zinc	23.960±1.943	22.126±0.953	23.611±1.959	35.802±1.647	22.425±1.885	25.585±5.763

(d) Inter-batch concentration of metals in HM5

Inter-batch concentration of metals in HM5						
	Location 1	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.417±0.021	0.423±0.034	0.388±0.010	0.336±0.032	0.386±0.034	0.390±0.035
cadmium	0.743±0.052	0.791±0.038	0.622±0.016	0.831±0.022	0.538±0.021	0.705±0.122
Chromium	4.530±0.354	4.081±0.340	4.791±0.155	5.605±0.403	4.555±0.347	4.712±0.561
Copper	63.416±2.557	56.200±3.553	50.181±4.046	59.060±3.013	57.017±3.654	57.175±4.806
Lead	6.240±0.565	5.952±0.361	5.576±0.421	5.574±0.437	6.194±0.409	5.907±0.322
Manganese	133.383±8.304	137.367±5.854	133.111±4.223	131.052±3.367	139.199±2.928	134.822±3.348
Nickel	3.145±0.193	2.999±0.177	2.912±0.283	2.957±0.197	2.727±0.169	2.948±0.152
Selenium	3.141±0.184	2.345±0.189	3.061±0.191	4.065±0.238	3.511±0.205	3.225±0.632
Zinc	89.147±7.631	88.272±4.984	82.434±7.468	93.682±5.264	77.909±4.818	86.289±6.161

(e) Inter-batch concentration of metals in HM6

Inter-batch concentration of metals in HM6						
	Location 1	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.301±0.019	0.225±0.019	0.233±0.017	0.275±0.018	0.215±0.014	0.250±0.037
cadmium	0.487±0.031	0.424±0.037	0.458±0.030	0.556±0.022	0.401±0.018	0.465±0.060
Chromium	0.728±0.047	0.778±0.027	0.730±0.030	0.676±0.048	0.776±0.034	0.738±0.042
Copper	10.937±0.495	11.840±0.999	11.146±0.522	11.441±0.581	11.348±0.711	11.343±0.339
Lead	2.913±0.189	2.601±0.107	2.395±0.035	2.801±0.138	2.923±0.088	2.727±0.226
Manganese	72.665±3.298	89.197±4.179	99.286±3.862	93.274±2.233	89.445±6.706	88.774±9.885
Nickel	1.597±0.040	1.226±0.106	1.885±0.058	1.901±0.127	1.919±0.076	1.706±0.299
Selenium	9.889±0.754	7.830±0.572	9.435±0.382	8.716±0.471	9.246±0.636	9.023±0.789
Zinc	21.115±1.324	19.149±1.569	19.873±1.431	18.393±1.258	17.896±0.978	19.286±1.269

(f) Inter-batch concentration of metals in HM7

Inter-batch concentration of metals in HM7						
Election	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.348±0.015	0.324±0.027	0.308±0.017	0.268±0.012	0.261±0.018	0.302±0.037
cadmium	0.469±0.026	0.487±0.036	0.504±0.027	0.477±0.020	0.427±0.024	0.473±0.029
Chromium	0.624±0.030	0.510±0.035	0.558±0.036	0.508±0.023	0.487±0.027	0.538±0.055
Copper	5.460±0.213	4.884±0.390	4.926±0.196	5.466±0.435	3.825±0.347	4.912±0.669
Lead	1.323±0.036	1.625±0.140	1.764±0.066	1.842±0.176	1.506±0.111	1.612±0.207
Manganese	43.511±3.590	37.124±2.084	38.610±3.420	39.460±2.285	34.517±2.017	38.644±3.305
Nickel	1.301±0.056	1.638±0.134	1.311±0.085	1.392±0.056	1.279±0.077	1.384±0.148
Selenium	5.414±0.280	5.362±0.460	5.051±0.147	4.942±0.242	5.269±0.457	5.208±0.203
Zinc	6.623±0.488	5.221±0.401	4.582±0.403	5.741±0.384	5.619±0.393	5.557±0.748

(g) Inter-batch concentration of metals in HM8

Inter-batch concentration of metals in HM8						
Element	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.430±0.022	0.330±0.021	0.373±0.013	0.261±0.013	0.356±0.023	0.350±0.062
cadmium	0.529±0.017	0.501±0.035	0.480±0.033	0.491±0.028	0.452±0.020	0.491±0.028
Chromium	2.982±0.103	3.842±0.148	3.205±0.088	3.970±0.227	3.678±0.135	3.536±0.424
Copper	37.281±3.759	38.613±2.084	37.169±2.280	38.944±3.183	39.829±1.927	38.367±1.134
Lead	2.910±0.131	3.029±0.185	3.245±0.099	2.855±0.188	3.568±0.115	3.122±0.291
Manganese	28.685±2.330	31.415±2.394	28.311±2.585	36.009±1.934	28.472±2.034	30.578±3.292
Nickel	8.059±0.758	8.171±0.368	8.689±0.257	8.464±0.430	7.785±0.283	8.234±0.352
Selenium	2.964±0.210	2.404±0.143	2.456±0.066	3.207±0.227	4.046±0.142	3.016±0.669
Zinc	95.438±7.164	91.219±2.913	87.111±6.193	96.634±3.403	94.947±3.443	93.070±3.898

(h) Inter-batch concentration of metals in HM9

Inter-batch concentration of metals in HM9						
Element	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.224±0.013	0.240±0.015	0.306±0.021	0.287±0.020	0.221±0.011	0.255±0.039
cadmium	0.499±0.019	0.492±0.036	0.481±0.021	0.389±0.020	0.430±0.024	0.458±0.047
Chromium	4.444±0.146	3.861±0.085	3.395±0.072	3.911±0.145	3.930±0.123	3.908±0.372
Copper	54.501±4.246	53.545±2.569	68.684±4.362	54.935±3.681	62.772±5.711	58.888±6.604
Lead	1.856±0.046	1.906±0.132	1.622±0.049	1.788±0.078	1.687±0.071	1.772±0.117
Manganese	91.353±5.581	93.667±4.569	82.663±5.457	94.540±4.577	93.112±7.011	91.067±4.840
Nickel	5.297±0.468	4.789±0.394	6.024±0.482	4.911±0.145	4.581±0.123	5.121±0.568
Selenium	2.808±0.174	2.634±0.085	3.075±0.093	2.137±0.211	1.965±0.119	2.524±0.463
Zinc	33.769±2.062	27.503±1.899	36.563±1.772	31.299±1.799	29.743±1.788	31.776±3.518

(i) Inter-batch concentration of metals in HM10

Inter-batch concentration of metals in HM 10						
Element	**Location 1**	**Location 2**	**Location 3**	**Location 4**	**Location 5**	**Average conc**
Arsenic	0.512±0.021	0.498±0.016	0.574±0.046	0.636±0.018	0.413±0.024	0.527±0.084
cadmium	0.492±0.021	0.526±0.046	0.542±0.014	0.496±0.026	0.427±0.029	0.497±0.044
Chromium	2.219±0.129	2.548±0.149	4.667±0.322	4.067±0.188	2.994±0.112	3.299±1.035
Copper	13.721±1.185	12.703±0.637	13.432±0.908	14.049±0.676	12.649±1.480	13.311±0.619
Lead	2.660±0.241	2.399±0.186	3.582±0.089	4.055±0.211	1.805±0.102	2.900±0.909
Manganese	63.877±4.541	64.366±2.984	63.988±3.243	61.198±2.877	62.329±3.443	63.151±1.341
Nickel	2.652±0.060	1.973±0.136	1.454±0.035	1.533±0.167	1.748±0.110	1.872±0.481
Selenium	3.917±0.351	4.380±0.338	2.754±0.149	4.797±0.472	4.404±0.286	4.050±0.789
Zinc	17.334±1.522	13.077±0.844	14.447±0.710	13.899±0.813	15.697±1.377	14.891±1.665

Appendix XIII: Table of P values for significance of intra-batch and inter-batch variability of heavy metals composition in HMs

(a) Intra-batch and inter-batch variability of heavy metals in HM2

HM2	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.031	Yes	0.451	No
Location 2	0.215	No		
Location 3	0.431	No		
Location 4	0.057	No		
Location 5	0.327	No		
Cadmium				
Location 1	0.177	No	0.211	No
Location 2	0.266	No		
Location 3	0.036	Yes		
Location 4	0.566	No		
Location 5	0.972	No		
Chromium				
Location 1	0.190	No	0.125	No
Location 2	0.140	No		
Location 3	0.028	Yes		
Location 4	0.399	No		
Location 5	0.238	No		
Copper				
Location 1	0.899	No	0.462	No
Location 2	0.172	No		
Location 3	0.660	No		
Location 4	0.011	Yes		
Location 5	0.304	No		
Lead				
Location 1	0.616	No	0.642	No
Location 2	0.096	No		
Location 3	0.485	No		
Location 4	0.022	Yes		
Location 5	0.061	No		
Manganese				
Location 1	0.074	No	0.528	No
Location 2	0.218	No		
Location 3	0.095	No		
Location 4	0.481	No		
Location 5	0.814	No		
Nickel				
Location 1	0.110	No	0.604	No
Location 2	0.345	No		
Location 3	0.142	No		
Location 4	0.019	Yes		
Location 5	0.011	Yes		
Selenium				
Location 1	0.390	No	0.390	No
Location 2	0.147	No		
Location 3	0.117	No		
Location 4	0.047	Yes		
Location 5	0.014	Yes		
Zinc				
Location 1	0.146	No	0.673	No
Location 2	0.098	No		
Location 3	0.148	No		
Location 4	0.357	No		
Location 5	0.442	No		

(b) Intra-batch and inter-batch variability of heavy metals in HM3

HM3	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.001	Yes		
Location 2	0.027	Yes		
Location 3	0.004	Yes		No
Location 4	0.007	Yes		
Location 5	0.002	Yes	0.114836	
Cadmium				
Location 1	0.003	Yes		
Location 2	0.016	Yes		
Location 3	0.012	Yes	0.481	No
Location 4	0.667	No		
Location 5	0.002	Yes		
Chromium				
Location 1	0.014	Yes		
Location 2	0.237	No		
Location 3	0.001	Yes	0.937	No
Location 4	0.785	No		
Location 5	0.013	Yes		
Copper				
Location 1	0.240	No		
Location 2	0.099	No		
Location 3	0.065	No	0.364	No
Location 4	0.073	No		
Location 5	0.914	No		
Lead				
Location 1	0.007	Yes		
Location 2	0.037	Yes		
Location 3	0.015	Yes	0.224	No
Location 4	0.138	No		
Location 5	0.002	Yes		
Manganese				
Location 1	0.062	No		
Location 2	0.029	Yes		
Location 3	0.259	No	0.077	No
Location 4	0.143	No		
Location 5	0.089	No		
Nickel				
Location 1	0.002	Yes		
Location 2	0.009	Yes		
Location 3	0.004	Yes	0.570	No
Location 4	0.224	No		
Location 5	0.007	Yes		
Selenium				
Location 1	0.007	Yes		
Location 2	0.002	Yes		
Location 3	0.002	Yes	0.281	No
Location 4	0.074	No		
Location 5	0.146	No		
Zinc		No		
Location 1	0.146	No		
Location 2	0.007	Yes		
Location 3	0.417	No		No
Location 4	0.582	No	0.606	
Location 5	0.006	Yes		

(c) Intra-batch and inter-batch variability of heavy metals in HM4

HM4	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.024	Yes	0.068	No
Location 2	0.001	Yes		
Location 3	0.573	No		
Location 4	0.297	No		
Location 5	0.996	No		
Cadmium				
Location 1	0.640	No	0.253	No
Location 2	0.301	No		
Location 3	0.049	Yes		
Location 4	0.561	No		
Location 5	0.412	No		
Chromium				
Location 1	0.202	No	0.092	No
Location 2	0.165	No		
Location 3	0.023	Yes		
Location 4	0.459	No		
Location 5	0.147	No		
Copper				
Location 1	0.004	Yes	0.719	No
Location 2	0.182	No		
Location 3	0.103	No		
Location 4	0.099	No		
Location 5	0.784	No		
Lead				
Location 1	0.015	Yes	0.154	No
Location 2	0.147	No		
Location 3	0.017	Yes		
Location 4	0.485	No		
Location 5	0.082	No		
Manganese				
Location 1	0.092	No	0.649	No
Location 2	0.083	No		
Location 3	0.096	No		
Location 4	0.656	No		
Location 5	0.228	No		
Nickel				
Location 1	0.988	No	0.522	No
Location 2	0.424	No		
Location 3	0.036	Yes		
Location 4	0.593	No		
Location 5	0.169	No		
Selenium				
Location 1	0.818	N0	0.818	No
Location 2	0.587	No		
Location 3	0.134	No		
Location 4	0.010	Yes		
Location 5	0.020	Yes		
Zinc				
Location 1	0.001	Yes	0.961	No
Location 2	0.060	No		
Location 3	0.041	Yes		
Location 4	0.250	No		
Location 5	0.900	No		

(d) Intra-batch and inter-batch variability of heavy metals in HM5

HM5	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.003	Yes	0.675	No
Location 2	0.001	Yes		
Location 3	0.430	No		
Location 4	0.268	No		
Location 5	0.767	No		
Cadmium				
Location 1	0.162	No	0.362	No
Location 2	0.333	No		
Location 3	0.019	Yes		
Location 4	0.312	No		
Location 5	0.423	No		
Chromium				
Location 1	0.741	No	0.896	No
Location 2	0.551	No		
Location 3	0.161	No		
Location 4	0.037	Yes		
Location 5	0.101	No		
Copper				
Location 1	0.423	No	0.146	No
Location 2	0.035	Yes		
Location 3	0.092	No		
Location 4	0.012	Yes		
Location 5	0.083	No		
Lead				
Location 1	0.897	No	0.873	No
Location 2	0.455	No		
Location 3	0.187	No		
Location 4	0.029	Yes		
Location 5	0.033	Yes		
Manganese				
Location 1	0.580	No	0.379	No
Location 2	0.132	No		
Location 3	0.376	No		
Location 4	0.418	No		
Location 5	0.218	No		
Nickel				
Location 1	0.481	No	0.288	No
Location 2	0.061	No		
Location 3	0.027	Yes		
Location 4	0.585	No		
Location 5	0.239	No		
Selenium				
Location 1	0.043	Yes	0.079	No
Location 2	0.230	No		
Location 3	0.011	Yes		
Location 4	0.328	No		
Location 5	0.056	No		
Zinc				
Location 1	0.604	No	0.876	No
Location 2	0.158	No		
Location 3	0.078	No		
Location 4	0.401	No		
Location 5	0.353	No		

(e) Intra-batch and inter-batch variability of heavy metals in HM6

HM6	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.001	Yes	0.351	No
Location 2	0.016	Yes		
Location 3	0.429	No		
Location 4	0.152	No		
Location 5	0.110	No		
Cadmium				
Location 1	0.019	Yes	0.256	No
Location 2	0.014	Yes		
Location 3	0.098	No		
Location 4	0.002	Yes		
Location 5	0.114	No		
Chromium				
Location 1	0.063	No	0.408	No
Location 2	0.731	No		
Location 3	0.018	Yes		
Location 4	0.001	Yes		
Location 5	0.036	Yes		
Copper				
Location 1	0.135	No	0.891	No
Location 2	0.446	No		
Location 3	0.036	Yes		
Location 4	0.034	Yes		
Location 5	0.158	No		
Lead				
Location 1	0.007	Yes	0.313	No
Location 2	0.147	No		
Location 3	0.001	Yes		
Location 4	0.163	No		
Location 5	0.037	Yes		
Manganese				
Location 1	0.122	No	0.027	Yes
Location 2	0.010	Yes		
Location 3	0.056	No		
Location 4	0.807	No		
Location 5	0.038	Yes		
Nickel				
Location 1	0.004	Yes	0.069	No
Location 2	0.149	No		
Location 3	0.676	No		
Location 4	0.004	Yes		
Location 5	0.004	Yes		
Selenium				
Location 1	0.481	No	0.594	No
Location 2	0.003	Yes		
Location 3	0.608	No		
Location 4	0.008	Yes		
Location 5	0.020	Yes		
Zinc				
Location 1	0.018	Yes	0.010	Yes
Location 2	0.035	Yes		
Location 3	0.038	Yes		
Location 4	0.473	No		
Location 5	0.005	Yes		

(f) Intra-batch and inter-batch variability of heavy metals in HM7

HM7	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.004	Yes	0.170	No
Location 2	0.005	Yes		
Location 3	0.728	No		
Location 4	0.087	No		
Location 5	0.758	No		
Cadmium				
Location 1	0.319	No	0.025	
Location 2	0.602	No		
Location 3	0.036	Yes		
Location 4	0.618	No		
Location 5	0.599	No		
Chromium				
Location 1	0.399	No	0.284	No
Location 2	0.455	No		
Location 3	0.039	Yes		
Location 4	0.579	No		
Location 5	0.525	No		
Copper				
Location 1	0.571	No	0.443	No
Location 2	0.358	No		
Location 3	0.337	No		
Location 4	0.197	No		
Location 5	0.020	Yes		
Lead				
Location 1	0.786	No	0.144	No
Location 2	0.701	No		
Location 3	0.621	No		
Location 4	0.968	No		
Location 5	0.180	No		
Manganese				
Location 1	0.129	No	0.457	No
Location 2	0.042	Yes		
Location 3	0.496	No		
Location 4	0.015	Yes		
Location 5	0.053	No		
Nickel				
Location 1	0.622	No	0.296	No
Location 2	0.262	No		
Location 3	0.038	Yes		
Location 4	0.879	No		
Location 5	0.674	No		
Selenium				
Location 1	0.804	No	0.041	Yes
Location 2	0.365	No		
Location 3	0.143	No		
Location 4	0.103	No		
Location 5	0.061	No		
Zinc				
Location 1	0.300	No	0.836	No
Location 2	0.494	No		
Location 3	0.267	No		
Location 4	0.242	No		
Location 5	0.070	No		

(g) Intra-batch and inter-batch variability of heavy metals in HM8

HM8	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.035	Yes	0.111	No
Location 2	0.049	Yes		
Location 3	0.320	No		
Location 4	0.076	No		
Location 5	0.978	No		
Cadmium				
Location 1	0.583	No	0.556	No
Location 2	0.081	No		
Location 3	0.051	No		
Location 4	0.474	No		
Location 5	0.562	No		
Chromium				
Location 1	0.107	No	0.645	No
Location 2	0.707	No		
Location 3	0.017	Yes		
Location 4	0.277	No		
Location 5	0.113	No		
Copper				
Location 1	0.187	No	0.204	No
Location 2	0.017	Yes		
Location 3	0.170	No		
Location 4	0.009	Yes		
Location 5	0.045	Yes		
Lead				
Location 1	0.057	No	0.005	Yes
Location 2	0.382	No		
Location 3	0.014	Yes		
Location 4	0.459	No		
Location 5	0.044	Yes		
Manganese				
Location 1	0.206	No	0.002	Yes
Location 2	0.020	Yes		
Location 3	0.023	Yes		
Location 4	0.012	Yes		
Location 5	0.045	Yes		
Nickel				
Location 1	0.686	No	0.298	No
Location 2	0.494	No		
Location 3	0.538	No		
Location 4	0.034	Yes		
Location 5	0.161	No		
Selenium				
Location 1	0.024	Yes	0.408	No
Location 2	0.707	No		
Location 3	0.014	Yes		
Location 4	0.254	No		
Location 5	0.078	No		
Zinc				
Location 1	0.943	No	0.854	No
Location 2	0.271	No		
Location 3	0.045	Yes		
Location 4	0.507	No		
Location 5	0.142	No		

(h) Intra-batch and inter-batch variability of heavy metals in HM9

HM9	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.003	Yes	0.695	No
Location 2	0.094	No		
Location 3	0.022	Yes		
Location 4	0.001	Yes		
Location 5	0.035	Yes		
Cadmium				
Location 1	0.008	Yes	0.573	No
Location 2	0.017	Yes		
Location 3	0.025	Yes		
Location 4	0.002	Yes		
Location 5	0.020	Yes		
Chromium				
Location 1	0.002	Yes	0.080	No
Location 2	0.045	Yes		
Location 3	0.003	Yes		
Location 4	0.405	No		
Location 5	0.002	Yes		
Copper				
Location 1	0.064	No	0.950	No
Location 2	0.074	No		
Location 3	0.023	Yes		
Location 4	0.154	No		
Location 5	0.001	Yes		
Lead				
Location 1	0.010	Yes	0.069	No
Location 2	0.089	No		
Location 3	0.004	Yes		
Location 4	0.166	No		
Location 5	0.026	Yes		
Manganese				
Location 1	0.431	No	0.923	No
Location 2	0.199	No		
Location 3	0.054	No		
Location 4	0.106	No		
Location 5	0.002	Yes		
Nickel				
Location 1	0.006	Yes	0.499	No
Location 2	0.006	Yes		
Location 3	0.429	No		
Location 4	0.025	Yes		
Location 5	0.071	No		
Selenium				
Location 1	0.127	No	0.923	No
Location 2	0.565	No		
Location 3	0.002	Yes		
Location 4	0.005	Yes		
Location 5	0.169	No		
Zinc				
Location 1	0.008	Yes	0.341	No
Location 2	0.196	No		
Location 3	0.034	Yes		
Location 4	0.150	No		
Location 5	0.003	Yes		

(i) Intra-batch and inter-batch variability of heavy metals in HM10

HM10	Intra-batch variability		Inter-batch variability	
	P- value	Sig.	P-value	Sig.
Arsenic				
Location 1	0.578	No	0.911	No
Location 2	0.337	No		
Location 3	0.034	Yes		
Location 4	0.072	No		
Location 5	0.831	No		
Cadmium				
Location 1	0.090	No	0.169	No
Location 2	0.312	No		
Location 3	0.161	No		
Location 4	0.305	No		
Location 5	0.385	No		
Chromium				
Location 1	0.264	No	0.983	No
Location 2	0.108	No		
Location 3	0.209	No		
Location 4	0.566	No		
Location 5	0.875	No		
Copper				
Location 1	0.194	No	0.246	No
Location 2	0.634	No		
Location 3	0.204	No		
Location 4	0.450	No		
Location 5	0.190	No		
Lead				
Location 1	0.201	No	0.264	No
Location 2	0.648	No		
Location 3	0.010	Yes		
Location 4	0.929	No		
Location 5	0.025	Yes		
Manganese				
Location 1	0.316	No	0.502	No
Location 2	0.210	No		
Location 3	0.461	No		
Location 4	0.413	No		
Location 5	0.894	No		
Nickel				
Location 1	0.014	Yes	0.475	No
Location 2	0.720	No		
Location 3	0.462	No		
Location 4	0.741	No		
Location 5	0.056	No		
Selenium				
Location 1	0.169	No	0.141	No
Location 2	0.760	No		
Location 3	0.027	Yes		
Location 4	0.045	Yes		
Location 5	0.370	No		
Zinc				
Location 1	0.247	No	0.179	No
Location 2	0.363	No		
Location 3	0.051	No		
Location 4	0.207	No		
Location 5	0.160	No		

Appendix XIV: Result of mercury analysis from Analytix laboratory

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Anglian Ruskin University Total Mercury Analysis in Herbal Medicine Samples

Introduction

The aim of this experiment was to determine mercury levels in herbal medicine using the Direct Mercury Analyser.

For the ease of use and practical laboratory purpose, the DMA-80 auto sampler system was used for this experiment. The DMA-80 shares the same technology as the DMA-1 manual single sample analysis system that is based on the principles of sample thermal decomposition, mercury amalgamation and atomic absorption detection.

Different types of sample boats such as nickel boats or quartz boats could be used. However, for this specific experiment, quartz boats were used. The analysis and determination of mercury in herbal medicine was carried out in-house at Analytix Ltd, based in Boldon.

Method

We ran three blank runs to ensure no mercury was left over. Four samples were analysed for total mercury using the DMA-80. A sample was weighed of 0.1 g from solid or 0.1 ml from liquid using a pipette of each of the herbal medicine sample using an analytical balance. The sample was weighed into a quartz boat, and its weight was recorded onto the DMA-80 terminal, EasyCONTROL software.

Sample boat was then loaded onto the built-in auto sampler and pressed 'START' on the touch screen terminal. The EasyCONTROL software provided full control over the analytical process.

A pneumatic arm carried the boat inside the decomposition furnace where the sample was combusted and mercury was released in vapour form. Purified air was used as a carrier gas which transported the mercury vapours to the catalyst. The catalyst filtered impurities and various states of mercury were reduced to elemental mercury. These mercury vapours were trapped on a gold amalgamator. The gold amalgamator flash heated to ~850°C and released the mercury in the atomic absorption spectrophotometer. Absorbance (peak height or peak area) was measured at 253.65 nm as a function of mercury concentration. The DMA-80 total time per analysis was 6 minutes.

Mercury concentrations results were automatically produced on the DMA-80 terminal EasyCONTROL software, saved automatically. These results were viewed, easily transferred using a USB memory stick and were converted into an Excel format for report writing purposes.

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Mercury standard solutions were used to check the validity of the calibration curve. A reference material (CRM2004 Taiwan Clay) was used to verify the accuracy of the results. Satisfactory results were obtained for all tests. Results are listed in the Table below.

Table 1 - Results of Total Mercury in Herbal Medicine Samples (ppb or ng)

Name	Hg [ng]	R	Weight	Unit
bb	0.0557	0.55698	0.1	[g]
bb	0.02656	0.26564	0.1	[g]
bb	0.01971	0.19708	0.1	[g]
Sample 1 body paint	0.79091	8.90664	0.0888	[g]
Sample 1 body paint	0.54841	6.03311	0.0909	[g]
Sample 1 body paint	0.45124	5.58462	0.0808	[g]
Sample 1 body paint	0.85089	10.05783	0.0846	[g]
Sample 2 male tonic	1.28362	14.48783	0.0886	[g]
Sample 2 male tonic	1.62097	14.16934	0.1144	[g]
Sample 2 male tonic	1.63495	18.79249	0.087	[g]
Sample 3 baby oku	0.03085	0.30848	0.1	[g]
Sample 3 baby oku	0.02742	0.2742	0.1	[g]
Sample 3 baby oku	0.01114	0.1114	0.1	[g]
Sample 3 baby oku	0.02742	0.2742	0.1	[g]
Sample 3 baby oku	0.02314	0.23136	0.1	[g]
Sample 4 herbal mixture	0.01885	0.18852	0.1	[g]
Sample 4 herbal mixture	0.02142	0.21422	0.1	[g]
Sample 4 herbal mixture	0.02057	0.20565	0.1	[g]

R = concentration value (ppb or µg/kg)

Table 2 - Average Results of Total Mercury in Herbal Medicine Samples (ppb or ng)

Sample Name	Average Hg (ng)	Average R (ppb or µg/kg)
Sample 1 Body pain	0.660	7.646
Sample 2 Male tonic	1.513	15.817
Sample 3 Baby oku	0.024	0.024
Sample 4 Herbal mixture	0.020	0.203

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Boldon, NE35 9PY
Tel 0844 800 4220 Fax 0844 800 4220
Web www.analytix.co.uk

Summary

1. Sample 1 Body Paint was analysed in quadruplicate and directly without dilution or any sample preparation. Each run was completed within 6 minutes.
2. Sample 2 Male Tonic was analysed in triplicate and directly without dilution or any sample preparation. Each run was completed within 6 minutes.
3. Sample 3 Baby Oku was analysed in quintuplet and directly without dilution or any sample preparation. Each run was completed within 6 minutes.
4. Sample 4 Herbal Mixture was analysed in triplicate and directly without dilution or any sample preparation. Each run was completed within 6 minutes.
5. The calibration was found to be stable. QCs were carried out using mercury standard and certified reference material (CRM2004 Taiwan Clay, 194 ± 18 ng g-1 Hg). Satisfactory results were obtained prior to sample analysis.

Conclusion

We could fully analyse all samples in multiple runs for mercury determination. Using the DMA-80, demonstrated mercury analysis as highly sensitive, accurate, precise and trouble free. The running cost and maintenance is reduced to a minimum.

Four samples were of completely different sample types; however, an analyst does not need to consider different methods for different sample types and all of the different sample types can be analysed using one calibration. Results are produced in under 6 minutes with working range required so you can easily cope with samples turnaround time.

I strongly believe we have fully demonstrated our market leading capabilities for our Direct Mercury Analysers.

If you have any questions or comments about the procedures, handling or systems please feel free to contact me.

Best Regards,

Aniket Divecha

Technical Sales

Analytix Ltd Unit 9 Boldon Court, Boldon Business Park, Boldon,
Tyne & Wear, NE35 9PY **Tel / Fax:** 0844 800 4220 (+44 191 519 4504 for outside UK)
Email: Aniket@analytix.co.uk
Web: www.analytix.co.uk

Appendix XV: Participant consent form

PARTICIPANT CONSENT FORM version one (5/09/15)

NAME OF PARTICIPANT:

Title of the project: PATTERN AND USE OF HERBAL MEDICINE IN EKITI STATE NIGERIA: IDENTIFICATION OF THE CHEMICAL CONSTITUENTS AND EPIDEMIOLOGICAL STUDY

Main investigator and contact details:
Aina olujimi olusola
Department of forensic and biomedical science
Anglia Ruskin University Cambridge

Members of the research team:

1. I agree to take part in the above research. I have read the Participant Information Sheet (version one (05/09/2015) for the study.
   I understand what my role will be in this research, and all my questions have been answered to my satisfaction.

2. I understand that I am free to withdraw from the research at any time, without giving a reason.

3. I am free to ask any questions at any time before and during the study.

4 I understand what will happen to the data collected from me for the research.

5. I have been provided with a copy of this form and the Participant Information Sheet.

6. I understand that quotes from me will be used in the dissemination of the research.

Data Protection: I agree to the University[1] processing personal data which I have supplied.

I agree to the processing of such data for any purposes connected with the Research Project

as outlined to me*

Name of participant (print)……..Signed……..….Date……

[1] "The University" includes Anglia Ruskin University and its Associate Colleges.

I WISH TO WITHDRAW FROM THIS STUDY.

If you wish to withdraw from the research, please speak to the researcher or email them at X stating the title of the research.

You do not have to give a reason for why you would like to withdraw.

Please let the researcher know whether you are/are not happy for them to use any data from you collected to date in the write up and dissemination of the research.

Appendix XVI: Questionnaire template

PATTERN AND USE OF HERBAL MEDICINE IN EKITI STATE NIGERIA

1. Age (Yrs): 18- 29 ☐ 30-49 ☐ 50 -69 ☐ 70 and above ☐

2. Sex: Male ☐ Female ☐

3. Level of Education: Uneducated ☐ Primary ☐ Secondary ☐ Tertiary ☐

4. Religion: ……

5. Occupation: …….

6. Annual income (Naira): Low Income (≤ 600,000) ☐

Middle income (601,000 to 2.4M) ☐ High Income (≥2.4M) ☐

7. Have you heard about herbal medicine: Yes ☐ No ☐

8. Do you know the difference between Government certified and uncertified herbal medicine: Yes ☐ No ☐

9. Have you used herbal medicine in the last 2 years? ☐ Yes ☐ No

10. If No why ? ……

If yes ticked to question 9 please continue with the rest of the questions.

11. Which type of herbal medicine have you used in the last 2 years: ☐ certified ☐ Uncertified ☐ Both

12. What are the names of the herbal medicine you used ……..

13. How many times have you used uncertified herbal medicine in the last 2 years?

Once-Twice ☐ 3- 10 times ☐ above 10 times ☐

14. Why do you prefer to use herbal medicine?

……

15. Why do you not prefer to visit the hospital? .....

16. Was the herbal medicine effective for the intended use: Yes ☐ No ☐

17. Do you think it is safe to take uncertified herbal medicine: Yes ☐ NO ☐

18. What side effect did you experience associated with herbal medicine use?

……

……

19. How did you manage the side effect?

……

20. How do you think the safety of uncertified herbal medicine can be achieved?

……

Appendix XVII: Picture of Selected HM samples

Sample No.	Sample name	Appearance	Sample No.	Sample name	Appearance
HM1	Body pain		HM2	Male tonic	
HM3	Supa A1		HM4	Wadco total blood cure	

HM5	Wadco pile& dysentry		HM6	M&T Capsule	
HM7	YK original malaria		HM8	Aromalegun	
HM9	Eroxy 5000		HM10	Original malaria/yellow fever and typhoid	

Appendix XVIII: Certificate of Certified Reference Material

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 1570a

Trace Elements in Spinach Leaves

This Standard Reference Material (SRM) is intended primarily for use in evaluating the reliability of analytical methods for the determination of major, minor, and trace elements in botanical materials, agricultural food products, and materials of similar matrix. A unit of SRM 1570a consists of 60 g of finely powdered dried spinach leaves.

Certified Mass Fraction Values: Certified mass fraction values for selected constituent elements, reported on a dry-mass basis, are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in its accuracy in that all known or suspected sources of bias have been investigated or taken into account [1].

Reference Mass Fraction Values: Reference mass fraction values of constituent elements, reported on a dry-mass basis, are provided in Table 2. Reference values are noncertified values that are the best estimates of the true values based on available data; however, the values do not meet NIST criteria for certification [1] and are provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Information Mass Fraction Values: Information mass fraction values for additional constituent elements are provided in Table 3. A NIST information value is a value that may be of interest to the SRM user, but insufficient information is available to asses the uncertainty associated with the value, therefore no uncertainty is provided [1]. Values are reported on a dry-mass basis.

Expiration of Certification: The certification of **SRM 1570a** is valid, within the measurement uncertainty specified, until **31 August 2023**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see "Instructions for Storage and Use"). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certified values before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of analytical measurements for the characterization of this SRM was performed by D.A. Becker of the NIST Chemical Sciences Division. Revision of this certificate was coordinated by K.E. Murphy, D.J. O'Kelly, and L.J. Wood of the NIST Chemical Sciences Division.

Analytical measurements at NIST were performed by current and former staff at NIST, E.S. Beary, D.A. Becker, C.M. Beck II, M.S. Epstein, J.D. Fassett, K.M. Garrity, R.R. Greenberg, R.M. Lindstrom, E.A. Mackey, P. Morales, K.E. Murphy, P.J. Paulsen, B.J. Porter, T.A. Rush, R. Saraswati, J.M. Smeller, G.C. Turk, R.D. Vocke, Jr., R.L. Watters, Jr., and L.J. Wood.

Additional elemental analyses were performed by D.L. Anderson (Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD), A.R. Byrne (Nuclear Chemistry Department, Jozef Stefan Institute, Ljubljana, Slovenia), and J. Kucera (Nuclear Physics Institute, Academy of Sciences of the Czech Republic, Rez, Czech Republic). Several elements were also measured in an International Atomic Energy Agency (IAEA, Vienna, Austria) interlaboratory comparison exercise.

Carlos A. Gonzalez, Chief
Chemical Sciences Division

Gaithersburg, MD 20899
Certificate Issue Date: 25 February 2014
Certificate Revision History on Page 5

Robert L. Watters, Jr., Director
Office of Reference Materials

Statistical analysis of the experimental data was performed by W.F. Guthrie, S.B. Schiller, and L.M. Gill of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

INSTRUCTIONS FOR STORAGE AND USE

Storage: The material should be kept in its tightly closed original bottle and stored in the dark at a temperature between 10 °C and 30 °C. It should not be exposed to intense sources of radiation. Ideally, the bottle should be kept in a desiccator under the conditions indicated above. Spinach leaves have a tendency to rapidly bleach and to turn a tan or light brown color in the presence of visible light. By monitoring SRM 1570, the original SRM, it was determined that there is no evidence of any change in elemental mass fractions as a result of the color change.

Instructions for Use: The contents of a bottle should be thoroughly mixed by rotating and/or rolling before each use. Allow the contents to settle for 1 minute prior to opening to minimize the loss of fine dust particles. A minimum sample mass of 150 mg of the material, dried as described in the section (see "Instructions for Drying"), should be used to relate analytical determinations to the certified values on this certificate. In some cases, especially for volatile elements such as mercury, it is preferable to analyze samples from the bottle without drying, determine the moisture content on a separate sample from the same bottle taken at the same time, and convert the analytical results to a dry-mass basis.

Digestion procedures should be designed to avoid loss of volatile elements, such as arsenic and mercury. Digestion of the SRM in nitric and perchloric acids was found to be incomplete, with a small residue of siliceous material remaining. This residue must be considered an integral part of this SRM and should be dissolved with a small amount of hydrofluoric acid to obtain total dissolution. All certified values are based on the total dissolution.

Instructions for Drying: Samples of this SRM must be dried by one of the following two procedures in order for certified values to be valid:

1. Drying in a desiccator at room temperature (approximately 22 °C) for 120 h over fresh anhydrous magnesium perchlorate. The sample depth should not exceed 1 cm.

2. Freeze-drying for 24 h at a pressure of 13.3 Pa or lower and a shelf temperature of –5 °C or lower after having frozen the sample (not to exceed 1 cm in depth) at –40 °C or lower for at least 1 h. At the end of the 24 h period, samples should be placed immediately in a desiccator with fresh anhydrous magnesium perchlorate. Samples should be weighed after allowing a minimum of 4 h to establish temperature equilibrium.

Note: An approximate mass loss on drying of 3.5 % was observed for the measurements reported here. Vacuum drying at room temperature and oven drying at elevated temperatures have resulted in excessive mass losses and therefore are **NOT** recommended.

SOURCE, PREPARATION, AND ANALYSIS[(1)]

Source and Preparation of Material: The material (approximately 2270 kg) for this SRM was obtained from commercial supplier Oregon Freeze-Drying Corp. (Albany, OR). It consists of U.S. Grade A chopped frozen spinach. The material was thawed, placed in a ribbon mixer, thoroughly mixed, and blended. After mixing, the spinach was freeze-dried. The freeze-dried material was then ground in a stainless steel grinder and shipped to NIST. At NIST, the freeze-dried material was sieved through a polypropylene sieve having openings of 0.25 mm (equivalent to a U.S. Series 60 standard sieve). The sieved material was then jet milled and air classified to a particle size of approximately 75 μm (200 mesh). After mixing in a large blender, the spinach was irradiated with cobalt-60 radiation to a minimum absorbed dose of approximately 27.8 kGy for microbiological control and was bottled.

[(1)] Certain commercial equipment, instruments or materials are identified in this certificate to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Elemental Analysis: Value assignment of the mass fractions of the elements in SRM 1570a was based on the combination of measurements from two or more different analytical methods at NIST and collaborating laboratories. NIST and collaborating laboratories provided measurements by using colorimetry (COLOR), cold-vapor atomic absorption spectrometry (CVAAS), flow injection hydride generation atomic absorption spectrometry (FI-HGAAS), inductively coupled plasma optical emission spectrometry (ICP), isotope dilution inductively coupled plasma mass spectrometry (IDICPMS), isotope dilution thermal ionization mass spectrometry (IDTIMS), instrumental neutron activation analysis (INAA), laser-excited atomic fluorescence spectrometry (LEAFS), prompt gamma activation analysis (PGAA), and radiochemical neutron activation analysis (RNAA). Data from an IAEA interlaboratory comparison exercise were also used where available. A list of analytical methods used for measurement of each element is provided in Appendix A.

Homogeneity Assessment: Samples from randomly selected bottles of SRM 1570a were tested for homogeneity. No evidence of statistically significant inhomogeneity was observed.

Certified Mass Fraction Values: Certified mass fraction values are weighted means of results from two or more different analytical methods combined using the DerSimonian-Laird procedure [2]. The uncertainty in the certified mass fraction values was calculated according to the methods in Supplement 1 to the ISO/JCGM Guide [3] and the results are consistent with the methods given in the ISO/JCGM Guide [4]. The uncertainty of each certified value is expressed as $U = ku_c$. The quantity u_c is the combined standard uncertainty, which accounts for the combined effect of within-method uncertainty from all potential sources and any bias between methods at the level of one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and a 95 % level of confidence for each analyte.

The certified values are reported on a dry-mass basis. For certified values to be valid, the material must be dried according to the instructions provided above. The measurand is the mass fraction of the element. The certified values are metrologically traceable to the SI unit of milligram per kilogram, expressed as percent.

Table 1. Certified Mass Fraction Values (Dry-Mass Basis) of Constituent Elements

Element	Mass Fraction (%)	Coverage Factor (k)
Calcium	1.526 ± 0.066	2.0299
Phosphorus	0.5187 ± 0.0067	1.9772
Potassium	2.900 ± 0.026	2.3226
Sodium	1.821 ± 0.023	1.9943

Element	Mass Fraction (mg/kg)	Coverage Factor (k)	Element	Mass Fraction (mg/kg)	Coverage Factor (k)
Aluminum	310 ± 15	2.0102	Mercury	0.0297 ± 0.0021	2.0492
Arsenic	0.068 ± 0.012	2.0561	Nickel	2.142 ± 0.058	1.9709
Boron	37.7 ± 1.2	2.0127	Selenium	0.1152 ± 0.0043	2.0371
Cadmium	2.876 ± 0.058	2.0464	Strontium	55.54 ± 0.50	2.5029
Cobalt	0.393 ± 0.030	2.0320	Thorium	0.0480 ± 0.0017	2.0053
Copper	12.22 ± 0.86	2.0488	Vanadium	0.568 ± 0.017	2.4938
Manganese	76.0 ± 1.2	1.9855	Zinc	82.3 ± 3.9	2.0136

Reference Mass Fraction Values: Each reference value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results provided by NIST and/or collaborating laboratories. The uncertainty in the reference mass fraction values is calculated as $U = ku_c$. The quantity u_c is the combined standard uncertainty calculated according to the ISO/JCGM Guide [3,4], which accounts for the combined effect of within-method uncertainty and for any bias between methods at the level of one standard deviation. The coverage factor, k, is determined from the Student's t-distribution corresponding to the appropriate associated degrees of freedom and a 95 % level of confidence for each analyte.

These reference values are reported on a dry-mass basis. In order for these reference values to be valid, the material must be dried according to the instructions provided above. The measurand is the mass fraction of the element as determined by the method indicated. The reference values are metrologically traceable to the SI unit of milligram per kilogram, expressed as percent.

Table 2. Reference Mass Fraction Values (Dry-Mass Basis) of Constituent Elements

Element	Mass Fraction (%)
Nitrogen (Total)	6.06 ± 0.20

Element	Mass Fraction (mg/kg)	Element	Mass Fraction (mg/kg)
Europium	0.0055 ± 0.0010	Rubidium	12.7 ± 1.6
Scandium	0.0055 ± 0.0006	Uranium	0.155 ± 0.023

Information Mass Fraction Values: Each information value, expressed as a mass fraction on a dry-mass basis, is an equally weighted mean of results provided by NIST and/or collaborating laboratories. Insufficient information is available to asses the uncertainty associated with the value, therefore no uncertainty is provided.

Table 3. Information Mass Fraction Values (Dry-Mass Basis) of Constituent Elements

Element	Mass Fraction (%)
Magnesium	0.9
Sulfur	0.5

Element	Mass Fraction (mg/kg)
Lead	0.2

REFERENCE

[1] May, W.; Parris, R.; Beck, C.; Fassett, J.; Greenberg, R.; Guenther, F.; Kramer, G.; Wise, S.; Gills, T.; Colbert, J.; Gettings, R.; MacDonald, B.; *Definitions of Terms and Modes Used at NIST for Value-Assignment of Reference Materials for Chemical Measurements*; NIST Special Publication 260–136, U.S. Government Printing Office: Washington, DC (2000); available at http://www.nist.gov/srm/upload/SP260-136.PDF (accessed Feb 2014).

[2] DerSimonian, R; Laird, N.; *Meta-Analysis in Clinical Trials*; Controlled Clin. Trials, Vol. 7, pp. 177–188 (1986).

[3] JCGM 101:2008; *Evaluation of Measurement Data – Supplement 1 to the "Guide to the Expression of Uncertainty in Measurement" - Propagation of Distributions using a Monte Carlo Method*; JCGM (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_101_2008_E.pdf (accessed Feb 2014).

[4] JCGM 100:2008; *Evaluation of Measurement Data - Guide to the Expression of Uncertainty in Measurement;* (GUM 1995 with Minor Corrections), Joint Committee for Guides in Metrology (2008); available at http://www.bipm.org/utils/common/documents/jcgm/JCGM_100_2008_E.pdf (accessed Feb 2014); see also Taylor, B.N.; Kuyatt, C.E.; *Guidelines for Evaluating and Expressing the Uncertainty of NIST Measurement Results*; NIST Technical Note 1297; U.S. Government Printing Office: Washington, DC (1994); available at http://www.nist.gov/pml/pubs/index.cfm (accessed Feb 2014).

Certificate Revision History: 25 February 2014 (Extension of certification period; updated certified values and uncertainties; removed reference and information values for proximates, calories, total dietary fiber, fatty acids, and nitrogen (organic and protein) due to instability of organic constituents; editorial changes); **08 October 2008** (Update of expiration date; editorial changes); **31 August 2001** (This technical revision reports the addition of reference and information values for proximates, calories, total dietary fiber, and fatty acids and a change from non-certified to reference and information values for several inorganic constituents); **15 July 1996** (Editorial changes); **20 October 1994** (Original certificate date).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at http://www.nist.gov/srm.

APPENDIX A

Methods Used in Elemental Determinations

Element	Method Code	Element	Method Code
Aluminum (Al)	ICP INAA	Nitrogen (N)	PGAA
Arsenic (As)	FI-HGAAS RNAA	Phosphorus (P)	COLOR ICP
Boron (B)	IDICPMS PGAA	Potassium (K)	IDTIMS INAA
Cadmium (Cd)	IDICPMS PGAA RNAA	Rubidium (Rb)	IAEA INAA
Calcium (Ca)	IDTIMS INAA	Scandium (Sc)	IAEA INAA
Cobalt (Co)	INAA RNAA	Selenium (Se)	FI-HGAAS INAA RNAA
Copper (Cu)	ICP RNAA	Sodium (Na)	PGAA INAA
Europium (Eu)	IAEA INAA	Strontium (Sr)	IDTIMS INAA
Lead (Pb)	IAEA IDICPMS	Sulfur (S)	PGAA IAEA
Magnesium (Mg)	IDICPMS	Thorium (Th)	INAA RNAA
Manganese (Mn)	INAA LEAFS	Uranium (U)	RNAA
Mercury (Hg)	CVAAS RNAA	Vanadium (V)	IDTIMS INAA
Nickel (Ni)	IDICPMS RNAA	Zinc (Zn)	ICP INAA

Key:

COLOR:	Colorimetry
CVAAS:	Cold-vapor atomic absorption spectrometry
FI-HGAAS:	Flow injection hydride generation atomic absorption spectrometry
IAEA:	Various methods from an IAEA interlaboratory comparison exercise.
ICP:	Inductively coupled plasma optical emission spectrometry
IDICPMS:	Isotope dilution inductively coupled plasma mass spectrometry
IDTIMS:	Isotope dilution thermal ionization mass spectrometry
INAA:	Instrumental neutron activation analysis
LEAFS:	Laser-excited atomic fluorescence spectrometry
PGAA:	Prompt gamma activation analysis
RNAA:	Radiochemical neutron activation analysis

Appendix XIX: Ethical Approvals

(a) Anglia Ruskin university ethics approval

Anglia Ruskin University

Cambridge | Chelmsford
London | Peterborough

Cambridge campus
East Road
Cambridge
CB1 1PT

T: +44 (0) 1223 363271

www.anglia.ac.uk
@angliaruskin
facebook.com/angliaruskin

Dr Olujimi Olusola Aina

26th October 2015

Dear Olujimi

Project Number: *FST/FREP/15/508*
Project Title: *Pattern and use of herbal medicine in Ekiti State Nigeria: Identification of the chemical constituents and Epidemiological study.*

Principal Investigator: *Olujimi Olusola Aina*

Thank you for supplying revisions to your application for ethical approval, as requested by the Faculty Research Ethics Panel (FREP) following its meeting on 17th September 2015.

I am pleased to inform you that your application has been approved by the Chair of the Faculty Research Ethics Panel under the terms of Anglia Ruskin University's *Research Ethics Policy*. Approval is for a period of three years from 26th October 2015.

It is your responsibility to ensure that you comply with *the Code of Practice for Ethical Approval at Anglia Ruskin University*, and specifically:

- The Participant Information Sheet and Participant Consent Form should be on Anglia Ruskin University headed paper.
- For online surveys it is recommended that the researcher turns off the IP logging software to ensure secure communication between the survey taker and server.
- The procedure for submitting substantial amendments to the committee, should there be any changes to your research. You cannot implement these changes until you have received approval from FREP for them.
- The procedure for reporting adverse events and incidents.
- The Data Protection Act (1998) and any other legislation relevant to your research. You must also ensure that you are aware of any emerging legislation relating to your research and make any changes to your study (which you will need to obtain ethical approval for) to comply with this.
- Obtaining any further ethical approval required from the organisation or country (if not carrying out research in the UK) where you will be carrying the research out. Please ensure that you send the FREP Secretary copies of this documentation.
- Any laws of the country where you are carrying the research out (if these conflict with any aspects of the ethical approval given, please notify FREP prior to starting the research).

- Any professional codes of conduct relating to research or research or requirements from your funding body (please note that for externally funded research, a project risk assessment must have been carried out prior to starting the research).
- Notifying the FREP Secretary when your study has ended.

Information about the above can be obtained on our website at:

http://web.anglia.ac.uk/anet/rdcs/ethics/index.phtml/ and or
http://www.anglia.ac.uk/ruskin/en/home/faculties/fst/research0/ethics.html

Please also note that your research may be subject to random monitoring by the Committee.

Please be advised that, if your research has not been completed within three years you will need to apply to our Faculty Research Ethics Panel for an extension of ethics approval prior to the date your approval expires. The procedure for this can also be found on the above website.

Should you have any queries, please do not hesitate to contact me. I wish you the best of luck with your research.

Yours sincerely,

Sue Short

Secretary to the Faculty Research Ethics Panel (FREP)
Faculty of Science and Technology
MAR325

Cc Dr Lata Gautam

(b) Ekit state hospital management board ethics approval

HOSPITALS' MANAGEMENT BOARD

PERMANENT SECRETARY

Doctors' Quarters Road, Similoluwa, Ado-Ekiti, Ekiti State, Nigeria.

All Communications should
be addressed to the Permanent Secretary

Our Ref. No: P. 5220/52

29th August, 2015

Dr. Olujimi Olusola Aina,
Department of Forensic & Biomedical Sciences,
Anglia Ruskin University,
Cambridge, CBI IPT,
United Kingdom.

Dear Sir,

RE: ETHICAL APPROVAL FOR YOUR RESEARCH PROJECT

I wish to refer to your letter dated 13th August, 2015 on the above subject matter i.e. Application for Ethical Approval for your Research Project.

I am glad to convey the approval of the Ekiti State Hospitals' Management Board of your request for Ethical Approval to access data regarding fatality and casualty with regards to use of Herbal Medicine.

This approval grants you full and unlimited access to every data you will require in any and all of our Hospitals.

Wishing you success in this your very important Research Project in the area of Herbal Medicine use that has become a big issue in Nigeria with proliferation of several Herbal Products including Herbal Alcohol Drinks.

Yours faithfully,

Dr. Adeyemi B. James,
Director Planning, Research & Statistics & Chairman Ethics Committee.
for: Permanent Secretary

...Ilẹ Iyì, Ilẹ Ẹyẹ

www.ekitistate.gov.ng

(C) Ekiti state University ethics approval

EKITI STATE UNIVERSITY TEACHING HOSPITAL
ADO-EKITI, NIGERIA.

ETHICS AND RESEARCH COMMITTEE

CLEARANCE CERTIFICATE

***PROTOCOL NUMBER*: EKSUTH /A67/2015/09/002**
***PROJECT TITLE* : HERBAL MEDICINE RELATED CASUALITY AND FATALITY IN EKITI STATE UNIVERSITY TEACHING HOSPITAL , ADO-EKITI.**
***INVESTIGATOR(S)*: AINA OLUJIMI OLUSOLA .**
***SUPERVISOR(S)*: DR LATA GAUTAM .**

DEPARTMENT: FORENSIC AND BIOMEDICAL SCIENCE .
INSTITUTION: ANGLIA RUSKIN UNIVERSITY , CAMBRIDGE, UK. CB1 1PT .
DATE CONSIDERED: 07\09\2015.

DECISION OF COMMITTEE: APPROVED

CHAIRMAN:* Dr. Obitade S. OBIMAKINDE *SIGNATURE & DATE: _____

DECLARATION BY INVESTIGATOR/PRINCIPAL INVESTIGATOR

PROTOCOL NUMBER (Please quote in all enquires) EKSUTH /A67/2015/09/002

To be completed in three copies and two copies returned to the Secretary; Ethics and Research Committee, University Teaching Hospital, Ado-Ekiti, Nigeria.

I/we fully understand the conditions under which I am/we are authorise to conduct the above-mentioned research and I/we guarantee that I/we will ensure compliance with these conditions. Should any departure be contemplated from the research procedure as approved, I/we undertake to resubmit the protocol to the Ethics and Research Committee.

Signature_____ Date:_____

NB: Any erasure, cancellation or alteration renders this certificate invalid.