The investigation and assessment of Nutritional and Traditional Supplement products for content validity, contamination and adulteration

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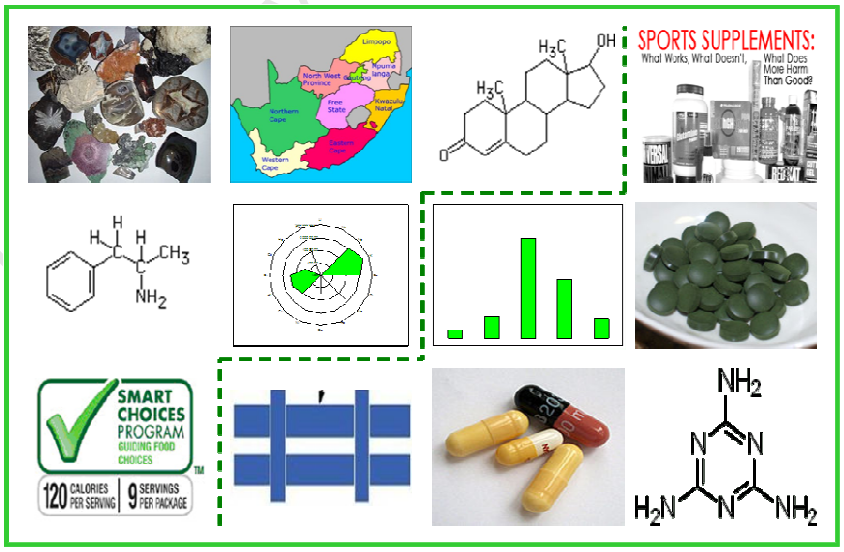
A dissertation submitted to the University of Cape Town in fulfilment of the requirements for the degree

Doctor of Philosophy in Pharmacology



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January 2013

Dedication

This thesis is dedicated to the efforts of,

Angeline Gabriels, whose tireless commitment and courage for decades in serving the teaching profession in impoverished communities, and providing children, education, knowledge and nourishment.

and,

the late **Alexander February**, whose humble birth in a rural setting, provided thorough and continuous debate, on principled sport development and participation, and the role it should play in contributing to a better society.

"What our South African society needs more than ever before is the development of a profound culture of philanthropy and volunteerism with a purpose, that will enhance people's dignity where inequality exists" – Gary Gabriels

Declaration

I, the undersigned , hereby declare that this document describes original work by the author and has not been submitted previously in any form, in its entirety or in part, to any other university for a degree. Where use was made of the work of others it has been duly acknowledged in the text. Copyright of thesis is hereby ceded in whole or in part in favour of the University of Cape Town for the purpose of research.

Name and Surname: Gary Gabriels

Date:

Signature:

Quotations

"In the middle of difficulty lies opportunity"

Albert Einstein

"What lies behind us and what lies before us are tiny matters compared to what lies within us."

Walt Emerson

"Success comes to those who know that life is first born in thought, who seek the vision before the deed, and conforms the deed to the vision."

B.Z. Bokser

"Success, real success, in any endeavour demands more from an individual than most people are willing to offer - not more than they are capable of offering."

James Roche

"The opportunity to become who we want lies in this very moment's behaviour."

David Reynolds

"Good work that leaves the world softer, and fuller, and better than ever before, is the stuff of which human satisfaction and spiritual values are made."

Joan Chittister

"Start by doing what's necessary, then what's possible, and suddenly you are doing the impossible."

Saint Francis of Assisi

Acknowledgements

I wish to express my gratitude to those who have made this project possible:

Professor Mike Lambert (Exercise Science and Sports Medicine, Department of Human Biology, University of Cape Town) for your willingness and acceptance to serve as supervisor for this project.

Professor Peter Smith (Division Clinical Pharmacology, Department of Medicine, University of Cape Town) for serving as supervisor, and for providing the environment to pursue this challenge.

Professor Gary Maartens (Division of Clinical Pharmacology, Department of Medicine, University of Cape Town) for the opportunity to pursue this project.

Professor Donavon Hiss (Department of Medical Biosciences, University of the Western Cape) for your proof reading and editorial assistance to this project, as well as your friendship, support and research collaboration over several years.

Dr Lubbe Wiesner (Division of Clinical Pharmacology, Department of Medicine, University of Cape Town) for your friendship and collegial support with respect to mass spectrometry method development and sample analysis to the project, without which the progress and timelines may not have been accomplished.

Shelly Meltzer Nutritionist and Dietician associated with the Sports Science Institute of South Africa, who was inspirational to the project concept and development in the formative period.

Dr Saskia Sterk at the RIKILIT – Institute for Food Safety, Wageningen, the Netherlands for providing, insight, guidance, experiences and requirements, relevant to global practices and standards for analytical analysis, related to the project.

The University of Cape Town Human Ethics Committee for endorsing this research project initiative.

Khalid Galant (Chief Executive Officer, South African Institute for Drug-Free Sport) for his interest in the project at the inception stage.

The South African Medical Research Council for contributing in part to the financial assistance of the project.

To Marilyn Gabriels, Kim Powell and Keenan Powell. Thanks for your contribution to my life's journey.

To my daughter Mikaela Gabriels. May you always be granted the courage to change the things you can, to accept the things you cannot change, and be provided with the wisdom to know the difference.

To my aunts and uncles for the guidance and wonderful role models you are.

To Sharon Wakefield from the Department of Medicine – UCT, for her timeous and constant inspirational spiritual messages.

To the support staff in the Division of Clinical Pharmacology, University of Cape Town, in particular Jessica Petersen, Ursula Smith, Linda Hartman, Sumaya Salie, Noor Salie, Afia Fredricks, Tracey Fourie, Marilyn Solomon, Ghakiema Jacobs, Katya Govender and Trevor Finch for their administrative support to the project.

Abstract

Background:

Nutritional supplements are used by competitive and recreational athletes of all ages. As a consequence the supplement industry has grown to meet the increasing demand. The regulation of the supplement industry is unrefined, which increases the risk of the nutritional supplements being contaminated. Contamination may be intentional, where the companies "spike" their products with an ergogenic aid, or unintentional. A consequence of contamination is that an athlete may fail a drug test after ingesting a contaminated supplement or there may be negative health consequences. Without adequate legislation it is difficult to control the industry and reduce the risk of contamination in the supplement.

Objectives:

To investigate the industry associated with commercially available nutritional and traditional supplements. These are in the five specific areas; (i) to review the regulations and legislations, and labelling and claims associated with nutritional products in the USA, European Union and South Africa, (ii) to assess the labelling and claims information on nutritional supplement products imported into and manufactured or assembled in South Africa, (iii) to assess using a survey questionnaire the container labelling and other sources of information that assist consumers of nutritional products in their purchasing decisions, (iv) to assess traditional commercial supplements for contamination and consistency of trace elements and heavy metals using Inductively Coupled Plasma-Mass Spectrometry, and (v) to assess the content of nutritional commercial supplements for steroids, stimulants and other compounds of interest using Tandem Liquid Chromatography-Mass Spectrometry.

Methods:

The thesis is divided into 6 Chapters. Chapter 1 describes the background to the problem and Chapter 2 reviews the existing legislation. In Chapter 3 the labelling and claims information on 40 nutritional supplements products are analysed, and the self-administered questionnaire determined what product label and other information influences consumers of nutritional supplements in their purchasing decisions. In Chapter 4 the consistency of trace elements and heavy metals are

analysed in selected nutritional supplements using Inductively Coupled Plasma Mass Spectrometry. In Chapter 5 selected nutritional supplements are analysed for steroids, stimulants and other compounds using Tandem Liquid Chromatography Mass Spectrometry. All the data of these sections are summarised in Chapter 6.

Results and conclusions:

The study identified shortcomings in the current South African regulations and legislation, labelling and claims, which detract from maintaining safety, quality, efficacy and enforcement of nutritional supplement products in the market place. Furthermore, there is a need to have statements such as "exclusion of use, and not a cure for disease", and the "presence of banned substances in the supplements" on the label. The self-administered questionnaire findings show the information that is pertinent for people who purchase nutritional supplements. The findings also show that trace element and heavy metals can be used as product specific markers to identify contamination, and product intra-and-inter-batch consistency, based on "finger-print" profiling, bar charts, and visual radar plots. The steroid and stimulant analysis findings reflect a high proportion of "positive" nutritional supplements, and provide sufficient evidence to emphasize the importance of regulation, monitoring and enforcement on an ongoing basis. However, the findings need to be verified, by re-extraction and analysis of identified "positives", to provide confirmation of results.

Publications, Workshops, Reports and Conference Presentations

The sections of the work presented in this thesis which have been published, presented as reports and at conferences are as follows:

Gabriels, G., Lambert, M. Nutritional Supplement Products: Does the label information influence purchasing decisions for the physically active. Nutrition Journal 2013 *(in print).*

Gabriels, G., Lambert, M., Smith, P., Wiesner, L., Hiss, D. Steroid contamination in Nutritional Supplement products – Addiction or Coincidence? South African Medical Research Council -6th Annual Research Conference – "Carrying the torch for health research" 24-25 October 2012- Cape Town

Gabriels, G., Lambert, M., Smith, P., Wiesner, L., Hiss, D. Nutritional supplements stimulant contamination – "FACELesS" by accident or design? 46th Annual Congress of the South African Society of Basic and Clinical Pharmacology 30th September - 02nd October 2012 University of Pretoria, Gauteng, South Africa.

Gabriels, G., Lambert, M., Smith, P Information on labels of nutritional supplements – time for legislation? South African Journal of Clinical Nutrition 2012; 25(1): 22-24

Gabriels, G., Lambert, M., Smith, P., Hiss, D. Will the new Consumer Protection Act prevent nutritional supplement users from harm? South African Sports Medicine Association. From Basics to Brilliance - World Class in Africa Congress 18th -20th October 2011 Sandton Convention Centre, South Africa.

Gabriels, G., Lambert, M., Smith, P., Hiss, D. Will the new Consumer Protection Act prevent nutritional supplement users from chemical contamination harm? ASSAf-DST-NRF Second Annual South African Young Scientists'Conference 26-28th September 2011 Diep in Die Berg Conference Centre, Pretoria, South Africa.

Gabriels, G., Lambert, M., Smith, P., Hiss, D. Will the new Consumer Protection Act prevent nutritional supplement users from harm? South African Medical Journal 2011; 101(8):543-545

58th International Conference on Mass Spectrometry and Allied Topics and Quantitative Mass Spectrometry Course 22nd-27th May 2010 Convention Centre Salt Lake City, Utah USA *(workshop attendance)*

Gabriels, G., Lambert, M., Smith, P. Nutritional Supplement, Trends, Perceptions and Product use, (including South African participants at the Olympic and Paralympic Games in Beijing 2008), 5th International Congress of

Pharmaceutical and Pharmacological Sciences, 23-26 September 2009, Potchefstroom, South Africa.

Vitafoods International Conference 2009, Geneva Palexpo Switzerland – Global Nutraceutical Event and Natural Foods Workshop 5th -7th May 2009

Gabriels, G., Smith, P. (2008) Trace element and heavy metal analysis of Commercial Ginseng and Hypoxis supplement products. WOCMAP IV World Conference on Medicinal and Aromatic Compounds 9th -14th November 2008, Cape Town, South Africa.

Gabriels, G., Govender, K., Smith, P., Spath, A (2007). Assessment of Commercial Ginseng and Hypoxis supplement products for trace element and heavy metal contamination. South African Pharmacology and Toxicology Congress 2-5th October 2007, Rustenberg, South Africa

Table of Contents

Chapter Two
Regulations and Legislation, Labelling and Claims

Chapter Three

Label content and information factors influencing consumers

Section 1

Nutritional supplement product label

Section 2

Labelling and other information that influences consumer purchasing decisions

Chapter Four
Analysis of Trace Elements and Heavy Metals in Ginseng and Hypoxis Products

Chapter Five

Analysis of Steroids and Stimulants in Nutritional Supplement Products

Chapter Six
Discussion, Conclusion, Perspectives

Abbreviations

AA	Aristolochic Acids
AAS	Anabolic Androgenic Steroids
ACC	American Consumer Council
ADRs	Alternative Dispute Resolution Schemes
ASA	Advertising Standards Authority
CC	Constitutional Court
CI	Consumer International
CRN	Council for Responsible Nutrition
CFSAN	Centre for Food Safety and Applied Nutrition
DEA	Drug Enforcement Administration
DSHEA	Dietary Supplement Health and Education Act
EC	European Commission
EU	European Union
EAS	European Advisory Services
EGA	Estrogens, Gestagens and Androgens
EPA	Environmental Protection Agency
ECC-Net	European Consumer Centers Network
EFSA	European Food Safety Authority
FDA	Food and Drug Administration
FOP	Front of Package
FVO	Food and Veterinary Office
GAP	Good Agricultural Practice
GDP	Gross Domestic Product
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practices
GSP	Good Supply Practice
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
HPA	Health Product Association
IOC	International Olympic Committee
ICP-MS	Inductively Coupled Plasma - Mass Spectrometry

LC-MS/MS	Liquid Chromatography-Mass Spectrometry
MVM	Multivitamin and Minerals
NCD	Non-Communicable Diseases
NCF	National Consumer Forum
NAMC	National Agricultural Marketing Council
OTC	Over-The-Counter
SCF	Scientific Committee on Food
SEM	Standard Error of the Mean
SOP	Standard Operating Procedures
STDEV	Standard Deviation
SANAS	South African National Accreditation System
SCVPH	Scientific Committee on Veterinary Measures relating to Public Health
TCM	Traditional Chinese Medicines
WADA	World Anti-Doping Agency
WHO	World Health Organisation

List of Figures

List of Tables

Chapter 1
Introduction

1.1 Introduction

There is increased use of nutritional supplements by athletes in competitive and recreational sport. There is also increased awareness of illegal substances used, and as such athletes have come under much media and public scrutiny, due to the increase in positive doping tests worldwide [1]. The situation is further exacerbated by the general pressure placed on certain groups to use supplements. For example, young sports participants who are engaged in developmental and competitive phases of sport, encounter peer pressure to use supplements [3].

Based on the World Anti-Doping Agency (WADA) Adverse Analytical Findings and Atypical Findings report (2009) from accredited laboratories for both Olympic and Non-Olympic Sport, there was an increase in number of samples analysed. For example, in 2003 there were 151 210 samples analysed, in 2008, 274 615 samples were analysed, and in 2009, 277 928 samples were analysed. The report shows a slight increase in Adverse Analytical Findings (AAFs) and Atypical Findings (AFs) from 1.84% (2008) to 2.02% (2009) [2].

Further, it has been suggested that the increase in positive tests is also due to unintentional exposure of athletes taking nutritional supplements which are contaminated or adulterated with performance enhancing compounds on the WADA list. These undeclared constituents may also have harmful short - and long-term health consequences. While some athletes have been guilty of deliberate misuse of banned substances, a growing number of *bona fide* athletes have blamed nutritional supplements as the primary cause of their positive doping test outcomes [4,5,6]. This *has* brought uncertainty to the minds of well-intentioned athletes, and has also compromised the development of the legitimate nutritional supplement industry.

1.2 History of Performance-Enhancing Substances

Through history, performance-enhancing substances have been part of competitive sport. As far back as 300 BC, athletes were disqualified from competing at the Olympics because they ingested mushroom or animal proteins which were banned at the time [7]. During the late 1900's the emphasis was placed on mainly using of steroids and amphetamines as ergogenic aids [7]. These agents were subsequently banned and athletes and scientists started searching for legal substances that would provide the competitive edge [7-10]. Whilst the general public has been targeted by the supplement industry for many years, as their big market, the shift in trend now in the marketing strategy has been to also target recreational athletes and children using the profile of athletes to increase sales.

During the late 1900s there was a large market focus on the role of nutrition in exercise and the use of "normal" dietary or nutritional supplements to enhance performance in competition and training [11]. However, now the emphasis has shifted and the use of sports nutritional supplements are no longer restricted to elite athletes, but are increasingly being used by recreational as well as professional athletes and children [12,13]. Consequently, the nutritional supplement industry has grown significantly, due to an increased trend to launch new supplement products that have a high level of "sports image" linked to increasing demand and market size. Further, in most countries there is not a clear distinction between "normal" and "sports" nutritional supplements, with many products and "small" players having their own sales channels. This approach leads to problems of controllability in the sector [14-16].

Global sporting organizations and anti-doping agencies, such as the World Anti-Doping Agency (WADA) often advise athletes to abstain from using supplements [2]. The South Africa Rugby Union, via there BokSmart programme (www.boksmart.com), makes a strong case against the use of supplements. Their recommendation states that supplements should not be used where nutrient needs can be met by normal foods and that nutritional supplements cannot compensate for poor dietary choices [17,18].

WADA's concern is that in many countries the manufacturing and labelling of supplements may not follow strict rules, which may lead to a supplement containing an undeclared substance that is prohibited under anti-doping regulations [2]. They have made it clear that failing a drug test as result of a poorly labelled dietary supplement product is not an adequate defense in a doping hearing [2].

It is therefore imperative, if the goal is to limit the risk of unintentional intake of forbidden substances, to have in place the relevant methods of assay analysis for supplement testing, verification and statistical evaluation [19, 20]. It is further important that research and testing should not be restricted to the prohibited substances, but that potential precursors of these prohibited substances should also be investigated in nutritional supplements and traditional indigenous food and medicine practices [21-27].

The media often raise the awareness to the general public about elite athletes who test positive in an anti-doping test. However the negative consequences of adulterated supplements should be of equal concern for the potential negative health ramifications of the broader general public who consume nutritional supplements in an unregulated environment. [28-30]. A doping problem in the context of sport performance can be defined as the knowing use and/or abuse of prohibited substance(s), or products that may contain these substances, that if consumed is not in the interest of fair play or the physical and mental health of the athlete. Instead it is to gain a competitive advantage over others due to performance-enhancing properties of the substance(s) [29]. A health problem, in the context of the broader general public, is the unknowing consumption of prohibited substance(s) in contaminated nutritional supplement products. Consuming these substances could cause both short and long term medical conditions [29].

1.3 Nutritional Supplement Industry

In the USA, the annual retail supplement sales increased from $8.8 billion in 1994 to $18.8 billion in 2003, an increase of 115%, with a sizable proportion that is spent on "sports supplements" [31]. A Global Industry Analysts Inc. report indicates that the herbal supplement market did not decline during the worldwide recession, but in fact exhibited steady growth over the period 2008 to 2009 [32]. It is anticipated that the market will reach US$93.15 billion by the year 2015. Also, the sales of vitamins and supplements at the retail level recorded a significant increase of more than 10 % in 2008, as compared to the previous year [32]. Further, the Nutrition Business Journal (NBJ) estimated in its Global Supplement & Nutrition Industry Report of February 2010 that the total global nutrition industry sales had increased by 8% to $270 billion in 2008. The definition includes dietary supplements, natural and organic foods and beverages, functional foods and beverages, and Natural and Organic Personal Care and Household Products (N&OPC) [32].

In the context of South Africa, the local turnover for nutritional supplements was estimated at R1.5 billion per year (Health Product Association survey 1998-2000) and has grown rapidly since then. In 2007 the estimates by the Health Product Association of Southern Africa was an average of R78.5 million for 39 companies, many with double digit growth rate in 1 year [33].

Drinks which provide energy form a large part of the supplement industry. There is a general belief that ingesting drinks containing electrolytes and carbohydrates before, during and after exercise has an ergogenic effect [33-38]. This however is a relatively new practice. For example, athletes, prior to 1969 were advised to avoid drinking during exercise, however by 1996 all athletes were advised to drink "as much as tolerable" to insure that they did not lose any weight during exercise – the "zero percent dehydration" doctrine [39]. The changing advice was met by a proliferation of products designed to accommodate the recommendations.

Publicly listed companies in the sports drink industry in the United States are required by law, to show a return on investment for any monies granted "altruistically". This requirement showed a financial return for the US sports drink

industry in an annual turnover from $217 million in 1985 to $2.69 billion in 2003. Thus encouraging the “staying ahead of thirst” concept implies that athletes would potentially increase consumption of sports energy drinks [39].

1.4 Use of Nutritional Supplement - Beliefs and Studies

Dietary supplements have been widely used by elite athletes who are convinced that these substances boost their physical and psychological abilities [29, 40]. The belief is fuelled by the supplement industry which markets their products in such a way as to create the impression that the ingestion of nutritional supplements is an essential prerequisite for optimal performance and rapid recovery from the rigors of competition and training [29]. The mechanisms underlying the proposed beneficial effects of these products are not clearly understood [8, 41-44]. Further, the issue is compounded by the fact that the nutritional supplements may be deliberately tainted with banned substances that are not listed in the ingredients advertised on the label. Furthermore, the supplements may be contaminated with traces of banned substances from lack of sterilisation or sanitation between batch preparations that could lead to adverse analytical finding [29, 45]. It has become common practice among elite athletes to request certification of batch purity and acceptance of liability from the nutritional supplement company in an attempt to decrease the risk of testing positive from contaminated supplements [29].

Several studies have also surveyed supplement use among athletes in general, or within specific types of sports. In these studies the use of supplements varied from 50-100% across most sports disciplines [15, 45, 46]. The studies showed that the most frequently used sources of information about supplements were from health care professionals, the internet, followed by magazines [15].

There is also concern among sports scientists and nutritionists that athletes and consumers make uninformed choices about sport and food supplements. Current literature suggests that information about supplements are obtained from coaches, parents, health store clerks, fitness clubs, books and magazines, and that this source of information may lack appropriate knowledge and accuracy [47-58].

1.5 Nutritional Supplements in health care

Nutritional supplements are also used, as example, to manage obesity. In South Africa, 30% of women are obese [59, 60]. Supplements are also used, as example to manage obesity, and in an unregulated environment, could however have large-scale disastrous consequences if contaminants are present that could adversely affect the initial treatment care and health management objectives [61-67].

Nutritional supplements are also used to treat deficiencies in nutrient intake in both the clinical and health promotion settings. There is global recognition that the environmental and climate changes may impact negatively on the sustainability of harvested products and natural indigenous food resources. Therefore, in response it makes nutritional supplement programmes attractive in particular regions where there is a need to overcome nutritional deficiencies [68, 69]. However, such good intentions could be compromised by the presence of contaminants in the nutritional supplement. The contaminants could be present in the nutritional supplement product unbeknown to the prescriber and/or patients due to lack of verifiable evidence of nutritional product quality [70-75].

1.6 Nutritional Supplement Contamination

Some nutritional supplements may exert their ergogenic effect as a result of contaminants such as steroids or other banned ergogenic substances which have been added intentionally by the manufacturers. In other cases the contaminants are added inadvertently. Research conducted in 2002 and 2004 identified that some supplements contained sufficiently high amounts of anabolic steroids to cause a positive drug test. Examples were 19-nor-4-androstenedione and 4-androstene-3,17, dione [76]. Some nutritional supplement products also contained hormones or prohormones not claimed on the label, and also contained substances other than those indicated by the manufacturer. In particular, nandrolone prohormones resulted in positive doping results for norandrosterone for several hours after ingestion [7, 76, 78].

Further, trace elements and heavy metals also forms part of the composition of nutritional supplement products, and might not be displayed on the product label. The trace elements and heavy metals could thus also be implicit in causing or

contributing to adverse reaction events [127]. Commercially available plant and nutritional supplement products are used extensively in self-medication, complementary and alternative medicine practices, and primary healthcare. Therefore, such products should be subject to rigorous routine quality control and scientific screening methods to verify their safety, quality and efficacy [142].

The book, "*The Coming of China Wars*" by Peter Navarro provides good insight into possibilities for contamination of supplement products [35]. This book explains that contamination can occur intentionally or inadvertently via the following marketing strategies, viz., (a) buyers market, (b) the underground market, (c) mislabelling market, (d) world-wide web marketing or a combination of stipulated marketing approaches [35].

1.7 Nutritional Supplements and Nanotechnology

The advent and widely acclaimed potential of nanotechnology has paved the way for innovation of nanoparticle usage in the nutritional supplement industry. This approach could have possible implications for nutritional supplement product formulations and nutrient content delivery systems and, consequently, may affect human metabolism and health. Thus, the great promise of nanoparticles could also be a peril, especially if contaminants cause undetermined or undesired adverse events and pathogenic effects. Therefore, the general use and application of nanotechnology in the nutritional supplement sector need to be carefully monitored within a legislative framework that specifies all the safety precautions and risks applicable to toxic and dangerous substances [92].

1.8 Laboratory Testing

The ideal situation for laboratory screen testing of nutritional supplements for contaminants requires simple, robust, sensitive and selective screening methods. These methods have to be established and/or modified from existing methods, but should also consider detecting a possible changing composition of the supplement on an ongoing basis. The analytical laboratory for testing and/or screening for drug analysis has evolved over time from High Performance Liquid Chromatography

(HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), to Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) [79-81].

Furthermore the issue is compounded by the fact that the nutritional supplements may be deliberately tainted with banned substances, that are not listed in the ingredients advertised on the label, or contaminated with traces of banned substances from lack of sterilisation or sanitation between batch preparations that could lead to adverse analytical finding. In targeted testing, the aim is to confirm compounds that are declared on the product label, and in untargeted testing the laboratory screen seeks to identify compounds that are not declared on the product label [29, 45]. Further, analysing supplement products with varying ingredients also presents potential pitfalls, due to potential matrix effects that could negatively contribute to a confirmatory qualitative determination.

1.9 Summary

While the media often raise the awareness of the public when an elite athlete tests positive for a banned substance, the negative consequences of adulterated supplements should be of equal concern for the broader general public who consume nutritional supplements in an unregulated environment [28-30]. To limit the risk of unintentional intake of forbidden substances it is imperative to have the relevant methods of assay analysis for supplement testing, verification and statistical evaluation [19, 20]. It is further important that research and testing should not pertain to the prohibited substances as defined only, but that potential precursors of these prohibited substances should also be investigated in nutritional supplements and traditional indigenous food and medicine practices [21-27]. In parallel to this there is a growing responsibility for health professionals and scientists to generate and circulate factual, evidence-based and practical advice about nutritional supplements [82-85].

1.10 Purpose of Research Study

This study seeks to evaluate the content and safety of commercially available nutritional and traditional supplements.

The key questions to be addressed are:

- What information is presented on the container labels of nutritional and traditional supplement products?
- What container labelling and other sources of information assist the consumers of nutritional supplement products in their purchasing decisions?
- How consistent is the batch-to-batch trace element and heavy metal composition of commercial traditional supplement products?
- Do nutritional and traditional supplement products contain undeclared contaminants and/or adulterants, such as steroids and stimulants that could compromise nutritional and traditional supplement usage and safety?

Scope of study

Aim

To firstly scrutinize popular commercially available traditional supplements for trace element and heavy metal consistency and safety. Secondly, to detect the presence of steroids, stimulants and other compounds of interest in nutritional supplements using qualitative and semi-qualitative techniques.

Objectives

1. To review regulations and legislation, and labelling and claims associated with nutritional supplement products in the USA, European Union and South Africa (Chapter 2).
2. To assess the labelling and claims information on nutritional supplement products imported into/and manufactured or assembled in South African (Chapter 3).
3. To assess using a survey questionnaire the container labelling and other sources of information that assist consumers of nutritional products in their purchasing decisions (Chapter 3).
4. To assess traditional commercial supplements for contamination and consistency of trace elements and heavy metals using Inductively Coupled Plasma-Mass Spectrometry (Chapter 4).
5. To assess the content of nutritional commercial supplements for steroids, stimulants and other compounds of interest using Tandem Liquid Chromatography-Mass Spectrometry (Chapter 5).

Chapter 2
Regulations and Legislation, Labelling and Claims

2.1 Introduction

The nutritional supplements (nutraceuticals) sector is generally encumbered with statutory laws in two extremes. Notably, those that govern medicines and those that govern foods. Legislation (statutory laws) is law promulgated by a legislature or a governing body for several purposes, including, to regulate, to sanction, and to declare or restrict. Regulations on the other hand are measures to control human or societal behaviour by rules or restrictions. These regulations, can take the form of legal restrictions or self-regulation. As such, medicine and food production, processing, distribution, retail, packaging and labelling in general is a multifaceted industry often governed by several laws, regulations, codes of practice and guidance, in different countries. This makes this a complex subject.

The nutritional supplement industry annual retail sales in the United States of America increased from $8.8 billion in 1994 to $18.8 billion in 2003, an increase of 115% of which a sizable proportion was spent on “sports supplements” [31,55]. In South Africa, the local turnover was estimated at R1.5 billion per year (Health Product Association survey 1998-2000) and has grown rapidly, if not exponentially since [9]. The increase in supplement sales can likely be attributed to aggressive marketing by manufacturers, rather than the development of more effective nutritional supplements [17]. As a result of the complex legislation governing supplements in most countries, including South Africa, the companies can make unsubstantiated claims about the efficacy of the supplement [83]. Furthermore, the accuracy of the labelling often goes unchallenged, therefore any effects of the supplement may be due to contaminants or adulterants such as 19-nor-4-androstenedione and androsten -3,17-dione in these products not reflected on the label [76,78, 86, 87]. The way in which the supplement industry is managed is in stark contrast to the drug industry which has strict legislation and control. Divergence between food and drug laws has generated “grey” areas with regard to the “voluntary” declaration of “all” content in a specific nutritional supplement product, making the product manufacture chain difficult to deal with or even subject to appropriate law enforcement.

Although some Consumer Protection and Anti-Doping Agencies have requested stricter report requirements for supplement manufactures and tougher penalties for repeat offenders, legislation remains unchanged in most countries [87].

In an attempt to heighten awareness about the lack of control in the supplement industry, this chapter will, (i) assess current peer-review literature with respect to nutritional supplements, (ii) provide examples of spurious claims and labelling practices as published in magazines aimed at the general population, and (iii) assess the regulation, legislation and consumer forums in the USA, the European Union of Nations and South Africa, pertaining to the nutritional supplement industry. This will facilitate, identify and expose current practice in the nutritional supplement sector. Improved processes and practices can as a result be formulated and enabled in the best interest of the consumer.

2.2 Current Issues in the Scientific Literature

2.2.1 Energy Drinks Claims and Negative Health Effects

Many energy drinks, are also considered as functional foods when they contain fortified ingredients that aid specific bodily functions. These energy drinks are marketed aggressively without always providing information about the type and quantity of ingredients on their labels. Some energy drinks promote the inclusion of natural ingredients which purport to supply energy, improve alertness and athletic performance. However, these claims are not always supported by published peer-review evidence [17]. Also there are concerns about the negative health effects associated with these products [37].

2.2.2 Health Claims on Nutritional Supplements

Nutritional supplements have received attention both from producers as a means of marketing the added value to health, and from consumers in terms of awareness, education and improved health. The nutritional supplements regulatory framework should thus be compatible with public health and safety, protect the consumer, promote fair trade, and encourage innovation [88-90].

The desired goal is for better control, for the claims on nutritional supplements in the same way that claims about medicines are controlled. At present unsubstantiated claims are often believed by the general public and vulnerable

groups of patients who are promised recovery or reduction in symptoms, despite the fact that there is no scientific basis for most of the claims [91].

Some of the marketing about the efficacy of nutritional supplements are not always supported by published peer-review evidence. This often leaves the general public confused as they are not able to distinguish between correct or false claims [10]. Another factor adding to the confusion is that the labelling on the containers does not always accurately reflect the contents [17]. This poses health concerns and also the risk of a competitive athlete failing a drug test, if a contaminant in the supplement is on the banned list. Indeed, there are examples in South Africa of nutritional supplements containing banned substances which would have resulted in a positive doping test [76]. Examples of spurious claims and labelling, published in magazines aimed at the general population are shown in Table 1. These examples are selected from popular magazines, such as, The South African Journal of Natural Medicine, Men's Health, Runners World, Bicycling, Muscle and Fitness and Iron Man. Examples have been selected which use pseudoscientific terminology designed to create an elevated impression about the product that is being advertised.

Table 1. *Examples of claims about effects and contents of supplements made in adverts published in popular magazines aimed at the general public.*

	Natural Medicine August 2010	Page	Comment
1.	*Manufactured to international pharmaceutical quality standards*	9	The quality of the raw materials however could be tainted.
2.	*Product manufactured under GMP conditions in an approved facility*	24	GMP or an approved facility does not necessary imply a quality untainted product.
3.	*Organic and 100% natural*	66	This implies that the product is contaminant free.
4.	*Where science and nature meets the effective natural way*	83	The use of the words "science" and "nature" implies quality validation. "Science" is used in a misleading way.
	Men's Health August 2010		
5.	*Has been scientifically formulated*	35	The product could still be contaminated. "Scientifically formulated" is a misleading term.
6.	*Advanced Nutrition*	93	The product could still be tainted.
	Runners World September 2010		
7.	*Originated from Nature. Perfected by Science*	33	The use of the words science and nature may imply quality validation.
	Bicycling 2008		
8.	*Scientifically Formulated*	22	The product could still be tainted. "Scientifically formulated" is a misleading term.
9.	*Supports Muscle Growth*	18	The product could still be tainted. What is the evidence to support these claims?
10.	*Stimulant Free*	22	What are the guarantees that the product is not contaminated?
	Muscle and Fitness 2010		
11.	*Based on University Research*	13	This is simplistic – University research does not automatically translate into believable valid research.
12.	*Natures ultimate lean Muscle Formula*	45	Use of the word "nature" may imply quality validation.
13.	*For faster muscle building and definition*	55	The product could still be tainted.
14.	*Powerful Anabolic Catalyst*	241	The "catalyst" could be a prohibited substance. Many anabolic substances are on the prohibited list.
	Ironman 2010		
15.	*Messenger Hormone Amplifiers*	33	The "messenger" could be a prohibited substance. This is cagey language designed to impress.
16.	*Professional Strength Anabolic Complex*	53	The "complex" could be a prohibited substance. Many anabolic substances are on the prohibited list and the language uses pseudoscience terminology.

2.3 Assessment of Legislation

Regulation of nutritional supplements in most countries, including South Africa, are usually embedded in other forms of legislation, in comparison to those governing and acted upon for medicine [93, 94]. The regulation and legislation of the nutritional supplement industry in the USA, the European Union of Nations and South Africa are used as examples of such legislation.

2.3.1 United States of America

The USA constitutes one of the world's most comprehensive and effective networks of public health and consumer protections, having a population of 305 million people. The Federal Food, Drug, and Cosmetic Act (FDC) of 1938 was passed after a legally marketed toxic elixir, killed 107 people, including many children [95]. The FDC Act completely overhauled the public health system in the USA at that time. Among other provisions, the law authorized the Food and Drug Administration (FDA) to demand evidence of safety for new drugs, issue standards for food, and conduct factory inspections. It was the vigilance of the FDA that prevented thalidomide being marketed in the USA after the tragedy of side-effects of the drug in Europe [95]. This strengthened the rules for drug safety, and required manufacturers to prove the effectiveness of their drugs.

The specific section of the USA FDC Act related to food and medicine (drugs) are (i) Chapter IV (Section 401-417) which covers food aspects in particular, and, (ii) Chapter V (Section 501-523) covers medicine (drugs) [95]. Both these sections of legislation may have aspects which apply to nutritional supplement products. Further, the Dietary Supplement Health and Education Act of 1994 (DSHEA) placed the responsibility on the manufacturer, to ensure that the dietary (nutritional) supplement is safe before marketing [95]. Prior to this act, dietary supplements were under the same regulatory requirement category as food.

The FDA thus ensures that products are honestly, accurately and informatively presented to the public. Some of the agency’s specific responsibilities that may also include nutritional supplement products are; Manufacturing Licensing and Standards, Testing Methods, Labelling, and Product Safety, not necessary as individual segments, but as overlapping roles. It is a requirement for domestic and

foreign suppliers to register their manufacture facilities with the FDA; however these manufactures and distributors are not required to register their products with the FDA where existing ingredients in the market are used. The exception is in the case of a new dietary ingredient, where pre-market review for safety data and other information are required by law [95].

In 2007 comprehensive regulations were published by the FDA for those companies which manufacture, package and hold dietary supplement products. The goal of these regulations was to ensure the identity, purity, quality, strength and composition of products [95].

According to these regulations any claims made about a product needs to be substantiated by adequate evidence. Manufacturers are allowed to make three types of claims for their dietary supplement products, (i) health, (ii) nutrient content claims, and (iii) structure/function claim, that also require a "disclaimer" indicating that the statement has not been evaluated by the FDA. The manufacturer is also required to provide label information that is "truthful" and not "misleading". To ensure that consumers are informed prior to purchasing of dietary products the FDA has developed a website (http://www.fda.gov/food/dietarysupplements) that provides tips to the consumer about making informed decisions and evaluating information provided by companies, and claims that can be made for conventional food and dietary supplements.

The FDA only acts on voluntary reporting where consumers may have identified or experienced adverse events. The responsibility of the FDA in such cases includes claims about product information, labelling, package inserts, and accompanying literature. Consumer nutritional supplement concerns and reporting needs to be dealt with and lodged to the specific manufacturing companies, in the first instance, and not to the FDA.

The FDA's Centre for Food Safety and Applied Nutrition (CFSAN), that has been in existence since 1906, has oversight for dietary supplements, and also ensures the monitoring in the market place for potential illegal products [95]. CFSAN also gathers information about products from agency inspectors, the dietary

supplement manufacturers and distributors, the internet, consumers and trade complaints. Laboratory analyses are done occasionally of selected products, in particular those associated with adverse events.

Due to limited resources the FDA has enforcement priorities that are only applied to products thought to be unsafe, have caused injury or illness, and are fraudulent or in violation of the law. The FDA's remaining funds are applied to the routine monitoring of products, based on it's own assessment or as the FDA determines. Consumers who would want to know or verify content of dietary supplements would need to engage the manufacturer directly and arrange for the analysis to be done in a commercial laboratory.

Although certain levels of legislation are in place in the USA to control the supplement industry, consumers are also often left confused about which regulatory agency is the most appropriate.

The alternate approaches for consumer concerns in the USA, is to solicit support via the American Consumer Council (ACC), which is dedicated to consumer education, awareness, and has an interest in quality products and services, and The Council for Responsible Nutrition (CRN) association representing dietary supplement manufacturers and ingredient suppliers[96,97].

2.3.2 Summary of the USA System

The FDA is by far the most comprehensive federal agency responsible for food and related products consumption and safety in the world today. The FDA's function is underpinned by the FDC Act that was promulgated as a direct result of proactive legislative requirement, in the context of the marketing of a toxic elixir and the thalidomide tragedy at the time. Whilst the regulation and laws have been developed, good proactive consumer activism has been thwarted by confusion when the need for engagement was required to debate the cross-cutting responsibility, authority and priorities of respective agencies in the area of nutritional (dietary) supplements. This perplexity was compounded, in particular by resource constraints that limited enforcement approaches and priorities. To

relieve the aspect of such constraints, the FDA has created guidance communications to the industry that does not confer rights to any party.

2.3.3 European Union

The European Union (EU) of Nations represents a population of 500 million people. It is a relatively recent development (1993), and has had to develop synergy across member nations to establish new legislation with reference to nutritional (food) supplements. This was accomplished via the European Community Treaties, which sets the EU constitutional basis for nations whom previously had their own legislative provisions as sovereign states. The Food and Veterinary Office (FVO) is the commission in the treaties that has as its primary responsibility the implementation of the legislation on food safety, animal health, plant health and animal welfare [98].

The current focus in the EU, as provided by the European Advisory Services on food and nutritional supplements relates to, (a) maximum levels of vitamins and minerals, (b) actions on “other ingredients”, (c) providing information to the consumer about labelling, (d) nutritional and health claims regulations, and (e) revision of novel food regulation [99]. The Directive 2002/46/EC of the European Parliament and Council of 10 June 2002 on the approximation of the laws of Member States relating to food supplements established harmonized rules for the labelling of food supplements, and introduced specific rules on vitamins and minerals in food supplements.

Food contamination generally has a negative impact on quality, and may imply a risk to human health. The EU has therefore taken measures to minimise contaminants in foodstuffs, by having the European Commission (EC) publish a fact sheet on food contaminants [100]. The European Commission regulates all nutritional and health claims on the product pack as well as all other communication materials intended for all consumers (promotions, advertising, leaflets). To assist the implementation of the necessary legislation and actions the European Food Safety Authority (EFSA) agency manages relations to ensure science-based risk management.

The alternate approach for consumer concerns in the European Union is via the European Consumer Centres Network (ECC-Net) [101]. This is designed to promote consumer confidence by advising on consumer rights, and ensuring easy access to redress with respect to cross-border information and assistance to individual member states who form part of the EU.

2.3.4 Summary of the European Union System

The development and formation of the EU of nations as a new phenomenon has implied that ideas and concepts for the provision of laws and regulations and the application thereof, and with specific reference to nutritional (food) supplements has been more current and relevant, although complex. This essentially meant that nations who now form part of the EU have to find synergy and collective harmonization of processes and requirements. Countries in the EU at the forefront of developments pertaining to adulteration and contamination of nutritional supplements are Germany, Belgium, The Netherlands, Austria and the UK.

Much of the work currently performed by the EU is covered and done in the form of Laws, Councils, Directives and Commissions, with the aim to have an integrated approach with adequate monitoring whilst ensuring effective functioning of the internal market of the EU. Whilst steady progress has been made in some areas, the initial time-lines by the EU in terms of firm accepted proposals and adoption of an implementation strategy are proving too complex to put into action [99].

2.3.5 South Africa

South Africa, with a population estimated at 49 million people has a constitution regarded as one of the most progressive in the world and enjoys high acclaim internationally [102]. There is a provision in the preamble of the constitution that states, *"Improve the quality of life of all citizens and free the potential of each person"*. Implicit in this statement is the assurance of improving quality of wellness and health of the nation. This has direct bearing on the topic of legislation of the nutritional supplementation industry where the risk of contamination of the products may impose health risks.

In South Africa the constitution has provided several acts which are associated with governance, in part, of nutritional supplements; (i) The Consumer Protection Act, (ii) The National Health Act, (iii) The Medicines and Related Substance and Amendment Act, (iv) The South African Institute for Drug-Free Sport and Amendment Act, and (v) The Medical Research Council Act [102].

The Consumer Protection Act, which is intended to serve the consumer, should have become operational incrementally on the 30th October 2010, but the implementation of the Act was postponed until April 2011.

This Act introduces consumer courts as a means of achieving protection and speedy enforcement of consumers rights. Consumer courts will however have limiting powers, and subservient to the Common Law Court, with respect to interpretation, ambiguity and the best spirit, that promotes the Consumer Act [103].

Within the context of the Act, in the event of harm (no-fault liability) being suffered as a result of the supply of any unsafe goods, product failure or inadequate instructions or warnings pertaining to hazardous use of any goods, the producer/importer/distributor or retailer is liable, irrespective of whether there was any negligence on the part of any of those persons [104,103]. This is in particular with respect to the manufacturer of medical products such pharmaceuticals. There will however be no-liability on unreasonable grounds, on the part of a distributor or retailer if they discover an unsafe product, and they did not form part of marketing of such products. Whilst the Consumer Protection Act is intended to serve the interest of the consumer it's intention will only become meaningful when challenges are lodged to the courts [103,104].

The National Health Act incorporates, The Medicine Control Council (MCC) who is charged with ensuring the safety, quality and effectiveness of medicines, and matters related. This requirement has however experienced difficulty considering that the MCC has registered less than half of the medicine applications to it, over the past 7 years [105]. This situation has become a life-threatening risk to patients and is now under legal threat from stake holders who claim it to be dysfunctional

[105]. Pharmaceutical companies and activist have also expressed “intense frustration“with the current process [105]. A problem is that the MCC is grossly understaffed resulting in inefficiency and the lack of appropriate delivery of requirement. The law enforcement division has also come under sustained fire, for failing to stem the flood of unscrupulous practices [105]. This point is further supported when one considers nutritional supplements may be tainted with conventional drugs (medicines) as in the case of the recently claimed natural supplement product Simply Slim, which was found to contain the prescription substance sibutramine [106]. This reinforces the concern that prohibited substances and stimulants may be embedded in nutritional supplements.

The Medicines and Related Substance and Amendment Act in part is to ensure the provision of registration of medicines intended for human or animal use, and providing licences to persons whom wish to compound and dispense, manufacture or act as a wholesaler or distributor for medicines. The South African Institute for Drug-Free Sport and Amendment Act ensures in part independent sample collection and testing programme, which may subject any sportsperson specifically to dope testing. The provision is thus specifically testing of the athletes and does not concern itself with the monitoring of nutritional supplements for tainted or contaminants that may affect the population adversely. The Medical Research Council Act makes provision for the establishment of the Medical Research Council (MRC) which has a mandate to promote the improvement of the health and quality of life of South Africans.

Further, providing safe food (nutritional supplements) to the consumer remains in part also the responsibility of the service provider. So whilst the Hazard Analysis Critical Control Point (HACCP) system for “food safety” has been gazetted as part of the South African Foodstuff, Cosmetics and Disinfectants Act, no sector to date has been obliged by law to introduce a functioning system or similar for nutritional supplements based on the principles of the HACCP [107].

The alternate approaches for consumer concerns in South Africa are to solicit support via National Consumer Forum (NCF), and the South African National Consumer Union (SANCU). These two leading consumer organisations should ensure in the interest of consumers, to (i) a wholesome environment, (ii) a

fundamental quality of life, and, (iii) good quality in the goods ***(including products such as nutritional supplements)*** and services provided by the private and public sectors alike [108,109].

The South African system of Acts and Bills have similarity to that of the USA with reference to Medicine and Food Law, maybe with the same intention, but not necessary the same intensity, rigour and enforcement as the FDC Act of the USA [110]. The lack of specificity pertaining to nutritional supplements may compromise enforcement, accountability and responsibility by respective authorities [88-90]. The content of nutritional supplement products in the market may also therefore, compromise the health of consumers, due to potential contamination and lack of appropriate labelling of products [37].

2.3.6 Summary of the South Africa System

South Africa's constitution embodies the principle of improving the quality of life, wellness and health of all it's citizens. In South African Law there are itemized sections in respective Acts and regulations that cover medicine and food that potentially relates to nutritional supplements and as indicated, closely resemble those of the USA. However, currently South Africa has no formalized guidance documentation on nutritional supplements and the industry, and many aspects of this sector are self-regulated. This applies to contamination or adulterated substances that may be present in nutritional supplements as well as provisions and requirements of labeling and respective claims.

Many of the Acts ensure compartmentalization of function, but are not necessarily directly related to nutritional supplements. However the link can be inferred. Although many aspects can be covered by this approach, in an environment of limited resources scant regard is often given to matters if they are not specifically defined. This creates "grey areas" for those who should take accountability and responsibility for appropriate enforcement. This also points to a lack of capacity that needs to be developed. These combination of factors, together with the increase in economy of scales and utilization of nutritional supplements in the

market, makes South Africa vulnerable for the download of goods from countries or regions where laws and regulations may have become more stringent.

2.4 Concluding Summary

A synopsis of the regulatory and legislation, claims and labelling provisions of the management of nutritional supplement products was described for the USA, the European Union and South Africa. The assessment has shown shortcomings across all the regions investigated, with inadequate provision to enforce legislation appropriately.

It is pivotal in the development of a sound nutritional supplement management system that consumer structures will have to play a greater role. This to ensure that quality products are maintained and knowledge awareness is created. Consumer forums will also need to contribute to ensuring appropriate legislation and regulation comes into place, that can appropriately be enforced where needed.

For South Africa it may be necessary to engage current Consumer Forums, and have them expand on their current objectives, to enable a more in-depth and ongoing scrutiny of nutritional supplement products in the best interest of consumers. There is also a need in South Africa to have an appropriate specific agency or intensified enforcement to monitor the quality of nutritional supplement products. This in the best interest of the consumer, considering that there is increased use of nutritional supplements that are being produced in a self-regulatory environment.

General observations also point to, (i) the need for synergy across sovereign regions with respect to regulatory and legislative frameworks, (ii) the need for clear and specific regulation and legislation for nutritional supplement sector without ambiguity, that is not embedded in legislation and regulations that may facilitate “grey” areas in interpretation and application, (iii) more attention to facilitate required resources needed to give effect to greater levels of policy development, enforcement and vigilance, and the need for a functional HACCP for nutritional supplements.

Chapter 3

Label content and information factors influencing consumers

This chapter consist of two sections. Section 1 focusses on the **content on** nutritional supplement product **labels**, and Section 2 focusses on what product label and other **information influences consumers** of nutritional supplement products in their purchasing decisions. Each of the sections has its own introduction, methodology, results, discussion, conclusion and recommendations.

Section 1

Nutritional supplement product labels

3.1 Introduction

Nutritional supplements have received attention both from manufacturers of food, as a means of marketing the added value to health, and from consumers in terms of awareness, education and improved health. The regulatory framework of nutritional supplements should thus be compatible with public health and safety, protect the consumer, promote fair trade, and encourage innovation [88-90].

To assist this process, it is important to have specific knowledge and understanding on the claims and labelling on nutritional supplements products used for general, and more specifically sports consumers. In particular, labelling information, including warnings, claims, disclaimers as well as proposed mechanisms of action need to be documented. All this information is needed for practical intervention, policy development, and possible appropriate legislation and regulation. Such information may also further provide and facilitate the design of guidelines on how nutritional supplement products may be marketed in future, and ensure that the consumer makes informed choices. In accordance with these points, the aim of this study was to select a group of nutritional products either imported into, or manufactured in South Africa and quantify the labelling and claims information on the labels.

3.2 Methodology

Nutritional supplement products were purchased at Century City Dischem Pharmacy in Canal Walk, Cape Town, Western Cape, South Africa. They consisted of internationally imported, and locally manufactured or assembled products, based on the list of top sellers as determined by purchased sales at the Dischem store, and within the available budget for the project.

Labelling information and claims made on the containers were assessed according to specific predetermined categories [48-50,110-114,125,126]. The categories included claims made on containers, general information, disclaimers, warnings, quality assurance, scientific pledge, and consumer/public relations categories, respectively. For the study, (i) a disclaimer was defined as any statement intended to specify or delimit the scope of rights and obligations that may be exercised and enforced by parties in a legally-recognized relationship, (ii) a warning is a caution to the consumer, (iii) quality assurance refers to the superiority of the product as stated by the manufacture, (iv) scientific pledge refers to the manufacturer's undertaking/guarantee of product, and (v) consumer/public relations refers to the facilitation of further contact between the consumer and the manufacturer/distributor. Figure 1 provides an illustration of product package content.

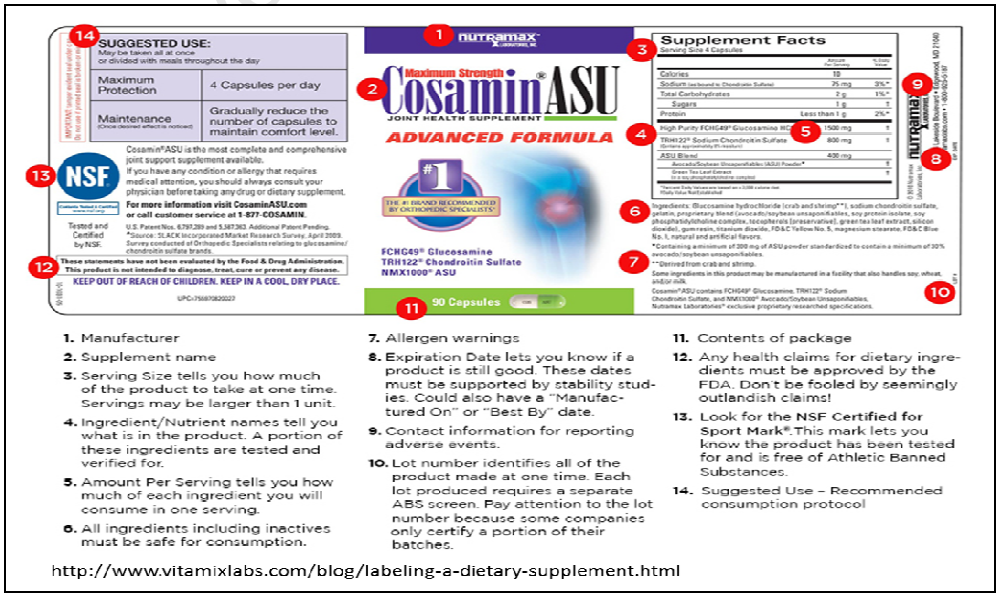


Figure 1. Illustration of product package label content

The supplements were assessed for each category and the information transcribed into a Widows-based Microsoft ® Office Excel 2003 SP 1 (Excel © 1985-2003 Microsoft Corporation) spreadsheet, for each of the nutritional supplement product labels, respectively. The statements were then captured as "yes" or "no" statements. The Excel file was imported to Statistica Version 10 (Stat Soft Inc. © 1984-2011 Stat Soft Inc.) to determine the percentages in the respective categories investigated.

3.3 Results

Forty products were selected for analysis of which, 21 (53%) were locally assembled or manufactured products, and 19 (48%) were international imported products. The formulations consisted of powders 16 (40%), capsules 13 (33%), tablets 8 (20%), and tablets and liquid in capsules 3 (8%). Only 4 (10%) of the product labels cited a literature reference, and of these 3 (75%) cited studies done in humans, and 12 (30%) of products had a statement on the labels indicating that the product was "contaminant free".

Thirty-eight (95%) of the nutritional supplements referred to a website on their labels. All the products provided information with the directions for use. 38 (95%) had a "best before" date and expiry date for the consumption of the specific product and 38 (95%) had batch numbers on the nutritional supplement labels. The mean number of ingredients/compounds described on each label was 28, with a range of 1 to 88. There was an average of 5 colours (range 3 to 9) on each label.

Thirty-eight (95%) of the products had some form of warning statement(s) on the label. The number of warnings ranged from no warnings, to 14 warnings per product, with a mean of 5 warnings. The warning categories assessed from the nutritional supplement label in this study are shown in Table 2. To give an illustration of how the various statements were collapsed for the warning description, the example, "Keep product out of reach of children" implies that the product is for adult consumption, and warns of the possible harm that may be incurred, if children were to consume product.

Table 2. *The 17 warning categories assessed on the product labels (n=40)*

Warning Descriptions	Total	Percentage of total %
Keep product out of reach of children	27	68
Importance of adherence to storage details	23	57
Use during pregnancy and lactation	17	43
Exceeding recommended dose	17	43
Importance of consultation with health care professionals	17	43
Age categories for consumption	12	30
Lower dose when experiencing adverse events	10	25
That nutritional supplements should not replace dietary requirements	10	25
Potentiate and suppress effect of drugs	10	25
Certain medical conditions and the use of supplements	10	25
Importance of staying hydrated	8	20
Time restriction consideration when consuming product	4	10
Maximum dose "dangers"	4	10
Presence of banned substances in the supplements	2	5
Presence of food items associated with allergies/intolerance	2	5
Product is gender specific	1	2
Exclusion of use, and not cure for disease states.	1	2

Thirty-four (85%) of the nutritional supplement products had a disclaimer on the label. Table 3 represents the disclaimers observed on the product labels in this study. To give an illustration of how the various statement were collapsed for the disclaimer descriptions, the example , "The product does not cure illness", implies that the product is not intended to treat illness and the disclaimer covers the manufacture in a broad context.

Table 3. *Disclaimers on product labels (n=40)*

Disclaimer Descriptions	Total	Percentage of total %
The product does not cure illness	21	52
The product is free of stimulants, colourants, flavourant and/or preservatives	16	40
The product, or claim made by the product, has not been evaluated by the FDA	12	30
The product should not replace medication	7	18

Thirty-two (98%) of the nutritional supplement products labels had some claim on the label. The different categories and numbers of supplements within each category are shown in Table 4. To give an illustration of how the various statement were collapsed for specific claims descriptions, the example , "Quality of the product emphasized ", implies that the manufacture wants to ensure the consumer of superiority of the product.

Table 4. *Description of the specific claims on the product labels (n=40)*

Claims Descriptions	Total	Percentage of total (%)
Quality of the product emphasized	22	45
Muscle mass changes	19	47
Provides increased energy	17	43
Use of pseudo–scientific terms	15	38
Will increase physical performance	15	37
Provides increased strength	13	33
Improves recovery	13	32
Supports general metabolism	12	30
Supports fat metabolism	7	18
Can have an effect on mood changes	7	18
Outrageous claims	7	17
Supports cognitive function	6	15
Serves as a weight loss/management product	5	12
Removal of metabolic by-products	5	12
Serves as an appetite suppressant	4	10
Supports glucose metabolism	4	10
Supports testosterone metabolism	4	10
Gives support to the immune system	4	10
Provides anti-oxidant properties	4	10
Research supporting evidence	4	10
Serves as a meal replacement	2	5
Relieves stress	2	5
Could alter libido	2	5

All the nutritional supplement products labels 40 (100%), presented some scientific indication and/or pledge, and 24 (60%) of the nutritional supplements had scientific and/or research claims on the labels. There was no evidence on the label that the claims made were peer-reviewed.

Sixteen (40%) of the supplements had pledges and/or indication of commitment to the nutritional supplements assessed and 14 (35%) made some pronouncement about a specific technology and/or specific patent used in the manufacture of the nutritional supplement. 13 (33%) of the nutritional supplement products had advertising on the label and 3 (8%) of the nutritional supplement products provided a linkage to social networks (eg. facebook, twitter).

Table 5 provides examples of selected categories as determined and assessed.

Table 5. *Examples of selected categories*

	Examples
Outrageous claims	(i) Instant muscle gratification, (ii) no loading, no cycling, no side effects, and (iii) the "last word" in ultra-enhancing sports supplements.
Pseudo Science	(i) Activates metabolism and shreds fat, (ii) ignites intense workouts, (iii) triggers anabolism and builds muscle, and (iv) natural thyroid support complex supports and enhanced metabolism.
Scientific, Research and Pledge terminology	(i) Scientifically formulated, (ii) Scientifically formulated for professional athletes, (iii) National Academy of Sciences, and (iv) New formula with the latest clinically researched ingredients.
Strong terminology	(i) Anabolic Hormone Enhancer, (ii) High Potency, and (iii) the Power of Science.
Specific technology and/or specific patent	(i) Advanced liquid delivery technology maximizes bioavailability, and (ii) Patent-pending Nano - Diffuse technology, and Patent creatine stabilization technology.
Claims	(i) Improves glucose metabolism, Lipotropic agent, Red blood cell and oxygen booster, (ii) we only use superior ingredients, sourced from international raw material suppliers, and (iii) to take your physical performance to the next level.
Disclaimers	(i) This product does not intend to prevent or cure any form of illness or disease and should not replace any medication, (ii) these statements have not been evaluated by the Food and Drug Administration, and (iii) the capsules contain no banned substances, illegal stimulants, caffeine, gluten, preservatives, colouring agents, yeast or lactose.
Indication of commitment	(i) With formulation based in the latest scientific research available our aim is to ensure that our products are unsurpassed in quality, potency and efficacy, (ii) copy of laboratories certificate of analysis for each lot can be supplied, and (iii) we make an unconditional pledge to you, our valued customer to continually supply you with products of highest quality and purity.
Advertising	(i) “Learn about our products, get diets and chat with members you can also win great prizes”, (ii) It is foundational, "core" nutrition that provides the basic nutritional framework for any lifter serious about iron warfare, and (iii) “If this product doesn’t meet with your standards for any reason, take the remainder back with your receipt to where you bought it for a full refund or exchange - No questions asked.”
Warnings	(i) Maximum dose “dangers”, (ii) keep product out of reach of children, and (iii) the importance of adherence to storage details.
Contaminant Free	(i) Stimulant free, (ii) ephedrine free, (iii) no artificial flavours, (iv) no artificial colourants, (v) no preservatives, and (vi) ingredients free of all impurities and by-products.

3.4 Discussion

This chapter has provided diverse examples of labelling of nutritional supplements. A limitation in this study is the sample selection that was based on the top nutritional supplement sellers at the time of purchase, and within the financial constraints of the study. This was done with specific reference to the

impact on elite athletes/consumers, and the impact the research outcome would have to educate, bring about awareness and advocacy.

The 40 nutritional supplement products assessed as part of this study yielded the following;

Twenty-eight (70%) of the nutritional supplement product labels had no indication or evidence that the product was “Contaminant Free”. This can be interpreted as either expressing confidence that the quality of the products are good and there is no concern for possible contamination, or that the manufacturer is not aware of possible contaminants in their products. This suggests that it is important to do screen testing of products to verify nutritional supplements for contaminants, with specific reference to steroids and stimulants that are investigated in this thesis. Even if “Contaminant Free” information is present on labels, this does not guarantee that the products are indeed free of contaminants, particularly considering that these products are produced in an environment without manufacturing regulations. Although the label may state that the product is stimulant free, there is no legislation which controls the accuracy of this statement. Furthermore, the word stimulant(s) may also be used in a generic way. Thus certain stimulants may be absent, whilst others may be present, either unintentionally or deliberately due to the manufacturing process [76, 86].

Of the nutritional supplements assessed 38 (95%) had warning statements. There were on average five warnings per supplement, with one supplement having as many as 14 warnings. These statement(s) often had a small font size, and were not immediately noticeable. The two most frequently used warning statements on the product labels assessed were, “keeping product out of reach of children” 27 (68%), and “the importance of adherence to storage details” 23 (57%). Both these warnings in particular alert the consumer of potential harm. The “children” alert often did not give a specific age. The warning statements that were present at lower frequency, but may in fact be of greater importance are, “exclusion of use, and not a cure for disease states 1 (2%)” and the “presence of banned substances in the supplements 2 (5%)”. For the former there may be “contaminants” in products that could directly or indirectly contribute to, or exacerbate specific disease states. The latter affects professional athletes who may test “positive” for a

banned substance after ingesting a supplement. For the general consumer the potential implication is the impact on long term health consequences, due to harmful, continuous intermittent use effects, or the accumulative effects over time.

In Australia and New Zealand new labelling initiatives pertaining to food standards, requires mandatory nutrition information panels. Substances that may cause adverse reactions have to be highlighted on the label. In context, this approach to labelling could also have similar application to nutritional supplement products [115].

Incorrect storage of the nutritional supplement product (eg. temperature) has the potential of "active" chemical compound(s) converting to other active or inactive chemical moieties, or completely harmful unknown compound(s) [76]. The consequence of consumption of such nutritional supplement products could lead to adverse events. The same logic applies to the consumption of gender specific products if not labelled adequately for a specific sex. Unintended consequences that impact on the physiological hormonal balance of a specific sex may occur if compounds present in the supplements are not declared on the label [8,116,117].

Further, consumers who fail to comply with the directions for use, could also predispose themselves to long term effects of elevated accumulative doses. This could cause adverse effects, due to abnormal physiological and, or normal cell biological disruptive activity.

Having an industry sector standardised batch number coding on nutritional supplement products with a trail of data from the manufacturing process to the consumer environment should be useful. Such an approach would serve as a reference to monitor good manufacturing practice (GMP) processes and also identify possible entry points of potential contaminants.

Although "Best before" and expiry date information is valuable information, in many cases it was not clearly visible on the label. In the context of warning statements, a recent study designed to determine nutrition knowledge, and understanding of nutritional information, concluded that only 27% of respondents

looked at package information before making a decision [118]. Another study suggested that older, compared to younger consumers detect information changes less readily [119]. Thus newly added information does not necessary reach all consumers, posing a potential problem to policy makers and scientists [119].

A study designed to determine the factors which draw the attention of the consumer to nutritional labels showed that the display size, colour scheme, familiarity with label, logo and its location on the front of the pack are key determinants [120]. The study showed that when the consumer had a better understanding of nutrition information and provided attention to label content, this impacted nutritional supplement choice [120].

Further, an off-label promotion study concluded that the media helps shape public perceptions and increases support for regulatory actions [121]. Off–label may be defined as drug or product used for indications not approved by the Food and Drug administration (FDA). This study concluded that the primary concerns about label content, included legal concerns, the lack of safety/efficacy data, and having no ethical, or clinical content. Having this type of information on the label would ensure that policy and decision makers, health care professionals, patients, and general public gather sufficient knowledge to make informed choices [121]. The findings in this study are supported by a study that suggests that nutrition education can be instrumental in encouraging, stimulating, motivating and providing technical help for the consumer [122]. Technical messages should include teaching consumers simple rules to interpret label information and to incorporate the information into planning [122]. Other alternative preventative approaches and improved label design in providing nutritional information, should be researched and implemented, if the results show it to be more effective [123].

It may be concluded from this study that “screen testing” nutritional supplement products on an ongoing basis is important. In particular the products would need to be tested via batch-to-batch analysis for contaminants, including steroids and stimulants. Also the presence and quantity of the ingredients on the label need to be verified. Peer-review scientific evidence would also ensure relevant engagement with the social media networks, and provide education and awareness to the consumer in an informed way. It also emphasizes the importance of relevant

screening of the "knowns" as per the number of ingredients on the label information (both qualitatively and quantitatively), and "unknowns" that may be determined or discovered, but are not reflected on the label information.

Whilst this study highlighted concerns about claims and labelling, the concerns are compounded by the lack of structures of governance and policy development that need to ensure the transition from self-regulation to regulation of nutritional supplement sector in an improved way. Thus the importance of screen testing for contaminants and adulterants, and that of steroids and stimulants, as intended in this thesis, should be apparent.

3.5 Conclusion and Recommendations

The following information needs to be regulated and enforced as part of the label to ensure that the consumer can make an informed choice;

- Highlighting the potential for adverse events needs to be presented on the label.

- Warning statements such as, "keeping product out of reach of children", and "the importance of adherence to storage details" should be maintained on the labels appropriately with acceptable font and visibility.

- Warning statements, such as, (i) "exclusion of use, and not a cure for disease states" and (ii) the, "presence of banned substances in the supplements", should be encouraged. These statements should be based on standardised laboratory screen methods.

- A nutritional supplement sector specific system and guideline with regards to the best before and expiry date information, batch numbers, and good (GMP), that will also facilitate batch-to-batch laboratory screen testing, and provision to ensure accurate content detail, should be determined and established.

- Future work in this area to assess claims and labelling could also include other specific supplements such those used in pregnancy (eg. Folates).

- Future studies, to distinguish claims and labelling comparatively for locally assembled or manufactured products, and international imported products into South Africa.

- An alternative label design, that would be beneficial to providing relevant information to the consumer, needs to be scientifically explored and researched.

Section 2

Labelling and other information that influences consumer purchasing decisions

3.6 Introduction

This section in Chapter 3 links to the content as presented in Chapter 2 of the thesis, and research work published by Gabriels et al. (2011 and 2012), which specifically dealt with Regulation and Legislation, and Labelling and Claims, pertaining to the nutritional supplement sector. A summary of observations made in the introduction of Chapter 2, stated that the nutritional supplement at present is generally laden between statutory laws in two extremes; (i) laws that govern medicines, and (ii) those that govern food at present. As discussed in Chapter 2 medicine and food production is a multifaceted industry often governed by several laws, regulations, codes of practice and guidance, in different countries. For the nutritional supplement sector, the resultant "grey" area, and the widening divergence wedged between medicine and food, that has been created due to the lack of specific laws and regulation for the nutritional supplement industry, makes this a convoluted subject.

In the United States of America (USA), and in more recent years in South Africa, sports supplements makes up a considerable proportion of the annual retail sales of nutritional supplement industry. In the USA in particular, the nutritional supplement sector has increased by $10 billion over a decade, amounting to $18.8 billion in 2003 [31, 55]. In South Africa, the local turnover was estimated at R1.5 billion per year (Health Product Association survey 1998-2000) and has grown swiftly, if not exponentially since. There is, however, no tangible and reliable information on annual retail sales in South Africa at present. The increase in retail sales is likely, not due to more effective nutritional supplements, but instead due to determined marketing by manufacturers [17].

Due to the complex legislation governing supplements in most countries, including South Africa, together with the aggressive marketing, companies can

make unproven claims about the effectiveness of the supplement [83]. Furthermore, the truthfulness of the labelling often goes uncontested, therefore any effects of the supplement may be due to contaminants or adulterants in these products not reflected on the label [76,78, 86, 87].

To provide an evidence-based solution to the problem it is important to determine how consumers of nutritional supplement products gather information to assist their purchasing decisions. Therefore, the approach for this study was to ask a range of people to complete a self-administered questionnaire which enquired about which container label information and information other than container labelling sources influenced their purchasing decisions for nutritional supplements.

3.7 Methodology

The self-administered questionnaire was approved by the University of Cape Town, Health Science Faculty Human Research Ethics Committee (HREC REF 346/2012) (Appendix 5b). Subjects who were present at the recruitment sites and gave informed consent were selected for the study. There was no exclusion criteria for participation. Subjects who were physically challenged, visually impaired or deaf, were assisted by the principal investigator or by professional support to ensure appropriate communication.

The questionnaire consisted of seven, closed and open-ended questions from a defined list of statements (Appendix 11). Question statements provided to the participants were basic and had the option of *yes* or *no* answers. From statements with defined options, participants could choose the respective categories ranging from, *strongly influenced* to *no influence*.

The questionnaire covered the following specific areas, (i) general information about participants such as gender, age and the level of physical activity, (ii) had participants purchased nutritional supplement in the last 12 months, (iii) what information on the container label influenced the purchase of nutritional supplements, and (vi) what had influenced the purchase of nutritional

supplements if it was not the information on the label. The participants were asked to complete the questionnaire as accurately as possible (truthfully and honestly), and to the best of their ability. The questionnaire took approximately 2-5 minutes to complete, and was administered in various similar classroom settings, over the period July to September 2012.

Written approval was obtained from the various institutions or organisation where target and convenient samples/groups were identified and followed up as part of the recruitment process. The specific sites were the University of Cape Town (UCT), the Cape Peninsula University of Technology (CPUT), and the University of the Western Cape (UWC) sport halls, and at the holding camps for the 2012 South Africa Olympic (Pretoria) and Paralympic (Johannesburg) teams, respectively, prior to their departure to the 2012 London Olympic Games.

The recruitment process, management, data capture and analysis of the questionnaire was done by the principal investigator. The compliance rate was 100% with 259 participants completing and returning questionnaires.

The data and processing of the returned questionnaires was captured using Windows-based Microsoft ® Office Excel 2003 SP 1 (Excel © 1985-2003 Microsoft Corporation). Statistica Version 10 (copyright © Stat Soft, Inc. 1984-2011) was used to calculate the descriptive statistics. Data are expressed as the mean ± standard deviation (SD).

3.8 Results

3.8.1 Level of physical activity and age of participants

Seventy-seven participants described their physical activity as moderate, whilst 181 of the participants stated that they are competitively active. Only one participant indicated that he was inactive and was therefore excluded from further analysis. The average age and standard deviation (SD) for the participants whose physical activity was classified as either moderate or competitive is presented in Table 6.

Table 6. *Level of physical activity and age participants*

Level of Physical Activity	No. of Participants	Participants'Age (years)
Moderate	77	29.5 ± 9.9
Competitive	181	27.6 ± 8.1
Total Group	258	28.2 ± 8.7

SD = standard deviation

3.8.2 Physical activity and gender comparison

Of the overall cohort (n=258), 159 were male and 99 were female participants. Fifty males indicated they were moderately active and 109 were competitively active. For the females 27 were moderately active and 72 were competitively active, respectively. The ages of the respective male and female groups based on level of physical activity are presented in Table 7.

Table 7. *Physical activity and gender comparison*

Level of Physical Activity	No. of Participants	Participants' Age (years)
Male (total)	159	29.5 ± 9.4
Moderate	50	31.0 ± 10.1
Competitive	109	28.8 ± 9.0
Females (total)	99	26.1 ± 6.9
Moderate	27	26.9 ± 8.7
Competitive	72	25.8 ± 6.1
All Groups (total)	258	28.2 ± 8.7

3.8.3 Purchase of nutritional supplements

The responses of the competitively physically active group indicated that 139 (74%) had purchased nutritional supplements in the previous 12 month, whilst 48 (26%) had not purchased nutritional supplements in this period. For the moderately physical active group, 61 (77%) indicated that they had purchased nutritional supplements, whilst 79 (23%) indicated that they had not purchased

nutritional supplements in the previous 12 months. These data are shown in Figure 2.

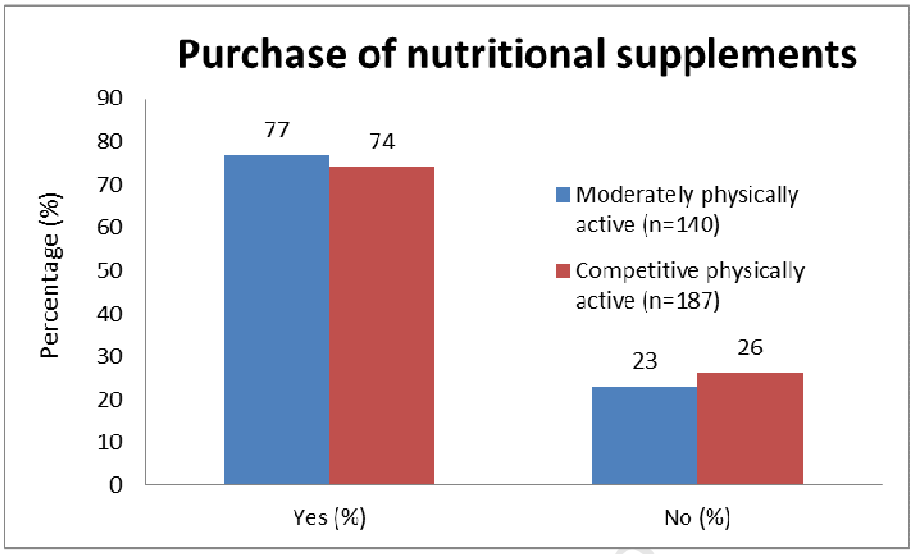


Figure 2. *Purchase of nutritional supplements in last 12 months*

3.8.4 Information on container label

When the data of the whole group (n=195) that had purchased nutritional supplement products were analysed, 132 (68%) indicated that they were influenced by information on the container label, and 63 (32%) indicated that their purchase of a nutritional supplement product was not based on information on the product label.

The results were similar (i.e. whether the information on the label of the supplement product influenced their purchase) when the moderately and competitively physically active groups were compared (Table 8).

Table 8. *Influence of container label in purchase*

Level of Physical Activity	No. of Participants	Yes	No
Whole Group	195 (100%)	132 (68%)	63 (32%)
Moderate	58 (100%)	37 (64%)	21 (36%)
Competitive	135 (100%)	93 (69%)	42 (31%)

Percentage (%) of respective groups in brackets

3.8.5 Information on the container label that influenced purchase of nutritional supplements

The self-administered questionnaire (Appendix 11) provides the five defined categories, (i) *absolutely no influence*, (ii) *partially no influence*, (iii) *uncertain*, (iv) *moderately influenced* and (v) *strongly influenced* that could be selected by participants for the respective container label information. The results are presented for, (a) brand name, (b) ingredients, (c) recommended dosage and directions for use, (d) claims, (e) disclaimers and warnings, (f) quality of product, and (g) free of banned substances.

3.8.5.1 Brand name

132 Respondents stated that brand name information on the container label influenced the purchase of nutritional supplement products. Thirteen % (n=17) stated the brand name had *no influence* on choice of purchase, whilst an increase of respondents in the defined respective categories, *partially no influence* 8% (n=10), uncertain 21% (n=27), *moderately influenced* 25% (n=31) and 36% (n=47), stated that the brand name had a *strong influence* on their purchase (Figure 3).

3.8.5.2 Ingredients

129 Respondents stated that ingredient information on the container label influenced the purchase of nutritional supplement products. Seven % (n=9) indicated the ingredient information had *absolutely no influence* and 7% (n=9)

stated *partially no influence* on choice of purchase, whilst an increase number of respondents in the other respective categories, being, *uncertain* 15% (n=19), *moderately influenced* 31% (n=40), and 40% (n=52), stated that the ingredient information had a *strong influence* on their purchase (Figure 3).

3.8.5.3 Recommended dosage and directions for use

132 Respondents stated that information about dosage and directions for use on the container label, contributed to their decision to purchase nutritional supplement products. The categories of influence were, 14% (n=18) who indicated that dosage and directions for use information had *no influence*, 13% (n=17) stated *partially no influence* on choice of purchase, whilst an increased number of respondents in the other respective categories, being, 25% (n=33) *being uncertain*, 27% (n=36) being *moderately influenced*, and 21% (n=28), stated that the dosage and directions for use information had a *strong influence* on their purchase (Figure 3).

3.8.5.4 Claims

127 Respondents stated that claims information on the container label influenced the purchase of nutritional supplement products. Twenty % (n=25) indicated that dosage and directions for use information had *no influence*, 21% (n=26) stated *partially no influence* on choice of purchase, 30% (n=38) being *uncertain* of this form of information, 22% (n=28) being *moderately influenced*, and 8% (n=10), stated that the dosage and directions for use information had a *strong influence* on their purchase (Figure 3).

3.8.5.5 Disclaimers and warnings

123 Respondents stated that disclaimers and warnings information on the container label influenced the purchase of nutritional supplement products. This ranged from 14% (n=17) stating that the disclaimers and warnings information had *no influence* on choice of purchase, whilst an increase number of respondents

in the defined respective categories stated, partially *no influence* 11% (n=14), *uncertain* 17% (n=21), *moderately influenced* 30% (n=35) and 29% (n=36) stated that disclaimers and warnings information had a *strong influence* on their purchase (Figure 3).

3.8.5.6 Quality of product

129 Respondents stated that the quality of product information on the container label influenced the purchase of nutritional supplement products. Six % (n=6) stated the quality of product information had *no influence* on their choice of purchase. The frequency of response tended to across the choices; *partially no influence* 2% (n=3), *uncertain* 11% (n=14), *moderately influenced* 30% (n=39) and 52% (n=67) stated that the quality of product information had a *strong influence* on their purchase (Figure 3)**.**

3.8.5.7 Free of banned substance(s)

129 Respondents stated that information declaring the supplement was free of banned substance(s) on the container label influenced the purchase of nutritional supplement products. Seven % (n=9) stated free of banned substance(s) information had *no influence* on the choice of purchase, whilst an increased number of respondents in the defined respective categories, *partially no influence* 4% (n=5), *uncertain* 9% (n=12), *moderately influenced* 11% (n=14), and 69% (n=89) stated that the free of banned substance(s) information had a *strong influence* on their purchase (Figure 3).

3.8.6 Information not on container label

The results are presented for, (a) coach, gym and/or fitness trainer, and fellow athletes, (b) supplement representatives, (c) pharmacist, dietician, nutritionist and doctors, (d) print media, (e) electronic media, and (f) social media and the internet. The categories were scored as previously described.

3.8.6.1 Coach, gym and/or fitness trainer, and fellow athletes

67 Respondents stated that information from coaches, gym and/or fitness trainer, fellow athletes influenced their purchase of nutritional supplement products. 30% (n=20) stated information from coaches, gym and/or fitness trainer, fellow athletes had *no influence* on the choice of purchase, whilst an increase number of respondents in the defined respective categories, *partially no influence* 10% (n=7), *uncertain* 15% (n=10), *moderately influenced* 21% (n=14), and 24% (n=16) stated that the information from coaches, gym and/or fitness trainer, fellow athletes had a *strong influence* on their purchase (Figure 3).

3.8.6.2 Supplement representatives

63 Respondents stated that information from supplement representatives influenced their purchase of nutritional supplement products. 60% (n=28) stated information from supplement representatives had *no influence* on the choice of purchase, whilst a decrease number of respondents in the defined respective categories, *partially no influence* 14% (n=9), *uncertain* 13% (n=8), *moderately influenced* 11% (n=7), and 2% (n=1) stated that the information from supplement representatives had a *strong influence* on their purchase (Figure 3).

3.8.6.3 Pharmacist, Dietician, Nutritionist and Doctors

60 Respondents stated that information from Pharmacist, Dietician, Nutritionist, and Doctor influenced their purchase of nutritional supplement products. 43% (n=26) stated information from Pharmacist, Dietician, Nutritionist, and Doctors had *no influence* on the choice of purchase**,** whilst a decrease number of respondents in the defined respective categories, *partially no influence* 23% (n=14), *uncertain* 12 % (n=7), *moderately influenced* 12 % (n=7), and 10% (n=6), stated that the information from Pharmacist, Dietician, Nutritionist, and Doctors had a *strong influence* on their purchase (Figure 3).

3.8.6.4 Print media

67 Respondents stated that information in the print media (eg. magazines, newspapers) influenced their purchase of nutritional supplement products. 39% (n=26) stated information in the print media had no influence on the choice of purchase, 13% (n=9), stated partially no influence on choice of purchase, 21% (n=14) being uncertain, 15% (n=10) being moderately influenced, and 12% (n=8), stated that the information in the print media had a strong influence on their purchase (Figure 3).

3.8.6.5 Electronic media

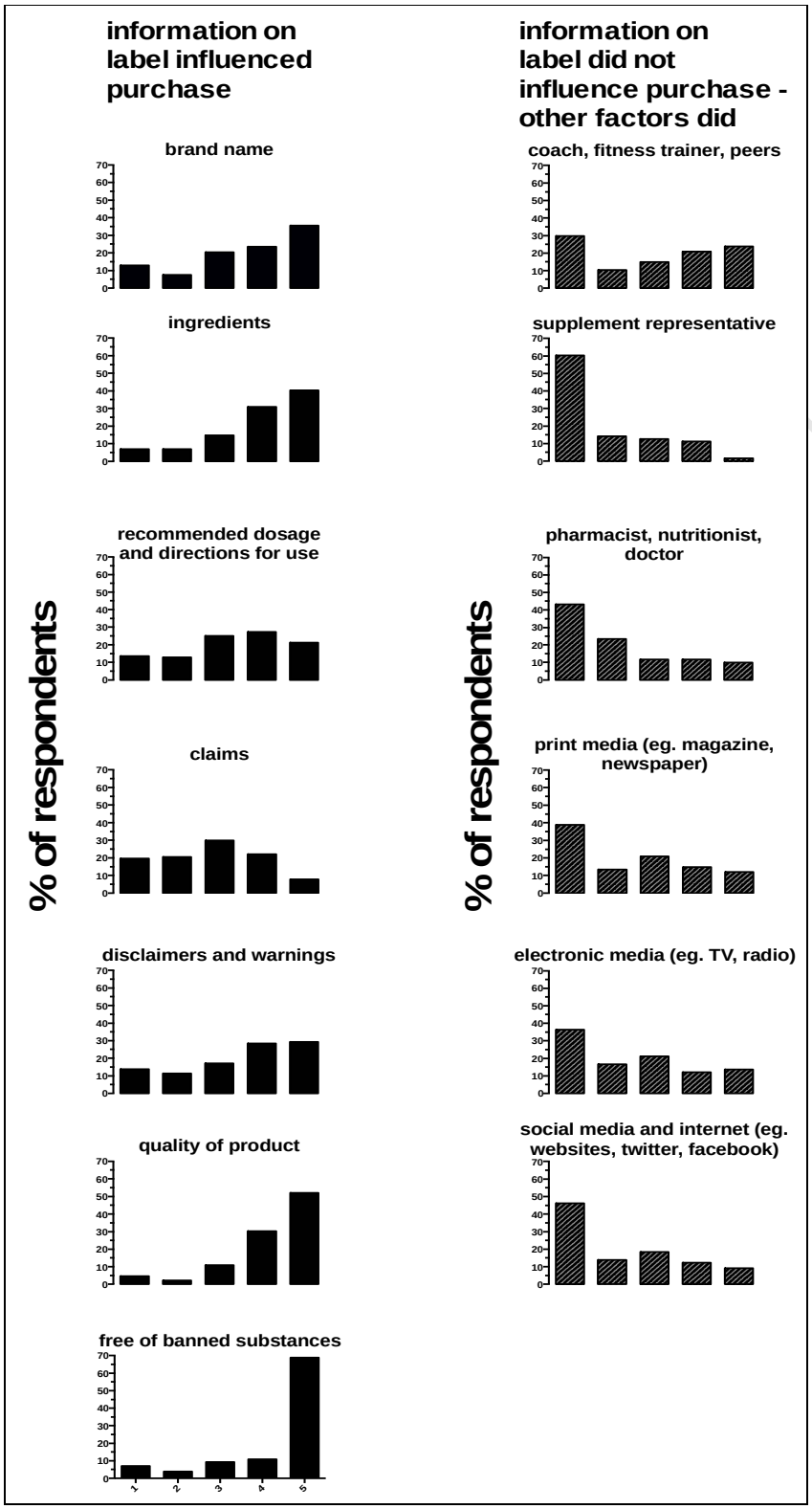
66 Respondents stated that information in the electronic media (eg. television, radio) influenced their purchase of nutritional supplement products. 36% (n=24) stated information in the electronic media had no influence on the choice of purchase, 17% (n=11), stated partially no influence on choice of purchase, 21% (n=14) being uncertain, 12 % (n=8) being moderately influenced, and 14% (n=9), stated that the information in the electronic media had a strong influence on their purchase (Figure 3).

3.8.6.6 Social media and the internet

65 Respondents stated that information from social media and the internet (eg. website, twitter, Facebook) influenced their purchase of nutritional supplement products. 46% (n=30) stated information from social media and the internet had *no influence* on the choice of purchase, 14% (n=9), stated *partially no influence* on choice of purchase, 19% (n=12) being *uncertain*, 12% (n=8) being *moderately influenced*, and 9% (n=6), stated that the information from social media and the internet had a *strong influence* on their purchase (Figure 3).

These data are summarized in Figure 3. The bar-charts on the left-hand side, illustrates the information on the container label that influenced the purchase of nutritional supplements. The bar-charts on the right-hand side illustrates other

factors that contributed to the purchase of nutritional supplements, rather than the information on the container label.



1- Absolutely no influence, 2- partially no influence, 3- uncertain, 4-moderately influenced 5- strongly influenced

Figure 3. *Information influencing purchasing choice*

3.9 Discussion

The main finding of this study was that close to 70% of the respondents who purchased supplements were ***strongly influenced*** by container label information that stipulated that the nutritional supplement product is free of banned substances. The second finding is that just over 50% of the respondents attach importance to the quality of the nutritional supplement product information on the container label. The third finding is that about 40% of the respondents were ***strongly influenced*** by the ingredients on the labels when they purchased nutritional supplements. Brand name (36%), disclaimers and warnings (29%), recommended dosage and directions for use (21%), and claims (8%) accounted for the other reasons influencing the purchase.

These findings are important as its shows the information that is pertinent to people who purchase nutritional supplements, and who base their purchasing decision(s) **on container label information.** The absence intentionally or unintentionally of specific information with specific reference to "free of banned substances" is an important determinant in the purchase of nutritional supplement products. Not presenting this information on the label, when in fact a product may contain a prohibited substance(s) may have dire consequence(s) to the health and wellness of the consumer of such products, or result in a competitive athlete testing "positive" for a prohibited substance by anti-doping agencies.

This information points to the level and degree of concern that consumers of nutritional supplements would have if not all product content is declared on the container label. Furthermore, concealing information about a nutritional supplement product being adulterated or contaminated either unintentionally or intentionally would impact the consumers decision to purchase a product. These findings therefore also point to the importance of independent laboratory screen testing of all nutritional supplement products for contaminants and/or adulterants on a regular, and batch-to- batch basis.

A recent paper showed that for the consumer to make informed choice there was a need to alert those consuming nutritional supplements of the potential for banned substances being present in products [222]. Of the products labels assessed in that particular study, only 5% had information on "The presence of banned substances in the supplements".

The findings **other than the container label information** raise awareness of the factors that ***strongly influences*** the purchasing of nutritional supplement products. An important finding was that coaches, gym and/or fitness trainers, and fellow athletes (24%) have a ***greater influence*** on the choice of nutritional supplement products than that of a Pharmacist, Dietician, Nutritionist and Doctors (10%). This is contrary to the recommendations of Meltzer et al [226], which provided a practical guide to the use of nutritional supplements in South Africa (2004), specifically stating that fitness coaches and conditioning staff should not prescribe supplements. It is important to note that supplement representatives (2%) did not have much influence with regards to the purchase of supplements.

The findings show that 46% of the respondents indicated that social media and the internet had ***absolutely no influence*** on their purchases. Print media and electronic media had ***absolutely no influence*** on their purchases, for 39% and 36% of the respondents, respectively.

The findings further show that for 14% of the respondents, electronic media had a ***strong influence*** on their purchase of nutritional supplement products. For print media and social media and the internet the number of respondents that were ***strongly influenced*** on their purchases were 12% and 9% of respondents, respectively.

These respective information areas could provide the opportunity for consumer education and awareness that will ensure informed choice, through improved knowledge.

In the context of this study, less than 15% of the study participants were influenced by the categories of electronic media, print media, social media and the internet to provide information on nutritional supplement products. What it does imply is that these are potential areas of growth to provide peer-reviewed evidenced based information.

3.10 Conclusion and Recommendations

The findings in this study (trends and/or emerging trends) thus provides;

- A mechanism, and/or requirements, that may be needed for practical intervention, policy development, and/or regulation.
- Facilitation, direction and guidelines of how nutritional products may be marketed in future.
- Ensures that the consumer makes informed choices with complete knowledge and understanding of all product content linked to container label content.

The evidence in assessing the labelling and claims information on nutritional supplement products, and assessing the impact of container labelling and other sources of information on consumer purchasing decisions highlights matters that require attention in the interest of both the nutritional supplement sector and the consumer. The evidence points to;

- Short-comings in current labelling information practices.
- It provides opportunities to improve label and non-label information and communication.
- The requirement for quality assurance laboratory “screening testing” for trace element and heavy metal consistency and safety.

- The requirement for quality assurance laboratory "screening testing" to detect for the presence of undeclared steroids, stimulants and other compounds of interest that could have negative consequences to the consumer.

The findings in chapter 3 with reference to the two specific study sections 1 and 2, thus forms the basis for the research gaps identified, and the laboratory experimental chapters that follow.

Chapter 4
Trace Elements and Heavy Metal Analysis
Experimental Study

4.1 Introduction

The composition of nutritional supplements also comprises trace elements and heavy metals. Trace element and heavy metals could therefore be implicit in causing or contributing to adverse reaction events [127]. Both trace elements and heavy metals would not necessarily be declared on the product label, and consequently becomes an important consideration for this study. The importance of measuring heavy metals and trace metals in particular, is presented in a Herbal Supplement book [227], which points to the contamination of herbal supplements from metals, pharmaceuticals and plant poisoning. One of the most serious concerns associated with using herbal supplements is the potential exposure to toxic substances, whether as an expected component of the formulation or as an unintended contaminant.

The presence of heavy metals is an excellent example of this concern as case reports have described adverse effects or death secondary to metal toxicity from supposedly safe, "all natural" products [227]. The detrimental health outcomes in the longer-term might arise as is illustrated by the following examples; (i) Cadmium (Cd) has a long biological half-life and ingestion over a lifetime results in substantial body burden due to storage in the liver and kidneys, (ii) Lead (Pb) replaces Calcium (Ca) leading to osteoporosis and may potentiate other genotoxins to induce cancer, (iii) Mercury (Hg) crosses the blood brain barrier and the placenta, inducing neurological damage, and (iv) Nickel (Ni) is a known carcinogen and shows wide variability to induce tumors [227].

There is no reason to suspect an offending herbal supplement for a risk of over exposure to heavy metals until the consumer shows signs of toxicity. Therefore, measuring the presence of these metals in nutritional supplement product could be used to verify their quality [227] and reduce the risk associated with unintentional or intentional metal exposure.

Further, the profiling of consumer products (nutritional supplement) for trace elements and heavy metals could have the potential to determine intra-and-inter

product batch consistency or inconsistency, or identifying a contaminant via quantitative bar charts or visual radar plots. In this context, the definition of a contaminant is any substance (element) that is either present in an environment where it does not belong, or is present at levels that might cause harmful effects to humans or the environment.

The World Health Organization (WHO) estimates that 60-80% of people in developing countries use traditional medicines [128]. Moreover, the use of over-the-counter (OTC) herbal and other forms of drugs derived from plants products has increased dramatically over the past decade, both in developing and developed countries [129]. This upsurge in the use of natural products was driven by many factors, including availability and cost effectiveness, perceived safety and lack of side effects and deteriorated belief in the efficacy and safety of conventional drugs. Furthermore, public tendency towards self-medication, and increased publicity and sensational journalism has also increased the public interest in the products [130]. In addition, many of the aforementioned products have intensively been marketed as nutritional supplements.

The next section will describe the experimental approach to the problem to extend the understanding of whether trace elements and heavy metals can be used to determine inter-and-intra product batch consistency or inconsistency. Following the review of the literature there will be a description of trace element and heavy metal analysis done. To serve as examples, Ginseng and Hypoxis products were used as sample products to do the trace element and heavy metal investigation. These products were selected because they are popular in complementary and alternative medicine practices [131]. However, this principle of trace element and heavy metal profiling as a measure of quality, could be applied to nutritional supplements in general, including multivitamins.

4.2 The Scientific Literature

4.2.1 Herbal-Botanical Synergistic Interactions

It is essential to recognize the potential risks that nutritional supplements and/or herbal-botanical preparations pose to consumers, especially those consumers on medication and who may be further exposed to adverse drug-herb interactions. Nutritional supplements that are either contaminated with trace elements and

heavy metals or do not have the concentrations of the ingredients declared on the label, could result in undesirable interactions with other drugs the person may be ingesting at the time. The adverse effects of drug-herb combinations may invariably be related to synergistic or additive interactions [4,128,132,133]. Synergy or additively is defined as interactions among biologically active agents, including chemicals, drugs, pharmaceuticals, supplements, carcinogens, environmental pollutants, toxins, and plant or herbal extracts (phytochemicals), such that their combination produces a greater intensity of response than the individual components [129]. A synergistic response is regarded as beneficial when the desired therapeutic effects outweigh the unwanted side effects. The same assumption also applies when consumers concomitantly use complementary or alternative medicines [129, 130,134-137].

4.2.2 Phytochemicals and Traditional Medicines

Plants have developed an array of antioxidant defences to protect themselves from environmental stress. Antioxidants, active substances, and trace element and heavy metal composition of plant-derived products also vary with the location or source [131,138-141]. It therefore follows that if these plants are ingested as food that the composition of the food will also vary. The use of traditional medicines or herbal preparations is commonplace in many societies and cultures. In South Africa, traditional medicines and herbs have multiple applications in various aspects of popular culture, including traditional healing practices related to ethnobotanical and ethnopharmacological folklore and knowledge, self-medication, and as new medicines and supplements in an ever-growing industry [110, 142-144]. The lack of public confidence in traditional medicines and herbal products is generally due to misidentification of plants, lack of standardization, poor manufacturing practices, botanical substitution, adulteration, and contamination. The therapeutic effects of plant-derived medicines are thought to be enhanced by essential trace elements [142]. However, some products may also contain excessive amounts of trace elements and heavy metals that may precipitate adverse effects in consumers. Consequently, chemical compositional analysis of active ingredients and substances in phytochemical mixtures has become increasingly important and relevant in view of the revival of interest in the development and discovery of new plant-based medicines, as well as their varying composition and unidentified toxicity spectrum [143].

4.2.3 Metal Content and Contamination in Supplements

A study on the composition of trace element and heavy metals of Ephedra containing nutritional supplements showed that the lowest concentration of metal were mostly found in single-ingredient supplements compared to multiple component supplements that generally had higher metal concentrations [127]. According to these data the multiple-component supplement products would have a greater probability of contributing to adverse effects [127]. Furthermore, this study showed that significant lot-to-lot variations were also observed in some of the supplement products [127].

Trace elements and heavy metals are also included in multivitamin and mineral supplements products. People use these products for many reasons, including to compensate for nutritional deficiencies, to promote and maintain health, as protection against the functional declines associated with aging, to reduce the risk of developing chronic disease such as cancer, heart disease, and to manage or treat diseases [145,146]. A review on herbal product contamination identified concerns that contribute to toxicity. These concerns were a lack of child-resistant packaging, contamination, proliferation of multiple ingredient products, excessive concentration, and discovery of new drug-herb interactions [129]. FDA analyses showed that the greatest contamination associated with metals has been observed in the course of inspection of herbal products imported from Asia [147]. In summary, quality assurance and batch-to-batch consistency of these products could theoretically be determined by using the trace elements and heavy metal profile markers.

4.2.4 Bioactive Properties of Plants

Although the effects and mechanisms of substances (compounds) in certain plant species may not be clearly understood, the plants often form an integral part of supplement products. The reason for their inclusion is often based on anecdotal evidence and observations of the plants eaten by wild animals such as chimpanzees, with the assumption that curative properties for disease in the plants eaten by these animals also applies to humans [156].

4.3 Literature from other Sources

The book, "The Coming of China Wars" by Peter Navarro highlights the origins of China's flood of contaminated, defective, and carcinogenic products on world markets [35]. The Chinese government's own reports expressed apprehension that many rivers in the region are so contaminated with heavy metals from industrial waste, by-products and pesticides, including DDT, that they are too dangerous to touch, and unsuitable for aquaculture or commercial fish farming (Washington Post) [35]. It follows that nutritional supplements, such as herbal and botanical products, may be constituted, grown or processed using contaminated water supplies and this will obviously affect the quality of the supplement. Stock farming processes may also be impeded by water contaminated with heavy metals through a chain of events, with potential adverse concentration-dependent effects at the human consumption stage [35]. The Aristolochia plant, with main bioactive ingredients such as aristolochic acids, is an example of a popular plant grown in China. These plants are used to manufacture nutritional supplements, slimming pills and Traditional Chinese Medicines [148,149].

4.4 Ginseng and Hypoxis

Commercial Ginseng and Hypoxis supplement products are regarded as herbal or plant-derived medicines used in primary healthcare, self-medication, and complementary and alternative medicine practices. Ginseng (prepared from the roots of the plant family *Araliaceae*, viz., *Panax quinquefolium* and *P. Schinseng*) is a popular traditional Chinese medicine used for its adaptogenic and restorative properties (an adaptogen is defined as a substance that balances the body, particularly during stress, generating mental arousal by either stimulating or relaxing mechanisms). Hypoxis (a member of the plant family *Hypoxidaceae*, *Hypoxis hemerocallidea*), also called the African potato, is used as a herbal supplement or in conjunction with conventional therapies. Hypoxis is acclaimed for its immune-boosting properties and beneficial effects against several diseases, including prostate cancer, rheumatoid arthritis, psoriasis, hypertension and, somewhat controversially, in terms of public debate, HIV/AIDS. The sterols and sterolins in extracts of Hypoxis have been implicated in their effects, but many other ingredients that are yet to be determined may also be involved.

"Finger print" profiling of the trace and heavy metal composition of supplements as will be used in this study, has the potential to be used as a reference standard against which subsequent manufactured batches can be validated. This should also be able to assist in the identification of trace element or heavy metal contamination. Radar plots could also be used as an additional visual tool to observe batch-to-batch discrepancies, insufficiencies or contaminations immediately after processing collective results. The proposed approach has the potential to contribute to standardising the production and quality of nutritional supplements and traditional herbal medicines. The trace elements and heavy metal respective concentration should be consistent for every brand type.

However, before the proposed system can be adopted, it needs to undergo evaluation to detect whether it is implementable. In accordance with this, the two products Ginseng and Hypoxis will be systemically analysed to determine trace element and heavy metal collective profiles.

This study will examine the repeatability of the trace element and heavy metal composition of the same and different batches of Ginseng and Hypoxis to determine whether their profiles can be used in operational quality control approaches applied to other nutritional supplements. The overall goal is to determine whether the trace element and heavy metal profiling of supplement product samples can determine the batch-to-batch consistency in a specific brand.

4.5 Methodology

4.5.1 Supplement Product Acquisition

Ginseng and Hypoxis (African potato) commercial product brands were purchased from various pharmacies and health stores in Cape Town, South Africa with the strategy where possible, of having different batch numbers of the same product. A total of 11 products, (Ginseng n= 6 and Hypoxis n = 5) were purchased. The definition used for trace element and heavy metals in this study was generic in contrast to the specific definitions used in analytical chemistry, biochemistry and geochemistry. There was no prior knowledge of the concentrations to be expected for the respective trace elements and heavy metals. The selection of these products allowed for inter-and-intra batch analysis of the trace elements and heavy metal composition.

4.5.2 Trace Element and Heavy Metals Investigated

The following trace elements and heavy metals were investigated:

Element	Abbreviation	Element	Abbreviation
Magnesium	Mg	Cobalt	Co
Potassium	K	Nickel	Ni
Calcium	Ca	Copper	Cu
Iron	Fe	Zinc	Zn
Manganese	Mn	Strontium	Sr
Lithium	Li	Cadmium	Cd
Sodium	Na	Mercury	Hg
Chromium	Cr	Lead	Pb

4.5.3 Inductively Coupled Plasma – Mass Spectrometry Methodology

The trace element and heavy metal content in the supplements was determined, using the analytical combination tandem system technique of ICP-MS (Inductively Coupled Plasma - Mass Spectrometry). ICP-MS is a powerful, accurate, fast and sensitive analytical technique. The Perkin-Elmer 6000 ICP-MS instrument and analytical sample preparation procedures were used for the above analysis (Courtesy: Department of Geochemistry, University of Cape Town) [150]. The ICP-MS comprises a sample aspiration system and spray chamber in the presence of argon (Figure 4). The inductively coupled plasma is attached to a radio frequency supply that results in the excitation of the metal ions under investigation being pumped through a selective plasma spectrometer interface, an ion lens into the mass filter quadropoles (quads) and a detector system (electron multiplier). All the experimental work and preparation, and data processing were done by the principal investigator.

4.5.4 Sample Preparation for ICP-MS Analysis

A 500 mg sample of each supplement was weighed. The digestion procedure was done in a wide mouth Erlenmeyer flask. All reagents were of analytical grade quality. The acid digestion was adapted from the Karbochem Research and Development Laboratory as follows: A 10 ml combination of HCL/HF (1:4) was added to the sample and the mixture heated to boiling point on a heating mantle in a fume hood. Then 10 ml nitric acid was added to the flask, allowed to boil until the sample volume was approximately 2 ml and then 5 ml perchloric acid added, followed by boiling until the sample volume reached 2 ml. The contents in the

Erlenmeyer flask was then transferred quantitatively to a 100 ml volumetric flask and filtered through Whatman No. 1 into a sample bottle.

Prior to analysis each sample was diluted with a solution of 5% nitric acid containing 4 internal standards consisting of Bi, Re, Rh and In, each at a concentration of 10 ppb, respectively. The analysis protocol consisted of running a blank and 3 calibration standards, made from artificial solutions of 10, 20 and 30 ppb for each of the respective trace elements and heavy metals as defined in the study. The instrument was recalibrated after the analysis of every 10 samples by re-running the blank and the 3 standards. Replicate analyses typically gave an overall procedural error of less than 3% relative standard deviation measured at ppm level, and total procedural blank measured at ppb level [150]. The duplicate samples were then submitted in a blind randomised (coded) way to the analyst for ICP-MS analysis. The content of trace elements and heavy metals in the samples was determined quantitatively from calibration curves, and the results decoded for the respective duplicate samples.

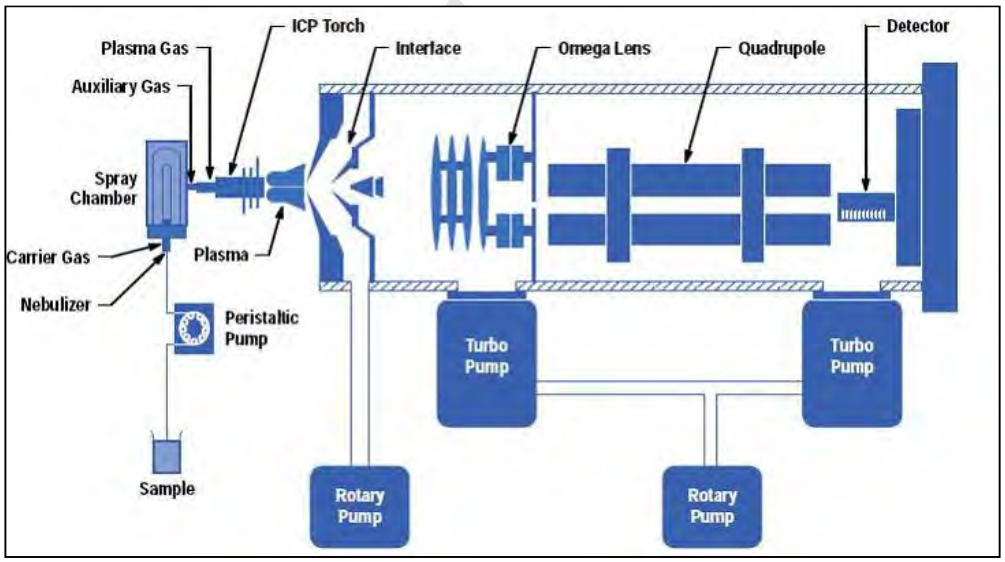


Figure 4. *Schematic diagram of an ICP-MS instrument (Source: Hewlett-Packard Journal)*

4.5.5 Statistical Analysis

Upon completion of data capturing and analysis of the respective investigations, the following computer-based programmes were used for statistical analysis and graphic representation: Windows-based Microsoft® Office Excel 2003 SP1

(Excel© 1985-2003 Microsoft Corporation), WinNonlin Standard Edition Version 1.5 (WinNonlin© 1984-1997, Scientific Consulting, Inc.), and GraphPad Prism® Version 2.01 (GraphPad Software© 1994, 1995, 1996 GraphPad Software, Inc.). Descriptive data were calculated as means and standard deviations and the coefficient of variation. Statistical significance was determined where the p value was ≤ 0.05. Consistency was accepted where both duplicate sample determination were $\leq 20\%$ CV for each trace and heavy metal investigated. The % CV was based on the upper limit of acceptable analytical precision, and within the permissible statistical window for bioequivalence assessment [151, 152, 153].

4.6 Results and Discussion

4.6.1 Products Investigated

Table 9 reflects the Ginseng and Hypoxis (African potato) samples investigated. The tables shows the product name, and batch number/date of purchase. There is also a column in the table clarifying whether the comparisons will be inter or intra batch comparisons. The products were evaluated for content validity and contamination for the following trace elements and heavy metals: Mg, K, Ca, Fe, Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg and Pb (see section 4.5.2 for full names of elements).

Table 9. *Ginseng and Hypoxis products investigated*

Code	Product	Batch number/date	Comparison
	Ginseng		
G1	Aspen Formule Naturelle Ginseng	B/N 403966	Inter
G2	Aspen Formule Naturelle Ginseng	B/N 403746	Inter
G3	Natrodale Formule Naturelle Ginseng	MNF 20 Dec04 E1	Intra
G4	Natrodale Formule Naturelle Ginseng	MNF 20 Dec04 E1	Intra
G5	Bettaway Ginseng	534521	Inter
G6	Bettaway Ginseng	539702	Inter
	Hypoxis products		
H1	Inkomfe Hypoxis	5092	Inter
H2	Inkomfe Hypoxis	5478	Inter/intra
H3	Inkomfe Hypoxis	5478	Inter/intra
H4	Trazure African Potato Hypoxis	B/N 303	Intra
H5	Trazure African Potato Hypoxis	B/N 303	Intra

4.6.2 Statistical Evaluation of Ginseng and Hypoxis Formulas

Samples of Ginseng and Hypoxis formulas (10 tablets or capsules) were randomly selected and evaluated for their mass (in mg) consistency. The unpaired-t-test was used to determine statistical significance for the comparison of mass. These data are shown in Table 10.

Table 10. *Comparison of mass for the Ginseng and Hypoxis samples (n = 10 in each).*

Code	Mass ± SD (mg)	Minimum (mg)	Maximum (mg)	CV (%)	Comparison (p value)
G1	619.2 ± 14.6	587.6	630.3	2.4	*0.903*
G2	620.0 ± 15.8	586.5	631.5	2.6	
G3	650.6 ± 21.2	608.2	612.8	3.3	*0.394*
G4	642.7 ± 19.3	612.8	663.9	3.0	
G5	438.3 ± 5.8	429.4	446.6	1.3	*0.042**
G6	432.6 ± 6.4	423.8	443.7	1.5	
H1	578.0 ± 13.7	547.6	598.2	2.5	*0.017**
H2	546.8 ± 34.8	497.2	594.1	6.4	
H4	513.8 ± 33.8	479.8	580.2	6.6	*0.894*
H5	516.0 ± 37.3	476.0	592.1	7.2	

The coding and details of each sample are shown in Table 9
The p-value comparison is for G1and G2, G3and G4, G5 and G6, H1 and H2, and H4 and H5

The coefficient of variation ranged from 1.3 to 7.2%. There was a significant difference between the average mass of G5 and G6 (Bettaway Ginseng different batches) (p = 0.042) and H1 and H2 (Inkomfe Hypoxis different batches) (p = 0.017). The samples from the same batches (Ginseng and Hypoxis) were not statistically significantly different.

4.6.3 Trace Element and Heavy Metals in Ginseng and Hypoxis

Tables 11 (a, b, c) and 12 (a, b) show the, (i) concentrations and (ii) mean total and percentage coefficient of variation (% CV) of replicate samples of trace element and heavy metals for Ginseng and Hypoxis products. These measurements indicate the inter-batch consistency. Tables 11 (a, b, c) and 12 (a, b, c) also show the (iii) mean and % CV of each duplicate sample of trace element and heavy metals. This gives an indication of the intra-sample batch consistency.

Table 11a, G1a and G1b are duplicate independent samples of the same batch of Ginseng formulas Naturelle product, and G2a and G2b are duplicate independent samples of a different batch of Ginseng formulas Naturelle product.

Table 11a. *Trace element and heavy metal concentration of Aspen Formule Naturelle Ginseng B/N403966 (G1) and 403746 (G2)*

	Sample 1 *(duplicates)*		**Mean**	**%CV**	**Sample 2** *(duplicates)*		**Mean**	**%CV**	***Mean***	***%CV***
	G1a	G1b	(G1a and G1b)		G2a	G2b	(G2a and G2b)		Samples 1 and 2 *(combined)*	
Mg*	5.28	4.54	4.91	10.7	7.20	7.04	7.12	1.6	*6.01*	*21.8*
K*	11.74	11.67	11.71	0.4	6.12	6.39	6.25	3.1	*8.98*	*35.1*
Ca*	79.11	75.56	77.33	3.2	68.84	68.61	68.72	0.2	*73.03*	*7.1*
Fe	3.71	7.14	5.43	44.7	4.81	0.00	2.40	141.4	*3.92*	*76.0*
Mn	59.57	63.80	61.69	4.8	92.13	45.44	68.79	48.0	*65.24*	*30.0*
Li	3.54	0.00	1.77	141.4	14.88	20.88	17.88	23.7	*9.82*	*99.0*
Na	0.00	0.00	0.00	-	113.98	0.00	56.99	141.4	*28.50*	*200.0*
Cr	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Co	1.00	2.26	1.63	54.4	1.44	1.38	1.41	2.7	*1.52*	*34.8*
Ni	1.88	0.00	0.94	141.4	4.47	0.00	2.23	-	*1.59*	*133.3*
Cu	2.11	0.00	1.06	141.4	3.99	2.03	3.01	46.0	*2.03*	*80.1*
Zn	7.00	0.00	3.50	141.4	11.04	5.18	8.11	51.1	*5.81*	*78.9*
Sr	45.54	38.89	42.22	11.1	57.25	99.29	78.27	38.0	*60.24*	*45.0*
Cd	0.02	0.06	0.04	61.2	0.12	0.40	0.26	76.7	*0.15*	*111.9*
Hg	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Pb	1.42	2.10	1.76	27.4	3.22	0.00	1.61	141.4	*1.69*	*79.9*

Values are expressed in mg/g for Mg, K, Ca and Fe and in µg/g for Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg, Pb
(*) % CV is ≤ 20% for the trace and heavy metal in each of Sample 1 and 2, respectively

In Table 11b, G3a and G3b are duplicate independent samples of the same batch of Ginseng formulas Naturelle product, and G4a and G4b are duplicate independent samples of the same batch of Ginseng formulas Natrodale product, both purchased at different geographical locations.

Table 11b. *Trace element and heavy metal concentration in Ginseng product Natrodale B/N MNF 20 Dec04 E1 (G3 and G4)*

	Sample 1 *(duplicates)*		**Mean**	**%CV**	**Sample 2** *(duplicates)*		**Mean**	**%CV**	***Mean***	***%CV***
	G3a	G3b	(G3a and G3b)		G4a	G4b	(G4a and G4b)		Samples 1 and 2 *(combined)*	
Mg	0.44	0.45	0.44	1.1	0.69	1.09	0.89	31.9	*0.67*	*45.7*
K*	8.43	7.63	8.03	7.1	8.55	10.26	9.41	12.9	*8.72*	*12.7*
Ca	0.31	0.53	0.42	36.9	1.08	2.02	1.55	42.8	*0.99*	*77.3*
Fe*	1.37	1.29	1.33	4.0	1.44	1.30	1.37	7.2	*1.35*	*5.1*
Mn*	30.55	28.92	29.73	3.9	28.83	29.29	29.06	1.1	*29.39*	*2.7*
Li	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Na	0.00	0.00	0.00	-	0.00	30.62	15.31	141.4	*7.65*	*200.0*
Cr	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Co	2.34	0.02	1.18	138.5	0.04	0.02	0.03	45.4	*0.60*	*191.2*
Ni	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Cu	0.00	5.96	2.98	141.4	1.56	0.00	0.78	141.4	*1.88*	*149.9*
Zn	0.00	0.00	0.00	-	0.21	0.00	0.11	141.4	*0.05*	*200.0*
Sr	44.32	8.40	26.36	96.4	15.51	27.51	21.51	39.4	*23.93*	*65.6*
Cd	0.37	0.01	0.19	130.7	0.03	0.01	0.02	73.2	*0.11*	*168.5*
Hg	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Pb	33.89	2.56	18.22	121.6	5.69	5.03	5.36	8.7	*11.79*	*125.4*

Values are expressed in mg/g for Mg, K, Ca and Fe and in µg/g for Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg, Pb
(*) % CV is ≤ 20% for the trace and heavy metal in each of Sample 1 and 2, respectively

In Table 11c, G5a and G5b are duplicate independent samples of the same batch of Ginseng formulas Naturelle product, and G6a and G6b are duplicate independent samples of a different batch of Ginseng Betterway product, respectively.

Table 11c. *Trace element and heavy metal concentration in Ginseng product Bettaway B/N 534521 (G5) and 539702 (G6)*

	Sample 1 *(duplicates)*		**Mean**	**%CV**	**Sample 2** *(duplicates)*		**Mean**	**%CV**	***Mean***	***%CV***
	G5a	G5b	(G5a and G5b)		G6a	G6b	(G6a and G6b)		Samples 1 and 2 *(combined)*	
Mg	1.78	0.91	1.35	45.6	1.40	0.96	1.18	26.5	*1.26*	*32.4*
K	3.03	9.89	6.46	75.2	3.23	8.26	5.74	61.9	*6.10*	*57.3*
Ca	4.00	2.51	3.26	32.4	4.71	3.25	3.98	25.9	*3.62*	*26.2*
Fe	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Mn	3.74	6.61	5.17	39.2	6.52	7.93	7.22	13.8	*6.20*	*28.4*
Li	0.00	2.47	1.24	141.4	0.00	0.00	0.00	-	*0.62*	*200.0*
Na	115.74	0.00	57.87	141.4	207.03	0.00	103.51	141.4	*80.69*	*124.4*
Cr	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Co	0.15	0.02	0.08	112.2	0.00	0.00	0.00	-	*0.04*	*173.5*
Ni	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Cu	0.00	0.00	0.00	-	1.16	0.00	0.58	141.4	*0.29*	*200.0*
Zn	0.00	0.00	0.00	-	17.19	11.73	14.46	26.7	*7.23*	*119.5*
Sr	57.11	26.49	41.80	51.8	54.36	22.93	38.64	57.5	*40.22*	*44.8*
Cd	0.07	0.03	0.05	51.8	0.25	0.15	0.20	34.4	*0.13*	*77.8*
Hg	4.14	0.00	2.07	-	1.90	0.00	0.95	-	*1.51*	*130.3*
Pb*	10.72	12.54	11.63	11.1	17.07	15.71	16.39	5.9	*14.01*	*20.7*

Values are expressed in mg/g for Mg, K, Ca and Fe and in µg/g for Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg, Pb
(*) % CV is ≤ 20% for the trace and heavy metal in each of Sample 1 and 2, respectively

In Table 12a, H1a and H1b are duplicate independent samples of the same batch of Hypoxis Inkomfe product. H2a and H2b, and H3a and H3b are duplicate independent samples, respectively of a different batches of Hypoxis Inkomfe products, respectively. All batches were purchased at different geographical locations.

Table 12a. *Trace element and heavy metal concentration in Hypoxis product Inkomfe B/N 5092(H1) and 5478 (H2 and H3)*

	Sample 1 *(duplicates)*		**Mean**	**%CV**	**Sample 2** *(duplicates)*		**Mean**	**%CV**	**Sample 3** *(duplicates)*		**Mean**	**%CV**	**Mean**	**%CV**
	H1a	H1b	(H1a and H1b)		H2a	H2b	(H2a and H2b)		H3a	H3b	(H3a and H3b)		Samples 1,2 & 3 *(combined)*	
Mg*	1.51	1.79	1.65	12.0	1.42	1.40	1.41	1.1	1.45	1.38	1.41	3.8	*1.49*	*10.3*
K*	11.31	11.67	11.49	2.2	11.36	10.83	11.10	3.4	4.67	4.14	4.41	8.5	*9.00*	*39.7*
Ca	1.42	1.91	1.67	20.7	1.50	1.83	1.66	13.7	1.29	1.85	1.57	25.1	*1.63*	*16.0*
Fe	0.00	0.00	0.00	-	0.00	0.00	0.00	-	0.00	0.15	0.07	141.4	*0.02*	*244.9*
Mn*	255.45	251.13	253.29	1.2	247.28	245.18	246.23	0.6	257.74	248.19	252.96	2.7	*250.83*	*2.0*
Li	0.00	5.82	2.91	141.4	0.00	0.00	0.00	-	48.87	15.79	32.33	72.4	*11.75*	*163.5*
Na	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.0	93.52	13.37	53.45	106.0	*17.82*	*210.3*
Cr	0.00	0.00	0.00	-	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Co	1.34	0.81	1.08	34.8	3.79	0.80	2.29	92.3	1.02	0.89	0.95	10.3	*1.44*	*81.0*
Ni	0.00	0.00	0.00	-	0.00	0.00	0.00	-	0.00	2.33	1.17	141.4	*0.39*	*244.9*
Cu	5.48	11.28	8.38	48.9	9.74	9.69	9.71	0.3	11.12	17.44	14.28	31.3	*10.79*	*35.9*
Zn*	33.32	30.00	31.66	7.4	59.14	46.02	52.58	17.7	43.28	36.00	39.64	13.0	*41.29*	*25.7*
Sr	27.34	34.19	30.76	15.7	77.34	63.98	70.66	13.4	96.77	26.40	61.58	80.8	*54.33*	*54.2*
Cd	0.18	0.19	0.19	2.8	0.58	0.49	0.54	12.5	0.72	0.14	0.43	96.1	*0.38*	*64.1*
Hg	0.00	0.18	0.09	141.4	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.03*	*244.9*
Pb	3.09	2.26	2.68	22.0	49.82	36.69	43.25	21.5	12.33	4.75	8.54	62.7	*18.16*	*111.2*

Values in are expressed in mg/g for Mg, K, Ca and Fe and in µg/g for Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg, Pb
(*) % CV is ≤ 20% for the trace and heavy metal in each of Sample 1,2and 3, respectively

In Table 12b, H4a and H4b are duplicate independent samples of the same batch of Hypoxis product Trazure, and H5a and H5b are duplicate independent samples of the same batch of Hypoxis product Trazure, both purchased at different geographical locations.

Table 12b. *Trace element and heavy metal concentration in Hypoxis product Trazure B/N 303 (H4 and H5)*

	Sample 1 *(duplicates)*		**Mean**	**%CV**	**Sample 2** *(duplicates)*		**Mean**	**%CV**	***Mean***	***%CV***
	H4a	H4b	(H4a and H4b)		H5a	H5b	(H5a and H5b)		Samples 1 and 2 *(combined)*	
Mg*	1.19	1.08	1.14	7.4	1.15	1.10	1.13	2.8	*1.13*	*4.6*
K*	4.26	3.91	4.09	6.1	4.56	4.24	4.40	5.2	*4.24*	*6.3*
Ca*	1.87	1.93	1.90	2.2	1.80	2.10	1.95	10.7	*1.93*	*6.5*
Fe	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Mn*	466.10	417.88	441.99	7.7	383.59	369.75	376.67	2.6	*409.33*	*10.5*
Li	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Na	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Cr	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Co	0.42	0.27	0.35	30.8	0.37	0.31	0.34	11.2	*0.34*	*19.2*
Ni	0.14	0.00	0.07	-	0.00	0.00	0.00	-	*0.04*	-
Cu	1.28	1.46	1.37	8.9	1.41	0.00	0.70	-	*1.04*	*67.0*
Zn	30.08	12.38	21.23	58.9	21.04	10.60	15.82	46.7	*18.52*	*48.3*
Sr*	26.34	22.90	24.62	9.9	32.58	28.54	30.56	9.3	*27.59*	*14.7*
Cd	0.33	0.08	0.21	84.5	0.26	0.04	0.15	103.6	*0.18*	*78.0*
Hg	0.00	0.00	0.00	-	0.00	0.00	0.00	-	*0.00*	-
Pb	1.21	0.00	0.61	141.4	1.57	0.00	0.79	141.4	*0.70*	*117.4*

Values in are expressed in mg/g for Mg, K, Ca and Fe and in µg/g for Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg, Pb
(*) % CV is ≤ 20% for the trace and heavy metal in each of Sample 1 and 2, respectively

Table 13 summarises the trace element and heavy metals that show intra-sample batch consistency, intra-batch variability, and inter-batch consistency. These trace elements and heavy metals can be used as markers, as part of specific product assessment and quality.

Table 13. *Trace element and Heavy metal Inter-and-Intra batch consistency*

		Intra-sample batch consistency	Intra-batch variability	Inter-batch sample consistency
Ginseng (G1,G2)	Aspen	Mg, K, Ca	Fe, Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg and Pb	Only for Ca
Ginseng (G3,G4)	Natrodale	K, Fe, Mn	Mg, Ca, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg and Pb	Only for K, Fe, Mn
Ginseng (G5,G6)	Bettaway	Pb	Mg, K, Ca, Fe, Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, and Hg	No
Hypoxis (H1,H2, H3)	Inkomfe	Mg, K, Mn, Zn	Ca, Fe, Li, Na, Cr, Co, Ni, Cu, , Sr, Cd, Hg and Pb	Only for Mg, Mn
Hypoxis (H4,H5)	Trazure	Mg, K, Ca, Mn, Sr	Fe, Li, Na, Cr, Co, Ni, Cu, Zn, Cd, Hg and Pb	Only for Mg, K, Ca, Mn, Sr

Assessment for consistency based on ≤ 20%CV values.

4.6.4 Trace Elements and Heavy Metals in Ginseng and Hypoxis

4.6.4.1 Ginseng Commercial Products

The Ginseng commercial products assessed showed that in the case of Aspen Formula Naturelle Pharmacare Ginseng, the highest concentrations measured

were: Mg (7.20 mg/g), K (11.74 mg/g), Ca (79.11 mg/g), Fe (7.14 mg/g), Mn (92.13 µg/g), Li (20.88 mg/g), Na (113.98 µg/g), Ni (4.47 µg/g), Sr (99.29 µg/g) and Cd (0.40 µg/g), whereas those for Natrodale Ginseng Vitality were Co (2.34 µg/g), Cu (5.96 µg/g) and Pb (33.89 µg/g). Bettaway Ginseng had the highest concentration for Zn (17.19 µg/g) and Hg (4.14 µg/g). Chromium was not detected in any of the Ginseng commercial products analyzed.

4.6.4.2 Hypoxis Commercial Products

The Hypoxis commercial products assessed showed that in the case of Hypoxis Inkomfe the highest concentrations measured were for Mg (1.79 mg/g), K (11.67 mg/g), Fe (0.15 mg/g), Li (48.87 µg/g), Na (93.52 µg/g), Co (3.79 µg/g), Ni (2.33 µg/g), Cu (17.44 µg/g), Zn (59.14 µg/g), Sr (96.77 µg/g), Cd (0.72 µg/g), Hg (0.18 µg/g) and Pb (49.82 µg/g). The highest concentrations for Ca (1.93 mg/g) and Mn 466 (µg/g) were present in Hypoxis Trazure. As for Ginseng, chromium was also not detected in any of the Hypoxis commercial products investigated.

Table 14 is a tabulated assessment of the highest trace elements and heavy metals measured overall, for both Ginseng and Hypoxis products.

Table 14. A*ssessment of highest element concentrations*

	Ginseng Product	C_{max} †	Hypoxis Product	C_{max} †
Mg	Aspen Formula Naturelle	7.20		
K	Aspen Formula Naturelle	11.74		
Ca	Aspen Formula Naturelle	79.11		
Fe	Aspen Formula Naturelle	7.14		
Mn			Trazure	466.10
Li			Inkomfe	48.87
Na	Aspen Formula Naturelle	113.98	Inkomfe	3.79
Cr				
Co	Natrodale Vitality	2.34	Inkomfe	3.79
Ni	Aspen Formula Naturelle	4.47		
Cu			Inkomfe	17.44
Zn	Aspen Formula Naturelle	59.14		
Sr	Aspen Formula Naturelle	99.29		
Cd			Inkomfe	0.72
Hg	Bettaway	4.14		
Pb			Inkomfe	49.82

† *C_{max} are the highest concentrations in mg/g for Mg, K, Ca and Fe and in µg/g for Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg, Pb measured for commercial Ginseng and Hypoxis products.*

4.6.5 Phytopharmaceutical Enhancing Indications and Toxicity

In the context of the recommended upper levels for selected trace elements and heavy metals presented in Table 14, the highest concentration in each product type were determined.

Table 15 summarizes selected trace elements and heavy metals measured in Ginseng and Hypoxis commercial products, their phytopharmaceutical enhancing indications, recommended daily upper limits and potential toxic effects in humans due to elevated levels [154, 155].

Table 15. *Phytopharmaceutical enhancing indications and toxicity*

Element	Phytopharmaceutical Enhancing Indication	UL*	Description and Toxicity
Ca	Ca relieves cough, chronic bronchitis, asthma, laxative, diuretic, cathartic, vermifuge.	2.5 g/day	These trace metals (Ca, Na, K, Mg) are macronutrients and are required in relatively large amounts in the human diet. In high doses they may be toxic to the body or produce deficiencies in other trace metals. Excessive intake of Ca result in deposits in soft tissue e.g., kidneys.
Na	Na is involved in extracellular and intracellular fluid balance, and maintenance of blood viscosity. Na also relieves urinary diseases.	2.3 g/day	Plasma Na levels above 152 mM may contribute to coma, paralysis of lung muscles and death.
K	K maintains homeostasis, influences cardiac muscle activity, relieves urinary diseases, fever, diarrhoea, cough, chronic bronchitis.	4.0 g/day	Individuals suffering from kidney diseases may suffer adverse health effects from consuming large quantities of dietary potassium
Mg	Mg is essential for the maintenance of cells, relieves dropsy, piles and boils. It is a purgative and diuretic.	0.350 g/day	Excessive intake of Mg leads to diarrhoea.
Fe	Immune-booster, diuretic, astringent, demulcent, antipyretic, relieves fever, chronic rheumatism and psoriasis.	45 mg/day	Fe is an important constituent of many enzymes. Has an important role in regulating immunocompetence. Known symptoms of iron toxicity include fatigue, dizziness, nausea, shortness of breath, headache, vomiting weight loss, anorexia, and greyish skin colour.
Cu	Expectorant, gastric disorders	10 mg/day	Constituent of more than eleven important oxidase enzymes and facilitates various principle metabolic functions. Cu is a heavy metal and is toxic in its unbound form. Symptoms of toxicity include hyperactivity, nausea, vomiting, diarrhoea, fatigue and apathy.
Zn	Relieves fever, ulcer, cough, chronic bronchitis; increases biopotency towards infectious diseases.	40 mg/day	Zn plays an important role as cofactor in several enzymatic reactions. High concentrations may precipitate stomach cramps, vomiting, nausea; skin irritations and anaemia may result.
Mn	Used as an astringent and to treat scabies, pneumonia, bronchitis, asthma, headache and spertamorrhea.	11 mg/day	Mn is an essential metal. Symptoms of poisoning include forgetfulness and nerve damage. A syndrome due to Mn toxicity has symptoms similar to Schizophrenia, dullness, weak muscle, headaches and insomnia.

**UL, upper limit*
Dietary Reference Intake (accessed 2012, July 2012). Available from *http://en.wikipedia.org/wiki/Dietary_Reference_Intake*

4.6.6 Comparative Highest Concentration Assessment

Table 16 illustrates the equivalent number of commercial Ginseng and Hypoxis product tablets or capsules that have to be consumed to achieve concentrations of Mg, K, Ca, Fe, Mn, Na, Cu and Zn that may potentially cause adverse events. The

equivalent number of tablets or capsules was derived from the highest concentration data summarized in Table 14 and the daily upper limit for trace elements and heavy metals defined in Table 15. It is particularly noteworthy from the results and in the context of Ginseng and Hypoxis commercial products investigated, for example, that at least 9 Ginseng tablets or capsules would need to be consumed to achieve the upper limit of 40 mg/day for iron (Fe). This is highly relevant since consumers can easily take higher dosages of product than recommended or prescribed. However, toxicity and adverse effects need to be carefully monitored and evaluated from the perspective of synergy among the product's ingredients, i.e., their individual and combined effect(s).

Table 16. *Illustrative equivalent upper limit tablet or capsule determination*

	Highest concentration (g/g)	Upper limit (g)	Mean tablet/ capsule (g)	Type	Brand	Factor	Quantity to consume (g)	Equivalent number of tablets or capsules
Mg	0.0072	0.350	0.62	Aspen	Ginseng	139	49	78
K	0.01174	4.0	0.62	Aspen	Ginseng	85	346	550
Ca	0.07911	2.5	0.62	Aspen	Ginseng	13	32	51
Fe	0.00714	0.04	0.62	Aspen	Ginseng	140	6	9
Mn	0.0004661	0.011	0.52	Trazure	Hypoxis	2145	24	46
Na	0.00011398	2.3	0.62	Aspen	Ginseng	8773	20179	32547
Cu	0.00001744	0.01	0.58	Inkomfe	Hypoxis	57339	573	992
Zn	0.00005914	0.04	0.62	Aspen	Ginseng	16909	676	1091

4.7 Fingerprint Profiles of Ginseng and Hypoxis Products

Using the trace element and heavy metal analysis data of the commercial Ginseng and Hypoxis products, comparative fingerprint plots were created using logarithmic concentration values. This allowed for comparative profiles of the complete data-set as well as an assessment of product and processing consistency.

All the trace elements and heavy metals assessed could be classified as contaminants, in the context that they were not described on the label content. However, certain trace elements and heavy metals, within given product sample batches showed consistency, being present in all replicate samples tested. These elements could be used as "markers" for product and batch –to– batch consistency testing.

4.7.1 Fingerprint Profile of Aspen Formula Naturelle Ginseng

The fingerprint profile (Figure 5) was established from quadruplicate samples from two respective batches of Aspen Formula Naturelle Ginseng. Based on the criteria of ≤ 20% CV and Table 13, Mg, K, and Ca showed intra-sample consistency. Ca was the only trace element with acceptable inter-batch sample consistency. Intra-batch variability was observed for Fe, Mn, Li, Na, Co, Ni, Cu, Zn, Sr, Cd, and Pb. No Cr and Hg were not detected in any of the specific samples.

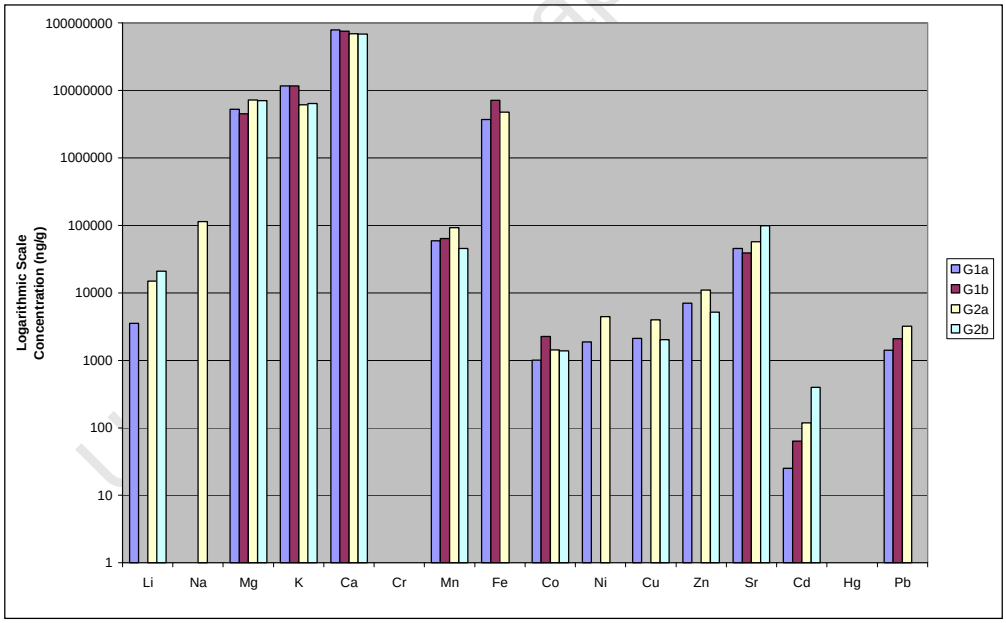


Figure 5. *Fingerprint profile of Aspen Formula Naturelle Ginseng*

4.7.2 Fingerprint Profile of Natrodale Ginseng Vitality

The fingerprint profile (Figure 6) was established from quadruplicate samples from two respective batches of Natrodale Ginseng Vitality. K, Fe and Mn showed intra-sample consistency. K, Fe, Mn also showed inter-batch sample consistency. Intra-batch variability was observed for Mg, Ca, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg and Pb. No Cr and Hg were not detected in any of the specific samples.

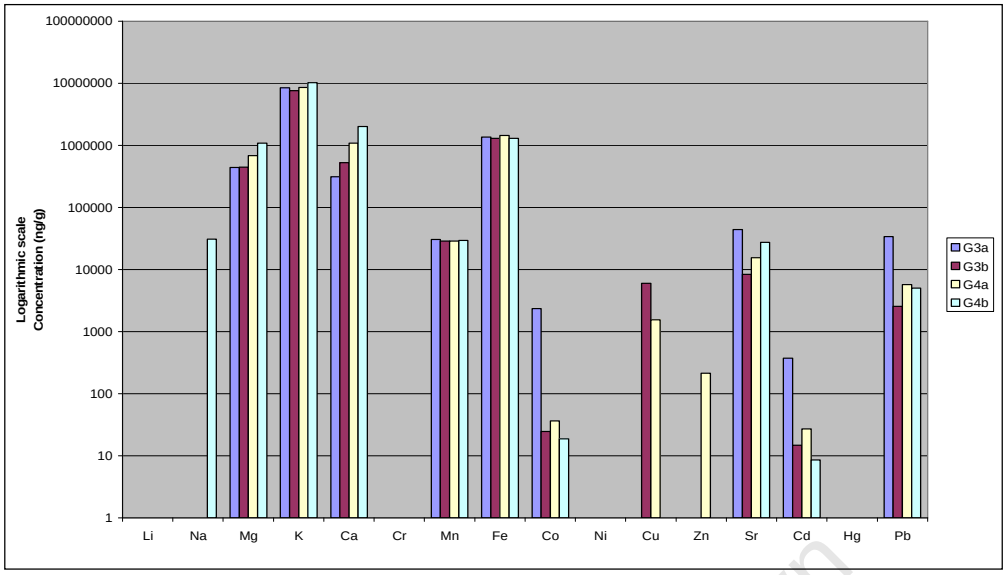


Figure 6. *Finger print profile of Natrodale Ginseng Vitality*

4.7.3 Fingerprint Profile of Bettaway Ginseng

The fingerprint profile (Figure 7) was established from quadruplicate samples from two batches of Bettaway Ginseng. Pb showed intra-sample batch consistency. Intra-batch variability was observed for Mg, K, Ca, Mn, Li, Na, Co, Ni, Cu, Zn, Sr, and Cd. No Cr, Fe and Ni were detected in the specific samples. None of the trace elements and heavy metals assessed showed inter-batch sample consistency.

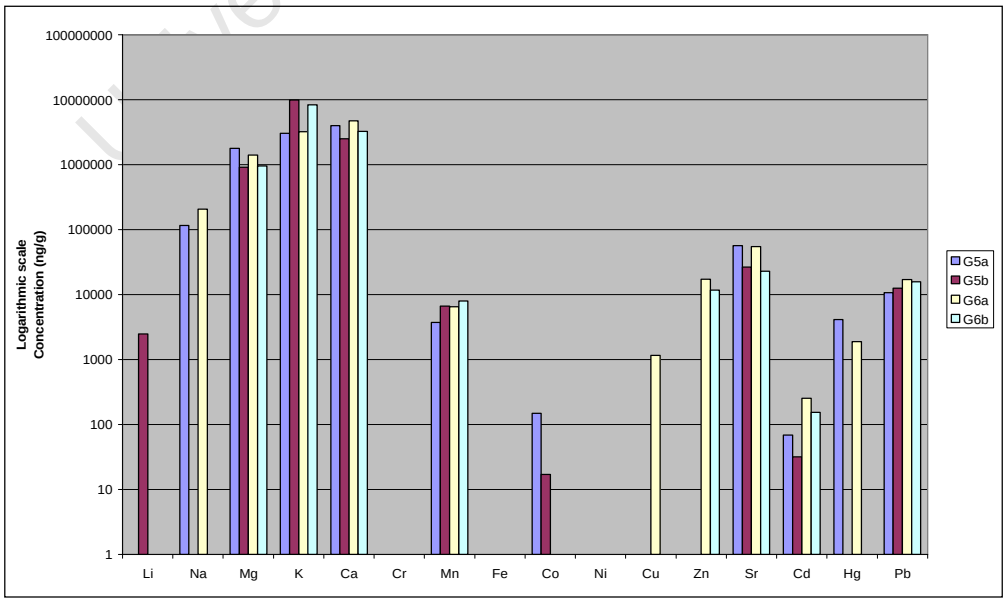


Figure 7. *Finger print profile of Bettaway Ginseng*

4.7.4 Fingerprint Profile of Inkomfe Hypoxis

The fingerprint profile (Figure 8) was established from six replicates from three respective batches of Inkomfe Hypoxis. Mg, K, Mn, Zn showed intra-sample batch consistency, and inter-batch sample consistency was observed for Mg and Mn. Intra-batch variability is observed for Ca, Fe, Li, Na, Co, Ni, Cu, Sr, and Cd. No Cr, Fe and Ni were detected in the specific samples.

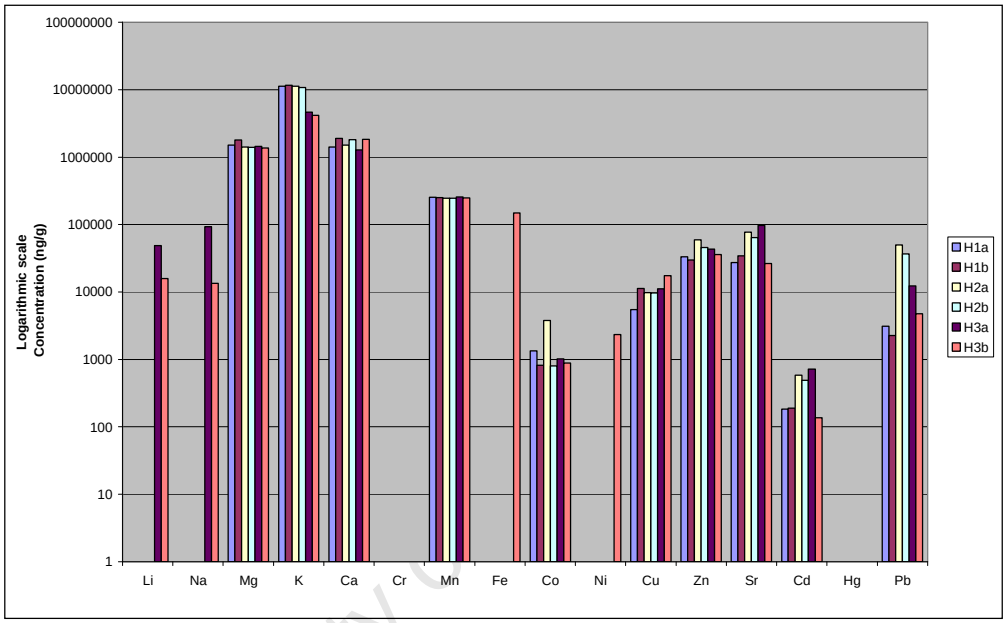


Figure 8. *Fingerprint profile of Inkomfe Hypoxis*

4.7.5 Fingerprint Profile of Trazure African Potato Hypoxis

The fingerprint profile (Figure 9) was established from quadruplicate samples from two respective batches of Trazure Hypoxis. Mg, K, Ca, Mn, Sr had intra-sample batch consistency, and inter-batch sample consistency was observed for Mg, K, Ca, Mn and Sr. Intra-batch variability was observed for Co, Ni, Cu, Sr, and Cd. Li, Na, Cr, Fe and Hg were not detected in the specific samples tested.

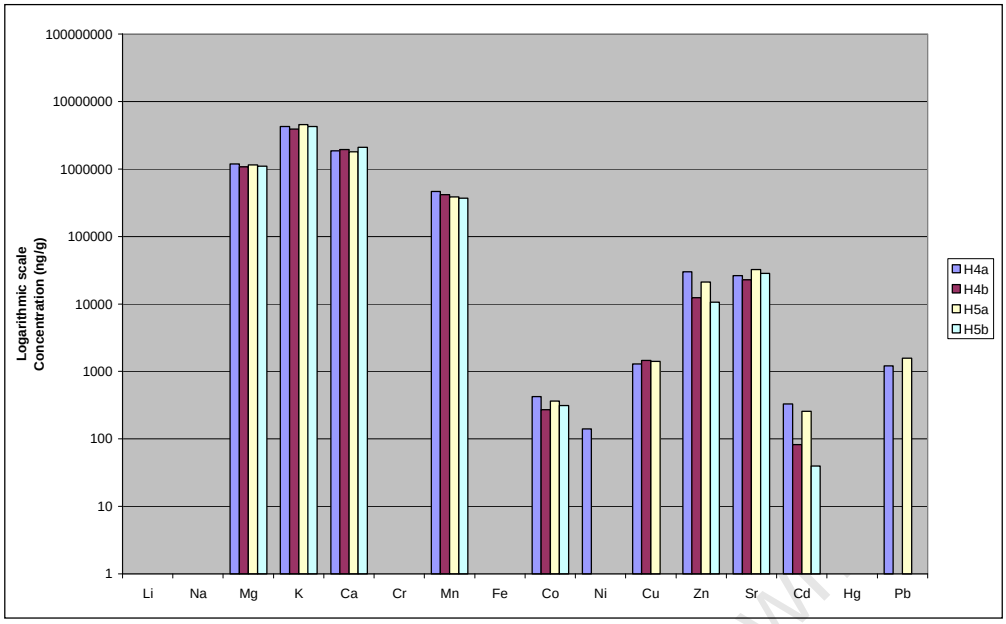


Figure 9. *Fingerprint profile of Trazure African Potato Hypoxis*

4.7.6 Summary of Commercial Ginseng and Hypoxis Fingerprint Plots

None of the commercial Ginseng and Hypoxis products investigated contained Cr. Of the 3 Ginseng and 2 Hypoxis products, intra-batch consistency based on trace element and heavy metal assessment was determined for specific products. All the products, except the Ginseng Bettaway product, provided inter-batch sample consistency, based on the assessment of certain trace elements and metals. The trace elements and heavy metals identified for each product, could be used as specific markers of inter and intra batch-to-batch consistency for the specific products.

4.8 Radar Plots of Ginseng and Hypoxis Commercial Products

Using the trace element and heavy metal analysis data of the commercial Ginseng and Hypoxis products, comparative radar plots were constructed using logarithmic concentration values. Each of the batches investigated were done in duplicate. This allowed a visual comparative profile of the complete data set as well as a visual assessment of the samples (product). A reflection of the overall collective manufacturing process, being from starting materials to final product is also accomplished. The results are represented in logarithmic concentrations ranging from nanogram (ng) to milligram (mg) trace element or heavy metal, depicted as

concentric circles in the plots. Figure 10 is an enlarged sample illustration, with respect to Figure 11 to Figure 15 for easy reference. The inner concentric circle being the lower concentration, and the outer concentric circle being the higher concentration of respective elements. No line from the centre for a given trace element or heavy metal respectively implies that it was not present at detectable level in the product sample investigated. In the case of Figure 10 this is Li, Cr, Ni, and Hg. The resultant image hence represents the trace elements and heavy metals, that are present in the product investigated.

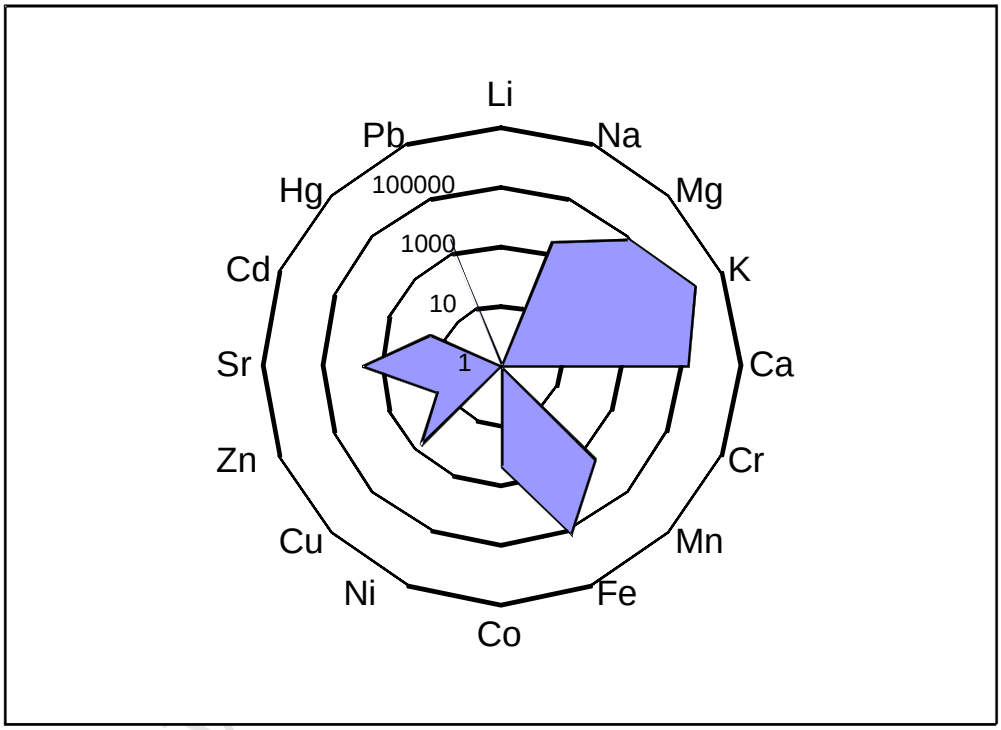


Figure 10. *Sample illustration of radar plot*

4.8.1 Radar Plots of Ginseng Aspen

In Figure 11 (Aspen Formula Naturelle Ginseng), the top left graph represents a composite radar plot of the concentration (logarithmic scale) of the trace element and heavy metal analysis of all Ginseng Aspen samples tested. This plot therefore gives the outer limits (all inclusive) for consistency. G1a and G1b, and G2a and G2b are duplicate samples of different batches of the same product brand. The composite radar concentration maximum (C_{max}) plot shows that there were no Hg and Cr present in the Ginseng Aspen samples tested. The duplicate representative radar plots of G1a and G1b, and G2a and G2b showed partial similarity, with respect to intra- and inter-sample batch association. There are, however,

indications of product inconsistency due to discrepancies in some of the samples for Na, Pb, Li, and Fe. This implies a lack of inter- and intra-sample homogeneity.

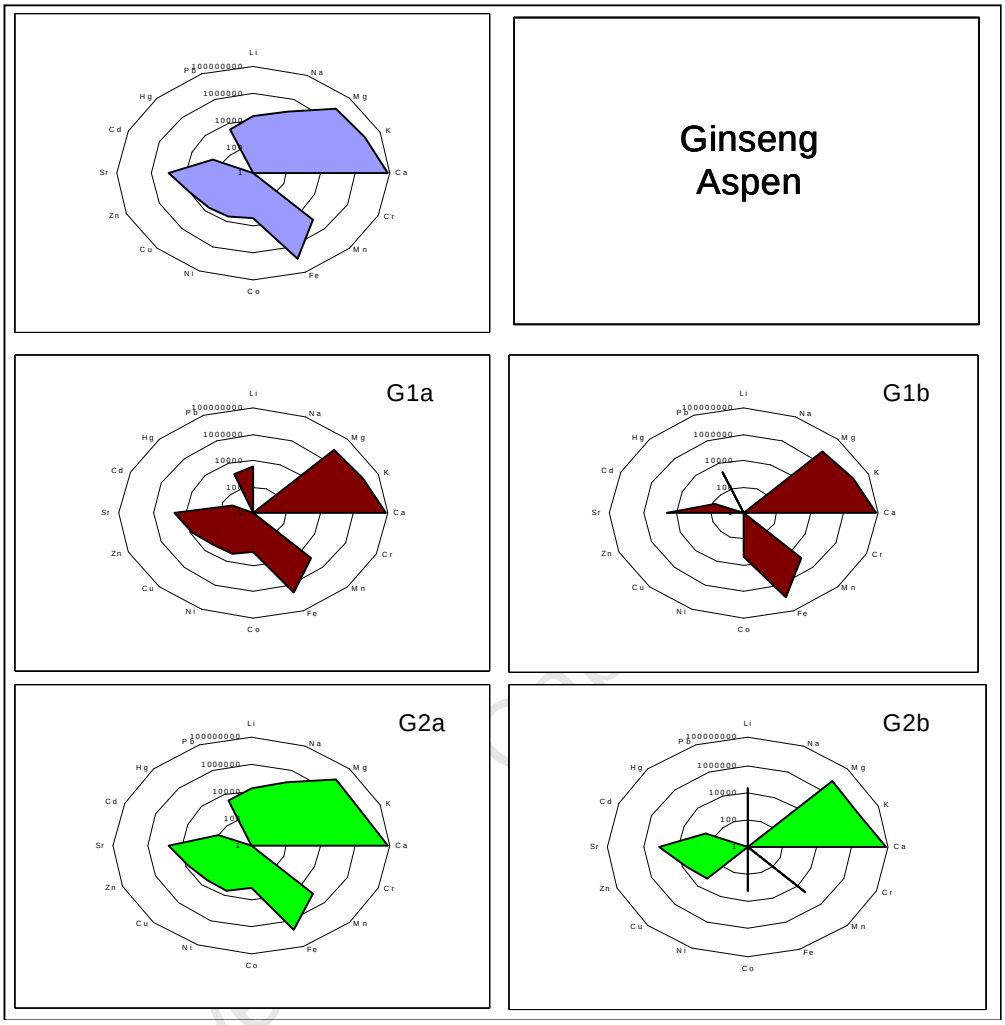


Figure 11. *Radar plots of Aspen Formula Naturelle Ginseng*

4.8.2 Radar Plots of Ginseng Vitality

In Figure 12 (Ginseng Vitality), the top left graph represents a composite radar plot of the concentration (log scale) of the trace element and heavy metal analysis of all Ginseng Vitality samples tested. This plot consequently gives the outer limits (all inclusive) for consistency. G3a and G3b, and G4a and G4b are duplicate samples of different batches of the same product type. The composite radar concentration maximum (C_{max}) plot suggests that there was no Hg, Cr, Li and Ni present in the Ginseng Vitality samples tested. The duplicate representative radar plots of G3a and G3b, and G4a and G4b showed excellent similarity, with respect to intra- and inter-sample batch association, except for Cu,

Zn and Na in some of the samples. This implies lack of inter- and intra-sample homogeneity.

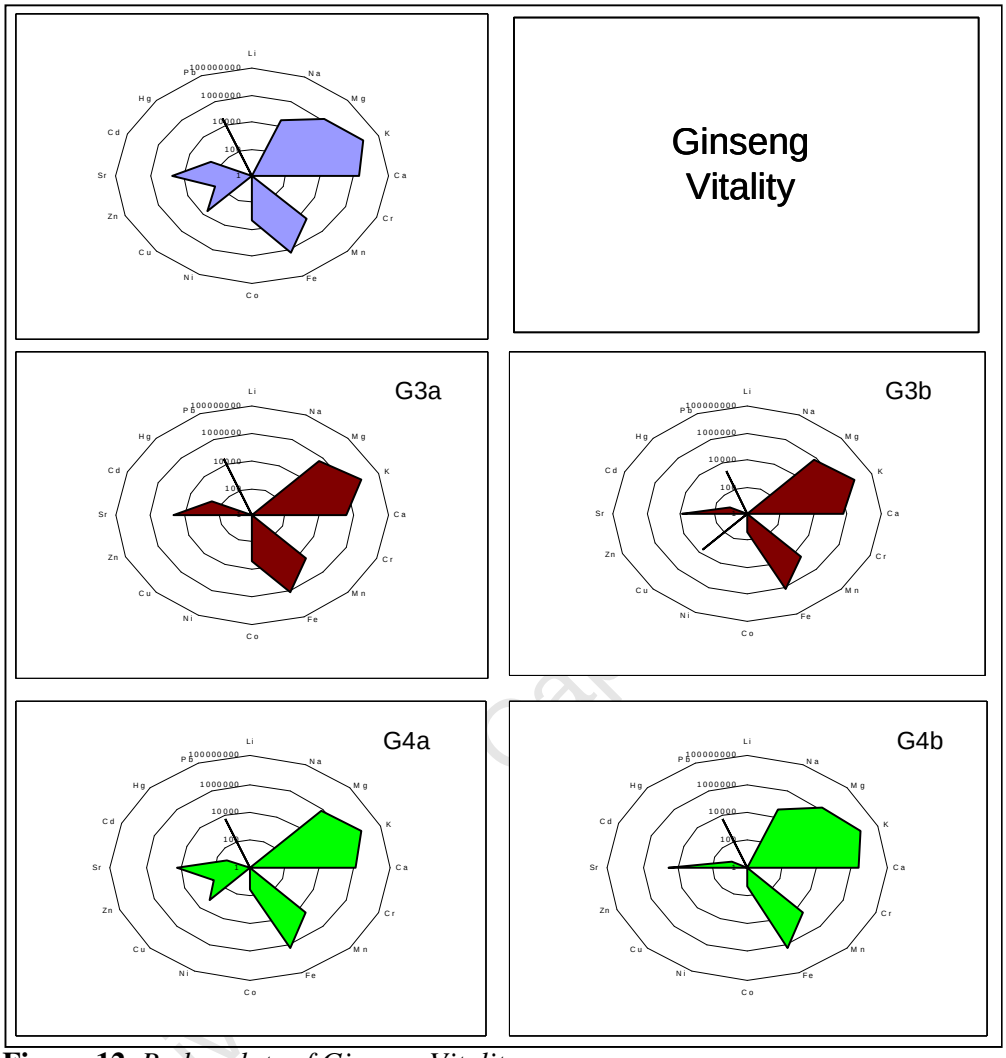


Figure 12. *Radar plots of Ginseng Vitality*

4.8.3 Radar Plots of Ginseng Bettaway

Figure 13 (Ginseng Bettaway) gives the outer limits (all inclusive) for consistency. G5a and G5b, and G6a and G6b are duplicate samples of different batches of the same product. The plot gives indicates that there was no Cr, Fe and Ni present in the Ginseng Bettaway samples tested. The duplicate representative radar plots of G5a and G5b, and G6a and G6b showed good similarity, with respect to intra- and inter-sample batch association, but not for some samples due to discrepancies in Co, Cu, Zn, Hg, Li and Na, pointing to lack of inter- and intra sample homogeneity.

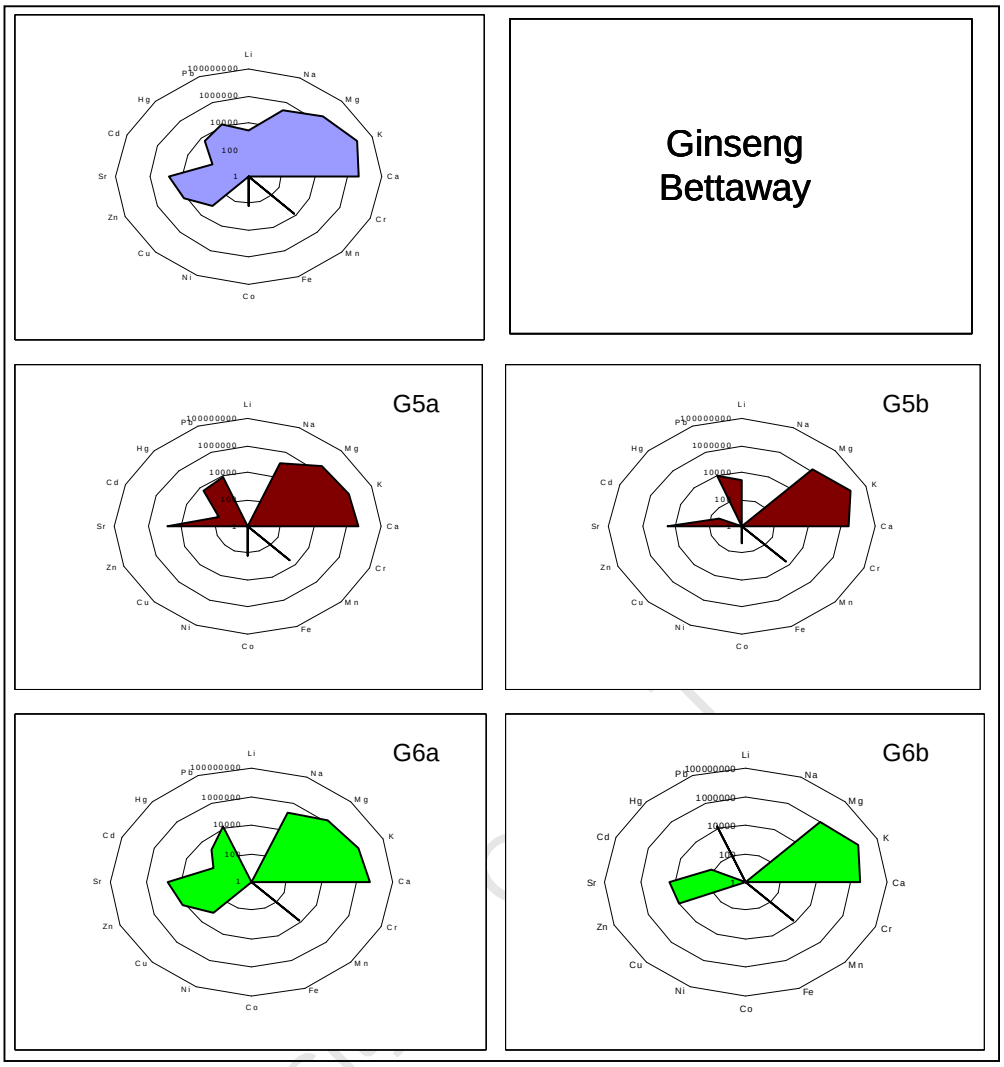


Figure 13. *Radar plots of Ginseng Bettaway*

4.8.4 Radar Plots of Hypoxis Inkomfe

In Figure 14 (Hypoxis Inkomfe), H1a and H1b, H2a and H2b, and, H3a and H3b, are duplicate samples of different respective batches of the same product type. The plot shows that Hg and Cr were absent in the Hypoxis Inkomfe samples tested. The duplicate representative radar plots of H1a and H1b, and H2a and H2b showed excellent similarity with respect to intra- and inter-sample batch association. H3a and H3b showed excellent intra-sample association, and partial similarity to H1 and H2 samples. There are, however, sample (product) inconsistency with specific reference to Ni and Fe content in H3 samples. This points to lack of inter and intra-sample homogeneity. H1 and H2, and H3 are consistent in trace element and heavy metal consistency for the respective duplicate samples tested, yet H3 is clearly different to H1 and H2. This may be

indicative of potentially different raw materials and/or manufacturing processes or protocols, or advertent additions.

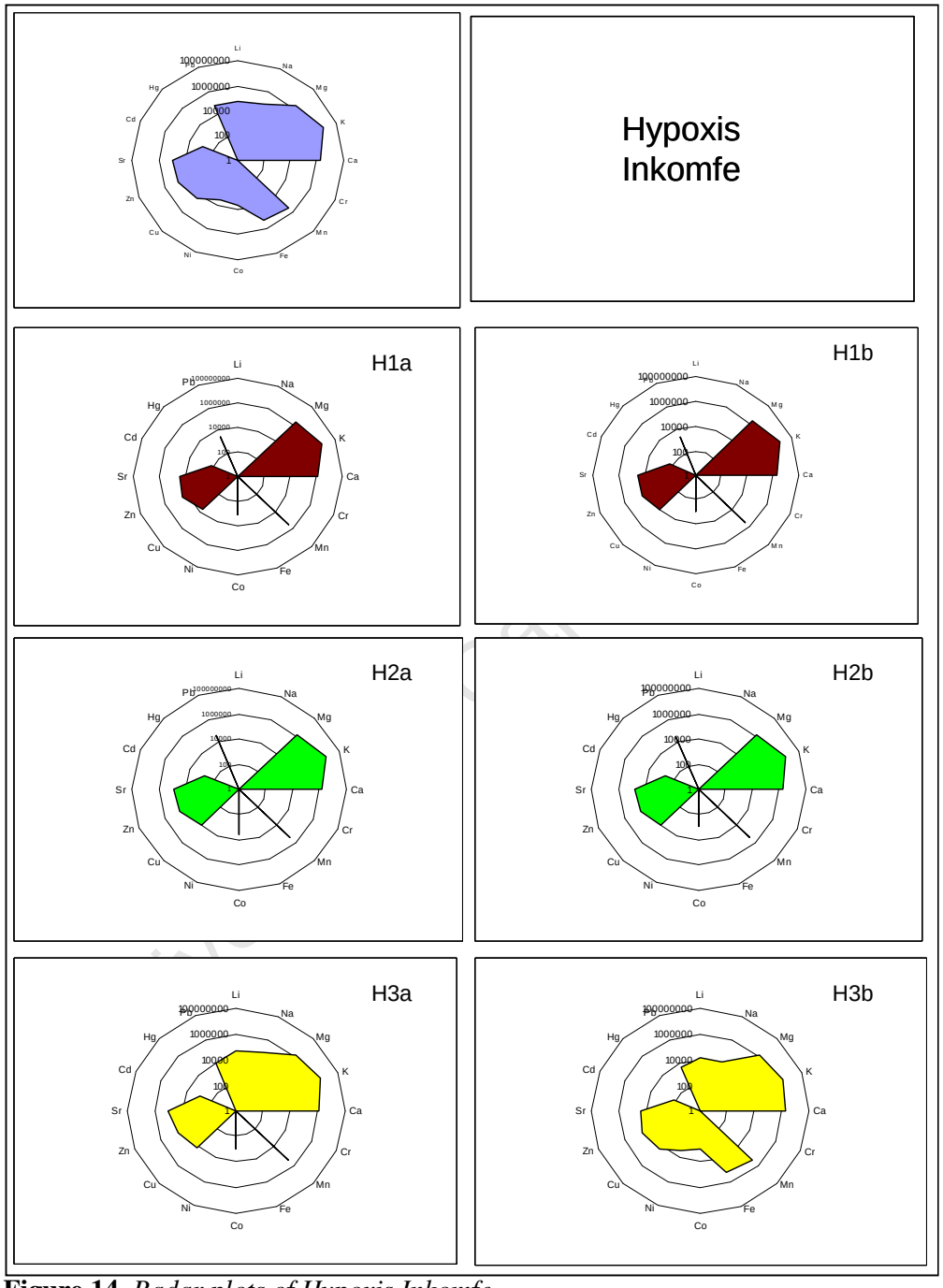


Figure 14. *Radar plots of Hypoxis Inkomfe*

4.8.5 Radar Plots of Hypoxis Trazure

In Figure 15 (Hypoxis Trazure), H4a and H4b, and H5a and H5b are duplicate samples of different batches of the same product type. The plot shows that no Hg, Cr, Li, Na and Fe were detected in the Hypoxis Trazure samples tested. The duplicate representative radar plots of H4a and H4b, and H5a and H5b showed excellent similarity, with respect to intra-and inter-sample batch association. Ni, Cu, and Pb results, point to lack of sample homogeneity.

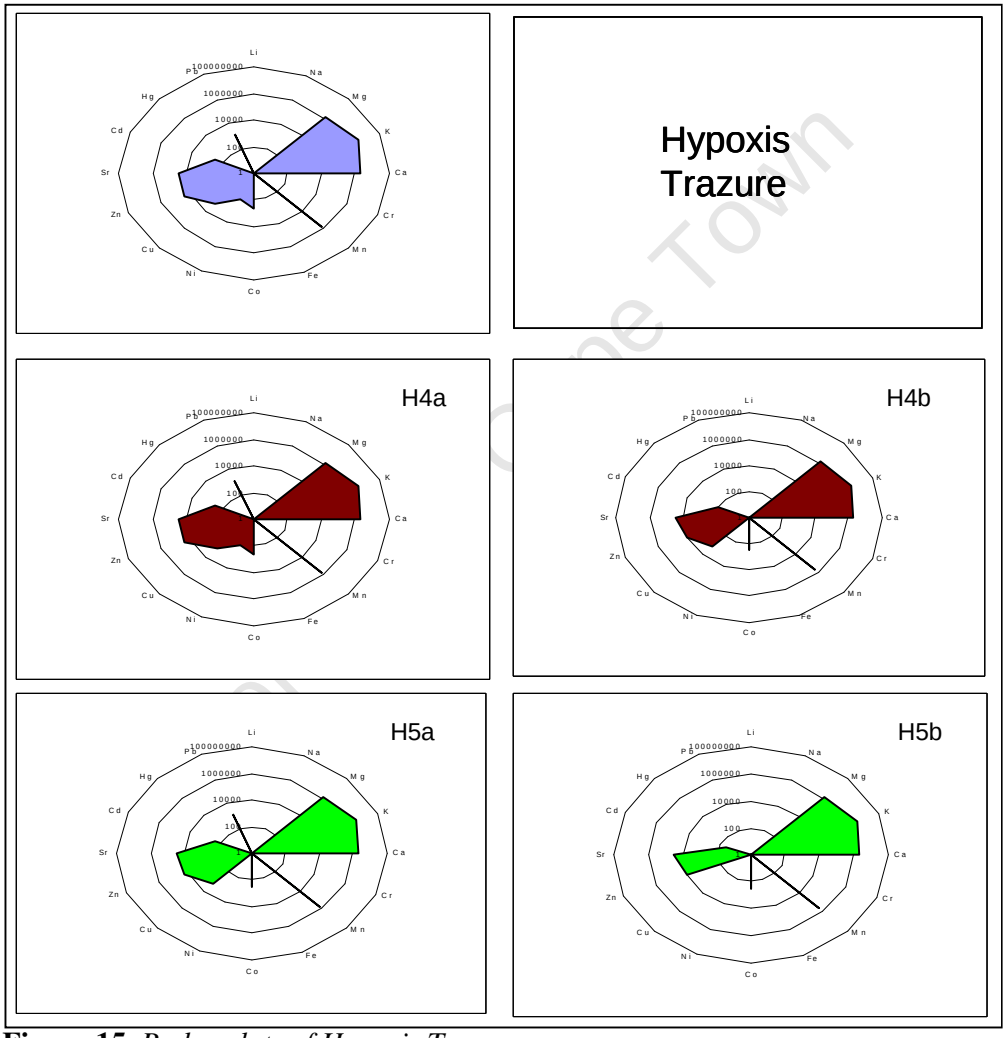


Figure 15. *Radar plots of Hypoxis Trazure*

4.8.6 Summary of Commercial Ginseng and Hypoxis Radar Plots

None of the commercial Ginseng and Hypoxis products investigated in this study contained Cr. The deviation from pre-determined, established and maintained visual radar plots when investigating and/or evaluating commercial products using the range of trace elements and heavy metals studied, imply the following: variation of product raw material source, variation in manufacturing processes, advertent additions/deletions and/or non-compliance with established protocols and/or operating procedures.

4.9 Summary of results

The descriptive data (Table 10) for the Ginseng and Hypoxis tablet or capsule samples generally showed good consistency for mass, as there was no significant difference between samples. However, there were significant differences in mass in the Bettaway Ginseng product batches G5 and G6 (p= 0.042), and Inkomfe Hypoxis product batches H1 and H2 (p= 0.017) respectively.

Table 11 (a, b, c) for Ginseng containing products, and Table 12 (a, b) for Hypoxis containing products, provide the results of two independent samples (Sample 1 and 2) each done in duplicate. The percentage coefficient of variation shows the extent of the inter-and-intra consistency for the respective trace element and heavy metals assessed.

Table 13 provides a summary of Ginseng and Hypoxis products assessed with respect to the trace element and heavy metals that show ***intra-sample*** batch consistency, those that show ***intra-batch*** variability, and those that show ***inter-batch*** sample consistency.

Table 14 provides a comparative assessment of the highest overall element concentrations in the respective Ginseng and Hypoxis products. These findings were used to establish Table 16, which illustrates the upper limit concentration of a given trace element or heavy metal individually that would be accomplished when consuming a given number of tablets or capsules. These findings also suggest the importance of the individual effects of the elements investigated, but also the combined, synergistic effect that may arise.

Further, the findings in this study show that; (i) none of the commercial Ginseng and Hypoxis products investigated contained Cr, (ii) the trace elements and heavy

metals identified for each specific product, could be used as specific markers for inter and intra batch-to-batch consistency, (iii) the Fingerprint and Radar Plots can be utilized as important visual tools to assist the process of establishing product quality and consistency on an ongoing basis, (iv) deviation from pre-determined and established visual radar plots implies the following individually or collectively for subsequent sample batches; (a) the variation of product raw material source and manufacturing processes, (b) advertent additions/deletions, and (b) non-compliance w.r.t. protocols and/or operating procedures.

4.10 Conclusion

Plant-derived phytochemicals, trace elements and minerals may potentially boost the therapeutic efficacy of complimentary and traditional medicines in foods (CTMF). The large potential of the phytopharmaceutical industry in South Africa may be jeopardized by lack of quality control testing procedures and efficient product regulation. It is also important to recognize the individual or collective (combined or synergistic) impact of various trace elements and heavy metals might have on human health and disease, particularly when use in conjunction with conventional drug treatment regimens.

There are many organizations and regulatory bodies that monitor and control the phytopharmaceutical industry in South Africa to ensure consumer confidence and satisfaction, including the Direct Selling Association and Health Products Association. Validation processes that are key to ensuring the quality, safety and efficacy of natural products include Standard Operating Procedures (SOPs) for Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practices (GMP). The aforementioned principles and fundamentals need to be established, implemented, regulated and monitored. The findings in this study reveal the need for such measures to be instituted.

The social and methodological contexts of this study are based on what is currently relevant in terms of the commercialization of Ginseng and Hypoxis supplement products, based on African traditional belief, anecdotal claims and absence of scientific evidence, for use as adaptogens, the treatment of HIV/AIDS as well as “all cure” for common ailments and other serious diseases.

This study emphasizes that prudent and novel approaches are necessary to ensure that commercially available nutritional supplement products comply with standard safety and efficacy requirements.

It further suggests that constituents such as trace elements and minerals, along with other constituents in supplement products, should have unique "fingerprints" and visual radar plot profiles to ensure GAP, GMP and GSP, in the interest of consumer safety and satisfaction.

It also emerged from this study that deviations from the initial pre-determined "fingerprint" levels and visual radar plots are reliable indicators to judge overall product quality and consistency, or lack thereof. Such analyses are imperative in the supplement industry.

That radar plots might be more accepting to consumers than written text on labels to assess quality, and could provide a vehicle for education, awareness, advocacy, research and enforcement, and for the manufacture will help in the process of ensuring continuous quality. These two aspects, (i) consumer driven requirement, and (ii) manufacturer requirement, will see that label design and content presentation of supplement products, will be presented differently to how information is currently provided.

4.11 Limitations and opportunities

- The current trace metal and heavy metal assessment was developed as a proof of concept, using Ginseng and Hypoxis based products.
- Further investigation for the presence of trace elements and heavy metals that were not consistently present in all of the Ginseng and Hypoxis, product requires replicate testing.
- In future studies the trace element and heavy metal method and application needs to be applied to nutritional supplement products to determine and evaluate product batch-to-batch consistency.

Chapter 5
Steroid and Stimulant Analysis
Experimental Study

5.1 Introduction

This chapter will describe the experimental approach in the context of steroids, stimulants and other compounds (drugs) of interest that may also be present in nutritional supplement products. Drug abuse in sport is often called doping. There are several suggestions explaining the different origins of the term “doping”. For example, the term may be derived from;

(i) the Dutch word “*doop*” which means “Christian baptism” [157],

(ii) the Dutch word "*doop*" as a thick dipping sauce that entered American slang to describe how robbers stupefied victims by mixing tobacco with the seeds of Datura stramonium [158],

(iii) from the Dutch word *dop* [159],

(v) a viscous opium juice, being the drug of choice of the ancient Greeks [160],

(vi) the name of an alcoholic beverage made from grape skins used by Zulu warriors to enhance their prowess in battle [159],

(v) an alcoholic drink used as a stimulant in ceremonial dances in 18th century Southern Africa [158],

(vi) the preparation of a thick viscous preparation of opium for smoking, and during the 1890s this extended to any stupefying narcotic drug [158],

(vii) dope, which was a word in the 1990’s to describe "*a preparation of drugs designed to influence a racehorse's performance*" [158].

The use of nutritional supplements by professional sportspersons, and serious and recreational participants in sport and physical activity is widespread [161]. This can partially be attributed to the ease with which supplements can be obtained. This has increased the temptation for the professional sportsperson, “weekend warriors”, and consumers to experiment with supplements [161].

"Supplement doping" may be rife at the local and international level based on evidence in a study showing the presence of small amounts of 19-nor-4-androstenedione, 4-androsten-3,17-dione, and another study showing the presence of prohibited anabolic androgenic steroids (prohormones) not declared on the label [76,162]. This type of doping is defined as the process whereby the supplement, contains ergogenic substances in addition to those substances which are described on the label. The addition of the ergogenic substance to the supplement may be intentional or inadvertent. An example of intentional doping is where a manufacturer deliberately adds a given anabolic steroid or stimulant, that is banned by the World Anti-doping agency (WADA), to the nutritional supplement without declaring this on the label. A professional athlete, who consumes the contaminated nutritional supplement product, could thus test "positive" if tested by anti-doping agency controls [35].

An example of inadvertent doping is where a manufacture is not aware of the steroid product contamination, due to lack of screen testing procedures and also specifically when ingredients of animal origin are used for the product, hormones, such as testosterone, may be present [35]. This may occur when raw materials are sourced from alternative suppliers or, where there are no or inadequate quality control procedures [35]. Using such a supplement may result in a "positive" drug test for the athlete if the added substance is on the banned list as defined by WADA [163].

Therefore, as described previously (Chapter 4), it is important to have measures for detection in place that will screen products for contaminants and consistency. Furthermore, to guard against "supplement doping" it is also important to screen nutritional supplements for prohibited steroids, stimulants and other compounds to ensure quality, clean and safe nutritional supplement products. The next section will present a review of the literature consisting of (i) drugs in supplements and

drug effects on performance and behaviour, (ii) analytical methods, and (iii) a summary of the experimental study.

5.2 The Scientific Literature

5.2.1 Drugs in supplements

WADA publishes an annual list of substances and practices prohibited at all time, in and out of competition. The original aim was to only list doping agents (drugs) known or suspected of improving performance in sports. However, the list is now defined by other reasons, such as the substance affecting the safety of athletes, being socially unacceptable, or attempting to mask the presence of a banned substance [164-166]. Reviews on the use of doping agents in sport and society have concluded that these agents, particularly anabolic androgenic steroids (AAS), are used widely [78,164,167].

There is evidence that in the USA, between 1 million and 3 million people have used AAS [167]. In Sweden the estimate is that 50 000-100 000 people among a population of 9 million have used AAS [164]. Further, a questionnaire investigation of 6000 Swedish people age 16-17 years revealed that 3.2% of males had used AAS [164]. A German study assessed the use of AAS among visitors to fitness centres by use of anonymous questionnaires. Fourteen percent reported that they had used AAS previously, of which four percent were women. Similar results for women were found in Great Britain and the USA [164].

There is a strong likelihood that some of the positive doping tests that have been reported may be as a result of inadvertent doping due to the athlete ingesting a prohibited substance that was present, but not stated, in a nutritional supplement product. An example would be an athlete testing positive for the steroid nandrolone as a result of inadvertent intake via a contaminated supplement [168,169]. In addition to the anabolic steroids other steroid products have appeared on the market that have never been marketed as approved drugs. An example would be numerous xenobiotic compounds that have been derived from

testosterone with the aim that the modification reduces androgenic properties while maintaining the anabolic potential. These products have been withdrawn from the legal market, but have been found to have been added to otherwise inefficient nutritional supplements [170].

Further, scientific investigations and developments since 1980s have highlighted the need for a well-balanced and well-controlled diet to optimise sports performance [215]. Although nutritionists and dieticians usually recommend whole food solutions to these and other problems first, some are starting to accept the use of supplements as an adjunct to nutrition [172,215]. However the marketing strategies of nutritional supplement manufacturers ensure that supplements are frequently presented as ergogenic aids. This means that supplements are intended to improve, or at least support performance in several aspects of physical activity [173,215]. Thus some may argue that using nutritional supplements, and particularly if these are contaminated with banned substances, may create or stimulate a doping "attitude" among consumers of these products.

5.2.2 Drug Effects on Performance and Behaviour

Table 17 represents examples of some drugs which may be included in nutritional supplement products. These drugs may be on the WADA prohibited list and may result in a “positive” doping test. More detailed description of each drug can be found in the references provided.

Table 17. *Illustration of drugs that could be included advertently or inadvertently in nutritional supplements*

Drug or Drug Combination	Drug Indication and Effects	Reference
Caffeine	• xanthine alkaloid that acts as a stimulant • increases lipolysis • no longer on WADA prohibited list. • the dose and frequency of consumption of products containing caffeine may have an effect on the metabolism and elimination of caffeine and other chemical moieties.	117,174-177
Anabolic-androgenic steroids (AAS)	• has the potential to alter myocardial textural parameters. • have anti-catabolic effect, used to increase muscle mass and strength	178,179
Cocaine	• is a stimulant of the central nervous system, an appetite suppressant. • use of such drugs on a daily basis can produce a clear, significant decrease in simple reaction times. • use is also associated with acute and chronic cardiovascular diseases.	180-182
Synephrine (Oxedrine), Octopamine, Tyramine, (sympathommimetic amines) and Caffeine, with St John's wort	• is a drug combination with a traditional medicine supplement. • use has been associated with the cause of acute myocardial infarction.	183
Testosterone	• is the principal male sex hormone biosynthesized from cholesterol. • phase 1 metabolism employs specific enzymes and pathways. • phase II metabolism and excretion follow more general patterns. • the effect is twofold: anabolic effect and androgenic (endogenous nature).	184
Amphetamines	• used in sports, which are characterised by short duration high intensity exercise, with the goal of delaying the onset of fatigue. • is addictive, and chronic abuse causes behavioural change and sometimes psychosis. • the mechanism of action is believed to be the relaxation of smooth muscles via the inhibition of cyclic guanosine monophosphate (GMP).	42,185,186
Sidenafil	• used for the treatment of erectile dysfunction. • at low dosage, the drug may be misconstrued to be safe hence the potential to be experimented with for improving sport or exercise performance. • it is believed the mechanism is the relaxation of smooth muscles via the inhibition of cyclic guanosine monophosphate (GMP).	187
Ephedrine and Ephedra	• ephedrine is classified as a sympathomimetic drug, and central nervous system stimulant, • ability to act as agonist and increase thermogenesis and to reduce fatigue and increase alertness. • used for weight-management or enhanced athletic performance, • the efficacy and safety of these compounds are uncertain • enhanced effectiveness is also coupled with both caffeine and aspirin. Ephedrine stimulates the synthesis of prostaglandins and serves as a prostaglandin blocker, and thereby may prevent inhibition of norepinepherine release	188-192

5.3 Analytical Methods

Laboratory testing and/or screening methods for drugs require analytical techniques and methods that can accomplish the desired multiple chemical compound (drug) separation, selectivity, and sensitivity with appropriate detection capability. Studies where the established analytical techniques of High Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS), and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) have been utilized for drug analysis are presented below.

5.3.1 High Performance Liquid Chromatography (HPLC)

Various High Performance Liquid Chromatography methods have been developed, to measure, as examples, iron chelators, drugs and metabolites in serum, urine and skin. The measurement is through ultraviolet (UV), florescence, or electrochemical detection [193-199]. Other published methods have also been developed for the quantification of ephedrines, and antiretrovirals using High Performance Liquid Chromatography [22, 200-203]. Useful methods have also been developed using preparative techniques measuring drugs from dried blood spots, prior to analysis by liquid chromatography [204].

5.3.2 Gas Chromatography-Mass Spectrometry (GC-MS)

Conventional testing for anabolic steroids has been done using the analytical method Gas Chromatography coupled with Mass Spectrometry [161]. For example, a review paper explaining the determination of Cannabinoids in biological samples, gave special attention to blood and alternative matrices like hair, saliva, sweat and meconium, using Gas Chromatography and Liquid Chromatographic procedures with mass spectrometry or dioarray detection [205]. Gas Chromatography with tandem mass spectrometry has also been used to detect and quantify strychnine in body fluids and tissues [206]. Other techniques in this category include isotope dilution tandem mass spectrometry to improve detection of compounds of interest [207-209, 228].

5.3.3 Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

LC-MS/MS advancement has enabled a more sensitive assay. This has subsequently provided an alternative analytical method to measure low levels of testosterone (e.g. at the low concentrations in women and children), that could previously not be detected and measured reliably by immunoassays methods [210].

Recent proteomics in mass spectrometry has enabled systematic analysis of cellular components in the ubiquitin pathway [211]. Matrix effects should also be taken in consideration when developing screening or validated methods for LC-MS/MS [212-214]. The analytical techniques as presented (5.3.1-5.3.3) have evolved from the use of HPLC with various drug detection systems, to more advanced combination analytical techniques of GC-MS and that of LC-MS/MS. In these techniques the mass spectrophotometer (MS) is used as the drug detection tool on it's own or to enhance specific drug detection using tandem combination techniques as in MS/MS [167,213,228].

5.4 Summary

The use of nutritional supplements has become widespread due to marketing and ease of availability of these products [161]. Supplements were initially used as an adjunct to nutrition, but more recently are being used to promote physical performance through their purported ergogenic properties [173,215]. Nutritional supplements may also contain steroids and stimulants. These may be included intentionally by the manufacturers, without any claims on the label, or inadvertently as a result of contamination. Concern about the efficacy and safety has been raised by the medical fraternity about the misuse of anabolic androgenic steroids (AAS) [78,164,167].

It is thus important to have detectable measures that can test nutritional supplements with specific reference to prohibited steroids and stimulants.

Analytical assay methods, which could serve this purpose, have evolved over time. Such analytical methods include HPLC, GC-MS, LC-MS to LC-MS/MS for various specific applications to achieve desired drug compound separation, selectivity, sensitivity and detection that require investigation [161,200,204, 207, 210, 216]. The analytical instrument technique used for this study was the analytical tool of LC-MS/MS [214].

5.5 Methodology

The analytical technique of Liquid Chromatography-Mass Spectrometry (LC-MS/MS) was used in this study to achieve the aim and objectives described in Chapter 1.This technique was selected because it offers the desired multiple chemical compound (drug) separation, selectivity, and sensitivity with appropriate detection capability. The methodology described below covers, (i) the steroids, stimulants, other compounds investigated in this study, and (ii) the analytical assay methodology for steroids, stimulants, and other selected compounds, using Liquid Chromatography-Mass Spectrometry (LC-MS/MS), and (iii) Nutritional supplement products purchase process.

The first consideration for the selection of compounds investigated in the study (Table 18, 19, 20) was whether they were discussed in the literature and deemed important. Thereafter compounds were considered if the reference compounds required for their analysis could be purchased and imported into South Africa, with the required inter-goverment written permission.

5.5.1 Steroid, Stimulants, and Other Compounds Investigated

Table 18 reflects the name, molecular formula, molecular weight, and chemical structure of steroids which fulfilled the selection criteria and which were investigated in this study.

Table 18. *Steroids investigated*

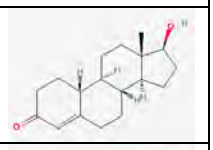
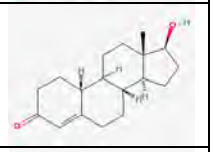
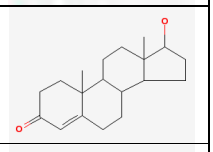
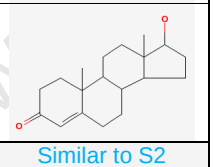
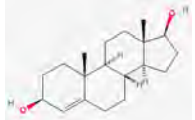
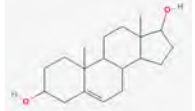
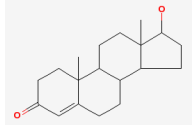
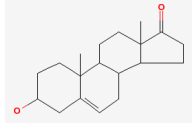
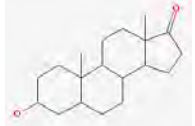
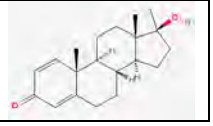
Code	Steroids	Molecular Formula	Molecular Weight (g/mol)	Chemical Structure
S1	17α-19 Nor testosterone	$C_{18}H_{26}O_2$	274.3	
S2	Nandrolone	$C_{18}H_{26}O_2$	274.3	
S3	17α-Testosterone	$C_{19}H_{28}O_2$	288.4	
S4	17ß-Testosterone	$C_{19}H_{28}O_2$	288.4	
S5	19-Nor-4-androstene-3ß, 17ß- diol	$C_{18}H_{28}O_2$	276.4	Similar to S2
S6	19-Nor-5-androstene-3ß, 17ß- diol	$C_{18}H_{28}O_2$	276.4	Similar to S2
S7	19-Nor-5-androstene-3,17 dione	$C_{18}H_{28}O_2$	272.4	Similar to S2
S8	4- Androstene-3ß,17ß- diol	$C_{19}H_{30}O_2$	290.4	
S9	5- Androstene-3ß,17ß- diol	$C_{19}H_{30}O_2$	290.4	
S10	4- Androstene-3,17-dione (AED)	$C_{19}H_{26}O_2$	286.4	Similar to S4
S11	1,4-androstediendione (ADD)	$C_{19}H_{24}O_2$	284.4	Similar to S2
S12	1-Androstedione	$C_{19}H_{26}O_2$	286.4	Similar to S2
S13	1-Testosterone	$C_{19}H_{28}O_2$	288.4	
S14	DHEA- Dehydroepiandrosterone	$C_{19}H_{28}O_2$	288.4	
S15	Epiandrosterone	$C_{19}H_{30}O_2$	290.4	
S16	Methyl- Boldenone	$C_{20}H_{28}O_2$	300.4	

Table 19 reflects the name, molecular formula, molecular weight, and chemical structure of the stimulants investigated.

Table 19. *Stimulants investigated*

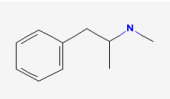
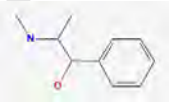
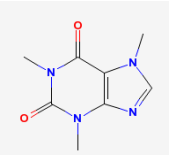
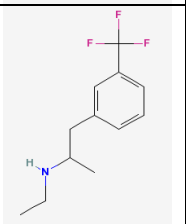
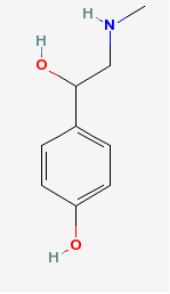
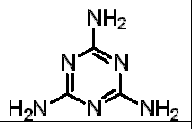
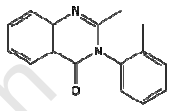
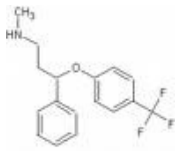
Code	Stimulants	Molecular Formula	Molecular Weight (g/mol)	Chemical Structure
T2	Methyl – amphetamine Commonly known as methamphetamine IUPAC N-methyl-1-phenyl-propan-2-amine	C10H15N	149.2	
T5	Ephedrine IUPAC 2-methylamino-1-phenyl-propan-1-ol	C10H15NO	165.2	
T10	Caffeine IUPAC 1,3,7-trimethylpurine-2,6-dione	C8H10N4O2	194.2	
T13	Fenfluramine IUPAC N-ethyl-1-[3-(trifluoromethyl)phenyl]-propan-2-amine	C12H16F3N	231.2	
T14	Synephrine IUPAC 4-(1-hydroxy-2-methylamino-ethyl)phenol	C9H13NO2	167.2	

Table 20 reflects the name, molecular formula, molecular weight, and chemical structure of other compounds of interest investigated in this study.

Table 20. *Other compounds of interest investigated*

Code	Other compounds of interest	Formula	Molecular Weight (g/mol)	Chemical Structure
O2.	Melamine IUPAC 1,3,5-triazine-2,4,6-triamine	C3H6N6	126.1	
O3.	Methaqualone IUPAC '2-methyl-3-o-tolyl-4(3H)-quinazolinone; 3,4-dihydro-2-methyl-4-oxo-3-o-tolylquinazoline; 2-methyl-3-(2-methylphenyl)-4-(3H)-quinazolinone	C16H14N2O	250.30	
O4.	Prozac (Fluoxetine) IUPAC N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propan-1-amine	C17H18F3NO	309.3	

5.6 Acquisition of Reference Standards and Permits

Steroid reference standards were purchased from Steraloids Incorporated, United States of America, after gaining the necessary import permit authorisation from the South African Government Department of Health (Appendix 6). Stimulants and other reference compounds were purchased from Sigma-Aldrich (Germany).

5.7 Chemical Reference Standard Preparations

Primary standards of the respective steroids, stimulants, and other compounds of interest, were prepared by recording the required weight (to 5 decimal positions) on a Sartorius analytical research electronic balance (R200D). The respective weighed samples were then reconstituted in ethanol for a concentration of 1000 µg/ml, for each of the compounds.

These primary standards were used to prepare secondary standards which were required to optimize the LC-MS/MS assay methodology. The concentration used

for the infusion optimization, through the mass spectrometer source was approximately 300 ng/ml of each of the respective steroids, stimulants and other compounds investigated, individually.

5.8 Liquid Chromatography-Mass Spectrometry Methodology

The South African National Accreditation System (SANAS) accredited laboratory and research support infrastructure in the Division of Clinical Pharmacology – University of Cape Town was used for the investigation of the steroids, stimulants and other compounds of interest in the nutritional supplements. The analytical instrumentation technique of Liquid Chromatography-Mass Spectrometers (LC-MS/MS) was used for the steroid, stimulant, and other compounds of interest investigated. All the experimental work and instrument data capture processing and integration was done by the principal investigator.

In the schematic below (Figure 16) the LC-MS/MS is comprised of a Liquid Chromatography component starting on the far left. This is interfaced into the source component of the Mass Spectrometer that is assembled alongside Quad 1, Quad 2 and Quad 3, with additional components in between, and a detector system to the far right. The quadropoles (quads) consist of four parallel rods (poles) that are equally spaced around a central axis. The principle of operation is that ions are introduced along the axis of the poles. By applying precisely controlled voltages (radio frequency and direct current to opposing sets of poles) a “mass filter” is created. Further, by ramping the voltage on each set of poles a complete range of masses can be passed to the detector. Only ions with a particular mass-to-charge ratio will pass through the filter to be detected at a particular applied voltage.

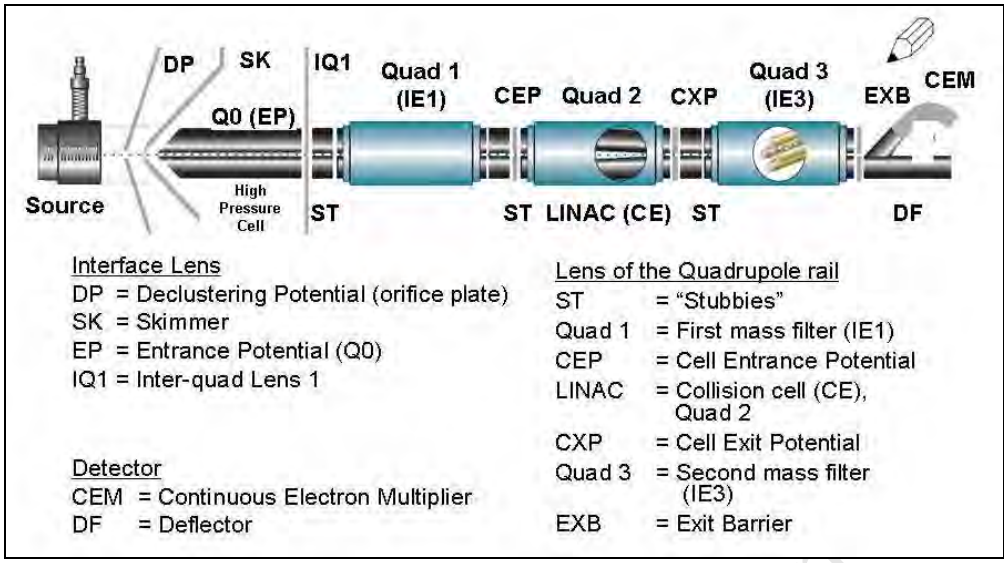


Figure 16. *Schematic diagram of a triple-quad LC-MS/MS instrument (Source Applied Biosystems - www.appliedbiosystems.com)*

5.9 Mass Spectrometry

A sensitive and selective LC- MS/MS assay method was developed to analyze for each of the respective steroids, stimulants, and other compounds of interest as stated in Tables 18, 19 and 20. The approach was to optimize (via infusion) the analytical instrumentation to detect on the Applied Biosystems Sciex API 3200 mass spectrometer for the respective steroids, stimulants and other compounds of interest.

The settings on the Agilent LC 1200 Binary Pump Method were; the minimum pressure (psi) 0.0, maximum pressure (psi) 5801.0, the dead volume (µl) 40.0, maximum flow ramp (ml/min^2) 100.0, maximum pressure ramp (psi/s) 290.0.

The settings on the MS/MS component of the instrument is summarized in Table 21, 23, and 24, and a representative Q1 (MS) and Q3 (MS/MS) spectra are presented in Figure 17-27.

Table 21. *Electronspray ionization settings*

	Steroids	Stimulants	Other Compounds
Curtain Gas (CUR)	20	20	20
Collision Gas (CAD)	5	5	5
Ion spray voltage (IS)	5500	5500	5500
Source temperature (TEMP)	400	475	475
Gas 1 (psi) (GS1)	50	50	50
Gas 2 (psi) (GS2)	60	60	60
Inter Heater (ihe)	On	On	On

For the MS/MS the scan type for steroids, stimulants, and other compounds was MRM, the polarity was in the positive mode, and the pause time was 5 milliseconds (ms) for the steroids, and 5 ms for stimulants, with a total run time of 3.0 minutes for steroids and 8 minutes for stimulants. The MS/MS instrumentation parameters for the steroids are reflected in Table 22, for stimulants in Table 23 and for other compounds of interest Table 24.

Table 22. *MS/MS instrumentation parameters for the steroids*

Code	Steroids	Q1 mass $[M+H]^+$	Q3 mass	Dwell time (ms)	Declustering potential (DP) (Volts)	Entrance potential (EP) (Volts)	Collision cell entrance potential (CEP) (Volts)	Collision energy (CE) (Volts)	Collision cell exit potential (CXP) (Volts)
S1	17α-19 Nor testosterone	275.2	109.1	50	56	4.5	16	39	4.0
S2	Nandrolone	275.2	81.0	50	56	4.5	14	39	4.0
S3	17α-Testosterone	289.2	109.1	50	61	4.5	20	35	4.0
S4	17ß-Testosterone	289.2	109.2	50	66	4.5	16	37	4.0
S5	19-Nor-4-androstene-3ß, 17ß- diol	277.0	216.8	50	51	4.0	14	21	4.0
S6	19-Nor-5-androstene-3ß, 17ß- diol	277.0	217.0	50	51	4.5	14	21	2.0
S7	19-Nor-5-androstene-3,17 dione	273.2	109.0	50	41	7.0	24	35	4.0
S8	4- Androstene-3ß,17ß- diol	293.0	137.0	50	41	5.0	18	35	4.0
S9	5- Androstene-3ß,17ß- diol	293.0	137.0	50	46	7.0	16	35	4.0
S10	4- Androstene-3,17-dione (AED)	287.2	97.1	50	51	7.0	16	31	4.0
S11	1,4-androstediendione (ADD)	285.2	121.1	50	41	11.0	20	29	4.0
S12	1-Androstedione	287.2	121.1	50	41	4.5	14	29	4.0
S13	1-Testosterone	289.2	109.1	50	51	4.0	24	35	4.0
S14	DHEA- Dehydroepiandrosterone	289.2	253.1	50	31	9.0	16	17	6.0
S15	Epiandrosterone	273.2	161.1	50	56	4.5	16	25	4.0
S16	Methyl- Boldenone	301.3	121.1	50	36	4.5	18	33	4.0

** an explanation of the coding is shown in Table 18*

Table 23. *MS/MS instrumentation parameters for the stimulants*

Code	Stimulants	Q1 mass $[M+H]^+$	Q3 mass	Dwell time (ms)	Declustering potential (DP) (Volts)	Entrance potential (EP) (Volts)	Collision cell entrance potential (CEP) (Volts)	Collision energy (CE) (Volts)	Collision cell exit potential (CXP) (Volts)
T5	Ephedrine	166.2	133.1	150	21	3.5	12	29	4
T6	Ephedrine	166.2	133.1	150	21	5.0	12	27	4
T10	Caffeine	195.1	138.1	150	51	5.0	12	27	4
T14	Synephrine	168.2	135.0	150	16	3.0	10	27	4
T2	Methyl – amphetamine	150.2	91.1	150	26	4.0	10	27	4
T2	Methyl – amphetamine	150.2	119.0	150	26	4.0	10	15	4
T13	Fenfluramine	232.2	159.1	150	36	5.5	14	31	4
T13	Fenfluramine	232.2	109.1	150	36	5.5	14	55	4

** an explanation of the coding is shown in Table 19*

Table 24. *Other compounds of interest*

Code		Q1 mass $[M+H]^+$	Q3 mass	Dwell time (ms)	Declustering potential (DP) (Volts)	Entrance potential (EP) (Volts)	Collision cell entrance potential (CEP) (Volts)	Collision energy (CE) (Volts)	Collision cell exit potential (CXP) (Volts)
O2	Melamine	127.1	68.1	150	41	9.5	10	37	4
O3	Methaqualone	251.2	132.1	150	61	5.5	16	37	4
O3	Methaqualone	251.2	91.0	150	61	6.5	16	53	4
O4	Prozac (Fluoxetine)	310.2	148.0	150	26	6.5	14	13	4
O4	Prozac (Fluoxetine)	310.2	117.2	150	26	6.5	14	69	4

** an explanation of the coding is shown in Table 20*

Figures 17–22 below represents Q1 and Q3 MS/MS spectra of selected steroids Nandrolone, Testosterone, Androstene, DHEA, Epiandrosterone and Methyl-Boldenone. For figures 17-27 the vertical (Y) axis has the measure of intensity (cps), and the horizontal (X) axis the m/z (amu). The display is to provide an illustration of MS/MS specific Q1 and Q3, respectively.

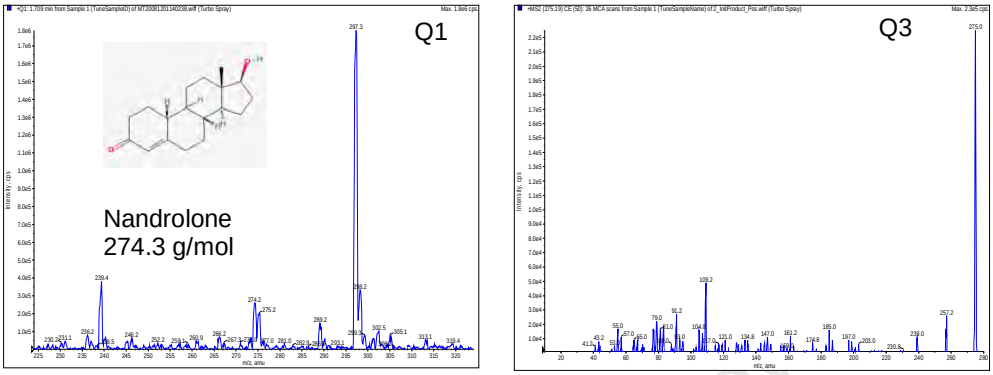


Figure 17. *Representative Q1 and Q3 Spectra of steroid Nandrolone*

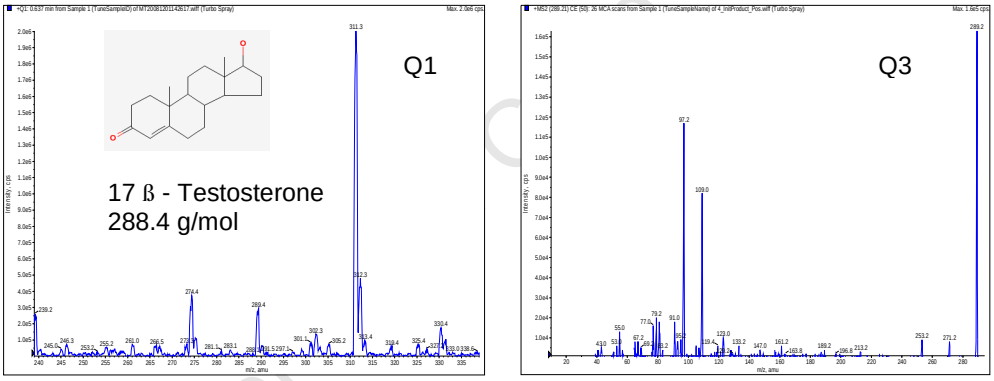


Figure 18. *Representative Q1 and Q3 Spectra of steroid 17 ß - Testosterone*

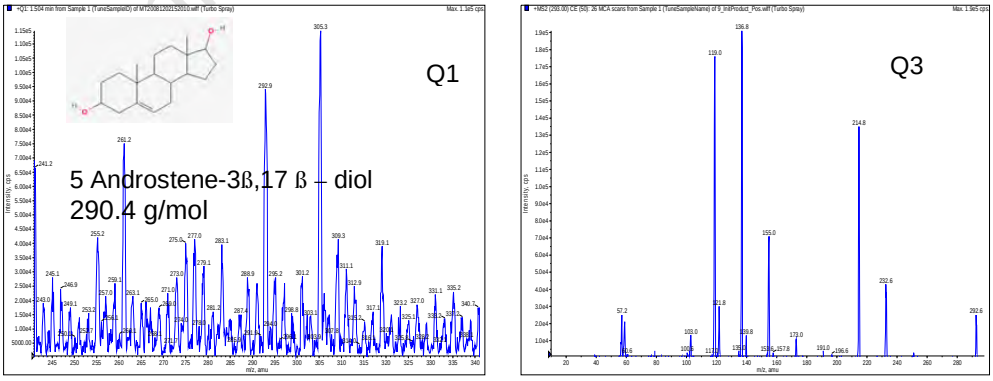


Figure 19. *Representative Q1 and Q3 Spectra of steroid 5 Androstene-3ß,17 ß – diol*

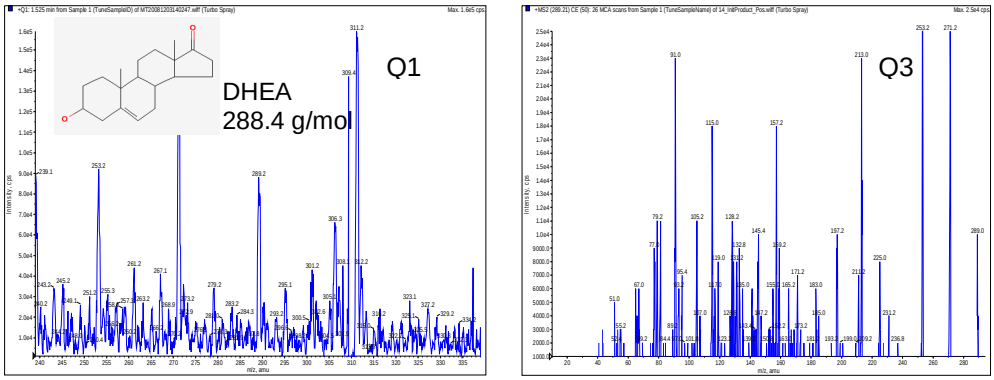


Figure 20. *Representative Q1 and Q3 Spectra of steroid DHEA*

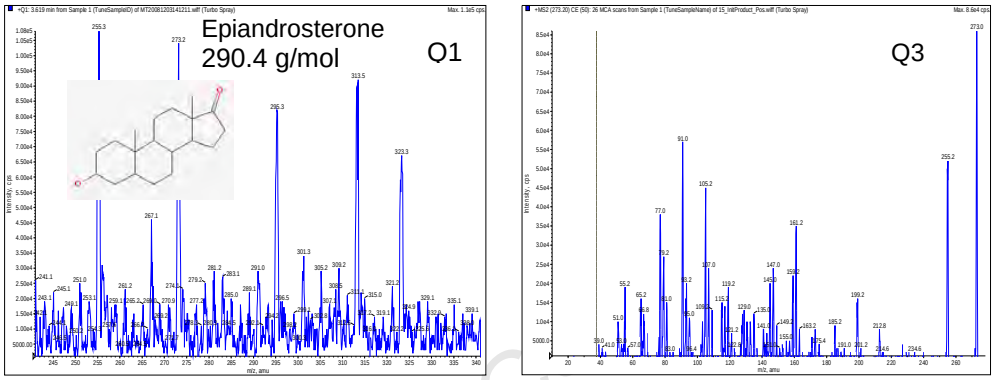


Figure 21. *Representative Q1 and Q3 Spectra of steroid Epiandrosterone*

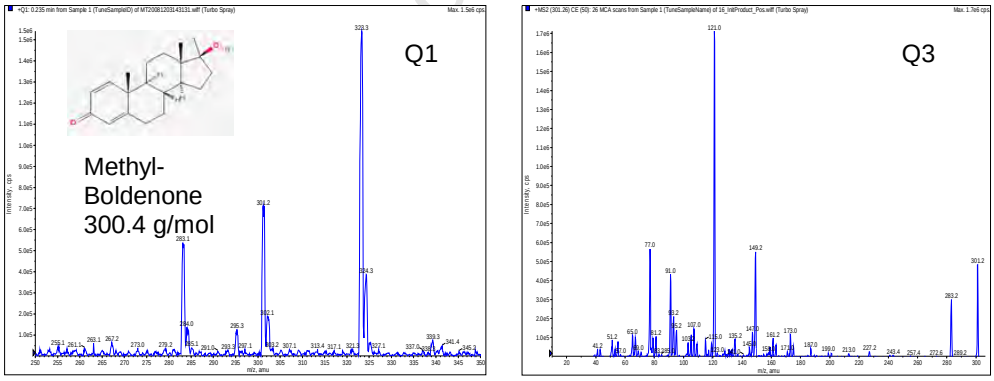


Figure 22. *Representative Q1 and Q3 Spectra of steroid Methyl- Boldenone*

Figures 23-27 below are representative Q1 and Q3 MS/MS spectra of selected stimulants, Ephedrine, Caffeine, Synephrine, Nor-ephedrine, pseudo-ephedrine.

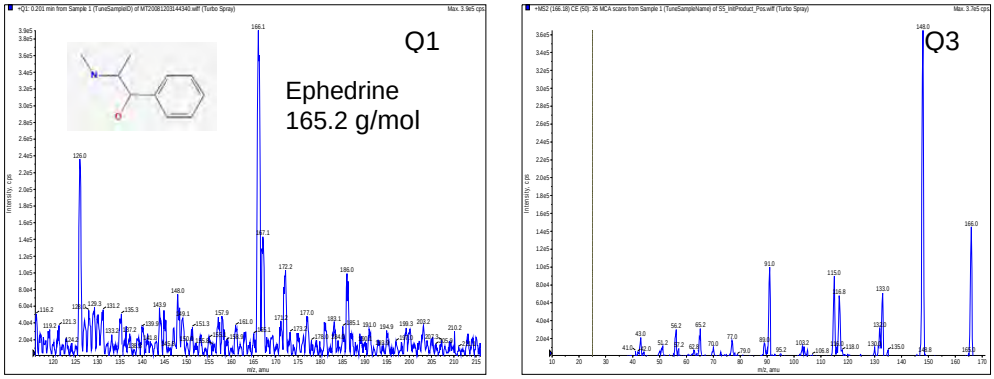


Figure 23. *Representative Q1 and Q3 Spectra of stimulant Ephedrine*

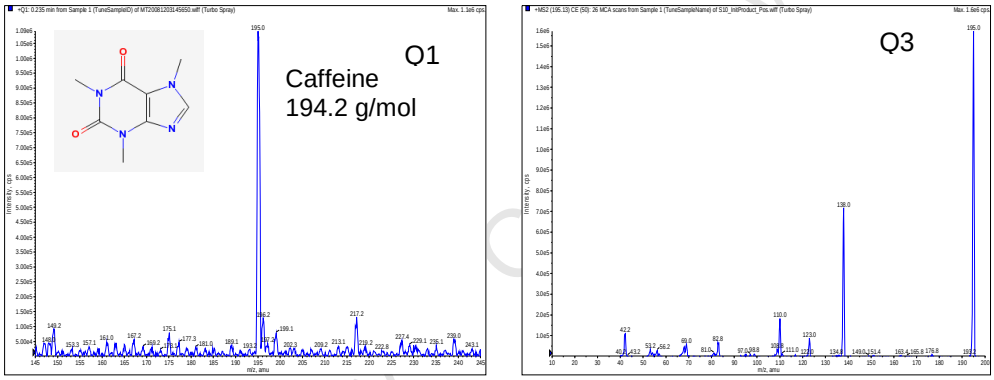


Figure 24. *Representative Q1 and Q3 Spectra of stimulant Caffeine*

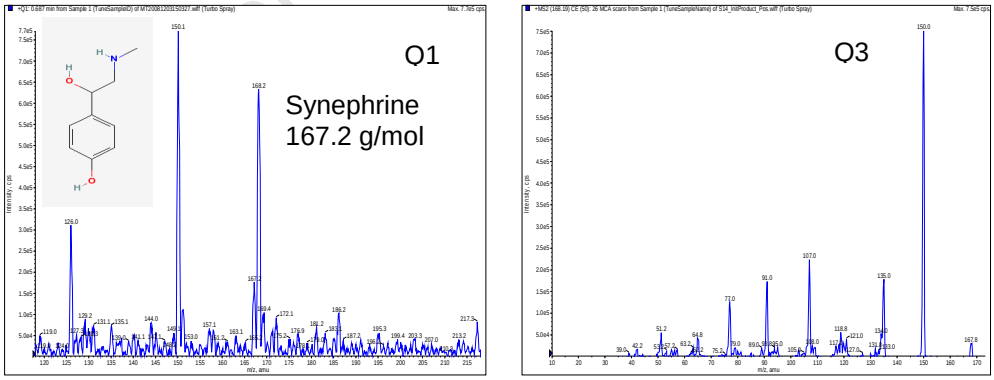


Figure 25. *Representative Q1 and Q3 Spectra of stimulant Synephrine*

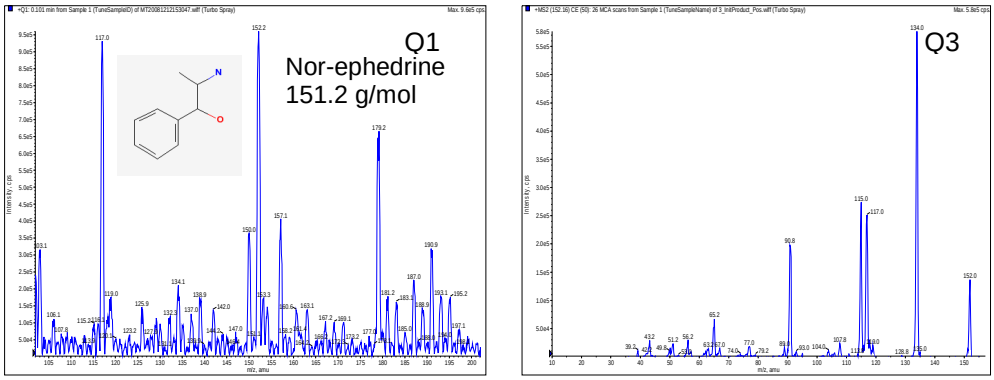


Figure 26. *Representative Q1 and Q3 Spectra of stimulant Nor-ephedrine*

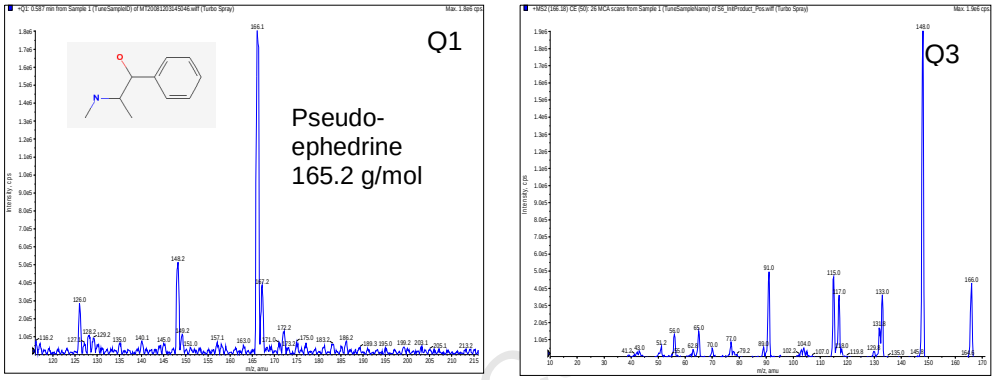


Figure 27. *Representative Q1 and Q3 Spectra of stimulant Pseudo-ephedrine*

The Chromatography method utilized prior to MS/MS detection, was performed using an Agilent 1200 series HPLC with a Phenomenex Gemini C18 NX (5µm, 110A, 50x2 mm) analytical column. The mobile phase consisted of Acetonitrile: 0.1% Formic acid (1:1, v/v) and was delivered at a constant flow rate of 0.5 ml/min. The column was kept in a column compartment at 35 °C [19, 78].

An autosampler injected 5 µl of sample onto the HPLC column. The injection needle was rinsed with mobile phase before each injection for 10 seconds using the flush port wash station. The samples were cooled to 5 °C while awaiting injection.

5.10 Chromatographs

Figures 28-31 below are representative chromatographs of selected steroids, stimulants and other compounds of interest at 125 ng/ml, and 5 µl and 20 µl injection volumes respectively. The vertical axis represents the arbitrary intensity values and the horizontal axis the retention time in minutes of respective chromatographic compounds.

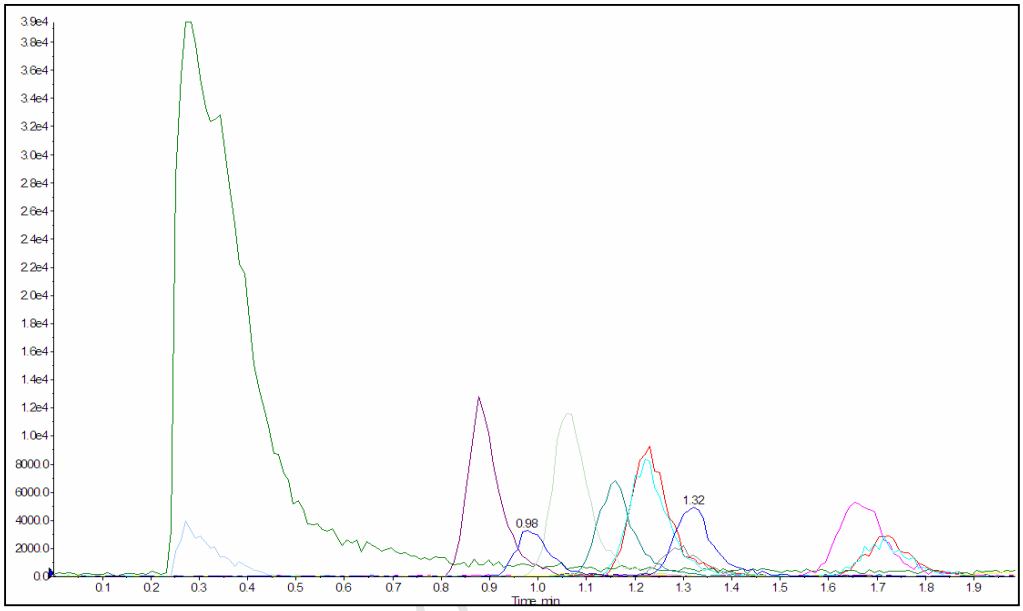


Figure 28. *Steroid chromatograph at 125 ng/ml (5µl injection)*

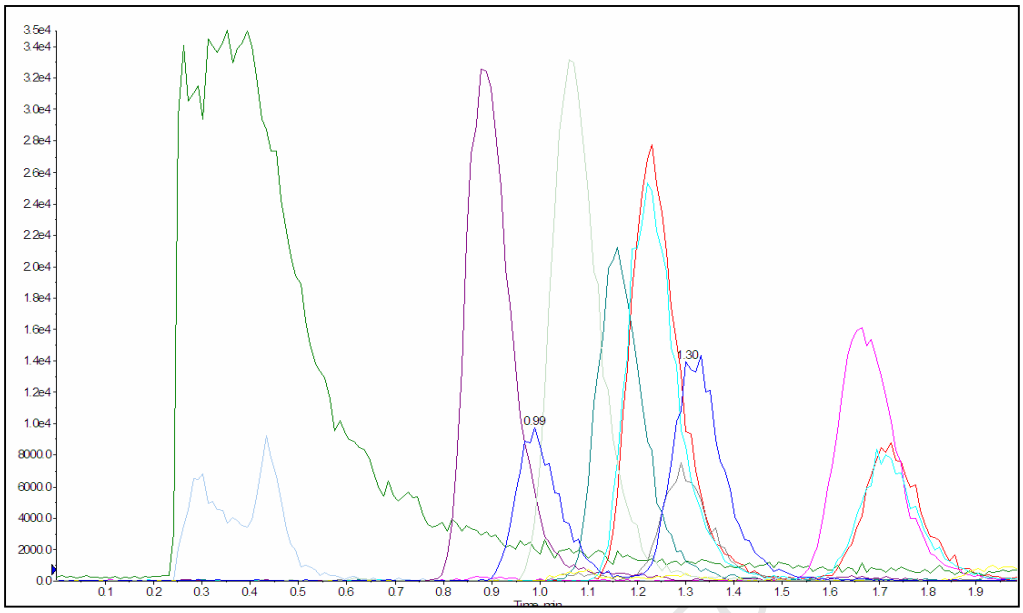


Figure 29. *Steroid chromatograph at 125 ng/ml (20 µl injection)*

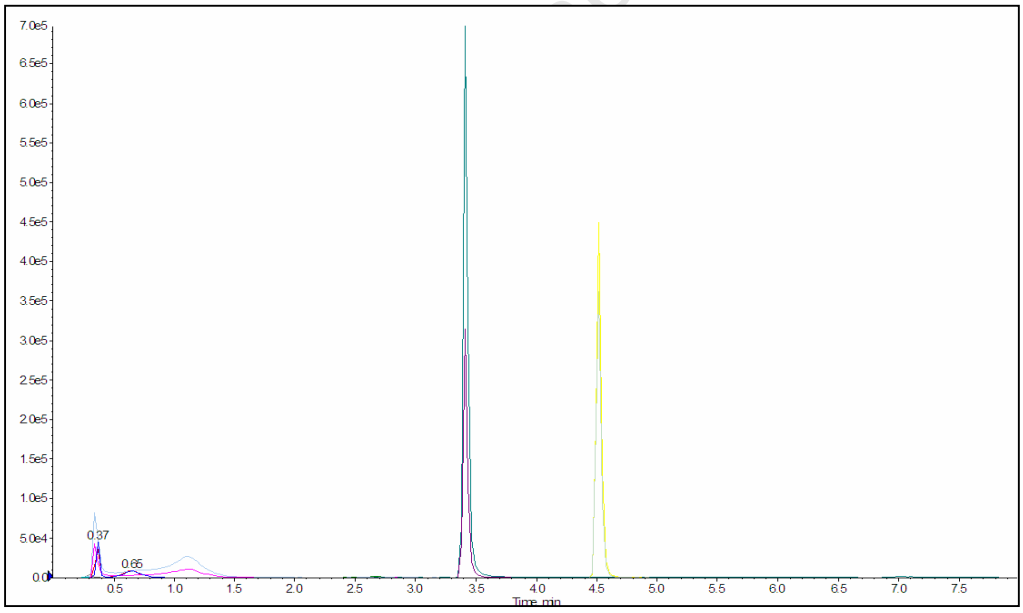


Figure 30. *Stimulants, (and other) chromatograph at 125 ng/ml (5 µl injection)*

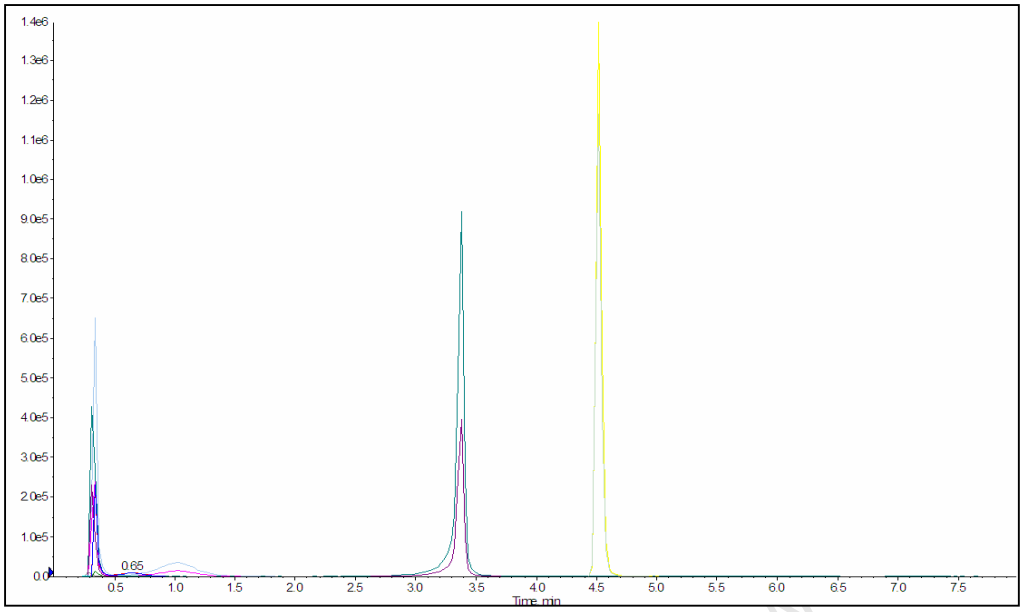


Figure 31. *Stimulants, (and other) chromatograph at 125 ng/ml (20 µl injection)*

5.11 Serial Dilution to Establish Calibration Curves

Figures 32-35 below are representative calibration curves of selected steroids, stimulants and other compounds of interest. The calibration range was established as a combined mixture of the investigated steroids and stimulants and other compounds of interest. Serial dilution using a prepared mixture of acetonitrile:0.1% formic acid (1:1) to establish concentrations starting from 1000 ng/ml to 1.953 ng/ml to establish the ng/ml range 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95.

The calibration analysis (primary and secondary stocks) was verified by repeat analysis in three cycles at both 5 µl and 20 µl, respectively to determine which volume would provide best results for the HPLC column configuration.

These primary and secondary stocks were stored in freezers at – 80 ^{o}C, and used in the sample batch analysis to serve as a system test, and establish calibration curves to determine estimation concentration in nutritional supplements.

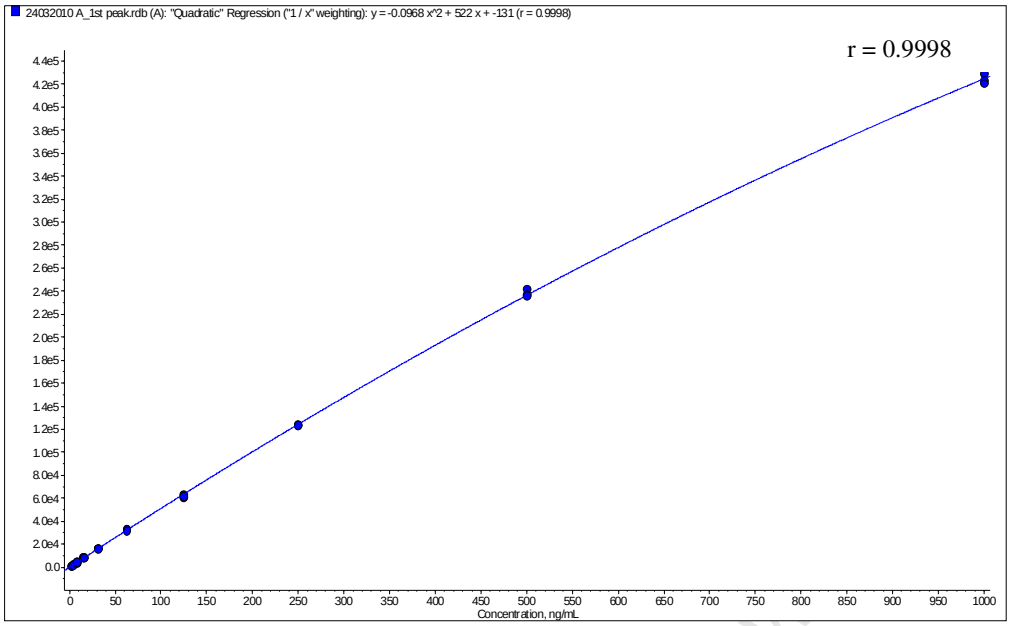


Figure 32. Calibration curve of steroid Testosterone 275.2/109.1 amu (n=3)

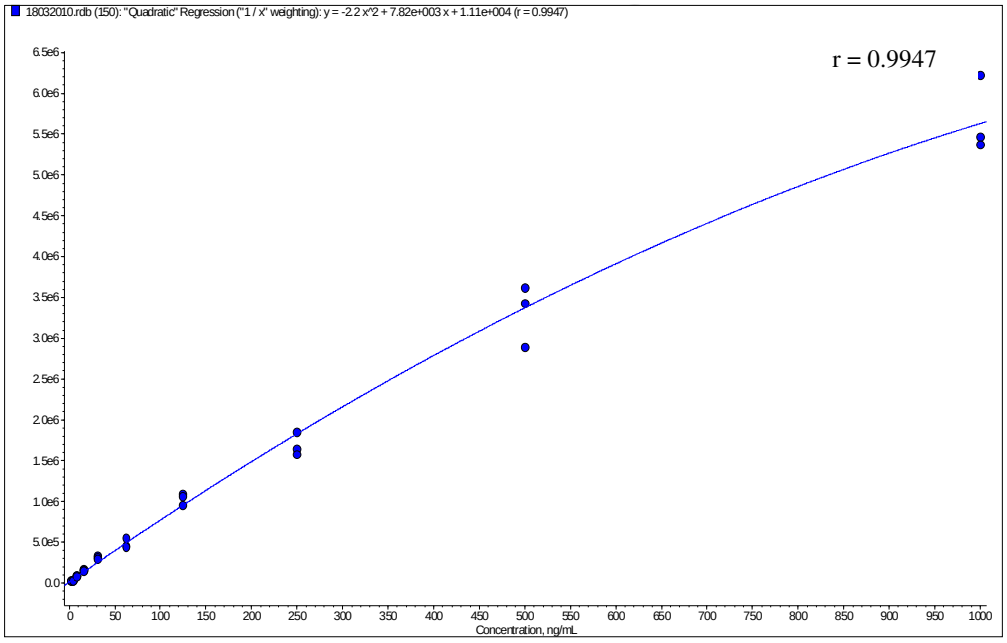


Figure 33. *Calibration curve of stimulant Fenfluramine 232.2/159.1 amu (n=3)*

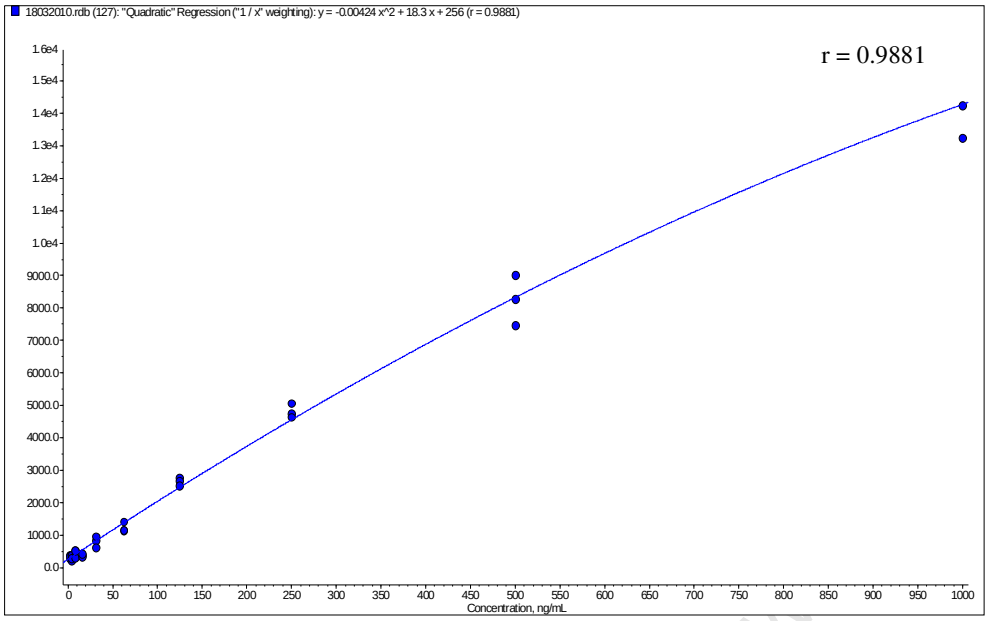


Figure 34. *Calibration curve of Melamine 127.1/68.1 amu (n=3)*

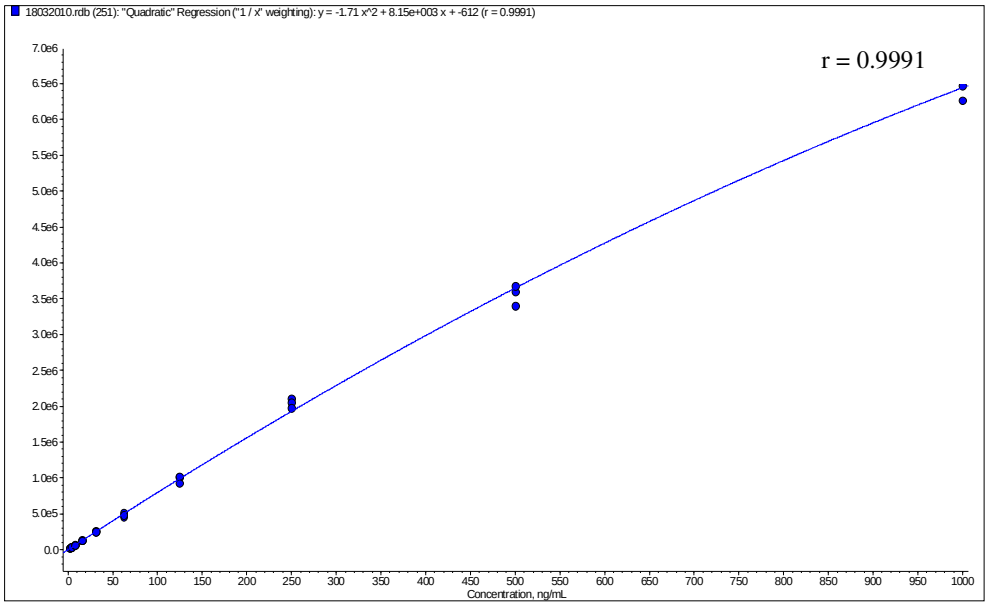


Figure 35. *Calibration curve of Methaqualone 251.2/132.1 amu (n=3)*

5.12 Nutritional Supplement Product Acquisition and Processing

The sample consisted of 138 nutritional supplements obtained from;

1. The returned questionnaire survey information based on 128 returned questionnaires from elite athletes (independent to this thesis) (unpublished research). Popular nutritional supplement brands that were consumed by

the study cohort assisted the process of sample acquisition as presented in point 2 below.

2. Direct purchases from shops, pharmacies and outlets which sold nutritional supplements. These supplements were either manufactured or assembled locally or were internationally imported brands. (n = 82)
3. The users of nutritional supplements. The samples were solicited through an active marketing campaign. (n = 41)
4. Suppliers, manufacturers and distributors of nutritional supplement products, and who were willing to supply nutritional supplement sample products for the research study. (n =15)

5.13 Nutritional Supplement Sample Storage and Management

Samples (n = 138) were stored in the dark, at ambient temperature in glass vials or as provided in the original product container, and appropriately labelled. For the nutritional supplement database register the following information was captured for record keeping and management of samples for laboratory analysis; (i) date when product/sample was obtained, (ii) provider of product/sample, (iii) contact details, (iv) product name, (v) product batch number, if available, (vi) other information that would be relevant to the product, and (vii) product study code.

5.14 Supplement sample formulation

Table 25 provides the breakdown of the 138 supplement samples tested. Supplements in the form of powders were the most common samples (39%).

Table 25. *Nutritional supplement formulation types*

Code	Type	n	%
P	Powder	54	39
P/T	Tablet ground to powder	42	30
P/C	Powder in capsule	30	22
L/C	Liquid in capsule	8	6
L	Liquid	4	3
Total		***138***	***100***

5.15 Sample Preparation for Assay Analysis for Steroids, Stimulants and Other Compounds

The sample preparation was in accordance with the requirements for the different formulations (Table 25). Depending on the formulation the required weight or volume (weighed) of sample was obtained using a Sartorius (R200D) analytical balance (Sartorius - Germany). The sample was then subjected to the necessary extraction procedure (see below). All reagent solvents used for extraction were Analytical Grade or LiChrosolv© quality.

5.16 Calibration standards and sample extraction methodology

5.16.1 Steroids

Figure 36 represents the extraction efficiency for triplicate samples (n=3) for the organic solvents Tertiary Butyl Methyl Ether (TBME), Diethyl Ether and 1-Chlorobutane respectively. The best extraction recovery was 74% for 1-Chlorobutane, 70% for TBME and 67% Diethyl Ether. Diethyl Ether gave the overall best solvent extraction recovery for the respective steroids that was investigated in this study.

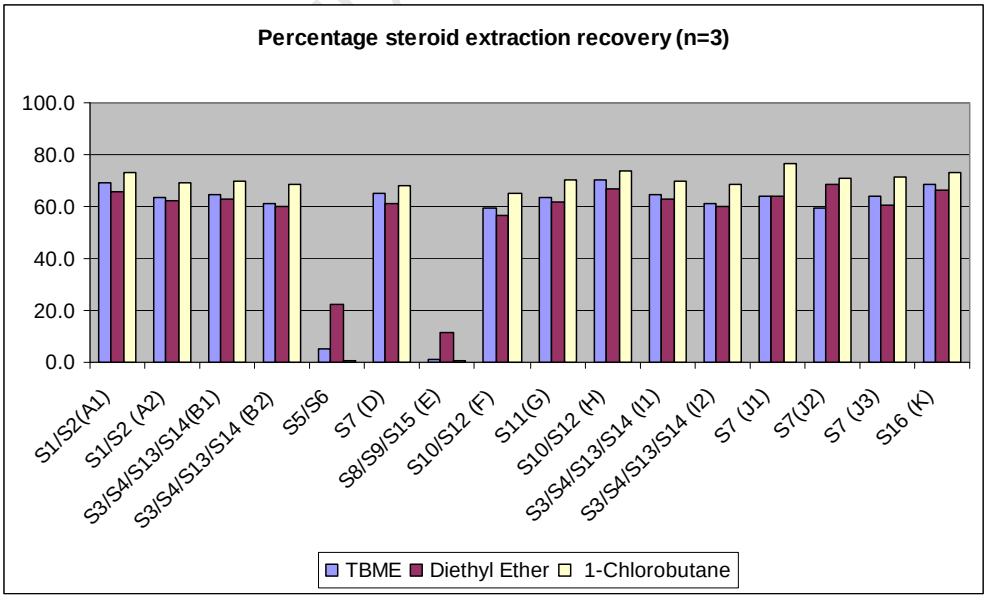


Figure 36. *Organic solvent tested for extraction recovery of steroids*

The specific extraction methodology for calibration curve standards and samples prior to analysis was thus as follows;

The extraction procedure for standards and samples were prepared on ice. Duplicate standards at each specific concentration were equally dispersed across the required concentration range.

- 200 µl of each prepared standard was pipetted and transferred into a 5 ml polypropylene tube. For the samples that were being tested, the accurately weighed amount of sample was mixed with 5 ml of Methanol in a 15 ml polypropylene tube. The samples were then vortexed vigorously for 1 minute. Then 200 µl of this mixture was used for the extraction procedure.
- 1 ml of the Diethyl Ether was added to all standards and samples, and vortexed vigorously for 1 minute.
- The standards and samples were then centrifuged at 5°C at a high speed (2500 rpm) for 5 minutes. Separation in the sample tube consisted of two phases, (i) organic layer (Diethyl Ether) as the upper layer, and (ii) the non-organic layer located below the organic content in the tube.
- Liquid nitrogen was used to freeze the bottom non-organic layer (standards and samples), thereby allowing quantitatively the transfer of the organic layer into clean 5 ml polypropylene tubes.
- The standards and sample were then evaporated in a nitrogen purge concentrator (Stuart sample concentrator –SBH CONC/1) at ambient temperature for 1 hour.
- The dried standards and samples were then reconstituted with 200 µl mobile phase (1:1) Acetonitrile and 0.1% Formic Acid
- Standards and samples were then vortexed for 30 seconds and then subjected to pipette transfer to 96 well polypropylene plates.
- The 96 well plate containing the standards and samples was then centrifuged at 5°C at 2500 rpm for 5 minutes.
- 20 µl was injected onto the HPLC column for analysis.

5.16.2 Stimulants and other drugs

Figure 37 represents the extraction efficiency for triplicate samples (n=3) for the organic solvents TBME, Diethyl Ether and 1- Chlorobutane respectively. The best extraction recovery was 108% for TBME, 102% for Diethyl Ether and 76% for 1-Chlorobutane. TBME gave the overall best solvent extraction recovery for the respective stimulants and other drugs that were investigated in this study.

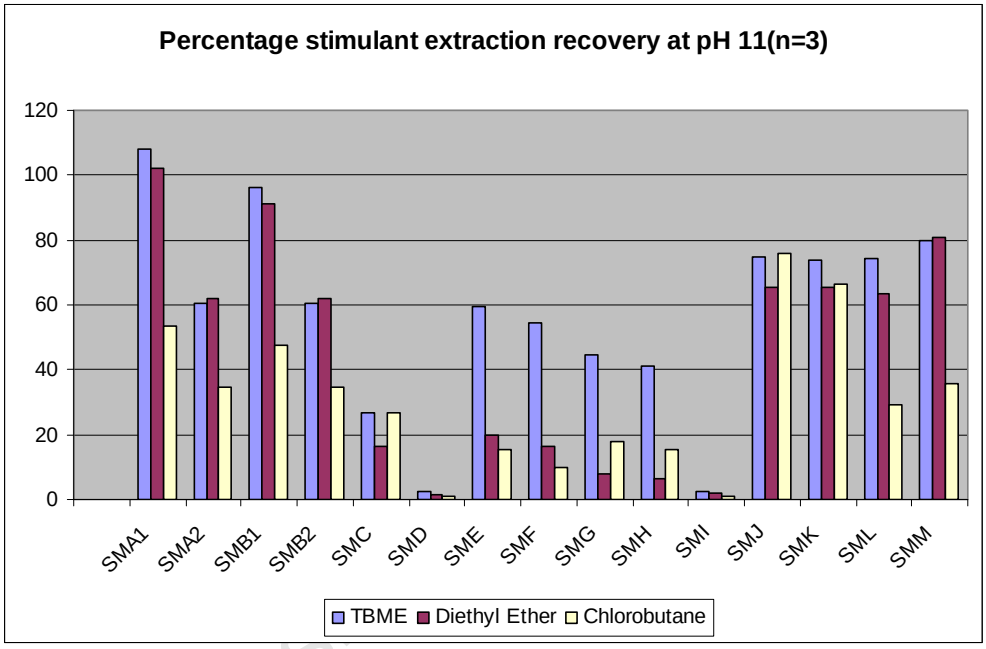


Figure 37. *Organic solvent tested for extraction recovery of stimulants*

- 200 µl of each prepared standards was pipetted and transferred into a 5 ml polypropylene tube. Each sample that tested was accurately weighed and was mixed with 5 ml of Methanol in a 15 ml polypropylene tube. The samples were then vortexed vigorously for 1 minute and then 200 µl of this mixture was used for the extraction procedure.
- 200 µl of Britton Robinson universal buffer (0.1 M, pH 11) was added to all standards and samples.
- 1 ml of the TBME was added to all standards and samples, and vortexed for 1 minute, vigorously.
- The standards and samples were then centrifuged at 5° C at a high speed (2500 rpm) for 5 minutes. This ensured good separation of the organic layer TBME. Separation in the sample tube consisted of two phases, (i)

organic layer (TBME) as the upper layer, and (ii) the non-organic layer located below the organic content in the tube.

- Liquid nitrogen was used to freeze the bottom non-organic layer (standards and samples). This allowed the organic layer to be transferred to clean 5ml polypropylene tubes.
- The standards and sample were then evaporated in a nitrogen purge concentrator (Stuart sample concentrator –SBH CONC/1) at ambient temperature for 1 hour.
- The dried standards and samples were then reconstituted with 200 µl (1:1) mobile phase Acetonitrile and 0.1% Formic Acid.
- Standards and samples were then vortexed for 30 seconds and then subjected to pipette transfer to 96 well polypropylene plates
- The 96 well plate containing the standards and samples was then centrifuged at 5° C at 2500 rpm for 5 minutes.
- 20 µl was injected onto the HPLC column for analysis.

5.17 Assay analysis approach methodology

The analysis of samples for the respective three categories (i.e. steroid, stimulants and other compounds), followed the steps below;

(1) Complete screen using LC-MS/MS detection for the assessment of the defined steroids and stimulants and other compounds of interest investigated.

(2) "Positive" tested supplements were then identified through the initial screen.

(3) The concentration (of contamination/ adulterant) was then estimated via calibration curve standards that formed part of the extraction and analysis run.

5.18 Statistical analysis and evaluation methodology

Upon completion of data capture and analysis the following computer-based programmes were used for the statistical analysis and graphic representation; Windows-based Microsoft ® Office Excel 2003 SP 1 (Excel © 1985-2003 Microsoft Corporation) and GraphPad Prism® Version 2.01 (GraphPad Software© 1994, 1995, 1996 GraphPad Software, Inc). Descriptive data were calculated as percentage positive samples, and the estimated concentration, median concentration, lowest limit of quantification (LLOQ) were determined.

5.19 Results and Discussion

Figure 38 is a representative illustration (eg. Steroid 1= 17α-19 Nor testosterone) of a bar plot overview of nutritional supplement samples investigated that tested positive for a given steroid, with estimate concentration in ng/g of sample. The tested sample was defined as positive when the result was greater than the lower limit of quantification (LLOQ) as presented in Table 26.

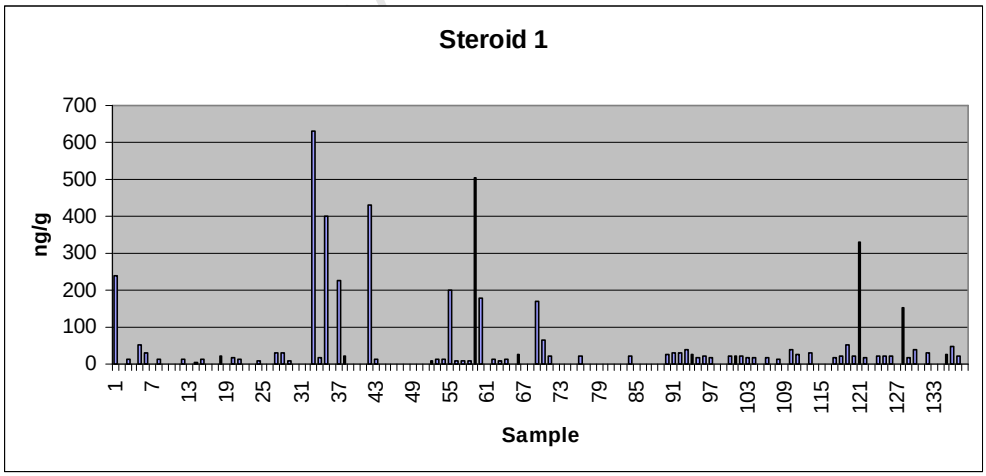


Figure 38. *Plot of samples that tested "positive" for steroids*

Figure 39 is a representative illustration (Stimulant 3 = Caffeine) of a bar plot overview of nutritional supplements samples investigated that tested positive for a given stimulant, with estimate concentration in ng/g of sample.

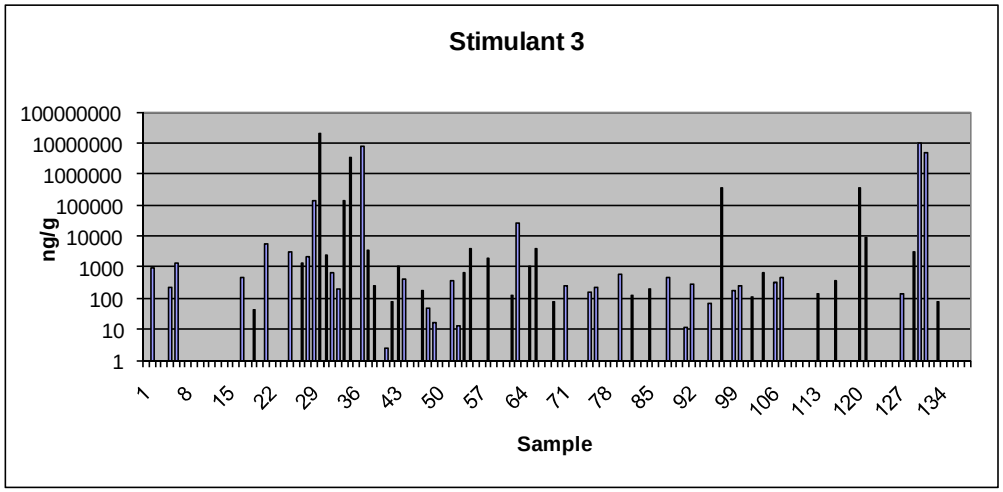


Figure 39. *Plot of samples that tested "positive" for stimulants*

5.20 Steroids

5.20.1 General overview of samples tested for steroids

Table 26, 27, 28 for steroids and Table 29, 30, 31 for stimulants and other compounds should be read and viewed as follows. The structure from left to right of each table consist of (first line of Table 26 used as example), (a) the steroid or stimulant (other compound) eg. S1/S2, (b) the MS/MS compound transition eg. 275.2/109.1, (c) Retention time in minutes of compound(s) eg. 1.02, (d) Total number of "positives" eg. 74, (e) Percentage of "positives" of the total 138 samples eg. 54%, (f) Lower limit of quantification (LLOQ) in ng/ml and ng/g equivalent in sample, (g) the percentage "positives" above and below LLOQ eg. 61 and 39 respectively, (h) the estimate concentration range ≥LLOQ eg. 19.65-630.41 ng/g, and the median concentration of the specific "positives" eg 31.01 ng/g. The two examples cited in the narrative describing each of the tables 26, 27, 28, 29, 30, and 31 are highlighted in blue in the steroid, and stimulant code column.

Table 26 provides a qualitative summary of all the samples (Table 25; n = 138) Steroid S7, 22 samples (16%) and (ii) Steroids S10/S12 118 samples (86%) based on overall qualitative assessment.

When sub-grouping the qualitative results (Table 26) in the examples (S7 and S10/S12) into "positive" tested samples greater than and equal to the lower limit of quantification (≥LLOQ) for S7, 55% (12) of the "positive" tested samples were ≥LLOQ and 45% (10) < LLOQ. For S7 the estimate concentration range was 19.42-149.37 ng/g with a median value of 55.04 ng/g. For S10/S12 this was ≥LLOQ 40% (47) and < LLOQ 60% (71). The S10/S12 estimate concentration range was 19.60-636.81 ng/g, with a median value of 34.38 ng/g.

Table 26. *Summary of Nutritional Supplement products investigated for steroids*

			Qualitative Assessment Screen								Estimate Concentration Range	
Steroid Code	Compound Transition	RT min.	No. Total	% Positive	LLOQ (ng/ml)	LLOQ (ng/g)	No. ≥ LLOQ	(%)	No. < LLOQ	(%)	≥ LLOQ (ng/g)	Median (ng/g)
S1/S2	275.2/109.1	1.02	74	54	1.95	19	45	61	29	39	19.65 - 630.41	31.01
S1/S2	275.2/109.1	1.36	42	30	1.95	19	21	50	21	50	19.13 - 579.28	25.85
S3/S4/S13/S14	289.2/109.1	1.27	31	22	1.95	19	13	42	18	58	19.60 - 584.80	43.09
S3/S4/S13/S14	289.2/109.1	1.76	85	62	1.95	19	32	38	53	62	19.34 - 88.92	25.79
S5/S6	277.0/216.8	0.30	44	32	3.91	39	40	91	4	9	53.18 - 316788.87	299.28
S7	273.2/109.0	1.33	22	16	1.95	19	12	55	10	45	19.42 - 149.37	55.04
S8/S9/S15	293.0/137.0	0.35	48	35	15.63	156	21	44	27	56	174.67 - 6603.40	504.73
S10/S12	287.2/97.1	1.71	53	38	1.95	19	7	13	46	87	28.17 - 655.09	125.10
S11	285.2/121.1	1.19	62	45	1.95	19	10	16	52	84	22.92 - 24331.91	166.46
S10/S12	287.2/121.1	0.92	118	86	1.95	19	47	40	71	60	19.60 - 636.81	34.38
S3/S4/S13/S14	289.2/109.1	1.27	34	25	1.95	19	16	47	18	53	20.92 - 689.62	28.19
S3/S4/S13/S14	289.2/109.1	1.77	68	49	1.95	19	5	7	63	93	20.20 - 700.65	21.54
S7	273.2/161.1	1.14	48	35	1.95	19	42	88	6	13	19.07 - 10682.16	190.39
S7	273.2/161.1	1.33	47	34	7.81	78	35	74	12	26	82.03 - 7842.95	246.68
S7	273.2/161.1	2.01	71	51	7.81	78	60	85	11	15	80.48 - 5453.52	192.00
S16	301.3/121.1	1.11	33	24	1.95	19	6	18	27	82	24.44 - 142.98	57.43

** Examples (a) = S7 and (b) =S10/S12*

5.20.2 South African Produced Nutritional Supplements

Table 27 provides a qualitative summary of the samples which were locally produced or assembled (n=27). Two examples are used to make the illustration (*), (i) for Steroids S3/S4/S13/S14, 4 samples (15%) and (ii) Steroids S10/S12 24 samples (89%).

When sub-grouping the qualitative results (Table 27) into "positive" tested samples greater than and equal to the lower limit of quantification (≥LLOQ) for S3/S4/S13/S14, 25% (1) "positive" tested sample was ≥LLOQ and 75% (3) < LLOQ. For S3/S4/S13/S14 the estimate concentration was 49.81 ng/g with a median value of 49.81 ng/g. For S10/S12 this was ≥LLOQ 42% (10) and < LLOQ 58% (14). The S10/S12 estimate concentration range was 20.20-315.19 ng/g, with a median value of 47.94 ng/g.

Table 27. *South African nutritional supplements investigated for steroids*

			Qualitative Assessment Screen								Estimate Concentration Range	
Steroid Code	Compound Transition	RT min.	No.	% Positive	LLOQ (ng/ml)	LLOQ (ng/g)	No. ≥ LLOQ	(%)	No. < LLOQ	(%)	≥ LLOQ (ng/g)	Median (ng/g)
S1/S2	275.2/109.1	1.02	17	63	1.95	19	13	76	4	24	19.65 - 630.41	32.54
S1/S2	275.2/109.1	1.36	12	44	1.95	19	8	67	4	33	19.20 - 82.39	23.50
S3/S4/S13/S14	289.2/109.1	1.27	4	15	1.95	19	1	25	3	75	49.81 - 49.81	49.81
S3/S4/S13/S14	289.2/109.1	1.76	18	67	1.95	19	11	61	7	39	19.34 - 64.96	27.35
S5/S6	277.0/216.8	0.30	4	15	3.91	39	4	100	0	0	191.97 - 316788.87	672.71
S7	273.2/109.0	1.33	8	30	1.95	19	6	75	2	25	30.59 - 149.37	74.88
S8/S9/S15	293.0/137.0	0.35	10	37	15.63	156	5	50	5	50	174.67 - 1171.47	374.48
S10/S12	287.2/97.1	1.71	9	33	1.95	19	1	11	8	89	125.10 - 125.10	125.10
S11	285.2/121.1	1.19	11	41	1.95	19	2	18	9	82	252.06 - 1377.25	814.66
S10/S12	287.2/121.1	0.92	24	89	1.95	19	10	42	14	58	20.20 - 315.19	47.94
S3/S4/S13/S14	289.2/109.1	1.27	11	41	1.95	19	4	36	7	64	20.92 - 48.84	26.58
S3/S4/S13/S14	289.2/109.1	1.77	16	59	1.95	19	2	13	14	88	21.14 - 21.57	21.35
S7	273.2/161.1	1.14	9	33	1.95	19	8	89	1	11	163.32 - 10682.16	1218.53
S7	273.2/161.1	1.33	10	37	7.81	78	7	70	3	30	390.21 - 7842.95	2001.15
S7	273.2/161.1	2.01	16	59	7.81	78	15	94	1	6	95.48 - 5453.52	256.61
S16	301.3/121.1	1.11	6	22	1.95	19	1	17	5	83	48.63 - 48.63	48.63

** Examples (a) = S7 and (b) =S10/S12*

5.20.3 South African Purchased Imported Nutritional Supplements

Table 28 provides a qualitative summary of all the samples tested for steroids (n=50) that were imported into South Africa. Two examples are used to make the illustration (*), (i) for Steroid S7, 6 samples (12%) and (ii) Steroids S10/S12 45 samples (90%). When sub-grouping the qualitative results (Table 28) into "positive" tested samples greater than and equal to the lower limit of quantification (≥LLOQ) for S7, 50% (3) of the "positive" tested samples were ≥LLOQ and 50% (3) < LLOQ. For S7 the estimate concentration range was 20.16-78.06 ng/g with a median value of 50.07 ng/g. For S10/S12 this was ≥LLOQ 44% (20) and < LLOQ 56% (25). The S10/S12 estimate concentration range was 19.67-509.64 ng/g, with a median value of 19.88 ng/g.

Table 28. *Imported nutritional supplement products investigated for steroids*

			Qualitative Assessment Screen								Estimate Concentration Range	
Steroid Code	Compound Transition	RT min.	No.	% Positive	LLOQ (ng/ml)	LLOQ (ng/g)	No. ≥ LLOQ	(%)	No. < LLOQ	(%)	≥ LLOQ (ng/g)	Median (ng/g)
S1/S2	275.2/109.1	1.02	26	52	1.95	19	17	65	9	35	19.67 - 178.33	25.99
S1/S2	275.2/109.1	1.36	18	36	1.95	19	9	50	9	50	22.14 - 579.28	31.06
S3/S4/S13/S14	289.2/109.1	1.27	15	30	1.95	19	10	67	5	33	20.51 - 584.80	38.13
S3/S4/S13/S14	289.2/109.1	1.76	27	54	1.95	19	18	67	9	33	19.40 - 88.92	24.43
S5/S6	277.0/216.8	0.30	27	54	3.91	39	24	89	3	11	53.18 - 6922.95	212.57
S7	273.2/109.0	1.33	6	12	1.95	19	3	50	3	50	20.16 - 78.06	50.07
S8/S9/S15	293.0/137.0	0.35	11	22	15.63	156	4	36	7	64	175.21 - 907.25	410.25
S10/S12	287.2/97.1	1.71	27	54	1.95	19	6	22	21	78	28.17 - 655.09	85.17
S11	285.2/121.1	1.19	30	60	1.95	19	7	23	23	77	24.61 - 24331.91	44.35
S10/S12	287.2/121.1	0.92	45	90	1.95	19	20	44	25	56	19.67 - 509.64	19.88
S3/S4/S13/S14	289.2/109.1	1.27	13	26	1.95	19	7	54	6	46	23.88 - 689.62	49.30
S3/S4/S13/S14	289.2/109.1	1.77	17	34	1.95	19	3	18	14	82	20.20 - 700.65	21.54
S7	273.2/161.1	1.14	20	40	1.95	19	19	95	1	5	25.48 - 1150.53	187.29
S7	273.2/161.1	1.33	21	42	7.81	78	17	81	4	19	82.03 - 2025.40	178.48
S7	273.2/161.1	2.01	21	42	7.81	78	19	90	2	10	80.48 - 1136.91	192.00
S16	301.3/121.1	1.11	16	32	1.95	19	4	25	12	75	45.86 - 142.98	66.62

** Examples (a) = S7 and (b) =S10/S12*

5.21 Stimulants

5.21.1 General overview of samples tested for stimulants

Table 29 provides a summary of all the samples tested for stimulants (n=138). Two examples are used to make the illustration (*); (i) for Stimulant T5, 18 samples (13%) and Stimulant T14 62 samples (45%). When sub-grouping the qualitative results (Table 29) into "positive" tested samples greater than and equal to the lower limit of quantification (≥LLOQ) for T5 72% (13) of the "positive" tested samples were ≥LLOQ and 28% (5) < LLOQ. For T5 the estimate concentration range was 19.35-2789.30 ng/g with a median value of 56.82 ng/g. For T14 this was ≥LLOQ 76% (47) and <LLOQ 24% (15). The T14 estimate concentration ranged from 161.00 ng/g to 19.53 mg/g, with a median value of 1025.55 ng/g.

Table 29. *Summary of Nutritional Supplement products investigated for stimulants*

			Qualitative Assessment Screen								Estimate Concentration Range	
Stimulant Code	Compound Transition	RT min.	No. Total	% Positive	LLOQ (ng/ml)	LLOQ (ng/g)	No. ≥ LLOQ	(%)	No. < LLOQ	(%)	≥ LLOQ (ng/g)	Median (ng/g)
T5	166.2/133.1	0.74	18	13	1.95	19	13	72	5	28	19.35 - 2789.30	56.82
T10	195.1/138.1	2.68	126	91	1.95	19	121	96	5	4	67.45 - 10369090.24	1637.76
T14	168.2/135.0	0.31	62	45	1.95	19	47	76	15	24	16100 - 19532746.07	1025.55
T2	150.2/91.1	1.39	10	7	1.95	19	9	90	1	10	19.75 - 1104.82	54.96
T13	232.2/159	3.45	3	2	3.91	39	1	33	2	67	42.26 - 42.26	42.26
O2	127.1/68.1	0.26	67	49	1.95	19	66	99	1	1	513.44 - 127029.61	5992.55
O3	251.2/132.1	4.46	6	4	15.63	156	3	50	3	50	30.37 - 1685.27	442.97
O4	310.2/148.0	4.53	75	54	1.95	19	72	96	3	4	360.96 - 525638.49	3889.99

* Examples (a) =T5 and (b) =T14

5.21.2 South African Produced Nutritional Supplements

Table 30 provides a summary of all the samples tested for stimulants (n=27) that were locally produced or assembled. Two examples are used to make the illustration (*), (i) Stimulant T5, 5 samples (19%) and (ii) Stimulant T14, 17 samples (63%).

When sub-grouping the qualitative results into "positive" tested samples greater than and equal to the lower limit of quantification (≥LLOQ) for T5, 100% (5) of the "positive" tested samples were ≥LLOQ and 0% (0) < LLOQ. For T5 the estimate concentration range was 19.35-282.34 ng/g with a median value of 41.17 ng/g. For T14 this was ≥LLOQ 88% (15) and < LLOQ 12% (2). The T14 estimate concentration range was 200.92 ng/g to19.53 mg/g, with a median value of 3516.67 ng/g.

Table 30. *South African nutritional supplements investigated for stimulants*

			Qualitative Assessment Screen								Estimate Concentration Range	
Stimulant Code	Compound Transition	RT min.	No. Total	% Positive	LLOQ (ng/ml)	LLOQ (ng/g)	No. ≥ LLOQ	(%)	No. < LLOQ	(%)	≥ LLOQ (ng/g)	Median (ng/g)
T5	166.2/133.1	0.74	5	19	1.95	19	5	100	0	0	19.35 - 282.34	41.17
T10	195.1/138.1	2.68	25	93	1.95	19	23	92	2	8	73.38 - 9766373.04	3430.73
T14	168.2/135.0	0.31	17	63	1.95	19	15	88	2	12	200.92 - 19532746.07	3516.67
T2	150.2/91.1	1.39	3	11	1.95	19	2	67	1	33	24.17 - 717.32	370.74
T13	232.2/159	3.45	-	-	3.91	39	-	-	-	-	- - -	-
O2	127.1/68.1	0.26	16	59	1.95	19	16	100	0	0	543.22 - 127029.61	10488.85
O3	251.2/132.1	4.46	1	4	15.63	156	1	100	0	0	1685.27 - 1685.27	1685.27
O4	310.2/148.0	4.53	18	67	1.95	19	17	94	1	6	554.22 - 431709.65	5193.39

**Examples (a) =T5 and (b) =T14*

5.21.3 South African Purchased Imported Nutritional Supplements

Table 31 provides a summary of all the imported nutritional supplement samples purchased in South Africa and tested for stimulants (n=50). Two examples are

used to make the illustration (*); (i) Stimulant T5, 9 samples (18%) and Stimulant T14 22 samples (44%). When sub-grouping the qualitative results into "positive" tested samples greater than and equal to the lower limit of quantification (≥LLOQ) for T5, 89% (8) of the "positive" tested samples were ≥LLOQ and 11% (1) < LLOQ. For T5 the estimate concentration range being, 24.96-2789.30 ng/g with a median value of 411.33 ng/g. For T14 this was ≥LLOQ 73% (16) and < LLOQ 27% (6). The T14 estimate concentration range was 161 ng/g to 342 µg/g, with a median value of 23.56 µg/g.

Table 31. *Imported nutritional supplement products investigated for stimulants*

			Qualitative Assessment Screen								Estimate Concentration Range	
Stimulant Code	Compound Transition	RT min.	No. Total	% Positive	LLOQ (ng/ml)	LLOQ (ng/g)	No. ≥ LLOQ	(%)	No. < LLOQ	(%)	≥ LLOQ (ng/g)	Median (ng/g)
T5	166.2/133.1	0.74	9	18	1.95	19	8	89	1	11	24.96 - 2789.30	411.33
T10	195.1/138.1	2.68	45	90	1.95	19	45	100	0	0	136.30 - 10369090.24	693313.37
T14	168.2/135.0	0.31	22	44	1.95	19	16	73	6	27	16100 - 342649.61	23557.25
T2	150.2/91.1	1.39	2	4	1.95	19	2	100	0	0	54.96 - 1104.82	579.89
T13	232.2/159	3.45	-	-	3.91	39	-	-	-	-	- - -	-
O2	127.1/68.1	0.26	23	46	1.95	19	22	96	1	4	1089.04 - 764919.21	12265.22
O3	251.2/132.1	4.46	-	-	15.63	156	-	-	-	-	- - -	-
O4	310.2/148.0	4.53	28	56	1.95	19	28	100	0	0	55193 - 104308.11	20051.35

** Examples (a) =T5 and (b) =T14*

5.22 Conclusion

This study described the percentage "positive" results of steroid and stimulant analysis in nutritional supplement products, based on the qualitative assessment screen results. The concentration range and the median values are presented as an estimate and not as an absolute quantitative assessment. It would be important for a subsequent study to verify the current findings by re-extraction and analysis.

Isomeric forms of steroids may not be able to be separated chromatographically (having similar retention time and similar molecular weight and similar fragmentation (transition) patterns. Hence results reflect steroid S3, S4, S13 and S14 individually or a combination of different proportions of S3, S4, S13, and S14 respectively. The results would therefore indicate a "positive" steroid sample, but the estimate concentration could be of individual steroid S3, S4, S13, S14 or collective proportional sum of S3, S4, S13, and S14.

The frequency of "positive" tested samples for the respective steroids assessed in the overall cohort ranged from 16-86%, for South African produced nutritional supplements 15-89%, and for imported products, bought in South Africa, 12-90%.

The frequency of " positives" tested samples for respective stimulants and other drugs assessed, in the overall cohort ranged from 2-91%, for South African produced nutritional supplements 4-93%, and for imported products, purchased in South Africa, 4-90%.

For the specific steroids investigated there was a notable high frequency in the overall sample cohort, of the nutritional supplements produced in South Africa for steroids, S10/S12, and the combination of S3, S4, S13, S14, and S1/S2. For the imported products, purchased in South Africa, it was the frequency of steroids S10/S12, S11, the combination of S3, S4, S13, S14, and S5/S6 compounds that was high in the overall sample cohort.

For the stimulants and other drugs investigated there was a notable high frequency of "positives" for T10 (Caffeine) 90-93%, T14 (Synephrine) 44-63%, and O4 (Fluoxetine) 54-67%, presented in the nutritional supplements categories assessed. For the specific stimulants and other drugs investigated there was a notable high frequency "positives" in the overall sample cohort. For imported products, purchased in South African this was T10, O4, and 02, and for South African produced products it was T10, O4 and T14.

The findings of the steroid and stimulant "positive" tested samples provide sufficient evidence for implementing a system for regulating, monitoring and enforcing the quality control of nutritional supplements.

5.23 Limitations and opportunities of current Steroid and Stimulant Screen

The current method was developed as a "Steroid and Stimulant Screen" respectively and thus results and data should be interpreted from a qualitative perspective. The quantitative assessment is only an estimate concentration and not absolute, due to potential matrix effects from the respective nutritional supplement products.

- Ion suppression or enhancement may/or would also contribute to varied concentration and hence the concentration should be viewed as an estimate and not in absolute terms.
- An improved method to the current "Screen Method" would be to use an appropriate Internal Standard (IS) to improve on the "estimate concentration approach", as done in this study.
- Another option to improve quantitative results and data is to use labelled isotopic compounds of interest.

Chapter 6
Discussion, Conclusion, Perspectives

6.1 Purpose of Research Study

The main purpose of the study was to evaluate commercially available, (i) traditional supplements for trace element and heavy metal consistency and safety (Chapter 4) and, (ii) in nutritional supplements to detect the presence of steroids, stimulants and other compounds of interest using qualitatively and semi-qualitative techniques (Chapter 5).

Prior to these experimental sections (Chapter 4 and 5) of the study the following studies were done;

- a comparative assessment of current Regulations, Legislation, Labelling and Claims applied in both South Africa and globally (as presented in Chapter 2),
- a study to assess the labelling and claims information on imported into/and South African manufactured or assembled nutritional supplements products (as presented in Chapter 3),
- a study to assess the impact of container labelling and other sources of information that currently assist consumers of nutritional supplement products in their purchasing decisions (as presented in Chapter 3).

The key questions of the overall study were;

- What information is presented on the container labels of nutritional and traditional supplement products?
- What container labelling and other sources of information assist the consumers of nutritional supplement products in their purchasing decisions?
- How consistent is the batch-to-batch trace element and heavy metal composition of commercial traditional supplement products?
- Do nutritional and traditional supplement products contain undeclared contaminants and/or adulterants, such as steroids and

stimulants that could compromise nutritional and traditional supplement usage and safety?

6.2 Discussion and Conclusion

Based on the literature review and personal global assessment of the nutritional supplement sector the gaps and "grey" areas in the industry were identified. These points formed the basis for the integrated approach to the thesis and covered the following; (i) regulations and legislation, and labelling and claims associated with nutritional supplement products in the USA, European Union and South Africa, (ii) assessing the labelling and claims information on nutritional supplement products imported into/and manufactured or assembled in South African, (iii) assessing how consumers were influenced when they purchased supplements information; the focus was on the labelling on the container and other sources of information (iv) assessing traditional commercial supplements for contamination and consistency of trace elements and heavy metals using Inductively Coupled Plasma-Mass Spectrometry, and (v) assessing the content of nutritional commercial supplements for steroids, stimulants and other compounds of interest using Tandem Liquid Chromatography-Mass Spectrometry.

The study thus makes an important contribution in assessing the current regulations and legislation (or lack thereof). This is the basic framework where fundamental changes should occur to ensure improved regulation and legislation of the nutritional supplement sector globally. Having scientific insight into the type and categories of information on the container of nutritional supplement will assist in determining what may be more appropriate, relevant and important. This will help to guide changes in label content in the best interest of the consumer.

The laboratory experimental work with respect to trace element and heavy metal profiling using Ginseng and African Potatoe products introduced a novel proof of concept. This method has the potential to ensure quality and consistency, and also contamination of nutritional supplement products from batch-to-batch. The laboratory experimental work with respect to assessing the content of nutritional

commercial supplements for steroids, stimulants and other compounds, exposed content that was not disclosed (intentionally or unintentionally) on the container label of nutritional supplement products. These could potentially be harmful to the consumer or have negative consequence if such banned substances or compounds are embedded in nutritional supplements.

The main findings and assessment for the respective chapters in the thesis are,

In **Chapter 1** the literature review identified the increased use of nutritional supplements by athletes in competitive and recreational sport. There has been an increase in media and public scrutiny because of the increase in positive doping tests worldwide in recent years [1]. Many athletes have blamed nutritional supplements as the primary cause of their positive doping test [2, 5, 6].

Chapter 1 further covered the history of Performance-Enhancing Substances, with evidence for their use going as far back as 300BC. Steroids and amphetamines became popular ergogenic aids more recently. When these substances where banned by WADA, the quest began for legal ergogenic substances that would provide the competitive edge [7-10]. This resulted in more interest in nutrition with the nutritional supplement industry growing at an exponential rate [14, 15].

Global sporting organizations and anti-doping agencies often advise athletes to abstain from using supplements because of the risk of ingesting a contaminated product that would result in a positive doping test [150,165,217]. However, this message has not had much impact, mainly due to athletes' belief that nutritional supplements boost their physical and psychological abilities [40]. This leads to the motivation for having a screening protocol for testing nutritional supplements for purity before they are available for commercial distribution [19, 20].

Chapter 1 also discussed the concern among sports scientists and nutritionists that athletes and consumers make uninformed choices about supplements. The concern that nutritional supplements may exert their effect by undeclared embedded contaminants such as steroids and stimulants was also highlighted [76, 78, 86].

The role of nutritional supplements in malnutrition, obesity, patient care and anti-aging was also presented and the consequences that may manifest due to contamination of consumed products was also discussed [45, 68, 72].

The literature review in Chapter 1 also presented a synopsis of current matters relating to regulation, de-regulation, re-regulation, law enforcement and consumer protection agencies [87, 93, 94]. An outline was also provided explaining how nutritional supplement product contamination may occur, including the conduit of counterfeiting and piracy [35]. The importance of laboratory screen testing of nutritional supplements, harmonization of interpretation of questionnaire surveys, and genetic pre-disposition profile of supplement users was given emphasis [79, 218-220].

Chapter 2 covered the global and local regulations, legislation, labelling and claims made for nutritional supplements. The chapter focussed on current laws in general, and then discussed the specific laws pertaining to the USA, the European Union (UK, Germany, Belgium, The Netherlands, and Austria in particular) and South Africa. This legal framework provided a reference to current practice and future potential development of relevant and appropriate policy.

In Chapter 2, a synopsis of the Current Laws and Regulation pertaining to nutritional supplements identified that infringes can only be interpreted or applied implicitly and not necessary stated specifically. The divergence of the laws between Food and Drug Law has created an increasingly “grey” area, which makes it difficult to institute appropriate law enforcement. This is particularly relevant to the “voluntary” declaration of “all” content in a specific nutritional supplement product. The need for basic research information and investigation is therefore important to maintain ongoing quality of nutritional supplement products.

Chapter 2 also identified the Consumer Forums and Councils of importance within specific regions. There was specific focus on Consumer International, The Council for Responsible Nutrition, The European Consumer Centres Network, The South African National Consumer Forum, and The South African National Consumer Union. The observations from this section provided supporting

evidence for the study, and shows additional requirements and guidelines to improve on the mandate and intention of consumer forums.

Chapter 3 discussed the assessment of specific knowledge and understanding on the claims and labelling on nutritional supplements products used by general, and more specifically sports consumers**.** The point was made of the importance for studying this information for practical intervention, policy development, and possible appropriate legislation and regulation. The information also provides guidelines on how nutritional supplement products may be marketed in future to ensure informed choice for the consumer. Secondly, Chapter 3 dealt with determining how consumers of nutritional supplement products gather information to assist their purchasing decisions. This was accomplished by administering a self-administered questionnaire to test which container label information, and information other than container labelling sources assist the consumer in making purchasing decisions.

The main finding in Chapter 3 showed that seventy percent of the nutritional supplement product labels had no indication or evidence that the product was "Contaminant Free". Chapter 3 further shows the importance of screen testing of products to verify nutritional supplements for contaminants, with specific reference to steroids and stimulants and other compounds that were investigated in this thesis. The three main findings with specific reference to the self-administered questionnaire were, (i) that close to 70% of the respondents were strongly influenced by container label information that stipulated that the nutritional supplement product is free of banned substances, (ii) 50% of the respondents attach importance to the quality, and (iii) 40% of the respondents where strongly influenced in their purchase of nutritional supplements based on ingredients. The specific finding in this thesis study related to "free of banned substances" information requirement, and the lack of information on product labels that the product is "contaminant free" should be of major concern to both consumer and government enforcement. Not presenting this information on the label, when in

fact a product may contain a prohibited substance(s), may have dire consequence(s) to both general consumer and competitive athletes, respectively. The main findings of the self-administered questionnaire study emphasises the importance of independent laboratory screen testing of all nutritional supplement products for contaminants and/or adulterants on a regular, and batch-to-batch basis.

These findings are further supported and extended by the published work from this thesis by Gabriels et al [222]. This study showed that in order for the consumer to make informed choice that there was a need to alert those consuming nutritional supplements of the potential for banned substances being present in products. Of the product labels assessed in that particular study, only 5% had information on "The presence of banned substances in the supplements". Brownell et al [223] conducted research work on the Food Industry (which is similar to the nutritional supplement sector). They describe the free-for-all approach different companies use and in many cases self-serving symbols to communicate how healthy their products are. The authors also point to the importance and evaluation of various classification models for front-of packaging labelling w.r.t to food packaging. They state that the effectiveness of any given system may vary with the population's nationality, culture, level of health literacy and socioeconomic status. A pertinent point, because of undeclared content on the labels, is that the industry may have proven itself untrustworthy [223].

At the legislative and regulatory level the published work of Gabriels et al [224] concluded that in South Africa, the new Consumer Protection Act 68 of 2008 (CPA) should promote greater levels of policy development, regular enforcement, and consumer education with respect to the supplement industry [122,224]. This work is supported by work published by Cohen et al that assessed supplement safety, stating specifically, if the FDA succumbs to industry pressure that the public health consequences will be significant, as hundreds of thousands

of Americans continue to turn to new supplements to sustain their health and treat their ailments [118, 225]. The importance of information on the container label linked to ingredient content is hereby emphasised.

The main finding in the category, **other than the container label information showed that** coaches, gym and/or fitness trainers, and fellow athletes (24%) have a greater influence to the consumer of nutritional supplement products than that of a Pharmacist, Dietician, Nutritionist and Doctors (10%), and nutritional supplement representatives (2%), respectively. A second finding is that electronic, print and social media and the internet, all showed an influence on respondents in the purchase of nutritional supplements, to less than 15%.

The findings supports the paper on practical guidelines to the use of nutritional supplement in South Africa (2004) [226]. These guidelines stated specifically that fitness coaches and conditioning staff should not prescribe supplements. The findings in this thesis (2012), shows that coaches, gym and/or fitness trainers, and fellow athletes have an influence on the consumer of nutritional supplement products. This influence may be construed as providing indirect-prescription for usage, and thus highlights an area where important focus with relevant peer-reviewed information should be directed to ensure educated and informed choice is provided to the athletes.

Further, work published by Berning et al showed that by identifying consumer preferences for nutrition information, the authors raise the possibility that alternative formats (platforms) of communication may become more effective [119,123]. In the context of this thesis, the findings show that less than 15% of the study participants were influenced by categories such as electronic, print, and social media and the internet. This finding suggests that these are potential areas of growth to provide peer-reviewed evidenced based information. This is in accordance with the work of Joshi et al which states that the news media helps

shape the public understanding of promotional practices of companies and their potential benefits and harms [121].

In conclusion, the findings in Chapter 3 provide a mechanism, and/or requirements, that may be needed for practical intervention, policy, regulation, and legislation development in South Africa. It may further provide and facilitate the direction and improved guidelines, and permissible marketing practices. This will ensure that the consumer can make informed choices about product content linked to container label content.

Chapter 4 described an Experimental Study of Trace Elements and Heavy Metal analysis in the natural commercially products Ginseng and Hypoxis. The World Health Organization (WHO) estimates, that 60-80% of people in developing countries use traditional medicines. This has resulted in over-the-counter plant drugs increasing dramatically over the past decade, in both developing and developed countries. The upsurge in the use of natural products has lead to these products being packaged in a similar way to nutritional supplement products in the market. This gives reason to scrutinize the quality content of natural products incorporated into the nutritional supplements for trace element and heavy metals.

Chapter 4 further highlights the importance of recognising the potential risk where consumers of nutritional supplements and/or Herbal- Botanicals who are receiving medical drug treatment, may be exposed to adverse drug-herb interaction that is synergistically induced [4,128,132,133]. The same reasoning may apply to the use of complementary/alternative medicine [129,130,134,135,221].

The findings of Chapter 4 includes the assessment of the trace elements and heavy metals, magnesium (Mg), potassium (K), calcium (Ca), Iron (Fe), manganese (Mn), Lithium (Li), sodium (Na), chromium (Cr), cobalt (Co), nickel (Ni), copper

(Cu), zinc (Zn), strontium (Sr), cadmium (Cd), mercury (Hg) and lead (Pb) using the analytical instrument methodology Inductively Coupled Plasma - Mass Spectrometry (ICP-MS).

The presence of these trace elements and heavy metals that would often not be declared on supplement product label, could have an impact when conventional medicine practice, is taken in conjunction with complementary or alternative medicine. Work published by Sirven et al [130], points to commonly tried botanical supplements as being garlic, gingko, soy, melatonin and kava. Linked to this Grippo et al, states that the lowest metal concentration were most single–ingredient botanical supplements, while multiple–component, ephedra containing dietary supplements generally had higher metal concentrations [127]. The presence of these metals could synergistically contribute to toxicity [4,132]. Work published by Singh and Dhananjay et al, points to the need for detailed investigations (screening) to identify clinically significant interactions that may cause adverse effects in herbal/botanical supplements [129,134,135,221].

The findings in this study also point to the important requirement for qualitative and quantitative assessment of these products, to minimize risk to health and wellness, and as suggested by Dasgupta et.al [227]. Herbal products are often exported to high-consumption markets, thus spreading the risk of heavy metal toxicity [227]. Whilst findings in this study, based on the Ginseng and Hypoxis products investigated is not cause for alarm, but allow for a marker of quality, this does not imply that other similar products may not present higher concentration levels as contaminants. The study described in Chapter 4, using Ginseng and Hypoxis supplements as examples shows the trace element and heavy metal content were not declared on label. This may have negative consequences as the metals accumulate in the same target organs (e.g. liver and kidney), suggesting that their respective toxicities may be additive. The number of case reports describing toxicity linked to the use of herbal products provides strong evidence

that the presence of heavy metals in alternative medicine is a matter of serious concern [227].

Further, the findings in Chapter 4 of this thesis established a proof of concept for measuring intra-sample consistency, intra-batch variability, and inter-batch sample consistency for the trace elements and heavy metal. Fingerprint profiles and novel Radar plots were established for each of the Ginseng and Hypoxis products investigated. The fingerprint profiles showed a reliable marker to evaluate overall product quality and consistency, and radar plot provided a concise visual analysis of the collective trace element and heavy metals. This novel approach has the potential to firstly determine the quality of products indirectly, secondly to assist in determining metal associated - drug interactions as required and concluded by other researchers [128].

Chapter 5 described an Experimental Study that laboratory screened for 16 steroids, 5 stimulants and 3 other compounds of interest. The requisite reference compounds were used for calibration in the analytical methodology Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). The study established a method with analytical detectable measures that has the potential to "screen test" supplements in the laboratory. In this study a total of 138 nutritional products were screened as part of the experimental study.

Findings in this thesis has illuminated the presence and extent of banned substances and stimulants in nutritional supplement products. The number of "positives" in the nutritional supplement products assessed ranged from 12-90% for the respective steroids. For stimulants and other drugs respectively, this was "positive" in 2-93% of the nutritional supplement products assessed. It was concluded that if this method was implemented on a larger scale it could be assured that the quality of the nutritional supplement could be verified to be "clean" and safe.

These results and findings contribute a substantial knowledge and insight about nutritional supplement products. The data confirms the requirement and importance of laboratory screen testing of nutritional supplements and harmonization of terminology. This will improve the quality of the supplement and also provide informed choice to consumers of nutritional supplement products through improved information provision.

Poor regulations contribute to contamination and/or adulteration of products such as metals, animal parts and conventional drugs [76,78,208,215,227]. The data from this thesis point to the need for expanded laboratory "screen testing", with continuous vigilance.

6.3 Perspectives – Future research work and recommendations

Chapter 2 suggests, that (i) there needs to be further work to achieve common international regulations and guidance policies for the nutritional supplement sector, (ii) greater awareness of concern needs to be created about nutritional supplements with respect to the potential problems, addressing enforcement difficulties and obstacles, (iii) there is the need to develop common global centres for laboratory screening for contaminants and maintaining research vigilance, with appropriate accountability, reporting and awareness measures, and (iv) there should be a global harmonization of protocols, including synergy in the use of terminology.

Chapter 3 suggests, (i) the importance of highlighting the potential for adverse events that may occur, should be presented on the nutritional supplement product label, (ii) that warning statements such as, "keeping product out of reach of children", and "the importance of adherence to storage details" should be maintained on the labels appropriately, with acceptable font and visibility, (iii) warning statements, such as; (a) "exclusion of use, and not a cure for disease states" and (b) the "presence of banned substances in the supplements", should be encouraged based on standardised laboratory screen methods as presented in

this study, (iv) the requirement to determine and establish a nutritional supplement sector specific system and guideline with regards to the best before and expiry date information, batch numbers, and Good Manufacturing Practices (GMP), that should also facilitate batch-to-batch laboratory screen testing, and (v) the need for scientifically explored and researched alternative label designs, that would be beneficial to providing relevant information to the consumer.

Chapter 4 suggests; (i) that trace elements and heavy metals are a good assessment tool to determine the consistency and a measure of quality for nutritional and traditional supplement products, (ii) that "finger print" profile of determined trace metals and heavy metals can be used as a reference standard to measure subsequent manufactured batches against, as well as identification of trace element or heavy metal contamination, (iii) that radar plots could be used as an additional visual tool, to observe discrepancies, deficiency and/or contaminants in replicate samples and respective batches, (iv) that the safety, quality and efficacy of these natural products need to be established according to the principles of Standard Operating Procedures (SOP), Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Supply Practice (GSP) and Good Manufacturing Practices (GMP), (v) the "finger print" profile and radar plot approach applied in this study, would have similar application for nutritional supplement products in general, and (vi) deviation from the initial pre-determined "fingerprint" levels and visual radar plot, would be a reliable measure of overall product quality and consistency.

Chapter 5 suggests; (i) that the current findings provide sufficient evidence that the nutritional supplement sector should be regulated and monitored on an ongoing basis, (ii) that "screen testing" should be applicable to existing products, when "new" products are developed and batch-to-batch testing of all nutritional supplement products in the market place, (iii) that the current "screen test" developed in this thesis has limitations and needs to be refined, as it is currently only able to provide a qualitative assessment with an estimate concentration component and not absolute concentration, (iv) the current study presents the

opportunity as a separate project to improve the current method by using ion-trap instrument technology to provide absolute quantitative determination for steroids and stimulants, (v) to expand the current "screen test" developed with additional chemical compound reference standard acquisition, and/or via LC-MS/MS software libraries, and (vi) that a further study should be conducted where the "positive" tested products could be consumed by subjects to test if the urine concentration would yield a "positive" doping result.

It is clear that a change in approach needs to occur in the nutritional supplement sector. There should be a requirement for "all" content in a specific product to be declared, rather than "voluntary" declaration of content only. The findings also suggest the need for enhanced capacity requirement, which will facilitate in part, the mandate of consumer forums and specific agencies, in South Africa. This requirement will ensure the relevant vigilance and continuous monitoring of the nutritional supplement sector. The data provides practical intervention for policy development, and possible appropriate legislation and regulation.

The laboratory screen testing for contamination and/or adulteration of steroids, stimulants and other drugs in nutritional supplement in this thesis highlights the extent of the problem. The study and the analytical guidelines provided, emphasises the importance and expansion for laboratory screen testing protocols. These processes and procedures will ensure informed choice to consumer of nutritional supplement products through improved peer-reviewed information provision.

In conclusion this thesis has shown that there should be concern about the quality of nutritional supplement products in the South African market place, based on the various aspects covered. Specific legislation and regulation needs to be drafted and enforced in the best interest for the wellness and health of the consumer of nutritional supplement products.

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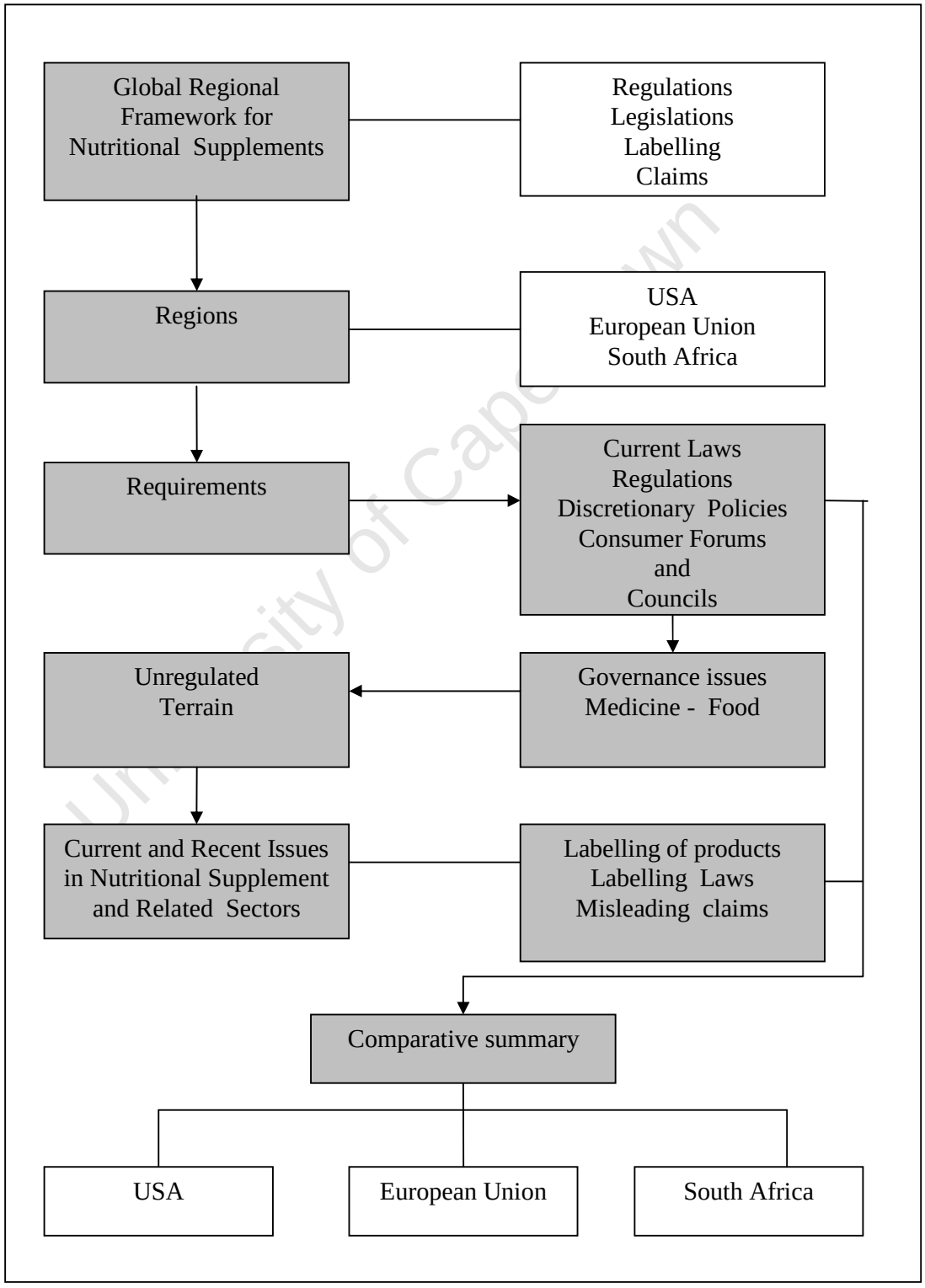
Appendices

Appendix 1

Action Plan 1

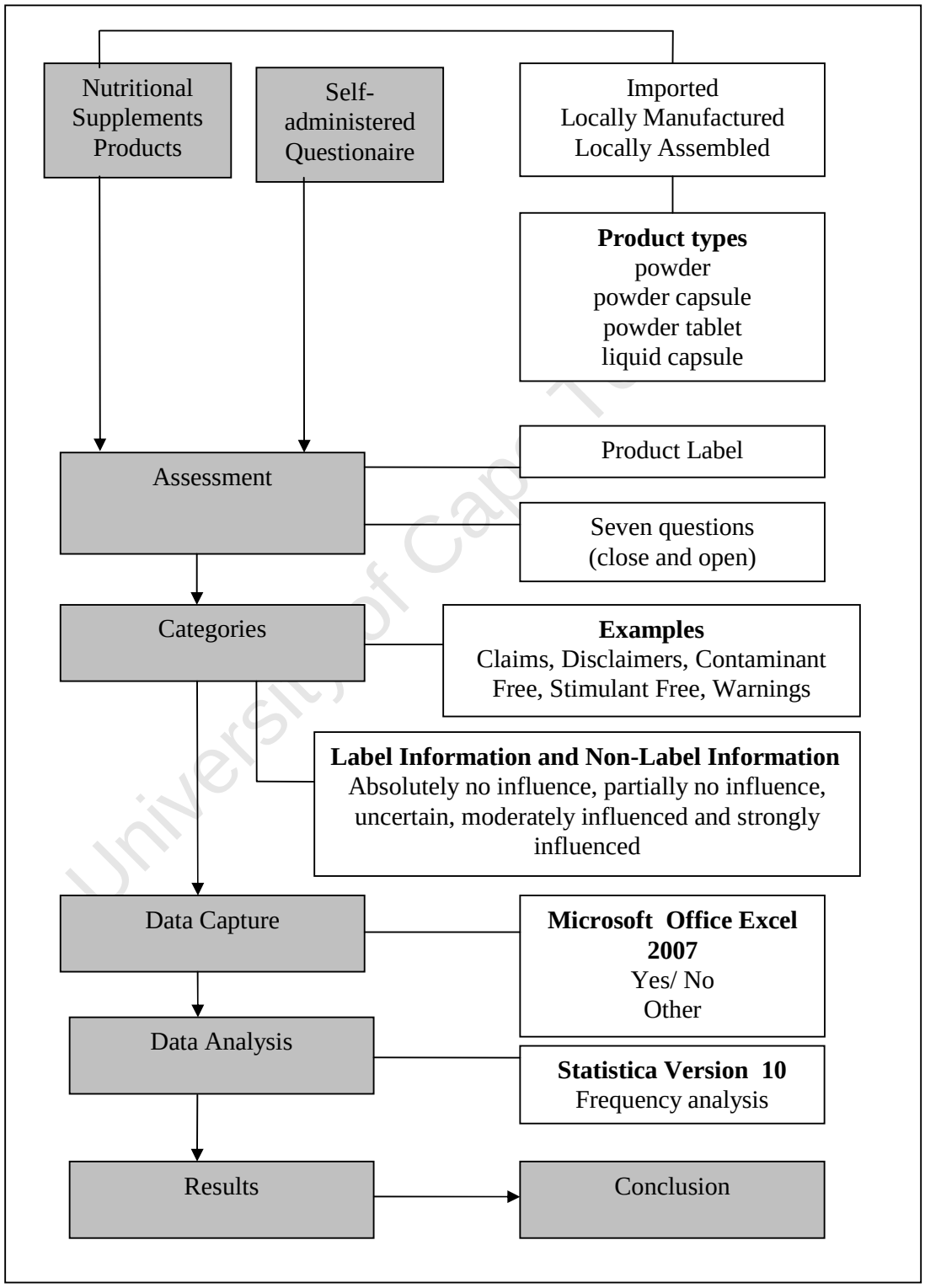
To accomplish objective 1 in scope of study with content in Chapter 2



Appendix 2

Action Plan 2

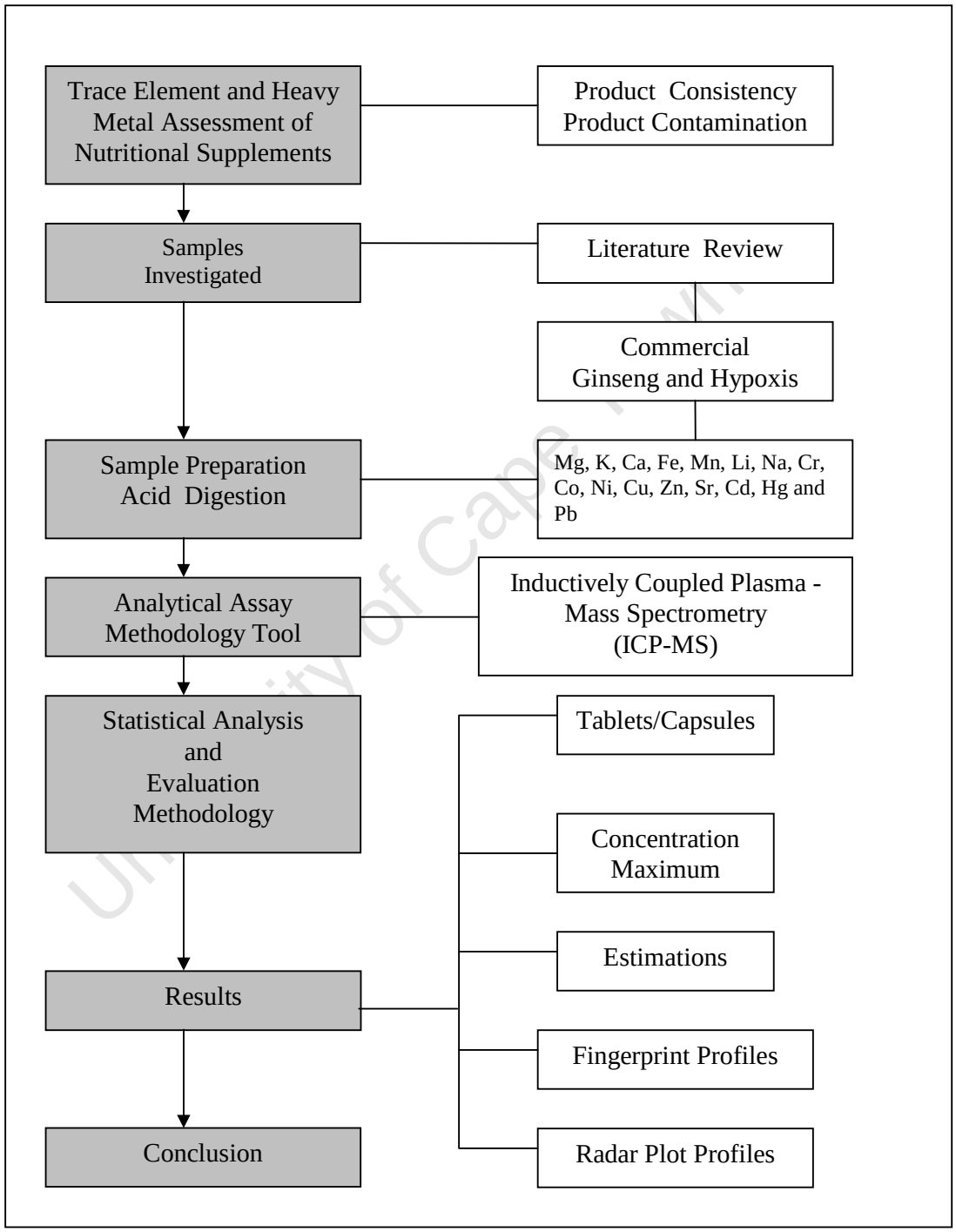
To accomplish objective 2 in scope of study with content in Chapter 3



Appendix 3

Action Plan 3

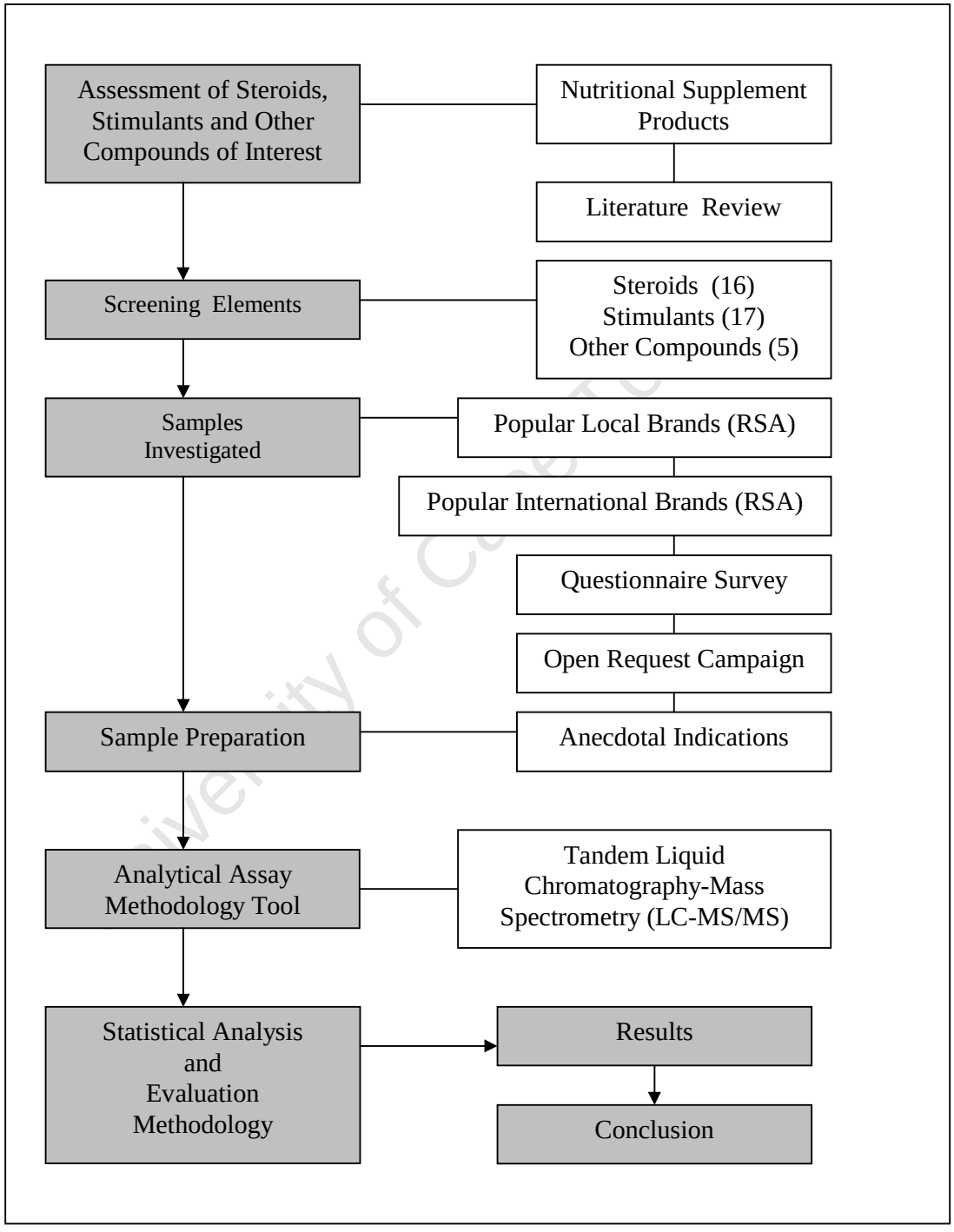
To accomplish objective 3 in scope of study with content in Chapter 4



Appendix 4

Action Plan 4

To accomplish objective 4 in scope of study with content in Chapter 5



Appendix 5a

Research Ethics Approval

UNIVERSITY OF CAPE TOWN

Health Sciences Faculty
Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: lameca.emjedi@uct.ac.za

18 July 2008

REC REF: 275/2008

Mr G Gabriels
Clinical Pharmacology
K Floor
OMB

Dear Mr Gabriels

PROJECT TITLE: THE INVESTIGATION AND ASSESSMENT OF NUTRITIONAL AND TRADITIONAL SUPPLEMENT PRODUCTS, FOR CONTENT VALIDITY, CONTAMINATION AND ADULTERATION

Thank you for submitting your study to the Research Ethics Committee for review.

It is a pleasure to inform you that the Ethics Committee has **formally approved** the above-mentioned study.

Approval is granted for one year till the 31st July 2009.

Please submit an annual progress report if the research continues beyond the expiry date. Please submit a brief summary of findings if you complete the study within the approval period so that we can close our file.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.

lemjedi

Appendix 5b
Research Ethics Approval

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
e-mail: shuretta.thomas@uct.ac.za

17 July 2012

HREC REF: 346/2012

Mr G Gabriels
c/o Prof M Lambert
Pharmacology
K-Floor
OMB

Dear Mr Gabriels

PROJECT TITLE: NUTRITIONAL SUPPLEMENT USE AND KNOWLEDGE OF LABELING INFORMATION (Sub study of HREC Ref 275/2008).

Thank you for responding to the issues raised by the Faculty of Health Sciences Human Research Ethics Committee in the e-mail received on 16th July 2012.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year till the 30th July 2013

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/research/humanethics/forms)

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the HREC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN ETHICS
Federal Wide Assurance Number: FWA00001637.

Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP) and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

s.thomas

Appendix 6

Example of Import For Controlled Substances

NO: A 40/2009

REPUBLIC OF SOUTH AFRICA
DEPARTMENT OF HEALTH
IMPORT AUTHORISATION FOR CONTROLLED SUBSTANCES

SUBSTANCES TO BE IMPORTED

ITEM NO	NAME OF SUBSTANCE / PREPARATION AND STRENGTH	QUANTITY	TOTAL AMOUNT OF CONTROLLED SUBSTANCE CONTENT
1	(-)-Deoxyephedrine D6787	1 g [one gram]	0.001 kg Deoxyephedrine
	NO OF ITEMS: 1 (one)		
e			

I certify that the importation by **Mr. G. A. Gabriels on behalf of The University of Cape Town, Division of Pharmacology, K-45, Old Main Building, Groote Schuur Hospital, Observatory, 7925, South Africa** being a duly analyst of the above mentioned substances from **Sigma-Aldrich Corporation, 3050 Spruce Street. St Louis, Mo 63103, USA** subject to compliance with the Medicines and Related Substances Act, 1965 (Act 101 of 1965), has been approved.

The importation is to be made through the port of **Cape Town International Airport** within six (6) months of the date of this permit.

I am satisfied that the above-mentioned substances are required for approved medicinal purposes or for licit research, scientific, analytical or educational purposes within the provisions of the said Act.

SIGNED AT PRETORIA

DIRECTOR-GENERAL
DEPARTMENT OF HEALTH

2009 -03- 16

Appendix 7

Conference Poster Presentation

2007 G.A. Gabriels, K. Govender, P.J. Smith, A.Spath. Assessment of Commercial Ginseng and Hypoxis supplement products for trace element and heavy metal contamination. South African Pharmacology and Toxicology Congress 2-5th October 2007, Rustenberg, South Africa

Assessment of Commercial Ginseng and Hypoxis supplement products for trace element and heavy metal contamination

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Introduction

The World Health Organization (WHO) estimates that 60-80% of people in developing countries use plant medicines as primary source of health care. The use of traditional medicines and over the counter plant drugs has increased dramatically over the past decade, in both developing and developed countries [1].
The upsurge in the use of natural products include; (i) cost effectiveness and availability, (ii) perceived safety and lack of side effects, (iii) deteriorated belief in the efficacy and safety of orthodox drugs, (iv) public tendency towards self – medication, (v) increase in the interest, publicity and sensational journalism [2].

Natural products extensive use as a source of primary health care requires routine quality control scientific testing methodologies to verify the safety, quality and efficacy of commercially available plant products. This will enhance public confidence in the use of such products in an unregulated environment, as adverse side effects often associated with the use of traditional/ herbal products, are generally due to misidentification of plants, lack of standardization and good manufacturing practices, botanical substitution, adulteration, and contamination. The therapeutic effect of plant medicine is alleged to be enhanced by essential trace elements. Some products may however also contain excessive amounts of trace elements and heavy metals.

For this study the focus was two product types: Ginseng as a popular traditional Chinese medicine used for its adaptogenic and restorative properties and African Potato products used as an immune booster for patients infected with HIV/AIDS.

Objective

To determine the trace and heavy metal content of selected, different commercial brands of Ginseng and African Potato products purchased from pharmacies and health stores in Cape Town.

Table 1 Benefits and risk of trace elements and metals in plant medicine

Chemical Element	Phyto pharmaceutical enhancing indication	* UL	Description and Toxicity
Ca	Relieves cough, chronic bronchitis, asthma, laxative, diuretic, sedative, vermifuge.	2.5g/day	These trace metals (Ca, Na, K, Mg) are macronutrients and are required in relatively large amounts in human diet. In high doses they may be toxic to body or produce deficiencies in other trace metals. Excessive intake of Ca result in deposits in soft tissue eg. kidneys. Excessive intake of Mg leads to diarrhoea. Plasma Na levels above 170mM may contribute to coma, paralysis of lung muscles and death. Fe is an important constituent of many enzymes, and thus plays a role in promoting immune competence. Symptoms of iron toxicity are fatigue, dizziness, nausea, vomiting, anaemia, headache, weight loss, shortness of breath, grayish colour skin. Constituent of eleven important oxidase enzymes that facilitate various principle metabolic functions. Cu is a heavy metal and is toxic in its unbound form. Symptoms of toxicity include: nausea, vomiting, diarrhoea, fatigue, apathy, hyperactivity. Zn plays an important role as co-fact in several biological enzymatic reactions. At high concentrations, stomach cramps, skin irritations, vomiting, nausea and anaemia may result. Mn is an essential metal. Symptoms of poisoning are, forgetfulness and nerve damage. A syndrome that is caused by Mn toxicity has symptoms such as Schizophrenia, dullness, weak muscle, headaches and insomnia.
Na	Extracellular and intracellular fluid balance and maintenance of blood viscosity, relieves urinary disease. Maintain homeostatic influences, cardiac muscle activity, relieves urinary disease, fever, diarrhoea, cough, chronic bronchitis. Maintenance of cells, relieves dropsy, piles and boils, purgative, diuretic		
K			
Mg			
Fe	Immune-booster, diuretic, astringent, demulcent, antipyretic, relieves fever, chronic rheumatism and psoriasis	45mg/day	
Cu	Hepatocure, gastric disorder.	10000µg/day	
Zn	Relieves fever, ulcer, cough, chronic bronchitis, increases tapotency towards infectious diseases	40mg/day	
Mn	Used as an astringent, treat scabies, pneumonia, bronchitis, asthma, headache and spermatorrhea.	11mg/day	

* Tolerable Upper Intake Levels (UL)

Methodology

Different commercial brands of Ginseng and African Potato products were purchased from pharmacies and health food stores in Cape Town for this study.

Evaluation of sample batch consistency from the same product brand type, as well across different brand types for the Ginseng and African Potato products respectively was investigated.

The products were evaluated for content validity and contamination for the following trace element and heavy metals, sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb) and mercury (Hg), Lithium (Li).

Digestion procedure prior to analysis followed the accurate weighing of the sample material. The acid digestion was adapted from the Karbochem Research and Development Laboratory. This followed, (i) combination of HCL/HF (1:4) , (ii) Nitric acid (iii) Perchloric acid as part of the procedure.

The instrument utilized for the trace element and heavy metal analysis was the combination, tandem system of Inductively Coupled Plasma - Mass Spectrometery (ICP-MS) that is a powerful, accurate, fast and sensitive, analytical technique.

Results

Figure 1 represents the statistical evaluation of tablets and capsules, individual mass of the Ginseng and African Potato products investigated respectively.

Statistics	A	B	C	D	E	F	G	H	I	J
Mean	0.6192	0.6200	0.4383	0.4323	0.6506	0.6427	0.5780	0.5468	0.5138	0.5160
Median	0.6270	0.6280	0.4389	0.4316	0.6590	0.6455	0.5798	0.5384	0.5090	0.5103
Minimum	0.5876	0.5965	0.4294	0.4238	0.6082	0.6128	0.5476	0.4972	0.4798	0.4760
Maximum	0.6303	0.6315	0.4466	0.4437	0.6706	0.6639	0.5982	0.5941	0.5802	0.5921
SD	0.0146	0.0159	0.0058	0.0064	0.0212	0.0193	0.0137	0.0349	0.0338	0.0373
SEM	0.0046	0.0050	0.0018	0.0020	0.0067	0.0061	0.0043	0.0110	0.0107	0.0118
p value	0.9031		0.0423		0.3942		0.0168		0.8936	

Fig 1. Ginseng - Formule Naturelle (A and B), Betterway (C and D), and Natrodale (E and F), African Potato - Inkomfe (G and H), and Trazure (I and J). n=10

Table 2. Element and Heavy metal content in selected Ginseng and Hypoxis products. Values are expressed in (mg/g) unless otherwise stated. * in ppm

	Na	Mg	K	Ca	Mn	Fe	Zn	Ni *	Cu *	Cr *	Pb *	Hg *	Li *
Tolerable Upper Intake Level (UL)	2300mg	420mg	4700mg	2500mg	11mg	45mg	40mg	1mg	15ppm	NR, 35µg (AI)	10ppm	0.5ppm	0.7mg
Naturelle (A)	54.12	3.06	7.40	21.36	0.06	0.17	0.05	4.55	6.20	13.90	0.44	0.07	17.00
Naturelle (B)	49.82	3.82	7.43	17.73	0.09	4.89	0.03	1.08	6.20	16.10	0.44	0.07	15.50
Betterway	60.53	1.23	11.12	13.29	0.02	0.80	0.03	4.27	7.61	24.60	8.03	0.00	42.60
Natrodale	35.67	0.46	9.64	5.48	0.03	1.69	0.02	2.00	5.66	16.20	10.6	0.34	9.00
Ginseng	21.85	2.72	2.82	41.25	0.22	1.04	0.22	2.47	6.28	7.84	0.27	0.04	6.51
Inkomfe (A)	45.68	2.13	14.56	1.85	0.26	0.07	0.09	3.95	16.10	21.10	13.80	0.60	26.00
Inkomfe (B)	32.20	1.27	9.08	1.50	0.15	0.35	0.01	2.41	11.00	13.20	4.79	0.51	14.80
Trazure (A)	41.25	1.76	12.35	8.45	0.40	0.46	0.05	3.22	8.31	13.00	5.68	0.55	35.10
Trazure (B)	49.77	1.76	5.59	17.75	0.40	0.53	0.05	2.67	8.81	13.00	0.61	0.10	15.60
FloraForce	36.84	0.14	0.51	2.77	0.01	0.46	0.03	8.80	6.78	0.17	2.28	0.64	22.80

Current results for the respective sub-section and group of Ginseng and African Potato products that were studied, show as general observation that the highest levels of Na, Mg, Ca, Fe, Zn, Cr, Hg and Li is present in Ginseng products, in comparison to African Potato products.

African Potato products investigated gave concentration highest for K, Mn, Ni, Cu, and Pb.

Conclusion

Trace elements and metals found in natural medicines may potentially **boost the therapeutic efficacy** of Complimentary and Traditional Medicines in Foods (CTMF) in general, and may facilitate the elimination of some toxic and plant chemical constituents.

The phyto-pharmaceutical industry in SA has huge potential which may be disadvantaged by lack of quality control testing and efficient product regulation. It is also important to recognize the impact that various trace element and heavy metals could have on its own, or collectively, in synergy, on various disease states, and with respect to drug treatment regimens.

There are many organizations and regulatory bodies and structures that already exist, such as the Direct Selling Association and Health Products Association that monitor and control the pharmaceutical industry in South Africa. Extensions of these entities, and having a **mechanism of enforcement** pertaining to the industry, may ensure better products for the consumer.

For the **safety, quality and efficacy** of these natural products standard operating procedures (SOP) for Good Agricultural Practice (GAP), Good Laboratory Practice (GAP), Good Supply Practice (GSP) and Good Manufacturing Practices (GMP) needs to be established, implemented and monitored.

References

1. **Oleszek, W.A.** Chromatographic determination of plant saponins. Journal of Chromatography A (2002); 967:147-162
2. **Grünwald, J.** The European Phytomedicines Market: figures, trends, analysis. HerbalGram (1995); 34:80-85.
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Acknowledgements

Thank you to the University of Cape Town for support and assistance.

Appendix 8

Conference Poster Presentation

2008 G.Gabriels, P.Smith. Trace element and heavy metal analysis of Commercial Ginseng and Hypoxis supplement products. WOCMAP IV World Conference on Medicinal and Aromatic Compounds 9th -14th November 2008, Cape Town, South Africa

Trace element and heavy metal analysis of Commercial Ginseng and Hypoxis supplement products

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Background

The World Health Organization (WHO) estimates that 60-80% of people in developing countries use plant medicines as primary source of health care. The use of traditional medicines and over the counter plant drugs has increased dramatically over the past decade, in both developing and developed countries [1].

The upsurge in the use of natural products include; (i) cost effectiveness and availability, (ii) perceived safety and lack of side effects, (iii) deteriorated belief in the efficacy and safety of orthodox drugs, (iv) public tendency towards self medication, (v) increase in the interest, publicity and sensational journalism [2]

Natural products extensive use as a source of primary health care requires routine quality control scientific testing methodologies to verify the safety, quality and efficacy of commercially available plant products. This will enhance public confidence in the use of such products in an unregulated environment as adverse side effects often associated with the use of traditional/ herbal products, are generally due to misidentification of plants, lack of standardization and good manufacturing practices, botanical substitution, adulteration, and contamination. The therapeutic effect of plant medicine is alleged to be enhanced by essential trace elements. Some products may however also contain excessive amounts of trace elements and heavy metals

For this study the focus was two product types. Ginseng as a popular traditional Chinese medicine, used for its adaptogenic and restorative properties, and African Potato products used as an immune booster for patients infected with TB/HIV/AIDS.

To determine the trace and heavy metal content of selected, different commercial brands of Ginseng and African Potato products purchased from pharmacies and health stores in Cape Town.

Table 1 Ginseng and Hypoxis products investigated

G1	Aspen Formule Naturelle Ginseng- BIN 400988
G2	Aspen Formule Naturelle Ginseng BIN 408746
G3	Natrodale Ginseng Vitality- MNF 20/Dec04 E1
G4	Natrodale Ginseng Vitality- MNF 20/Dec04 E1
G5	Bettaway Ginseng - 534521
G6	Bettaway Ginseng - 539702
H1	Inkomfe Hypoxis 5092
H2	Inkomfe Hypoxis 5478
H3	Inkomfe Hypoxis 5478
H4	Trazure African Potato Hypoxis BIN 303
H5	Trazure African Potato Hypoxis BIN 303

Materials and Methods

Different commercial brands of Ginseng and African Potato products were purchased from pharmacies and health food stores in Cape Town for this study.

Evaluation of sample batch consistency from the same product brand type, as well across different brand types for the Ginseng and African Potato products respectively was investigated

The products were evaluated for content validity and contamination for the following trace element and heavy metals, magnesium (Mg), potassium (K), calcium (Ca), iron (Fe), manganese (Mn), Lithium (Li), sodium (Na), chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), strontium (Sr), cadmium (Cd), mercury (Hg) and lead (Pb)

Digestion procedure prior to analysis followed the accurate weighing of the sample material. The acid digestion was adapted from the Karbochem Research and Development Laboratory. This followed, (i) combination of HCL/HF (1:4) , (ii) Nitric acid (iii) Perchloric acid as part of the procedure.

The instrument utilized for the trace element and heavy metal analysis was the combination, tandem system of Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) that is a powerful, accurate, fast and sensitive, analytical technique.

Results and Discussion

Figure 1 represents the statistical evaluation of tablets and capsules, individual mass of the Ginseng and African Potato products investigated respectively

Statistics	A	B	C	D	E	F	G	H	I	J
Mean	0.6192	0.6200	0.4383	0.4323	0.6606	0.6427	0.5780	0.5468	0.5138	0.5160
Median	0.6270	0.6260	0.4389	0.4316	0.6690	0.6455	0.5796	0.5384	0.5080	0.5103
Minimum	0.5876	0.5865	0.4294	0.4238	0.6082	0.6128	0.5476	0.4972	0.4798	0.4760
Maximum	0.6303	0.6315	0.4466	0.4437	0.6706	0.6639	0.5982	0.5941	0.5802	0.5921
SD	0.0146	0.0158	0.0058	0.0064	0.0212	0.0193	0.0137	0.0348	0.0338	0.0373
SEM	0.0046	0.0050	0.0018	0.0020	0.0067	0.0061	0.0043	0.0110	0.0107	0.0118
p-value	0.9031		0.0423		0.3942		0.0108		0.8836	

Fig 1. Ginseng - Formule Naturelle (A and B), Bettenway (C and D), and Natrodale (E and F), **African Potato** - Inkomfe (G and H), and Trazure (I and J). n=10

* Values are expressed in mg/g for Mg, K, Ca and Fe and in µg/g for Mn, Li, Na, Cr, Co, Ni, Cu, Zn, Sr, Cd, Hg, Pb

* BTB- Batch-to-batch consistency

Table 2 . Trace element and heavy metal content in selected Ginseng products.

	G1a	G1b	G2a	G2b	G3a	G3b	G4a	G4b	G5a	G5b	G6a	G6b
Mg	5.70	4.54	7.70	7.04	0.44	0.47	0.69	1.06	1.18	0.91	1.40	0.56
K	11.74	11.67	8.12	6.56	8.43	7.55	6.55	19.28	3.09	5.60	3.23	9.26
Ca	59.11	75.55	58.64	60.68	0.11	0.53	1.08	2.97	4.00	2.51	4.71	3.25
Fe	3.15	2.14	4.81	-	1.10	1.05	1.48	1.38	-	-	-	-
Mn	59.57	63.80	93.13	45.44	36.56	38.83	38.83	39.59	8.76	6.61	6.53	9.63
Li	3.54	-	14.68	20.86	-	-	-	-	-	2.47	-	-
Na			110.98					39.62	116.74		207.03	
Cr	-	-	-	-	-	-	-	-	-	-	-	-
Co	1.00	2.26	1.44	1.38	2.04	0.02	0.04	0.02	0.15	0.02	-	-
Ni	1.88	-	4.47	-	-	-	-	-	-	-	-	-
Cu	2.11	-	3.99	2.83	-	1.90	1.36	-	-	-	1.16	-
Zn	7.00		11.01	8.18				0.21			17.19	11.78
Sr	45.54	38.80	57.35	66.95	44.10	8.40	15.11	37.51	57.11	26.40	54.96	22.50
Cd	0.02	0.06	0.12	0.10	0.07	0.01	0.03	0.01	0.07	0.03	0.25	0.15
Hg	-	-	-	-	-	-	-	-	4.14	-	1.90	-
Pb	1.49	2.10	3.22		53.89	9.56	6.46	5.05	10.72	13.54	17.07	15.71
p value	0.8844		0.9061		0.1374		0.2947		0.5311		0.3284	
BTB	0.2259				0.1079				0.3402			

Table 3 . Trace element and heavy metal content in selected Hypoxis products.

	H1a	H1b	H2a	H2b	H3a	H3b	H4a	H4b	H5a	H5b
Mg	1.59	1.76	1.42	1.49	1.45	1.36	1.69	1.68	1.55	2.10
K	19.31	11.67	11.36	10.88	4.67	4.14	4.76	3.99	4.16	4.74
Ca	1.49	1.81	1.59	1.63	1.79	1.65	1.87	1.93	1.80	2.10
Fe						9.15				
Mn	255.45	251.13	247.28	245.18	257.74	248.19	466.19	417.88	383.59	389.45
Li		5.83			48.87	15.76				
Na					63.53	13.37				
Cr	-	-	-	-	-	-	-	-	-	-
Co	1.56	0.81	3.79	0.20	1.02	0.86	0.42	0.23	0.37	0.51
Ni	-	-	-	-	-	7.13	0.14	-	-	-
Cu	3.48	11.20	6.74	9.89	11.12	15.44	1.28	1.48	1.81	
Sr										
Cd										
Hg										
Pb										
p value	0.9741		0.0440		0.0996		0.1865		0.1097	
DTB	0.1087						0.3569			

Ginseng products as reflected in Table 2 showed intra-sample and batch-to-batch consistency for Mg, K, Ca, Mn, Fe, Sr, Cd, Pb, and could be used as "markers" . K and Mg had comparable concentration per gram equivalent level for all Ginseng products investigated. For Ca the concentration was highest for Formule Naturelle, followed by Bettaway and Natrodale. Mn was highest in Formulle Naturelle, followed by Natrodale and Bettaway. Fe was highest in the Naturelle product, followed by Natrodale. Sr was comparable for both Naturelle and Bettaway, and lower in Natrodale. Cd was in similar, but in low concentration. Pb was more prominent at higher levels in the Bettaway product, than that of Natrodale and Naturelle.

Hypoxis products as reflected in Table 3 showed intra-sample and batch-to-batch consistency for Mg, K, Ca, Mn, Co, Cu, Zn, Sr, Cd, Pb, and could be used as "markers". Mg Ca, Mn, Zn, Cd and Sr, had comparable concentration per gram equivalent level for all Hypoxis products investigated. K had higher levels in the Inkomfe, to that of the Trazure product respectively. Cu and Pb was elevated in the Inkomfe product compared to Trazure product.

Conclusion

Trace elements and metals found in natural medicines may potentially **boost the therapeutic efficacy** of Complimentary and Traditional Medicines in Foods (CTMF) in general, and may facilitate the elimination of some toxic and plant chemical constituents.

The phyto-pharmaceutical industry in SA has huge potential which may be disadvantaged by **lack of quality control testing** and efficient product regulation. It is also important to recognize the impact that various trace element and heavy metals could have on its' own, or collectively in synergy, on various disease states, and with respect to drug treatment regimens.

There are many organizations and regulatory bodies and structures that already exist, such as the Direct Selling Association and Health Products Association that monitor and control the phyto-pharmaceutical industry in South Africa. Enhancing the function of these entities, and having a **mechanism of enforcement** pertaining to the industry, may ensure better products for the consumer.

For the **safety, quality and efficacy** of these natural products standard operating procedures (SOP) for Good Agricultural Practice (GAP), Good Laboratory Practice (GAP), Good Supply Practice (GSP) and Good Manufacturing Practices (GMP) needs to be established , implemented and monitored.

References

1. **Oleszek, W.A.** Chromatographic determination of plant saponins. Journal of Chromatography A (2002); 967:147-162
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Acknowledgements

Thank you to the University of Cape Town and the Medical Research Council for support and assistance

Appendix 9a
Peer-Reviewed Publication

Gabriels, G., Lambert, M., Smith, P., Hiss, D. Will the new Consumer Protection Act prevent nutritional supplement users from harm? South African Medical Journal 2011; 101:543-545

Go to the link below to see the article in electronic format (and PDF)
http://www.samj.org.za/index.php/samj/article/viewFile/4946/3309

Will the new Consumer Protection Act prevent harm to nutritional supplement users?

Gary Gabriels, Mike Lambert, Pete Smith, Donavon Hiss

Background. There is no clear distinction between the regulation of food, supplements and medicines in South Africa. Consequently, grey areas exist in implementing the legislation, particularly in the supplement industry. The increase in supplement sales in South Africa can be attributed to aggressive marketing by manufacturers whose claims are not always supported by published peer-reviewed evidence. Such claims often go unchecked, resulting in consumers being mislead about the role of supplements. As a result of poor regulation, contaminants or adulterants in supplements may also cause insidious effects unrelated to the listed ingredients.

Aim. To assess the regulations, legislation, and claims associated with nutritional supplement products in South Africa.

Method. Peer-reviewed literature and the relevant South African statutes were consulted.

Results. The National Health Act incorporates the Medicine Control Council, which is charged with ensuring the safety, quality and effectiveness of medicines, and related matters, including complementary/alternative medicines. The South African Institute for Drug-Free Sport and Amendment Act provides for testing athletes for using banned substances, but currently does not concern itself with monitoring nutritional supplements for contaminants or adulterants that may cause a positive drug test, which has implications for sports participants and also the health of the general population. The implementation of the Consumer Protection Act 68 of 2008 (CPA) could protect consumer rights if it is administered and resourced appropriately.

Conclusion. The CPA should promote greater levels of policy development, regulatory enforcement, and consumer education of South Africa's supplement industry.

S Afr Med J 2011;101:543-545.

There is no clear distinction between food, supplements and medicines in South Africa. As these are regulated differently, grey areas exist in implementing the legislation, particularly in the supplement industry.

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The increase in supplement sales in South Africa can be attributed partly to aggressive marketing by manufacturers. Claims made by the companies selling supplements are not always supported by published peer-review evidence. Such claims often go unchecked, resulting in consumers being misled about the role of supplements. Contaminants or adulterants in supplements may also cause insidious effects unrelated to the listed ingredients. The Consumer Protection Act 68 of 2008 (CPA) could promote greater levels of policy development, regulatory enforcement, and consumer education of South Africa's supplement industry.

Nutritional supplement annual retail sales in the USA increased from $8.8 billion in 1994 to $18.8 billion in 2003 – an increase of 115%, of which much was spent on 'sports supplements'.[1,2] South Africa's supplement turnover was estimated at R1.5 billion per year (Health Product Association Survey 1998 - 2000) and continues to grow rapidly.[3] The increase in supplement sales is more probably due to aggressive marketing by manufacturers, rather than the

Appendix 9b
Peer-Reviewed Publication

Gabriels, G., Lambert, M., Smith, P Information on labels of nutritional supplements – time for legislation? South African Journal of Clinical Nutrition 2012; 25(1): 22-24

Go to the link below to see the article in electronic format (and PDF)
http://www.sajcn.co.za/index.php/SAJCN/article/view/559/842

Information on nutritional supplement labels: time for legislation?

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Keywords: nutritional, supplements, contaminants, claims, legislation, labelling

Abstract

Background: Nutritional supplements have received attention both from food manufacturers, as a means of marketing the added value to health; and from consumers, in terms of awareness, education, and improved health. To assist this process, it is important to have specific knowledge and understanding of the claims made on labels of nutritional supplement products used for general, and more specifically, for sports consumers. The industry is not regulated, and therefore the claims that are made may not always be accurate.

Method: The aim was to describe the labelling and claims information on the labels of a select group of nutritional supplements, either manufactured in, or imported into South Africa. Specific predetermined categories of labelling and claims made on the containers were assessed and summarised.

Results: Forty products were selected for analysis, of which 21 (53%) were locally assembled or manufactured products, and 19 (48%), international imported products. Ninety-five per cent of products contained a warning statement on the label. Eighty-five per cent of the nutritional supplement products had a disclaimer on the label. Ninety-eight per cent of the nutritional supplement product labels included some claim on the label.

Conclusion: The following information, in particular, needs to be regulated and enforced as part of the labelling process, to ensure that the consumer can make an informed choice. This includes highlighting the potential for adverse events, encouraging warning statements pertaining to "exclusion of use, and "not a cure for disease states", and alerting consumers of the potential for the presence of banned substances, based on laboratory screen methods.

Peer reviewed. (Submitted: 2011-08-10. Accepted: 2012-01-01.) © SAJCN S Afr J Clin Nutr 2012;25(1):22-26

Introduction

Nutritional supplements have received attention, both from manufacturers as a means of marketing the added value to health; and from consumers, in terms of awareness, education, and improved health. A supplement is a product taken orally that contains a "dietary ingredient" intended to supplement the diet. The "dietary ingredients" in these products may include vitamins, minerals, herbs, or other botanicals, amino acids, and substances, such as enzymes, organ tissues, glandulars, and metabolites.[1]

The regulatory framework of nutritional supplements should be compatible with public health and safety, protect the consumer, promote fair trade, and encourage innovation.[2-4] Consumer structures need to play a greater role in the development of a sound nutritional supplement management system, to ensure that appropriate legislation and regulation are developed and enforced on a sustainable basis, in line with the Consumer Protection Act (CPA) 68 of 2008.[5]

Section 29 of the CPA specifically states that a producer, importer, distributor, retailer or service provider, must not market any goods or services in a manner that is reasonably likely to imply a false or misleading representation, and that is misleading, fraudulent or deceptive in any way. Section 41 of the CPA specifically provides, and outlines, how claims that have been made could be affected.[5]

The South African Medical Control Council published a August 2011 guideline for comment, with regard to the quality, safety, and efficacy of complementary medicine. This guideline and its implementation could have relevance, and apply to nutritional supplements when it becomes law. If the specific scheduled complementary medicine or active ingredients that have an established identity and tradition of use, determined or known, are not declared on the label, or when claims are made, and are not clearly substantiated by peer-review scientific processes, a legal challenge could result.[6]

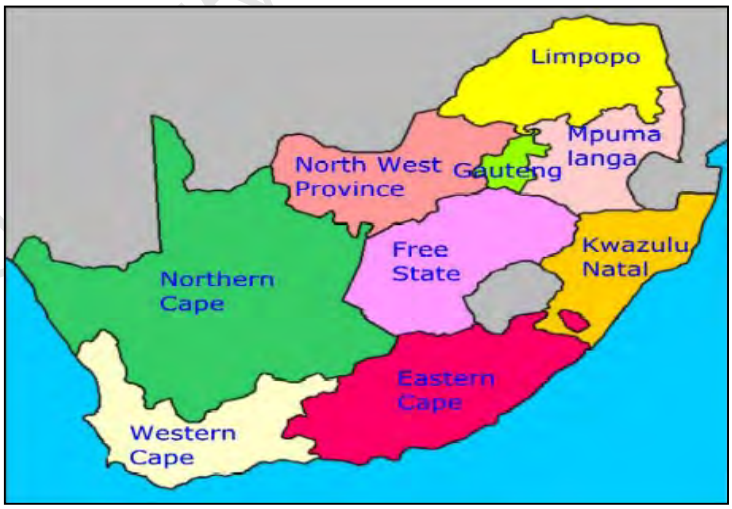
The concern among health professionals is that the supplement industry in South Africa is currently not regulated sufficiently. These

Appendix 10
Questionnaire Survey (Unpuplished Research)

The investigation and assessment of Nutritional and Traditional Supplement products.

Survey Questionnaire

Gary Gabriels



July 2008

Questionnaire

Please complete the questionnaire as accurately (truthfully and honestly) to the best of your ability and write /mark legibly please.

Section A - General Information (GI)

1. Date when completing questionnaire *(dd:mm:yy)*
2. State your following details. Your age (in years), estimated height (m) and weight (kg).

Age (in years)		Height (m)		Weight (kg)	

3. State your ethnicity. …….
4. Educational Experience – Which category reflects your highest educational experience.

Informal only		Primary School		High School	
Technical College		University Degree		Other *(state)*	

5. City of residence ……..
6. Province (in South Africa)

Western Cape		Eastern Cape		Kwazulu-Natal	
Gauteng		Free State		Mpumalanga	
Northern Cape		North West		Limpopo	

7. Occupation …….
8. Exact name of event where this questionnaire is being completed.

   …….

9. What is your Nationality……..
10. What is your country of origin……..

11. What is your marital status:

Single		Married		Divorced	

12. Do you have any children

Yes		No	

Section B - Health Status (HS)

13. How would you rate your own health at this moment. *(tick the relevant box with ×)*

Very Poor		Poor		Reasonable		Good		Very good	

14. How would you rate the quality of your own diet overall(**excluding** the use of supplements). *(tick the relevant box with ×)*

Very Poor		Poor		Reasonable		Good		Very good	

15. How many hours per week in total do you estimate you engage in some form of physical activity.

2 hours		4 hours		6 hours		8 hours		More than 8 hours	

16. Do you exercise in a gym. If yes, state the name of the gym.

Yes		No		Name of gym (state)	

17. Do you smoke cigarettes. If yes, state how many cigarettes you smoke per day.

Yes		No		How many cigarettes per day	

18. What best describes your alcohol consumption over a given week. *(tick the relevant box with ×)*

Do not consume alcohol at all	
Two glasses (2)	
Three glasses (3)	
More than three glasses	
Only in moderation on special occasions	

19. State which exercise(s) you participate in, and frequency of participation over the last three(3) months.

Type of Exercise	No	**Yes**	If **yes,** how many days per week	If **yes**, how many minutes per day
Bodybuilding/ toning (eg. weights, circuits)				
Cardiovascular machines/ track (eg. running, cycling)				
Cardiovascular classes (eg. Taibo, V-box, spinning)				
Flexibility classes (eg. Yoga)				

20. What are your goal(s) for doing exercise training? (*tick the relevant box with* ×)

	Strongly Disagree				Strongly Agree
Muscle building and strength	1	2	3	4	5
To stay healthy	1	2	3	4	5
To lose weight	1	2	3	4	5
To stay fit	1	2	3	4	5
To look good	1	2	3	4	5
To impress others	1	2	3	4	5
Other (*state*)…….....	1	2	3	4	5

21. List the organised sport types you currently participate in competitively, in order of priority.

1 (most important)	
2	
3	
4	
5	

Section C - Supplement Use/ Non Use (SU/NU)

Please read the DEFINTION of dietary supplement before proceeding further with the questionnaire.

Dietary Supplement Health and Education Act of 1994 (DSHEA) defines a dietary supplement as a "product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: (a) a vitamin; (b) a mineral; (c) a herb or other botanical; (d) an amino acid; (e) a dietary substance for use by man to supplement the diet by increasing the total dietary

intake; or (f) a concentrate, metabolite, constituent, extract, or combination of any ingredient described in clause (a), (b), (c), (d), or (e)".

Please proceed with the questionnaire - Thanks

ONLY ANSWER THIS QUESTION IF YOU DO NOT USE ANY SUPPLEMENTS

22. Why do you NOT use supplements? *(tick the relevant boxes with ×)*

	Strongly Disagree				Strongly Agree
Supplements are not necessary	1	2	3	4	5
Supplements do not work	1	2	3	4	5
I do not know enough about supplements	1	2	3	4	5
Supplements are too expensive	1	2	3	4	5
Supplements use can give you side effects	1	2	3	4	5
Supplements may contain banned substances	1	2	3	4	5
Other (*state*)…….....	1	2	3	4	5

If you <u>do not use supplements</u> and answered the <u>above</u> question then you may proceed to the end of the questionnaire. (Section F - Question 44)

If <u>you use supplements</u> , then proceed further with the remaining questions and sections of the questionnaire <u>below</u>.

23. Which phase of the season do you use supplements in general. (*tick the relevant box with ×*)

Base phase - (Pre- Season)	
Competition Phase	
Both- (Base Phase and Competition Phase)	

24. When do you use supplements in general. *(tick the relevant box with ×)*

Only on training days	
Unrelated to training days	
Competitions days	
Daily	

25. **Supplements Group A -** Mark which of the listed supplement(s) you used during the past 6 months and state the brand name in the column provided. *(tick the relevant boxes with ×*)

Example	×	Brand / Name Eg. vital
Multi vitamin and mineral		
Multi vitamin with no minerals		
Vitamin C		
Vitamin E		
Vitamin B complex		
B-carotene/Vitamin A		
Effervescent tablets (eg. berroca)		
Energy tonic (eg. bioplus/vita-thion		
Immune support (eg. viral guard)		
Anti-oxidants		
Zinc		
Magnesium		
Calcium		
Iron		
Selenium		
Boron		
Vanadium		
Chromium		
Procydin		
Spirulina		
Echinacea		
Herbal mix		
Astragalus		
Chorella		
Bee Pollen		
Flaxseed oil		
Pycnogenol		
Probiotics/ Prebiotics		
Essential fatty acids		
Inosine		
Other supplement(s) *(Name)*		

26. **Supplements Group B -** Mark which of the listed supplement(s) you used during the past 6 months and state the brand name of the supplement in column provided. *(tick the relevant boxes with* ×)

Example	×	Brand / Name Eg. Fast Fuel
Creatine		
Hydroxymethylbutyrate (HMB)		
Glutamine		
Arginine(eg alpha nitrox)		
Pretraining (arginine, creatine, caffeine)		
Lactic buffers/ bicarbonate		
Cramp attack/ cramp stop		
VO2max/ VO2peak/Rhodiola		
Endurox/Enduraid		
Carnitine		
Ribose		
Pyruvate		
ZMA		
Testosterone Enhancers		
DHEA- Dehydroepiandrosterone		
Fat burners (eg. phedracut)		
Glucosamine		
Condroitin Sulfate		
CLA- Conjugated linoleic acids		
Ornithine		
Androstenedione		
Ginseng		
Ginko biloba		
Green tea extract (EGCG)		
Caffeine/Guarana		
Yohimbine		
Ephedra/ Ma Huang		
Tribulus Terrestris		
Chrysin		
Indole-3 Carbinol		
Gamma oryzanol		
Simlax		
Mummio		
Saw palmetto		
Any other supplement(s) you use that is not listed above *(Name)*		

27. **Supplements Group C -** Mark which of the listed supplement(s) you used during the past 6 months and state the brand name in the column provided. *(tick the relevant boxes with ×*)

Example	×	Brand / Name Eg. USN muscle fuel
Energy drinks (eg. energade)		
Corn syrup		
Energy gels (eg. GU or Vooma)		
Carboloader		
Energy bars (eg. powerbar)		
Protein bars		
Protein powder (eg. whey/soy)		
Amino acid combo		
Weight gainer (eg. mega mass)		
Meal replacement (nutrin active)		
Recovery drink (eg. recovery max)		
Any other supplement(s) you use that is not listed above *(Name)*		

28. Indicate by rating with × which statement(s) describes your general use of supplements.

	Strongly Disagree				Strongly Agree
Obtain energy	1	2	3	4	5
Reduces fatique/ tiredness	1	2	3	4	5
Improve your performance	1	2	3	4	5
Prevent/treat illness	1	2	3	4	5
Improve general health	1	2	3	4	5
To build muscle	1	2	3	4	5
Compensate for less optimal diet	1	2	3	4	5
To meet unusual nutrient demands induced by exercise	1	2	3	4	5
Just like the taste	1	2	3	4	5
For rehydration	1	2	3	4	5
Enhance recovery	1	2	3	4	5
My diet does not provide what I need	1	2	3	4	5
To decrease body fat	1	2	3	4	5
To improve exercise recovery	1	2	3	4	5
To increase energy levels	1	2	3	4	5
To protect my joints	1	2	3	4	5
To prevent muscle cramps	1	2	3	4	5
Do not know	1	2	3	4	5
Other (*state*)…….....	1	2	3	4	5

29. What monetary estimate do you spend and/or are sponsored on nutritional supplements on a monthly basis. *(tick the relevant box with* ×)

Less than R500		Greater than R500		Greater than R1500	

30. How often do you purchase and/or sponsored most of your supplements products. *(tick the relevant boxes* ×)

Weekly		Monthly		Quarterly		As may be needed		At Promotions	

Section D - Perceptions, information sources and knowledge (SR)

31. How would you rate your knowledge and understanding of supplements.

Very Poor		Poor		Reasonable		Good		Very good	

32. How do you gain your sources of information to choose the supplements you use, or might intend using? *(tick the relevant boxes with* or ×)

	Strongly Disagree				Strongly Agree
Gym trainer	1	2	3	4	5
Fellow athletes	1	2	3	4	5
Supplement representative	1	2	3	4	5
Pharmacist	1	2	3	4	5
Dietician/ nutritionist	1	2	3	4	5
Doctor	1	2	3	4	5
Family	1	2	3	4	5
Friends	1	2	3	4	5
Magazines	1	2	3	4	5
Television	1	2	3	4	5
Radio	1	2	3	4	5
Internet	1	2	3	4	5
Other (*state*)…….....	1	2	3	4	5

33. Where and from whom do you usually buy/get your supplements. *(tick the relevant boxes with ×*)

	Strongly Disagree				Strongly Agree
Grocery store	1	2	3	4	5
Pharmacy	1	2	3	4	5
Health food store	1	2	3	4	5
Provided free of charge	1	2	3	4	5
Sport shop	1	2	3	4	5
Gym	1	2	3	4	5
Fitness Centre	1	2	3	4	5
Expo	1	2	3	4	5
Home based business	1	2	3	4	5
Nutritionist	1	2	3	4	5
Trainer/Coach	1	2	3	4	5
Mail Order	1	2	3	4	5
Internet	1	2	3	4	5
Other (*state*)…….....	1	2	3	4	5

34. List any negative experiences/ adverse effects, you believe associated with using supplements you have indicated previously. *(tick the relevant boxes with ×*)

	Strongly Disagree				Strongly Agree
Stomach cramps	1	2	3	4	5
Fever	1	2	3	4	5
Headache	1	2	3	4	5
Dehydration	1	2	3	4	5
Pain	1	2	3	4	5
Nervousness	1	2	3	4	5
Insomnia	1	2	3	4	5
Laxative effect	1	2	3	4	5
Increased heart rate	1	2	3	4	5
Loss of energy	1	2	3	4	5
Water retention	1	2	3	4	5
Reduced aerobic capacity	1	2	3	4	5
Diarrhoea	1	2	3	4	5
None	1	2	3	4	5
Other (state)…….	1	2	3	4	5

35. Do you consciously seek more knowledge and understanding of supplement products.

Yes		NO	

36. What are your overall perceptions of the quality and consistency of all the supplements you use. *(tick the relevant box with* ×)

Acceptable		Sometimes suspicious		Always suspicious	

37. Are you comfortable in general that all the supplement products you use are of good quality and not contaminated in any form. *(tick the relevant box* with ×)

	Strongly Disagree				Strongly Agree
I am confident	1	2	3	4	5

Section E - Marketing and Labelling (ML)

38. How do you read the labels/information on supplements products. *(tick the relevant boxes with* ×)

	Strongly Disagree				Strongly Agree
I look at the name	1	2	3	4	5
I look at the ingredients	1	2	3	4	5
I look at the directions for use	1	2	3	4	5
I look at the warnings for side effects	1	2	3	4	5
I look at the recommended dosage	1	2	3	4	5
I look at the nutritional information/ RDA	1	2	3	4	5
Other (*state*)…….....	1	2	3	4	5

39. Do you usually follow the dosage instructions on the supplement container label. *(tick the relevant box with* ×)

I normally **use less than** the dose the label states	
I normally use the **exact dose** as the label states	
I normally use **more than** the dose the label states	

40. Do you use supplement products that are often on promotion relative to your supplement needs.

Yes		NO	

41. Do you use supplement products that others have referred you to use.

Yes		NO	

42. Do you use any of these supplement products that is promoted for joint health and protection against ageing and overuse. *(tick the relevant boxes with ×*)

Antioxidants	
Essential fatty acids	
Vitamins: Niacin (B3)	
Pantothenate (B5),	
Minerals- boron, calcium	
Proteolytic enzymes	
Glucosamine	
Chondroitin	
Methyl sulphonylmethane (MSM)	
S – Adenosyl methionine (SaMe)	
Type 2 Collagen	
Hyaluronic acid	
Soy isofalvones	
None of the above	
Any other supplement for this condition. (*state)*	

43. What attracts you predominantly to the supplement products you use.

	Strongly Disagree				Strongly Agree
Taste	1	2	3	4	5
Product colour	1	2	3	4	5
The packaging	1	2	3	4	5
Friends and family referred	1	2	3	4	5
Labelling colour	1	2	3	4	5
Reputable and quality brand	1	2	3	4	5
Good manufacturing practice (GMP)	1	2	3	4	5
Sufficient/ adequate information and support.	1	2	3	4	5

Section F - General Comments (GC)

44. Any further comments on supplements you wish to provide.

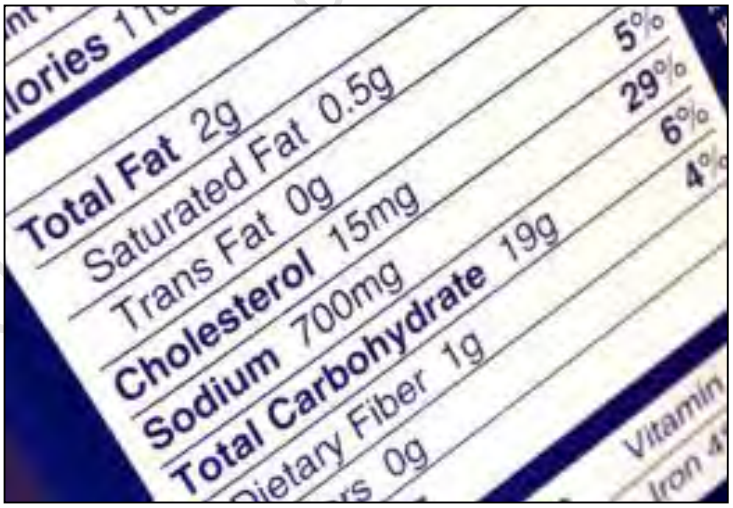
Thank you for your time and contribution in completing this survey

Appendix 11
Questionnaire Survey (Unpuplished Research)

Nutritional supplement use and knowledge of labelling information

Questionnaire

Gary Gabriels



June 2012

Questionnaire

Please complete the questionnaire as accurately (truthfully and honestly) to the best of your ability and write /mark legibly.

Question 1

Have you purchased a nutritional supplement in the last 12 months *(mark the relevant box with X)*

YES		NO	

Question 2

If you answered YES to Question 1, did the information on the container label influence your purchase? *(mark the relevant box with X)*

YES		NO	

Question 3

If you answered YES in Question 2, please rate each type of information on the nutritional supplement product container label that influenced your choice: *(tick the relevant box with X)*

	Absolutely no influence on my choice				Strong influence on my choice agree
Brand name	1	2	3	4	5
Ingredients	1	2	3	4	5
Recommended dosage and directions for use	1	2	3	4	5
Claims	1	2	3	4	5
Disclaimers and warnings	1	2	3	4	5
Quality of product	1	2	3	4	5
That the product is free of banned substance(s)	1	2	3	4	5
Other (state).....	1	2	3	4	5

Question 4

If you answered NO in Question 2, state what information contributed to you purchasing a nutritional supplement product: *(tick the relevant box with X)*

	Absolutely no influence on my choice				Strong influence on my choice agree
Coach, gym and/or fitness trainer, fellow athletes	1	2	3	4	5
Supplement representative	1	2	3	4	5
Pharmacist, Dietician, Nutritionist, Doctor	1	2	3	4	5
Information in print media – eg. magazine, newspaper	1	2	3	4	5
Information in electronic media – eg. television, radio	1	2	3	4	5
Information in internet and social media eg. website, twitter, facebook	1	2	3	4	5
Other *(state)*.....	1	2	3	4	5

Question 5

What is your age? (in years)

Question 6

What is your gender? (eg. male/female)

Male		Female	

Question 7

Do you regard yourself as:

Sedentary		Moderately physically active		Competitive sportsperson	