Complementary and Alternate Medicines:

A Forensic Analysis of the Potential Adulteration of Over-The-Counter Anorectics and "Lifestyle" Medicines in South Africa

By

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DECLARATION

I, Sandra Catterson, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Background:

Complementary and Alternate Medicines (CAMs) in South Africa are not yet subjected to the same rigorous testing required for allopathic (prescription) medication, yet they are freely available as over-the-counter medicines. Past research has shown the presence of a banned drug, sibutramine in natural anorectics and a schedule 6 prescription drug, sildenafil, found in natural erectile dysfunction preparations.

Methods: Initially, 26 exhibits (18 erectile dysfunction medicines and 8 anorectics) were screened for active pharmaceutical ingredients using high performance liquid chromatography tandem mass spectrometry. An AB SCIEX 3200 TRAP® linear ion-trap quadrupole mass spectrometer was used to detect and subsequently quantitate these active pharmaceutical ingredients using a targeted multiple reaction monitoring mode. Samples were extracted with 50% v/v methanol in water. A method for the quantitation of sildenafil was subsequently partially validated. The intra- and inter-assay precisions were evaluated and the linearity of the method was investigated in the range of 20 ng/mL to 2000 ng/mL. The method was then successfully applied to a random selection of CAMs. A random sample (n=61) of erectile dysfunction CAMs were selected for quantitation from two different clusters. Cluster 1 comprised of supermarkets and cluster 2 of pharmacies.

Results: The validation method for sildenafil showed that the limit of detection was 1.09 ng/mL and the limit of quantitation was 20 ng/mL. The correlation co-efficient and bias were less than 20%. Initial screening of the 26 exhibits indicated that sildenafil was present in 12 of the 18 samples tested and sibutramine in 6 of the 8 anorectics. Of the later 61 exhibits tested, 43 tested positive for sildenafil. The mass of sildenafil per sample ranged from 1.09 ng/mL to 123.7 mg/sample.

Conclusion: The lack of label content, regulation and legislation exposes the consumer to the risk of consuming an active pharmaceutical ingredient which may very likely have an adverse effect on their health. There is a need to raise public awareness to the potential dangers of unregulated CAMs, encourage doctors to become more aware of their patients' consumption of CAMs and to motivate the Medicines Control Council to follow through with their deadlines for the regulation of CAMs.

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ABBREVIATIONS

AER	Adverse Events Report
AIFA	The Italian Medicines Agency
API	Active pharmaceutical ingredient
CAMs	Complementary and Alternate Medicines
CDER	Centre for Drug Evaluation and Research
cGMP	Cyclic guanosine monophosphate
DBRUP	Division of Reproductive and Urologic Products
DoH	Department of Health
EMA	European Medicines Agency
FDA	American Food and Drug Administration
HPA	Health Products Association
IMPACT	International Medical Products Anti-Counterfeiting Taskforce
IS	Internal Standard
ISMH	International Society of Medical Hydrology and Climatology
LOD	Limit of Detection
LOQ	Limit of Quantitation
MCC	Medicines Control Council
NAION	Non-arteritic anterior ischemic optic neuropathy
OTC	Over-The-Counter
PDE-5	Phosphodiesterase type-5
RASFF	Rapid Alert System on Food and Feed
rpm	revolutions per minute
SA	South Africa
TCM	Traditional Chinese Medicine
TRC	Toronto Research Chemicals Inc.
WCG	Western Cape Government

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CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Worldwide, health problems such as erectile dysfunction (ED) and obesity are increasing in prevalence. For numerous reasons these conditions are being self-treated with Complementary and Alternate Medicines (CAMs) instead of visiting a physician (Sucar *et al.* 2002 ; Lee *et al.* 2011; Venhuis *et al.* 2012; Ponnuru *et al.* 2012; Li *et al.* 2013; Rocha, 2016). Complementary implies in addition to prescription/allopathic/orthodox/western drugs and alternate means used instead of these drugs. The term CAM also includes practices such as spiritual, yoga and acupuncture as well as traditional remedies – African, Chinese and Ayurveda, these can include plants and animal parts. CAMs are untested by the South African Medicines Control Council (MCC) and as such may place the consumer at risk (MCC*,* 2016).

Erectile dysfunction is a disease of sexual impotence that affects approximately 150 million men worldwide (Aytaç *et al.* 1999). Men have been affected by ED for centuries and remedies have been provided since as early as 1600 BC (Schrameck *et al.* 2014). In the 1990's, a worldwide solution to ED came in the form of a drug called; Viagra® which contains the active ingredient sildenafil (Pfizer, United States of America). It was approved by the American Food and Drug Administration (FDA) in April 1998 and was made available to the public in 1999, resulting in 30 million men diagnosed medically with ED seeking treatment with Viagra® (FDA, 2016). Consequently, other drugs such as tadalafil and vardenafil amongst others, have since been approved for the treatment of this disease (FDA, 2016). Drugs have also become the approved treatment of choice for other global health issues.

A public health issue affecting millions of people is obesity. It is a chronic disease, the incidence of which has been increasing in recent years (Sucar *et al.* 2002; Ponnuru *et al.* 2012; Li *et al.* 2013). Obesity is associated with an increased risk of cardiovascular disease, hypertension, diabetes, high cholesterol, sleep deprivation, and cancer. In 2014, Ng et al estimated overweight and obesity to have caused 3.4 million deaths. These authors reported that the highest rate of obesity among adults in sub-Saharan Africa is found in South Africa (SA) with a rate amongst women at 42%. Medical solutions for obesity have surfaced in the form of prescription drugs such as sibutramine, phentermine and fenfluramine (FDA, 2016).

Prescription drugs, whilst beneficial, are not always tolerated by each individual (Lee *et al.* 2015) who then seek alternative treatment to medication (Ventola, 2010). Lifestyle changes such as exercise and healthy eating, as an alternative to medication, can also reduce ED and obesity, however, this takes both time and effort. In order to avoid lifestyle

changes or orthodox medicine (OM) and to obtain rapid results, an increased number of people are turning to what they perceive to be a safe, quick alternative in the form of CAMs (Rocha, 2016; Lee *et al.* 2011; Venhuis *et al.* 2012; Lee *et al.* 2013). These herbal medicines are freely and conveniently available as Over-The-Counter (OTC) medicines and they do not require a doctor's visit or a prescription. This makes them an appealing alternative to OM (Ventola, 2010).

CAMs are, however, not subjected to the same stringent testing applicable to OM exposing them to possible adulteration which means Active Pharmaceutical Ingredients are hidden in these products(API's) (Department of Health, 1965). Most consumers are unaware of this and therefore, rely on the product label for information on the contents, side effects and special precautions (Novella, 2010). CAMs by their very nature and by law should only contain natural ingredients (Department of Health, 1965). The product label on CAMs states this and in some cases, even confirms that they do not contain any API's (MCC*,* 2016). The problem is that the label, if present, is based on unregulated ingredients and is likely to be inaccurate in many ways.

This lack of regulation together with the demand and the huge economic value ascribed to the global trade of CAMs has made them prone to adulteration with API's (Wheatley & Spink , 2013). The consumer's health is therefore at risk when they consume CAMs which have been illegally advertised as "all natural" (Li *et al.* 2013). These products therefore, need to be analyzed quickly and efficiently for API's and the public must be made aware of the outcomes.

For purposes of quality control and product and public safety, it has become necessary to establish analytical procedures which can selectively detect and quantify any API possibly unlawfully concealed in a CAM (Rocha *et al.* 2016). High Performance Liquid Chromatography tandem Mass Spectrometry (HPLC-MS/MS) is an accurate analytical method which can identify and quantitate API's (Montesano *et al.* 2014). Whilst many studies have been performed worldwide to detect API's hidden in CAMs, a validated method for the quantitation of API's in ED and anorectic CAMs is yet to be established in products available in SA (Pittler, Ernst, 2002 & 2003;Menniti-Ippolito & Mazzanti *et al.* 2005; Gallo, Giocaliere *et al.* 2012, Neergheen-Bhujun 2013;Posadzki, Watson *et al.* 2013). The next section defines CAMs and discusses their popularity and dangers of adulteration.

1.2 Complementary and Alternate Medicines (CAMs)

1.2.1 Definition

Non-conventional (herbal) medicines or CAMs include medicines conveniently and freely available to the public OTC without a prescription and/or license. In 1993, the World Health Organization (WHO) defined CAMs as all forms of healthcare not included in the official health sector (Eisenberg et al. 1993). In 2008, the National Institutes of Health (NIH) defined CAMs as a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine. Natural alternatives to OM are appealing to the public and their consumption has been increasing.

1.2.2 Popularity, Consumption and Impact on the Economy

According to a WHO survey, non-conventional medicine was used by over 70% of the world's population (WHO, 2008). Most of these populations, however, reside in developing countries where access to OM may be limited (Akerele,1993). According to a popular science website, there has been a growing trend which, unusually shows Western countries also beginning to rely increasingly on CAMs (Farran, 2017). Anecdotal evidence predicts the global market for CAMs to reach an estimated marketing value of US$107 billion by 2017 (San Jose, 2012). In 1993, a survey conducted by Eisenberg *et al.*, claimed that in 1990 Americans made an estimated 425 million visits to providers of non-conventional therapy. This was 37 million more visits than to all US primary care physicians and at a cost of approximately US $13.7 billion. By 2010, 40% of the US population was using some form of CAM (Ventola, 2010). The use of CAMs in SA has also increased, with the market for CAMs and health products at consumer level increasing from approximately R1.9 billion in 2003 to an approximate R8 billion in 2014 (Thomson & Tucker, 2007; Kahn, 2014). The reasons for this increase in consumption of CAMs are numerous.

1.2.3 Reasons for Increase in Procurement and Consumption

Ventola and colleagues (2010) reported that with the increased availability of information on the Internet there was an increase in the general dissatisfaction of the public with OM together with a growing distrust and frustration with the health care system. OM is becoming less popular as it is not able to cure all diseases, patients may have side effects

from OMs or their current medication may preclude their use (Ventola, 2010). According to the Nemours Foundation (2016), factors which play a role are expense, convenience and consumers enjoying taking responsibility for their own health. CAMs are perceived to be cheaper than OM (Ventola, 2010) and there is a universal and pervasive belief that CAMs are a safer alternative to allopathic medicine (Novella, 2010). Marketing promises rapid results with a safe, natural and side effect free product. CAMs need to be effective in order to sell, which increases the seller's profit (Rocha *et al.* 2016). A scientific website points out that anorectics (weight-loss dietary supplements), for example, will not be used by consumers unless they actually realize rapid initial effects (Anderson, 2017). In order to have an effective product which works, manufacturers are resorting to illegally hiding API's in their all-natural products which exposes the consumer to risks which could be severe.

1.2.4 Dangers of Consumption and Adulteration

The huge economic value ascribed to the global trade of CAMs has made them prone to adulteration for economic reasons and profit increases which puts the consumers' health in jeopardy (Wheatley & Spink 2013). The Pharmaceutical Security Institute (2002) stated that the trading of counterfeit CAMs is international and continually increasing. The WHO has estimated that 30% of CAMs on sale in Africa, Southeast Asia and Latin America are adulterated (WHO, 2008). The majority of CAMs consumed are those for weight-loss, muscle building/sport performance and sexual performance enhancement purposes, these are therefore the most prone to adulteration (Rocha *et al.* 2016). Miller, Zhang and colleagues (2012), define adulteration of CAMs as a fraudulent practice that includes adulteration with inessential, improper or inferior products. A cheap filler, whilst unscrupulous, should be less harmful but adulteration with an API can have serious repercussions (Ernst, 2003; Li *et al.* 2013). In a systematic review by Ernst (2002), 2 600 samples of traditional Chinese medicine from Taiwan were analyzed and 24% were found to be adulterated with API's. Overall, at least one death and six potentially life-threatening complications were reported in this review. Low *et al.* (2009) state that ten deaths had been recorded in Singapore by August 2009 as a direct result of the consumption of CAMs adulterated with medication. A considerable amount of literature has been published

showing herbal medicines to contain adulterated, banned or scheduled products, the most prevalent API's are sibutramine, phentermine and fenfluramine in the anorectics and sildenafil, vardenafil, tadalafil and their analogues in ED medications (Appendix A Table 1.1 and Table 1.2) (Rocha *et al.* 2016). Alongside the growth of CAMs, there has been a decrease in good manufacturing practice (Gratz *et al.* 2004). This had raised concerns at the time with regard to the quality and safety of these products (Li *et al.* 2003). Although tighter regulations of CAMs have become a worldwide movement, the safe use of CAMs is still a challenge. It has been suggested that this may be due to the limited accessibility of reliable information and misconceptions concerning CAMs that are widespread among consumers (Damiano *et al.* 2014). Tighter regulations and legislation are vital to protect the consumer.

1.2.5 Legislation in South Africa

The MCC controls medicines in SA and claims to apply standards laid down by the Medicines and Related Substances Act, which governs the manufacture, distribution, sale, and marketing of medicines (MCC, 2016, Department of Health, 1965). Whilst SA is one of the few nations that have made significant progress to integrate traditional and complementary medicine into the legislative framework for health practitioners, CAMs are still mostly unregulated (NIH, 2008). By 2013, no testing or regulation had been introduced and in fact, the MCC instead were overwhelmed by the backlog of medications to be tested. The Health Minister Dr. Aaron Matsoaledi in October 2010 stated that approximately 155 thousand submissions for complementary medicines had been received by the Department of Health (DoH) since February 2002. Motsoaledi noted that none of these complementary medicines were evaluated for safety, quality and efficacy as the process, procedures and guidelines, were not yet in place (Meyer, 2013). In November 2013, the MCC issued a mandate requiring all CAMs to be regulated. In terms of the government gazette 37032, Notice R.870 of 15th November 2013 a timeline was produced (Appendix B). Products which contained "banned" or scheduled substances had to be removed with immediate effect. All slimming products as well as sexual

stimulation (“lifestyle”) products needed to be tested by November 2015. The intention of the MCC is to have all CAMs regulated by 2019 (Department of Health, 1965).
These requirements of the MCC are even more stringent than the FDA and there are therefore, lobbyists against the testing. There is a concern that free public choice will be taken away if CAMs are removed (Viall, 2014). Until all CAMs have been regulated the product label is therefore the only source of information for the consumer.

1.2.6 Product Labelling

If a CAM is untested, according to the MCC, in terms of section 14/2 of the Medicines Act, wholesalers and retailers, importers and manufacturers can either remove the untested product or provide a label which states that the product has not been approved as a medicine by the MCC and can therefore make no medical claim in any form (MCC*,* 2016). This could consequently result in many products when tested having to be withdrawn from the market at a loss of billions of SA Rand.

Labelling alone does not necessarily protect the consumer. Some products actually do state on the label that they contain an API but they often actually do not or contain much more than is stated on the label (Stecher *et al.* 2010). At the International Society of Medical Hidrology and Climatology (ISMH) World Congress, 2010, the UK authorities seized over 200 products obtained from the internet which were labelled as containing sildenafil, the active ingredient in Viagra® which is a phosphodiesterase type-5 (PDE-5) inhibitor and a prescription drug used for ED. On analysis of these products 85% were found to be counterfeit, either containing no sildenafil, sildenafil and tadalafil (also a PDE-5 inhibitor) or much more than the amount stated on the label (Stecher *et al.* 2010). Consumers therefore need to be educated that they have the right to know what they are taking.

1.2.7 Education and Research

Education and research are vital in combatting the adulteration of CAMs. Yet, despite the high prevalence of ED in SA, only five SA articles have addressed this since 1970 (Campbell & Stein, 2014). The core claim of CAMs is that they are marketed and labelled

as 100% natural (Singh *et al.* 2009) and ubiquitously assumed to be safe (Novella, 2010). Their appeal is also amplified by the fact that CAMs are now being sold widely and in SA, are easily obtainable in supermarkets, health shops and reputable pharmacy outlets. Consumers often rely on what the product label states and do not do any further research. This leaves the consumer vulnerable to the consumption of an API without their knowledge which could potentially have severe if not lethal side effects or interactions with other medicines already being administered. Ernst stated that as these herbal medicinal products are regularly associated with serious adverse cardiovascular events, it is vital for the consumer to be vigilant (Ernst, 2003).

International collaboration is necessary with authorities and regulators working together to curb the illicit adulteration of CAMs. Numerous countries are already making progress in this regard. The Italian Medicines Agency (A.I.F.A.) is a member of the "International Medical Products Anti-Counterfeiting Taskforce" (I.M.P.A.C.T.) proposed by WHO in 2007. I.M.P.A.C.T is active in reinforcing international collaboration to seek solutions to the global challenge of counterfeit medical products and to raise awareness of their dangers (Damiano *et al.* 2014). In the US the submission of a voluntary adverse events report (AER) allows the FDA to monitor safety issues that require intervention, these include adverse reactions to CAMs (GAO.Gov 2013) and in the EU, the rapid communication system on food and feed (RASFF) is available in an effort to encourage collaboration between the public and authorities. In SA, numerous websites provide an informal platform for the public to discuss issues surrounding CAM adulteration and usage (Lee, 2014). A more formal platform and international collaboration should increase public awareness and assist in protecting the consumer.

The use and abuse of CAMs is extensive but consumers have very little knowledge of their potential adverse effects (Giveon *et al.* 2004) and as such should inform their doctor of all CAMs consumed. Problematically, consumers, assuming CAMs are safe do not deem it necessary to report their usage to their doctor. Giveon and colleagues (2004) suggested that the public needs to be educated on the necessity of reporting such usage

to the family physician. The medical practitioner, however, also has the responsibility to question the patient on their CAMs practise.

1.2.8 Role of the Medical Practitioner

As early as 1993 Eisenberg *et al.* found in a survey that 72 percent of the respondents who used non-conventional therapy did not inform their doctor (Eisenberg *et al.* 1993). This was corroborated by Penson *et al.* (2001) mainly because consumers believe CAMs fall outside the rubric of conventional medicine or because physicians do not ask (Giveon *et al.* 2004). Penson and colleagues (2001) suggest physicians need to be informed about CAMs and be attuned to the psychosocial needs of patients. In a review by Cohen and Ernst *et al.* (2010), the authors place emphasis on the responsibility of the doctor to garner from the patient all possible information of their CAMs consumption. It seems critical that caregivers be aware of the potential risk of patient's use of CAMs (Bagnis, Deray *et al.* 2004). An article published in 2010 highlights the need for doctors to provide patient counselling (Ventola *et al.* 2010) and their use of CAMs should be an integral part of the history taking (Giveon *et al.* 2004). This would unquestionably go a long way in educating the public and possibly prevent/identify health risks, side effects, toxicity, overdoses or contra-indications. It may also assist at autopsies in distinguishing whether CAMs may have caused or contributed to an individual's death.

1.2.9 Forensic Toxicology

Undisclosed CAMs consumption is a reality and this consumption, especially if the product is adulterated, could place the consumer in a potentially lethal situation. The adulterated CAMs could be partly or fully responsible for the cause of death and this may go undetected (Skerrett *et al.* 2010). It would be possible to find sildenafil, sibutramine and/or their metabolites in the blood and urine of decedents who had not been prescribed either drug. Forensic toxicologists who have detected these drugs and/or their metabolites should suggest that medicolegal death investigators look both for prescriptions for these drugs and for CAMs at the death scene and among the

decedent's possessions. Information regarding a decedent's use of adulterated CAMs may aid the coroner or medical examiner in determining cause and manner of death. Attempts to regulate CAMs are likely to face public opposition. The public needs to be informed of the widespread adulteration of CAMs not only because of immediate safety concerns but also because the producers of CAMs for erectile dysfunction and obesity have little faith in the efficacy of their natural ingredients.

ED and obesity are public health issues affecting millions of people who are all looking for a solution.

1.3 Erectile Dysfunction

ED is a condition affecting men resulting in them being unable to achieve or maintain an erection. Over 18 million men in the US are affected by ED (Medscape, 2016). The prevalence of ED in the US is higher in men with cardiovascular disease (50%) and diabetes (51%), and is increased with such lifestyle factors as smoking (13%) and obesity (22%) (Selvin *et al.* 2007). SA men are becoming some of the world's biggest consumers of black market/illegal CAMs for ED. According to a newspaper report, between April 2014 and March 2015 an estimated R21 million worth of black market drugs for sexual enhancement were seized at SA ports of entry (Citizen, 2016). Medication has been successfully used to treat ED.

1.3.1 Sildenafil

1.3.1.1 Mechanism of Action

Sildenafil citrate is the API in Viagra® (Pfizer) (Figure 1.1). Sildenafil works to promote erection by inhibiting the enzyme PDE-5 in the corpus cavernosum of the penis. Sildenafil has a similar structure to cyclic guanosine monophosphate (cGMP) and the PDE-5 inhibitor binds to sildenafil instead of degrading the cGMP. This increases the nitrogen

dioxide and cGMP concentration which facilitates penile erection by the smooth muscle relaxation of helicine arteries resulting in an increase in blood flow (Singh *et al.* 2009). Tadalafil (Cialis®, Eli Lilly) and vardenafil (Levitra®, Bayer) are very similar to sildenafil but tadalafil has a longer duration and milder effect than sildenafil so it is, therefore, becoming the more popular choice (Doggrel, 2005).

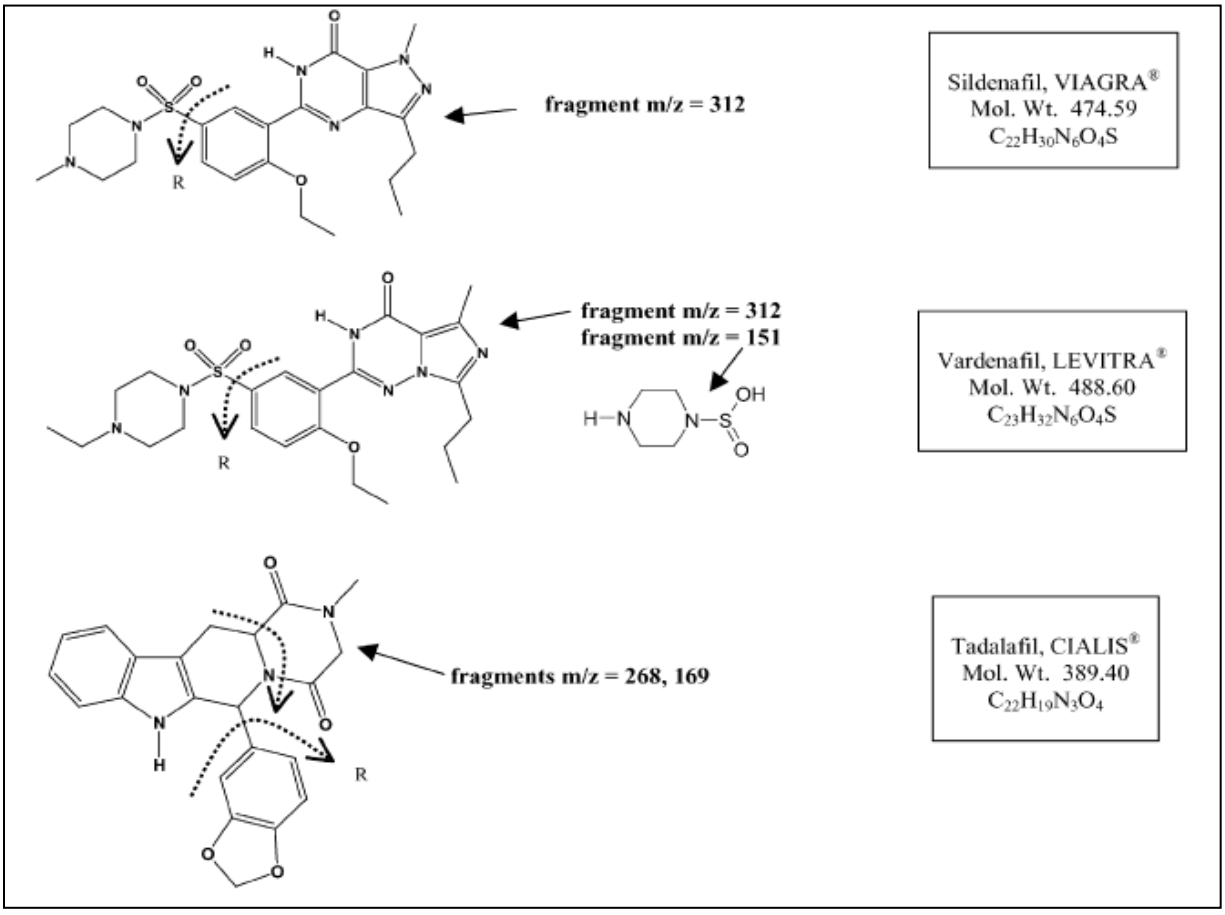

Figure 1.1. The molecular structures of sildenafil, vardenafil and tadalafil showing possible fragmentation patterns (Gratz *et al.* 2005)

Whilst sildenafil has been of huge benefit to the male population, incorrect use of this drug and its abuse may have dangerous consequences.

1.3.1.2 Dangers of Sildenafil Consumption

In SA, Viagra® is a schedule 6 drug which can only be dispensed with an original prescription (written out by the doctor after a consultation) as it has numerous side effects such as syncope and is contra-indicated with blood pressure medications (Pfizer.ca 2013). Kontaras and colleagues state that sildenafil itself is not a dangerous drug and they advocate that if used appropriately it does not seem to increase the risk of myocardial infarction or sudden cardiac death (Kontaras *et al.* 2008). Similarly, Jun Park *et al.* in their 2008 study could also not find any significant detrimental side effects when Viagra® was taken in hypertensive patients on antihypertensive drugs. Another study showed how the use of sildenafil citrate not only reduced male impotence but additionally improved self-esteem, confidence and relationships (Althof, O'Leary *et al.* 2006). These studies, however, fail to highlight the dangers of the interaction of these PDE-5 inhibitors with other medication, for example nitrates, which could lead to fatal hypotension (Doggrel, 2005). People suffering from heart disease, diabetes, high blood pressure or high cholesterol are often prescribed nitrates. Although Kontaras *et al.* advocated the safety of Viagra®, they did concede it can be potentially toxic (Kontaras *et al.* 2008). The product monograph for Viagra® warns that the use of organic nitrates is absolutely contraindicated. This is for either regular and/or sporadic consumption and in any form (e.g. oral, sublingual, transdermal, by inhalation) (Pfizer.ca, 2013). In addition, it states that anyone with pre-existing cardiovascular risk factors, who consumes a PDE-5 inhibitor, runs the risk of myocardial infarction, sudden cardiac death, transient ischaemic attack and/or hypotension (Pfizer.ca, 2013). Rocha *et al.* (2016) added headaches, flushing, dyspepsia, nasal congestion, and visual disorders to the side effects. The authors also included α-blockers as well as the above-mentioned nitrates, as being contraindicated with PDE-5 inhibitor use (Rocha *et al.* 2016). Sildenafil is also contra-indicated in patients who have experienced loss of vision because of a problem with blood flow to the nerve in the eye (non-arteritic anterior ischemic optic neuropathy or NAION)

(Ema.europa.eu, 2016). Given the side effects and contraindications for Viagra®, the use of an all-natural product as an alternative becomes very appealing.

1.3.2 Complementary and Alternate Medicines for Erectile Dysfunction

Men are often embarrassed to tell their doctors about their ED condition and this together with the lure of an all-natural, easily obtainable, OTC product, with no side effects has resulted in the increased use of CAMs (Citizen, 2016). Venhuis *et al.* (2012) claim that sales of "lifestyle" product CAMs rivals and possibly exceeds legitimate sales of ED medication. They estimate that in Europe, in 2009, for every two legitimate prescriptions of an ED medicine, an additional third was purchased as a CAM. This means that every year, six million purchases of ED medication occur outside of the official distribution (Venhuis *et al.* 2012). Although CAMs are unregulated and could pose potential health risks, they have become a popular choice (Singh *et al.* 2009). The increased CAMs use for ED has permeated sexually healthy male communities who are looking for increased sexual enhancement (Kim *et al.* 2002). Sometimes these "lifestyle" products are used by young men without ED in order to enhance sexual performance for recreational purposes (Bechara *et al.* 2010). Frequently in the gay community, it has been reported that natural ED CAMs are used to counteract the effects of illicit drugs on achievement of erection with detrimental effects (Kim *et al.* 2002). In Bangladesh, the increased use of ED medication has resulted in a rise in sexual violence and perversion (Podder *et al.* 2014). The increased popularity of CAMs for ED has exposed them to adulteration for economic gain by dishonest manufacturers (Lee *et al.* 2015; Jeong *et al.* 2016).

1.3.3 Adulteration of Complementary and Alternate Medicines for Erectile Dysfunction

The popularity of ED CAMs has made them prone to adulteration (Rocha *et al.* 2016). Leaving the consumer at risk of contra-indications or overdose. Also, a small group may exist for whom sildenafil may be toxic. Mark Hirsch, a medical team leader at the Centre for Drug Evaluation and Research Division of Reproductive and Urologic Products

(CDER) warned that the undisclosed presence of prescription drug ingredients can lead to serious side effects in users (FDA.gov, 2016).

Viagra® is available in 25 mg, 50 mg and 100 mg doses taken as needed only once per day (Medlineplus.gov, 2016). Sildenafil overdose can cause priapism/prolonged erection, which if not treated immediately, may result in penile tissue damage and/or permanent loss of potency (Medlineplus.gov, 2016).

It has also been reported that CAMs have been adulterated with analogues of the original prescription drug (Oh *et al.* 2006; Patel at al 2014; Jeong *et al.* 2016; Lee *et al.* 2015; Lee at al 2013). The illegal addition of these analogues has increased tenfold over the last ten years (Lee *et al.* 2014). These analogues are created by subtly modifying the chemical structures of pharmaceuticals making them extremely difficult to detect and are a way for producers to evade regulatory inspection (Damiano *et al.* 2014). As no pharmacological studies are available for these analogues, it makes them very dangerous as they have not been tested formally for quality, safety and efficacy (Racho *et al.*; Yeun *et al.* 2007). In addition, they can be easily created from patent literature which is publicly accessible (Venhuis *et al.* 2012). CAMs for weight loss are also being adulterated with synthetic anorectics.

1.4 Anorectics: Sibutramine

1.4.1.1 Mechanism of Action

Serotonin is a chemical neurotransmitter linked to a number of brain functions, including a feeling of satiety. Sibutramine (Figure 1.2) acts as an anorectic by inhibiting the reuptake of serotonin and noradrenaline into neurons of the brain, resulting in higher concentrations of these compounds at the synaptic clefts, thus leading to a reduction in appetite (Deconinck *et al.* 2014). Sibutramine is derived from amphetamines and was approved by the FDA in 1997 (Zou *et al.* 2007). Phentermine and fenfluramine are also

popular synthetic anorectics which are scheduled drugs and even banned in certain countries. Fenfluramine works by releasing extra amounts of serotonin into the body. However, as with sibutramine this can lead to an excess of serotonin and serotonin syndrome can result. Too much serotonin can be fatal. Fenfluramine or dexfenfluramine has been used together with phentermine (a stimulant) in a combination known as “fen-phen” which doctors have mixed as an “off label” product (in a way not intended by the manufacturers) (Futures of Palm Beach FL Addiction Treatment, Rehab, and Detox Centre 2016). These anorectics have many side effects and may even result in death if abused.

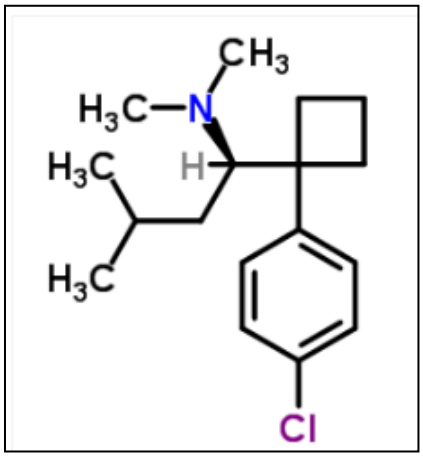

Figure 1.2: The molecular structure of sibutramine (Chemspider.com)

1.4.1.2 Dangers of Sibutramine Consumption

Ozdemir *et al.* (2013) listed headache, dry oropharyngeal mucosa, anorexia, constipation, and insomnia as the most commonly reported side effects of sibutramine use whereas, tachycardia, hypertension, headache, and dizziness are reported toxic effects of overdose. Serotonin syndrome can result if sibutramine is taken in conjunction with other serotonin reuptake inhibitors or if the herbal/natural product is adulterated with more than the recommended dose of sibutramine. This has been reported to present as anxiety, agitation, dizziness, confusion, excess sweating, disorientation, painful joints, hyper excitement, fever, loss of co-ordination, loss of consciousness, shivering, tachycardia, tremor and weakness (MedicineNet, 2016). Sibutramine was removed from the market in

the US in 2010 due to the high risk of heart attacks and strokes, especially in patients with a history of cardiovascular disease (Csupor *et al.* 2013). The European Medicines Agency (EMA) considered that the drug's benefits did not justify the potential risk of heart attacks, so in the same year; they issued a statement for the removal of sibutramine from the European market (EMA, 2010). In SA, sibutramine was banned on the 8th October 2010 because of concerns around an increased risk of heart attack and stroke (MedicineNet, 2016). Arterburn *et al.* conducted a systematic review in 2004 to assess the quality of published and unpublished evidence for sibutramine as a weight-loss agent and to quantify its benefits and harms (Arterburn *et al.* 2004). Their results were largely inconclusive except to claim that weight-loss does result from the use of sibutramine. They could not say conclusively if metabolic and cardiovascular risk increased. However, the FDA found that the negative effects (insomnia, confusion, high blood pressure, tremors, coma and even death) far outweighed any positive benefits and that is why sibutramine was banned (FDA, 2016). Natural herbal supplements are a popular alternative to anorectics as they should be free of API's and are marketed as having no side effects, however, CAMs for weight loss in order to work rapidly are being adulterated with API's.

1.5 CAMs for Weight-Loss and their Adulteration

Even with bans in place for sibutramine, both scientific studies and regulatory agency controls indicated that this drug continued to be fraudulently added to CAMs (FDA, 2016). Of the 416 alerts by the public to the FDA, between 2010 and 2015, 37% corresponded to adulterated weight-loss products and 87% of these cases involved the illegal addition of sibutramine (FDA, 2016). Lam *et al.* (2012), documented a case study of a 21-year-old woman who presented with somnolence, sinus tachycardia, generalised increase in tone, hyper-reflexia and clonus more prominent in the lower limbs after an intentional overdose of a non-prescription slimming product. The product was found to contain over seven times the normal dose of sibutramine (Lam, Leung *et al.* 2012). In another case in Turkey in 2012, sibutramine was suspected to be hidden in a pepper pill when a 17-year-old girl presented with palpitations, dizziness, anxiety, and insomnia. She had taken three herbal

slimming pills 4 hours earlier. Sibutramine intoxication was suspected (Gunaydin *et al.* 2015). In 2007, a retrospective study was conducted on patients admitted to a Hong Kong hospital between 2004 and 2006, on suspicion of side effects from using herbal slimming tablets (Yeun *et al.* 2007). Almost two thirds of patients had taken slimming products found to be adulterated with sibutramine or fenfluramine or their analogues.

One case necessitated a liver transplant and another presented acute psychosis. The patient had been taking the tablets for a week (Yuen *et al.* 2007). Chen *et al.* (2008) also describe the identification of sibutramine, fenfluramine and fenfluramine analogues in slimming products with adverse psychotic effects on the user. Cohen *et al.* (2012) assessed the prevalence of use and associated side effects of '*Pai You Guo'*. This weight-loss dietary supplement manufactured in China was found to be adulterated and had been recalled by the FDA in late 2009. A safety alert was sent to consumers because of concerns associated with the product (Cohen *et al.* 2012). The authors did not actually analyse the samples but the FDA had previously found sibutramine in '*Pai You Guo'*. It was reported that 61% of users purchased the dietary supplement after the FDA recall and none of the respondents were aware of the FDA alert (Cohen *et al.* 2012). Subsequently, Cohen *et al.* in 2014 evaluated the presence of banned drugs in 27 dietary supplements which had been purchased at least 6 months after being recalled by the FDA. The authors found that 67% remained adulterated. Of the 18 adulterated products, 12 still contained the same products and 6 a new product or analogue of the original (Rocha *et al.* 2016). With the reality of adulteration and with public safety at risk, it is imperative to have a rapid, specific and reliable method to screen for and quantitate API's and their analogues in CAMs.

1.6 Analytical Methods

1.6.1 Previous and Current Analytical Methods

The adulteration of CAMs is a dangerous and criminal practice. If this activity is to be stopped or at the very least if public awareness is to be increased, it is necessary to have

a rapid and accurate method to detect and quantify these adulterants. This tool will assist regulatory agencies to monitor the fraudulent practice of CAM adulteration (Rocha *et al.* 2016). Fortunately, it has become possible to detect these drugs and their derivatives as adulterants and counterfeits by using modern sensitive and selective analytical techniques [e.g., liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Koster *et al.* 2014), Fourier transform (FT) with near infrared spectrometry, and FT with Raman spectroscopy] (Singh *et al.* 2009). Extremely specific methods such as high performance liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), liquid chromatography tandem mass-spectrometry (LC–MS/MS), nuclear magnetic resonance (NMR) spectroscopy, vibrational spectroscopy, liquid chromatography-Fourier transform ion cyclotron resonance-mass spectrometry (LC–FT-ICR-MS), liquid chromatography-hybrid triple quadrupole linear ion trap mass spectrometry with information dependent acquisition, ultra high performance liquid chromatography-time of flight-mass spectrometry (UHPLC–TOF-MS), ion mobility spectroscopy (IMS) have all been employed and assessed by Patel *et al.* (2013) and Deconinck *et al.* (2013). These different methods of analysis have been employed in order to determine accurately if CAMs contain banned or scheduled medicines, to identify the API and to quantitate the amounts of these present in exhibits.

Chromatography is the best choice for chemical separation and mass spectrometry for identification and confirmation (Yamamoto, Sumioka *et al.* 2011 December). LC-MS/MS is becoming the most popular analytical method of choice, as in forensic chemistry and toxicology it is excellent for the screening and quantitation of a wide range of compounds, often with reduced sample preparation requirements (Montesano, Johansen & Nielsen, 2014). This method is highly selective as it uses two or more fragments (a quantifier and qualifier ions) to simultaneously confirm the presence of a substance as well as to calculate the concentration of the compound with use of a certified reference material (Koster *et al.* 2014). Research has been done to validate or partially validate the method for the quantitation of sildenafil in solid dose form but this has not been reported in SA research literature (Tseng *et al.* 2001; Kim *et al.* 2014; Zhu *et al.* 2005; Jeong *et al.* 2016; Lee *et al.* 2013; Lee; Lee *et al.* 2013).

With the recent advent of novel analogues of approved drugs being used as adulterants, detection of these drugs has become particularly difficult (Patel *et al.* 2014). Lee *et al.* in their study of Korean products advertised as enhancing male sexual performance, used LC-MS/MS to find more than 46 analogues present as illegal adulterants (Lee *et al.* 2013). Screening methods have been developed to detect analogues of sildenafil and vardenafil, which would otherwise be invisible. Analogues are expected to have different elution profiles and characteristic mass fragments and thus would not be detected in routine drug screening (Ng *et al.* 2010). In the course of the last decade liquid chromatography diode array detector tandem mass spectrometry (LC-(DAD)–MS) techniques have rapidly become the principle technique to screen for analogues (Singh *et al.* 2009). Venhuis *at al.* (2012) acknowledge the importance of MS techniques but say that even considering their importance, one cannot neglect the fact that good chromatography is imperative, especially for forensic purposes where an unknown product is being identified, particularly if there are compounds with similar or exact masses and fragments. The authors also highlight the necessity of keeping LC methods up to date (Blok-Tip *et al.* 2004; Venhuis *et al.* 2012). HPLC-MS/MS has reported to be an optimal method to accurately target, detect and quantify sibutramine, fenfluramine, phentermine, sildenafil, vardenafil and/or tadalafil hidden as adulterants in CAMs.

1.6.2 Gap in South Africa

Tables 1.1 and 1.2 in Appendix A (adapted from Rocha *et al.*) show all the methodologies previously employed to detect and quantitate API's in CAMs for anorectics and ED. Notably, none of these studies have been used in research on herbal products sold in SA to the author's knowledge. Springfield *et al.* (2005) performed HPLC fingerprinting on South African herbal products but only determined their quality and did not attempt to identify API's (Springfield, Eagles *et al.* 2005). Snyman and others studied two cases in SA where traditional herbal medicines had resulted in a child having a seizure and a woman aborting her foetus. It was identified using high-performance liquid chromatography with a photodiode array detector and gas chromatography-mass spectrometry that the traditional remedies had been adulterated with western medicine

for seizures, an anaesthetic and an anti-inflammatory (Snyman, Michael et al. 2005). To the best of the author's knowledge, to date no studies have tested South African CAMs for weight loss and/or erectile dysfunction in the solid dosage form, using a partially validated HPLC-MS/MS method. This was deemed a vital exercise to assist the MCC in understanding the urgency and necessity of regulation of some CAMs in SA. It was also suggested to be imperative for consumers, doctors and producers to be educated around the fact that adulteration of natural products may be occurring, and if identified that the possible dangers and risks linked to the consumption of CAMs is reported.

1.7 Rationale for the study

ED and obesity are worldwide problems which for various reasons may be treated by the public themselves with OTC CAMs (Schrameck *et al.* 2014; Rocha *et al.* 2016; Iuns.org, 2015). The main suggested reason for this is that self-medication with herbal OTC medicines is believed to be safe (Novella, 2010). The CAMs industry is worth billions and is on the rise (San Jose, 2012). With the substantial increase in CAMs consumption, it is evident that consumers are taking responsibility for their own health by using OTC medicines (The Nemours Foundation, 2016). Unfortunately, this increased consumption of CAMs and the huge economic gain has exposed CAMs for ED and weight loss to the unscrupulous and illegal adulteration with API's (Wheatley & Spink 2013). ED products are reported to be spiked with the PDE-5-inhibitors sildenafil, tadalafil or vardenafil and weight loss preparations with sibutramine, phentermine or fenfluramine or their analogues (Appendix A). The targeted detection and quantitation of these API's may be best achieved through the analytical technique of HPLC-MS/MS. Other techniques have not proved as rapid or specific, nor has the method for the validation of sildenafil in solid dose been assessed in SA research literature.

The dangerous criminal activity of adulteration has left the consumer vulnerable to serious side effects and even the possibility of fatal consequences (Skerrett *et al.* 2010). CAMs are still mostly unregulated and whilst the MCC in SA is endeavouring to rectify this, currently the only information available to the public is via a product label (*MCC,* 2016

and NIH 2008). Despite growing awareness of the importance of sexual health, SA-based scientific research on sexual dysfunction is still limited (Campbell & Stein 2014). The general public, looking for quick effective solutions may not stop to think about the lack of regulation of CAMs and their possible adulteration. There is therefore, a need for increased awareness among the public and the medical professionals about the emerging threat of CAMs adulteration and the resulting consequences (Yuen *et al.* 2007). This can be strengthened with further research into the identification and quantitation of any API's detected in CAMs. Throughout the world research has been done and is continuing in this field. Very little research, however, has been done on the identification and quantitation of the API's sildenafil and sibutramine in CAMs for ED and weight loss available in SA.

In SA, in particular it is still *caveat emptor* for the consumer. i.e. the person who buys a product is responsible for checking the quality and suitability thereof (Skerrett 2010). This situation deems it necessary and urgent to raise the awareness of possible adulteration of CAMs to the general public, health sector and government, if this is in fact occurring in SA. In addition, in the interest of public safety, the development of a method which is both accurate and sensitive, to screen and confirm the illegal adulterants in CAMs and food products is of urgent priority and will be of benefit to this research (Mokhatar *et al.* 2016).

1.8 Aim and Objectives

Aim

The aim of this study was to determine whether CAMs contain APIs, particularly in the form of sibutramine or sildenafil, through analysis of a number of commercially available over-the-counter products available in Cape Town, SA, and to quantitate any API's detected.

Objectives

The aim of this project would be achieved, through the following objectives:

1) To purchase random samples of CAMs in Cape Town.
2) To extract any potential API's from each sample.
3) To perform HPLC- MS/MS analysis on each random sample.
4) To analyse the results in order to ascertain which products (if any) contain the API's sibutramine or sildenafil.
5) To validate the method for the quantitation of any API's found.
6) To quantitate any API's found in the samples.

CHAPTER 2: MATERIALS AND METHODS

2.1 Materials

2.1.1 Certified Reference Standards

Certified Reference Standards of sibutramine, sildenafil and sildenafil-d_3, were obtained from Toronto Research Chemicals Inc. (Toronto, Canada). Sildenafil-d_3 (1 mg) was used as the internal standard (IS). Ultrapure water was prepared using a Synergy Water Purification System (Millipore, Bedford, MA, USA). Analytical grade methanol and ethanol as well as ammonium acetate and acetonitrile were obtained from Merck (Darmstadt, Germany).

2.1.2 Exhibit Samples

Simple random cluster sampling was used to obtain slimming tablets (anorectics) and/or tablets for ED (sexual enhancement) treatment. In total, 87 samples were obtained, which included 79 ED samples and 8 anorectics. The exhibit samples were bought from two clusters of stores in Cape Town, namely, supermarkets and pharmacies. Exhibit samples were randomly selected and purchased based on availability at the stores (Table 2.1). See Appendix C for store and exhibit sample identification tables.

Table 2.1: Clusters and number of exhibit samples purchased over-the-counter for erectile dysfunction and weight loss

Store Code	Number of Erectile Dysfunction Exhibit Samples	Number of Anorectic Exhibit Samples
Cluster 1 Supermarket/store 1:C1S1	39	4
Cluster 1 Supermarket/store 2:C1S2	12	2
Cluster 2 Chain pharmacy/store 1:C2S1	19	-
Cluster 2 Private pharmacy 1/store 2:C2S2	7	2
Cluster 2 Private pharmacy 2/store 3:C2S3	2	-
Total	**79**	**8**

The samples obtained were in the form of either tablets, capsules or large spherical solid lumps (Figure 2.1).

(a) Tablets **(b) Capsules**

(c) Solid Lumps

Figure 2.1: Photographs showing the different forms of exhibit samples purchased: (a) tablets, (b) capsules, (c) solid lumps (Images courtesy of Sandra Catterson)

2.1.3 Sample storage and management

All samples were purchased in sealed packages and transported to the Pharmacology Laboratory at the Division of Pharmacology at the University of Cape Town. Once the samples had been extracted, the solutions were stored at -80^{0}C in the access controlled laboratory. The Certified Reference Standards and IS were kept at -20^{0}C.

2.2 Method for Preparation of Standards, Exhibit Samples and Controls

2.2.1 Standards

The standards of sildenafil citrate (1 mg) and sibutramine (1 mg) were made up to a concentration of 1 mg/mL by adding 1 mL of 50% v/v methanol/water followed by briefly vortexing and sonicating for 10 minutes until all the solute was dissolved. Working stock solutions of 10 µg/mL of sildenafil and sibutramine were made up in Kimble tubes by diluting 100 µL of the 1 mg/mL solutions with 9900 µL of methanol/water (50% v/v).

2.2.2 Internal Standard

Deuterated sildenafil, sildenafil-d_3, was used as the IS. A solution of 1 mg/mL of IS was made up by adding 1 mL of 50% v/v methanol/water to 1 mg of sildenafil-d_3 IS followed by brief vortexing then sonication for 10 minutes. This 1 mg/mL solution was diluted to 1 µg/mL with 50% v/v methanol/water by adding 10 µL of the 1 mg/mL IS to 9990 µL of methanol/water (50% v/v) in a Kimble tube. Sufficient stock solution was made up so that it could be used for the entire validation process.

2.2.3 Preparation of Exhibit Samples for Screening

Preliminary Screening

Initially, 26 exhibit samples (18 ED samples and 8 anorectics) were screened for any adulterated API's by HPLC-MS/MS using a Shimadzu Prominence High Performance Liquid Chromatography System and API 3200 Qtrap Mass Spectrometer. One sample (capsule/tablet/solid lump) from each exhibit was randomly selected for analysis. In some cases, more than one sample was analysed from the same pack. Initially, each sample was weighed to obtain the total mass of each tablet/capsule/solid lump. The samples were homogenized individually using a pestle and mortar for the tablets and capsule contents and the solid lump was mechanically homogenized with a few drops of 50% v/v methanol/water mixture. A small portion of each sample was then weighed out into a Kimble tube for extraction. A Kimble tube was used to prevent splashing as the Eppendorf tubes proved too small.

The samples were then extracted by adding 5 mL of a 50 % v/v methanol/water mixture to each weighed-out portion of the sample. The concentration of each sample was calculated from the weighed-out portion dissolved in 5 mL of the 50% v/v methanol/water. The solutions were vortexed, sonicated for 15 minutes and then centrifuged at
13 000 rpm (9.464g) for 10 minutes. In order to make up a solution of 1 mg/mL, a calculated amount of the supernatant was transferred to a Kimble tube and then diluted with 50% v/v methanol/water (Appendix C: Table 2.2). Following this, 10 µL of each 1 mg/mL sample solution was then diluted further with 990 µL of 50% v/v methanol/water to produce
10 µg/mL working solutions for injection into the instrument. All 26 exhibit samples were then screened for seven adulterated APIs using a previously developed method discussed in 2.2.4 below.

Following the outcome of these preliminary screening experiments, as reported in the results chapter, it was ascertained that sildenafil should be targeted in the ED exhibit

samples and quantitated. Following validation procedures described below, sildenafil was targeted in the remaining 61 exhibit samples for ED and quantitated if detected.

2.2.4 High performance Liquid Chromatography Tandem Mass Spectrometry (HPLC-MS/MS)

HPLC-MS/MS analysis was performed on a Shimadzu Prominence High Performance Liquid Chromatography System (HPLC) (Shimadzu Corporation, Kyoto, Japan) coupled to an API 3200 Qtrap Mass Spectrometer (MS) (ABSCIEX, Toronto, Canada). Chromatographic separation was achieved with a Kinetex C18 column (50 mm x 3 mm, id 2.6 µm) (Phenomenex, California, USA) at 40°C with mobile phase A as 10 mM ammonium acetate and mobile phase B as acetonitrile methanol. The system was run in a linear gradient from 5 % organic phase to 95 % organic phase in 3 minutes. The organic phase was then decreased back to 5 % from 3.10 to 6.90 minutes. The total run time was 7 minutes at a constant flow of 0.4 mL/min.

The mass spectrometer was equipped with an electrospray ionization probe, and ionization was performed in positive mode. The ion spray voltage was set to 5500 V and the source temperature was 500 °C. Direct infusions of reference solutions at 10 µg/mL diluted in 50 % mobile phase A and 50 % mobile phase B were performed and from the spectra obtained, precursor ions were selected and fragmented. Two multiple reaction monitoring (MRM) transitions were evaluated for sildenafil – the most abundant was used as the quantifier ion and the other as the qualifier ion. The MRM transitions, declustering potentials, collision exit potential and collision energies are shown in Table 2.3.

Table 2.3: Transitions and MS/MS conditions for each analyte and internal standard

Analyte	Q^1 (m/z)	Q^3(m/z)	DP (V)	CEP (V)	CE (V)
Sildenafil	475.20	58.30 (quantifier) 283.1 (qualifier)	101.0 100.0	22.17 22.17	51.00 50.00
Sildenafil-d_3(IS)	478.20	61.20	96.00	22.28	57.00
Tadalafil	390.10	268.10	50.00	19.00	25.00
Vardenafil	489.10	151.20	80.00	22.69	40.00
Sibutramine	280.10	125.10	60.00	14.90	50.00
Phentermine	150.10	91.10	80.00	10.06	40.00
Fenfluramine	232.20	159.20	80.00	13.12	50.00
Amphetamine	136.00	119.00	60.00	9.54	50.00

Q^1 – precursor ion, Q^3 – product ion, m/z – mass to charge ratio DP – declustering potential, CEP - collision cell exit potential, CE – collision energy, V-volts

Chromatograms were analyzed with Analyst 1.6.2 (ABSCIEX, Toronto, Canada). One ionic transition was used for sildenafil-d_3 and sibutramine (as this was only screened and not quantitated as indicated in section 2.2.3). Flow injection analysis was used to optimize the mass spectrometry parameters for seven analytes prior to this project: three PDE-5 inhibitors; sildenafil, tadalafil, vardenafil and four anorectics; phentermine, fenfluramine, sibutramine and amphetamine.

2.2.5 Controls

Various quality controls (QC's) were prepared for bias (accuracy) and precision analyses using the same working solution of 10 µg/mL of sildenafil standard and working solution of 1 µg/mL of IS. The QC's were distinguished following the validation procedures described in 2.3. Each of these concentrations were prepared six times. Low concentration (QC low) was approximately three times the lowest end of the working range of the method (LOQ) and high concentration (QC high) was within approximately 80% of the highest end of the working range of the method. Medium concentration (QCmedium) was near the midpoint of the low and high concentrations. The

following quality controls were made up LOQ (20 ng/mL); QC Low (60 ng/mL), QC medium (400 ng/mL) and QC medium/high (800 ng/mL) and QC high (1600 ng/mL) by pipetting the calculated aliquot into a Kimble tube and diluting with 50% methanol/water and vortexing (Table 2.4).

Table 2.4: Volumes required to prepare quality controls for bias and precision analyses

Concentration of Standard (ng/mL)	Volume of 10µg/mL stock used (µL)	Volume (µL) of methanol/water (50% v/v) used for dilution
20 (LOQ)	2	998
60 (QC low)	6	994
400(QC med)	40	960
800(QCmed/high)	80	920
1600 (QChigh)	160	840

2.3 Method Validation for Sildenafil Quantitation

The method for the detection and quantitation of sildenafil was validated by using a model adopted from both SWGTOX and the FDA guidelines (SWGTOX, 2013). A calibration model, bias and precision, limit of quantitation (LOQ), limit of detection (LOD), inter and intra-day variation, exhaustive extractions (not recoveries), carry over and partial stability studies were performed as well as autosampler stability.

2.3.1 Calibration Model

Haque *et al.* (2014) in earlier studies to quantitate sildenafil, used a working range from as low as 1 ng/mL up to 1000 ng/mL. For the purposes of this study, a working range of 20 ng/mL to 2000 ng/mL was investigated as the therapeutic dose of sildenafil in Viagra® starts at 25 mg and it was deemed sufficient to detect even trace amounts in the samples. To evaluate this model, a series of standard solutions were prepared from the reference material, by dilution of the common stock solution of the standard to cover the reasonable range of signal response from the instrument. Nine calibrator samples were prepared as standard solutions and analyzed to establish the calibration model. The nine calibrators were chosen to span the range of concentrations

expected 20; 50; 100; 250; 500; 750; 1000; 1500 and 2000 ng/mL. The concentrations were made up in duplicate as shown in Table 2.5.

Table 2.5: Concentrations of standard calibrators made up for evaluation of the standard curve

Concentration of standard (ng/mL)	Volume of 10 µg/mL stock used (µL)	Volume (µL) of methanol/water (50% v/v) used for dilution
20	2	998
50	5	995
100	10	990
250	25	975
500	50	950
750	75	925
1000	100	900
1500	150	850
2000	200	800

Every standard and duplicate was spiked with 50 µL of the 1 µg/mL stock of IS (sildenafil-d_3). A double blank (methanol/water 50% v/v without IS) and a blank (methanol/water 50% v/v with IS) were made up in duplicate and added to each run. MRM was performed on the entire working range. Each calibrator was analyzed in duplicate, twice on the same day and once the following day. Fresh batches were made up in duplicate each time. All data points from the six runs were plotted (using a statistical software package-Analyst) to establish the calibration model and regression co-efficient. The origin was not included as a calibration point. Calibration curves were obtained by plotting the peak area of analytes to internal standard versus the analyte concentration to internal standard using a weighted (1/x) quadratic regression model.

2.3.2 Method for determining Bias (Accuracy) and Precision (%CV)

Bias according to SGTOX, is the closeness of agreement between the mean of the results of measurements of a measurand and the true (or accepted true) value of a measurand (SWGTOX, 2013) which is reported as a percent difference. The terms accuracy or trueness may also be used

to describe bias. Precision is described by SWGTOX as the measure of the closeness of agreement between a series of measurements obtained from multiple samplings of the same homogenous sample; it is expressed numerically as imprecision (SWGTOX 2013). Precision is expressed as the coefficient of variation (% CV), also known as the relative standard deviation (RSD). The mean and standard deviation (s) of the response is calculated for each concentration to determine the % CV.

$$\%\mathrm{CV} = \frac{\mathrm{s}}{\text{mean response}}$$

The acceptance criteria for these were based upon SWGTOX guidelines and used as follows in this study: bias must not exceed ±20% and the % CV shall not exceed 20% for each concentration. A fresh calibration curve was prepared for each run using the working range established from the calibration model. MRM was performed on each QC six times on two separate days (inter-day) and on the same day (intra-day) to show reproducibility. The mean for each concentration was calculated and statistical analysis was performed to determine the standard deviation, %CV (RSD) and % bias (accuracy). Precision was expressed as the coefficient of variation (% CV). The mean and standard deviation (s) of the response was calculated for each concentration to determine the % CV.

2.3.3 Method for determining Limit of Quantitation

The LOQ was set as the value of the lowest non-zero calibrator. Duplicates of the lowest calibrator were analyzed over three runs to demonstrate that all detection, identification, bias, and precision criteria were met: % CV < 20% and bias of ±20 %. The LOQ was determined to be ten times the signal to noise ratio (S/N) and all peaks were required to be Gaussian in shape.

2.3.4 Method for determining Limit of Detection

The LOD was determined by using analyzing the background noise. To determine the LOD using this approach, the results from the previously generated calibration curve were used. The LOD

was determined as three times the background noise. The background noise was determined from the double blank and the height of the analyte (LOD) was then calculated as three times the amplitude of the noise.

2.3.5 Exhaustive Extractions

To ensure that all the API had been extracted, an exhaustive extraction was performed on the exhibit samples. The exhaustive extraction was repeated three times for 11 of the exhibit samples. The supernatant was pipetted out (5 mL) and diluted with 5 mL 50% v/v methanol/water. This was then briefly vortexed, sonicated for 15 minutes and centrifuged at 13 000 rpm for 10 minutes. The sample was run on the mass spectrometer to determine the peak area of sildenafil. The process was repeated for a second and third wash, each time adding 5 mL 50% v/v methanol/water to the original sample and the procedure repeated to determine the efficiency of the extractions. Recovery was not fully investigated in this study and warrants further investigation in future.

2.3.6 Carry Over

Analyte carry over into a subsequent sample may lead to an inaccurate qualitative or quantitative result when using instrumental methods. To ensure that there was no carry over i.e. contamination of a sample from preceding samples, water blanks were run on the instrument in between each sample and then analysed. Carry over after the highest calibrator must not exceed 10% of the signal of the lowest calibrator**.**

2.3.7 Stability Studies

2.3.7.1 Storage Temperature Stability

Low, medium and high quality controls were left on the bench (n=12), at -20^{0}C (n=12) and at -80^{0}C (n=6) for 24 hours and then run on the 3200 Qtrap to investigate 24-hour stability. The QC's were run six times, the samples stored at – 20 ^{0}C were run four times and the samples stored at – 80 ^{0} C were run twice.

2.3.7.2 Autosampler Stability

The standard curve and QC's were re-injected for six runs after remaining in the autosampler at room temperature for two days to determine the stability of the autosampler.

2.4 Sample Preparation of Exhibit Samples for Quantitation

For the quantitation of sildenafil in the exhibit samples, the exact procedure used for the screening method was followed. This time all 61 samples were purchased for ED only (separate to the 26 screened). This was because from the screening method, the API for ED, sildenafil, was far more prevalent that the API in the anorectics, sibutramine. There was also a much larger abundance of samples available to purchase for ED. Of the 61 samples, 36 samples were from the supermarket cluster; 28 were from store 1, 8 were from store 2. From the pharmacy cluster, there were 25 samples; 19 were from store 1; 4 from store 2 and 2 from store 3 (Table 2.6).

The 61 exhibit samples for ED were screened for sildenafil and where it was present the amount of sildenafil in those samples was quantitated. From the calibration curves, the concentration of sildenafil was calculated by the instrument in each 10 µg/mL solution. From this value, the percentage of sildenafil in each solution was calculated by dividing the concentration from the instrument (in µg/mL) by 10 µg/mL and multiplying by 100. i.e.

$$\% \text{ sildenafil} = \frac{concentration\ in\ \mu g/mL}{10\ \mu g/mL} \times 100$$

This percentage was then used to calculate the amount of sildenafil in each dose (tablet/capsule/solid lump) of exhibit sample from the total original mass of each dose. i.e.

$$\text{Mass of sildenafil/dose} = \frac{percentage\ sildenafil\ in\ solution}{100} \times \text{total mass of dose.}$$

For example, for BG B1C1, the concentration of sildenafil calculated by the instrument is 1728 ng/mL which is 1.78 µg/mL. The percentage of sildenafil in the 10 µg/mL solution is calculated as follows:

% sildenafil = $\frac{1.78\ \mu g/mL}{10\ \mu g/mL}$ x 100 = 17.8 %

Therefore, the mass of sildenafil in the 310.82 mg dose is:

Mass of sildenafil/dose = $\frac{17.28}{100}$ x 310.82 = 53.71 mg.

Table 2.6: Data for the Preparation of Exhibit Samples for Quantitation

Table 2.6.1: Data for exhibit samples from C1S1

Sample Name	Mass of tablet/capsule/ lump (mg)	Mass weighed out (mg)	Concentration after adding 5 mL water/methanol (mg/mL)	Volume required to make 1mg/mL solutions (µL)
1. BG B1C1	310.82	53.35	10.70	93.72
2. BG B1C2	331.22	51.01	10.20	98.00
3. Xi B1 T1	599.64	65.25	13.05	76.63
4. Xi B1T2	612.05	50.34	10.10	99.00
5. CJ B1T1	684.73	67.14	13.43	74.47
6. CJ B1T2	677.02	52.37	10.50	95.00
7. CJ B2T1	672.71	54.01	10.07	99.30
8. MK B1C1	483.33	67.08	13.42	74.54
9. MK B1C2	479.09	51.00	10.20	98.00
10. MK B2C1	486.22	49.72	9.94	100.60
11. MK B2C2	422.24	54.22	10.84	92.30
12. St L1	6122.44	107.78	21.56	46.39

13. St L2	6122.44	75.20	15.04	66.70
14. Yi B1T1	461.76	55.25	11.05	90.50
15. Yi B1T2	454.07	49.20	9.84	101.6
16. CC B1C1	271.88	48.28	9.66	103.56
17. CC B1C2	262.61	50.20	10.04	99.60
18. BB B1C1	245.42	54.08	10.82	92.46
19. BB B1C2	236.52	50.93	10.19	98.00
20. YA L1	3941.85	78.81	15.76	63.44
21. YA L1	3941.85	70.58	14.12	70.80
22. YP L1	4704.34	74.70	14.94	66.93
23. YP L2	4701.21	89.10	17.82	56.00
24. GD B1C1	355.50	41.96	8.39	119.16
25. GD B1C1	355.50	50.34	10.07	99.30
26. GD B1C2	381.38	49.28	9.86	101.4
27. GG B1C1	322.59	50.49	10.10	99.00
28. GG B1C2	336.89	53.38	10.68	93.60

Table 2.6.2: Data for exhibit samples from C1S2

Sample Name	Mass of tablet/capsule/ lump (mg)	Mass weighed out (mg)	Concentration after adding 5 mL water/methanol (mg/mL)	Volume required to make 1mg/mL solutions (µL)
29. AK47 B1C1	267.32	49.75	9.95	100.00
30. AK47 B1C2	263.15	54.02	10.80	92.60
31. ES B1C1	256.66	52.63	10.53	95.00
32. ES B1C2	233.48	50.11	10.00	100.00
33. RD B1T1	641.71	52.73	10.55	94.80
34. RD B1T2	649.72	52.92	10.58	94.10
35. Qu B1C1	275.73	51.98	10.40	96.20
36. Qu B1C2	428.69	51.70	10.34	96.70

Table 2.6.3: Data for exhibit samples from C2S1

Sample Name	Mass of tablet/capsule/ lump (mg)	Mass weighed out (mg)	Concentration after adding 5 mL water/methanol (mg/mL)	Volume required to make 1mg/mL solutions (µL)
37. MK8 B1C1	428.22	53.45	10.69	93.54
38. MK8 B1C2	400.33	51.51	10.30	97.00
39. CJ1 B1T1	752.08	45.15	9.03	110.74
40. SP4 B1T1	745.95	63.02	12.60	79.34
41. SP4 B1T2	743.92	50.95	10.19	98.00
42. SP1 B1T1	752.08	53.78	10.70	94.00
43. MK1 B1C1	435.29	80.11	16.02	62.41
44. MK1 B2C1	496.1	50.41	10.08	99.00
45. MKB1 B1T1	562.27	60.39	12.08	82.80
46. MKB4 B1T1	577.73	64.31	12.86	77.75
47. MKB1 B2T1	555.61	51.66	10.33	96.80
48. MKSC B1C1	306.87	52.67	10.53	95.00
49. MKSC B2C1	562.27	53.28	10.66	93.80
50. Vi B1C1	554.92	50.03	10.00	100.0
51. Pa B1C1	426.07	49.46	9.89	101.11
52. An B1C1	597.55	55.20	11.04	90.58
53. An B1C2	596.23	52.79	10.56	94.70
54. Re B1C1	769.44	49.65	9.93	100.7
55. Re B1C2	762.60	50.81	10.16	98.40

Table 2.6.4: Data for exhibit samples from C2S2

Sample Name	Mass of tablet/capsule/ lump (mg)	Mass weighed out (mg)	Concentration after adding 5 mL water/methanol (mg/mL)	Volume required to make 1 mg/mL solutions (µL)
56.Am B1C1	749.03	39.30	7.86	127.23
57. HR B1C1	704.97	34.95	6.99	143.06
58. TYG B1T1	345.71	36.77	7.35	135.98
59. TYS B1T1	497.82	38.20	7.64	130.89

Table 2.6.5: Data for exhibit samples from C2S3

Sample Name	Mass of tablet/capsule/ lump (mg)	Mass weighed out (mg)	Concentration after adding 5 mL water/methanol (mg/mL)	Volume required to make 1 mg/mL solutions (µL)
60. CJ B3T1	770.70	51.82	10.36	96.5
61. CJ B3T2	817.49	46.89	9.38	106.6

CHAPTER 3: RESULTS

3.1 Instrumental Analysis Optimization

Several studies have illustrated the use of HPLC-MS/MS as a sensitive, rapid, accurate and specific technique for determination and quantitation of adulterants in herbal dietary supplements, illicit drugs, or counterfeits (Tseng *et al.* 2001; Oh *et al.* 2006; Liang *et al.* 2006; Abdel-Hamid, 2006; Singh *et al.* 2009; Lau *et al.* 2003). This technique provides mass selectivity of the detector and baseline separation of the analytes through chromatography**.** The MRM transitions**,** DP, CEP and CE were optimized on the instrument.

The optimized method (completed prior to the study) for the analytes sildenafil, tadalafil, vardenafil, phentermine, fenfluramine, sibutramine and amphetamine resulted in the formation of structurally useful fragment ions while maintaining sufficient $[M+H]^+$ response. The optimum HPLC-MS/MS transition parameters for the analytes are indicated in Table 3.1.

Table 3.1: The optimum HPLC-MS/MS transition parameters for the analytes (PDE-5 inhibitors and anorectics) together with the retention times for sildenafil and sildenafil-d_3, $[M+H]^+$ and fragment ions

Analyte	Q^1 (m/z)	Q^3(m/z)	DP (V)	CEP (V)	CE (V)	RT (min)
Sildenafil	475.20	58.30 (quantifier) 283.1 (qualifier)	101.0 100.0	22.17 22.17	51.00 50.00	3.50
Sildenafil-d_3(IS)	478.20	61.20	96.00	22.28	57.00	3.51
Tadalafil	390.10	268.10	50.00	19.00	25.00	-
Vardenafil	489.10	151.20	80.00	22.69	40.00	-
Sibutramine	280.10	125.10	60.00	14.90	50.00	-
Phentermine	150.10	91.10	80.00	10.06	40.00	-
Fenfluramine	232.20	159.20	80.00	13.12	50.00	-
Amphetamine	136.00	119.00	60.00	9.54	50.00	-

Q^1 – precursor ion $[M+H]^+$, Q^3 – product ion, m/z – mass to charge ratio, DP – declustering potential , CEP - collision cell exit potential, CE – collision energy, V-volts, RT-Retention time. (RT were only established for sildenafil and sildenafil-d_3)

The identification of precursor and product ions, and preliminary optimization of the instrument settings were conducted prior to the initiation of the project. MRM transitions were monitored in the positive ESI modes. In addition, chromatographic conditions including mobile phase composition and gradient were optimized prior to the study. Figure 3.1 represents a proposed HPLC-MS/MS fragmentation pathway for the PDE-5 inhibitor sildenafil.

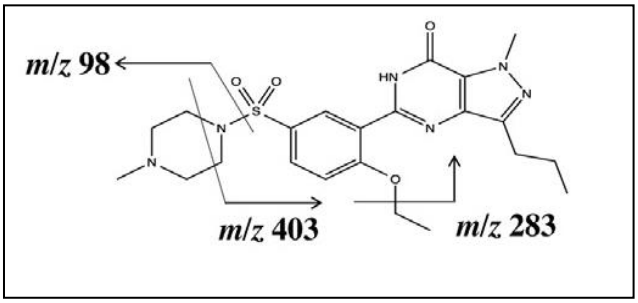

Figure 3.1: Proposed fragmentation pattern of the parent ion m/z 475 $[M+H]^+$ into a precursor ion m/z 283 (Mokhatar *et al.* 2015).

3.2 Sample Preparation Optimization

The samples were extracted in a 50:50 v/v methanol/water solution, followed by sonication and centrifugation. Excellent recovery of analytes was shown in prior studies using this simple extraction technique (Tseng *et al.* 2001; Oh *et al.* 2006; Liang *et al.* 2006; Abdel-Hamid, 2006; Singh *et al.* 2009; Lau *et al.* 2003). Most of the analytes due to their polar nature extract very well in this mixture.

3.3 Screening of Exhibits for Active Pharmaceutical Ingredients (API's)

Initially, 26 samples (18 ED samples and 8 anorectics) were randomly purchased and then screened on HPLC-MS/MS to identify whether there were active pharmaceutical ingredients present using the method developed for the analytes (Table 3.2). From the products purchased, samples were randomly selected from each pack. From the first supermarket (C1S1) every sample tested for ED (*n*=11) contained sildenafil and every sample for weight-loss (*n*=4) contained

sibutramine. The second supermarket (C1S2) showed one out of four ED products contained sildenafil and both slimming tablets contained sibutramine. None of the samples (*n*=5) from one of the pharmacies in the second cluster (C2S2) had any sildenafil or sibutramine present. Capsules and tablets were as prone as each other to be tainted. Table 3.2 shows the API's found in the first 26 samples that were screened and Table 3.3 summarizes the data (Full product names in Appendix C).

Table 3.2: Samples screened for API's and the API's detected.

Cluster 1 Store 1 : C1S1

Sample Name	API detected
1.BG	Sildenafil
2.Xi	Sildenafil
3.CJ	Sildenafil
4.MK	Sildenafil
5.St	Sildenafil
6.Yi	Sildenafil
7.CC	Sildenafil
8.BB	Sildenafil
9.YA	Sildenafil
10.YP	Sildenafil
11.GD	Sildenafil
12. Kangmei(slimming)	Sibutramine
13. Kangmei(slimming)	Sibutramine
14. Kangmei(slimming)	Sibutramine
15. Kangmei(slimming)	Sibutramine

Cluster 1 Store 2: C1S2

Sample Name	API detected
16. Chinaga	None
17. TYG	None
18. Power up	None
19. CJ	Sildenafil
20. Kangmei(slimming)	Sibutramine
21. Kangmei (slimming	Sibutramine

Cluster 2 Store2: C2S2

Sample Name	API detected
22. TYS	None
23. High Rise	None
24. Passion flower	None
25. Slimming dragees (slimming)	None
26. G.I lean Hunger Buster (slimming)	None

Table 3.3: Summary of API's detected in the 26 samples screened.

	Tablet	Capsule	Solid Lump
C1S1:			
Erectile Dysfunction (# tested):	3	5	3
Sildenafil detected	3	5	3
Anorectics (# tested):		4	
Sibutramine detected		4	
C1S2:			
Erectile Dysfunction (# tested):	2	2	
Sildenafil detected	2	1	
Anorectics (# tested):			
Sibutramine detected		2	
		2	
C2S2			
Erectile Dysfunction (# tested):	1	1	
Sildenafil detected	0	0	
Anorectics (# tested):	1	2	
Sibutramine detected	0	0	

Following the preliminary screening of the exhibits, it was decided that the rest of the project would focus on the quantitation of sildenafil in additionally purchased exhibit samples. This was because there was an abundance of ED CAMs available but few CAMs for weight-loss. The method validation for sildenafil included LOD, LOQ, calibration

model, precision, accuracy, carry over, exhaustive extractions and partial stability studies (Haque *et al.* 2014).

The mass spectra (MS) for sildenafil and sibutramine were used to determine the fragmentation patterns of the precursor and product ions. For sildenafil, the precursor ion was at m/z 475.20 and product ions were at m/z 58.3 and 283.1. Figures 3.2 and 3.3 show the mass spectra obtained for sildenafil and sibutramine together with the precursor and product ions for sildenafil.

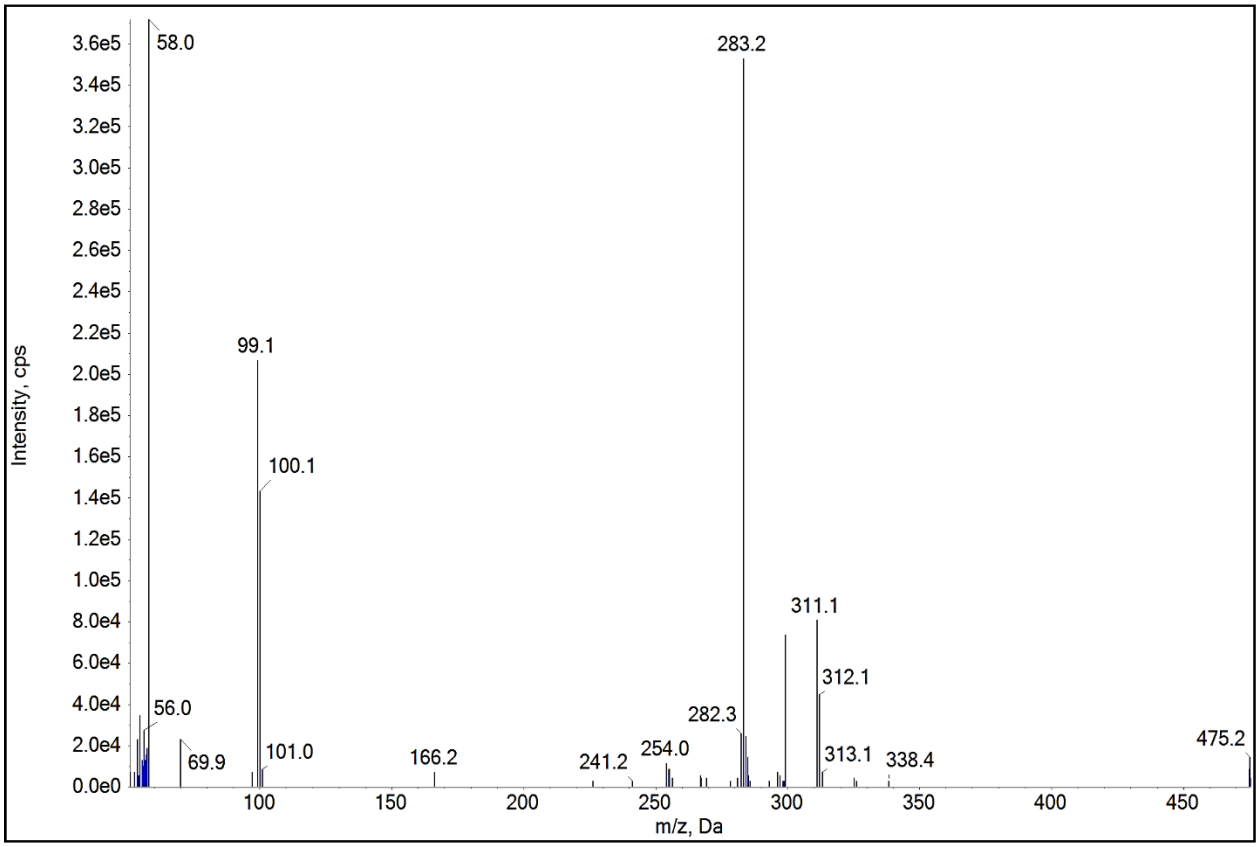

Figure 3.2: Mass spectrum fragmentation patterns for sildenafil. The precursor ion is at m/z 475.20 and product ions at m/z 58.3 and 283.1)

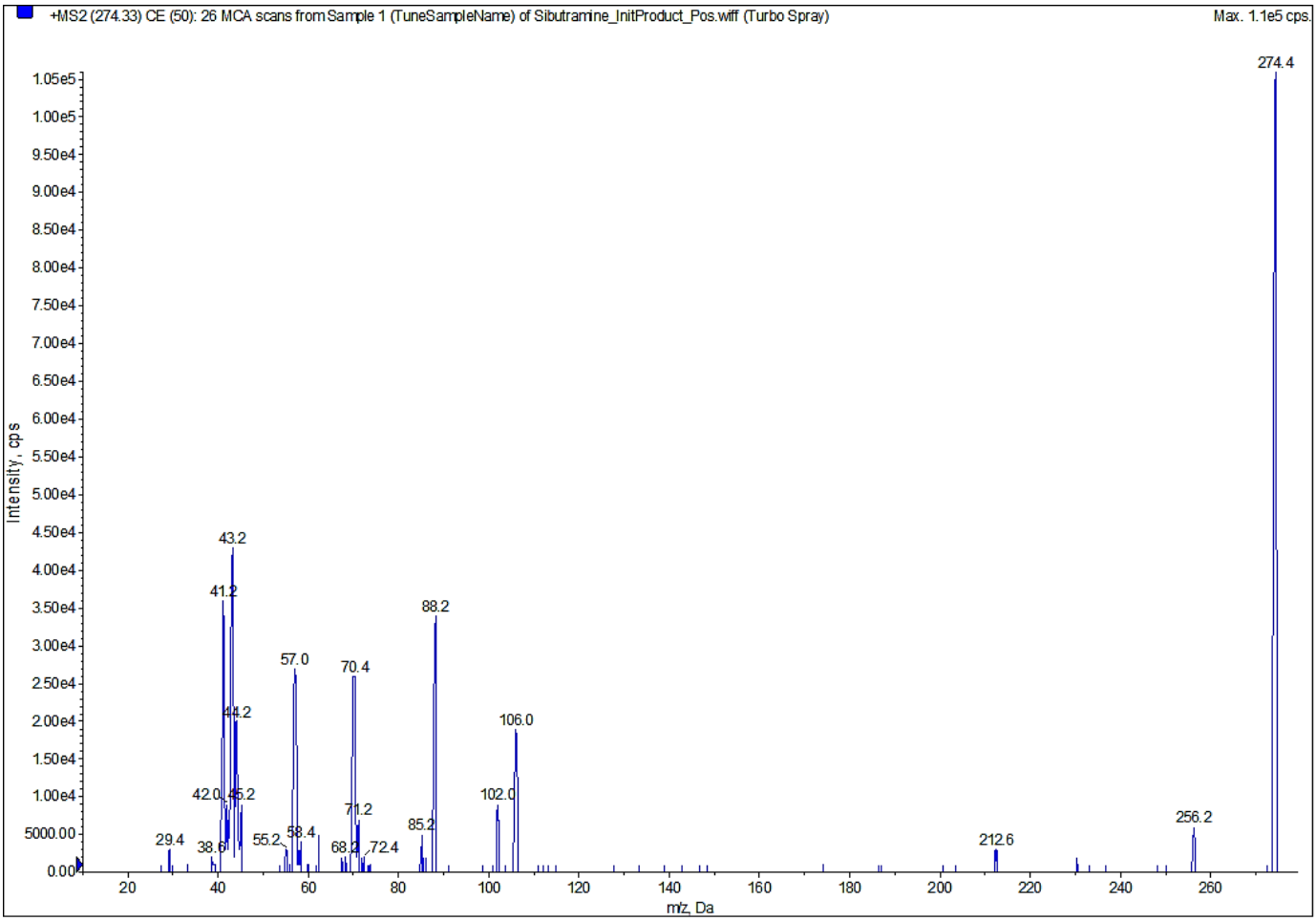

Figure 3.3: Mass spectrum fragmentation patterns for sibutramine

Total ion chromatograms (TIC) were obtained for sildenafil from the method validation and together with the MS fragmentation spectra and ion ratios were compared to those from the exhibit samples in order to quantitate the amount of sildenafil present in these samples. TIC's are shown below for a representative calibrator (250 ng/mL) and one of the exhibit samples which tested positive for sildenafil. Figures 3.4-3.8 show the mass spectrum and chromatograms obtained for the 250 ng/mL calibrator of sildenafil and Figures 3.9-3.11 show the chromatograms for the sildenafil found in one of the exhibit samples. The retention time, the mass spectra and fragmentation patterns, and the ion ratios were compared between the exhibits and the standards, indicating a positive detection.

Figure 3.4 shows the total ion chromatogram for sildenafil at a concentration of 250 ng/mL. The sildenafil-d_3 IS and the precursor ion at m/z 475.20 ($[M+H]^+$) and product ions at m/z 58.3 and 283.1 are shown on the same chromatogram. The corresponding mass spectrum is shown in Figure 3.5. Figures 3.6-3.8 show the expanded chromatograms for the two fragment ions and the IS for the 250 ng/mL calibrator.

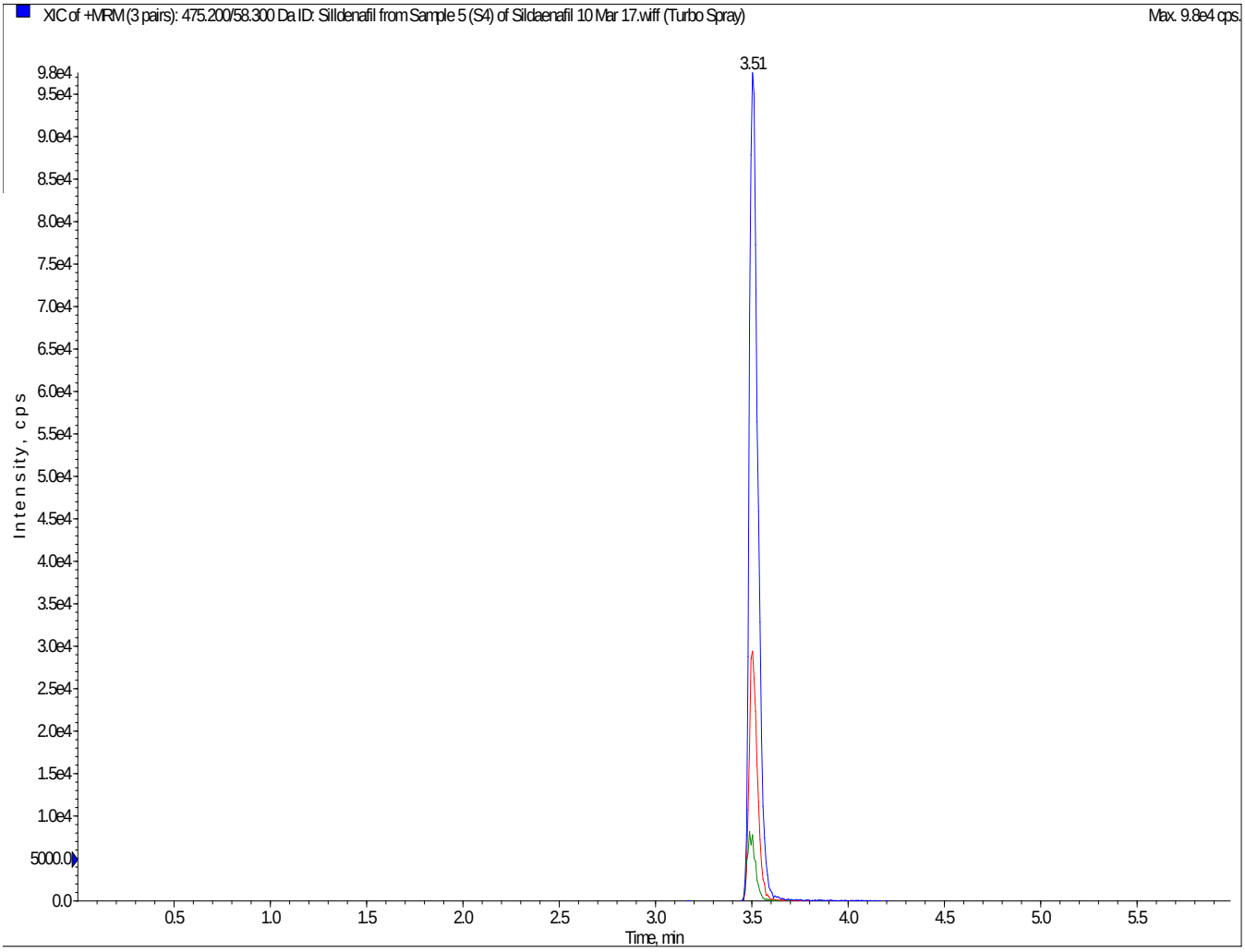

Figure 3.4: TIC for sildenafil at 250 ng/mL. The precursor and product ions are shown at a retention time of 3.51 minutes

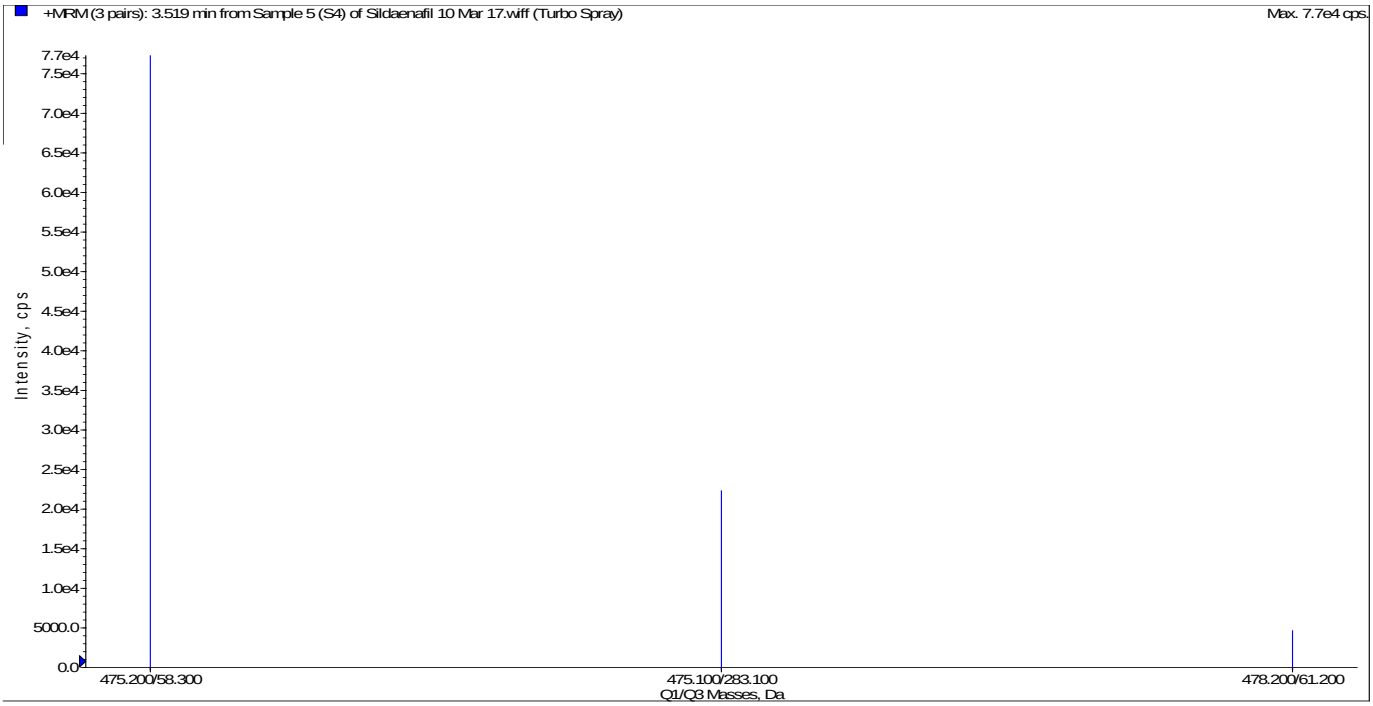

Figure 3.5: Mass spectrum fragmentation patterns for sildenafil in a standard calibrator of 250 ng/mL. The precursor ion is at m/z 475.20 and the previously established product ions at m/z 58.3 and 283.1.

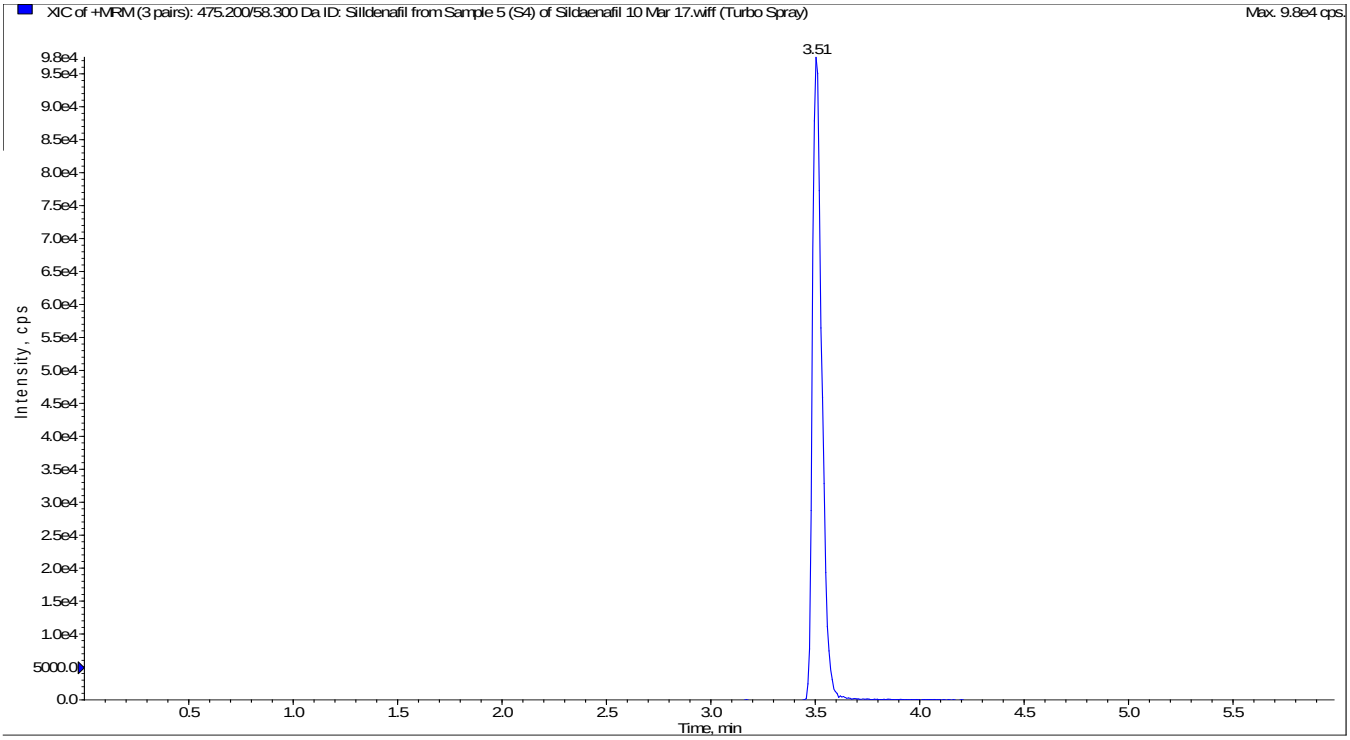

Figure 3.6: The TIC for sildenafil in a standard calibrator of 250 ng/mL showing the peak obtained for the fragment ion at m/z 58.3

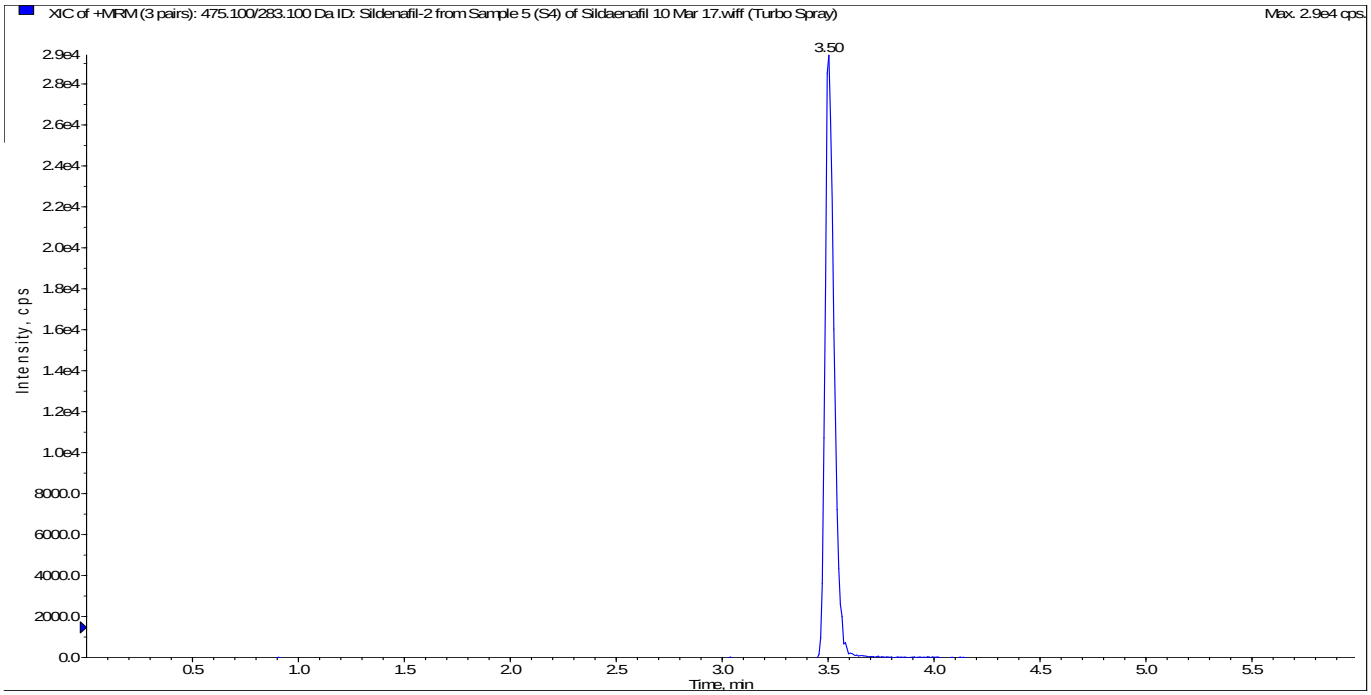

Figure 3.7: The TIC for sildenafil in a standard calibrator of 250 ng/mL showing the peak obtained for the fragment ion at m/z 283.1.

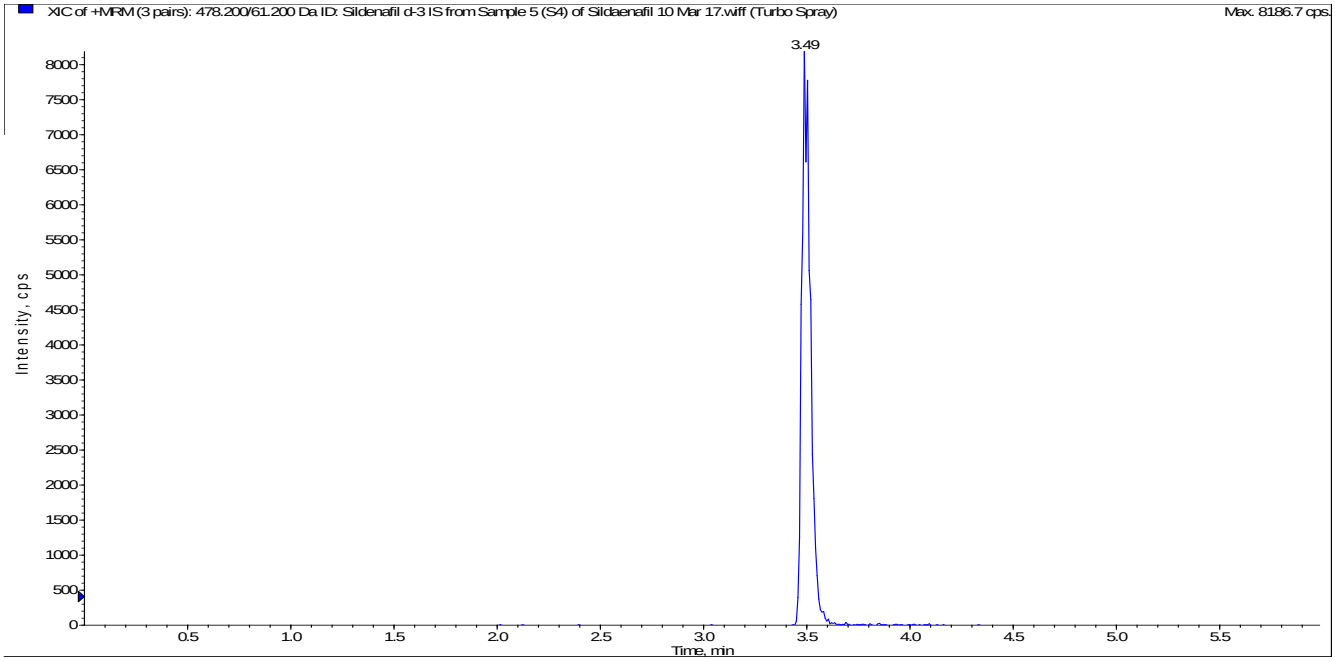

Figure 3.8: The TIC for sildenafil in a standard calibrator of 250 ng/mL showing the peak obtained for the IS, sildenafil-d_3 ion at m/z 61.2.

HPLC-MS/MS measurements for the exhibit samples which tested positive for sildenafil revealed a protonated precursor ion $[M+H]^+$ at m/z 475.20 and product ions at m/z 58.3 and 283.1 with a retention time of 3.5 minutes. Figures 3.9-3.11 show the chromatograms of the precursor and fragment ions of sildenafil found in an exhibit sample which tested positive for sildenafil. All figures are for the same exhibit sample.

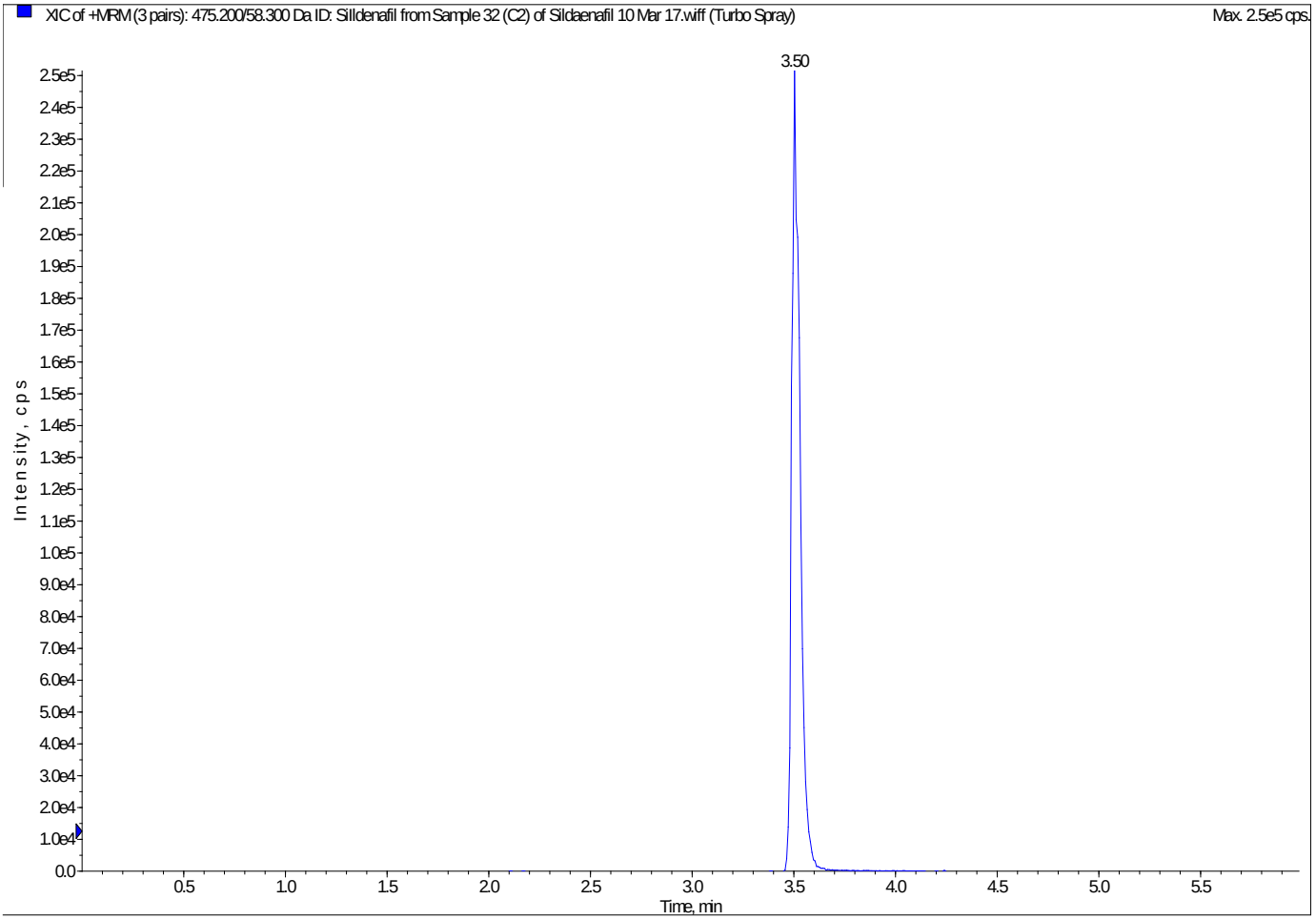

Figure 3.9: The TIC for sildenafil found in an exhibit sample showing the peak obtained for the fragment ion at m/z 58.3

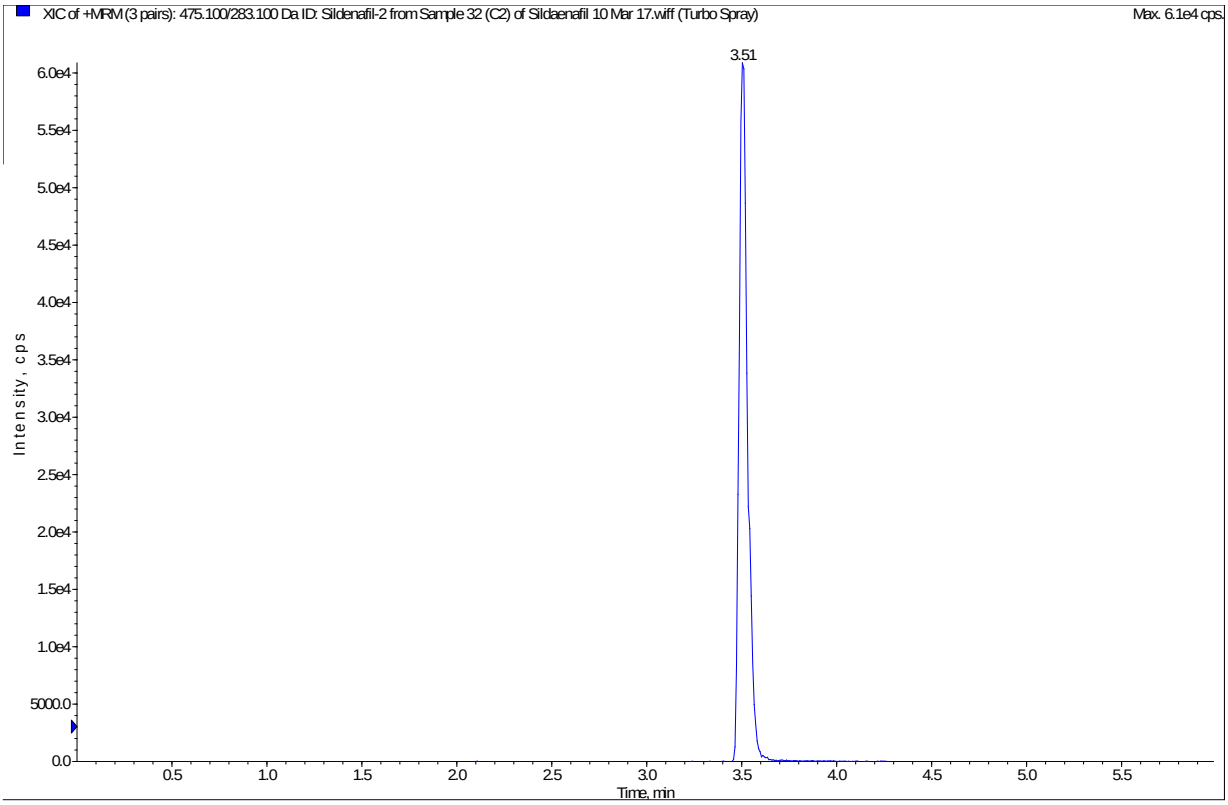

Figure 3.10: The TIC for sildenafil found in an exhibit sample showing the peak obtained for the fragment ion at m/z 283.1

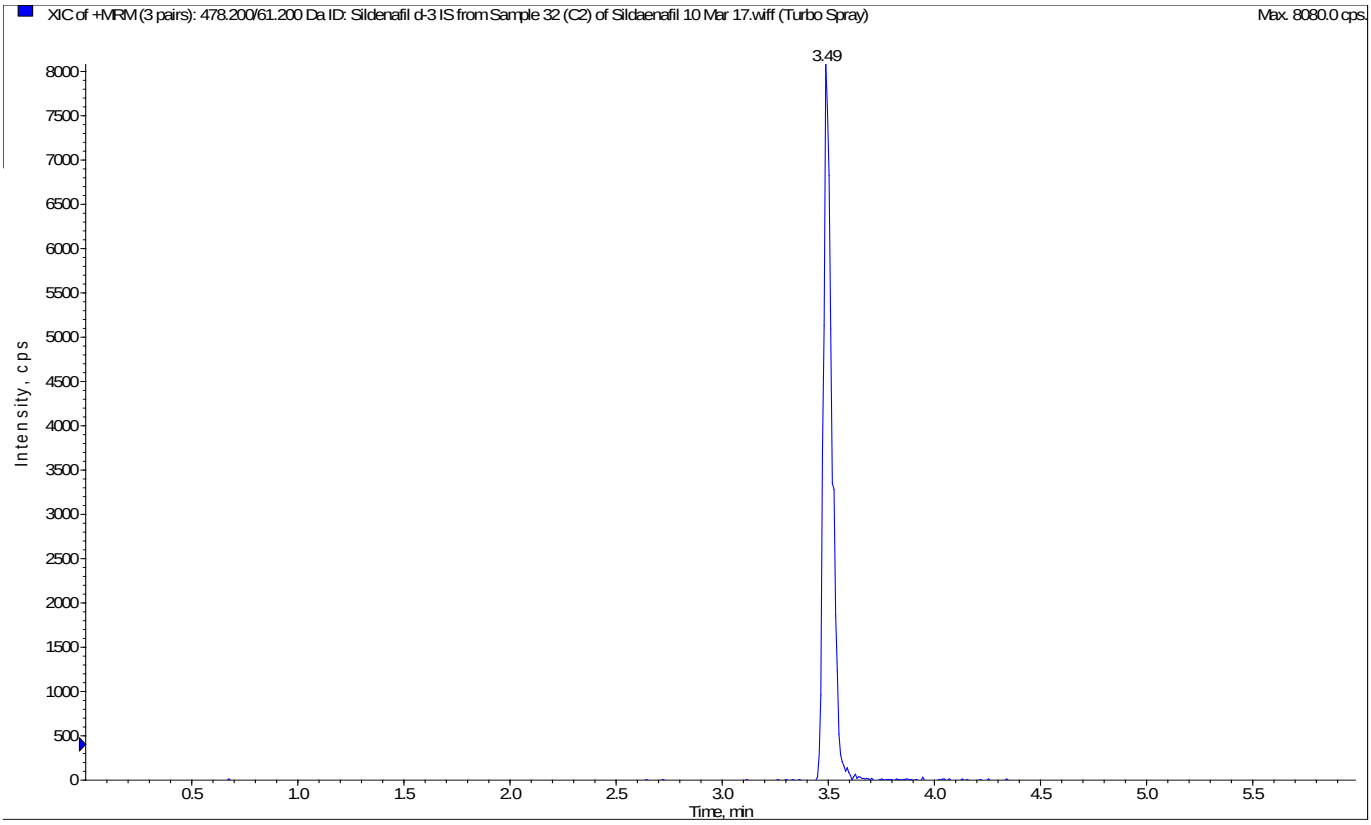

Figure 3.11: The TIC illustrating the sildenafil d_3 IS at m/z 61.2 in an exhibit sample.

Figure 3.12 shows the enlarged chromatograms for the 20 ng/mL standard used as the LOQ. A Gaussian peak can be seen at 3,51 minutes and the IS at 3,50 minutes.

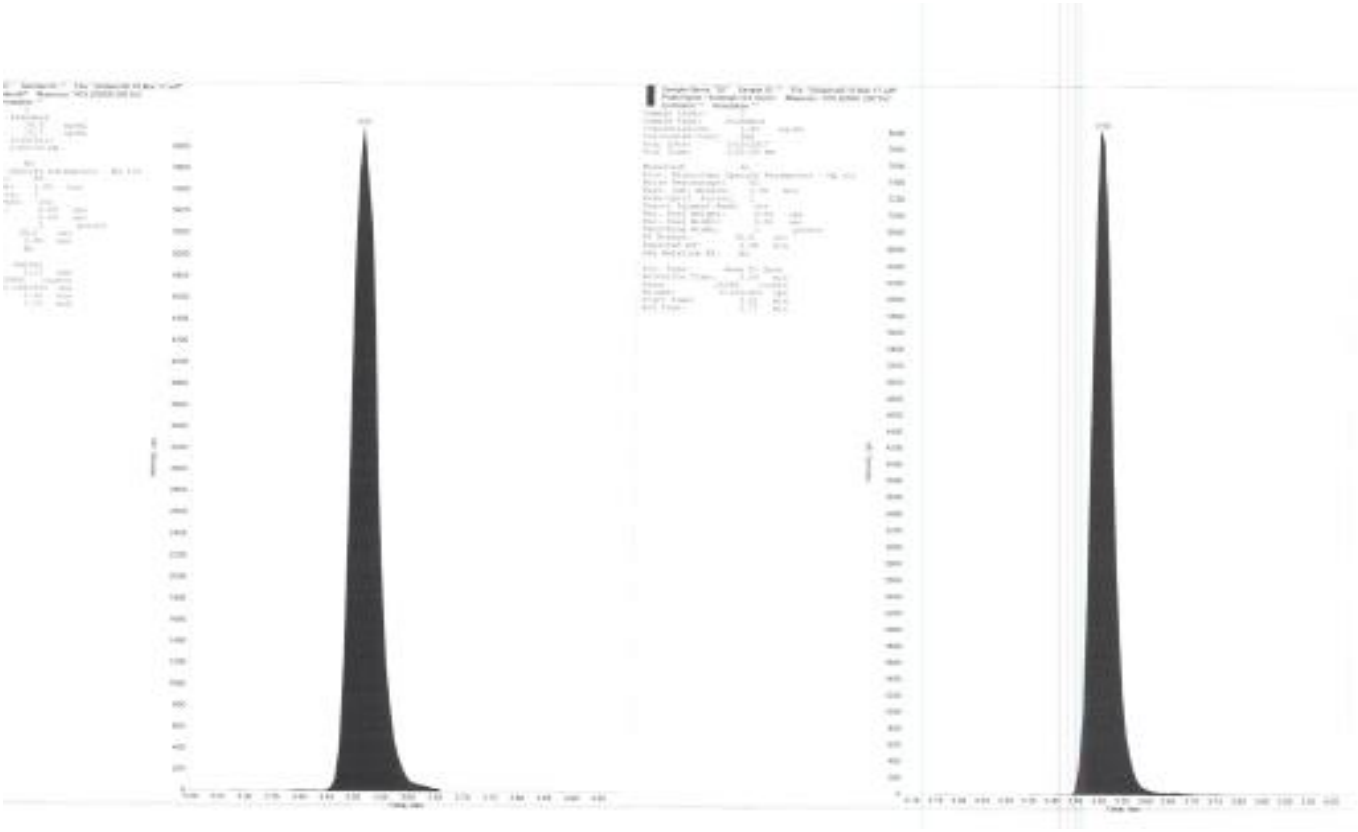

Figure 3.12: Chromatograms for the LOQ (20ng/mL) standard calibrator and IS

3.4 Results for Method Validation of Sildenafil

3.4.1 The Calibration Model

The calibration model for sildenafil was assessed and a quadratic model was decided upon with the range of 20 ng/mL to 2000 ng/mL. Each calibrator was analyzed in duplicate, twice on the same day (intra-day precision) and once the following day (inter-day precision), equating to six runs in total.

Table 3.4: Table of calibration data for the six runs showing the peak areas and ratio of sildenafil peak area to the peak area of the IS.

	Run 1			Run 2 (Dup)			Run 3			Run 4 (dup)			Run 5			Run 6 (dup)		
Conc (ng/mL)	Sildenafil Analyte Peak Area (counts)	IS Analyte Peak Area (counts)	ratio	Sildenafil Analyte Peak Area (counts)	IS Analyte Peak Area (counts)	ratio	sildenafil Analyte Peak Area (counts)	IS Analyte Peak Area (counts)	ratio	sildenafil Analyte Peak Area (counts)	IS Analyte Peak Area (counts)	ratio	sildenafil Analyte Peak Area (counts)	IS Analyte Peak Area (counts)	ratio	sildenafil Analyte Peak Area (counts)	IS Analyte Peak Area (counts)	ratio
20	20000	50100	0.40	36400	104000	0.35	22100	85500	0.26	25300	91800	0.28	16400	19400	0.85	17700	21900	0.81
50	36700	48800	0.75	67900	97200	0.70	59300	90100	0.66	69400	86500	0.80	50800	23200	2.19	41400	22600	1.83
100	80100	49900	1.61	144000	93700	1.54	133000	81800	1.63	154000	93600	1.65	95100	23400	4.06	84300	25200	3.35
250	221000	52300	4.23	410000	95200	4.31	345000	87400	3.95	406000	90200	4.50	231000	24300	9.51	224000	20700	10.82
500	511000	49400	10.34	859000	95400	9.00	768000	84000	9.14	815000	94800	8.60	470000	22200	21.17	431000	23700	18.19
750	709000	54000	13.13	1340000	88400	15.16	1170000	78700	14.87	1270000	90200	14.08	700000	22800	30.70	670000	21100	31.75
1000	999000	54400	18.36	1760000	90300	19.49	1480000	82300	17.98	1720000	86800	19.82	861000	24200	35.58	884000	23100	38.27
1500	1420000	50200	28.29	2520000	89600	28.13	2220000	76800	28.91	2520000	85800	29.37	1320000	23000	57.39	1280000	21500	59.53
2000	1860000	53500	34.77	3270000	88200	37.07	3000000	78600	38.17	3170000	84900	37.34	1810000	21400	84.58	1730000	23400	0.81

The standard curves were run for the calibrators at concentrations of 20, 50, 100, 250, 500, 750, 1000, 1500 and 2000 ng/mL in order to establish linearity. The calibrators were tested for intra-day variation by running them in duplicate on the same day and inter-day precision was found by running them in duplicate on the following day. A quadratic regression was found with excellent regression co-efficients for all of the six runs. The standard curves for the six runs are shown in Figures 3.13-3.15. Figure 3.13 shows the curves for the first run and repeat, Figure 3.14 shows the curves obtained when the calibrators were run for a second time (in duplicate) on the same day (intra-day precision). Figure 3.15 shows the curves obtained when the calibrators were run for a third time (in duplicate) on the following day (inter-day precision).

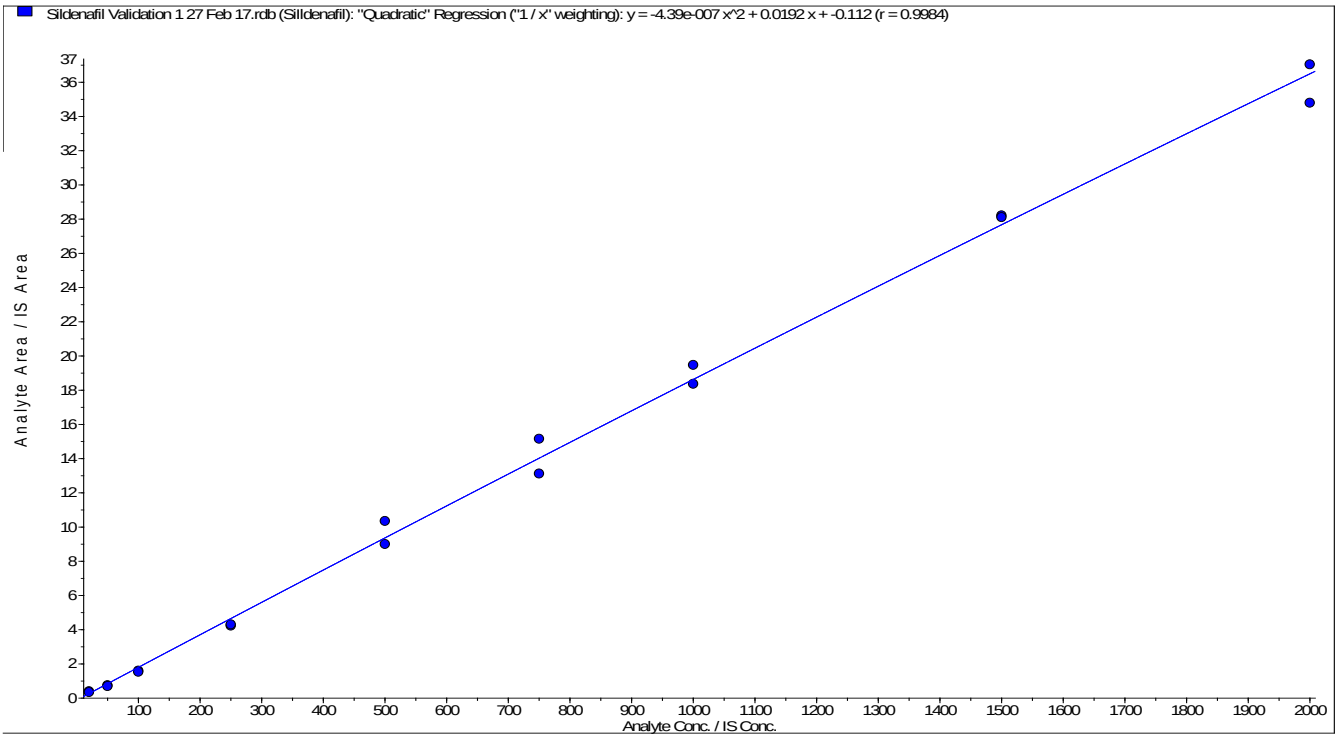

Figure 3.13: The standard curves for the first run and repeat

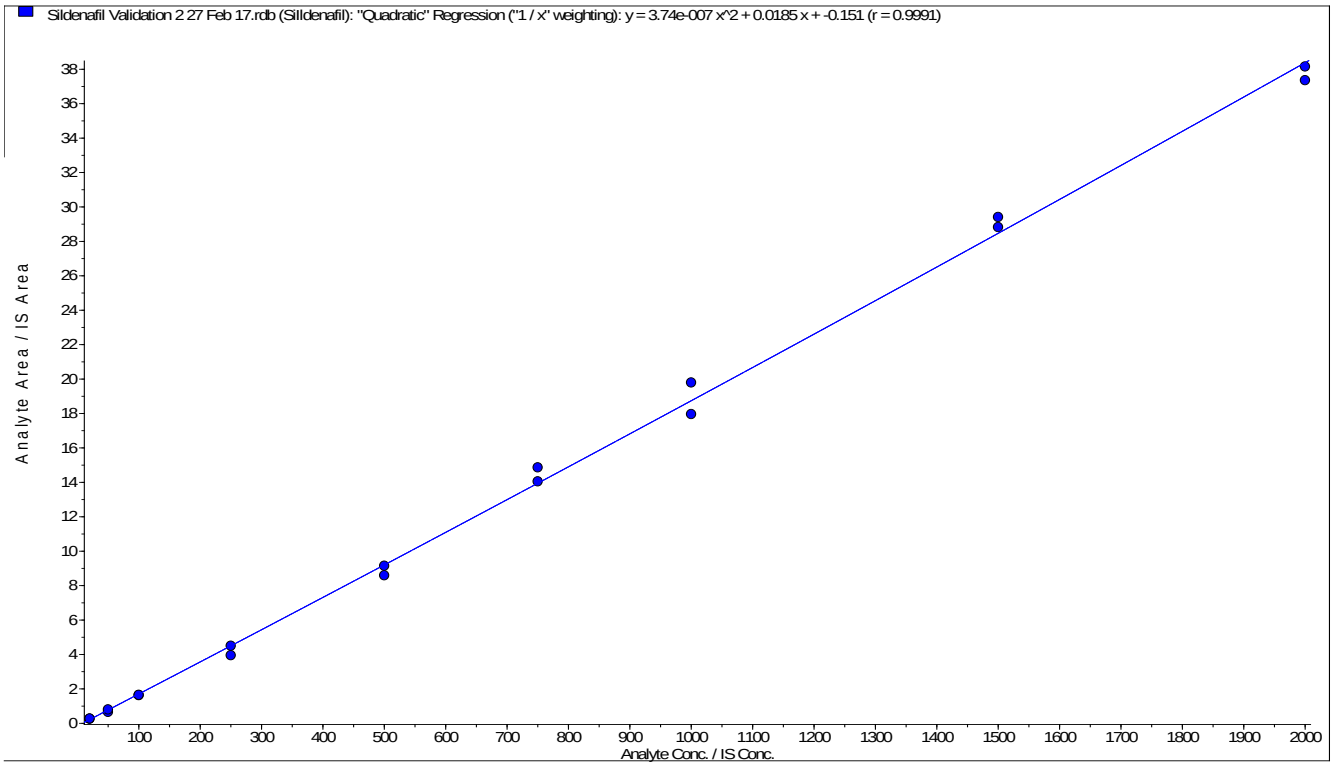

Figure 3.14: The standard curves for second run and repeat (Calibrators were re-run on the same day and in duplicate)

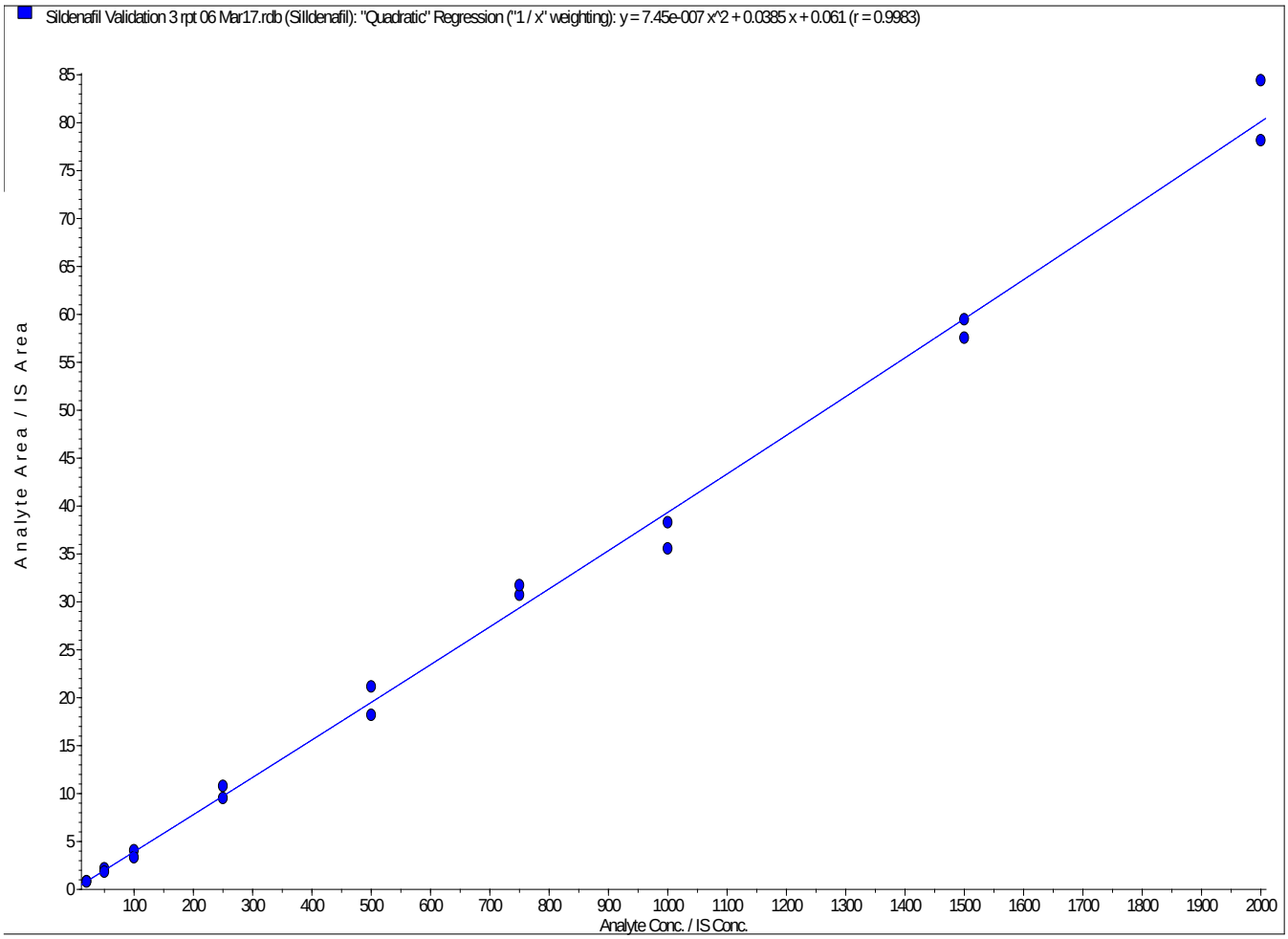

Figure 3.15: The standard curves for the third run and repeat (Calibrators were run on the following day in duplicate)

The data from all six runs were summarized on one graph to show the overall quadratic regression and linearity. Figure 3.16 shows the results of all six standard curves plotted on the same axes. A quadratic regression was obtained with r = 0.9985.

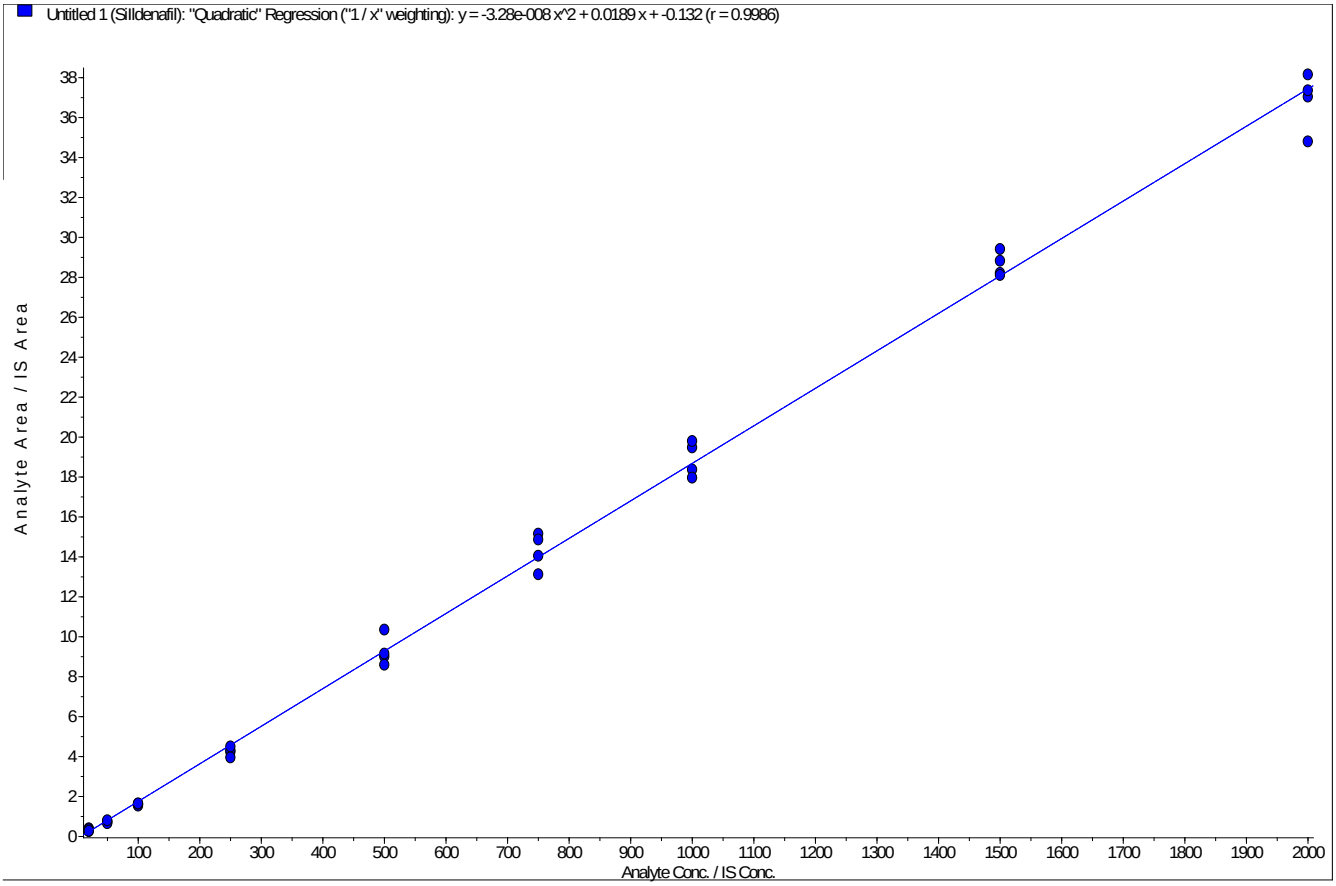

Figure 3.16: Standard curves from all six runs on the same axes.

3.4.2 Bias (Accuracy) and Precision (% CV)

To establish the bias and precision for the method the four quality controls were run in duplicate, twice on the same day and once on the following day. All QC's showed a bias and precision of < 20%. The results are shown in Appendix D-Table 3.5. A summary of all the data for bias and precision showing the mean, standard deviation, % CV and bias are represented in Table 3.6.

Table 3.6: Summary of the data for bias and precision showing the mean, standard deviation, % CV and bias.

Expected concentration ng/mL	Sample name	Number of analyses run	Mean	Standard Deviation	% CV Precision	Bias
20	LOQ	18	20.87	4.072	19.5	4.35
60	QC low	18	57.84	5.279	9.13	15.68
400	QC medium	18	373.89	72.79	19.47	-6.75
800	QCmed/high	18	811.05	52.45	6.47	1.38
1600	QC high	18	1552.52	230.50	14.85	-2.96

3.4.3 Limit of Detection

The LOD was approximated using the criteria of acceptance as three times the S/N ratio. The height of the analyte was determined to be three times the amplitude of the noise. The chromatogram of the double blank shown in Figure 3.17 shows the noise at 113 counts per second (cps). The LOD is equivalent to three times this value. The 20 ng/mL calibrator had an intensity of 6200 cps, therefore the LOD was suggested to be approximately 1.09 ng/mL. This was deemed appropriate for the purpose of this preliminary study.

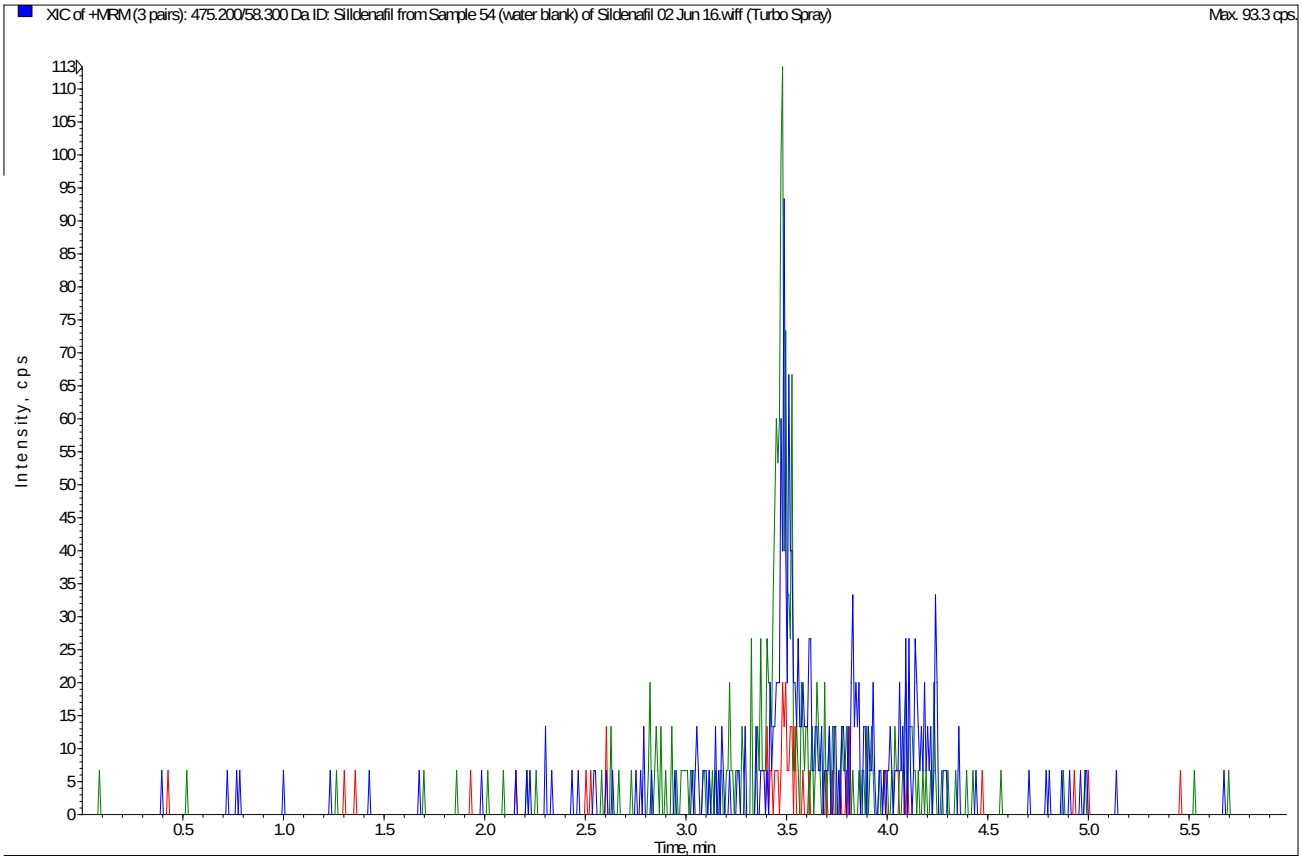

Figure 3.17: The chromatogram for the double blank showing a noise count of 113cps.

3.4.4 Limit of Quantitation

The LOQ was set as the value of the lowest non-zero calibrator namely 20 ng/mL.

After duplicates of the lowest calibrator were analyzed over three runs it was demonstrated that all detection, identification, bias, and precision criteria were met. These criteria were: % CV < 20% and bias of ±20% (Table 3.6). The LOQ was over ten times the S/N ratio and all peaks were Gaussian in shape. The LOQ was therefore set at 20 ng/mL for the purposes of this study.

3.5 Quantitation of Sildenafil in the Exhibit Samples

HPLC-MS/MS was subsequently utilized to quantify sildenafil in the 61 exhibit samples which were different to the first 26 screened exhibits. The standard curve for the exhibit samples is shown in Figure 3.18. All calibrators were freshly prepared and run in duplicate. The % CV and bias were both < 20 % and the correlation co-efficient was 0.9985.

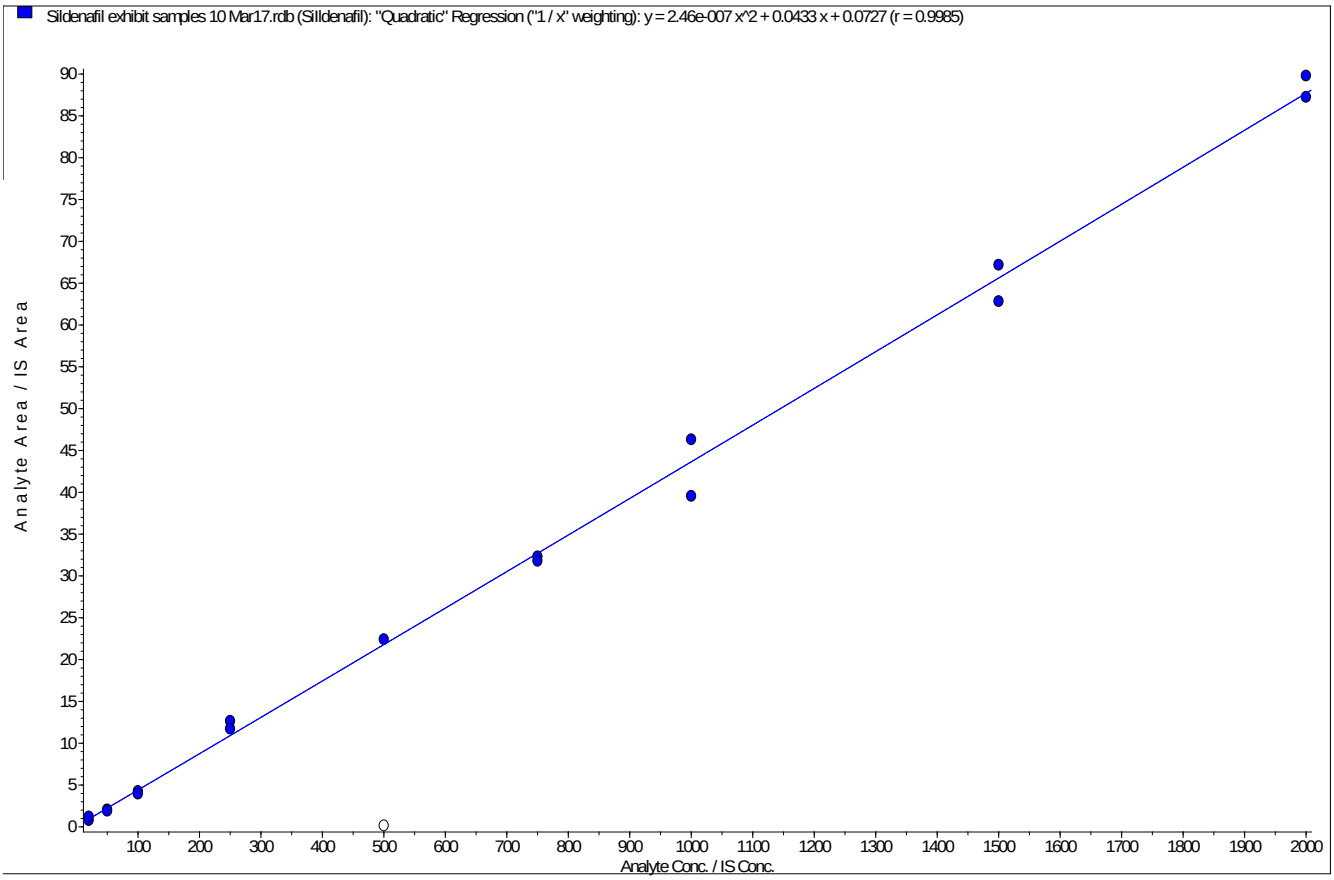

Figure 3.18: The Standard Curve for the Exhibit Samples.

The findings from the analysis of the samples showed that 100% of the random samples selected (*n*=28) for erectile dysfunction from the Chinese supermarket (C1S1) contained sildenafil and all (*n*=4) of the slimming tablets contained sibutramine. The average amount of sildenafil was 55.5 mg/dose. Four products contained approximately more than the maximum dose of sildenafil (more than 100 mg)

The average amount of sildenafil in the products (n=6) from the second supermarket, the R5 store (C1S2) was 38.5 mg.The products for erectile dysfunction tested from the reputable pharmacy chain (C2S1) contained an average of 10.1 mg of sildenafil per dose. None of the products tested from the independent pharmacy 2 (C2S2) contained sildenafil. Both the tablets tested from the third pharmacy (C2S3) contained sildenafil at an average of 32.5 mg/tablet

The Chinese supermarket was the highest in the samples tested of all the clusters. Although the averages from the other store and pharmacies was lower, the odd product had high levels of sildenafil; 63.0 mg from the R5 store, 66.9 mg from the large pharmacy chain and 53.1 mg from the private pharmacy 3. This indicates the variation between and within exhibits, which warrants further investigation.

Figures 3.19-3.22 summarize the data showing the amount of sildenafil found/dose in all of the exhibit samples which tested positive for sildenafil. The therapeutic dose of sildenafil in Viagra ® is 25 mg and is shown in red on the graphs. Anything above the line shows exhibit samples which contained above the minimum dose of sildenafil in Viagra ®. From C1S1, 25 out of 28 samples had more than the minimum dose of sildenafil in Viagra ®, 6 out of 8 samples had above the minimum dose from C1S2, 4 out of 7 samples that tested positive for sildenafil from C2S1 had above the minimum dose. Both samples from C2S3 tested positive for sildenafil but only one was above the minimum dose.

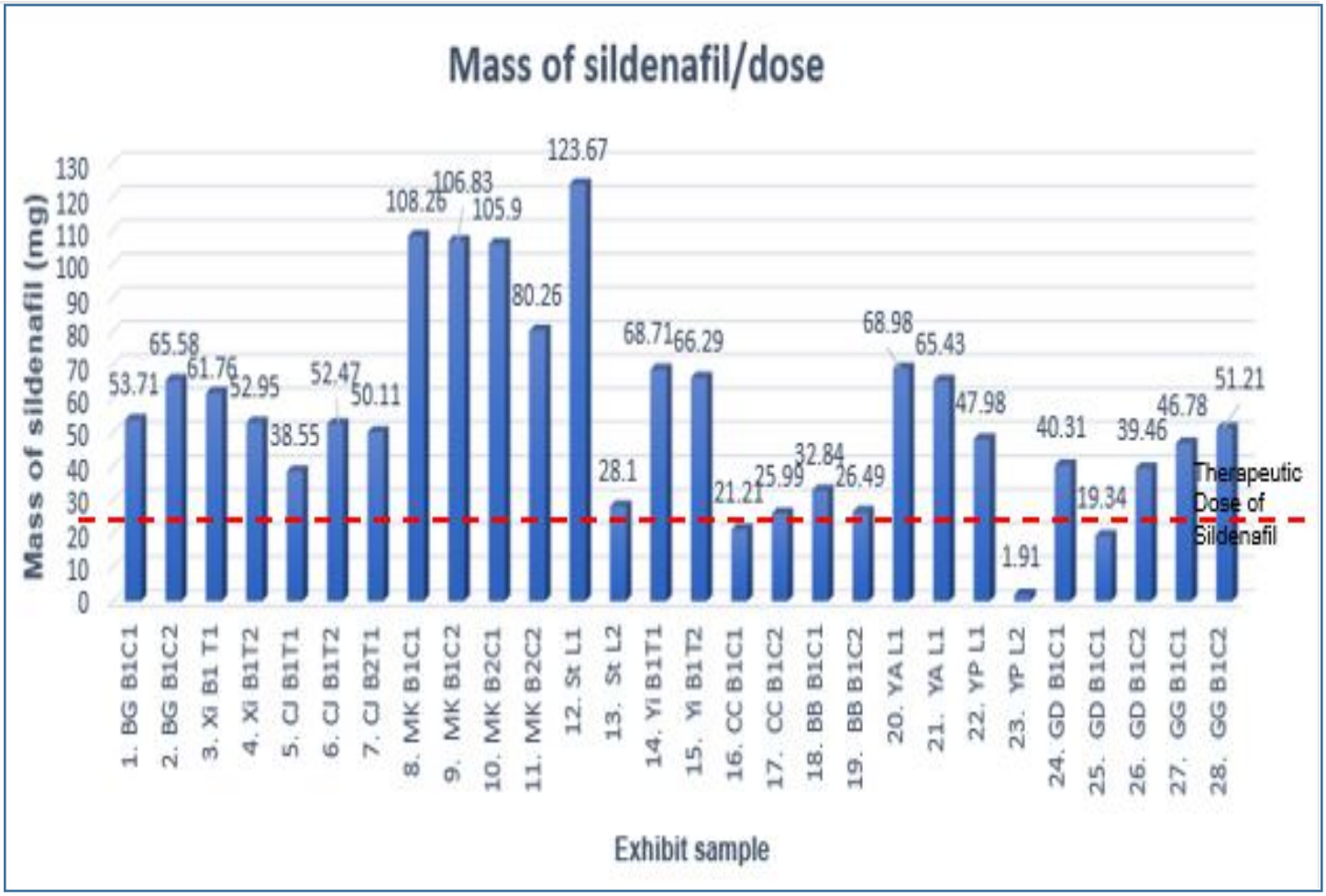

Figure 3.19: Mass of sildenafil found/dose from C1S1. The amount of sildenafil found ranged from above the maximum recommended dose of sildenafil to trace amounts with 89% of samples containing above the minimum dose.

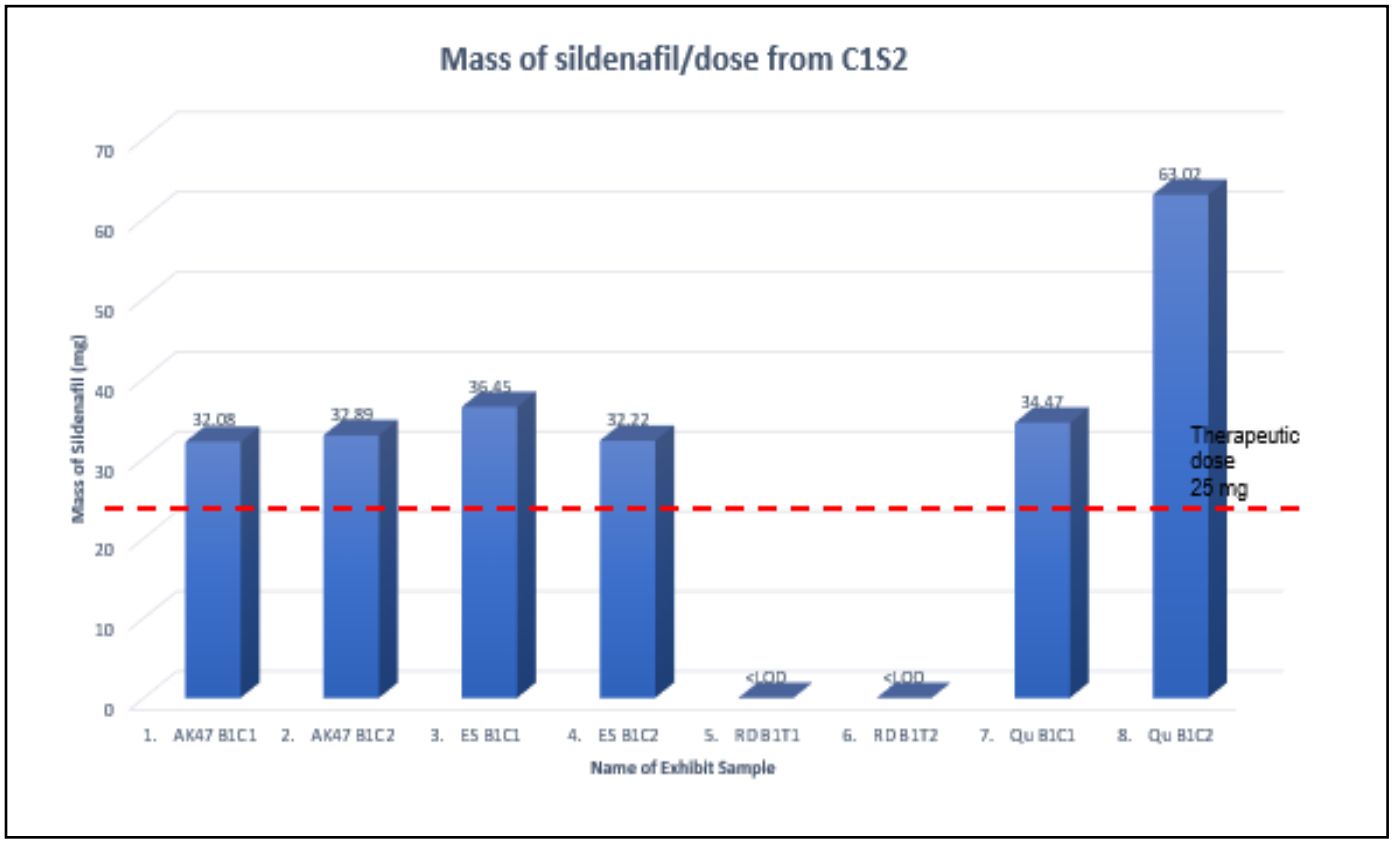

Figure 3.20: Mass of sildenafil found/dose from C1S2 with 75% of the samples containing above the minimum recommended dose of sildenafil.

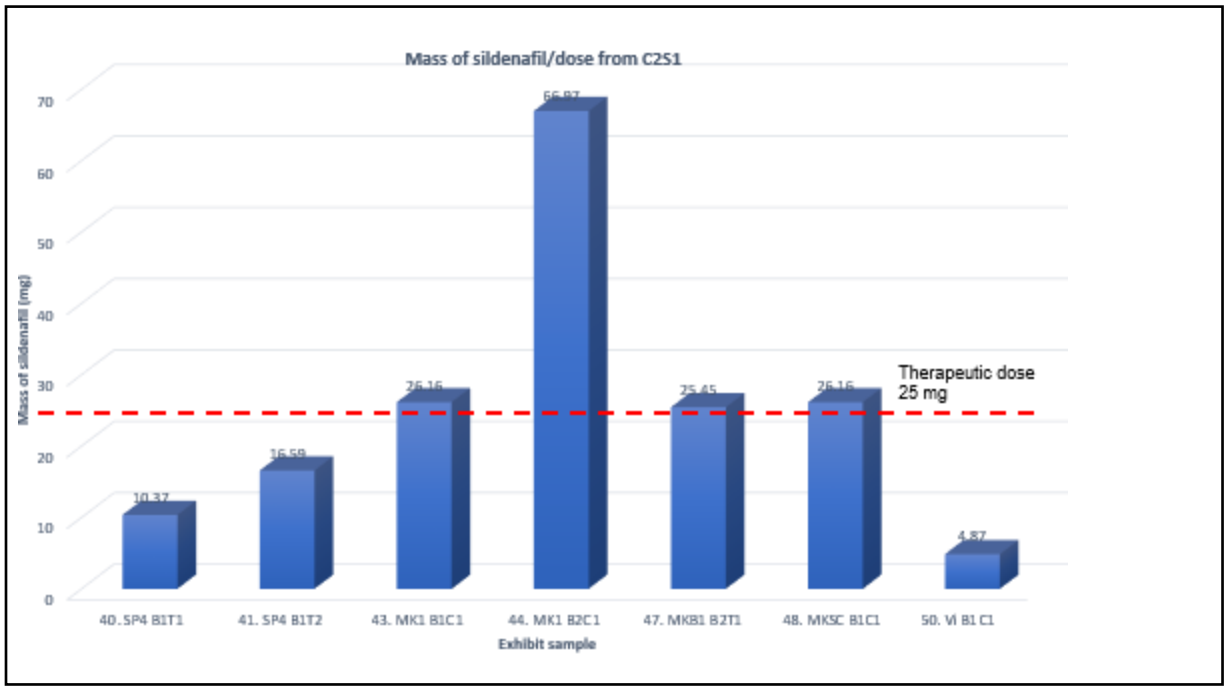

Figure 3.21: Mass of Sildenafil/dose found in samples from C2S1with 57% of products containing above the minimum dose of sildenafil.

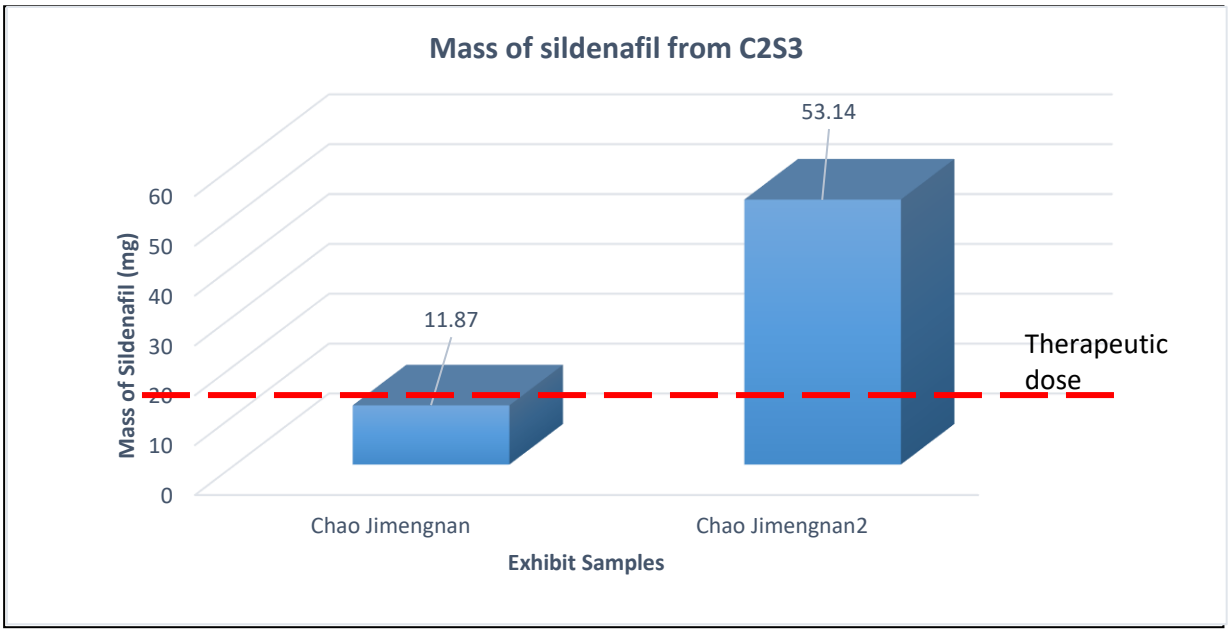

Figure 3.22: Mass of sildenafil found/dose from C2S3 with both of the samples containing sildenafil, one was above the average recommended dose of sildenafil.

All the results were collated from the clusters and a summary is shown in Table 3.8. The number of positive results in each dosage form is shown as well as the mean and mean amount of sildenafil per tablet/capsule/lump. The supermarket clusters (C1) contain more products with sildenafil than the pharmacy cluster (C2). There was not much variation between the amount of sildenafil found in capsules and tablets, however not enough samples within each product were analysed and this should be investigated further.

Table 3.8: Summary of results showing how many samples tested positive for sildenafil, the mean and median as well as the number of capsules, tablets or lumps which tested positive and the mean amount of sildenafil in each.

Store	Total number of samples	Total positive for sildenafil (n, %)	Mean mass of sildenafil detected (mg)	Median mass	Total number of capsules	Caps positive for sildenafil	Mean mass of sildenafil/ cap	Total number of tabs	Tabs positive for sildenafil	Mean mass of sildenafil/ tab	Total number of lumps	Lumps positive for sildenafil	Mean mass of sildenafil/ lump
C1S1	28	28 (100)	55.53	51.84	15	15	54.75	7	7	55.83	6	6	56.01
C1S2	8	6 (75)	38.52	32.56	6	6	38.52	2	0	0	0	0	0
C2S1	19	7 (37)	10.06	25.45	11	3	5.20	8	4	17.92	0	0	0
C2S2	4	0 (0)	0	0	2	0	0	2	0	0	0	0	0
C2S3	2	2 (100)	32.5	32.51	0	0		2	0	32.5	0	0	0

3.6 Results from Exhaustive extractions

The exhaustive extractions were a preliminary investigation into how much sildenafil was relatively extracted after one wash in a 50%v/v methanol/water mixture, in order to ascertain whether this would be an efficient extraction technique. The results showed that the approximate range of sildenafil extracted, was from a relative maximum of 97.5% to an approximate minimum of 80% (See Table 3.9) and Figure 3.23. These are relative percentages as full recovery was not assessed from an equivalent blank matrix.

Table 3.9: Exhaustive extractions of compounds show the peak area of sildenafil after 1, 2 and 3 washes.

Sample Name	Analyte Peak Name	Analyte Peak Area (counts) 1st wash	% sildenafil present after 1st wash	Analyte Peak Area (counts) 2nd wash	% sildenafil present after 2nd wash	Analyte Peak Area (counts) 3rd wash	% sildenafil present after 3rd wash
1.BG	sildenafil	5810000	97.5	128000	2.20	20000	0.30
2.Xi	Sildenafil	2930000	87.0	379000	12.9	34000	1.16
3.CJ	Sildenafil	2370000	92.5	177000	7.50	24100	1.00
4.MK	Sildenafil	6580000	94.0	396000	6.00	65100	0.99
5.St	Sildenafil	293000	84.0	45600	15.6	46800	15.9
6.Yi	Sildenafil	6310000	96.0	260000	4.00	44000	0.007
7.CC	Sildenafil	4840000	94.0	287000	5.90	24600	0.50
8.BB	Sildenafil	4100000	84.0	648000	15.8	294000	7.20
9.YA	Sildenafil	980000	90.0	101000	10.0	74400	7.60
10.YP	Sildenafil	793000	80.0	162000	20.0	99200	2.50
11.GD	sildenafil	3670000	89.0	404000	11.0	332000	9.00

The second and third washes analyte peak areas were used to calculate the percentage of sildenafil left in the supernatant as a ratio of the first wash. This remaining amount less 100% determined the amount of sildenafil initially extracted. This was not intended to be a quantitative recovery method but rather an indication that most but not all of the sildenafil had been extracted. It was decided that since most of the sildenafil was extracted from the first wash – for the purpose of this preliminary study only one extraction was used. This would underestimate

the quantity of sildenafil in the sample. All the exhibit samples were therefore extracted using only one wash.

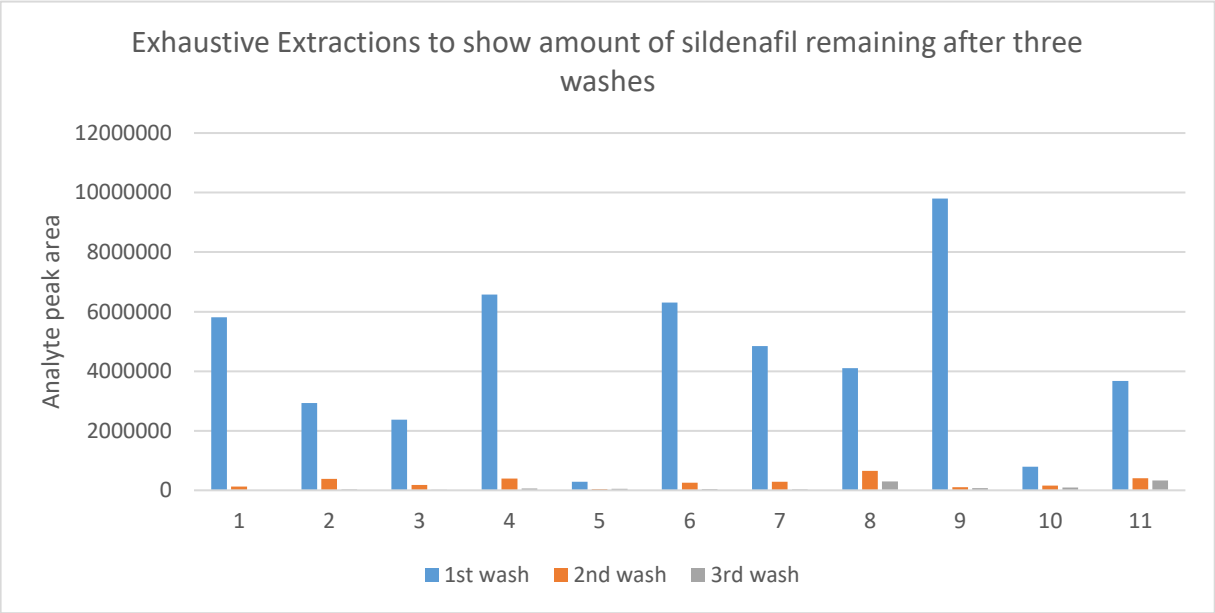

Figure 3.23: Bar graph showing results of exhaustive extractions by the third wash trace amounts of sildenafil remained, most of the sildenafil had been extracted after the first wash.

3.7 Results from Carry over and Stability Studies

By running a water blank between each sample, it was confirmed that there was no relevant carry over. All extracted samples remained stable on the bench at room temperature, at -20 ^{0}C and -80 ^{0}C after 24 hours. Table 3.10 shows the results obtained from the 24-hour stability studies at room temperature, -20 ^{0}C and -80 ^{0}C.

Table 3.10: Results from 24-hour stability studies for exhibit sample solutions left at room temperature, -20 ^{0}C and -80 ^{0}C.

Concentration (ng/mL)	Sample	Number of runs	Mean	Standard Deviation	% CV	Accuracy
20	LOQ (room temp)	6	19.74	3.46	17.54	98.7
60	QC low (room temp)	6	55.2	4.69	8.49	92.0
400	QC med (room temp)	6	396.47	12.72	3.2	99.12
800	QC med/high (room temp)	6	758.17	12.67	1.67	94.8
1600	QC high (room temp)	6	1574.43	75.79	4.81	98.4
60	QC low (-20^{0}C)	4	60.65	4.57	7.54	101.1
400	QC med (-20^{0}C)	4	387.42	19.39	5.01	96.85
1600	QC high (-20^{0}C)	4	1149.7	431.73	37.55	71.86
60	QC low (-80^{0}C)	2	67.39	1.23	1.82	112.31
400	QC med (-80^{0}C)	2	412.35	13.57	3.29	103.1
1600	QC high (-80^{0}C)	2	1525.94	50.9	3.34	95.37

CHAPTER 4: DISCUSSION AND CONCLUSION

4.1 Discussion

Herbal products for ED and weight-loss in SA are prone to adulteration as not all medications are subjected to regular, rigorous testing. Little research has been done on the adulteration of CAMs sold in SA, placing the consumer in a vulnerable, uninformed and possibly dangerous position. The current study aimed to determine whether CAMs for weight-loss and ED contain sibutramine or sildenafil in a number of commercially available over-the-counter (OTC) products sold in Cape Town, SA, and to quantitate any sildenafil found.

The exhibits obtained in this study were analyzed at the University of Cape Town in the Division of Clinical Pharmacology. The labels on the samples purchased claimed to contain only natural contents such as fur seal penis, stag antlers and various other plant and animal products.

This study indicated that scheduled medication in the form of sibutramine and sildenafil were present in OTC medicines in several stores in Cape Town. Sildenafil in ED medication, was found to be more prevalent than sibutramine in the anorectics as there were more products available for ED than for weight-loss (Table 2.1). The partially validated method for the quantitation of sildenafil permitted the approximate calculation of the amount of sildenafil within each sample. A fully validated method, looking at recovery studies and matrix effects would have added more value; however, previous authors have shown excellent recovery and no effect from the herbal matrices (Zhu *et al.* 2005; Liang *et al.* 2005; Lee; Lee at al 2013).

The amount of sildenafil found allowed comparisons to be made to therapeutic doses of sildenafil in Viagra®. However, not every tablet/capsule or lump was tested in every pack of exhibit sample, therefore further investigations would be warranted. Further studies would have been beneficial to determine whether other active pharmaceutical ingredients (API's) besides sildenafil and sibutramine, were present in both ED medication and

anorectics. The study would have been more robust had tadalafil and vardenafil been detected, however, standards of these were not available. Screening and quantitating for analogues would also have added breadth to the findings of this study. This was a preliminary study to investigate whether further testing of these products in more controlled environments is warranted.

4.1.1 Previous Research

Adulteration of herbal products has been a problem for many years particularly within Africa, Southeast Asia and many countries in Latin America (Deconinck *et al.* 2012). With the concurrent increase in the consumption of CAMs, there has been an increase in their adulteration, rendering many scheduled or toxic substances being unwittingly consumed (Gratz *et al.* 2004; Oh *et al.* 2006; Lee *et al.* 2014; Patel *et al.* 2013). Products available in Cape Town from both supermarkets and certain pharmacies have been shown in this study to contain the API's sildenafil and sibutramine.

Lifestyle CAMs for obesity and ED are amongst the most popular, and it has been suggested that individuals without ED are using these drugs for recreational purposes (Patel *et al.* 2013). Both ED and obesity can be partially treated with OM. Lifestyle changes such as exercise and healthy eating, as an alternative to medication, can likewise reduce these conditions. However, in order to avoid lifestyle changes or OM, individuals may turn to what they perceive to be safe, quick alternatives in the form of CAMs (Rocha, 2016).

Sildenafil (Viagra®, Pfizer) was the first approved drug for ED, followed by vardenafil (Levitra®, Bayer) and tadalafil (Cialis®, Elli Lilly). These PDE -5 inhibitors increase the amount of cGMP which leads to smooth muscle relaxation and penile erection (Singh *et al.* 2009). CAMs for ED are therefore being adulterated with these PDE-5 inhibitors, with sildenafil being amongst the most prevalent (Zhu *et al.* 2005; Lee *et al.* 2013; Oh *et al.* 2006; Abdel-Hamid 2006; Liang *et al.* 2005; Singh *et al.* 2009; Patel *et al.* 2013; Tseng *et al.* 2001; Lee *et al.* 2014; Deconinck *et al.* 2013; Jeong *et al.* 2016; Venhuis *et al.* 2013; Lee; Lee *et al.* 2013). Tadalafil was, however, found to be more prevalent than sildenafil in two studies (Lee *et al.* 2013; Jeong *et al.* 2016). This study found sildenafil in the ED CAMs which tested positive for adulteration.

Anti-obesity drugs such as sibutramine, fenfluramine and phentermine are being added to CAMs for weight-loss (Kim *et al.* 2014). Sibutramine was the most frequently found anorectic in a study done by Kim and colleagues in 2014. These anorectics have many side effects and may even result in death if abused (Csupor *et al.* 2013). Even although sibutramine is banned in SA it was detected in six of the anorectics tested.

All-natural CAMs are an appealing alternative to prescription medication, as many OM's require prescriptions or are contra-indicated with medication already being used (Jeong *et al.* 2016). A CAM adulterated with a PDE-5 inhibitor may therefore pose a severe health risk to the consumer (Singh *et al.* 2009; Oh *et al.* 2006; Zhu *et al.* 2005; Lee *et al.* 2014; Patel *et al.* 2013). It has, therefore become imperative to monitor and regulate these adulterants, particularly through instrumental analyses (Liang *et al.* 2005).

Various instrumental methods have been validated for the best chromatographic separation and detection of APIs in herbal and medicinal products. Patel *et al.* (2013) have employed and assessed various methods (Deconinck *et al.* 2013). Each have their own merits but it was found by numerous authors that overall, HPLC-MS/MS was the most selective and specific, rapid and reliable method able to detect numerous targeted adulterants in one run (Lee *et al.* 2013; Oh *et al.* 2006; Abdel-Hamid 2006; Liang *et al.* 2005; Singh *et al.* 2009; Patel *et al.* 2013; Tseng *et al.* 2001; Eerkes *et al.* 2002; Liang *et al.* 2006; Haque *et al.* 2014; Gou *et al.* 2016). The method to detect and quantitate sildenafil had been previously validated in numerous studies (Tseng *et al.* 2001; Gratz *et al.* 2004; Liang *et al.* 2005; Zhu *et al.* 2005; Abdel-Hamid 2006;Venhuis *et al.* 2008Lee *et al.* 2013; Lee; Kim *et al.* 2013; Jeong *et al.* 2014; Wollein *et al.* 2015) as well as stability studies and dilution integrity (Haque *et al.* 2014). High Performance Liquid Chromatography tandem Mass Spectrometry (HPLC-MS/MS) proved to be a rapid, reliable and accurate method to detect and quantitate API's in solid dose form in CAMs for ED and anorectics.

The complex matrices of herbal preparations, consisting of unusual fillers, pose the risk of interference and false positives of scheduled API's, however, Zhu *et al.* (2005) found that the complex herbal matrices of CAMs did not interfere with the detection of the API's when LC-MS/MS

technique was used. It has been suggested that novel analogues of scheduled APIs are being developed by clandestine chemists (Patel *et al.* 2013). These analogues have similar structures and functions to the registered drug substance and a highly specific method is needed to identify each individually (Venhuis *et al.* 2013). New methods are being developed as novel analogues are continuously being synthesized, with over 50 unapproved analogues of PDE-5 inhibitors currently on the market (Lee *et al.* 2014; Patel *et al.* 2013). In the interest of public safety collaboration, education and research is needed worldwide in order to curb this illegal practice (Venhuis *et al.* 2013; Lau *et al.* 2002; Deconinck *et al.* 2013; Lee *et al.* 2013; Oh *et al.* 2006; Jeong *et al.* 2016; Patel *et al.* 2013; Kim *et al.* 2014).

4.1.2 Current and Future Research

A robust screening method was required in order to identify the adulterant amongst the other interfering compounds**.** HPLC-MS/MS was the analytical technique chosen as it was the best method identified from previous research to quantitate PDE-5 inhibitors in solid dose. This method was adopted for its specificity and it also allowed for the quantitation of any sildenafil detected and has been used successfully in previous studies (Singh *et al.* 2009; Oh *et al.* 2006; Jeong *et al.* 2016).

The method for the quantitation of sildenafil was partially validated following guidelines from previous studies (Tseng *et al.* 2001; Gratz *et al.* 2004; Liang *et al.* 2005; Zhu *et al.* 2005; Abdel-Hamid 2006; Venhuis *et al.* 2008; Lee *et al.* 2013; Lee; Lee *et al.* 2013; Jeong *et al.* 2014; Wollein *et al.* 2015; Appendix A). Controls were prepared in pools large enough to provide samples for the entire study**.** The LOD was found to be 1.09 ng/mL and the LOQ was set at 20 ng/mL (Table 3.6). A quadratic regression showed good linearity for nine calibrators from 20-2000 ng/mL with a regression coefficient of 0.999 (Figure 3.16). Validation data also showed the method to be accurate and precise with %CV < 20% and bias < 20%. There was no carry over and extracted samples were stable at room temperature, -20^0 C and -80^0 C for 24 hours. These results compared favourably to previous studies (Tseng *et al.* 2001; Gratz *et al.* 2004; Liang *et al.* 2005; Zhu *et al.* 2005; Abdel-Hamid 2006; Venhuis *et al.* 2008; Lee *et al.* 2013; Lee; Lee *et al.* 2013; Jeong *et al.* 2014; Wollein *et al.* 2015; Appendix A).

Interference studies and recoveries were not performed within this preliminary investigation, but previous authors had shown no interference from herbs and excellent recoveries (Zhu *et al.* 2005; Liang *et al.* 2005; Lee; Lee at al 2013) and the highly specific method of HPLC-MS/MS compensated for these effects. However, if these herbal products require further investigation by the MCC, the validation would have to be extended to include matrix effects, ion suppression and enhancement studies, and possibly investigating a linear curve over a smaller range.

The developed method allowed for the screening of 26 exhibit samples for hidden API's and sibutramine and sildenafil were detected in weight-loss preparations and ED products respectively (Table 3.2). These products were not manufactured in SA and this study did not look at traditional herbal medicines made in SA. Employing HPLC-MS/MS, the API's sildenafil and sibutramine were found in CAMs for ED and weight-loss respectively and the detected sildenafil was quantitated using this partially validated but very specific method. Out of the 26 exhibit samples for screening, 12 were positive for sildenafil and 6 for sibutramine. This indicates that there are products available in Cape Town that contain scheduled drugs that consumers are unaware of using.

A consumer in Cape Town, SA, who buys an OTC natural product for ED or weight-loss, may run the risk of consuming an API that has been hidden in the product and that is not declared on the product label, this could have serious health consequences. The herbal products available for ED were more widely available in the market than those for weight-loss. It was therefore, decided to proceed with investigating ED CAMs and to quantitate any scheduled substance, such as sildenafil, found in the products. However, it is advisable that the MCC investigate weight-loss products available in Cape Town, as there are some containing sibutramine as was identified by the preliminary screening component of the project.

Out of the 61 additional ED exhibit samples tested, 43 (70%) tested positive for sildenafil. The dose of sildenafil ranged from trace amounts to 123.7 mg/tablet/capsule/lump. Of the capsules 24 out of 32 (75%) tested positive for sildenafil and 13 out of 19 (68%) of the tablets contained sildenafil. All six of the solid lumps tested were positive for sildenafil. Capsules, at 75% were more frequently

adulterated than tablets at 68%. Although all the lumps tested were adulterated they were far less available. However, not all tablets and capsules were tested within the 61 purchased packets of exhibits. Intra-exhibit variability warrants further investigation, as there may be a variation in adulterant within samples from the same exhibit.

These results are similar to previous studies which quantitated PDE-5 inhibitors, although this study focused only on sildenafil found in commercially available OTC products. Only this study differentiated between samples from clusters of supermarkets and pharmacies. Liang *et al.* (2005) screened for nine different adulterants with 74 out of 200 samples testing positive. Of these 28 out of 81 products for ED were adulterated with sildenafil. Gratz and colleagues in 2005 tested for tadalafil and an analogue of sildenafil as well as sildenafil. They found that nearly half of the botanical products they tested for PDE-5 inhibitors contained therapeutic levels of sildenafil, tadalafil and homosildenafil. Similarly, Reeuwijk *et al.* (2013) tested various PDE-5 inhibitors and found that 18 out of 23 herbal products on the Dutch market contained one of nine PDE-5 inhibitors. They also concluded that the levels were sufficient to have a pharmacological effect. In a further study, 52 natural ED products were tested for 38 PDE-5 inhibitors and their analogues, with 87% containing the targeted compound (Lee; Lee at al 2013). CAMS for ED are being adulterated with sildenafil, it's analogues and various other PDE-5 inhibitors. Sildenafil was reported as the most prevalent in these studies and often detected in pharmacologically toxic amounts.

Viagra® is a schedule 6 medicine and only available with a doctor's prescription. It is available in 25mg, 50mg and a maximum dose of 100mg tablets, and is usually prescribed at the lowest dose of 25 mg (Pfizer.com). Sildenafil is the API in Viagra®. The findings from the analysis of the samples showed that 100% of the samples (*n*=28) for erectile dysfunction from the Chinese supermarket (C1S1) contained sildenafil and all (*n*=4) of the slimming tablets contained sibutramine (a banned API in anorectics in SA). The average amount of sildenafil was 55.5 mg/sample, which is above the average recommended dose of Viagra® and more than double the minimum starting dose. Four products contained more than the maximum dose of sildenafil per sample which may be of concern to public safety. A consumer in Cape Town, who buys a natural sexual enhancement product from a Chinese supermarket, runs a high risk of consuming a dose of Viagra®.

The average amount of sildenafil in the products (*n*=6) from the second supermarket, the R5 store (C1S2) was 38.5 mg. Also, an appreciable amount of sildenafil and above the minimum dose.

The pharmacies in cluster 2 did not have as many tainted samples and those that were adulterated had on average less than those doses found in the first cluster of supermarkets. There is a significant difference in the amount of sildenafil found in the products from the supermarkets compared to the pharmacies as the pharmacies have tighter regulations. The products for erectile dysfunction tested from the reputable pharmacy chain (C2S1) contained an average of 10.1 mg of sildenafil per dose. Whilst this is below the lowest recommended dose, the consumer who is self-medicating may take more than one tablet. If the consumer is sensitive or allergic to sildenafil, even a small dose may be harmful. Sildenafil should not be in a natural product in even trace amounts given its scheduled nature. None of the products tested from the independent pharmacy 2 (C2S2) contained sildenafil. Both the tablets tested from the third pharmacy (C2S3) contained sildenafil at an average of 32.5 mg/tablet, again above the minimum dose. Pharmacies may have a better reputation than the supermarkets and the consumer may be more trusting of the products bought from pharmacies.

Sibutramine was found in a previous study as ranging from 0.03 mg/g to 132.40 mg/g (Kim *et al.* 2014). Tadalafil was found in amounts from 0.08 -138.69 mg/g (Jeong *et al.* 2014) and from 0.08 -138.69 mg/g (Lee *et al.* 2013). Lee *et al.* (2013) found sildenafil ranging from 0.03 - 369.93 mg/g. In this study, the amount of sildenafil found ranged from trace amounts to 123.7 mg/dose. These studies indicate the variability in the adulteration of different samples. This indicates the limited nature of controlled manufacturing of these products, which further indicates the risk to the consumer of these OTC products. Sibutramine was not quantitated as being a banned substance just being present in trace amounts is problematic.

At an average of 55.5 mg/dose from the Chinese supermarket, the level of sildenafil that was found in the CAMs is appreciable and the highest of all the clusters. Although the averages from the other store and pharmacies was lower, the odd product contained high levels of sildenafil; 63.0 mg from the R5 store, 66.9 mg from the large pharmacy chain and 53.1 mg from the private pharmacy 3

(Tables 3.7.1-3.7.5). Again, this depends on the sample randomly tested from the exhibit packets (which often contained more than one sample), and further investigation into the variation of sildenafil between samples within the exhibits is warranted.

The results showed that the supermarkets generally had more adulterated samples, although substantial amounts of sildenafil were found in the pharmacy cluster, too. This poses a very real risk to the consumer as sildenafil may produce side/toxic effects following consumption. The dosage may also need to be adjusted in certain situations due to drug interactions or for special populations e.g. men with heart disease or hypertension (Pfizer.com; Zhu *et al.* 2005; Singh *et al.* 2009). There can be contraindications, side effects, over dosage and the need for precautions (Pfizer.com; Zhu *et al.* 2005; Singh *et al.* 2009). The amount of sildenafil hidden in the CAMs from the Chinese supermarket was higher than the samples tested from the reputable pharmacy chain (bearing in mind that not all samples were analysed from each exhibit). While sildenafil detected in the samples analysed from the pharmacy chain on average was low, it should not be detected at all given the nature of its legal scheduling and it is to be noted that the odd tablet/capsule showed amounts of sildenafil nearly three times above the minimum recommended dose.

Patterns and Relationships

Man King was available from both supermarkets and adulterated with a high amount of sildenafil in the tablets tested. It was also found at the reputable pharmacy chain but not all tablets in the packs were adulterated. A similar pattern was found for Chaojemengnan but it was marketed as 'superpowerful man pills' at the large pharmacy chain although the packaging and tablets were identical.

When tablets within the same box were tested, most tablets had the same amount of sildenafil but there were a few occasions where one tablet had a high dose of sildenafil and a second tablet had a much lower dose. This was illustrated in the lumps, for example one lump of Stag contained 123.7 mg of sildenafil whilst a second lump only had 28.1 mg (Figures 3.19-3.22). It is evident from

these findings that there is variability between samples within the same package, and further investigation into this variability, particularly from a statistical viewpoint is necessary.

Unexpected Findings

It was unexpected to find that so many products (37%) from the large pharmacy chain were adulterated. Most of the adulterated products were manufactured in China or Thailand but distributed and marketed in SA (Gauteng). Except for one product which had trace amounts of sildenafil, all other products manufactured in SA were unadulterated. A few (*n*=3) of the products (only from the large pharmacy chain) have already been classified by the MCC as category D (complementary medicines) and this was printed on the label. These did not contain sildenafil. Only three products contained the MCC label that the product had not been evaluated by the MCC. The tablets from the Chinese supermarket which had the highest dose of sildenafil stated on the label that it was safe to use for patients with high blood pressure or cardiac disease or after drinking. All, in fact, are contra-indicated conditions (Pfizer.com). Another capsule also testing high for sildenafil states, “it is absolutely made of the Chinese herbs and is safe to take.”

Limitations of Design

This study was firstly limited by a small sample size of stores; therefore, the results cannot be inferred to all other samples and a wider sample size would present a more representative sample and have increased statistical significance. This would then allow us to analyse increased numbers of exhibits; however, an appreciable number of samples within the stores tested positive for sildenafil and other studies have tested as little as one sample (Tseng *et al*. 2001). It is imperative that in future studies, multiple or all samples within an exhibit are tested to distinguish variability within the packet. This study did not look at herbal products manufactured in SA. These are generally traditional African herbal medicines. In a previous study, these were shown to be adulterated with an anaesthetic and anti-convulsant (Snyman et al. 2005). Traditional medicines manufactured in SA for ED and weight-loss were not in the scope of this study.

The method was not validated for analogues of sildenafil nor other PDE-5 inhibitors and no method was validated for sibutramine or other anorectics as this was not within the scope of this study, in addition the method was robust enough for a partial validation. Exhaustive extractions were performed but not actual recovery tests. These showed that most but not all the sildenafil had been extracted, therefore, the actual mass of sildenafil/ sample would in fact be higher than that calculated which would only add more significance to these results. Although no matrix effects were tested, previous authors have shown them to not have interfered in the quantitation.

A full method validation with recoveries and stability studies would have made this project more robust, however these results were sufficient for the scope of this project and a full validation was not deemed necessary as both full and partial validations have been done in prior studies with equally robust results (Lee *et al.* 2013; Tseng *et al.* 2001; Kim *et al.* 2014; Zhu *et al.* 2005). The aim of the project was to distinguish whether scheduled APIs could be detected in these products, and if so whether they could be quantified, which was achieved.

Implications for the Consumer

In SA, Viagra® is a schedule 6 drug. This means it cannot be given on repeat prescription but requires a doctor's visit, together with a new script, for each prescription. This is necessary as the sildenafil in Viagra® may produce side and/or toxic effects upon administration.

Sildenafil is contraindicated with the concomitant use of other drugs and alcohol and in certain population groups (McCullough 2002; Krenzelok 2000). Sildenafil also has adverse reactions, side effects and there is a possibility of overdosing and even death (Pfizer.com 2016; Krenzelok 2000).

The labelling on all the CAMs in this study claim that the products only contain natural ingredients and the detection of sildenafil in these products is of legal and safety concern. For regulatory purposes, drug enforcement agencies (MCC in SA) often need to screen for western drug ingredients in CAMs. The use of a validated analytical technique which is rapid and specific such as HPLC-MS/MS in scheduled multiple reaction monitoring (MRM) will assist in this regard. Anecdotal evidence claims that the CAMs industry in SA is worth billions, so

although the mandate is clear that these products, until tested must carry a label, the complementary medicines industry seems reluctant to label their medicines as untested by the MCC (Africa Check, 2013). The primary reason for the reluctance of the CAM industry to withdraw their products for labelling is because they claim that this may result in billions of rands of lost revenue, loss of jobs and an adverse effect on the economy (Rocha *et al.* 2014). The complementary medicines lobby says people have the right to take responsibility for their own health, diagnose themselves and choose their medication. The Health Products Association (HPA), an industry body for complementary medicine businesses, agrees and estimates that the losses to the economy could be as much as R50 billion (Kahn, 2014). Most recently a South African pharmacy chain refused to remove GNC herbal products for not containing what the label claims, yet they have been removed from certain states in America (Kahn, 2015). However, not all members of the CAM industry disagree with labelling, for example, it has been suggested that some CAMs may need to carry warnings because," as a consumer I want to know that what the label says is what I get" (Viall, 2014).

Product labelling

The product label, if available, provides the consumer with a list of ingredients that illustrate what the product contains. Certainly, for the samples in which sildenafil was detected in this study, the label did not warn the consumer that the product contains an API like sildenafil or sibutramine and the dangers thereof. In most cases the label encouraged use because of the natural, safe products that are used. In some cases, there was only a label stating that the product has not been declared a medicine by the MCC, which at present is the only labelling requirement of the MCC.

The product is untested and so what is stated on the label may bear no resemblance to the actual ingredients. The consumer is unaware of what they are taking but conveniently believes it is all natural, especially if they are deriving the desired effect from the product. Admittedly, clever marketing does lure the consumer into the false belief that the product is safe as it claims to contain only natural, harmless ingredients.

Dosage

For most patients, the recommended dose of Viagra® (sildenafil) is 50 mg taken, as needed, approximately 1 hour before sexual activity. Based on individual factors, drug efficacy and possible tolerance, the doctor may increase the dose to a maximum of 100 mg or decrease it to 25 mg. The maximum recommended dosing frequency is once per day, with or without food (Pfizer.com, 2016). Viagra is more potent than tadalafil and vardenafil and not as safe (Zhu *et al.* 2005). In the elderly, it is prescribed at the minimum 25 mg dose which often, even at this low dose, may cause severe side effects (McCullough, 2002). Sibutramine in anorectics, being banned in SA should not be in any product in any amount.

Dangers

Numerous products contained above the therapeutic dose of Sildenafil and a few contained more than the maximum dose, this may produce toxicity. Sildenafil can have adverse reactions for people with certain medical conditions. The product monograph for Viagra® instructs the consumer to alert their doctor of any of the following conditions before taking Viagra®: heart problems such as a heart attack, irregular heartbeat, angina, chest pain, narrowing of the aortic valve or heart failure; heart surgery within the last 6 months,
pulmonary hypertension, previous stroke, low blood pressure, or high blood pressure that is not controlled, a deformed penis shape, an erection that lasted for more than 4 hours, problems with blood cells such as sickle cell anemia, multiple myeloma, or leukemia, retinitis pigmentosa, a rare genetic (runs in families) eye disease, severe vision loss, including an eye problem called non-arteritic anterior ischemic optic neuropathy (NAION), bleeding problems, stomach ulcers, liver problems, kidney problems or are having kidney dialysis (Pfizer, 2016).
Sildenafil is contraindicated with the concomitant use of certain medications and has various side effects (See Appendix F for a comprehensive list).
Sibutramine can cause tremors, insomnia, coma and even death (Csupor 2013).

Consequences

If a consumer unwittingly consumes sildenafil or sibutramine in an herbal product, they will be unaware of any of the contraindications, dangers, side-effects, precautions and warnings which would usually be given by the doctor prescribing sildenafil in Viagra®. The product is purchased OTC with no counselling or advice given. The consumer may be inclined to take more of the product as it is assumed to be natural and therefore harmless. Toxicity becomes a very real possibility in this case. The consumer, however, will not even be aware that they may have overdosed on too much adulterated product. Previously, it was assumed that the older population with prior heart conditions were the most at risk to sildenafil. Researchers, however, at Cedars-Sinai Medical Center in Los Angeles analysed the adverse events reports made to the FDA and in March 2000 found that from 1473 reports 822 men had died. Mostly these deaths were due to cardiovascular reasons. Unusually, all of the men were under 65 years old and none had previous cardiac issues (Psa-rising.com, 2016).

Although the consumer may be unaware of possible adulteration, it is imperative for the healthcare provider to be told about all the medicines taken, including prescription and over-the-counter medicines, vitamins, and herbal supplements. The doctor should also be asking the correct questions and themselves be aware of the possibility of the adulteration of CAMs.

The consumer should be aware that their CAM consumption must be made known to medical practitioners and in a medical emergency. Ironically an unintentional potentially fatal interaction can occur in a medical emergency of severe syncope (possible side effect of sildenafil) where the administration of the necessary nitrates will worsen the situation if the patient has taken sildenafil.

To protect the consumer, screening for adulterants in CAMs is the obvious necessary first step and important, as adulterants should not be present in even trace amounts. However, quantitation gives more rigour to the analysis in order to make comparisons between the amount of product available in a prescription dose compared to the

adulterated dose as shown in this study. The quantitation of the sildenafil found in the samples in this study showed many having a higher quantity of sildenafil than the minimum dose. Sibutramine, as a banned substance in SA, should not be present at all in the samples screened.

Although the economy is adversely affected by death CAMs manufacturers and suppliers, believe that if they are made to withdraw their product from the market, this will create a job deficit and the decreased contribution to the economy will also have a large negative impact (Viall, 2014)

Suggested ways forward

The general consumer needs to be made of aware of the possible adulteration of herbal remedies as illustrated in this research. Research by the consumer is helpful but there should also be an input from the health care practitioners. With the widespread popularity of CAMs, the gap between knowledge and communication for health care professionals has become apparent. Ventola, (2010) believes that due to a lack of formal training, there is not enough evidence-based information about efficacy, safety, and drug interactions with CAM therapies.

The government and MCC should be assisting with intensive surveillance, better border control, testing, product labelling and regulation of CAMs. Legislation, whilst impressive in theory, needs to be controlled practically (the major pharmaceutical companies) and deadlines must be met. This may necessitate increased funding and man-power within that sector of the pharmaceutical industry in SA. These requirements may be weighed up against the possible harms to consumers; however, these findings may generate increased pressure on the regulatory companies to pursue this testing further.

The media should be highlighting the possibility of CAM adulteration and more public forums and websites should be set up in order to educate the consumer and increase the awareness of possible adulteration and the dangers thereof. This could at least provide

insight into what may be consumed when using these kinds of CAMs, and the consumer can then make a some-what educated decision in their use.

Products bought by pharmacies should be done with discretion and from reputable manufacturers and suppliers. It might be necessary that pharmacies themselves control and regulate further, the products they are selling.

CAMs have been growing in SA, as elsewhere in the world, over the last couple of decades and it is reported that CAMs manufacturers have a different theory to the issue of adulteration (Rees *et al.* 2016). CAMs manufacturers believe that 'big pharma' (the major pharmaceutical companies) are responsible for removing CAMs from the marketplace (Rees *et al.* 2016). Anecdotal evidence states that CAMs manufacturers are under the impression that “big pharma” either want to make CAMs illegal or alternatively too expensive for small manufacturers by forcing CAMs to be regulated in the same way as drugs are; or where feasible, to buy out the successful CAMs companies and incorporate CAMs into their suit of products (natural health alliance, 2014). As many of the big pharmaceutical industries are in the EU, there is also a belief that these regulatory laws are based on EU laws (natural health alliance, 2014). CAMs manufacturers believe the laws will only leave ineffectual products available to the public. The reality is that tainted products are entering the market, putting the consumer at risk. This is why they need to be tested and possibly removed from the market.

Popular science magazines may claim that CAMs manufacturers see the laws of the DoH as pernicious and suspect and which they believe will destroy the CAMs industry in SA, however, the findings of this paper cannot be ignored. Whilst some aspects of the new laws may seem overly vigilant, the consumer must be protected from the danger of the possible and very real adulteration of CAMs.

Further research is warranted into testing products from more clusters and more of the larger more reputable pharmacy chains, where the consumer is more likely to believe they are getting a legitimate product. There is a higher level of trust in the product from a

pharmacy chain with a good reputation. The consumer genuinely believing that they have purchased a natural product may be more inclined to increase the dose especially if the product works, which it will do if it contains the API for erectile dysfunction or weight-loss.

Future research could include the matric effects as well as a full method validation and a screening which could include tadalafil and vardenafil. This study could be extended to adulteration of traditional herbal medicines which are manufactured in SA.

Analogues of PDE-5 inhibitors are constantly being synthesized. These structures, whilst similar, to sildenafil and other PDE-5 inhibitors are unregulated and undetectable as certified reference standards are not always available and their side effects are unknown (Lee *et al.* 2013). Rapid screening and quantitation of PDE-5 inhibitors and their analogues, as well as anti-obesity CAMs with possible banned APIs, is therefore imperative. This information also needs to be disseminated to the public, health sectors, pharmacies and stores purchasing CAMs. Collaboration is also necessary between the regulating authorities and all stakeholders. Research studies such as this may assist in providing further incentive to medicine regulation in South Africa, particularly when it comes to scheduled or illegal active compounds being detected in OTC products. SA needs some forum for reporting adverse reactions and this could be monitored for insight into side/toxic effects using these products. The genuine risk to the health of the general public when consuming an unregulated product cannot be ignored.

4.2 Conclusion

Consumers around the world, including South Africans are taking more responsibility for their health by using OTC herbal preparations. The main reason is because they are assumed to be harmless yet effective and they are conveniently available and often cheaper. This has caused a substantial increase in the use of OTC CAMs, particularly those for sexual dysfunction and weight-loss. Consumer knowledge is limited and consumers rely on product labels for information about the product. Unfortunately, most consumers are unaware that the product label does not contain information that has been

verified by regulatory bodies and the consumer has no guarantee of the safety or efficacy of the product. Almost none of the labels in this study were accurate. This lack of regulation and testing has exposed CAMs to adulteration with API's. The adulteration of CAMs is both illegal and a crime, yet it is a fact that it is happening. In Cape Town, SA, CAMs for ED and weight-loss investigated in this study were adulterated with sildenafil and sibutramine which could have toxic effects for the consumer which could be dire and may even be fatal. The amount of sildenafil in samples of ED CAMs was appreciable and mostly above the minimum therapeutic dose found in Viagra®. The method used to quantitate sildenafil can be used for further research and as a platform to detect other PDE-5 inhibitors, anorectics and analogues in these OTC medications. In view of the potential harm to the public, regulation, backed by education and research, is needed to improve the quality and quality use of CAMs. Urgent action is required to prevent harm to the consumer and to keep them out of danger. It is imperative that not only the general public but the health sector and government are also made aware of this situation. Solutions lie in regulatory and enforcement actions using suggested analytical techniques such as HPLC-MS/MS and very simple liquid-liquid sample preparation techniques, for robust and high performance testing. Education via the media, social media, published research findings, regulators, health care professionals and global alliance will go a long way to increase public awareness. To date, there is very little order in the CAM industry and the safest option is for the consumer to educated and aware and to take ultimate responsibility until such time as regulations are fully in place.

REFERENCES

Accessdata.fda.gov. 2016. Drugs@FDA: FDA Approved Drug Products. [online] Available at: https://www.accessdata.fda.gov/scripts/cder/drugsatfda/ [Accessed 14 Oct. 2016].

Africa Check. 2013. Claim that traditional medicines will be tested is churnalism not journalism - Africa Check. [online] Available at: https://africacheck.org/reports/new-testing-for-traditional-medicines-the-claim-is-misleading/#sthash.O7fRqC0c.dpuf) [Accessed 4 Oct. 2016].

Akerele, O.1993. Nature's medicinal bounty: don't throw it away. World Health Forum. 14:390-395.

Althof, S.E., O'Leary, M.P., Cappelleri, J.C., Hvidsten, K., Stecher, V.J., Glina, S., King, R. & Siegel, R.L. 2006. Sildenafil Citrate Improves Self-Esteem, Confidence, and Relationships in Men with Erectile Dysfunction: Results from an International, Multi-Center, Double-Blind, Placebo-Controlled Trial. The Journal of Sexual Medicine. 3(3):521-529.

Anderson, L. Drugs.com. 2017. Side Effects of Weight Loss Drugs (Diet Pills). [online] Available at: http://www.drugs.com/article/side-effects-weight-loss-drugs.htmL [Accessed 16 Jan. 2017].

Anzanello, M.J., Fogliatto, F.S., Ortiz, R.S., Limberger, R. & Mariotti, K. 2014. Selecting relevant Fourier transform infrared spectroscopy wavenumbers for clustering authentic and counterfeit drug samples. Science & Justice. 54(5):363-368.

Anzanello, M.J., Ortiz, R.S., Limberger, R. & Mariotti, K. 2014. A framework for selecting analytical techniques in profiling authentic and counterfeit Viagra and Cialis. Forensic Science International. 235:1-7.

AOAC International, "Appendix K: Guidelines for Dietary Supplements and Botanicals, Part 1 AOAC Guidelines for Single-Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals", 2013. [Accessed 3 Feb.2017]. http://www.eoma.aoac.org/app_k.pdf

Aronson, J.K. (2014). 76 - Plant Poisons and Traditional Medicines. In Manson's Tropical Infectious Diseases (Twenty-Third Edition). J. Farrar, and others, Eds. London: W.B. Saunders. 1128-1150.e6.

Asher, G.N. 2010. Herbal products review: what do we really know? Journal of the American College of Cardiology. 56(11):903; author reply 905-9.

Asif, M. 2012. A brief study of toxic effects of some medicinal herbs on kidney. Advanced Biomedical Research. 1:44-9175.100144. Epub 2012 Aug 28.

Atavwoda, A.T. & Gabriel, A.A. 2012. Assessment of pharmacists knowledge, attitude and practices regarding herbal drug information services. Journal of Basic and Clinical Pharmacy. 3(3):317-322.

Awortwe, C., Bouic, P.J., Masimirembwa, C.M. & Rosenkranz, B. 2014. Inhibition of major drug metabolizing CYPs by common herbal medicines used by HIV/AIDS patients in Africa-- implications for herb-drug interactions. Drug Metabolism Letters. 7(2):83-95.

Aytaç, I.A.; Mckinlay, J.B.; & Krane, R.J. 1999. The likely worldwide increase in erectile dysfunction between 1995 and 2025 and some possible policy consequences. BJU International, 84(1):50-56.

Baretic, M. 2013. Obesity drug therapy. Minerva Endocrinologica. 38(3):245-254.

Bechara, A., Casabé, A., De Bonis, W., Helien, A. & Bertolino, M.V. 2010. Recreational Use of Phosphodiesterase Type 5 Inhibitors by Healthy Young Men. The Journal of Sexual Medicine. 7(11):3736-3742.

Bertero, E. & Montorsi, F. 2014. Safety of Sildenafil Citrate: Review of 67 Double-Blind Placebo-Controlled Trials and the Postmarketing Safety Database. The Journal of Sexual Medicine. 11(4):885-887.

Blok-Tip, L., Zomer, B., Bakker, F., Hartog, K.D., Hamzink, M., Ten Hove, J., Vredenbregt, M. & De Kaste, D. 2004. Structure elucidation of sildenafil analogues in herbal products Food Additions and Contaminants. 21:737-748.

Bogusz, M., Hassan, H., Al-Enazi, E., Ibrahim, Z. and Al-Tufail, M. (2006). Application of LC–ESI–MS–MS for detection of synthetic adulterants in herbal remedies. Journal of Pharmaceutical and Biomedical Analysis, 41(2), pp.554-564.

Brantley, S.J., Argikar, A.A., Lin, Y.S., Nagar, S. & Paine, M.F. 2014. Herb-drug interactions: challenges and opportunities for improved predictions. Drug Metabolism and Disposition: The Biological Fate of Chemicals. 42(3):301-317.

But, P. & TomLinson, B. 1996. Adulteration of herbal products can cause poisoning. Br Med J. 313:117.

Byard, R.W. 2010. A Review of the Potential Forensic Significance of Traditional Herbal Medicines. Journal of Forensic Sciences. 55(1):89-92.

Campbell, M. and Stein, D. 2014. Sexual dysfunction: A systematic review of South African research. S Afr Med J, 104(6), p.440.

Cannistra, L.B. & Cannistra, A.J. 1998. Regression of multivalvular regurgitation after the cessation of fenfluramine and phentermine treatment. New England Journal of Medicine. 339(11):771-771.

Cassileth, B. 2011. Complementary therapies, herbs, and other OTC agents. Bromelain. Oncology (Williston Park, N.Y.). 25(2):195.

Chan, K. Sep 2003. Some aspects of toxic contaminants in herbal medicines. Chemosphere. 52(9):1361-1371.

Chen, M.C., Lai, J.N., Chen, P.C. & Wang, J.D. 2013. Concurrent Use of Conventional Drugs with Chinese Herbal Products in Taiwan: A Population-based Study. Journal of Traditional and Complementary Medicine. 3(4):256-262.

Chen, S.P., Tang, M.H., Ng, S.W., Poon, W.T., Chan, A.Y. & Mak, T.W. 2010. Psychosis associated with usage of herbal slimming products adulterated with sibutramine: a case series. Clinical Toxicology (Philadelphia, Pa.). 48(8):832-838.

citizen.co.za/lifestyle/420057/sa-men-bolster-erectile-dysfunction-drugs-black-market/

Cohen, P. & Ernst, E. 2010. Safety of Herbal Supplements: A Guide for Cardiologists . Cardiovascular Therapeutics. 28:246-253.

Cohen, P.A., Benner, C. & McCormick, D. 2012. Use of a pharmaceutically adulterated dietary supplement, Pai You Guo, among Brazilian-born women in the United States. Journal of General Internal Medicine. 27(1):51-56.

Colson, C.R.D. & De Broe, M.E. 2005. Kidney injury from alternative medicines. Advances in Chronic Kidney Disease. 12(3):261-275.

Connoley, I.P., Liu, Y., Frost, I., Reckless, I.P., Heal, D.J. & Stock, M.J. 1999. Thermogenic effects of sibutramine and its metabolites. British Journal of Pharmacology. 126(6):1487-1495.

Coulter, I.D. & Willis, E.M. 2004. The rise and rise of complementary and alternative medicine: a sociological perspective Medical Journal of Australia. 180(11):587-9.

Csupor, D., Boros, K., Danko, B., Veres, K., Szendrei, K. & Hohmann, J. 2013. Rapid identification of sibutramine in dietary supplements using a stepwise approach. Die Pharmazie. 68(1):15-18.

Damiano, F., Silva, C., Gregori, A., Vacondio, F., Mor, M., Menozzi, M. & Di Giorgio, D. 2014. Analysis of illicit dietary supplements sold in the Italian market: Identification of a sildenafil thioderivative as adulterant using UPLC–TOF/MS and GC/MS. Science & Justice. 54(3):228-237.

David E. Arterburn, MD, MPH, Paul K. Crane, MD, MPH & David L. Veenstra, PharmD, PhD 2004. The Efficacy and Safety of Sibutramine for Weight Loss: A Systematic Review. JAMA Internal Medicine. 164(9):994-1003.

Davis, S.A., Feldman, S.R. & Taylor, S.L. 2014. Use of St. John's Wort in potentially dangerous combinations. Journal of Alternative and Complementary Medicine (New York, N.Y.). 20(7):578-579.

Deconinck, E., Andriessens, S., Bothy, J.L., Courselle, P. & De Beer, J.O. 2014. Comparative dissolution study on counterfeit medicines of PDE-5 inhibitors. Journal of Pharmaceutical Analysis. 4(4):250-257.

Department of Health, Government Gazette, 1965; http://www.mccza.com/documents/cfbd5477Gov_Gazette_37032_15_11_2013_Regulations_Act101.pdf.[Accessed 24 September 2016].

Drugs.com. (2016). Sildenafil Citrate Monograph for Professionals - Drugs.com. [online] Available at: https://www.drugs.com/monograp.h/sildenafil-citrate.htmL [Accessed 6 Dec. 2016].

Djuv, A., Nilsen, O.G. & Steinsbekk, A. 2013. The co-use of conventional drugs and herbs among patients in Norwegian general practice: a cross-sectional study. BMC Complementary and Alternative Medicine. 13:295-6882-13-295.

Doggrell, S. 2005. Comparison of clinical trials with sildenafil, vardenafil and tadalafil in erectile dysfunction. Expert Opinion on Pharmacotherapy. 6(1):75-84.

Dumestre-Toulet, V., Cirimele, V., Gromb, S., Belooussoff, T., Lavault, D., Ludes, B. & Kintz, P. 2002. Last performance with VIAGRA®: post-mortem identification of sildenafil and its metabolites in biological specimens including hair sample. Forensic Science International. 126(1):71-76. DOI:http://dx.doi.org.ezproxy.uct.ac.za/10.1016/S0379-0738(02)00012-9.

Duraz, A.Y. & Khan, S.A. 2011. Knowledge, attitudes and awareness of community pharmacists towards the use of herbal medicines in muscat region. Oman Medical Journal. 26(6):451-453.

Eerkes,A; Addison,T; Naidong,W. 2002 Simultaneous assay of sildenafil and desmethylsildenafil in human plasma using liquid chromatography–tandem mass spectrometry on silica column with aqueous–organic mobile phase, Journal of Chromatography B, 768(2), pp 277-284.

Eisenberg, R.C., Kessler. C, Foster. F.E, Norlock. D.R, Calkins. T.L & Debanco 1993. Unconventional medicine in the United States. Prevalence, costs, and patterns of use. New England J. Med. 328(4): pp. 246–252.

El-Mallakh, R.S. 2014. Chapter 3 - Lithium. Side Effects of Drugs Annual. 36:27-36. DOI:http://dx.doi.org.ezproxy.uct.ac.za/10.1016/B978-0-444-63407-8.00003-4.

Ema.europa.eu. (2016). European Medicines Agency - Find medicine - Viagra. [online] Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/000202/human_med_001136.jsp&mid=WC0b01ac058001d124 [Accessed 3 Oct. 2016].

ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/001080/WC500068025.pdf

Ernst, E. 2002. Adulteration of Chinese herbal medicines with synthetic drugs: a systematic review (Review Article). . Journal of Internal Medicine. 252:107-113.

Ernst, E., Pittler, M., Wider, B. & Boddy, K. 2006. The desktop guide to complementary and alternative medicine, 2nd edn. ed. Edinburgh: Elsevier Mosby.

Ernst, E. 2002. Adulteration of Chinese herbal medicines with synthetic drugs: a systematic review. Journal of Internal Medicine. 252(2):107-113.

Ernst, E. 2000. Adverse effects of herbal drugs in dermatology. British Journal of Dermatology. 143(5):923-929.

Ernst, E. 2003. Cardiovascular adverse effects of herbal medicines: a systematic review of the recent literature. Canadian Journal of cardiology. 19(7):818-827.

Ernst, E. 2003. Serious adverse effects of unconventional therapies for children and adolescents: A systematic review of recent evidence. European Journal of Pediatrics. 162(2):72-80.

Ezuruike, U.F. & Prieto, J.M. 2014. The use of plants in the traditional management of diabetes in Nigeria: pharmacological and toxicological considerations. Journal of Ethnopharmacology. 155(2):857-924.

Farran, A.G. SciDev.Net. (2017). Integrating modern and traditional medicine: Facts and figures. [online] Available at: http://www.scidev.net/global/indigenous/feature/integrating-modern-and-traditional-medicine-facts-and-figures.htmL [Accessed 16 Jan. 2017].

Fasinu, P.S., Bouic, P.J. & Rosenkranz, B. 2012. An overview of the evidence and mechanisms of herb-drug interactions. Frontiers in Pharmacology. 3:69.

FDA, Consumer Health Information, Feb-21, 2009. “Hidden Risks of Erectile Dysfunction Treatments Sold Online”, Viagra ingredients found, dangerous interactions and preventive measures.

FDA, gov. (2016). Hidden Risks of Erectile Dysfunction 'Treatments' Sold Online. [online] Available at: http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm048386.htm [Accessed 3 Oct. 2016].

FDA, gov. (2016). U S Food and Drug Administration Home Page. [online] Available at: http://www.fda.gov/default.htm [Accessed 4 Oct. 2016].

Fejős, I., Neumajer, G., Béni, S. & Jankovics, P. 2014. Qualitative and quantitative analysis of PDE-5 inhibitors in counterfeit medicines and dietary supplements by HPLC–UV using sildenafil as a sole reference. Journal of Pharmaceutical and Biomedical Analysis. 98:327-333.

Fried, R. 2014. Chapter 8 - Arginine and Arginine-Combinations in Treatment of Erectile Dysfunction. In Erectile Dysfunction As a Cardiovascular Impairment. R. Fried, Ed. Boston: Academic Press. 231-258.

Futures of Palm Beach FL Addiction Treatment, Rehab, and Detox Center. (2016). Harmful Effects of Diet Pills and Supplements - Futures of Palm Beach FL Addiction Treatment, Rehab, and Detox Center. [online] Available at: https://www.futuresofpalmbeach.com/healthy-diet-exercise/harmful-effects-diet-pills-supplements/ [Accessed 1 Nov. 2016].

Gallo, E., Giocaliere, E., Benemei, S., Bilia, A.R., Karioti, A., Pugi, A., di Pirro, M., Menniti-Ippolito, F. *et al.*. 2012. Anything to declare? Possible risks for patients' health resulting from undeclared plants in herbal supplements. British Journal of Clinical Pharmacology. 73(3):482-483.

Gao.gov. (2016). Dietary Supplements: FDA May Have Opportunities to Expand Its Use of Reported Health Problems to Oversee Products. [online] Available at: http://www.gao.gov/products/GAO-13-244. [Accessed 14 Oct. 2016].

Gardin, J.M., Schumacher, D., Constantine, G., Davis, K.D., Leung, C. & Reid, C.L. 2000. Valvular abnormalities and cardiovascular status following exposure to dexfenfluramine or phentermine/fenfluramine. Jama. 283(13):1703-1709.

Gilard, V., Balayssac, S., Tinaugus, A., Martins, N., Martino, R. & Malet-Martino, M. 2015. Detection, identification and quantification by 1H NMR of adulterants in 150 herbal dietary supplements marketed for improving sexual performance. Journal of Pharmaceutical and Biomedical Analysis. 102:476-493.

Giveon SM, Liberman N, Klang S, Kahan E. 2004. Are people who use “natural drugs” aware of their potentially harmful side effects and reporting to family physician? Patient Educ Counselling. 53(1):5-11.

Globalissues.org. 2017. Obesity — Global Issues. [online] Available at: http://www.globalissues.org/article/558/obesity [Accessed 16 Jan. 2017].

Goldstein, I. 2014. The 15th Anniversary of the First Oral Therapy for Erectile Dysfunction. The Journal of Sexual Medicine. 11, Supplement 2:115-136. DOI:http://dx.doi.org.ezproxy.uct.ac.za/10.1111/jsm.12434.

Gou, X.-., Zhao, X.-., Gao, X., Zhou, M.-. & Liu, W.-. 2016, "Simultaneous determination of ten phosphodiesterase-5 inhibitors in health foods by ultra performance liquid chromatography with tandem mass spectrometry", Journal of Chinese Mass Spectrometry Society, vol. 37, no. 6, pp. 554-561.

Gqalen. N, Moodley. I, Kruger. H, Ntuli. A & McLeod.H 2007. Traditional and complementary medicine. Available: www.hst.org.za/uploads/files/chap12_07.pdf.

Grundlingh, J., Dargan, P.I., El-Zanfaly, M. & Wood, D.M. 2011. 2,4-dinitrophenol (DNP): a weight loss agent with significant acute toxicity and risk of death. Journal of Medical Toxicology: Official Journal of the American College of Medical Toxicology. 7(3):205-212.

Gupta, S.K., Kaleekal, T. & Joshi, S. 2000. Misuse of corticosteroids in some of the drugs dispensed as preparations from alternative systems of medicine in India. Pharmacoepidemiology and Drug Safety. 9(7):599-602.

Haque, A.,Kumar, N. 2014 Method Development And Validation Of LC-MS/MS Method For The Estimation Of Sildenafil And Its Metabolite Piperazine N-Desmethyl Sildenafil In Human Plasma. International Journal of Pharmacy and Pharmaceutical Sciences. 6(5) 677-682.

Hughes, G.D., Aboyade, O.M., Clark, B.L. & Puoane, T.R. 2013. The prevalence of traditional herbal medicine use among hypertensives living in South African communities. BMC Complementary and Alternative Medicine. 13:38-6882-13-38.

Hughes, G.D., Puoane, T.R., Clark, B.L., Wondwossen, T.L., Johnson, Q. & Folk, W. 2012. Prevalence and predictors of traditional medicine utilization among persons living with AIDS (PLWA) on antiretroviral (ARV) and prophylaxis treatment in both rural and urban areas in South Africa. African Journal of Traditional, Complementary, and Alternative Medicines: AJTCAM / African Networks on Ethnomedicines. 9(4):470-484.

Hundal, Ø. 2007. Major depressive disorder viewed as a dysfunction in astroglial bioenergetics. Medical Hypotheses. 68(2):370-377.

Ignarro, L.J. 2014. Nitric Oxide. In Reference Module in Biomedical Sciences. Elsevier.

Ingram, K.T. & Kennewell, P.D. 2014. Major Drug Introductions☆. In Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Elsevier.

Isnard Bagnis, C., Deray, G., Baumelou, A., Le Quintrec, M. & Vanherweghem, J.L. 2004. Herbs and the kidney. American Journal of Kidney Diseases. 44(1):1-11.

Iuns.org. (2015). The Global Challenge of Obesity and the International Obesity Task Force · International Union of Nutritional Sciences. [online] Available at: http://www.iuns.org/resources/the-global-challenge-of-obesity-and-the-international-obesity-task-force/ [Accessed 14 Oct. 2016].

J. Anzanello, M., S. Ortiz, R., Limberger, R. & Mariotti, K. 2014. Performance of some supervised and unsupervised multivariate techniques for grouping authentic and unauthentic Viagra and Cialis. Egyptian Journal of Forensic Sciences. 4(3):83-89.

Jin, G. & Wong, S.T.C. 2014. Toward better drug repositioning: prioritizing and integrating existing methods into efficient pipelines. Drug Discovery Today. 19(5):637-644..

Johansson, M., Fransson, D., Rundlöf, T., Huynh, N. & Arvidsson, T. 2014. A general analytical platform and strategy in search for illegal drugs. Journal of Pharmaceutical and Biomedical Analysis. 100:215-229..

Jolly, K., Gammage, M.D., Cheng, K.K., Bradburn, P., Banting, M.V. & Langman, M.J. 2009. Sudden death in patients receiving drugs tending to prolong the QT interval. British Journal of Clinical Pharmacology. 68(5):743-751.

Jones, G.R. 2013. Postmortem Specimens. In Encyclopedia of Forensic Sciences. J.A.S.J.S.M. Houck, Ed. Waltham: Academic Press. 270-274.

Jones, R.E. & Lopez, K.H. 2014. Chapter 8 - The Human Sexual Response. In Human Reproductive Biology (Fourth Edition). R.E. Jones & K.H. Lopez, Eds. San Diego: Academic Press. 135-157.

Jurado López, A.R. 2014. Tratamiento farmacológico de la eyaculación precoz. SEMERGEN - Medicina De Familia. 40, Supplement 3:16-21.

Kahn, T. 2014 http://www.bdlive.co.za/national/health/2014/02/27/complementary-medicine-industry-resists-Chan,ge. Available: http://www.bdlive.co.za/national/health/2014/02/27/complementary-medicine-industry-resists-Chan,ge.

Kahn, T. 2015. Clicks stands by its herbal products despite US findings. Available: http://www.bdlive.co.za/business/retail/2015/02/05/clicks-stands-by-its-herbal-products-despite-us-findings.

Kang, W., Bae, K. & Noh, K. 2010. Enantioselective determination of sibutramine and its active metabolites in human plasma. Journal of Pharmaceutical and Biomedical Analysis. 51(1):264-267..

Kim, A.A., Kent, C.K. & Klausner, J.D. 2002. Increased risk of HIV and sexually transmitted disease transmission among gay or bisexual men who use Viagra, San Francisco 2000–2001. Aids. 16(10):1425-1428.

Kirsch, D.R. 2014. Chapter 23 - Therapeutic Drug Development and Human Clinical Trials. In Biotechnology Entrepreneurship. C. Shimasaki, Ed. Boston: Academic Press. 315-330.

Kontaras, K., Varnavas, V. & Kyriakides, Z.S. 2008. Does Sildenafil Cause Myocardial Infarction or Sudden Cardiac Death? . American Journal of Cardioivascular Drugs. 8(1):1-7.

Kossat, J. & Vetter-Höltershinken, C. 2014. 13 - Niere, Harn- und Samenwege, Elektrolythaushalt. In Praxisleitfaden Allgemeinmedizin (7. Auflage). S. Gesenhues, R.H. Zieschè & A. Breetholt, Eds. Munich: Urban & Fischer. 747-800.

Koster, R., Alffenaar, J., Greijdanus, B., VanDernagel, J.E.L. & Uges, D.R.A. 2014. Fast and highly selective LC-MS/MS screening for THC and 16 other abused drugs and metabolites in human hair to monitor patients for drug abuse. Therapeutic Drug Monitoring. 36(2): 234-243.

Krenzelok, E.P. 2000. Sildenafil:Clinical Toxicology profile, Journal of Toxicology, Clinical Toxicology. 38:645-651

Krivohlavek, A., Ivešic, M., Žuntar, I. & Šikic, S. 2014. Presence of sildenafil, tadalafil and avanafil in food supplements determined by validated high-pressure liquid chromatography–electrospray tandem mass spectrometry method. Toxicology Letters. 229, Supplement:S185.

Labeling.pfizer.com. (2016). [online] Available at: http://labeling.pfizer.com/ShowLabeling.aspx?id=652#S4.1 [Accessed 6 Dec. 2016].

Lai, J.-., Tang, J.-. & Wang, J.-. 2013. Observational studies on evaluating the safety and adverse effects of traditional Chinese medicine. Evidence-Based Complementary and Alternative Medicine. 2013.

Lam, P.K., Leung, K.S., Wong, T.W., Lee, H.H., Tang, M.H. & Mak, T.W. 2012. Serotonin syndrome following overdose of a non-prescription slimming product containing sibutramine: a case report. Human & Experimental Toxicology. 31(4):414-417.

Langenstroer, P. 2007. Erectile Dysfunction. In xPharm: The Comprehensive Pharmacology Reference. S.J. Enna, & D.B. Bylund, Eds. New York: Elsevier. 1-3.

Lebel, P., Gagnon, J., Furtos, A. & Waldron, K.C. 2014. A rapid, quantitative liquid chromatography-mass spectrometry screening method for 71 active and 11 natural erectile dysfunction ingredients present in potentially adulterated or counterfeit products. Journal of Chromatography A. 1343:143-151.

Lee, J.H., Kim, N.S., Han, K.M., Kim, S.H., Cho, S. & Kim, W.S. 2013. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. Epub 2013 Sep 2. Monitoring by LC-MS/MS of 48 compounds of sildenafil, tadalafil, vardenafil and their analogues in illicit health food products in the Korean market advertised as enhancing male sexual performance. Food Additives & Contaminants: Part A. 30(11):1849-1857.

Lee, E., Lee, J.H., Han, K.M., Kim, J.W., Hwang, I.S., Cho, S., Han, S.Y. & Kim, J. 2013. Simultaneous determination of 38 phosphodiestrase-5 inhibitors in illicit erectile dysfunction products by liquid chromatography–electrospray ionization-tandem mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis. 83(0):171-178.

Lee, J.H.; Kim, J.W.; Jung, E.N.; Kim, J.Y.; Cho, S.H.; Do, J.; Yoon, C; Cho, S.; Kim, W.S. 2014. Identification and screening of a tadalafil analogue found in adulterated herbal products Journal of the Korean Society of Food Science and Nutrition , 2014 (10)

Leonard, B., Huff, H., Merryweather, B., Lim, A. & Mills, E. 2004. Knowledge of safety and herb-drug interations amongst HIV+ individuals: a focus group study. The Canadian Journal of Clinical Pharmacology = Journal Canadien De Pharmacologie Clinique. 11(2):e227-31.

Li, G.Q., Duke, C.C. & Roufogalis, B.D. 2003. The quality and safety of traditional Chinese medicines. Australian Prescriber. 26(6):128-130+151.

Liang, Q., Qu, J., Luo, G. & Wang, Y. 2006. Rapid and reliable determination of illegal adulterant in herbal medicines and dietary supplements by LC/MS/MS. Journal of Pharmaceutical and Biomedical Analysis, 40(2), pp.305-311

Lin, H.W., Pickard, A.S., Mahady, G.B., Karabatsos, G., Crawford, S.Y. & Popovich, N.G. 2010. An instrument to evaluate pharmacists' patient counseling on herbal and dietary supplements. American Journal of Pharmaceutical Education. 74(10):192.

Li, J., Zhang. Z., Liu, X., Yan, H., Han, S., Zhang, H., Zhang, S., Cheng, J. 2013. Analysis of fourteen β-Agonists in weight-reducing dietary supplements using QuEChERS-Based extraction followed by high resolution UHPLC-MS. Food Analysis Methods.

Lis-Balchin, M. 1999. Possible health and safety problems in the use of novel plant essential oils and extracts in aromatherapy. Journal of the Royal Society for the Promotion of Health. 119(4):240-243.

Liu, Y., Kam, W.R., Ding, J. & Sullivan, D.A. 2014. One man's poison is another man's meat: Using azithromycin-induced phospholipidosis to promote ocular surface health. Toxicology. 320:1-5.

Low, M.-., Zeng, Y., Li, L., Ge, X.-., Lee, R., Bloodworth, B.-. & Koh, H.-. 2009. Safety and quality assessment of 175 illegal sexual enhancement products seized in red-light districts in Singapore. Drug Safety. 32(12):1141-1146.

Low, M.-., Zeng, Y., Li, L., Ge, X.-., Lee, R., Bloodworth, B.-. & Koh, H.-. 2009. Safety and quality assessment of 175 illegal sexual enhancement products seized in red-light districts in Singapore. Drug Safety. 32(12):1141-1146.

MacLennan, A.H., Myers, S.P. & Taylor, A.W. 2006. The continuing use of complementary and alternative medicine in South Australia: costs and beliefs in 2004 Medical Journal of Australia. 184(1):27-31.

Mahmood, G., Mei, Z., Hojjat, H., Pace, E., Kallakuri, S. & Zhang, J.S. 2014. Therapeutic effect of sildenafil on blast-induced tinnitus and auditory impairment. Neuroscience. 269:367-382.

Man, C.N. 2009. Identification of sildenafil, tadalafil and vardenafil by gas chromatography–mass spectrometry on short capillary column. Journal of Chromatography A. 1216(47):8426-8430.

Marson, L., Brotto, L.A. & Romanzi, L.J. 2014. Current Literature Review. The Journal of Sexual Medicine. 11(8):1892-1897.

Mathon, C., Ankli, A., Reich, E., Bieri, S. & Christen, P. 2014. Screening and determination of sibutramine in adulterated herbal slimming supplements by HPTLC-UV densitometry, 31:1, 15-20, DOI: 10.1080/19440049.2013.861934. Food Additives & Contaminants: Part A. 31(1):15-20.

Mazzari, A.L. & Prieto, J.M. 2014. Herbal medicines in Brazil: pharmacokinetic profile and potential herb-drug interactions. Frontiers in Pharmacology. 5:162.

MCC,. 2016. Medicines Control Council. [ONLINE] Available at: http://www.mccza.com/About. [Accessed 24 September 2016].

McCullough, A.R. 2002 Four-Year Review of Sildenafil Citrate. Reviews in Urology. 4(Suppl 3):S26-S38.

McMonagle, L. 1987. Private rights to adulterated/misbranded articles. AIDS & Public Policy Journal. 2(2):33-49.

MedicineNet. 2016. sibutramine (Meridia): Drug Facts, Side Effects and Dosing. [online] Available at: http://www.medicinenet.com/sibutramine/article.htm [Accessed 14 Oct. 2016].

Medlineplus.gov. 2016. Sildenafil: MedlinePlus Drug Information. [online] Available at: https://medlineplus.gov/druginfo/meds/a699015.htmL [Accessed 3 Oct. 2016].

Medscape. (2016). Dietary Supplements for Male Sexual Enhancement. [online] Available at: http://www.medscape.com/viewarticle/853044 [Accessed 1 Nov. 2016].

Menniti-Ippolito, F., Mazzanti, G., Firenzuoli, F., Bianchi, A. & Raschetti, R. 2005. Pilot study for the surveillance of adverse reactions to herbal preparations and dietary supplements. Annali Dell'Istituto Superiore Di Sanita. 41(1):39-42.

Miller, L., Hume, A. & Harris, I. 2001. Adulteration: its various meanings. Pharmacotherapy. 21(6):770-771.

Millet, B., Vanelle, J. & Benyaya, J. 2014. 40 - Stratégies thérapeutiques médicamenteuses devant les effets indésirables des psychotropes. In Prescrire les psychotropes (2e édition). B. Millet, J. Vanelle & J. Benyaya, Eds. Paris: Elsevier Masson. 333-349.

Mills, E., Cooper, C., Seely, D. & Kanfer, I. 2005. African herbal medicines in the treatment of HIV: Hypoxis and Sutherlandia. An overview of evidence and pharmacology. Nutrition Journal. 4:19.

Mittleman, M.A., Carrier, S. & Seftel, A.D. 2014. Cardiovascular Outcomes Among Sildenafil Users: Results of the International Men's Health Study. The Journal of Sexual Medicine. 11(4):880-884..

Modern Medicine Network. 2010. Most Doctors not knowledgeable about Herbals. [ONLINE] Available at: http://www.modernmedicine.com/%5Bnode-source-domain-raw%5D/news/clinical/clinical-pharmacology/most-doctors-not-knowledgeable-about-he. [Accessed 1 October 2016].

Mokhatar, S.U. Mokhtar; Chin, S.T.; Kee, C.L.; Low, M.Y.; Drummer, O.H.; Marriott,P.J. Rapid determination of sildenafil and its analogues in dietary supplements using gas chromatography–triple quadrupole mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis 121 (2016) 188–196

Montesano, C., Johansen, S.S. & Nielsen, M.K.K. 2014. Validation of a method for the targeted analysis of 96 drugs in hair by UPLC–MS/MS. Journal of Pharmaceutical and Biomedical Analysis. 88: 295-306.

Monti, G.A., Chattah, A.K. & Linck, Y.G. 2014. Chapter Four - Solid-State Nuclear Magnetic Resonance in Pharmaceutical Compounds. Annual Reports on NMR Spectroscopy. 83:221-269.

Mothupi, M.C. 2014. Use of herbal medicine during pregnancy among women with access to public healthcare in Nairobi, Kenya: a cross-sectional survey. BMC Complementary and Alternative Medicine. 14:432-6882-14-432.

Musicki, B., Bivalacqua, T.J., Champion, H.C. & Burnett, A.L. 2014. Sildenafil Promotes eNOS Activation and Inhibits NADPH Oxidase in the Transgenic Sickle Cell Mouse Penis. The Journal of Sexual Medicine. 11(2):424-430..

Mustazza, C., Borioni, A., Rodomonte, A.L., Bartolomei, M., Antoniella, E., Di Martino, P., Valvo, L., Sestili, I. *et al.*. 2014. Characterization of Sildenafil analogs by MS/MS and NMR: A guidance for detection and structure elucidation of phosphodiesterase-5 inhibitors. Journal of Pharmaceutical and Biomedical Analysis. 96:170-186.

naturalhealthalliance.co.za/Odyssey-CAMs-01082014.pdf

Neergheen-Bhujun, V.S. 2013. Underestimating the toxicological challenges associated with the use of herbal medicinal products in developing countries. BioMed Research International. 2013:804086.

The Nemours Foundation. 2016. KidsHealth > For Teens > Complementary and Alternative Medicine. [ONLINE] Available at: http://kidshealth.org/en/teens/alternative-medicine.htmL#kha_22. [Accessed 24 September 2016]).

Newman, B. & Caplan, J. 2014. Cystic Lung Lesions in Newborns and Young Children: Differential Considerations and Imaging. Seminars in Ultrasound, CT and MRI. 35(6):571-587.

Nivison-Smith, L., Zhu, Y., Whatham, A., Bui, B.V., Fletcher, E.L., Acosta, M.L. & Kalloniatis, M. 2014. Sildenafil alters retinal function in mouse carriers of Retinitis Pigmentosa. Experimental Eye Research. 128:43-56.

Ng, C., Law, T., Cheung, Y., Ng, P. and Choi, K. (2010). Development of a screening method for the detection of analogues of sildenafil and vardenafil by the use of liquid chromatograph coupled with triple quadrupole linear ion trap mass spectrometer. Analytical Methods, 2(7), p.890.

Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, *et al*. 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 384(9945):766-81.

NIH. 2008. The Use of Complementary and Alternative Medicine in the United States. [ONLINE] Available at: https://nccih.nih.gov/research/statistics/2007/CAMsurvey_fs1.htm. [Accessed 24 September 2016]).

Novella, S. 2012. Why Do People turn to Alternative Medicine . Available: https://www.sciencebasedmedicine.org/why-do-people-turn-to-alternative-medicine/.

Novo-Matos, J., Hurter, K., Bektas, R., Grest, P. & Glaus, T. 2014. Patent ductus arteriosus in an adult cat with pulmonary hypertension and right-sided congestive heart failure: hemodynamic evaluation and clinical outcome following ductal closure. Journal of Veterinary Cardiology. 16(3):197-203.

Ortiz, R.S., Mariotti, K.d.C., Holzschuh, M.H., Romão, W., Limberger, R.P. & Mayorga, P. 2013. Profiling counterfeit Cialis, Viagra and analogs by UPLC–MS. Forensic Science International. 229(1–3):13-20..

Oshikoya, K.A., Oreagba, I.A., Ogunleye, O.O., Oluwa, R., Senbanjo, I.O. & Olayemi, S.O. 2013. Herbal medicines supplied by community pharmacies in Lagos, Nigeria: pharmacists' knowledge. Pharmacy Practice. 11(4):219-227.

O'Sullivan, L.F., Brotto, L.A., Byers, E.S., Majerovich, J.A. & Wuest, J.A. 2014. Prevalence and Characteristics of Sexual Functioning among Sexually Experienced Middle to Late Adolescents. The Journal of Sexual Medicine. 11(3):630-641.

Ozdemir, B., Sahin, I., Kapucu, H., Celbis, O., Karakoc, Y., Erdogan, S. & Onal, Y. 2013. How safe is the use of herbal weight-loss products sold over the internet? Human & Experimental Toxicology. 32(1):101-106.

Özgür, B.C., Telli, O., Yuceturk, C.N., Sarici, H., Ozer, E., Surer, H., Kılınc, A.S., Hucumenoglu, S. *et al.*. 2014. The Effect of Sildenafil and Udenafil on Testicular Damage Following Ischemia-Reperfusion Injury in Rats. The Journal of Urology. 192(4):1272-1277. DOI:http://dx.doi.org.ezproxy.uct.ac.za/10.1016/j.juro.2014.04.011.

Pamukcu Gunaydin G1, Dogan NO2, Levent S3, Kurtoglu Celik G1 2015. Herbal weight loss pill overdose: sibutramine hidden in pepper pill. Case Rep Emerg Med. 2015 ;2015: Epub. 213874

Park, H.J., Park, N.C., Shim, H.B., Park, J.K., Lee, S.W., Park, K., Kim, S.W., Moon, K.H. *et al.*. 2008. An Open-Label, Multicenter, Flexible Dose Study to Evaluate the Efficacy and Safety of Viagra® (Sildenafil Citrate) in Korean Men with Erectile Dysfunction and Arterial Hypertension who are Taking Antihypertensive Agents. The Journal of Sexual Medicine. 5(10):2405-2413.

Patel, D.N., Low, W.-., Tan, L.L., Tan, M.-.B., Zhang, Q., Low, M.-., Chan,, C.-. & Koh, H.-. 2012. Adverse events associated with the use of complementary medicine

and health supplements: An analysis of reports in the Singapore Pharmacovigilance database from 1998 to 2009. Clinical Toxicology. 50(6):481-489.

Patel, D.N., Li, L., Kee, C., Ge, X., Low, M. & Koh, H. 2014. Screening of synthetic PDE-5 inhibitors and their analogues as adulterants: Analytical techniques and challenges. Journal of Pharmaceutical and Biomedical Analysis. 87(0):176-190.

Patel, J.K. 2014. Chapter 4 - Drugs of Abuse. Side Effects of Drugs Annual. 36:37-52.

Penson, R., Castro, C., Seiden, M., Chabner, B. & Lynch TJ Jr. 2001. Complementary, alternative, integrative, or unconventional medicine? Oncologist. 6(5):463-473.

Perlman, A.I., Lebow, D.G., Raphael, K., Ali, A. & Simmons, L.A. 2013. A point-of-sale communications campaign to provide consumers safety information on drug-dietary supplement interactions: a pilot study. Health Communication. 28(7):729-739.

Pfizer. (2013). VIAGRA* (sildenafil citrate) Product Monograph. [online] Available at: http://www.pfizer.ca/sites/g/files/g10017036/f/201410/VIAGRA_PM_E_165139_26August2013.pdf [Accessed 1 Oct. 2016].

Pifarré, P., Gutierrez-Mecinas, M., Prado, J., Usero, L., Roura-Mir, C., Giralt, M., Hidalgo, J. & García, A. 2014. Phosphodiesterase 5 inhibition at disease onset prevents experimental autoimmune encephalomyelitis progression through immunoregulatory and neuroprotective actions. Experimental Neurology. 251:58-71.

Pistos, C., Papoutsis, I., Dona, A., Stefanidou, M., Athanaselis, S., Maravelias, C. & Spiliopoulou, C. 2008. Off-line HPLC method combined to LC–MS for the determination of sildenafil and its active metabolite in post-mortem human blood according to confirmation criteria. Forensic Science International. 178(2–3):192-198.

Pittler, M.H. & Ernst, E. 2003. Systematic review: hepatotoxic events associated with herbal medicinal products. Alimentary Pharmacology & Therapeutics. 18(5):451-471.

Pizarro, A. 2014. Uso de sildenafilo y aumento del riesgo de melanoma en varones. Revista Clínica Española. 214(8):475-476.

Podder, A.K., Chakrobarty, J.K. & Faroque, A.B.M. 2014. Qualitative and Quantitative Analysis Of Sildenafil In Traditional Medicines And Dietary Supplements. Asian Journal of Pharmaceutical and Clinical Research. 7(7 (SUPPL. 2)):25-30.

Poels, S., Bloemers, J., van Rooij, K., Koppeschaar, H., Olivier, B. & Tuiten, A. 2014. Two novel combined drug treatments for women with hypoactive sexual desire disorder. Pharmacology Biochemistry and Behavior. 121:71-79.

Ponnurua, V., Challab, B. & Nadendla, R. 2012. Quantification of sibutramine and its two metabolites in human plasma by LC–ESI-MS/MS and its application in a bioequivalence study. Journal of Pharmaceutical Analysis;. 2(4):249–257.

Poole, D.C. & Erickson, H.H. 2014. 31 - Heart and vessels: Function during exercise and training adaptations. In Equine Sports Medicine and Surgery (Second Edition). K.W. Hinchcliff, A.J. Kaneps & R.J. Geor, Eds. W.B. Saunders. 667-694.

Posadzki, P., Watson, L. & Ernst, E. 2013. Herb-drug interactions: an overview of systematic reviews. British Journal of Clinical Pharmacology. 75(3):603-618. DOI:10.1111/j.1365-2125.

Posadzki, P., Watson, L. & Ernst, E. 2013. Contamination and adulteration of herbal medicinal products (HMPs): an overview of systematic reviews. European Journal of Clinical Pharmacology. 69:295+.

Provenza, N., Calpena, A.C., Mallandrich, M., Halbaut, L. & Clares, B. 2014. Design and physicochemical stability studies of paediatric oral formulations of sildenafil. International Journal of Pharmaceutics. 460(1–2):234-239.

Psa-rising.com. (2016). Viagra Deaths In Younger Men With No Reported Heart Problems, Study Finds. [online] Available at: http://www.psa-rising.com/medicalpike/viagracardiodeaths031500.htm [Accessed 6 Dec. 2016].

psi-inc.org [Accessed June 2017].

Product Monograph- Pfizer. (2016). [online] Available at: http://www.pfizer.ca/sites/g/files/g10023411/f/201506/VIAGRA_PM_E.pdf [Accessed 6 Dec. 2016].

Puzzo, D., Loreto, C., Giunta, S., Musumeci, G., Frasca, G., Podda, M.V., Arancio, O. & Palmeri, A. 2014. Effect of phosphodiesterase-5 inhibition on apoptosis and beta amyloid load in aged mice. Neurobiology of Aging. 35(3):520-531.

Q. Li, G., Duke, C.C. & Roufogalis, B.D. 2003. The quality and safety of traditional traditional Chinese medicines . Australian Prescriber. 26(6):128-130.

Quan, L., Ishikawa, T., Hara, J., Michiue, T., Chen, J., Wang, Q., Zhu, B. & Maeda, H. 2011. Postmortem serotonin levels in cerebrospinal and pericardial fluids with

regard to the cause of death in medicolegal autopsy. Legal Medicine. 13(2):75-78.

Rapôso, C., Luna, R.L.d.A., Nunes, A.K.S., Thomé, R. & Peixoto, C.A. 2014. Role of iNOS-NO-cGMP signaling in modulation of inflammatory and myelination processes. Brain Research Bulletin. 104:60-73.

Raskovic, A., Cvejic, J., Stilinovic, N., Golocorbin-Kon, S., Vukmirovic, S., Mimica-Dukic, N. & Mikov, M. 2014. Interaction between different extracts of Hypericum perforatum L. from Serbia and pentobarbital, diazepam and paracetamol. Molecules (Basel, Switzerland). 19(4):3869-3882.

Rau, N.H., Rau, P., Chin, A.S., Provost, Y., Stevens, L., Noiseux, N. & Chartrand-Lefebvre, C. 2014. Coronary artery bypass graft imaging with 256-slice MDCT: surgical concepts, current techniques, and interpretation. Clinical Imaging. 38(5):571-579.

Rees, A. ; Schonken, S. 2016 Sham Cam Regulatory Workshop With Medicines Control Council, The Traditional and Natural Health Alliance. http://www.tnha.co.za/april-2016-news/

Rightdiagnosis.com. 2017. Statistics by Country for Impotence - RightDiagnosis.com. [online] Available at: http://www.rightdiagnosis.com/i/impotence/stats-country.htm [Accessed 16 Jan. 2017].

Rocha, T., Amaral, J. S. & Oliveira, M.B.P.P. 2016. Adulteration of Dietary Supplements by the Illegal Addition of Synthetic Drugs: A Review doi: 10.1111/1541-4337.12173. Comprehensive Reviews in Food Science and Food Safety. 15:43-62.

Rocha, M., Aguiar, F. & Ramos, H. 2014. O uso de esteroides androgénicos anabolizantes e outros suplementos ergogénicos – uma epidemia silenciosa. Revista Portuguesa De Endocrinologia, Diabetes e Metabolismo. 9(2):98-105.

Said, M.M., Gibbons, S., Moffat, A.C. & Zloh, M. 2014. Rapid detection of sildenafil analogue in Eurycoma longifolia products using a new two-tier procedure of the near infrared (NIR) spectra database. Food Chemistry. 158:296-301.

San Jose, C.A (PRWEB) March 07, 2012. http://www.prweb.com/releases/herbal_supplements/herbal_remedies/prweb9260421.htm. [Accessed 24 September 2016]

Santosa, A., Ng, P.S.L. & Teng, G.G. 2014. Traditional Chinese medication for rheumatoid arthritis: more than what meets the eye. Rheumatology International.

Sazlina, S.G. & Zaiton, A. 2009. Cushing's syndrome secondary to adulterated complementary and alternative medicine. Malaysian Family Physician. 4(2-3):12.

Schmidt, H.M., Munder, T., Gerger, H., Frühauf, S. & Barth, J. 2014. Combination of Psychological Intervention and Phosphodiesterase-5 Inhibitors for Erectile Dysfunction: A Narrative Review and Meta-Analysis. The Journal of Sexual Medicine. 11(6):1376-1391.

Schramek, N., Wollein, U. & Eisenreich, W. 2014. Identification of new synthetic PDE-5 inhibitors analogues found as minor components in a dietary supplement. Journal of Pharmaceutical and Biomedical Analysis. 96:45-53.

Schurmann, P.A. & Levine, G.N. 2014. Chapter 17 - Non–ST Segment Elevation Acute Coronary Syndrome. In Cardiology Secrets (Fourth Edition). G.N. Levine, Ed. Philadelphia: W.B. Saunders. 124-134.

Scientific Working Group for Forensic Toxicology (SWGTOX) Standard Practices for Method Validation in Forensic Toxicology. 2013. Journal of Analytical Toxicology. 37:452-474.

Scully, C. 2014. 34 - Substance dependence. In Scully's Medical Problems in Dentistry (Seventh Edition). C. Scully, Ed. Oxford: Churchill Livingstone. 749-773.

Scully, C. 2014. 4 - Signs and symptoms. In Scully's Medical Problems in Dentistry (Seventh Edition). C. Scully, Ed. Oxford: Churchill Livingstone. 97-122.

Selvin, E., Burnett, A.L., & Platz, E.A. 2007. Prevalence and risk factors for erectile dysfunction in the U.S. American Journal of Medicine. 120:151-157.

ShamLoul, R. & Bella, A. 2014. Complementary and Alternative Medicine (CAM) for Sexual Dysfunction. The Journal of Sexual Medicine. 11(4):1097-1098.

Shang, N., Shao, Y., Cai, Y., Guan, M., Huang, M., Cui, W., He, L., Yu, Y. *et al.*. 2014. Discovery of 3-(4-hydroxybenzyl)-1-(thiophen-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one as a phosphodiesterase-5 inhibitor and its complex crystal structure. Biochemical Pharmacology. 89(1):86-98.

Shi, F., Guo, C., Gong, L., Li, J., Dong, P., Zhang, J., Cui, P., Jiang, S. *et al.*. 2014. Application of a high resolution benchtop quadrupole-Orbitrap mass spectrometry for the rapid screening, confirmation and quantification of illegal adulterated phosphodiesterase-5 inhibitors in herbal medicines and dietary supplements. Journal of Chromatography A. 1344:91-98.

Shimasaki, C. 2014. Chapter 12 - Understanding Biotechnology Business Models and Managing Risk. In Biotechnology Entrepreneurship. C. Shimasaki, Ed. Boston: Academic Press. 161-174.

Shindel, A.W. 2014. A Systematic Review Assessing the Economic Impact of Sildenafil Citrate (Viagra®) in the Treatment of Erectile Dysfunction: Martin AL, Huelin R, Wilson D, *et al.* (United BioSource Corporation, Lexington, MA) J Sex Med 10:1389-1400, 2013§. Yearbook of Urology. 2014:111-112.

Silverman, R.B. & Holladay, M.W. 2014. Chapter 1 - Introduction. In The Organic Chemistry of Drug Design and Drug Action (Third Edition). R.B. Silverman & M.W. Holladay, Eds. Boston: Academic Press. 1-17.

Simiele, M., Pensi, D., Pasero, D., Ivaldi, F., Rinaldi, M., Di Perri, G., Ranieri, V., D'Avolio,A. (2015). Development and validation of an ultra performance liquid chromatography tandem mass method for sildenafil and N-desmethyl sildenafil plasma determination and quantification. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1001(5 September 2015), pp.35-40

Singh, S., Prasad, B., Savaliya, A.A., Shah, R.P., Gohil, V.M. & Kaur, A. 2009. Strategies for characterizing sildenafil, vardenafil, tadalafil and their analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs. TrAC Trends in Analytical Chemistry. 28(1):13-28.

Skerrett, P.J. 2010. Are Drugs lurking in your dietary supplements? Available: http://www.health.harvard.edu/blog/are-drugs-lurking-in-your-dietary-supplements-20101004551.

Smith, C.A., Priest, R., Carmady, B., Bourchier, S. & Bensoussan, A. 2011. The ethics of traditional chinese and Western herbal medicine research: views of researchers and human ethics committees in australia. Evidence-Based Complementary and Alternative Medicine: ECAM. 2011:256915.

Snyman, Stewart, M., Grove, A. & Steenkamp, V. 2005. Adulteration of South African traditional herbal remedies. Therapeutic Drug Monitor. 27:86-89.

Sorensen, J.M. 2002. Herb-drug, food-drug, nutrient-drug, and drug-drug interactions: mechanisms involved and their medical implications. Journal of Alternative and Complementary Medicine (New York, N.Y.). 8(3):293-308.

Springfield, E.P., Eagles, P.K.F. & Scott, G. 2005. Quality assessment of South African herbal medicines by means of HPLC fingerprinting. Journal of Ethnopharmacology. 101(1–3):75-83.

Stecher Vera J., Jackson Graham, Banks Ian, Arver Stefan, & Greenall Wendy October 2010. Analysis of Pharmaceuticals Seized by Authorities in the United Kingdom for Suspicion of Being Counterfeit VIAGRA® (Sildenafil Citrate) : ISMH World Congress 2010 Abstract 125. Journal of Men's Health. 7(3):321.

Sternitzke, C. 2014. Drug repurposing and the prior art patents of competitors. Drug Discovery Today. 19(12):1841-1847.

Sucar, D., Sougey, EB., Neto, JB. 2002. Psychotic episode induced by potential drug interaction of sibutramine and finasteride. Rev Bras Psiquiatr. 24:30–33.

Suresh, V., Ponnuru, P., Challa, B. & Nadendla, R. 20121. Quantificationofsibutramineanditstwometabolites in humanplasmabyLC–ESI-MS/MSanditsapplication in abioequivalencestudy Journal ofPharmaceuticalAnalysis. 2(4):249–257.

Szewczyk, K. & Zidorn, C. 2014. Ethnobotany, phytochemistry, and bioactivity of the genus Turnera (Passifloraceae) with a focus on damiana—Turnera diffusa. Journal of Ethnopharmacology. 152(3):424-443.

Tachjian, A., Viqar, M. & Jahangir, A. 2010. Use of Herbal Products and Potential Interactions in Patients with Cardiovascular Diseases . Journal of American College of Cardiology. 55(6):515-525.

Tavel, M.E. 2014. The Placebo Effect: the Good, the Bad, and the Ugly. The American Journal of Medicine. 127(6):484-488.

Taylor, M.J., Rudkin, L., Bullemor-Day, P., Lubin, J., Chukwujekwu, C. & Hawton, K. 2014. Estrategias para el tratamiento de la disfunción sexual inducida por la medicación antidepresiva. Revista Médica Clínica Las Condes. 25(1):166-167.

Thevis, M., Sigmund, G., Schiffer, A. & Schänzer, W. 2006. “Determination of N-desmethyl- and N-bisdesmethyl metabolites of Sibutramine in doping control analysis using liquid chromatography-tandem mass spectrometry”. European Journal of Mass Spectrometry. 12(2):129-136

Thomson Prentice, Lina Tucker. 2007. World Health Report 2007. [ONLINE] Available at: http://www.hst.org.za/publications/world-health-report-2007. [Accessed 24 September 2016])

Toomey, V.M., Litzau, J.J. & Flurer, C.L. 2012. Isolation and structural characterization of two tadalafil analogs found in dietary supplements. Journal of Pharmaceutical and Biomedical Analysis. 59(0):50-57.

Venhuis, B.J. & de Kaste, D. 2012. Towards a decade of detecting new analogues of sildenafil, tadalafil and vardenafil in food supplements: A history, analytical aspects and health risks. Journal of Pharmaceutical and Biomedical Analysis. 69(0):196-208.

Venhuis, B.J. & de Kaste, D. 2012. Towards a decade of detecting new analogues of sildenafil, tadalafil and vardenafil in food supplements: A history, analytical aspects and health risks. Journal of Pharmaceutical and Biomedical Analysis. 69(0):196-208.

Venhuis, B.J. & de Kaste, D. 2012. Towards a decade of detecting new analogues of sildenafil, tadalafil and vardenafil in food supplements: A history, analytical aspects and health risks. Journal of Pharmaceutical and Biomedical Analysis. 69:196-208.

Venhuis, B.J., Zwaagstra, M.E., Keizers, P.H.J. & de Kaste, D. 2014. Dose-to-dose variations with single packages of counterfeit medicines and adulterated dietary supplements as a potential source of false negatives and inaccurate health risk assessments. Journal of Pharmaceutical and Biomedical Analysis. 89:158-165.

Ventola, C.L. 2010. Current Issues Regarding Complementary and Alternative Medicine (CAM) in the United States: Part 1: The Widespread Use of CAM and the Need for Better-Informed Health Care Professionals to Provide Patient Counseling. Pharmacy and Therapeutics. 35(8):461-468.

Viall, J.2014 leave-complementary-medicines-alone-. Available: http://www.iol.co.za/capetimes/leave-complementary-medicines-alone-1.1714417#.VVc_qPmqqkp.

Wang, L., Chopp, M., Szalad, A., Zhang, Y., Wang, X., Zhang, R.L., Liu, X.S., Jia, L. *et al.* 2014. The role of miR-146a in dorsal root ganglia neurons of experimental diabetic peripheral neuropathy. Neuroscience. 259:155-163.

Wang, X., Xue, Y., Zhou, T. & Sakuma, T. 1997. Characterization of traditional Chinese medicine by liquid chromatography/atmospheric pressure ionization mass spectrometry. Journal of Food and Drug Analysis. 5(4):337-346.

Weinmann, W., Bohnert, M., Wiedemann, A., Renz, M., Lehmann, N. & Pollak, S. 2001. Post-mortem detection and identification of sildenafil (Viagra) and its metabolites by LC/MS and LC/MS/MS. International Journal of Legal Medicine. 114(4-5):252-258.

Weinmann, W., Lehmann, N., Müller, C., Wiedemann, A. & Svoboda, M. 2000. Identification of lorazepam and sildenafil as examples for the application of

LC/ionspray-MS and MS–MS with mass spectra library searching in forensic toxicology. Forensic Science International. 113(1–3):339-344.

Wells, C.L. 2014. Chapter 45 - Pulmonary diseases. In A Comprehensive Guide to Geriatric Rehabilitation (Third Edition). T.L. Kauffman, and others, Eds. Oxford: Churchill Livingstone. 315-325.

Werner, S.M. 2014. Patient safety and the widespread use of herbs and supplements. Frontiers in Pharmacology. 5:142.

Wheatley, V. M. & Spink, J. 2013. Defining the Public Health Threat of Dietary Supplement Fraud. Comprehensive Reviews in Food Science and Food Safety. 12: 599–613.

Wolleina,U; Schecha,B; Hardt; J ; Schramek, N. Determination and quantitation of sildenafil and its major metabolite in the breast milk of a lactating woman. Journal of Pharmaceutical and Biomedical Analysis 120 (2016) 100–105.

World Health Organisation (WHO) Counterfeit Drugs Kill. 2008 http://apps.who.int/iris/bitstream/10665/42851/1/WHO_TRS_917.pdf [Accessed June 2017].

Woo,C.S.J., Lau, ,J.S.H. & El-Nezami, ,H. Herbal Medicine. Toxicity and Recent Trends in Assessing Their Potential Toxic Effects.

Yamamoto, S., Sumioka, S., Fujiyoka, M., Mikami, E. & Miyamoto, K. 2011 December. A Study on Detection of Drugs in Slimming Health Foods Using GC-MS/MS.

Ye, M. & Guo, D.A. 2005. Analysis of bufadienolides in the Chinese drug Chan,Su by high-performance liquid chromatography with atmospheric pressure chemical ionization tandem mass spectrometry. Rapid Communications in Mass Spectrometry: RCM. 19(13):1881-1892.

Yi, E., Kim, J., Rhee, Y., Kim, S., Lee, H., Park, C. & Park, E. 2014. Preparation of sildenafil citrate microcapsules and in vitro/in vivo evaluation of taste masking efficiency. International Journal of Pharmaceutics. 466(1–2):286-295.

Yuen, Y.P., Lai, C.K., Poon, W.T., Ng, S.W., Chan, A.Y. & Mak, T.W. 2007. Adulteration of over-the-counter slimming products with pharmaceutical analogue--an emerging threat. Hong Kong Medical Journal = Xianggang Yi Xue Za Zhi / Hong Kong Academy of Medicine. 13(3):216-220.

Zhang, J., Wider , B., Shang, H., Li , X. & Ernst, E. 2012. Quality of herbal medicines: challenges and solutions. . Complementary Therapeutic Medicine. 20(1-2):100-106.

Zhang, K., Xu, B., Liu, D., Wang, X., Zhu, J., Deng, C., Jin, J. & Jiang, H. 2014. Sildenafil Improves Erectile Hardness in Chinese Men With Erectile Dysfunction: A Real-life Study Analyzed on Age Stratification. Urology. 83(4):831-836.

Zhuang, X., Long, M., Li, F., Hu, X., Liao, X. & Du, Z. 2014. PDE5 inhibitor sildenafil in the treatment of heart failure: A meta-analysis of randomized controlled trials. International Journal of Cardiology. 172(3):581-587.

Zou, P., Oh, S., Kiang, K., Low, M. & Bloodworth, B. (2007). Detection of sibutramine, its two metabolites and one analogue in a herbal product for weight loss by liquid chromatography triple quadrupole mass spectrometry and time-of-flight mass spectrometry. Rapid Communications in Mass Spectrometry, 21(4), pp.614-618.

Zou, P., Oh, S., Hou, P., Low, M. & Koh, H. (2006). Simultaneous determination of synthetic phosphodiesterase-5 inhibitors found in a dietary supplement and pre-mixed bulk powders for dietary supplements using high-performance liquid chromatography with diode array detection and liquid chromatography–electrospray ionization tandem mass spectrometry. Journal of Chromatography A, 1104(1-2), pp.113-12

APPENDICES

Appendix A:

Table 1.1. Summary of studies performed on the analysis of adulterants in weight-loss dietary supplements and used methodologies

Adulterants of interest	Formulation type	Method	Adulterated samples /total samples	Reference
Sibutramine	Capsules	HPLC-DAD; GC-MS	1/27	Jung and others (2006)
Anorectics (sibutramine,N-di-desmethylsibutramine,N-mono-desmethylsibutramine, fenfluramine), antiobesity drug (orlistat), laxative (phenolphthalein)	Capsules, teabags	LC-ESI-MS		Wang and others (2008)
Untargeted screening of adulterants	Capsules, tablets, powder	DOSY ^{1}H-NMR; MS/MS	14/20	Vaysse and others (2010)
Anorectics (sibutramine, fenfluramine), stimulants (ephedrine, norpseudoephedrine), diuretic (clopamide) and others (natural laxatives rhein, emodin, chrysophanol)	Tea powder, capsules, tablets	HPLC-ESI-MS/MS	12/12	Shi and others (2011)
34 Compounds including anorectics (amfepramone, phentermine, rimonabant, 2,4-dinitrophenol, fenfluramine, sibutramine), stimulants (amphetamine, caffeine, synephrine, ephedrine, pseudoephedrine), laxative (phenolphthalein), diuretics (althiazide, bumetanide, furosemide, spironolactone, triamterene) and antidepressant (fluoxetine)	Capsules, tablets, powders	UHPLC-DAD	20/20	Rebiere and others (2012)
Anorectics (amfepramone, sibutramine, fenproporex) and antidepressants (fluoxetine, paroxetine, sertraline, bupropion)	Not referred	Capillary Electrophoresis	4/106	De Carvalho and others (2012)
N-desmethylsibutramine	Not referred	LC-PDA; LC/MS	1/27	Park and others (2012)
Anorectics (sibutramine,N-desmethylsibutramine,N-didesmethylsibutramine), laxative (phenolphthalein)	Capsules, tablets	FI-MS/MS (confirmation using LC-MS/MS)	11/17	Song and others (2014)
29 Drugs including anorectics (sibutramine, desmethylsibutramine, didesmethylsibutramine,	Capsules, powder, tablet, granule and liquid	LC–MS/MS	62/188	Kim and others (2014)

diethylpropion, fenfluramine, mazindol, phentermine), stimulants (caffeine, ephedrine, pseudoephedrine, phendimetrazine), antidepressants (bupropion, fluoxetine, paroxetine, sertraline), laxatives (bisacodyl, phenolphthalein, sennosides)				
Anorectic drug (lorcaserin)	Capsules	NMR; MS/MS	1/1	Hachem and others (2014)
Anorectics (sibutramine, desmethylsibutramine, didesmethylsibutramine, rimonabant) and laxative (phenolphthalein)	Capsules, tablets, powder sachets	HPLC-DAD-MS/MS	24/50	Reeuwijk and others (2014)
Sibutramine	Powder and encapsulated liquids	HPTLC-UV densitometry; TLC–MS interface	28/52	Mathon and others (2014)
96 compounds including anorectics (fenfluramine, phentermine, rimonabant, sibutramine, topiramate), stimulants (amphetamine, β-methylphenethylamine, 1,3-dimethylamylamine, evodiamine, norephedrine, methamphetamine, cathine, ephedrine), antiobesity drug (orlistat), antidepressants (fluoxetine, sertraline), anxiolytic (diazepam), diuretics (hydroflumethiazide, bumetanide, chlorthalidone, hydrochlorothiazide, indapamide, methyclothiazide, metolazone)	Tablets, capsules, softgels and liquids	UHPLC–Q-orbitrap MS	3/23	Vaclavik and others (2014a)
Anorectics (benfluorex, phentermine, phenmetrazine, phendimetrazine, fenfluramine, fencanfamine, mephentermine, sibutramine), stimulants (ephedrines, caffeine)	Powder, oily capsules and tablets	LC/HRMS	3/36	Strano-Rossi and others (2015)

Table 1.2. Summary of studies performed on the analysis of PDE-5 inhibitors in dietary supplements and methodologies

Adulterants of interest	Formulation type	Method	Adulterated samples/total samples	Reference
Sildenafil	capsules	LC-MS/MS	1/1	Tseng and others (2001)
Phenylbutazone,caffeine,oxyphenbutazone	Sachets of powder	LC-MS/MS	Not referred	Lau and others (2002)
Sildenafil and analogues	Herbal supplements and soft drinks	HPLC-DAD-MS/MS	29/73	Reeuwijk and others (2013)
Sildenafil, Vardenafil and Tadalafil	Oral liquid, wine, beverage	HPLC-ESI-MS/MS	8/ 9	Zhu and others (2005)
Sildenafil, famotidine, Ibuprofen, Promethazine, Diazepam, Nifedipine, Captopril, Amoxicillin, Dextro methorphan	Capsules, tablets, oral solutions	LC-MS/MS	75/229	Liang and others (2005)
Sildenafil, Vardenafil and Tadalafil	Capsulesand tablets	HPLC-ESI-MS/MS	3 /3	Abdel-Hamid (2006)
Sildenafil, Vardenafil and Tadalafil	Bulk powders	LC-ESI-MS and LC-UV	20/40	Gratz and others (2004)
Sildenafil, Vardenafil and Tadalafil and analogues	Powders, capsules, tablets, film	LC-ESI-MS/MS	80/188	Jeong and others (2016)
Tadalafil analogue	Powders, capsules, tablets, liquid and pill	HPLC-DAD-Q-TOF/MS and NMR	10 /91	Lee and others (2014)
Sildenafil, Vardenafil and Tadalafil and analogues	Powders, capsules, tablets, film	LC-MS/MS	77/164	Lee and others (2013)
Sildenafil analogues	Bulk powders	HPLC-DAD-MS/MS and MNR and IR	Not referred	Oh and others (2006)
Sildenafil, Vardenafil and Tadalafil and analogues	Pills, capsules and powders	LC-ESI-MS/MS	45/52	Lee, Lee and others (2013)
Sildenafil	Capsules	HPTLC	3/3	Abourashed and others (2007)

35 Compounds including 2 PDE-5 inhibitors and methyltestosterone	Capsule, tablets, pill, granules, oral solution	QTRAP LC-MS/MS	11/29a	Chen and others (2009)
2 PDE-5 inhibitors, 5 sildenafil analogs and 1 vardenafil analog	Not referred	LC-MS/MS	some products/>100b	Ng and others (2010)
3 PDE-5 inhibitors	Capsule, tablets, herbal oils	LC-MS/TOF; HPLC-DAD	1/85c	Savaliya and others (2010)
3 PDE-5 inhibitors and yohimbine	Pill, soft capsules, hard capsules, oral drinks	HPLC-MS/MS	21/26	Zhang and others (2010)
3 PDE-5 inhibitors	Capsules, tablets	FI-MS/MS	1/13	Song and others (2012)
2 Tadalafil analogs	Capsules	LC-ESI-MS^n	2/2	Toomey and others (2012)
18 Compounds including PDE-5 inhibitors, analogs and yohimbine	Capsules, tablets	UFLC-ESI-MS/MS	9/16	Ren and others (2012)
2 Sildenafil analogs	Capsules	NMR; LC-MS; MS/MS; IR	1/1	Vaysse and others (2012)
PDE-5 inhibitors and analogs	Capsules, tablets, pills	HPLC-DAD-ESI-MS	74/91	Campbell and others (2013)
3 PDE-5 inhibitors and 10 analogs	Bulk powder, capsules	HPLC-CAD	13/24	Poplawska and others (2013)
1 Vardenafil analog (Hydroxythiovardenafil)	Capsules	LC-UV-MS/MS; NMR; IR	1/1	Jankovics and others (2013)
3 PDE-5 inhibitors, 24 sildenafil analogs, 5 tadalafil analogs and 5 vardenafil analogs	Pills, soft capsules, hard capsules, bulk powders	LC-ESI-MS/MS	45/52	Lee and others (2013a)
6 PDE-5 inhibitors, 26 sildenafil analogs, 7 tadalafil analogs, 5 vardenafil analogs; 4 other drugs for ED	Capsules, tablets, powder, film, liquid	LC-MS/MS	77/164	Lee and others (2013b)
Sildenafil and analogs	Capsules, tablets, liquids	LC-DAD-MS/MS	23/71	Reeuwijk and others (2013)
Diethylaminopretadalafil	Capsules	LC-UV; NMR; HRMS	1/1	Zhang and others (2014)
4 Sildenafil analogs	Capsules	LC-DAD; LC-MS; NMR	1/1	Schramek and others (2014)
82 Compounds including 71 erectile dysfunction active substances (PDE-5 inhibitors and analogs)	Tablets, liquid-gel capsules, oral liquids, herbal samples	LC-MS/MS	-/35d	Lebel and others (2014)
3 PDE-5 inhibitors and 11 analogs	Tablets, capsules	HPLC-UV	5/-	Fejos and others (2014)

3 PDE-5 inhibitors and 2 analogs	Tablets, capsules, powders, soft capsules	UPLC-TOF-MS; GC-MS	3/11	Damiano and others (2014)
3 PDE-5 inhibitors	Powders, oily capsules, tablets	LC–HRMS	4/36	Strano-Rossi and others (2015)
21 Compounds including 3 PDE-5 inhibitors and 5 analogs, yohimbine, phentolamine and mesylate	Capsules, tablets, powders, granules, drinkable liquids, chewing-gums, strips, gel	^{1}H NHR	104/150 (some samples with multiple compounds)	Gilard and others (2015)

1. [a]Given values comprise only samples related with ED adulterants; in this study 35 adulterated samples were detected among 105 PFS samples.
2. [b]More than 100 samples of health supplements and Chinese herbal drugs samples were analyzed with analogs being detected in some products (number not reported).
3. [c]Herbal formulations.
4. [d]The number of positive samples was not reported; samples included herbal medicines, dietary supplements, and legitimate or counterfeited trademark products.
5. CD_3CN:D_2O, deuterated acetonitrile: deuterated water; TSP, sodium 2,2,3,3- tetradeutero-3-(trimethylsilyl)propanoate; MeOH, methanol; ACN, acetonitrile; PFTE, polytetrafluoroethylene; PVDF, polyvinylidene difluoride.

Appendix B: Roadmap for the registration of complementary medicines

PSSA Perspectives
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In November 2013, the Minister of Health published amended regulations to the Medicines and Related Substances Act 101 of 1965. In December, this was followed by the Medicines Control Council's (MCC) roadmap for the regulatory control of complementary medicines for human and/or veterinary use. The following is an extract from the roadmap. The MCC has noted that there is an increasing number of medicines that are frequently called complementary or alternative medicines (CAMs) that are being sold in South Africa, for which claims of safety, quality and efficacy are being made without the products being registered by the MCC.

2002 notice On 22 February 2002, the MCC published a notice in the Government Gazette, in order to gain an understanding of the number and types of CAMs that were already on the market, or about to enter the market by August 2002. Although this objective was achieved, submissions of notification of new products continued to be made to the Department of Health and to the MCC. The 2002 notice led to considerable uncertainty among importers, manufacturers, wholesalers, retailers and consumers with regard to the legal status of these products, as companies who submitted such applications were often under the misconception that these submissions were serving as applications for registration, rather than simple notifications.

2013 amendment of regulations The amended regulations call for the legislative control of these CAMs. Category D has been created for complementary medicines, and will be subdivided into a number of different disciplines.

Antiviral agents, oral hypoglycaemic medicines, cardiac medicines and cytostatic agents In April 2012, the MCC resolved that, based on a risk assessment, an application for evaluation should be submitted to the MCC for CAMs that make claims about and purport to treat, diagnose and modify conditions of human immunodeficiency virus and acquired immune deficiency syndrome, diabetes, hypertension and cancer. This is in accordance with the requirements of the general guideline on complementary medicine registration. CAMs falling within the pharmacological classifications of antiviral agents, oral hypoglycaemic medicines, cardiac medicines or cytostatic agents were subject to registration from 15 November 2013. Medicines in these categories that are already available for sale in South Africa must be submitted for registration within six months from this date, while new medicines must be registered before they can be sold.

Similarly, all slimming or weight-reduction products and sexual stimulation medicines, so-called "lifestyle" products, will be called in for assessment within 24 months of publication of the regulations. This will also apply to complementary or alternative medicines that are currently on the market, as well as to new CAMs products.

Immune boosters, medicines acting on the muscular system and certain sports supplements All immune boosters, medicines acting on the muscular system and sports supplements that make any medicinal claims and/or which contain supplements in excess of the identified upper limit of vitamins or minerals, will be called in for evaluation assessment within 30 months of the publication of the regulations.

Immediate withdrawal from the market Products that contain "banned" substances, such as yohimbine, damiana, kava kava and apiol, must be withdrawn from the market with immediate effect. In addition, those that contain scheduled substances that are currently listed in the Schedules to the Act have to be withdrawn from the market with immediate effect. These include:

- Glucosamine when used for arthritis (S3)
- Silymarin, as contained in "milk thistle" (S3), Vitamin D in amounts greater than 500 IU/day (S3), and phenylephrine [also known as m-synephrine, that is contained in Citrus aurantium or bitter orange (S1)]
- Dehydroepiandrosterone [also known as DHEA and prasterone (S5)], and dimethylaminoethanol [also known as DMAE and deanol (S5)].

Call in of other pharmacological classifications The call up of products will continue until such time as all pharmacological classifications for CAMs have been called in. This should be completed by November 2019.

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Licensing of manufacturers and wholesalers All manufacturers and wholesalers of CAMs must be licensed in terms of section 22C(1)(b) of the Medicines and Related Substances Act 101 of 1965.

Labelling of complementary medicines All medicines falling into category D must comply with the labelling requirements in so far as the product label, the package insert and the patient information leaflet are concerned. In addition, these documents must also identify the discipline in which the complementary medicine is used. If the medicine has not been registered with the MCC, the following disclaimer must be included: "This medicine has not been evaluated by the Medicines Control Council. This medicine is not intended to diagnose, treat, cure or prevent any disease".

Registration procedure The MCC acknowledges that a significant number of CAMs, despite their long tradition, do not fulfil the requirements of a well established medicinal use with recognised efficacy and an acceptable level of safety, and have therefore not been eligible for registration.

The Pharmaceutical Society of South Africa (PSSA) welcomes the following pharmacists who joined the Society in November and December 2013. We trust that you will be welcomed into your branches and sectors, and that you will find great value in your membership: Risqah Allie, Veronica Aucamp, Janet Barry, Steven Peter Beetge, JJN Bester, Maureen Susan Botes, Pieter Cornelis Bruwer, Lauren Burger, Corne Buys, Andisiwe Canca, Marion Joy Cornelissen, Marlie de Ridder, Orla de Wet, Jean Deschamps, Minette Dippenaar, Melinda du Plessis, Daniel Mawunyega Korsi Ekar, Rosalina Beverley Esselaar, Mogamat Shafique Fakier, Teresa Faver, Verdell Jeanine Fensham, Catherine Theresa Forbes, Kim Ruth Germiquet, Famida Ghulam Hoosain, Magashree Govender, Zahra Harneker, Linda Kathleen Henderson, Ashmita Jayaram, Zaheeda Khan, Johannes Jakobus Kruger, Liezel Latsky, Maryka Jacolene le Roux, Morola Maria Lekalakala, Pearl Phologe Lentsoane, Pamela Levesque, Raymond Philip Maddock, Trinia Ntshebotse Mafarafara, Moyagabo Dorcas Mailula, Thato Jameson Makanyana, Hanlie Matthee, Naeema Mayet, Mosebiadi Veronica Mmako, Sabelo Mngomezulu, Paul Mohlala, Phuti Edward Mohlala, Sylvester Kabelo Mojatau, Raesetsa Christinah Mokou, Vincent Molele, Kamseelan Moodley, Kovanya Moodley, Marlene Rose Moonsamy, Tracy Motubatse, Brijlal Motwani, Jimson Mbongeni Mshengo, Gracious Lindiwe Msi, Patricia Musokotwane, Derisha Natalie Naidoo, Shalendra Naidoo, Nondumiso Nala, Luthando Nduna, Bosede Bisola Nkana, Nontobeko Noluthando Nzama, Irsadhusen Ibrahim Patel, Tinyika Patience Phaswana, Margherita Manuela Phillips, Gregory Purcell, Nagien Nivneat Ranchod, Shaeen Rawat, Hybre Rochelle Reeding, Lizette Schoeman, Dirk Cornelis Kleinsmit Serfontein, Deenash Singh, Lisa Marie Solomons, Maudrey Spelman, Nerita Suckhoo, Shirley Teffu, Titus Letsogile Tejane, Annatjie Conradie Theron, Jan Hendrik Petrus van Rooyen, Daleen van Schalkwyk, Mohesh Vason Bawjee, Theana Wessels, Carlien Wierenga and Wendy Anne Wilson. Student members We are also delighted to welcome the following student members: Louise Baartzes, Mpho Baliki, Faith Banda, Phiona Banda, Riaan Barnard, Aguhpi Beja, Asanda Biyana, Sunet Botha, Gaboelwe Botshelo, Ngawonke Bulala, Lauren Campbell, Hitekane Beryl Chauke, Amber Cheng, Josephine Tafadzwa Chirimanyemba, Andrea Lauren Connacher, Dewald Eugene Coutts, Charity T Dandira, Charity Bantle David, Njengele Galelo Didiza, Thembekile Veronica Dladla, Nhlakanipho Fairhope Dlamini, Linda Rompel Eijbers, Laycan Essex, Sithembelihle Tracy Falala, Jaco Fourie, Zaheer Gaida, Elandri Gerber, Kayleigh Cynthia Halgreen, Rushenda Maxine Hoffman, Kiryagana Peter Isabirye, Kristin Kate Jansen, Michelle Anne Jansen, Antonia Joao, Andrew Mearns Johnston, Bianca Kabasa, Kesolofetse Onicah Keakile, Yasmine Michelle Khan, Andile Kheswa, Onneile Khumalo, Sandile Comfort Khwela, Casey Courtney Amber Lee, Daliwong Gift Lungu, Samuel Lupe, Mpho Given Mabalanganye, Zizo Majwede, Tapiwa Matibiri, Aseza Momelezi Matolengwe, Ogochukwu Mbachu, Esona Mbiko, Meagan Claire Meyer, Thembinkosi Thobani MLawu,
Olekantse Moikwathai, Lebogang Molefhi, Keshana Moodaley, Viola Tuduetso Mositiemang, Lubalethu Mpabanga, Gofaone Mpundu, Tabitha Nalugwa, Aida Mary Nankumba, Mayi Nanyonga, Veli Wonder Nhlapho, Paul Herve Noudem, Pache Oranje, Abongile Petela, Kemo Terry Ramakau, Yumna Ramjan, Johannes Rungwe, Kirti Salaye, Chan,gu Same Sebeela, Nompumemelo Sepopa, Avikaar Sewpersad, Dumisani Jeffrey Sifelani, Mzuyanda Michael Sizani, Jenna Maree Stickells, Lana Strydom, Alysha Transell, Moemedi Tshauwe, Jacklyn Kate van Dyk, Michelle van Niekerk, Larita van Wyk, Samantha van Wyk, Martin Weder, Alexander West and Chris-Gere Zeelie.

In memoriam The PSSA extends its sincere condolences to the family and friends of the following members who passed away in November and December 2013: ER Berry, Southern Gauteng Branch; Rufus Frank Carklin, Southern Gauteng Branch; Allan Albert Graham, Southern Gauteng Branch; and George Richard Smith, Pretoria Branch.

The Pharmaceutical Society of South Africa 69th Annual General Meeting Notice in terms of the Constitution (Section 22.2) To: All members of the General Council of the Pharmaceutical Society of South Africa You are hereby notified that the 69th Annual General Meeting of the General Council will take place at The Boardwalk Convention Centre, Summerstrand, Port Elizabeth on Saturday, 10 May 2014, commencing at 14h00 Issued by: Ivan Kotzé, Executive Director Pharmaceutical Society of South Africa January 2014

Appendix C:

Summary Tables showing the Identification of the Stores and Exhibit Samples

Identification of Stores

Identification	Name of Store
Cluster 1 Supermarket/store 1:C1S1	Chinese Supermarket
Cluster 1 Supermarket/store 2: C1S2	R5 Store
Cluster 2 Chain pharmacy/store 1: C2S1	Dischem
Cluster 2 Private pharmacy 1/store 2: C2S2	Steenberg Pharmacy
Cluster 2 Private pharmacy 2/store 3: C2S3	Rivetts Pharmacy

Identification of Exhibit Samples C1S1C					
Name of Sample	**ID**	**Tablet No**	**Box No**	**Lump No**	**Capsule No**
1. Black Gold 1st capsule same box	BG B1C1		B1		C1
2. Black Gold 2nd capsule same box	BG B1C2		B1		C2
3. Xianggantianlongshenwu 1st tablet	Xi B1 T1	T1	B1		
4. Xianggantianlongshenwu (2nd tablet)	Xi B1T2	T2	B1		
5. Chao Jimengnan (1 tablet first box)	CJ B1T1	T1	B1		
6. Chao Jimengnan (2nd tablet first box)	CJ B1T2	T2	B1		
7. Chao Jimengnan (1 tablet second box)	CJ B2T1	T1	B2		
8. Man King (1 capsule 1st box)	MK B1C1		B1		C1
9. Man King (2nd capsule first box)	MK B1C2		B1		C2
10. Man King (1 capsule 2nd box)	MK B2C1		B2		C1
11. Man King (2nd capsule 2nd box)	MK B2C2		B2		C2
12. Stag (1st lump)	St L1			L1	
13. Stag (2nd lump)	St L2			L2	

14. Yilishen (1st tablet)	Yi B1T1	T1	B1		
15. Yilishen (2nd tablet same box)	Yi B1T2	T2	B1		
16. Cattle Capsule (1st capsule)	CC B1C1		B1		C1
17. Cattle Capsule (2nd capsule same box)	CC B1C2		B1		C2
18. Blue box(vigorous) (1st capsule)	BB B1C1		B1		C1
19. Blue box(vigorous) (2nd capsule same box)	BB B1C2		B1		C2
20. Yellow Ant (1 lump)	YA L1			L1	
21. Yellow Ant (more of same lump)	YA L1			L1	
22. Yellow and purple box (1st lump)	YP L1			L1	
23. Yellow and purple box (2nd lump)	YP L2			L2	
24. Gold and red dragons (1st capsule)	GD B1C1		B1		C1
25. Gold and red dragons (more of 1st capsule)	GD B1C1		B1		C1
26. Gold and red dragons (2nd capsule)	GD B1C2		B1		C2
27. Gold Gun (1st capsule)	GG B1C1		B1		C1
28. Gold Gun (2nd capsule same box)	GG B1C2		B1		C2

C1S2

29. Dr Lee AK 47(1 of 3 capsules)	AK47 B1C1		B1		C1
30. Dr Lee AK 47(2 of 3 capsules-same pack)	AK47 B1C2		B1		C2
31. Extreme Striker (1 of 3 capsules)	ES B1C1		B1		C1
32. Extreme Striker (2 of 3 capsules-same pack)	ES B1C2		B1		C2
33. Red dragon (1 of 4 tablets)	RD B1T1	T1	B1		
34. Red dragon (2 of 4 tablets-same pack)	RD B1T2	T2	B1		
35. Quickie (1 of 3capsues)	Qu B1C1		B1		C1
36. Quickie (2 of 3 capsules-same pack)	Qu B1C2		B1		C2

C2S1

37. Man King (1 of 8 capsules)	MK8 B1C1		B1		C1
38. Man King (2nd of 8 capsules-same box)	MK8 B1C2		B1		C2
39. Chao Jimengnan (pack of 1 tablet)	CJ1 B1T1	T1	B1		
40. Super powerful man pills (Pack of 4)	SP4 B1T1	T1	B1		
41. Super powerful man pills (2nd pack of 4)	SP4 B1T2	T2	B1		
42. Super powerful man pills (single tablet)	SP1 B1T1	T1	B1		
43. Man King (pack of 1 capsule)	MK1 B1C1		B1	C1	
44. Man King (2nd pack of 1 tablet)	MK1 B2C1		B2		C1
45. Man King blue (pack of 1 tablet)	MKB1 B1T1	T1	B1		
46. Man King blue (pack of 4 tablets)	MKB4 B1T1		B1	T1	
47. Man King blue (2nd pack of 1 tablet)	MKB1 B2T1		B2	T1	

48. Man King (single capsule)	MKSC B1C1		B1		C1
49. Man King (2nd single capsule)	MKSC B2C1		B2		C1
50. Viriya	Vi B1C1		B1		C1
51. Panalt	Pa B1C1		B1		C1
52. Anela (1 of 2 capsules)	An B1C1		B1		C1
53. Anela (2nd of 2 capsules)	An B1C2		B1		C2
54. Revitalizer 700 (1st of 2 capsules)	Re B1C1		B1		C1
55. Revitalizer 700 (2nd of 2 capsules)	Re B1C2		B1		

C2S2

56. Amandla	Am B1C1		B1		C1
57. High Rise	HR B1C1		B1		C1
58. Tong Yong Gold	TYG B1T1	T1	B1		
59. Tong Yong silver	TYS B1T1	T1	B1		

C2S3

60. Chao Jimengnan (1st of 4 tablets)	CJ B3T1	T1	B3		
61. Chao Jimengnan (2nd of 4 tablets-same box)	CJ B3T2	T1	B3		

Table 2.2: Data for the Preparation of the Exhibit Samples for Screening for API's.

C1S1

Sample Name	Mass of tablet/ capsule/ lump (mg)	Mass weighed out (mg)	Concentration after adding 5 mL water/methanol (mg/mL)	Volume required to make 1mg/mL solns (µL)
1.BG	310.82	53.37	10.67	93.72
2.Xi	599.64	42.00	8.4	119.05
3.CJ	684.73	46.88	9.38	106.66
4.MK	486.34	42.58	8.52	117.43
5.St	6122.44	44.04	8.81	113.53
6.Yi	462.13	37.06	7.41	134.92
7.CC	271.88	39.78	7.96	125.69
8.BB	237.57	48.65	9.73	102.78
9.YA	3941.85	45.92	9.18	108.89
10.YP	4704.34	54.71	10.94	91.39
11.GD	355.50	42.05	8.41	118.91
12. Kangmei(slimming)	387.67	40.01	8.00	124.97
13. Kangmei(slimming)	414.86	51.2	10.2	98.0
14. Kangmei(slimming)	402.71	55.03	11.0	90.9
15. Kangmei(slimming)	387.67	48.63	9.73	102.82

C1S2

Sample Name	Mass of tablet/ capsule/ lump (mg)	Mass weighed out (mg)	Concentration after adding 5 mL water/methanol (mg/mL)	Volume required to make 1mg/mL solns (µL)
16. Chinaga	362.17	49	9.8	102
17. TYG	340.82	34	6.8	147.05
18. Power up	452.14	54	10.8	92.6
19. CJ	670.24	47	9.4	106.4
20. Kangmei(slimming)	390.84	38	7.6	131.5
21. Kangmei(slimming	392.05	50.61	10.12	98.8

C2S1

Sample Name	Mass of tablet/ capsule/ lump (mg)	Mass weighed out (mg)	Concentration after adding 5 mL water/methanol (mg/mL)	Volume required to make 1mg/mL solns (µL)
22. TYS	341.98	43	8.6	116.2
23. High Rise	320.68	28	5.6	178.5
24. Passion flower	412.33	40	8.0	125.0
25. Slimming dragees	124.32	39	7.8	128.2
26. G.I lean Hunger Buster (slimming)	406.84	36	7.2	138.8

Appendix D

Table 3.5: Quantitative results of bias and precision runs. All concentrations are in ng/mL.

LOQ (20 ng/mL)	Run 1 Concentration (ng/mL)	Run 2	Run 3	Run 4	Run 5	Run 6
Rep 1	19.2	20.8	22.6	21.4	16.5	22.4
Rep 2	18.1	15.0	22.5	17.8	18.0	28.9
Rep 3	26.2	16.6	26.7	19.1	17.4	26.5
Low (60 ng/mL)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	58.3	55.0	60.5	59.1	56.0	64.1
Rep 2	64.9	61.8	66.5	58.0	54.5	51.1
Rep 3	48.4	63.0	52.4	62.2	52.7	52.7
Med (400 ng/mL)	**Run1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	191	418	411	450	412	357
Rep 2	398	414	189	411	380	357
Rep 3	408	406	434	394	363	337
Med/high (800 ng/mL)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run3**	**Run 6**
Rep 1	771	802	831	798	924	765
Rep 2	871	790	794	884	741	739
Rep 3	864	770	825	817	857	756
High (1600 ng/mL)	**Run 1**	**Run 2**	**Run 3**	**Run 4**	**Run 5**	**Run 6**
Rep 1	1660	1680	1620	1400	1510	1650
Rep 2	1460	1690	1680	1640	1600	1620
Rep 3	1400	1720	1630	1610	1610	1560

Appendix E: Results of all exhibit samples to show the mass of sildenafil calculated

The results of all the exhibit samples are shown in Tables 3.7.1-3.7.5 showing the total mass of the tablet/capsule/lump, the % sildenafil present and the calculated mass of sildenafil per tablet/capsule/lump.

Table 3.7.1: The API detected and the calculated mass of sildenafil in each dosage form (tablet, capsule or lump) from C1S1.

Sample Name	Mass of tablet/capsule/lump (mg)	API detected	Calculated concentration from calibration curve (ng/mL)	%sildenafil	Mass of sildenafil/tablet/capsule /lump (mg)
1. BG B1C1	310.82	sildenafil	1728	17.28	53.71
2. BG B1C2	331.22	sildenafil	1980	19.8	65.58
3. Xi B1 T1	599.64	sildenafil	1030	10.3	61.76
4. Xi B1T2	612.05	sildenafil	721	7.21	52.95
5. CJ B1T1	684.73	sildenafil	563	5.63	38.55
6. CJ B1T2	677.02	sildenafil	775	7.75	52.47
7. CJ B2T1	672.71	sildenafil	745	7.45	50.11
8. MK B1C1	483.33	sildenafil	2240	22.4	108.26
9. MK B1C2	479.09	sildenafil	2230	22.3	106.83
10. MK B2C1	486.22	sildenafil	2180	21.8	105.9
11. MK B2C2	482.24	sildenafil	1900	19.0	80.26
12. St L1	6122.44	sildenafil	202	2.02	123.67
13. St L2	6122.44	sildenafil	45.9	0.459	28.10
14. Yi B1T1	461.76	sildenafil	1488	14.88	68.71
15. Yi B1T2	454.07	sildenafil	1460	14.6	66.29

16. CC B1C1	271.88	sildenafil	780	7.80	21.21
17. CC B1C2	262.61	sildenafil	990	9.90	25.99
18. BB B1C1	245.42	sildenafil	1338	13.38	32.84
19. BB B1C2	236.52	sildenafil	1120	11.2	26.49
20. YA L1	3941.85	sildenafil	175	1.75	68.98
21. YA L1	3941.85	sildenafil	166	1.66	65.43
22. YP L1	4704.34	sildenafil	102	1.02	47.98
23. YP L2	4701.21	sildenafil	4.07	0.047	<LOD
24. GD B1C1	355.50	sildenafil	1134	11.34	40.31
25. GD B1C1	355.50	sildenafil	544	5.44	19.34
26. GD B1C2	381.38	sildenafil	956	9.56	36.46
27. GG B1C1	322.59	sildenafil	1450	14.5	46.78
28. GG B1C2	336.89	sildenafil	1520	15.2	51.21

Table 3.7.2: The API detected and the calculated mass of sildenafil in each dosage form (tablet, capsule or lump) from C1S2.

Sample Name	Mass of tablet/capsule/lump (mg)	API detected	Calculated concentration from calibration curve (ng/mL)	%sildenafil	Mass of sildenafil/tablet/capsule /lump (mg)
29. AK47B1C1	267.32	sildenafil	1200	12.0	32.08
30. AK47B1C2	263.15	sildenafil	1250	12.5	32.89
31. ES B1C1	256.66	sildenafil	1420	14.2	36.45
32. ES B1C2	233.48	sildenafil	1380	13.8	32.22
33. RD B1T1	641.71	sildenafil	9.74	0.097	<LOD
34. RD B1T2	649.72	sildenafil	4.15	0.0041	<LOD
35. Qu B1C1	275.73	sildenafil	1250	12.5	34.47
36. Qu B1C2	428.69	sildenafil	1470	14.7	63.02

Table 3.7.3: The API detected and the calculated mass of sildenafil in each dosage form (tablet, capsule or lump) from C2S1.

Sample Name	Mass of tablet/capsule/lump (mg)	API detected	Calculated concentration from calibration curve (ng/mL)	% sildenafil	Mass of sildenafil/tablet/ capsule/lump (mg)
37.MK8 B1C1	428.22	sildenafil	<LOD	<LOD	<LOD
38.MK8 B1C2	400.33	sildenafil	4.88	0.048	<LOD
39. CJ1 B1T1	752.08	sildenafil	8.88	<LOD	<LOD
40. SP4 B1T1	745.95	sildenafil	139	1.39	10.37
41. SP4 B1T2	743.92	sildenafil	223	2.23	16.59
42. SP1 B1T1	752.08	sildenafil	24.7	<LOD	<LOD
43. MK1 B1C1	435.29	sildenafil	601	6.01	26.16
44. MK1 B2C1	496.1	sildenafil	1350	13.5	66.97
45. MKB1 B1T1	562.27	sildenafil	<LOD	<LOD	<LOD
46. MKB4 B1T1	577.73	sildenafil	<LOD	<LOD	<LOD
47. MKB1 B2T1	555.61	sildenafil	458	4.58	25.45
48. MKSC B1C1	306.87	sildenafil	937	9.37	26.16
49. MKSC B2C1	562.27	sildenafil	4.74	0.047	<LOD
50. Vi B1C1	554.92	sildenafil	87.7	0.087	4.87
51. Pa B1C1	426.07	sildenafil	4.15	0.041	<LOD
52. An B1C1	597.55	sildenafil	4.21	0.042	<LOD
53. An B1C2	596.23	sildenafil	2.85	0.028	<LOD
54. Re B1C1	769.44	sildenafil	4.96	0.049	<LOD
55. Re B1C2	762.60	sildenafil	4.96	0.049	<LOD

Table 3.7.4: The API detected and the calculated mass of sildenafil in each dosage form (tablet, capsule or lump) from C2S2.

Sample Name	Mass of tablet/capsule/lump (mg)	API detected	Calculated concentration from calibration curve (ng/mL)	%sildenafil	Mass of sildenafil/tablet/ capsule/lump (mg)
56.Am B1C1	749.03	none	<LOD	<LOD	<LOD
57. HR B1C1	704.97	sildenafil	<LOD	<LOD	<LOD
58. TYG B1T1	345.71	sildenafil	<LOD	<LOD	<LOD
59. TYS B1T1	497.82	sildenafil	<LOD	<LOD	<LOD

Table 3.7.5: The API detected and the calculated mass of sildenafil in each dosage form (tablet, capsule or lump) from C2S3.

Sample Name	Mass of tablet/capsule/lump (mg)	API detected	Calculated concentration from calibration curve (ng/mL)	%sildenafil	Mass of sildenafil/tablet/ capsule/lump (mg)
60. CJ B3T1	770.70	sildenafil	154	1.54	11.87
61. CJ B3T2	817.49	sildenafil	650	6.50	53.14

Appendix F: Contraindications and side effects of sildenafil (Pfizer.com)

Contraindications

The sildenafil in Viagra® may affect the way other medicines work, and other medicines may affect the way sildenafil works causing side effects. Anyone taking any of the medicines listed below, should not be taking sildenafil:

- medicines called nitrates (such as nitroglycerin)
- medicines called guanylate cyclase stimulators, such as riociguat (Adempas)
- street drugs called "poppers" such as amyl nitrate or amyl nitrite, and butyl nitrate
- medicines called alpha blockers such as Hytrin (terazosin HCl), Flomax (tamsulosin HCl), Cardura (doxazosin mesylate), Minipress (prazosin HCl), Uroxatral (alfuzosin HCl), Jalyn (dutasteride and tamsulosin HCl), or Rapaflo (silodosin). Alpha-blockers are sometimes prescribed for prostate problems or high blood pressure. In some patients, the consumption of sildenafil with alpha-blockers can lead to a drop in blood pressure (syncope) or to fainting. The drop in blood pressure can even be fatal.
- medicines called HIV protease inhibitors, such as ritonavir (Norvir), indinavir sulfate (Crixivan), saquinavir (Fortovase or Invirase) or atazanavir sulfate (Reyataz)
- some types of oral antifungal medicines, such as ketoconazole (Nizoral), and itraconazole (Sporanox)
- some types of antibiotics, such as clarithromycin (Biaxin), telithromycin (Ketek), or erythromycin
- other medicines that treat high blood pressure
- other medicines or treatments for ED
- sildenafil is the same medicine found in another drug called REVATIO. REVATIO is used to treat a rare disease called pulmonary arterial hypertension (PAH). Sildenafil should not be used with REVATIO or with other PAH treatments containing sildenafil or any other PDE5 inhibitors (such as Adcirca [tadalafil]).

Sildenafil can also have an adverse effect with the simultaneous intake of alcohol and certain individuals may be hypersensitive or even allergic to it. The safety of Viagra® is unknown in patients with bleeding disorders and patients with active peptic ulceration (Pfizer, 2016).

Side effects

The sildenafil in Viagra® can cause serious side effects. Reported, although rare side effects include:

- an erection that will not go away (priapism). If an erection lasts more than 4 hours, medical help is required urgently. If it is not treated right away, priapism can permanently damage the penis.
- sudden vision loss in one or both eyes. Sudden vision loss in one or both eyes can be a sign of a serious eye problem called non-arteritic anterior ischemic optic neuropathy (NAION). Viagra® must be immediately stopped and the healthcare practitioner advised of vision loss.
- sudden hearing decrease or hearing loss. Some people may also have ringing in their ears (tinnitus) or dizziness. If there are these symptoms, Viagra® must be stopped and a doctor contacted right away.

The most common side effects of sildenafil are:

- headache
- flushing
- upset stomach
- abnormal vision, such as Chan,ges in color vision (such as having a blue color tinge) and blurred vision
- stuffy or runny nose
- back pain
- muscle pain
- nausea
- dizziness

- rash

In addition, heart attack, stroke, irregular heartbeats and death have happened rarely in men taking Viagra®. Most, but not all, of these men had heart problems before taking Viagra®. It is not known if Viagra® caused these problems.

The product monograph for Viagra® warns the consumer to not take it more than once a day. It also advises to call your doctor or go to the nearest hospital emergency room right away if too much Viagra® has been taken. In the elderly, higher plasma levels were found and this may increase the incidence of adverse reactions, a starting dose of 25 mg should be considered in older subjects. The same holds true for patients suffering from renal or hepatic impairment (Drugs.com 2016).