QUALITY AND SAFETY ASSESSMENT OF SEXUAL PERFORMANCE ENHANCEMENT HERBAL MEDICINES

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LIST OF PUBLICATIONS

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2. Li L, Low MY, Ge X, Bloodworth BC, Koh HL. 2009b. Isolation and structural elucidation of dapoxetine as an adulterant in a health supplement used for sexual performance enhancement. J Pharm Biomed Anal 50: 724-728.

3. Low MY, Zeng Y, Li L, Ge XW, Lee R, bloodworth BC, Koh HL. 2009. Safety and quality assessment of 175 illegal sexual enhancement products seized in red-light districts in Singapore. Drug Saf 32: 1141-1146.

4. Häberli A, Girard P, Low MY, Ge X. 2010. Isolation and structural elucidation of an interaction product of aminotadalfil found in an illegal health product. J Pharm Biomed Anal 53: 24-28.

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LIST OF POSTER PRESENTATIONS

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LIST OF ABBREVIATIONS

a.k.a	also know as
ADR	Adverse Drug Reaction
ANH-Intl	Alliance for Natural Health International
BP	British Pharmacopoeia
CE	Collision Energy
CP	Chinese Pharmacopoeia
CPM	Chinese Proprietary Medicines
CSM	Committee on Safety of Medicines, UK
CXP	Collision Cell Exit Potential
DAD	Diode Array Detection
DP	Declustering Potential
DSHEA	Dietary Supplement Health and Education Act, USA
EBF	European Benefyt Foundation
EHPA	European Herbal Practitioners Association
EMA	European Medicines Agency
EP	Entrance Potential
EPI	Enhanced Product Ion
ESI	Electrospray Ionization
FTMS	Fourier Transform Mass Spectrometry
GACP	Good Agricultural and Collection Practices
GMP	Good Manufacturing Practices

GC	Gas Chromatography
HKSAR	Hong Kong Special Administrative Region
HM	Herbal medicines
HMPC	Herbal Medicinal Products Committee formed under EMA
HSA	Health Sciences Authority, Singapore
HPLC	High Performance Liquid Chromatography
HSDD	Hypoactive Sexual Desire Disorder
ICP	Inductively Coupled Plasma
IDA	Information-dependent Acquisition
IMS	Ion Mobility Spectrometry
IR	Infrared Spectrometry
LC	Liquid Chromatography
LTQ	Quadrupole Linear Ion
MS/MS	Tandem Mass Spectrometry
LOD	Limit of Detection
MRM	Multiple reaction monitoring
MS	Mass Spectrometry
NAION	Nonarteritic Anterior Ischemic Optic Neuropathy
NMR	Nuclear Magnetic Resonance Spectroscopy
OTC	Over-The-Counter
PDE-5	Phosphodiesterase Type 5 enzyme
Q1	First Quadrupole

Q2	Third Quadrupole
RT	Retention Time
THMP	Traditional Herbal Medicinal Products
TM	Traditional Medicines
TCM	Traditional Chinese Medicine
USP	US Pharmacopoeia
WHO	World Health Organization

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SUMMARY

In recent years, there is increasing interest in the use of herbal health products as an alternative to erectile dysfunction drugs to enhance sexual performance. Adverse events associated with the consumption of herbal products for sexual performance enhancement and treatment of erectile dysfunction have been reported. In Singapore, four illegal sexual performance enhancement health products have been reported to be adulterated with sildenafil and a very high dose of glibenclamide. These products have caused severe hypoglycaemia leading to 10 deaths in Singapore.

The objectives of this study are to assess the safety and quality of sexual performance enhancement herbal health products illegally sold in the red-light districts in Singapore and to develop analytical methods to enhance the adulterant testing capability in a national quality control laboratory.

The first part of the study assessed the safety and quality of the sexual performance enhancement herbal products in Singapore. This part of the work involved adulterant screening of 247 illegal sexual enhancement health products seized by Health Sciences Authority (HSA) during the period Feb to Dec 2008 from makeshift stalls in red-light districts of Singapore and a shipment at the Singapore Customs. The extent of adulteration, contamination and content level of the adulterants were assessed. The risks associated with the consumption of

such products were also evaluated. The extensive list of adulterated products tested and the assessment results will be useful to consumers, regulators and the industry players.

The second part of the project involved the development of a LC-Hybrid Tandem MS method for the detection of PDE-5 inhibitors and their related analogues in herbal health products marketed for sexual performance enhancement. The method developed was able to screen for the 3 approved PDE-5 inhibitor drugs (Sildenafil, Tadalafil and Vardenafil) and their related 22 analogues in sexual enhancement health products in 20 min. The developed screening method was validated using 11 blinded samples (consisting positive and negative products previously tested by HSA's laboratory) and the validation results showed that the screening method was rapid, sensitive, specific and was able to simultaneously detect the PDE-5 inhibitors and related analogues present in the samples. To the best of our knowledge, this is thus far the only method that can provide such comprehensive screening of the PDE-5 inhibitors and their related analogues.

The final part of the project involved the structural elucidation of an unknown adulterant detected in a herbal health product claimed for female sexual performance enhancement. The product was sent by a client to HSA for testing. The chemical structure of the unknown compound with molecular mass of 390 was structurally elucidated using LC-DAD, LC-LTQ Orbitrap XL FTMS, NMR and IR analysis. The unknown adulterant was confirmed to be flibanserin, a non-

hormonal treatment drug developed for pre-menopausal woman with hypoactive sexual desire disorder (HSDD). The New Drug application for flibanserin was rejected by FDA Advisory Committee for Reproductive Health Drugs as the efficacy was deemed not sufficiently robust to justify the risks, concerns over the safety signals and potential drug interactions. The results of this study enabled the local regulator to stop the adulterated health product from entering Singapore's market and raised the awareness of the possible adulteration of flibanserin in health products claimed for female sexual performance enhancement.

The work presented in this thesis is useful to consumers, regulators, health care professionals and industry players. The method developed and validated has helped to enhance the testing capability of the national regulatory control laboratory in screening and detection of PDE-5 inhibitors, their analogues and other unknown adulterants found in health products claimed for sexual performance enhancement.

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Chapter 1

Introduction

1.1 Quality and safety of herbal medicines

Herbal medicines are plant-derived materials or preparations with therapeutic or other health benefits, containing either raw or processed ingredients from one or more plants (Barnes 2003a, WHO 2005). Herbal supplements are a form of herbal medicines.

Herbal medicines are widely used around the world. About 1.5 billion people worldwide use traditional Chinese herbal medicines (Hosbach et al. 2003). A survey conducted in 2005 (Jordan et al. 2010) revealed that 71% of Canadian used natural health products, which include herbal medicines, vitamins and minerals. 11% of the people surveyed used herbal remedies and algal/fungal products. In the United States, about 19% of the adult population was using herbal medicines as of 2002 (Jordan et al. 2010, Kennedy 2005). Another study showed that about $17.8 billion was spent in the U.S. on dietary supplements with $4.2 billion of this amount on herbs (Dobos et al. 2005, NBJ 2002).

Although herbal medicines are widely considered to be of lower risk compared to synthetic drugs, they are not completely free from the possibility of toxicity or other adverse effects (De Smet 2004). While inherent toxicity of certain herbs

such as Ephedra and Aristolochia is well known, adverse effects from the use of herbal medicines may also result from contamination of products with toxic metals, adulteration with pharmacologically active synthetic drugs, misidentification or substitution of herbal ingredients, or improperly processed products. Interactions may also occur between drugs, food and other herbal medicines taken concomitantly (Jordan et al. 2010). The following sections discuss various quality and safety issues related to herbal medicines.

1.1.1 Plant misidentification and substitution

The correct identification of plant materials during collection and processing is critical for the quality control of herbal medicines. Substitution with more toxic herbs may occur due to misidentification of plant species or deliberately for economic reasons when a cheaper herb is supplied to replace a safe, more expensive one. Several cases of incorrect plant substitution or mis-identification have been reported. One example which resulted in significant morbidity, in particular, renal failure and renal cancer following the use of slimming products contaminated with *Aristolochia* species first surfaced in the 1990s, initially from Belgium, France and later UK (Barnes et al. 2002, EMEA 2002, Jordan et al. 2010). This was due to the substitution of nontoxic herbs, including *Stephania tetrandra* and *Clematis armandii*, with toxic *Aristolochia* species.

Koren et al. reported a case of maternal and neonatal androgenisation associated with a herbal product in 1990. The mother experienced increased hair growth on her head, forehead and public area. At birth, the male child had thick hair in the pubic region, and on the forehead, along with red swollen nipples. The mother had been taking a commercial product labeled as containing "Siberian ginseng" during her pregnancy and 2 weeks of breast feeding (Koren et al. 1990). Awang later investigated the case and found that the raw materials used did not contain authentic Siberian ginseng (*Eleutherococcus senticosus* (Rupr. And Maxim.) Maxim.), but were likely to contain silk vine (*Periploca sepium* Bunge, Apocynaceae) instead. It had previously been known that some *Periploca* species had been substituted for *Eleutherococcus* in some imported products, possibly due to confusion of the Chinese names of the two plants (Awang 1991, Jordan et al. 2010). The Chinese name for *Eleutherococcus senticosus* is "Ci-wu-jia" (刺 五 加), while the Chinese name for *Periploca sepium* is "wu-jia" (五 加), which may explain the source of confusion (Boon and Smith 2004).

A similar case was reported in 2004. A 60-year-old man was diagnosed with kidney failure and cancer of the urinary tract in Hong Kong (Zhao et al. 2006, Liang et al. 2006). He had been taking a herbal prescription called "Bai Mao Teng" (白毛藤). Investigation by HKSAR Department of Health revealed that the patient had been mistakenly given *Aristolochia mollissima* Hance (Xun Gu Feng, 寻 骨 风). *Aristolochia mollissima* Hance is known to contain aristolochic acid. Further investigation revealed that *Aristolochia mollissima* Hance was

erroneously substituted for *Solanum lyratum* Thunb (Bai Ying, 白 英) at the wholesale level as both the crude drugs have the same common names “Bai Mao Teng” (白毛藤). It was also found that there is recurring confusion with regard to the names Xun Gu Feng, Bai Ying and Bai Mao Teng. In light of this, the HKSAR Department of Health called for a suspension of the use of these 3 Chinese herbs (Liang et al. 2006).

1.1.2 Plant toxicity

There is a common misconception that because herbs are natural, they are entirely ‘safe’. Clearly, this is not the case (some plants are inherently poisonous), and plants used medicinally do, in some cases, cause adverse effects. Paracelsus, sometimes called the Father of Toxicology, had also in his famous quote, “Poison is in everything, and nothing is without poison; only the dose permits something not to be poisonous” said that substances considered toxic are harmless in small doses, and conversely an ordinarily harmless substance can be deadly if over-consumed. This applies to the toxicity of herbal medicines. In general, little is known regarding the adverse effect of most herbal medicines.

Digitalis species, *Rauvolfia serpentine* (L.) Benth. ex Kurz. (Apocynaceae), *Atropa belladonna* and *Strychnos nux vomica* L. (Strychnaceae), among others, are toxic plants but they are useful therapeutic agents when used appropriately and when administered in suitable doses. On the other hand, there are medicinal

plants that persistently evoke moderate to severe reactions, and should not be employed in any medical therapy. Plants including species of *Sebecio*, *Crotalaria*, and *Symphytum*, which contain pyrrolizidine alkaloids having an unsaturated 1,2-double bond in the pyrrolizidine ring, should be avoided due to the hepatotoxic effect of these compounds (Denham 1996, Fong 2002).

Aristolochia species are examples of plants containing toxic chemical constituents that should not be used medically. Aristolochic acid I, found in all species of *Aristolochia* investigated to-date, has been identified as a potent carcinogen and nephrotoxin (Fong 2002, Menges and Stotzem 1993, Menges and Stotzem 1992, Vanherweghem et al. 1993). Renal failure, nephritis, and urinary tract neoplasm have been associated with the use of Chinese and Kampo herbal medicines that contain *Aristolocia* species (Chen 2000, Fong 2002, Hashimoto et al. 1999, Nortier et al. 2000, Vanherweghem et al. 1993).

In 2001, 30 cases of hepatotoxicity, ranging from abnormal liver function to liver failure, associated with the use of kava extracts were reported in Germany and Switzerland. One case was fatal and five others required liver transplants. Although it is difficult to assess causality in these cases as the evidence is complicated by other factors such as the use of concomitant drugs which may also cause liver toxicity. However, by July 2002, the Medicines Control Agency (MCA) in UK had received 68 case reports of hepatotoxicity worldwide, including the UK (Barnes 2003b). The Committee on Safety of Medicines (CSM)

in UK revealed that the benefit-risk profile of kava appeared to be negative and on 13 January 2003, a statutory order came into effect in UK prohibiting the sale, supply and import of unlicensed medicines containing kava (Barnes 2003b). Singapore has also banned the sale of kava products.

1.1.3 Interactions with conventional drugs

In recent years, it has become increasingly clear that even therapeutically safe herbs can manifest toxic effects as a result of herb-drug interaction, when administered concomitantly with synthetic pharmaceutical agents. Since 1999, evidence emerged of pharmacokinetic interactions between St John's wort (*Hypericum perforatum*) products and certain drugs, including anticancer agent (imatinib and irinotecan), anti-HIV (e.g indinavir, lamivudine and nevirapine), anti-inflammatory agents (e.g ibuprofen and fexofenadine), antimicrobial agents (e.g erythromycin and voriconazole), cardiovascular drugs (e.g digoxin, ivabradine, warfarin, verapamil, nifedipine and talinolol), central nervous system agents (e.g amitriptyline, buspirone, phenytoin, methadone, midazolam, alprazolam, and sertraline), hypoglycaemic agents (e.g tolbutamide and gliclazide), immuno-modulating agents (e.g cyclosporine and tacrolimus), oral contraceptives, proton pump inhibitor (omeprazole), respiratory system agent (theophylline), statins (e.g atorvastatin and pravastatin) (Di et al. 2008, Zhou et al. 2004, Barnes et al. 2001a). Both pharmacokinetic and pharmacodynamic components play a role in the interactions of drugs with St John's wort (Di et al.

2008). As St John's wort is a potent inducer of cytochrome P450s (e.g CYP2C9 and 3A4) and P-glycoprotein (P-gp), it is not surprise that many drugs that interact with St John's wort are substrates of CYP3A4, CYP2C9 and P-gp. The combined use of St John's wort with such drugs may enhance or reduce the bioavailability of the drugs. Interactions leading to synergistic therapeutic effects may lead to unfavorable toxicities and complicate the dosing regimen of long-term medications. Antagonistic interactions will result in decreased efficacy and therapeutic failure (Di et al. 2008, Zhou et al. 2004).

Panax ginseng has been reported to interact with phenelzine and other monoamine inhibitors causing central nervous system (CNS) stimulation (Chan 2003, Jones and Runikis 1987). The anticoagulant effect of warfarin was decreased when *Panax ginseng* was also taken simultaneously (Chan 2003, Janetzky and Morreale 1997). Digoxin levels were elevated in a patient who took a preparation labeled as Siberian ginseng, *Eleutherococcus senticosus*, but there was no sign of toxic effects (Chan 2003, McRae 1999). Danshen (*Salvia miltiorrhiza*) affected both the pharmacodynamics and pharmacokinetics of warfarin in rats (Chan 2003, Lo et al. 1992). Danshen prolonged the prothrombin time of warfarin, an indicator of anti-coagulation in the rat (Lo et al. 1992) ; increased the bioavailability and decreased the elimination of warfarin in the rat (Chan et al. 1995) ; inhibited CYP1A1, CYP2C6 and CYP2C11-mediated warfarin metabolism both in vitro and in vivo in the rats (Wu and Yeung 2010). Danggui (*Angelica sinensis*) affected the pharmacodynamics but not the

pharmacokinetics of warfarin in rabbits (Chan 2003, Lo et al. 1995). There was no significant variation in the single dose pharmacokinetic parameters of warfarin were observed after Danggui treatment. However, prothrombin time of warfarin was significantly lowered after 3 days co-treatment with Danggui (Lo et al. 1995).

Drug-herb interaction must be considered and monitored to promote a safe integration of efficacious herbal medicine into conventional medical practices. With a worldwide rise in the use of herbal preparations, more clinical data regarding herb-drug interactions are needed and herbs should be properly labeled to alert consumers of the potential interactions when concomitantly used with drugs.

1.1.4 Standardization

The therapeutic components of plants vary depending on the part of the plant used, age, geographic area where the plant is grown processing methods, and storage conditions. Therefore, batch-to-batch and manufacturer-to-manufacturer variation in preparations of the same herb will occur. With increased awareness in quality control of herbal medicines, some manufacturers now produce standardized herbal extracts as an approach to ensure batch-to-batch consistency. With standardization, products will contain a specific quantity of active constituent(s) and in some cases, unwanted or toxic constituents are removed. For example, standardized extracts of *Ginkgo biloba* contain 22-27% ginkgo flavonoid

glycosides, 5-7% terpene lactones, and less than 5 parts per million of ginkgolic acids, which are known to be allergenic (Barnes 2003a).

Although standardization is an important step for quality control, it is a challenge for many herbs whereby their active constituents are not known. In these cases, products may be standardized based on the content of certain "marker" compounds (chemicals characteristic of the herb, or present in large quantities). However, this approach assumes that the unknown active constituents are standardized by standardizing the "marker" compounds (Barnes 2003a).

Specifications for the quantities of the marker compounds for some herbal medicinal ingredients are set out in pharmacopoeias such as the United States Pharmacopoeia (USP 2010), the British Pharmacopoeia (BP 2010) and Chinese Pharmacopoeia (Committee of National Pharmacopeia of PR China 2010), which are recognized as the official compendia in their respective countries. WHO has also published guidelines on the quality of herbal medicines on Good Manufacturing Practices for herbal medicines (WHO 2007) and on Good Agricultural and Collection Practices (GACP) for medicinal plants (WHO 2003), and on quality control methods for medicinal plant materials (WHO 1998).

1.1.5 Contamination

Contamination of herbal medicines with high level of heavy metals, pesticides residues and micro-organisms are of safety concern. Heavy metal contamination can occur at the cultivation, post-harvest treatment, or product manufacturing stages. A review article (Koh and Woo 2000) reported excessive toxic heavy metals in Chinese proprietary medicines (CPM) in Singapore between 1990 and 1997. Lead, arsenic and mercury have also been detected in CPM using ICP-MS and atomic absorption spectroscopy (Au et al. 2000). Heavy metals contamination in herbal medicinal plants and products were reported by Gasser and Street (Gasser et al. 2009, Street et al. 2008). Heavy metals may be intentionally added to products within specific traditional health paradigms such as Ayurveda (Ernst 2002, Cooper et al. 2007, Jordan et al. 2010, Saper et al. 2008) and contamination may occur through inadequate quality control.

Microbial contamination can occur during the collection and the processing of ingredients or finished products. Microbial species may be introduced due to poor quality control or hygiene practices (Jordan et al. 2010, Sagoo et al. 2009). Microbial contamination of herbal medicines including the health implications has been reviewed (Guédon et al. 2007a). The potential for microbial contamination of herbs may be increased by the use of manures in agriculture, including those which may contain toxic strains of *Escherichia coli*. The drying of herbs shortly after harvest will lessen the potential of the growth of

microorganisms (WHO 2003). Fungal attack of plants can introduce mycotoxins in herbal medicines (Guédon et al. 2007b, Roy et al. 1988).

A relatively limited number of reports exist about the presence of pathogenic microorganisms in herbal plants. Czech et al. has screened 138 medicinal herbal drugs for a broad spectrum of pathogens and indicator germs. It was shown that these microorganisms are relatively rarely found, with exceptions of *Bacillus cereus* and *Clostridium perfringens*. However, these two spore-formers usually do not present in magnitudes representing a real toxicity potential (Czech et al. 2001). Moulds like *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Cladosporium* and *Aeruobasidium spp.* were reported to be found quite often in herbal drugs (Kneifel et al. 2002). Considerable risk levels of aflatoxins were detected in several herbal medicinal samples of different taxa (Kneifel et al. 2002). Findings revealed that environmental conditions (climate, humidity, hygiene etc) largely contributed to the microbial contamination problem (Kneifel et al. 2002).

1.1.6 Adulteration

One of the greatest safety concerns is the adulteration of herbal medicines with undeclared pharmaceutical drugs or their analogues, in illicit attempts to evade detection. It represents another problem in product quality and is one of the major causes for adverse events. In Taiwan, a large scale effort was initiated in 1992 to screen traditional Chinese medicines that were suspected of adulteration with

synthetic therapeutic substances (Huang et al. 1997). A total of 2,609 samples were collected from eight major general hospitals. Samples were collected through physicians' referrals during patient visits. The study revealed that 23.7% of the samples collected were adulterated. More than half (52.8%) of the adulterated traditional Chinese medicines contained two or more adulterants.

Herbal products for weight loss, erectile dysfunction and sexual performance enhancement are considered high risk products and are commonly found to be adulterated. Cases of weight loss products adulterated with sibutramine and fenfluramine were reported (Corns and Metcalfe 2002, Jung et al. 2006). In Singapore, a 42-year-old female developed fulminant hepatic failure in 2002 after consuming a weight-reducing herbal product "Slim 10" containing fenfluramine, nitrosofenfluramine and thyroid gland extract over a period of approximately 4 months (Lau et al. 2004). In Japan, there were 12 cases of acute liver injury associated with the use of weight-reducing herbal products "Chaso" and "Onshido", which were also found to be adulterated with nitrosofenfluramine. Two patients developed fulminant hepatic failure. One died and the other underwent a liver transplant (Adachi et al. 2001). The danger of consuming adulterated herbal products is obvious.

The adulteration of health products for the treatment of sexual dysfunction and to improve sexual performance with undeclared pharmaceutical drugs such as PDE-

5 inhibitors and their analogues will be further discussed in detail in Section 1.3 of this Chapter.

1.1.7 ADRs reporting

There is a general misconception that "natural" always means "safe" and that remedies from natural origin are harmless. However, some medicinal plants are inherently toxic. As with all medicines, herbal medicines are expected to have side effects, which may be of an adverse nature. Some adverse events reported in association with herbal products are attributable to problems of poor quality. Major cases of such events are adulteration of herbal products with undeclared other medicines and potent pharmaceutical substances. Adverse events may also arise from the misidentification of medicinal plants, incorrect dosing, errors in the use of herbal medicines both by health-care providers and consumers, interactions with other conventional drugs, and use of products contaminated with potentially hazardous substances, such as toxic metals, pathogenic microorganisms and agrochemical residues (WHO 2004).

Pharmcovigilance for herbal medicines is in its infancy. Generally, there is a lack of clinical trial data for herbal medicines and, in any case, controlled clinical trials have the power only to detect common, acute adverse effects. Adverse events thus far reported in relation to herbal products are frequently attributable either to poor quality or to improper use of herbal medicines, and it is therefore difficult to

distinguish genuine adverse reactions to herbal medicines and products until the cause of such events have been identified (WHO 2004). However, one of the recognized limitations includes the poor quality of some reports, and the difficulty in establishing causality (Barnes 2003b).

Adverse events for herbal medicines are usually under reported, unlike those for conventional drugs. Due to the belief that herbal medicines are natural and safe, consumers may not associate ADRs with their use (De Smet et al. 1997). Furthermore, users of herbal medicines may be reluctant to report ADRs associated with these products to their doctors or pharmacists (Barnes et al. 1998), and some healthcare professionals may be unaware of the ADR reporting scheme (Barnes 2001b). Healthcare professionals may also hesitate to report ADRs as the causality is often difficult to establish.

In recognizing safety monitoring as a critical component of quality control, WHO has upon the request by the members countries, developed the WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems (WHO 2004).

1.2 Regulatory environment for herbal medicines

A regulatory framework for herbal medicines provides consumers greater assurance that there has been an assessment of the safety, quality and efficacy of the products prior to granting of market authorization, and that the product is free from adulteration and within tolerance limits for contaminants. Requirements for Good Manufacturing Practices (GMP) provide a framework for assuring quality. Adverse reaction reporting facilitate the detection of any noxious and unintended responses from marketed health products.

WHO conducted a global survey on the regulations of Traditional Medicine (TM), Complementary/Alternative Medicine (CAM) and Herbal Medicine (HM) (WHO 2005). A total of 141 Member States participated in the survey. The survey revealed that a total of 92 Member States (65%) had laws or regulations for herbal medicines. The survey also indicated that most of the Member States (74%) treated herbal medicines as Over-the-counter medicines (O.T.C), which are allowed to be sold over-the-counter without doctor's prescription. The finding revealed the difference in regulatory status in different countries. In addition, the safety assessment of the herbal medicines is usually based on the demonstrated safe traditional use or reference to documented scientific research on similar products (WHO 2005).

Four different regulatory frameworks, particularly from USA, Europe, China and Singapore are summarized below, to illustrate some common similarities and differences in the regulation of herbal medicines.

1.2.1 Regulatory control of herbal medicines in USA

Herbal medicines and supplements are regulated by US FDA under a category called “dietary supplements”. In recognition of vast consumer usage of dietary supplements, the US Congress passed the Dietary Supplement Health and Education Act (DSHEA) in 1994. The US Congress defined dietary supplements as products taken by mouth that contains "dietary ingredients" intended to supplement the diet (US FDA 2010a). The "dietary ingredients" in these products may include vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites. Dietary supplements can also be extracts or concentrates, and may be found in many forms such as tablets, capsules, softgels, gelcaps, liquids, or powders. They can also be in other forms, such as a bar, but if they are, information on their label must not represent the product as a conventional food or a sole item of a meal or diet. Whatever their form may be, DSHEA places dietary supplements in a special category under the general umbrella of "food", not drugs, and requires that every supplement be labeled a “dietary supplement” (US FDA 2010a). The US FDA regulates dietary supplements like food and under a different set of regulations

from those concerning the conventional prescription and OTC drugs (US FDA 2010a).

Under the DSHEA, the dietary supplement manufacturer is responsible for ensuring that a dietary supplement is safe (free from dangerous contaminants, adulterants or unsafe ingredients) and product label information is truthful and not misleading before it is marketed. There is no provision in the law for FDA to "approve" dietary supplements for safety or effectiveness before they reach the consumer. Hence, manufacturers do not need to register their products with FDA nor get FDA approval before producing or selling dietary supplements. Except in the case of a new dietary ingredient (a dietary ingredient which was not sold in the U.S. in a dietary supplement before October 15, 1994), where pre-market review for safety data and other information is required by law, the manufacturer has to notify and provide FDA with information, including any citation to published articles, which is the basis on which the manufacturer has concluded that a dietary supplement containing such dietary ingredient will reasonably be expected to be safe under the conditions of use recommended or suggested in the label of the product (US FDA 2010b). US FDA is responsible for taking action against any unsafe dietary supplement product after it reaches the market. FDA will have to show that a dietary supplement is "unsafe" before it can take action to restrict the product's use or removal from the market (US FDA 2010b).

Manufacturers will need to register themselves pursuant to the Bioterrorism Act with FDA before producing or selling supplements. In June, 2007, FDA published comprehensive regulations for Current Good Manufacturing Practices for those who manufacture, package or hold dietary supplement products (US FDA 2010d). These regulations focus on practices that ensure the identity, purity, quality, strength and composition of dietary supplements.

FDA's post-marketing responsibilities include monitoring safety, e.g. voluntary dietary supplement adverse event reporting, and product information, such as labeling, claims, package inserts, and accompanying literature. The Federal Trade Commission regulates dietary supplement advertising.

FDA regulations also require that certain information appear on dietary supplement labels. Information that must be on a dietary supplement label includes: a descriptive name of the product stating that it is a "supplement;" the name and place of business of the manufacturer, packer, or distributor; a complete list of ingredients; and the net contents of the product. In addition, each dietary supplement (except for some small volume products or those produced by eligible small businesses) must have nutrition labeling in the form of a "Supplement Facts" panel. This label must identify each dietary ingredient contained in the product.

1.2.2 Regulatory control of herbal medicines in Europe

In Europe, herbal medicines are considered a category of products used for therapeutic purposes that are derived from plants and plant materials. Herbal medicines are generally sold as food supplements and a common regulatory status in the various European countries does not exist. In March 2004, the European Directive 2004/24/EC on Traditional Herbal Medicinal Products (THMP) was released by the European Parliament and Council of Europe (Gioacchino 2008, Silano 2004). This directive is the basis of regulation for the future use of herbal medicines in Europe. The full application of the Directive in Europe is scheduled 7 years after its release, and is currently in force.

The Directive establishes that herbal medicines released in the market need authorization by the national regulatory authorities of each European country and these products must have a recognized level of safety and efficacy. (Gioacchino 2008). The safety of herbal medicinal products will be evaluated on the basis of existing scientific literature (data from clinical studies, case reports, pre-clinical studies). The Directive also classified herbal medicines into two categories : (i) well established herbal medicinal products ; and (ii) traditional use herbal medicinal products. It requires the traditional use medicinal products to demonstrate at least 30 years of safe traditional use, including 15 years in the European Community (Dobos et al. 2005, Gioacchino 2008). Taking into consideration the long medicinal use of these traditional products in the European

Community, only a special simplified registration is required. This is based on specific standards of safety and quality, agreed indications based on traditional usage, and systematic patient information allowing the safe use of the product (Gioacchino 2008, Jordan et al. 2010).

The Directive also requires that herbal products are produced according to GMP to ensure the quality of the product and also demonstrate safety and that these products should carry indications for use that are limited to minor medical conditions (Dobos et al. 2005).

A new committee, the Herbal Medicinal Products Committee (HMPC), has been formed inside the European Medicines Agency (EMA) with responsibility for herbal medicinal products. HMPC members are generally but not exclusively nominated by the national regulatory agencies. HMPC prepares and releases documents useful to the implementation of the Directive such as list of herbal substances, preparations and combinations for use in traditional medicinal products and herbal monographs that provide scientific summary of all data available on the safety and efficacy of a herbal preparation intended for medicinal use. Information such as clinical indications, posology, method of administration, contraindications, precautions, interactions etc is included (Gioacchino 2008).

The goal of harmonizing the legislative framework for market authorization of herbal medicines in Europe will help to contribute to the quality and safer use of

herbal medicines. However, the THMP Directive has led to a widespread industry opposition amongst herbal producers, practitioners and the public. The European Herbal Practitioners Association (EHPA) had submitted their response to the EMEA Consultation held on 30 September 2005, pointing out that the quality control guidelines proposed to be applied to traditional herbal medicinal products were unworkable for multi-herbal preparations and the costs of implementation of the Directive may see the demise of many small to medium sized herbal suppliers and manufacturers. In addition, the Directive discriminated against non-European Herbal Traditions by requiring at least 15 out of 30 years of usage within the European Community, as the basis for proving long established, traditional usage. This provision was deemed to seriously disadvantage Ayurveda, Tibetan and Traditional Chinese Medicines (EHPA 2005). Two non-government organizations, the Alliance for Natural Health International (ANH-Intl) and the European Benefyt Foundation (EBF) developed a joint position paper, stating a range of concerted actions which include improvement of the food supplement regime, judicial review of the European Directive 2004/24/EC on THMP and facilitation of a new regulatory framework for traditional medicinal products (Verkerk and Dhaenens 2010). An online petition was also called by ANH-Intl to challenge the THMP Directive (ANH-Intl 2011).

A forum was organized by members of Greens/European Free Alliance on 21 Jun 2011 in the European Parliament to seek clarification on THMP Directive and the future of herbal medicines within European. The European Commission (EC)

argued in the forum that the drug registration system offered by the EU herbal directive provides no additional obstacles for products of non-European traditions, as compared with European ones. In addition, THMP Directive applies only to medicinal products, it does not regulate holistic traditions. Despite the efforts of clarification, participants were disappointed as they felt that their questions were not clearly addressed and explained in details. ANH-Intl issued an open letter to EC three days after the forum to demand for immediate response and actions to revise the THMP Directive. It also plans to file a legal challenge at the High Court in London (ANH-Intl 2011).

1.2.3 Regulatory control of herbal medicine in China

Herbal medicines in China are classified into 2 groups: functional foods and drugs. Each differs in its applications, approval standards, production management, labeling, specifications, advertising, and overall supervision. There have been no comprehensive laws or regulations in China that standardize the manufacturing, processing, and marketing of functional foods such as health foods or supplements, except some aspects of these foods have been regulated in the Provisional Law of the People's Republic of China on Food Hygiene (Liu and Salmon 2010).

Crude traditional Chinese medicinal materials (plants, animal parts and minerals) and Chinese proprietary medicines (CPM) (the final dosage forms) are classified as drugs and the Drug Administration law requires these drugs to be evaluated for safety and efficacy. Drugs will have to be produced according to GMP standards, distributed and stored according to good distribution and storage practices (Liu and Salmon 2010). It is worthwhile to note that in China, synthetic drugs in CPM are allowed if the information is made known to the health authorities at the time of registration and is indicated in the package insert, subject to approval (Koh and Woo 2000).

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The Pharmacopoeia of the People's Republic of China defines the country's drug standards. The 2010 edition contains 3 volumes. Volume I includes monographs for crude traditional Chinese medicinal materials, Chinese crude drug preparations (single ingredients) and Chinese proprietary medicines. Volume II deals with monographs of chemical drugs, antibiotics, biochemical preparations, radiopharmaceuticals and excipients for pharmaceutical use. Volume III contains monographs for biological products (Committee of National Pharmacopeia of PR China 2010). Volume I of the 2010 edition includes 2165 monographs, of which 1019 are new entries compared with the previous 2005 edition. These monographs describe the sources, identification methods, and preparation processes for the traditional Chinese medicinal materials and proprietary medicines along with their utilization and major indications for use, dosages, and cautions (Committee of National Pharmacopeia of PR China 2010).

China has integrated herbal medicines into its primary health care system and is exploring new ways to bring the ancient practice of Chinese medicine in line with modern standards (Liu and Salmon 2010). The crucial issues relating to the safety of the herbal medicines in China are monitoring and enforcement (Liu and Salmon 2010).

1.2.4 Regulatory control of herbal medicines in Singapore

In Singapore, there are national regulations on herbal medicines in Singapore. A subgroup of herbal remedies is the Chinese proprietary medicines (CPM), which is defined as any medicinal product in any dosage form used in the system of therapeutics according to the traditional Chinese method (The Statutes of the Republic of Singapore, 2005).

The legislations governing the control of CPM in Singapore includes the Medicines Act and its subsidiary legislations; the Poisons Act and its rules; the Medicines (Advertisement and Sales) Act and its legislations; and the Sale of Drugs Act and its regulations (The Statutes of the Republic of Singapore, 1985a, 1985b, 1985c, 1999). In future, the regulation of health products, defined as any substance, preparation and device intended for use by human principally for health related purpose, will be governed by the Health Products Act (HSA 2011).

This will include drugs, medical devices, cosmetics and complementary medicines. Currently only medical devices and cosmetics are controlled under the Health Products Act. The control of CPM has been implemented since 1 September 1999 to promote safety and quality of CPM available in Singapore. Only products which meet the required standards can be listed by the CPM Unit, Health Product Regulation Group, Health Sciences Authority (HSA) and allowed to be manufactured, imported, supplied or sold (Yee et al. 2005, Koh and Woo 2000).

Under the regulatory framework, CPM importers, wholesalers, manufacturers and re-packers must be licensed and CPM products assessed by the health authority before they are allowed for sale. CPM must comply with the legal permissible limits for mercury, arsenic, lead and copper, which are set at 0.5, 5, 20 and 150 ppm respectively. The microbial contamination is also controlled : total aerobic microbial count, no more than 10^5 per g or ml; yeast and mould count, no more than 5 x 10^2 per g or ml; and *Escherichia coli*, *Salmonellae*, *Staphylococcus aureus*, absent in 1 g or ml. Manufacturers or distributors must also ensure that CPM is free from prohibited toxic phytochemicals and drug substances controlled under the Poisons Act (Chapter 234) (HSA 2011, Yee et al. 2005, Koh and Woo 2000).

The labels, packaging and package inserts of CPM shall not make references to any of the 19 serious diseases or medical conditions (blindness, cancer, cataract,

drug addiction, deafness, diabetes, epilepsy or fits, hypertension, insanity, kidney diseases, leprosy, menstrual disorders, paralysis, tuberculosis, sexual function, infertility, impotency, frigidity and conception and pregnancy) specified in the First Schedule to the Medicines Act (HSA 2011, Yee et al. 2005, Koh and Woo 2000).

There are currently no registration requirements for herbal medicines and none are included on a national essential drug list. However, a listing system has been established for CPM products. The post-marketing surveillance system for all herbal medicines has included adverse-effect monitoring since 1993. There are no restrictions on sale of herbal medicines, as long as they comply with the national regulations (WHO 2005, Yee et al. 2005, Koh and Woo 2000,).

1.3 An Emerging Threat : Adulteration of herbal medicines with PDE-5 Inhibitors and their related analogues

The market success of the three approved synthetic phosphodiesterase type-5 (PDE-5) inhibitors for the treatment of erectile dysfunction has led to the development of a large market for herbal medicines claimed to be natural alternatives to these synthetic drugs. As mandatory requirements regulating the manufacture and sale of herbal medicines are much less stringent than those related to pharmaceuticals, these herbal sexual enhancement products are heavily advertised on the internet and are freely available for purchase without prescription. Furthermore, adulteration of these supposedly natural products is a very common and serious phenomenon. These adulterated products are usually characterized by wildly exaggerated claims and sold to the public by unscrupulous manufacturers, without evidence of safety or effectiveness. The adulteration has also extended to the analogues of the three approved synthetic PDE-5 inhibitors and this is an emerging threat to public safety. Table 1.2 shows the list of reports on herbal health products, claimed to be useful for sexual performance enhancement and treatment of erectile dysfunction, that were found adulterated with PDE-5 inhibitors, related analogues and other synthetic drug substances from the year 2002 based on the literature searches using PubMed and SciFinder Scholar.

As shown in Table 1.2, there is an increase in reports on sexual performance enhancement health products adulterated with PDE-5 inhibitors and their related analogues over the years. The first PDE-5 inhibitor analogue, homosildenafil was reported by Shin in 2003 (Shin et al. 2003) and since then, the list of PDE-5 inhibitor analogues found in herbal health products continues to grow. Creating drug analogues for unregistered use is an old problem. For example, nitroso-fenfluramine, an analogue of the antiobesity drug, fenfluramine, has been found adulterated in slimming supplements resulting in several acute liver injury cases and one death each in Singapore and Japan (Adachi et al. 2001, Lau et al. 2004). PDE-5 inhibitor analogues are merely new comers. It is believed that adulteration of a health product with a drug analogue instead of the parent compound amounts to an attempt to evade regulatory inspection. Since analogues are structurally modified from the parent drugs, these analogues may escape detection by the routine laboratory screening methods. It is important for the regulator to recognize this emerging threat so as to introduce effective surveillance system and control measures to detect them.

More recently, Venhuis has reported a new analogue, a nitrosated prodrug of the PDE-5 inhibitor aildenafil, in a dietary supplement (Venhuis et al. 2011). The presence of the nitrosamine moiety may generate nitrite oxide and enhance the vasodilating effect of a PDE-5 inhibitor with the risk of a dangerous drop in blood pressure (Oliver et al. 2009, Webb et al. 1999). In addition, nitrosamines are also known carcinogens. Consumption of nitrosamines poses a health risk.

It is also worthwhile to note the outbreak of severe hypoglycaemia in 2008 associated with the consumption of several sexual illegal performance health products reported in both Singapore and Hong Kong (Lim et al. 2009, Poon et al. 2009). A total of 10 deaths and 3 deaths were reported in Singapore and Hong Kong respectively. The presence of glibenclamide in such sexual performance enhancement health products was puzzling. It is speculated that glibenclamide was wrongly used during the manufacturing process, suggesting the lack of stringent good manufacturing practice and inadequate quality control on the part of the manufacturer.

The proliferation of the natural sexual enhancement health products, emerging threat of adulteration with PDE-5 inhibitors and analogues, and the poor quality control by the manufacturers warrant immediate attention and action from health authorities in different countries. Prompt education of the public, which must include warnings to avoid sexual performance enhancement health products of dubious origin will help to reduce the demand of illegal health products. Information sharing and co-operation among regulatory bodies will also be necessary to trace their ultimate source and eliminate these illegal products. Finally, enhancing the testing capabilities of regulatory laboratories in screening and structural elucidation of unknown drug adulterants will help in the early detection and regulatory control of these potentially lethal products.

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011)

No.	Year	Adulterants detected	Type of herbal products	Uses	References
1	2002	Sildenafil	Herbal medicine capsules from China	Male tonic, sexual dysfunction	Ku et al. 2002
2	2002	Sildenafil	A soft drink collected from Nagoya City (Japan) markets by Aichi prefecture drugs inspectors in 2001	Roborant nutrition	Mikami et al. 2002
3	2002	Sildenafil	Pink and cream capsule with dark brown powder dietary supplement in Taiwan	Male tonic, sexual dysfunction	Tseng and Lin 2002
4	2002	Sildenafil	Thirteen herbal medicine samples collected by State Food and Drug Administration (SFDA) of China	Male tonic, sexual dysfunction	Zhang et al. 2002
5	2003	Homosildenafil	An imported miscellaneous beverage submitted for inspection in Korea	Male tonic, sexual dysfunction	Shin et al. 2003
6	2004	Acetildenafil (a.k.a : hongdenafil)	A commercial herbal drink submitted to Korea Food and Drug Administration (KFDA) for testing	Male tonic, sexual dysfunction	Shin et al. 2004
7	2004	Acetildenafil, homosildenafil and hydroxyhomosildenafil	Three traditional Chinese medicine samples submitted by the Dutch Food and Consumer Product safety Authority and Dutch Health Care Inspectorate	Male tonic, sexual dysfunction	Blok-Tip et al. 2004
8	2004	Sildenafil, tadalafil and homosildenafil	Nineteen herbal samples collected in US market by US FDA	Sexual Performance enhancement	Gratz et al. 2004

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011) (Cont.)

No.	Year	Adulterants detected	Type of botanical health products	Uses	References
9	2005	Homosildenafil and acetildenafil	Two dietary supplements in Japan	Male tonic	Takako et al. 2005
10	2005	Sildenafil	Two dietary supplements Actra-Rx and YiliShen sold on websites	Sexual performance enhancement	US FDA 2005
11	2005	Sildenafil and tadalafil	Two herbal products purchased via the internet or at Canadian health food stores	Male tonic, treatment of sexual dysfunction	Fleshner et al. 2005
12	2005	Vardenafil	One herbal medicine in oral liquid formulation purchased in a supermarket	Male tonic, sexual dysfunction	Zhu et al. 2005
13	2006	Acetildenafil	A dietary supplement in Taiwan market	Treatment of erectile dysfunction	Lai et al. 2006
14	2006	Hydroxyacetildenafil	One dietary supplement submitted to HSA of Singapore for testing	Sexual performance enhancement	Hou et al. 2006
15	2006	Aminotadalafil and hydroxyhomosildenafil	One herbal medicine submitted to HSA of Singapore for testing	Sexual performance enhancement	Zou et al. 2006a
16	2006	Sildenafil, tadalafil, vardenafil, acetildenafil, homosildenafil and hydroxyhomosildenafil	Pre-mixed bulk powder submitted to HSA of Singapore for testing	Sexual performance enhancement	Zou et al. 2006b

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011) (Cont.)

No.	Year	Adulterants detected	Type of botanical health products	Uses	References
17	2006	Piperidenafil (a.k.a : Pseudovardenafil)	An herbal dietary supplement purchased on the internet.	Sexual performance enhancement	Reepmeyer and Woodruff 2006
18	2006	Acetildenafil, homosildenafil and hydroxyhomosildenafil	Two herbal supplements submitted to HAS of Singapore for testing	Sexual performance enhancement	Oh et al. 2006
19	2006	Sildenafil	Herbal supplements purchased by SFDA of China	Improving both male and female sexual function	Liang et al. 2006
20	2006	Sildenafil and tadalafil	Three marketed herbal products	Sexual performance enhancement	Abdel-Hamid 2006
21	2006	Sildenafil and tadalafil	Four herbal products in the market of Saudi Arabia	Nourish the body and fortify the male sexual function	Bogusz et al. 2006
22	2006	Aminotadalafil, piperidino acetildenafil (a.k.a : Piperiacetildenafil), hydroxyacetildenafil and Piperidino vardenafil (a.k.a : Piperidenafil, Pseudovardenafil)	Several “all-natural” herbal products	Sexual performance enhancement	Gratz et al. 2006

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011) (Cont.)

No.	Year	Adulterants detected	Type of botanical health products	Uses	References
23	2006	Sildenafil, hydroxyhomosildenafil and yohimbine	Two herbal food supplements in Taiwan market	Sexual performance enhancement	Lin et al. 2006
24	2007	Pseudovardenafil and hydroxyacetildenafil	Two dietary supplement in South Korea market	Sexual performance enhancement	Park et al. 2007
25	2007	Acetildenafil, piperidenafil, hydroxyhomosildenafil, hydroxyacetildenafil	Twenty-six dietary supplements bought in Hong Kong market	Male tonic, treatment of sexual dysfunction	Poon et al. 2007
26	2007	Noracetildenafil	An herbal dietary supplement submitted to US FDA for testing	Male tonic, treatment of sexual dysfunction	Reepmeyer and Woodruff 2007a
27	2007	Methisosildenafil (a.k.a : Aidenafil)	An herbal dietary supplement submitted to US FDA for testing	Sexual performance enhancement	Reepmeyer et al. 2007b
28	2007	Imidazosagatriazinone	A dietary supplement in Taiwan market	Treatment of erectile dysfunction	Lai et al. 2007a
29	2007	Piperidenafil (a.k.a : Pseudovardenafil)	A dietary supplement in Taiwan market	Treatment of erectile dysfunction	Lai et al. 2007b
30	2007	Sildenafil	Chinese herbal/patent medicines collected from New York City's Chinatown	Male tonic	Miller and Stripp 2007

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011) (Cont.)

No.	Year	Adulterants detected	Type of botanical health products	Uses	References
31	2007	Desmethylacetildenafil	A herbal product	Male tonic	Zou et al. 2007
32	2007	Thiosildenafil, piperidenafil	"True Man Sexual Energy", "Energy Max"	Sexual performance enhancement	US FDA 2007
33	2008	Thioquinapiperifil, thiosildenafil	One dietary supplement in Japan	Sexual performance enhancement	Uchiyama et al. 2008
34	2008	A tadalafil analogue, aminotadalafil and hydroxyhomosildenafil	One dietary supplement purchased on the internet	Male tonic, treatment of sexual dysfunction	Hasegawa et al. 2008
35	2008	Aminotadalafil and its stereoisomers	A health food in Japan	Male tonic	Kurita et al. 2008
36	2008	Imidazosagatriazinone	A new herbal health product purchased in Hong Kong market	Sexual performance enhancement	Lam et al. 2008
37	2008	Thiosildenafil and thiohomosildenafil	Two health supplements submitted to HSA of Singapore for analysis	Sexual performance enhancement	Zou et al. 2008a
38	2008	Xanthoanthrafil (a.k.a : Benzamidenafil)	A natural herbal supplement sent to HSA of Singapore for testing	Sexual performance enhancement	Zou et al. 2008b

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011) (Cont.)

No.	Year	Adulterants detected	Type of botanical health products	Uses	References
40	2008	Gendeafil	Dietary supplements in Taiwan	Energy enforcement	Lin et al. 2008
41	2008	Hydroxyvardenafil and methisosildenafil	A health food seized by KFDA	Male tonic	Choi et al. 2008
42	2008	Homosildenafil and thiohomosildenafil	Marihuana sample in the Netherlands	Male tonic	Venhuis and de Kaste 2008b
43	2008	Acetildenafil, piperidenafil and hydroxyacetildenafil	Three herbal aphrodisiacs	Sexual performance enhancement	Venhuis et al. 2008a
44	2008	Thiohomosildenafil	A herbal aphrodisiac	Sexual performance enhancement	Venhuis et al. 2008c
45	2008	Xanthoanthrafil	A dietary supplement in Japanese market	Male tonic	Kumasaka et al. 2008
46	2008	Hydroxyhomosildenafil	A dietary supplement tested by US FDA	Treatment of erectile dysfunction	US FDA 2008
47	2009	Sildenafil and tadalafil	Twenty-five food products and herbal preparations	Sexual performance enhancement	Man et al. 2009
48	2009	Sildenafil, homosildenafil, tadalafil, thiosildenafil, piperidenafil, methisosildenafil, thiomethisosildenafil	Twenty-six herbal products	Sexual performance enhancement	Gryniewicz et al. 2009

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011) (Cont.)

No.	Year	Adulterants detected	Type of botanical health products	Uses	References
49	2009	Hydroxythiohomosildenafil (a.k.a : thiohydroxyhomosildenafil)	Two dietary health supplements bought on-line	Sexual performance enhancement	Li et al. 2009a
50	2009	Dapoxetine	A health supplement submitted to HSA of Singapore for testing	Sexual performance enhancement	Li et al. 2009b
51	2009	Sulfoaildenafil (a.k.a : thiomethisosildenafil)	A bulk material, labeled as an ingredient for a dietary supplement, submitted to US FDA for testing	Sexual performance enhancement	Gratz et al. 2009
52	2009	Sildenafil, tadalafil, vardenafil, hydroxyhomosildenafil and thiomethisosildenafil	Seventeen commercial formulations of herbal drugs or dietary supplements	Treatment of sexual dysfunction	Balayssac et al. 2009
53	2009	Sildenafil and vardenafil	Five different cosmetic creams obtained from Internet websites	Treatment of male erectile dysfunction, for male and female genitals stimulation	De Orsi et al. 2009
54	2009	Thiohydroxyhomosildenafil (a.k.a : hydroxythiohomosildenafil) and hydroxyhomosildenafil	A herbal dietary supplement acquired from a distributor	Sexual performance enhancement	Reepmeyer and D'Avignon 2009b

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011) (Cont.)

No.	Year	Adulterants detected	Type of botanical health products	Uses	References
55	2009	Thiosildenafil and thiomethisosildenafil (a.k.a : sulfoaildenafil)	Herbal aphrodisiacs appropriated from distributors' warehouses	Male tonic, treatment of sexual dysfunction	Reepmeyer and D'Avignon 2009a
56	2009	Cyclopentynafil and *N*-Octylnortadalafil	A dietary supplement in Japanese market	Treatment of erectile dysfunction	Hasegawa et al. 2009
57	2009	Sildenafil and glibenclamide	Twenty-five sexual enhancement products obtained from patients	Sexual performance enhancement	Poon et al. 2009
58	2009	Sildenafil and glibenclamide	An illegal sexual enhancement, Power 1 walnut, in Singapore market	Sexual performance enhancement	Lim et al. 2009
59	2009	Sildenafil, tadalafil, vardenafil,piperidenafil, acetil acid, thiosildenafil, thiohomosildenafil, glibenclamide, naproxen, chloramphenicol, sibutramine and lidocaine	Hundred and seventy five illegal sexual enhancement health products from seven raids conducted by HSA of Singapore.	Sexual performance enhancement	Low et al. 2009
60	2010	A tadalafil analogue	A health food product submitted to HSA of Singapore for testing	Male tonic	Haberli et al. 2010
61	2010	Sildenafil, tadalafil, vardenafil and yohimbine	Twenty-six natural dietary supplements bought at Changsha, China	For male sexual health	Zhang et al. 2010

Table 1.1 Undeclared synthetic drugs found in herbal products to treat sexual dysfunction or to enhance sexual performance (2002 – 2011) (Cont.)

No.	Year	Adulterants detected	Type of botanical health products	Uses	References
62	2010	Acetil-acid	A commercial herbal supplement in Hong Kong market	Sexual performance enhancement	Ng et al. 2010
63	2010	Hydroxythiohomosildenafil	"Magic Power coffee" tested by US FDA	Male tonic	US FDA 2010c
64	2010	Sildenafil	85 herbal formulations purchased from different cities of India	Sexual performance enhancement	Savaliya et al. 2010
65	2011	Acetylvardenafil	A dietary supplement, MEGATON, imported from USA to Incheon Airport in South Korea	Carbohydrate metabolism and energy production	Lee et al. 2011
66	2011	Nitroso-prodenafil	A herbal dietary supplement tested by National Institute for Public Health and the Environment, The Netherlands	Libido enhancement	Venhuis et al. 2011

Chapter 2

Rationale and Objectives

Herbal remedies promising to enhance sexual performance or for treatment of erectile dysfunction have been promoted over the years. These products are usually characterized by widely exaggerated claims and sold to the public by unscrupulous manufacturers, without evidence of safety and effectiveness. Consumers might have been lured into buying them because the social stigma attached to their problem makes them shy away from talking to the doctors. Many of these products are marketed as health supplements. The mandatory requirements regulating the manufacture and sale of these products are much less stringent than those related to medicinal products. Adulterated products are less commonly found among herbal products classified as Chinese Proprietary Medicines (CPM) in Singapore as they require pre-marketing approvals (in the form of product listing) and safety assessment which include screening for contaminants and adulterants. For health supplements, no pre-marketing approval is currently needed.

To protect the safety and health of consumers from the emerging threat of adulteration with western drugs and their analogues, more stringent and effective control of such high risk herbal products is needed. Health authorities must have effective legislations, quality and safety assessment and monitoring, which

include the testing of these herbal health products for inherent toxic ingredients, contaminants and adulteration.

To enhance the testing capability of herbal medicines in the national quality control laboratory, the Health Sciences Authority (HSA) in Singapore has collaborated with the Department of Pharmacy, National University of Singapore on this study with the objectives to :

(1) assess the quality and safety of sexual enhancement herbal health products illegally sold in the red-light districts in Singapore. The assessment will create greater public awareness of the danger in consuming such illegal health products and hence help to reduce the demand of such products ;

(2) develop a sensitive and rapid LC-Hybrid MS Tandem MS method to simultaneously screen for PDE-5 inhibitors and their analogues in sexual enhancement herbal supplements. The developed method will help to enhance the screening capability of the regulatory laboratory in the detection of synthetic PDE-5 inhibitors and their analogues present in herbal supplements ;

(3) detect, isolate and structurally elucidate an unknown adulterant in a health product claimed for female sexual performance enhancement, which was sent to HSA for pre-market testing. The study allows the regulatory

laboratory to develop its capability in structural elucidation of unknown adulterants and the findings will allow the regulator to take appropriate legal actions against the supplier of the product.

Chapter 3

Safety and quality assessment of 247 illegal sexual performance enhancement products seized by Health Sciences Authority in Singapore

3.1 Introduction

Erectile dysfunction (ED) is a common and important medical condition. Erectile dysfunction is defined as the consistent inability to obtain or maintain an erection for satisfactory sexual relations. More than 18 million men in United States over age 20 are affected by ED (Selvin et al. 2007). The prevalence of ED was strongly linked to age, cardiovascular disease, diabetes and a lack of physical activity. The U.S. Food and Drug Administration (USFDA) approved phosphodiesterase Type 5 enzyme (PDE-5) inhibitor drugs to treat ED are Sildenafil (Viagra®), Tadalafil (Cialis®) and Vardenafil (Levitra®). Despite the wide use and effectiveness of these drugs, clinically significant adverse side effects such as headaches, facial flushing and visual disorders, cardiovascular events etc have been reported. Habek and Petravić have reported a case with multiple strokes in the posterior cerebral circulation in close temporal association with regular sildenafil use (Habek and Petravic 2006). Spontaneous post-marketing reports of adverse visual events, including nonarteritic anterior ischemic optic neuropathy (NAION) and cardiovascular events associated with the use of PDE-5 inhibitor have also been

reported (Gorkin et al. 2006, Kontaras et al. 2008, Laties and Sharlip 2006, Laties 2009, Sobel and Reynolds 2008, Wooltorton 2006). Significant hypotension can be found in patients who are concurrently taking nitrates with PDE-5 inhibitor (Reffelmann et al. 2008). Associations between sildenafil and neurologic, emotional, or psychological disturbances, amnesia or loss of consciousness or aggressive behavior have also been reported (Milman and Arnold 2002).

The advent of these highly successfully drugs has spurred the marketing of herbal dietary supplements as natural alternatives for the enhancement of sexual performance. The growing trend for consumers to turn to natural herbal treatment and supplements may be attributed to the assumption that "natural means safe". However, this is not necessarily true. Factors affecting the safety of such products include intrinsic toxicity, adulteration, substitution, contamination, misidentification, lack of standardization, incorrect preparation and/or dosage and inappropriate labeling. Adulteration of health products with synthetic PDE-5 inhibitors and their analogues with claims to enhance sexual performance has been reported (Blok-Tip et al. 2004, Bogusz et al. 2006, Hasegawa et al. 2009, Lam et al. 2008, Oh et al. 2006, Reepmeyer and Woodruff 2006, Shin et al. 2003, Zou et al. 2006a, Zou et al. 2008a). More recently, four illegal sexual performance enhancement products have been reported to be adulterated with sildenafil and a very high dose of glibenclamide (HSA 2008b). These products have caused severe hypoglycaemia leading to 10 deaths as of 30 Oct 2008 (HSA 2008a, 2008c).

In this study, we report the findings on the screening of 247 illegal sexual enhancement health products for western drug adulterants. These products are claimed to be natural supplements for enhancement of sexual performance and they were seized by Health Sciences Authority from make-shift stalls at the red-light district areas in Singapore and Singapore Customs during the period February to December 2008.

3.2 Objective

The objective of this work is to evaluate the extent of adulteration of health products with synthetic PDE-5 inhibitors and other drugs, and the risks of consuming such illegal sexual enhancement products.

3.3 Experimental

3.3.1 Materials

All chemicals used were analytical grade or better. Acetonitrile and methanol (HPLC grade) were purchased from LAB-SCAN. Water for HPLC was treated with a PURELAB Ultra water purification system (ELGA). Sildenafil citrate, piperidenafil, acetil acid, sulfosildenafil and sulfohomosildenafil were supplied by

TLC PharmaChem., Inc. (Vaughan, Ontario Canada). Tadalafil was supplied by Eli Lilly Company (Indianapolis, IN, USA). Vardenafil hydrochloride was supplied by Bayer Corporation (West Haven, CT, USA). Lignocaine was supplied by Sigma (Sigma-Aldrich, USA). Sibutramine hydrochloride monohydrate was supplied by Abbott Laboratories (Abbott Park, Illinois, USA). Chloramphenicol, glibenclamide and naproxen were supplied by United State Pharmacopeia (USP, Rockville, USA).

3.3.2 Samples and standards preparation

A total of 247 illegal sexual performance enhancement health products that were claimed to be natural supplements for the enhancement of sexual performance were seized by Health Sciences Authority from 7 major raids that involved 17 make-shift stalls at the red-light districts in Singapore and a shipment to Singapore through Singapore Customs during the period February to December 2008. The products were screened for the presence of western drug adulterants.

For sample preparation, 10 ml of methanol was added to 1 g of the powdered sample. For liquid sample, 1 g of the liquid was diluted with 10 ml of methanol. The powdered or diluted sample was sonicated for 10 minutes and filtered through 0.45 μm membrane filter for preliminary screening by reversed phase high-performance liquid chromatography (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS) with in-house and commercial mass spectral

libraries, namely Wiley7 (John Wiley & Sons Pte Ltd., United States) and Nist05 (National Institute of Standards and Technology, United States).

Confirmation of adulterants was performed by liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS). The sonicated sample was further diluted from 10 to 1000 times with methanol, depending on the concentration level of the adulterants and filtered through 0.45 μm membrane filter for direct injection.

Quantitative analysis was performed using the above high-performance liquid chromatography (HPLC-DAD) system. 10 ml of methanol was directly added to about 0.25 g of the homogenized powdered or liquid sample. The powdered or diluted sample was sonicated for 10 minutes and filtered through 0.45 μm membrane filter for quantitation analysis. Standard solutions (0.0125 mg/ml, 0.025 mg/ml, 0.05 mg/ml, 0.125 mg/ml and 0.25 mg/ml) of the adulterants detected were prepared for calibration purposes. Further dilution was performed for those adulterants detected at higher concentrations than their respective standard calibration range.

3.3.3 Screening by HPLC-DAD

A Hewlett Packard (HP) series 1100 quaternary gradient pump, photo-diode array detector and HP series 1100 autosampler (Palo Alto, CA, USA) were used. System control, data acquisition and process, and auto-library search were

performed with an in-house ultraviolet spectra library using HP ChemStation software for LC 3D. A BDS Hypersil reverse phase (RP) C18 200 x 4.6 mm x 5 μm column from Thermo Scientific, United States, was used.

Gradient elution (acetonitrile/phosphate buffer) was performed as follows: Solvent A - sodium dihydrogen phosphate buffer (25 mM, pH 3.2) ; Solvent B – acetonitrile ; step gradient is from 10% to 30% v/v of B over 10 min, then to 50% of B over another 10 min and finally to 70% v/v of B over 10 min and maintained for 5 min. Total chromatographic duration was 35 min. The equilibration time between two consecutive injections was set at 5 min (total cycle time 40 min). The flow-rate of the mobile phase was 1 ml/min. Injection volume was 10 μl. The detection wavelengths were set at 220, 254 and 280 nm. The UV spectra from 200 to 400 nm were recorded on-line during the chromatographic run.

A HSA in-house ultraviolet (UV) spectra library of 420 drug substances was used for the screening test. An autolibrary search for adulterants was conducted. Unknown UV spectra were compared with those UV spectra in the UV spectra library. A library match score of 1000 represents a perfect match.

3.3.4 Screening by GC-MS

HP 6890 series of GC system fitted with HP 6890 series injection and HP 5973 series mass selective detector (Palo Alto, CA, USA) were used in this analysis.

The analytes were separated with a HP-5 MS capillary column (5% phenyl-95% methyl siloxane; 25 m x 0.2 mm internal diameter capillary) with the carrier gas (helium) set at 1 ml min $^{-1}$. A 1.0 µl volume of the sample was injected using the splitless mode. The data acquisition system was controlled by MS ChemStation. Full scan mass spectra were collected between 50 and 550 amu at 1.53 scan s^{-1}. The MS was operated in the electrospray ionization mode. The initial oven temperature was set at 80^0C. It was then increased to 300^0C at 10^0C min^{-1}. The final temperature of 300^0C was held for 10 min. The total running time was 32 min. The Wiley standard chemical MS library (McLafferty et al. 1998) and spectra of reference standards were used in the drug identification.

3.3.5 Confirmation by LC-ESI-MS/MS

Confirmation of the adulterants in the samples was performed by the use of LC-ESI-MS/MS. An API 2000 Triple Quadrupole LC/MS/MS mass spectrometer from MDS Sciex/Applied Biosystems (Foster City, CA, USA) coupled to a Shimadzu LC system (LC-10ADVP, Nakayo-ku, Japan) was used. Data acquisition and processing were performed using the Analyst software from Applied Biosystems (Foster City, CA, USA). Separation was carried out using a BDS Hypersil C18 reversed-phase column (150 mm x 2.1 mm I.D, 5 µm). Gradient elution was performed as follows : Solvent A – 0.1% formic acid in water ; Solvent B – acetonitrile ; step gradient was from 20% to 95% of B over 5 min, held for 2 min, return to 20% of B and maintained for 5 min. The flow rate

of the mobile phase was 0.25 ml/min. The injection volume was 10 μL. The mass spectrometer was coupled with the LC system in splitless mode. An electrospray ionization interface with positive mode was employed. The ESI conditions were as follows : declustering potential, 65 V; focus potential, 400V; entrance potential, 10.0 V; curtain gas, 40 (arbitrary units); collision gas, 7 (arbitrary units) ; ion spray voltage, 4.5 kV; source temperature, 350 ^{0}C; ion source gas 1, 40 (arbitrary units); ion source gas 2, 60 (arbitrary units); and MS full scan range : m/z 50-550. Both quadrupoles were maintained at unit resolution. For MS^2 run, collision energy was set at 50 V for all analyses.

3.3.6 Quantification by LC-DAD

Quantitative analysis was conducted using the above LC-DAD and conditions for all adulterants accept for the analogues of PDE-5 inhibitors (piperidenafil, acetil acid, sulfosildenafil and sulfohomosildenafil). The detector was set at 254 nm.

The previously reported and validated methods (Liu et al. 2001) were used with slight modification. Technical assistance from Dr Zeng Yun in this study is acknowledged.

3.4 Results and Discussion

3.4.1 Safety Assessment

Details of the 247 illegal health products and the chemical analysis results are presented in Appendix I.

The results showed that 190 samples (76.9%) were adulterated with western drugs or their analogues. Among the 190 samples found to contain western drug adulterants, 150 samples contained only 1 adulterant while 40 samples contained more than 1 adulterant. A majority of these samples (165, 86.8%) were found to be adulterated with sildenafil. 22 samples were adulterated with tadalafil (11.6%). 3 samples were found to be adulterated with vardenafil (1.6%) and 42 samples contained other adulterants (22.1%). In addition, 4 analogues of PDE-5 inhibitors were also detected. They were piperidenafil, acetil acid, thiosildenafil and thiohomosildenafil. Figure 3.1 summarized the adulterants detected in the 190 adulterated samples. Six counterfeit drugs of the 3 approved PDE-5 inhibitors, Cialis®, Levitra® and Viagra® in different dosage forms and batches had also been seized and screened for adulterants. Sildenafil was detected in all of these products. Increasing, the counterfeit product labelled as "Cialis® Tadalafil 80 mg" was not found to contain tadalafil. No vardenafil was detected in the products labelled as "Levitra® 20".

92 samples (55.8%) of the products adulterated with sildenafil were found to contain more than 100 mg per dosage unit of sildenafil (Figure 3.2). The therapeutic dosage of sildenafil, Viagra® (Pfizer), for erectile dysfunction is 25 mg to 100 mg taken not more than once per day. Two products, namely "Power 1 Walnut 动力一号核桃素片" (product number 135 in Appendix 1) and "Zhong Hua Niu Bian 中華牛鞭" (product number 244 in Appendix 1) were found to contain high doses of glibenclamide, 98.9 mg per tablet and 13.1 mg per capsule respectively. In addition, 11 of the products (5.8%) were found to contain trace amounts of sibutramine.

3.4.2 Product labels and claims

Twenty of the products screened are targeted for female consumers. Among these products, 3 (15%) were found to contain lignocaine. Unlike those for male consumers, most of these products (13/20, 65%) are in solution or cream form.

Many products are claimed to be extracts of natural herbs or are produced by modern techniques. Many did not state their manufacturing dates. Most of them have simple labels with indications, side effects and dosage. No information on drug interactions and toxicity is provided. Only a few products bear information to advise consumers to consult with a physician before taking the products and some of them offer "antidotes" for side effects: drinking a large volume of cold water. Many products are labelled to be safe for consumers with heart diseases, high blood pressure and with concomitant consumption of alcohol.

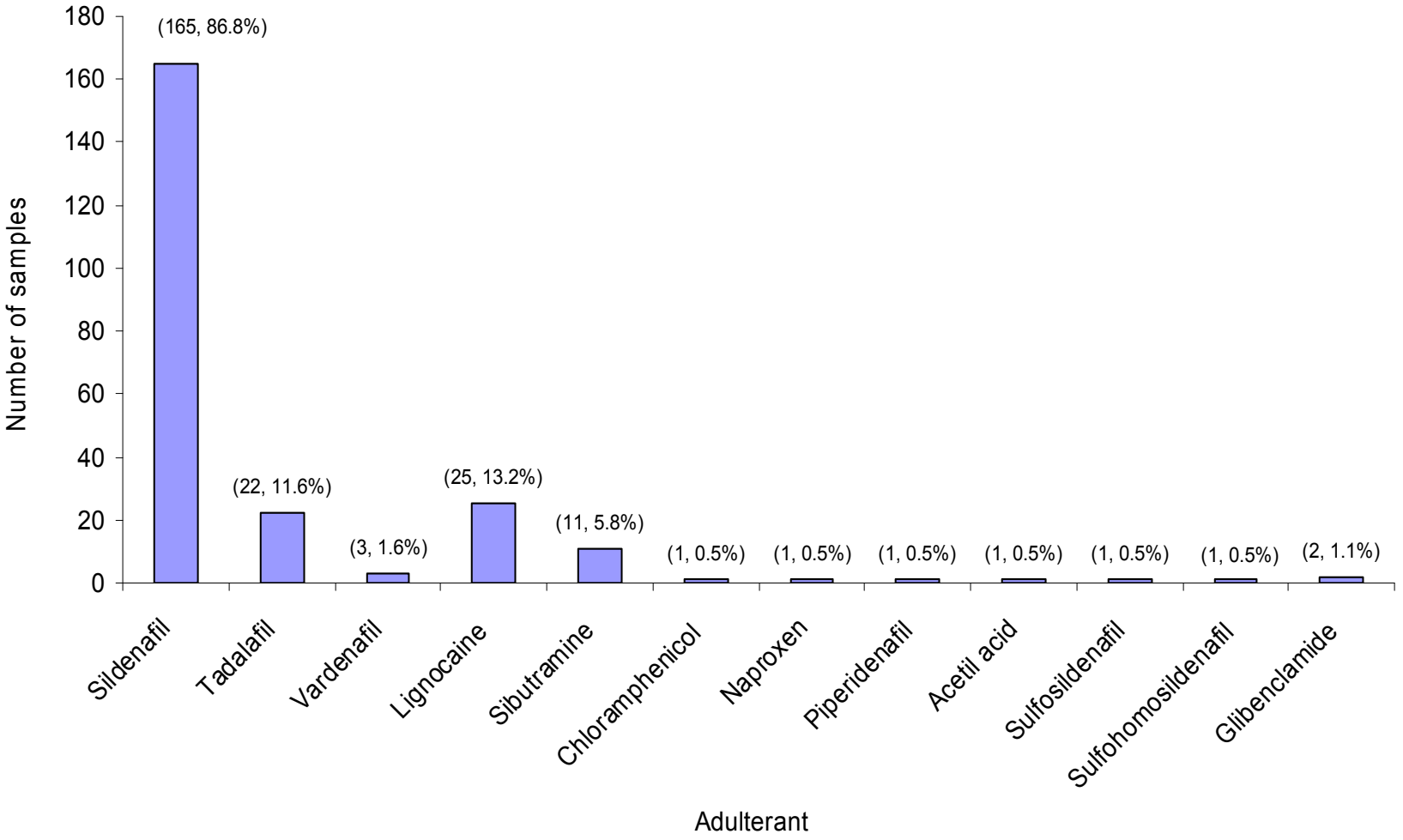


Figure 3.1 No. of times (with percentage) that each adulterant was detected in the 190 samples that were found to be adulterated with western drugs. Some samples contained more than one adulterant

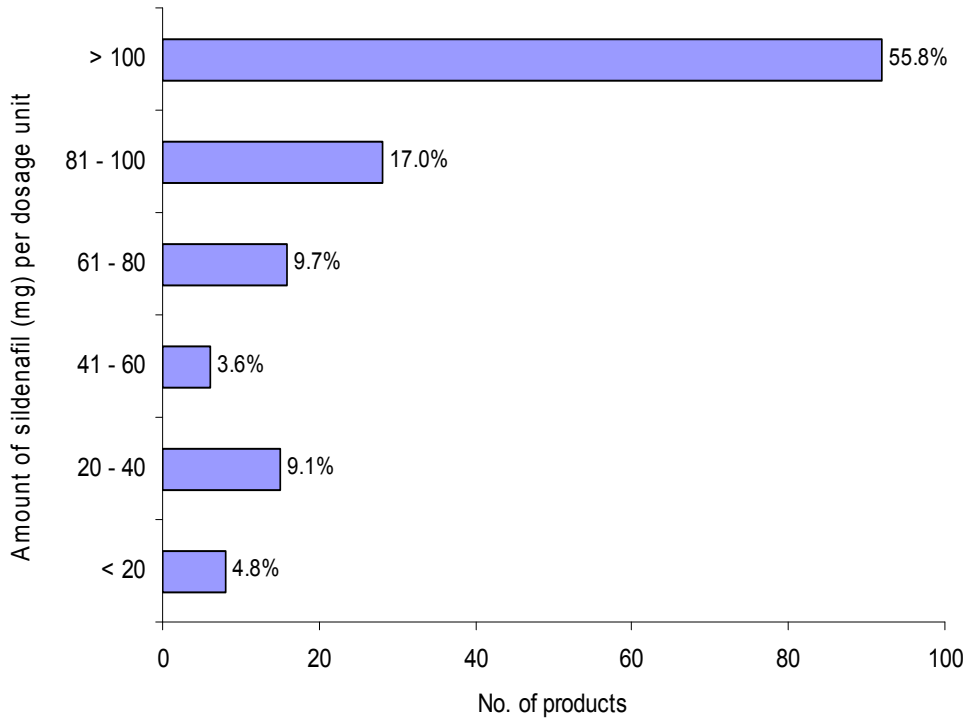


Figure 3.2 Levels of sildenafil detected per dosage unit in the 165 products that were found to be adulterated with sildenafil

3.4.3 Discussion

The easy accessibility and lower pricing of the sildenafil raw material from illegal sources may be one of the reasons for the high incidence of adulteration with sildenafil. Tadalafil and vardenafil were comparatively less encountered as an adulterant in such illegal health products. Sildenafil, tadalafil and vardenafil are inhibitors of PDE-5 and are used as oral therapy for erectile dysfunction (Sweetman 2007). These drugs are rapidly absorbed after oral dose. Peak plasma concentrations are attained within 30 – 120 minutes for sildenafil and vardenafil

and within 2 hours for tadalafil. The terminal half-lives of sildenafil and vardenafil are about 4 – 5 hours while the mean half –life of tadalafil is about 17.5 hours (Sweetman 2007). The much shorter time required to attain peak plasma concentrations may be the reason for the preference of sildenafil over tadalafil as adulterant in illegal health products. In this study, 6 counterfeit products of approved PDE-5 inhibitor drugs were detected. Such counterfeit drugs are poor in quality with ingredients that are different from their label claims.

The presence of prescription drugs in such products posed severe risk to consumer. Consumers taking nitrate medications for hypertension, hyperlipidemia and ischemic heart diseases may experience drastic drop in blood pressure if they consume such illegal health products adulterated with the synthetic PDE-5 inhibitors (Gorkin et al. 2006, Habek and Petravic 2006, Kontaras et al. 2008, Laties and Sharlip 2006, Laties 2009, Milman and Arnold 2002, Nencini et al. 2008, Sobel and Reynolds 2008, Wooltorton 2006). In addition, the high concentration of sildenafil (more than 100 mg per dosage unit) in about half of the illegal health products adulterated with sildenafil is believed to be the approach taken by dishonest manufacturers to ensure the efficacy of the products. However, an overdose of sildenafil and its misuse can be dangerous in view of the various adverse events reported

More alarmingly, the presence of glibenclamide in high doses is puzzling as well as potentially fatal. The levels of glibenclamide were 0.7 to 40 times above its

therapeutic dose of 2.5 – 20 mg for daily treatment of diabetes (Sweetman 2007). Glibenclamide is a sulfonylurea antidiabetic drug. Glibenclamide is readily absorbed from gastrointestinal tract, with peak plasma concentrations usually occurring within 2 to 4 hours, and it is extensively bound to plasma proteins. Glibenclamide has a relatively long duration of action of up to 24 hours and appears to cause severe hypoglycaemia more often than shorter-acting sulfonylureas such as tolbutamide (Sweetman 2007). These 2 products were responsible for the severe hypoglycaemia cases leading to 10 deaths in Singapore as at 30 Oct 2008 (HSA 2008c). Pharmacologically, glibenclamide is not known to improve sexual performance and based on the batch number of the adulterated product, Power 1 Walnut 动力一号核桃素片, together with the period of adverse drug reaction reporting, there is a possibility that glibenclamide was wrongly used during the manufacturing process in Jan 2008. From our analyses, previous batches of "Power 1 Walnut 动力一号核桃素片" manufactured prior to the year 2008 were free from glibenclamide. The drug adulteration resulted in fatality and is likely to be a result of the lack of stringent GMP. GMP prohibits any changes of formulation according to the free will of the manufacturer. Inadequate quality control of the health products will definitely be associated with high incidence of health risks when these products are consumed on a regular basis (Low et.ah 2010).

With regards to product label claims, many of them are incorrect, misleading and potentially dangerous to consumers. The manufacturers should bear the legal

consequences of posting potential danger to the consumers and it is important for National Authorities to collaborate/work together to curb the supply sources. One limitation is that the manufacturers on the labels often do not exist. The presence of lignocaine in female consumer products, largely topical preparations for vaginal use, may be explained by lignocaine's local anaesthetic effect to relieve pain, burning and itchy sensations during sexual intercourse.

For products that were seized from the raids in red light districts, they were openly presented for sale at make-shift stalls. After a couple of raids, the operators started hiding the products along drains, in beer carton boxes etc while some chose to display empty product boxes instead. Some products were hidden in motorcycles and were only retrieved for sale when there were interested customers. The 247 illegal sexual enhancement products were seized from 7 major raids in red light districts and a shipment to Singapore through Singapore Customs. Such illegal sexual enhancement products are available worldwide and it is important to alert potential consumers, health professionals and regulators of the possibility of adulteration, counterfeiting and presence of high levels of sildenafil and glibenclamide.

The data in this report in general may be affected by the narrow region where products were seized - all within the red-light district areas in Singapore and shipments to Singapore. A survey that extends outside of this region or a world-

wide survey may give different results e.g. perhaps not such a domination of adulteration with sildenafil.

3.5 Conclusion

In conclusion, 190 (77%) illegal health products out of 247 products for sexual performance enhancement and treatment of erectile dysfunction seized from red-light district areas in Singapore and shipments to Singapore through the Singapore Customs were found to be adulterated with western drugs and their analogues.

The extent of the adulteration in part explained the effectiveness of these illegal health products in sexual performance enhancement. Hence, with the low price and efficacy, the demand of these products remains high, despite the many severe and sometimes fatal cases of adverse drug reactions reported. It is conceivable that such illegal sexual enhancement products might work in showing their effectiveness of sexual enhancement on the principle of the adulterated drug, and not so on the pharmacognostic nature of the natural herbs. Besides, in the presence of a readily available source with affordable price, the adulterated drug can easily be manipulated in multiple health product manufacturing, which ultimately cause severe and sometimes fatal cases of adverse drug reactions so often reported in the region.

To safeguard public health, greater public awareness of the danger of consuming such illegal products and the lack of quality control of these illegal sexual performance enhancement health products, is important. An effective public education programme will help to reduce the demand of such illegal health products and complement the enforcement actions taken in the control of the supply of such illegal health products in the market. Healthcare professionals, regulators and the media play an important role in effective public education, especially in the outreach to high-risk groups, including foreign workers.

Chapter 4

Screening of PDE-5 Inhibitors and their analogues in sexual performance enhancement health products by Liquid Chromatograph Hybrid Tandem Mass Spectrometer

4.1 Introduction

Sildenafil (Viagra; Pfizer, NY, USA), tadalafil (Cialis; Elli Lilly, IN, USA) and vardenafil (Levitra; Bayer Pharmaceuticals Co., Wuppertal, Germany) are three phosphodiesterase-5 (PDE-5) inhibitors licensed for the treatment of erectile dysfunction (ED) (Gratz et al. 2004). The prevalence of erectile dysfunction, high cost of treatment, sensitivity of the condition, presence of side effects, adverse events and the requirement for medical supervision of the therapy have led to the promotion of herbal supplements as substitutes in enhancing male sexual function, often under the premise that natural products are safer. While there are many such herbal products sold at local stores or over the internet which claim to enhance sexual performance, there have been reports of some "natural" products with synthetic PDE-5 inhibitors and their analogues (Haberli et al. 2010, Lee et al. 2011, Ng et al. 2010, Savaliya et al. 2010, US FDA 2010c, Venhuis et al. 2011, Zhang et al. 2010). Manufacturers of these products usually claimed that the effect of their products came from "purely natural substances". In addition, the

safety of these adulterated products has not been clinically tested and may cause unpredictable health effects on consumers. The challenge for the drug regulatory control agencies to effectively assess and regulate such adulterated products is to have a robust, high-throughput, sensitive and reliable test method to simultaneously screen for the synthetic PDE-5 inhibitors and their analogues adulterated in such herbal supplements.

Since 2004, there were reports of LC-MS and LC-MS-MS screening methods for simultaneous detection of the 3 approved PDE-5 inhibitors, namely sildenafil, tadalafil and vardenafil (Abdel-Hamid 2006, Gratz et al. 2004, Savaliya et al. 2009, Zhang et al. 2010, Zhu et al. 2005). With the emerging threat from analogues of PDE-5 inhibitors, four screening methods were reported to simultaneously detect the PDE-5 inhibitors and their related analogues. However, these methods are restricted to only a few analogues. Zou et al. has developed a LC-ESI-MS/MS method for simultaneous detection of the sildenafil, vardenafil, tadalafil and three related analogues (homosildenafil, hydroxyhomosildenafil and acetildenafil) (Zou et al. 2006b). Balayssac et al. has developed a 2D and 3D DOSY 1H NMR method to screen for sildenafil, tadalafil, vardenafil and three related analogues (hydroxyhomosildenafil, thiosildenafil and thiomethisosildenafil) (Balayssac et al. 2009). Gryniewicz et al. has developed an ion mobility spectrometry (IMS) method to screen for sildenafil, tadalafil, vardenafil and five related analogues (methisosildenafil, homosildenafil, piperidenafil, thiosildenafil and thiomethisosildenafil) (Gryniewicz et al. 2009).

Ng et al. has developed an LC-MS-MS screening method based on precursor ion scan to specifically detect analogues of sildenafil, vardenafil and acetildenafil (Ng et al. 2010). This method is however limited to analogues that have the same selected precursor ions for sildenafil, vardenafil and acetildenafil.

In this study, a LC-Hybrid Tandem MS method was developed to simultaneously screen for a total of 25 PDE-5 inhibitors and related analogues in a single run of 20 minutes. Samples were extracted by methanol and directly screened by the system. A multiple reaction monitoring (MRM) as survey scan and an enhanced product ion (EPI) scan as dependent scan were performed in an information-dependent acquisition (IDA) experiment. Finally, drug identification was carried out by library search with a newly developed MS/MS library based on EPI spectra at 3 different collision energies in positive ionization mode. To the best of our knowledge, this is thus far the only method that can provide such comprehensive screening of the PDE-5 inhibitors and related analogues.

4.2 Objective

The objective of this study is to develop a LC-Hybrid Tandem MS method to simultaneously screen for a total of 25 PDE-5 inhibitors and related analogues.

4.3 Experimental

4.3.1 Materials

All chemicals used were analytical grade or better. Acetonitrile and methanol (HPLC grade) were purchased from LAB-SCAN. Water for HPLC was treated with a PURELAB Ultra water purification system (ELGA). Sildenafil citrate, were supplied by TLC PharmaChem., Inc. (Vaughan, Ontario Canada), tadalafil was supplied by Eli Lilly Company (Indianapolis, IN, USA) and vardenafil hydrochloride was supplied by Bayer Corporation (West Haven, CT, USA). The other 22 PDE-5 inhibitors analogues reference standards (acetil-acid, acetildenafil, aminotadalafil, carbodenafil, chloropretadalafil, dimethylsildenafil, gendenafil, homosildenafil, hydroxyacetildenafil, hydroxyhomosildenafil, hydroxythiohomosildenafil, imidazosagatriazinone, N-desethylacetildenafil, N-desmethylsildenafil, normeosildenafil, piperiacetildenafil, thiodimethylsildenafil, thiohomosildenafil, thiosildenafil, udenafil and xanthoanthrafil) were synthesized by TLC PharmaChem., Inc. (Vaughan, Ontario Canada) for this study.

4.3.2 Sample preparation for validation

Eleven validation samples, which were previously tested by HSA, were supplied by HSA and chosen by A/P Koh out of 140 samples as blind samples for the validation study. The details of the blind samples and previous HSA test results,

which were not made known to the analyst prior to the validation, were summarized in Table 4.1. 1 g samples were extracted by 10 ml methanol. The extraction samples were further diluted 500-fold with methanol. The diluted sample solutions were directly screened by the LC-Hybrid Tandem MS System. For cases that showed overloaded signals, the diluted sample solutions were further diluted 20-fold and re-screened by the LC-Hybrid Tandem MS System.

The limit of detection (LOD) for the 25 PDE-5 inhibitors and analogues were determined based on 3 times the signal-to-noise ratio through the injection of their respective 50 ppb standard solutions.

4.3.3 Instrumentation and chromatographic conditions for screening test

The HPLC analysis was performed with an autosampler, column oven and a binary pump (HP1200 series LC, Agilent, Waldbrown, Germany). For the separation, an Dionex Acclaim® 120 C18 RP analytical column (2.1mm x 150mm, 3μm) was used. A gradient starting with 20% mobile phase B (acetonitrile with 0.1% formic acid (v/v) and 0.1% ammonium formate (v/v) was increased linearly to 80% B over 10 min, kept constant at 80% B for 5 min, and then decreased to 20% B in 0.5 min and re-equilibrated for 5 min. Mobile phase A consisted of aqueous solution containing 0.1% formic acid (v/v) and 0.1% ammonium formate (v/v). The flow rate was 0.3 ml/min, injection volume was 5 μl and oven temperature was 30^0C.

For the MS/MS screening, an API QTrap4000 with Cliquid™ Software and a TurboIonSpray source (Applied Biosystems, Ontario, Canada) was used. Infusion of reference standards of the 25 PDE-5 inhibitors and analogues was performed to identify the MRM transition parameters (Q1 and Q3 at optimized DP, EP, CE, CXP). TEM was set at 500^0C with a curtain gas 20psi, nitrogen collision gas (CAD) set to high, GS1 40 psi and GS2 70 psi. The current was 4KV with the interface heater on. The declustering potential (DP), entrance potential (EP), collision energy (CE) and collision cell exit potential (CXP) were optimized for each of the 25 PDE-5 inhibitors and analogues. The survey scan containing 25 MRM transitions for the 25 PDE-5 inhibitors and analogues was summarized in Table 4.2. Each transition was performed with a 50 ms dwell time and a pause time of 2 ms at the optimized CE. Total scan time was 1.3 s for all 25 transitions.

The IDA scan intensity threshold was set to 1000 counts per second (cps). The dependent scan was an EPI scan, which was carried out at 3 different CEs : 20, 35 and 50 eV. Fill time of the trap (Q3) was set to 50 ms, linear ion trapping (LIT) scan rate was 4000 amu/s. Three EPI scans were performed before switching back to MRM mode. The maximum cycle time (MRM + three EPI scans) was 3.4s. Dynamic exclusion, which defines the time for which a transition is excluded after acquiring an EPI scan, was set to 18s to allow the detection of coeluting substances. The resulting EPI spectra were then searched against the newly developed MS/MS library.

4.3.4 MS/MS library

The MS/MS library was developed based on the EPI spectra of the 25 PDE-5 inhibitors and related analogues reference standards at three different CEs (20, 35 and 50 eV) in positive ionization mode. The concentration of the reference standards used was 50 ppb. Mass tolerance and purity threshold for library search were set at 0.50 and 10%.

4.3.5 Confirmation of adulterants

Confirmation test was performed to ascertain the presence of those adulterants suggested by the library of the screening system developed. Confirmation was performed by injecting the reference standards of the suspect adulterants (at concentration similar to the unknown) into the same the LC-Hybrid Tandem MS Screening System. The retention times (RT) and EPI spectra of the reference standards of the suspect adulterants were compared against the retention time (RT) and EPI spectrum of the unknown peak.

Table 4.1 Details of the eleven blind samples for validation study

Sample Code	Product Description	Batch Number	Manufacture	Dosage Form	Ingredients/quantity	Adulterant Detected
1	America Viagia 美国战神	-	海南新贝乐生物科技有限公司	Brown tablet	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子等。	Sildenafil Vardenafil Piperidenafil (trace)
2	Wei Ge 99 偉哥 99	Q/LZB-003-2003	西藏拉萨市力龙生物制品公司	Blue and white capsule with "力龙生物" imprint	虎鞭，海狗鞭，牛鞭，人参，淫羊藿等	Sildenafil
3	Wei Bao Capsules 胃 宝 胶 囊	B102	Yi Shi Yuan Pte Ltd	Transparent capsule with light brown powder	Cortex Cinnamomi, Fructus Amomi Rotundus, Fructus Amomi, Rhizoma Pinelliae Praeparata, Herba Menthae, Pericarpium Citri Retiiculatae, Radix Aucklandiae, Radix Et Rhizome Glycyrrhizae, Rhizoma Cyperi, Flos Caryophylli, Fructus Aurantii Praeparata, Endoconche Sepine, Pericarpium Zanthoyli	ND
4	Qiang Li Wei Ge Wang 强力威哥王	-	-	Blue tablet with "800mg" marking	-	Sildenafil Tadalafil
5	Liu Wei Di Huang Jiao Nang 六 味 地 黄 胶 囊	BD1902090112	SingCo Pharma Pte Ltd	Bright blue/light blue capsule with brown powder	Radix Rehmanniae Praeparata, Cortex Moutan, Poria, Fructus Corni, Rhizoma Dioscoreae, Rhizoma Alismatis, Tartrazine	ND
6	Polygonati Rhizoma Capsule	-	Jiansen Engineering Pte Ltd	Transparent capsule with brown powder	-	Aminotadalafil

Table 4.1 Details of the eleven blind samples for validation study (Cont.)

Sample Code	Product Description	Batch Number	Manufacture	Dosage Form	Ingredients/quantity	Adulterant Detected
7	Zeng Ye Jiao Nang 增 液 胶 囊	DB1212081111	SingCo Pharma Pte Ltd	Purple/black capsule with greyish brown powder	Radix Scrophulariae, Radix Ophiopogonis, Radix Rehmanniae, Tartrazine	ND
8	Nan Gen Jing Hua Su 男根精华素	-	香港金黄金科技开发有限公司	Blue and white capsule	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子，肉桂	Sildenafil
9	XTRABIG	-	Incontech Pte Ltd	Red capsules	-	Dimethylsildenafil, Thiodimethylsildenafil
10	San Ti Niu Bian Bo Dong Li 三體牛鞭勃动力	Q/WS9-95	安徽三体医药保健品公司	Blue and white capsule with "勃动力" imprint	黄牛鞭，鹿茸，人参，五味子，枸杞子，菟丝子，淫羊藿，肉苁蓉，龙眼肉等	Sildenafil
11	Refine Yin Qiao For Cold Capsules (Concentrated) 精 制 银 翘	E903T	Union Chemical & Pharmaceutical Pte Ltd	Dark green/light green capsule with brown powder	Flos Lonicerrae, Fructus Forsythiae, Radix Platycodi, Fructus Arctii, Herba Schizonepetae, Herba Lophatheri, Semen Sojae Preparatum, Mentholx, Radix Glycyrrhizae	ND

Legend :

ND Not Detect

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues

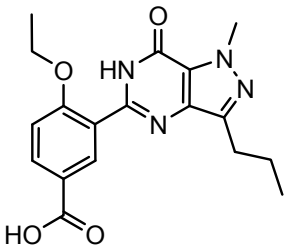
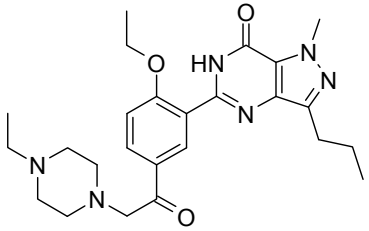
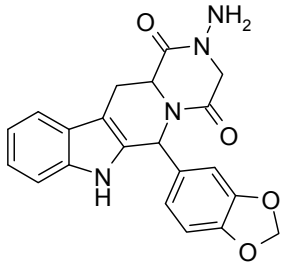
S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
1	Acetil-Acid		$C_{18}H_{20}N_4O_4$	356.1485	7.97	357.2	329.2	93.0	10.0	33.0	8.0	127.1
2	Acetildenafil		$C_{25}H_{34}N_6O_3$	466.2692	5.35	467.4	111.3	135.1	5.0	41.1	6.4	7.4
3	Aminotadalafil		$C_{21}H_{18}N_4O_4$	390.1328	7.19	391.4	269.1	90.0	9.8	18.2	4.8	14.9

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues (Cont.)

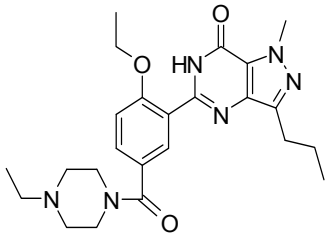
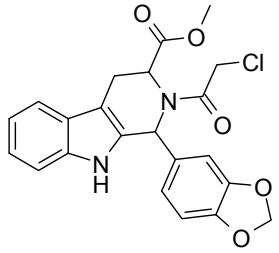
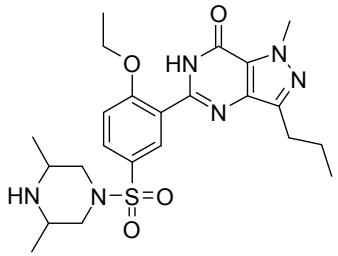
S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
4	Carbodenafil		$C_{24}H_{32}N_6O_3$	452.2536	4.91	453.1	311.1	143.3	6.0	45.2	5.8	8.0
5	Chloropretadalafil		$C_{22}H_{19}ClN_2O_5$	426.0982	10.58	427.1	135.3	115.6	12.1	26.1	7.9	13.1
6	Dimethylsildenafil		$C_{23}H_{32}N_6O_4S$	488.2206	6.20	489.3	99.1	127.2	5.0	55.6	5.4	8.6

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues (Cont.)

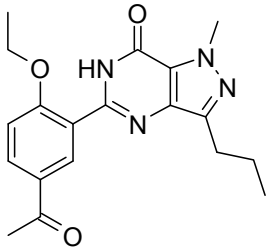
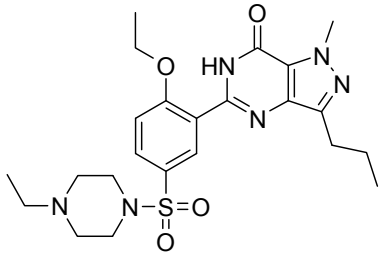
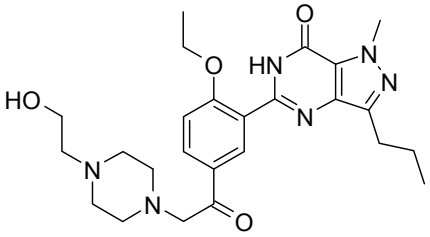
S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
7	Gendenafil		$C_{19}H_{22}N_4O_3$	354.1692	9.62	355.5	285.1	115.8	4.5	40.1	4.8	6.3
8	Homosildenafil		$C_{23}H_{32}N_6O_4S$	488.2206	6.00	489.4	113.0	136.4	4.2	42.2	5.2	14.6
9	Hydroxyacetildenafil		$C_{25}H_{34}N_6O_4$	482.2642	5.01	483.4	127.3	158.5	9.7	42.1	7.9	12.2

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues (Cont.)

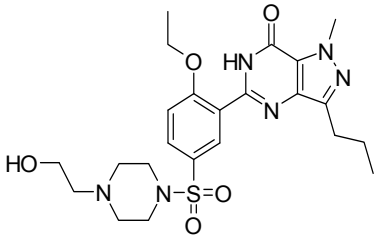
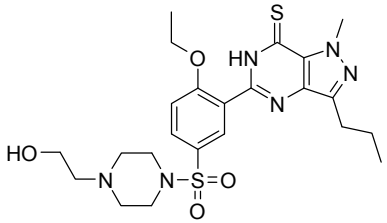
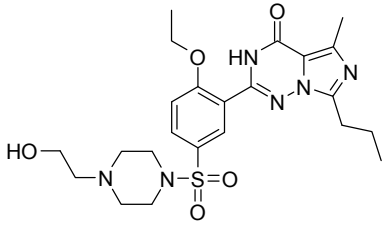
S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
10	Hydroxyhomosildenafil		$C_{23}H_{32}N_6O_5S$	504.2155	5.85	505.1	99.0	131.1	4.3	60.7	2.4	10.5
11	Hydroxythiohomosildenafil		$C_{23}H_{32}N_6O_4S_2$	520.1926	7.78	521.4	129.3	160.2	8.5	43.2	8.3	18.3
12	Hydroxyvardenafil		$C_{23}H_{32}N_6O_5S$	504.2155	4.92	505.4	151.1	135.8	6.3	71.1	9.9	8.9

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues (Cont.)

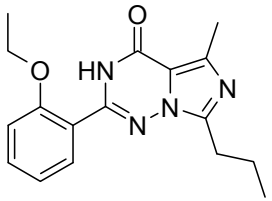
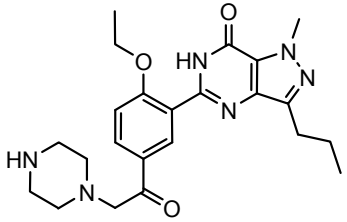
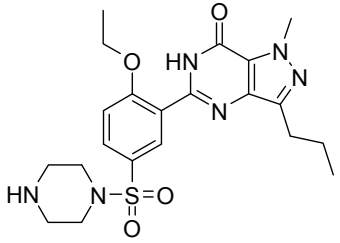
S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
13	Imidazosagatriazinone		$C_{17}H_{20}N_4O_2$	312.1586	11.49	313.4	285.0	116.9	8.4	34.0	5.0	5.2
14	N-Desethylacetildenafil		$C_{23}H_{30}N_6O_3$	438.2479	4.95	439.2	99.1	149.1	8.0	44.0	5.5	5.1
15	N-Desmethylsildenafil		$C_{21}H_{28}N_6O_4S$	460.1893	5.85	461.3	283.3	125.6	6.3	49.1	4.6	7.9

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues (Cont.)

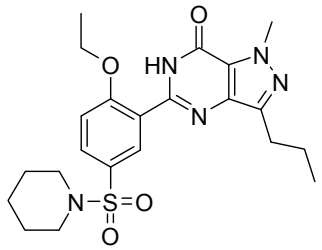
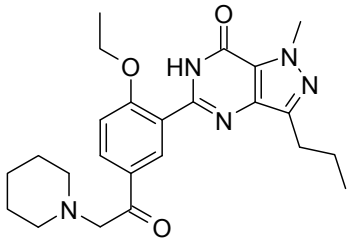
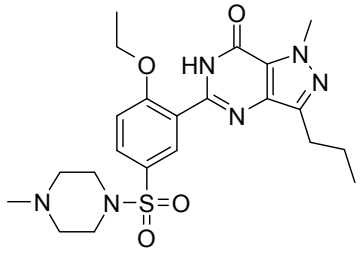
S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
16	Norneosildenafil		$C_{22}H_{29}N_5O_4S$	459.1940	11.66	460.4	283.1	146.9	6.7	46.9	19.8	100
17	Piperiacetildenafil		$C_{24}H_{31}N_5O_3$	437.2427	5.80	437.9	98.2	71.3	7.9	48.1	5.3	8.2
18	Sildenafil		$C_{22}H_{30}N_6O_4S$	474.2049	5.90	475.3	283.4	111.0	5.0	55.0	6.0	10.9

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues (Cont.)

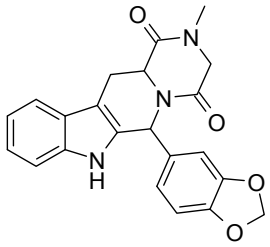
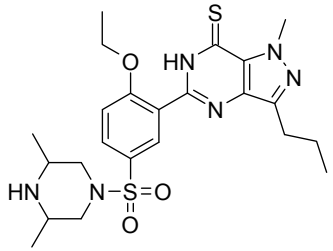
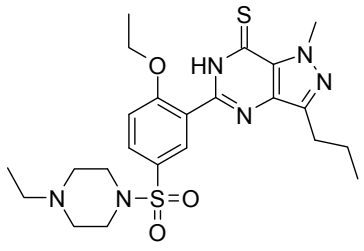
S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
19	Tadalafil		$C_{22}H_{19}N_3O_4$	389.1376	8.00	390.3	267.9	68.0	9.9	16.0	16.0	3.1
20	Thiodimethylsildenafil		$C_{23}H_{32}N_6O_3S_2$	504.1977	8.32	505.1	327.1	121.1	6.8	41.4	6.4	7.3
21	Thiohomosildenafil		$C_{23}H_{32}N_6O_3S_2$	504.1977	8.18	505.4	299.1	109.9	6.4	55.2	5.8	69.4

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues (Cont.)

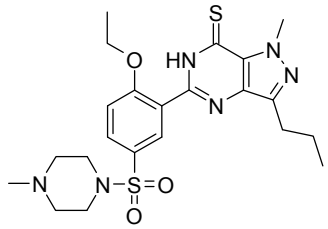
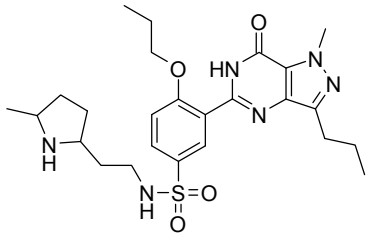
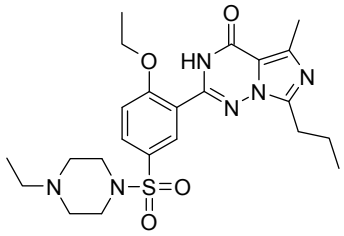
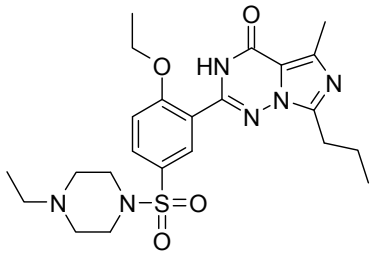
S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
22	Thiosildenafil		$C_{22}H_{30}N_6O_3S_2$	490.1821	7.98	491.3	100.3	124.1	7.6	43.8	5.5	34.6
23	Udenafil		$C_{25}H_{36}N_6O_4S$	516.2519	6.40	517.0	325.3	60.8	8.0	51.7	5.8	5.5
24	Vardenafil		$C_{23}H_{32}N_6O_4S$	488.2206	5.07	489.3	151.2	111.0	6.0	62.0	11.0	14.6

Table 4.2 MRM transitions, infusion data and LOD for PDE-5 inhibitors and their related analogues (Cont.)

S/N	Compound Name	Structure	Compound Formula	MW (amu)	RT (min)	Q1 Mass (amu)	Q3 Mass (amu)	DP (V)	EP (V)	CE (eV)	CXP (V)	LOD (ppm)
25	Xanthoanthrafil		$C_{19}H_{23}N_3O_6$	389.1587	8.24	390.4	150.9	55.5	9.8	18.4	10.7	5.3

Legend :

DP	Declustering potential	CE	Collision energy	EP	Entrance potential	CXP	Collision cell exit potential
Q1	First quadrupole	Q3	Third quadrupole				

4.4 Results and discussion

The analytical procedure is summarized in Figure 4.1. The developed method was successfully applied to the 11 validation samples which were obtained from the HSA investigative cases and selected by A/P Koh. Dynamic exclusion of detected MRM transition was used to detect coeluting compounds. This is especially important for adulteration cases, where unknown analogues with similar structures may be present.

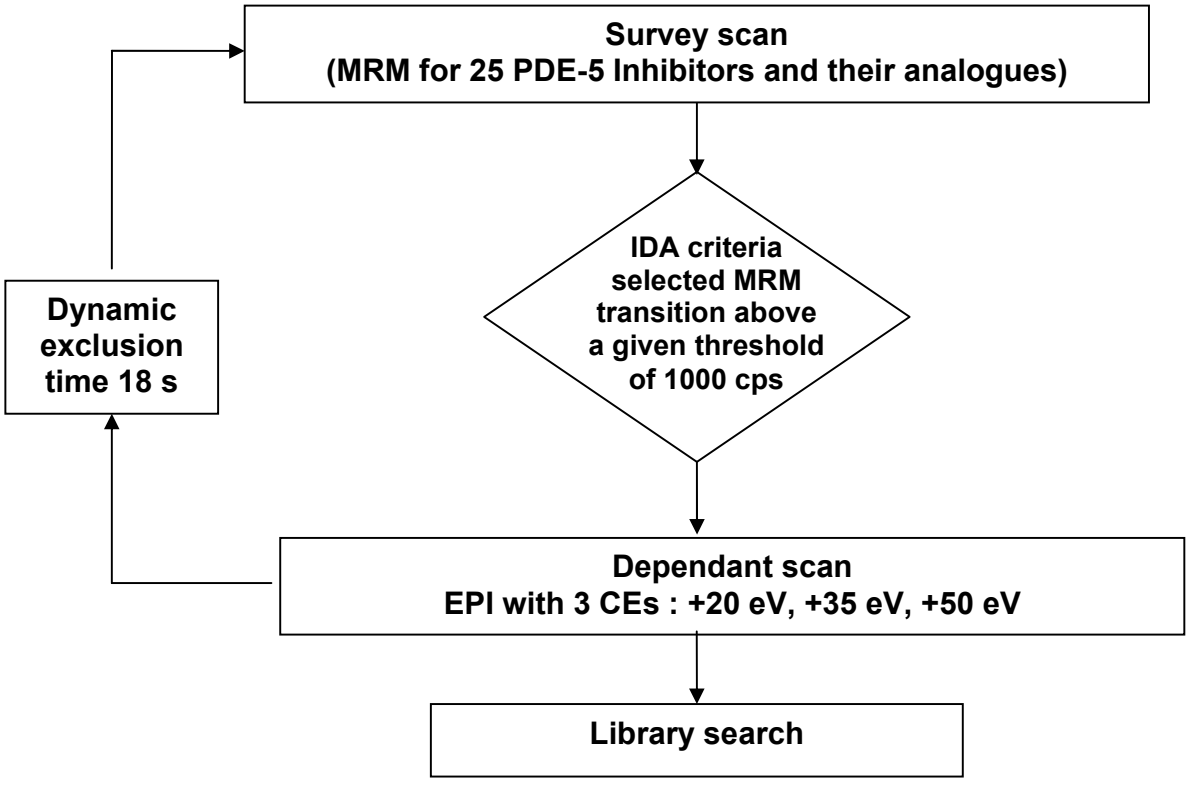


Figure 4.1 Analytical procedure for MS/MS screening

Figure 4.2 shows the total ion chromatogram of the extracted validation sample No. 6 whereby aminotadalafil, an analogue of tadalafil, was detected. A typical library search result in this case for validation sample No.6, is as shown in Figure 4.3. The purity is a measure of the similarity of the library spectrum and the unknown spectrum and it falls in the range 0 to 100 (Stein and Jordon 1994).

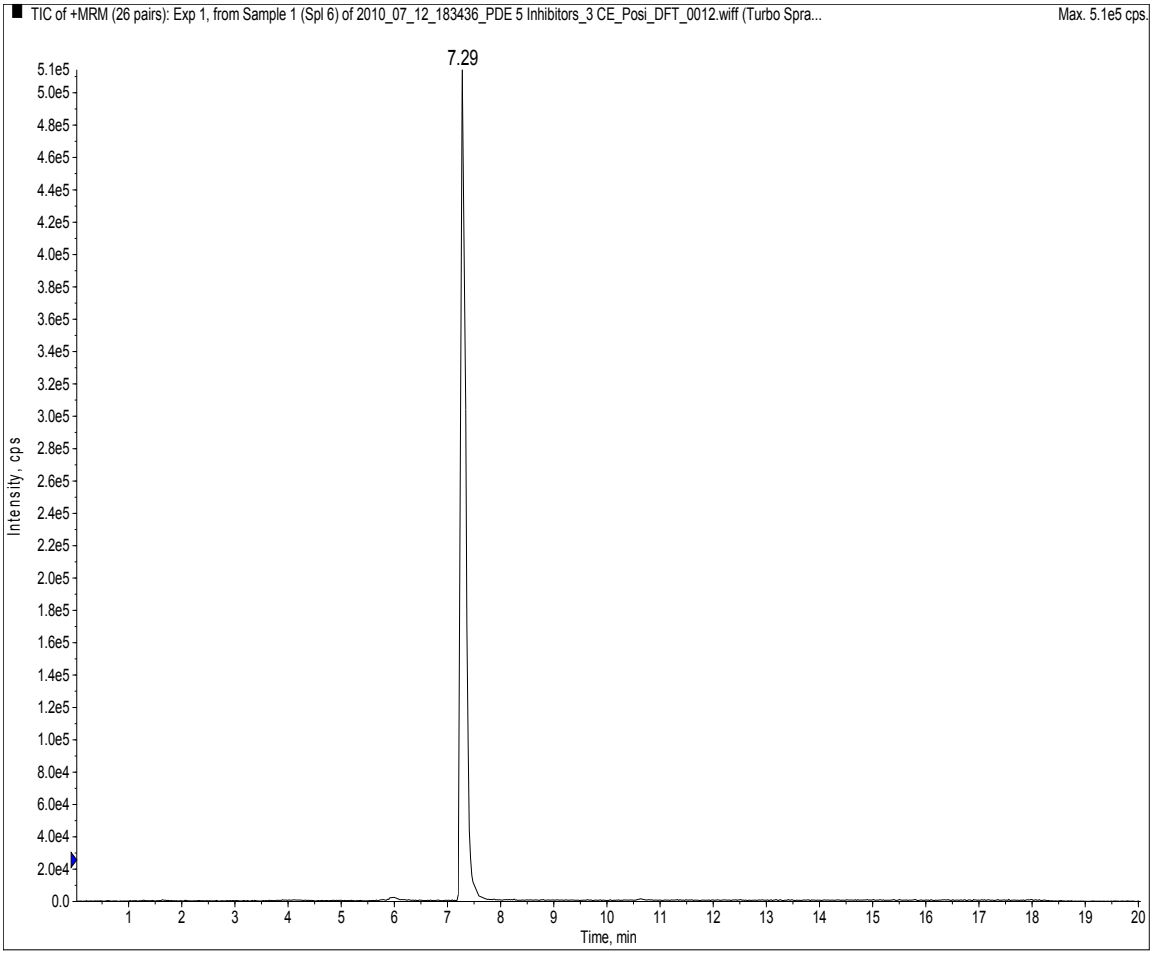
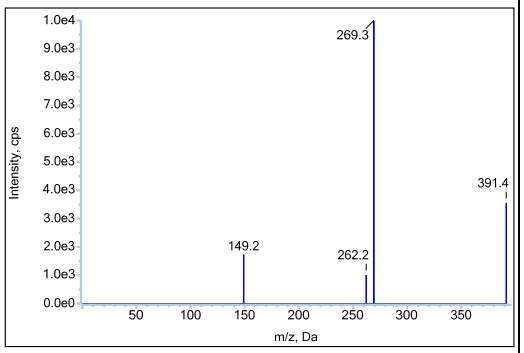
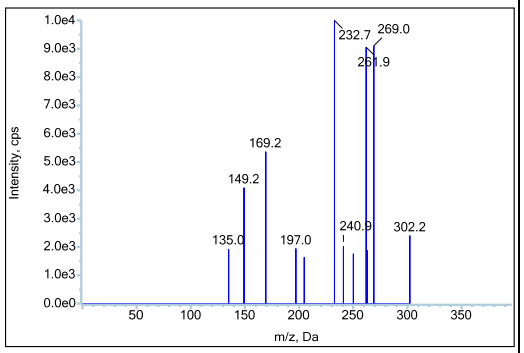
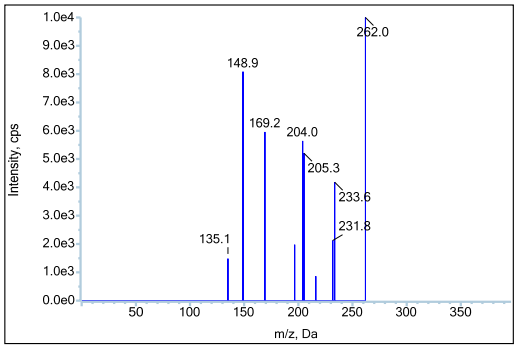
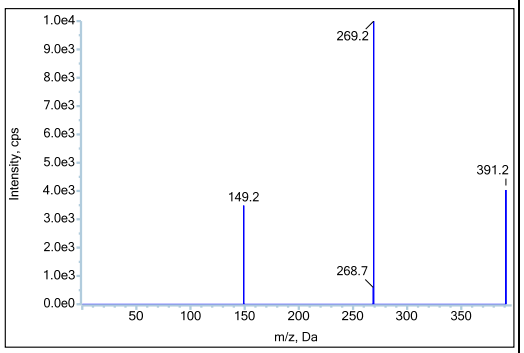
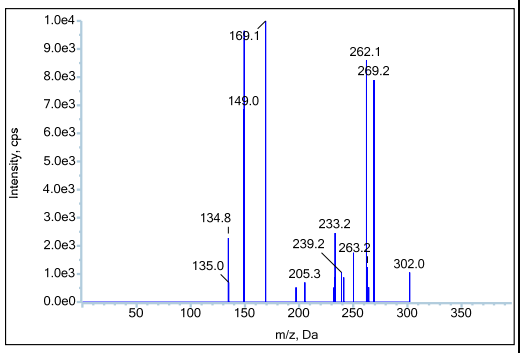
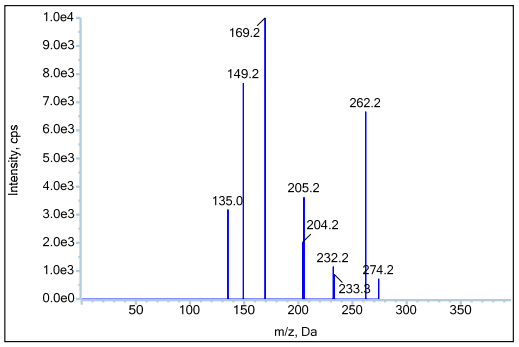


Figure 4.2 Total ion chromatogram : validation sample No.6, aminotadalafil is detected at 7.29 min.

Retention Time: 7.24 minutes **Q1/Q3:** 391.4/269.1 amu **Area:** 127000 counts **Date:** 7/12/2010 11:04:41 PM

	(a) Collision Energy = 20 eV	(b) Collision Energy = 35 eV	(c) Collision Energy = 50 eV
Acquired			
Library			

	Compound Name	Purity (%)	Compound Name	Purity (%)	Compound Name	Purity (%)
1	PDE-5-Inhibitor Analogues_Aminotadalafil	94.3	PDE-5-Inhibitor Analogues_Aminotadalafil	83.7	PDE-5-Inhibitor Analogues_Aminotadalafil	74.3

Figure 4.3 Library search results for validation sample no. 6 - acquired analyte sprectra and library spectra at CE (a) 20 eV, (b) 35 eV and (c) 50 eV showing purity match from library search at 3 different CE.

The LOD of the 25 PDE-5 inhibitors and related analogues based on 3 times the sign-to-noise ratio (ICH 1996) are summarized in Table 4.2.

The validation results are summarized in Table 4.3. The validation results were compared to the HSA previous testing results obtained by LC-DAD screening. The developed LC-Hybrid Tandem MS screening method was able to pick up all the PDE-5 inhibitors and analogues adulterated in these validation samples except for the trace piperidenafil in Sample 1. The purity match for the positive detection at the 3 CEs ranged from 68.2% to 100.00% for all positive adulterants detected except for tadalafil in sample 4, which has a library purity match of 30.7% at CE 50. This was attributed to the matrix interferences from this sample at CE 50, which resulted in low purity match.

For sample 1, trace amount of piperidenafil was previously detected by HSA using LC-DAD screening method. However, for the LC-Hybrid Tandem MS screening system developed, the sample injected to the system is 500 times more dilute than the LC-DAD screening method, in view of the high sensitivity of the LC-Hybrid Tandem MS screening system. Piperidenafil, which is present in trace amount, was not detected after such a dilution. On the other hand, traces of homosildenafil was detected by the new LC-Hybrid Tandem MS screening method and was not detected previously by the LC-DAD screening method. The HSA LC-DAD screening system was unable to detect traces of homosildenafil as the small peak of homosildenafil co-eluted with the big peak of sildenafil.

However, in the LC-Hybrid Tandem MS system, the trace homosildenafil was detected as the mass pairs for sildenafil and homosildenafil are different. This demonstrates the usefulness of the selectivity and specificity of the LC-Hybrid Tandem MS method developed.

For sample 4, trace amount of vardenafil was detected by the LC-Hybrid Tandem MS screening system. The method is more sensitive in detecting vardenafil compared to the LC-DAD screening system.

The validation study revealed some discrepancies in both screening results (LC-Hybrid Tandem MS and LC-DAD) for trace contaminants. This is attributed to the difference in the detection sensitivity of these methods towards the trace contaminants. As the targeted analytes for adulterants screening are usually present at therapeutic level, it is advisable to retain the sample preparation of this study (i.e. 1 g sample to be extracted by 10 ml methanol, follow by 500 times dilution with methanol). Without the 500 times dilution step, there is a risk of system overload and contamination by the main adulterants, which are usually present in large quantities. In addition, severe mass shift due to overloaded signal contributed by the main adulterants may be experienced and this will affect the detection of the adulterants through library search and match. In general, the LC-Hybrid MS screening method is rapid, selective and capable of simultaneous detection of PDE-5 inhibitors and their related analogues adulterated in herbal medicines.

Table 4.3 Validation results for the LC-Hybrid Tandem MS Screening System

Sample Code	RT (min)	Library Search Result									Confirmation Result	Remark
		CE 20			CE 35			CE 50				
		Suggestion	RT (Std)	Purity	Suggestion	RT (Std)	Purity	Suggestion	RT (Std)	Purity		
1	5.11	Homosildenafil	6.16	98.00	Homosildenafil	6.16	92.90	Vardenafil	5.26	82.40	Vardenafil	Same finding as HSA except for piperidenafil in trace was not detected. Additional adulterant, homosildenafil was detected in trace amount, as a result of the higher sensitivity of the LC-Tandem MS screening system.
		Dimethylsildenafil	6.29	97.80	Dimethylsildenafil	6.29	74.80	Homosildenafil	6.16	31.80		
		Vardenafil	5.26	93.50	Vardenafil	5.26	74.80	Dimethylsildenafil	6.29	26.50		
	5.96	Sildenafil	6.00	100.00	Sildenafil	6.00	100.00	Sildenafil	6.00	89.30	Sildenafil	
	6.08	Dimethylsildenafil	6.29	100.00	Dimethylsildenafil	6.29	90.40	Homosildenafil	6.16	70.30	Homosildenafil	
		Homosildenafil	6.16	100.00	Vardenafil	5.26	90.40	Dimethylsildenafil	6.29	58.10		
		Vardenafil	5.26	96.00	Homosildenafil	6.16	86.70	Vardenafil	5.26	57.00		
2	5.94	Sildenafil	6.00	100.00	Sildenafil	6.00	100.00	Sildenafil	6.00	89.30	Sildenafil	Same finding as HSA.
3	Nil										Nil	Sample not adulterated. Same finding as HSA.
4	5.13	Homosildenafil	6.16	100.00	Dimethylsildenafil	6.29	100.00	Vardenafil	5.26	86.50	Vardenafil	Same finding as HSA. Trace vardenafil was detected by LC-Hybrid Tandem MS system but not in HSA LC/DAD system.
		Vardenafil	5.26	99.30	Vardenafil	5.26	100.00	Homosildenafil	6.16	39.40		
		Dimethylsildenafil	6.29	84.30	Homosildenafil	6.16	95.60	Dimethylsildenafil	6.29	25.60		
	5.96	Sildenafil	6.00	84.80	Sildenafil	6.00	100.00	Sildenafil	6.00	89.90	Sildenafil	
	8.00	Tadalafil	8.05	90.60	Tadalafil	8.05	93.30	Tadalafil	8.05	30.70	Tadalafil	

Table 4.3 Validation results for the LC-Hybrid Tandem MS Screening System (Cont.)

Sample Code	RT (min)	Library Search Result									Confirmation Result	Remark
		CE 20			CE 35			CE 50				
		Suggestion	RT (Std)	Purity	Suggestion	RT (Std)	Purity	Suggestion	RT (Std)	Purity		
5	Nil										Nil	Sample not adulterated. Same finding as HSA.
6	7.24	Aminotadalafil	7.24	94.30	Aminotadalafil	7.24	83.70	Aminotadalafil	7.24	74.30	Aminotadalafil	Same finding as HSA.
7	Nil										Nil	Sample not adulterated. Same finding as HSA.
8	5.94	Sildenafil	6.00	92.40	Sildenafil	6.00	95.30	Sildenafil	6.00	89.70	Sildenafil	Same finding as HSA.
9	6.26	Dimethylsildenafil	6.29	100.00	Dimethylsildenafil	6.29	68.20	Dimethylsildenafil	6.29	83.00	Dimethylsildenafil	Same finding as HSA.
		Homosildenafil	6.16	100.00	Vardenafil	5.26	68.20	Homosildenafil	6.16	68.20		
		Vardenafil	5.26	97.80	Homosildenafil	6.16	63.90	Dimethylsildenafil	6.29	66.60		
								Vardenafil	5.26	22.90		
	8.26	Thiohomosildenafil	8.29	94.80	Thiohomosildenafil	8.29	96.80	Thiohomosildenafil	8.29	66.80	Thiodimethylsildenafil	
		Hydroxyvardenafil	4.99	94.80	Hydroxyvardenafil	4.99	96.80	Hydroxyvardenafil	4.99	18.70		
		Thiodimethylsildenafil	8.60	94.80	Thiodimethylsildenafil	8.60	96.80	Thiodimethylsildenafil	8.60	80.70		
		Hydroxyhomosildenafil	6.06	94.80	Hydroxyhomosildenafil	6.06	84.00					

Table 4.3 Validation results for the LC-Hybrid Tandem MS Screening System (Cont.)

Sample Code	RT (min)	Library Search Result									Confirmation Result	Remark
		CE 20			CE 35			CE 50				
		Suggestion	RT (Std)	Purity	Suggestion	RT (Std)	Purity	Suggestion	RT (Std)	Purity		
10	5.94	Sildenafil	6.00	100.00	Sildenafil	6.00	94.60	Sildenafil	6.00	84.50	Sildenafil	Same finding as HSA.
11	Nil										Nil	Sample not adulterated. Same finding as HSA.

4.5 Conclusion

With the increasing trend in adulteration of sexual enhancement health supplements with novel PDE-5 inhibitors and their analogues, screening for adulterants in sexual enhancement health products should not be restrictive to the 3 approved PDE-5 inhibitors (sildenafil, tadaladil and vardenafil). The developed LC-Hybrid Tandem MS method is useful for the rapid and simultaneous screening of a total of 25 PDE-5 inhibitors and related analogues in herbal supplements. The screening capacity of the system can be further expanded with new PDE-5 inhibitor analogues, which are not covered in this study.

Chapter 5

Isolation and structural elucidation of Flibanserin as an adulterant in a health supplement used for female sexual performance enhancement

5.1 Introduction

Sexual dysfunction is more prevalent for women (43%) than men (31%) and is associated with various demographic characteristics, including age and educational attainment (Laumann et al. 1999). Hypoactive sexual desire disorder (HSDD) is a form of Female Sexual Dysfunction (FSD). HSDD is defined as a persistent or recurrent deficiency (or absence) of sexual fantasies/thoughts and desire for sexual activity causing marked distress or interpersonal difficulty (American Psychiatric Association 2000). Up to 10% of women in the USA suffer from FSD of which HSDD is the major subindication (Shifren et al. 2008). Sexual Desire Disorders are generally under-diagnosed as many women are reluctant to discuss sexual issues with their physicians and have low expectations concerning the prospects for help (Marwick 1999, Shifren et al. 2008). Currently, no pharmacological therapy is approved by US FDA for the treatment of HSDD. Some hormonal therapies have been used off-label mainly in postmenopausal women. With accumulating evidence indicating that low-dose testosterone treatment is efficacious in women with HSDD, more research into testosterone treatments designed specifically for HSDD is prompted (Abdallah and Simon 2007, Krapf and Simon 2009, Kronawitter et al.

2009, Simon 2005b, Simon et al. 2005a). A wide range of testosterone formulations, including injections, implants, transdermal patches, cream, gel and tablets have been studied, with varying results (Simon 2005b). Some of these therapies are in their late-stage clinical trials.

Other than hormonal therapies, a wide range of pharmacologic agents have been evaluated for their efficacy in the treatment of HSDD. Some of these drugs have shown little potential for clinical use, whereas others have exhibited promise. Numerous investigations have examined whether vasoactive drugs used to treat erectile dysfunction in men might also improve sexual function in women (Berman et al. 2003, Segraves 2008). In general, no promising results are obtained from the studies of such agents (including phosphodiesterase inhibitors and alprostadil). Research focusing on dopaminergic drugs (such as levodopa, pergolide, and apomorphine) has yielded mixed results in women with HSDD (Segraves 2008, Segraves et al. 2001).

With no approved HSDD therapies available to women, women with reservations to seek help from their physicians may turn to natural health products for help. As a result, health supplements for treating FSD have become increasingly popular in recent years as they are believed to be natural with no side effects. Many of these products claim to be libido enhancer that stimulates blood flow, circulation and hormone activity which result in a more enjoyable orgasm experience for women.

5.2 Objective

The objective of this study is to detect, isolate and identify an unknown compound in a health product claimed for sexual performance enhancement using LC-DAD, LC-FTMS, IR and NMR.

5.3 Experimental

5.3.1 Materials

A health supplement, named MMP, was sent to the Health Sciences Authority (HSA) of Singapore for testing. It is presented as transparent capsules with white powder. Methanol (AR grade) and acetonitrile (HPLC grade) were supplied by Lab-Scan Analytical Sciences (Patumwan, Bangkok, Thailand). Methanol (HPLC grade) was supplied by RCI Labscan Limited (Thailand). Dimethylsulfoxide (DMSO) was supplied by Sigma-Aldrich (Steinheim, Germany). 0.45 μm nylon membrane filters were supplied by Whatman International Ltd. (Maidstone, UK). Ultrapure water was obtained using a Elga Purelab Water Purification System. Sodium dihydrogen phosphate was supplied by Sigma-Aldrich (Steinheim, Germany). Deuterated formic acid and deuterated chloroform used for NMR analysis were purchased from Sigma-Aldrich (St. Louis, MO, USA).

5.3.2 Extraction of sample

For LC-DAD, 1 g of the capsular contents (white powder) were ultrasonically extracted in 10 ml methanol (AR grade) for 30 min. 1 ml extract was further diluted to 10 ml with methanol (AR grade) and filtered through the 0.45 μm PTFE membrane filter.

For LC-LTQ Orbitrap XL FTMS, NMR and IR analyze, 3.3 g of the capsular contents were ultrasonically extracted with 40 ml methanol (AR grade) for 30 min. The extract was filtered and the solvent was evaporated off using a rotary evaporator. The residue was reconstituted in 1.5 ml DMSO and filtered through 0.45 μm PTFE membrane filter. The filtered sample was further purified using preparative HPLC and the purified compound of interest was analyzed by LC-LTQ Orbitrap XL FTMS, NMR and IR.

5.3.3 Preparative HPLC

A Shimadzu HPLC system with two preparative pumps (LC-8A, Kyoto, Japan) and an automatic fraction collector (FRC-10A, Kyoto, Japan) were used. An Agilent ZORBAX SB-C18 reversed phase semi-preparative column (250 × 9.4 mm i.d., 5 μm) was used for the sample separation. The mobile phases were water and acetonitrile (HPLC grade). The gradient elution profile was as follows: acetonitrile was increased from 20% to 90% in 15 minutes and maintained for 3 minutes. The flow rate of mobile phase was 4 ml/min and injection volume was 50 μl. Column oven temperature was set at 40 ^{0}C. The UV and visible spectra from 190–600 nm were recorded on-line during the chromatographic run.

Fractions containing the target compound were collected by the automatic fraction collector based on UV detection (254 nm). The solvent was removed using a rotary evaporator and the residue was freeze dried using Christ Alpha 2-4 LD Plus Freeze Dryer (Osterode, Germany).

5.3.4 LC-DAD analysis

An Agilent 1100 series HPLC chromatograph with diode-array detector (Palo Alto, CA, USA) was employed. A BDS Hypersil C18 column (200 mm × 4.6 mm i.d., 5 μm) from Thermo Scientific (USA) was used. Mobile phase A consisted of 25 mM sodium dihydrogen phosphate in water, adjusted to pH 3.2 ± 0.1 with phosphoric acid. Mobile phase B was acetonitrile (HPLC grade). The gradient elution profile was as follows: 0–30 minutes, acetonitrile rose from 10% to 70% (v/v), and maintained for 5 minutes. The flow rate of mobile phase was 1 ml/min. The injection volume was 10 μL. The UV spectra from 200 to 400 nm were recorded on-line during the chromatographic run. The chromatograms of both original methanol extract and purified compound were recorded at the wavelength 254 nm.

5.3.5 LTQ Orbitrap XL FTMS analysis

The isolated compound was dissolved in methanol (HPLC grade) at a concentration of 0.5 μg/ml and was injected into Thermo Fischer Scientific LTQ Orbitrap XL™ hybrid FTMS System (Bremen, Germany) controlled by Xcalibur (Version 2.0.7) software. The ESI

ionization source was operated in the positive ionization mode with spray voltage set at 3 kV, sheath gas flow rate at 40 arb, auxiliary gas flow rate at 10 arb, capillary voltage and temperature at 39 V and 250 ^{0}C respectively. The tube lens were set at 120 V, mass range was set at 200 to 500 Da at a resolution of 30000. The high-resolution MS spectrum was acquired by direct infusion with a flow rate of 5 µl/min. The $[M+H]^{+}$ was selected as a precursor ion and the MS^{2} spectrum was acquired with Collision energy (CE) set at 45V under HCD (High energy Collision Dissolution) mode.

5.3.6 NMR and IR analyses

The isolated compound was dissolved in deuterated chloroform ($CDCl_3$) for NMR analysis. ^{13}C and DEPT spectra were recorded on a Bruker AMX 500 spectrometer (Bruker BioSpin, Rheinstetten, Germany). ^{1}H, COSY, HMQC and HMBC spectra were recorded on a Bruker DRX 500 spectrometer (Bruker BioSpin, Rheinstetten, Germany). Chemical shifts were reported in ppm using the solvent peak as an internal standard. IR spectrum in KBr disc was recorded on a Nicolet 6700 FTIR spectrometer (Madison, Wisconsin, United States) and recorded over the spectral range 4000–400 cm^{-1}.

5.4 Results and Discussion

Approximately 10 mg of white amorphous powder was isolated from 3.3 g of MMP powder.

As shown in Figure 5.1, the UV spectrum of the isolated unknown compound in methanol showed maximal absorbances at 210 nm, 230 nm, 250 nm and 280 nm. The retention times for the compound in both the extract and purified sample were 17.2 min and 17.4 min respectively under the LC-DAD chromatographic conditions [Figure. 5.2 (A) and (B)].

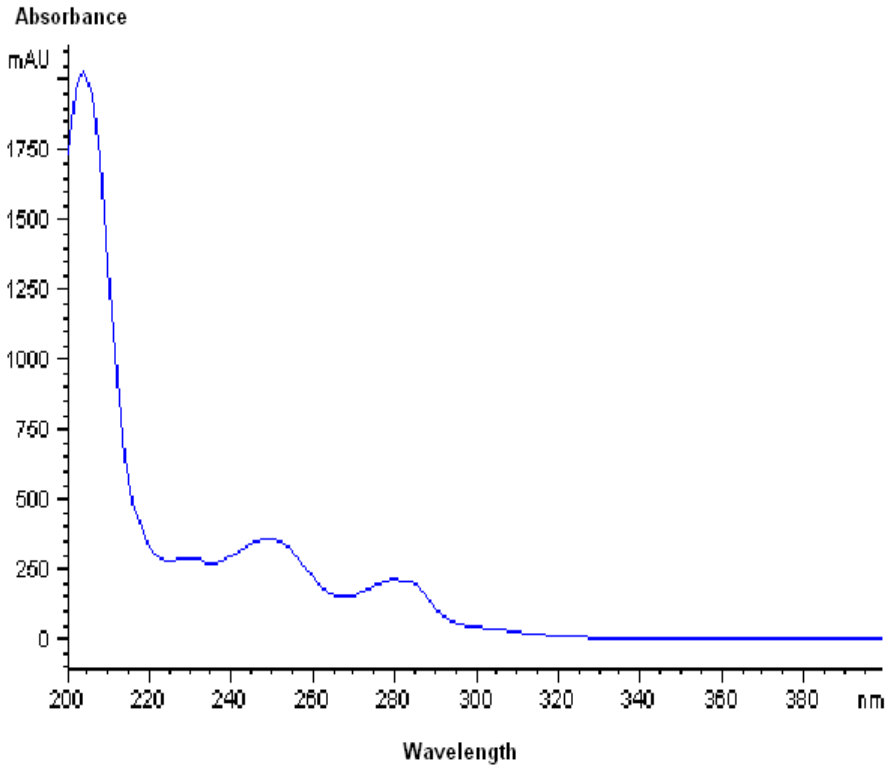
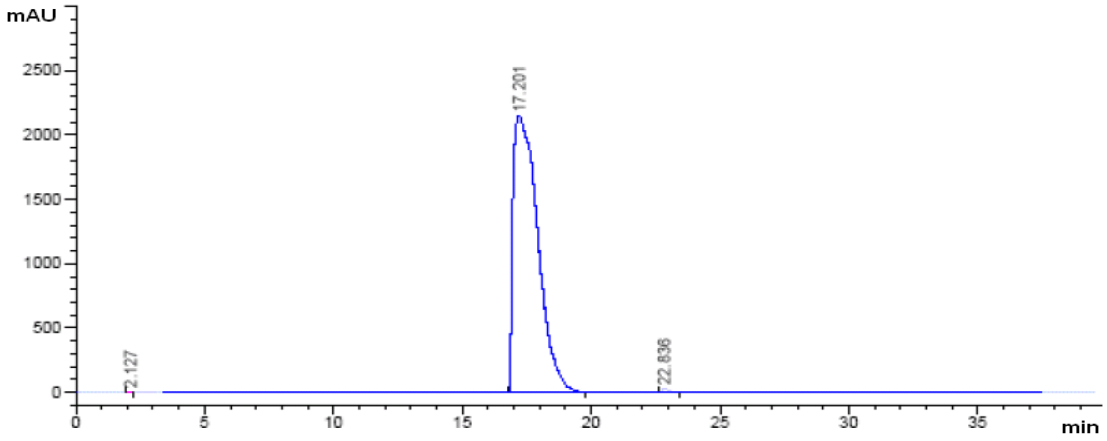


Figure 5.1 UV-vis spectrum of unknown compound in methanol, scanned from 200 nm to 400 nm, showing the maximal absorbances at 210 nm, 230nm, 250 nm and 280 nm.

(A)



(B)

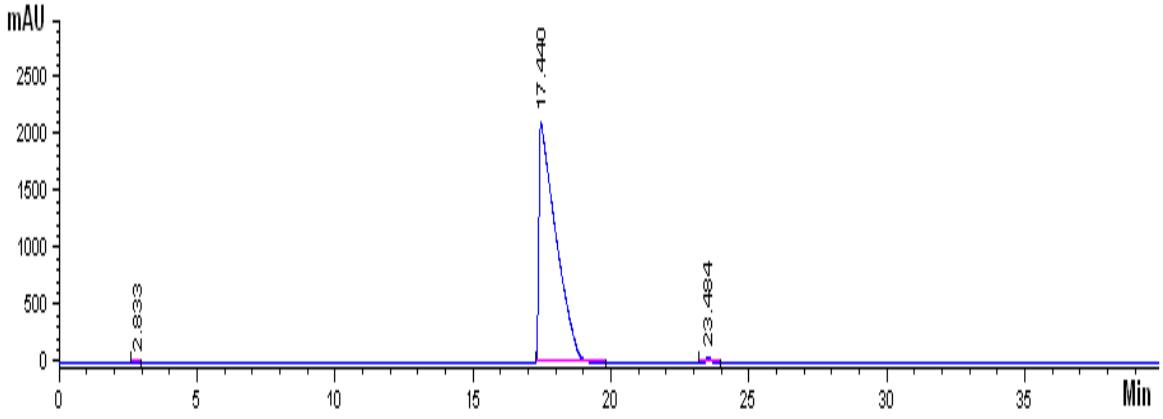


Figure 5.2 HPLC chromatograms of (A) methanol extract of MMP and (B) purified unknown compound at wavelength 254 nm.

High-resolution ESI-MS spectrum (Figure 5.3) of the compound revealed $[M+H]^+$ at m/z 391.17310, suggesting a molecular formula of $C_{20}H_{21}F_3N_4O$. The error between observed mass and theoretical mass of $[M+H]^+$ was -2.352 ppm.

The high-resolution MS^2 spectrum by high-resolution ESI-MS/MS in Figure 5.4 showed the major fragments at m/z 161.07045, 204.11269, 243.10999, 257.12555 and 371.16727. The proposed chemical structure of the unknown is shown in Figure 5.5.

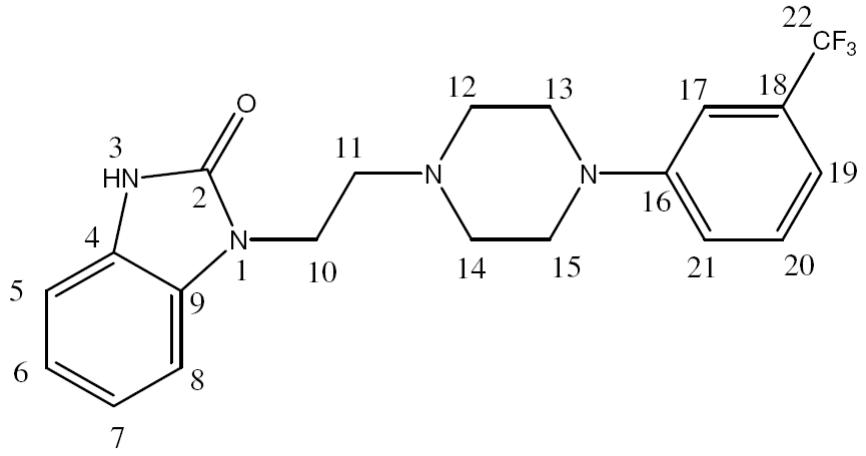


Figure 5.5 Proposed chemical structure of unknown compound

Based on the proposed chemical structure, the fragmentation process proposed by Mass Frontier™ 5.0 (HighChem, Ltd., Slovak Republic) is shown in Figure 5.6.. All proposed fragments have mass errors below 5 ppm.

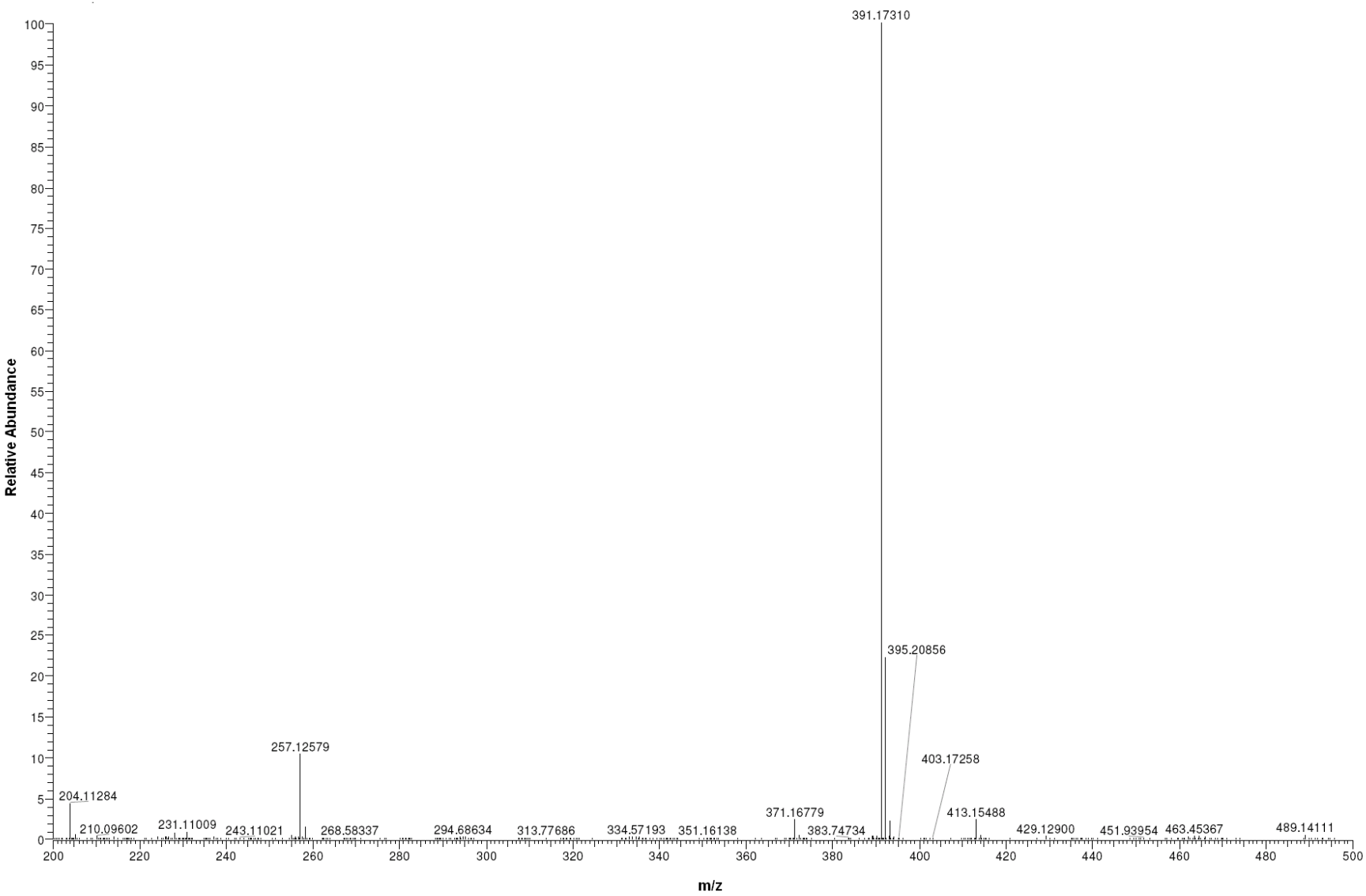


Figure 5.3 High resolution MS spectrum of unknown compound

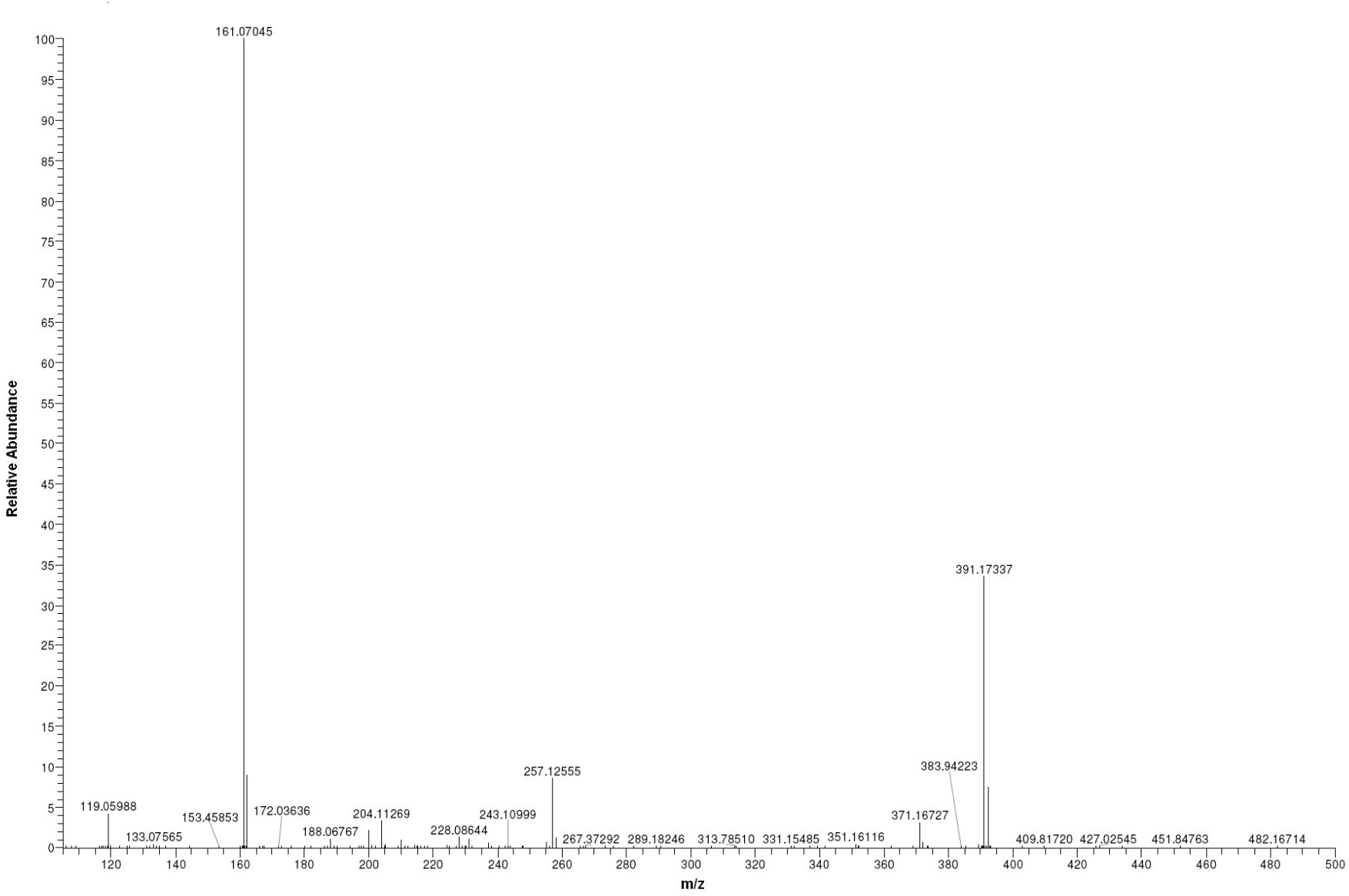


Figure 5.4 High resolution ESI MS/MS spectrum of unknown compound

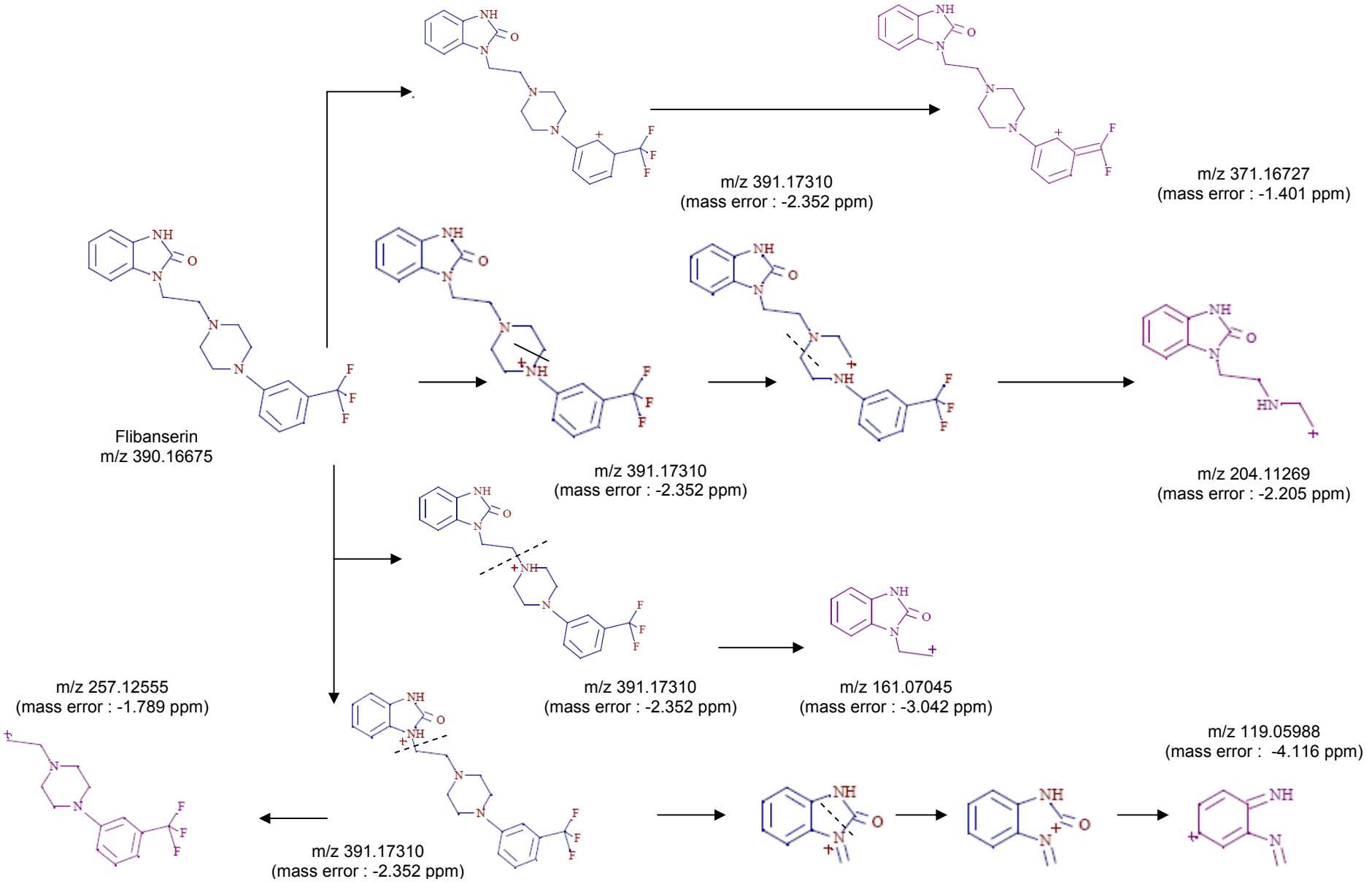


Figure 5.6 Proposed ESI-MS/MS fragmentation of the protonated molecules of unknown compound ($[M+H]^+$ m/z 391.17310)

This proposed formula and structure was further confirmed by ^{1}H and ^{13}C NMR data which indicated the presence of 20 carbon atoms and 21 protons. 1D and 2D NMR (Table 5.1) data were used to assign the protons and carbon atoms. In the ^{1}H NMR spectrum (Table 5.1), a sharp singlet at 10.00 ppm integrating for one proton is a typical proton attached to nitrogen. HMBC correlated this proton to C-2, C-4, and C-9 suggesting that it was H-3. Complex signals were observed between 7.00 to 7.31 ppm, integrating for eight protons. A triplet at 7.31 ppm, integrating for a proton has a coupling constant of 8.0 Hz. HMBC correlated this proton with C-16, C-19, and C-21 suggesting that it was H-20. A *double-doublet* splitting pattern at chemical shift 7.11 ppm, integrating for a proton, has coupling constants of 6.3 Hz and 1.6 Hz. HMBC correlated this proton to C-6, C-7, and C-9 showing that it was H-8. Overlapped signals were observed from 7.04 ppm to 7.10 ppm, integrating for five protons. A *double-doublet* splitting pattern at 7.01 ppm with coupling constant 8.0 Hz and 2.0 Hz, integrating for a proton was observed. HMBC correlated this proton to C-17 suggesting that it was either H-19 or H-21. Four triplet signals were also observed from 2.73 ppm to 4.08 ppm, integrating for a total of twelve protons. Two of these triplet signals at 2.74 ppm and 3.22 ppm integrated for four protons each, suggesting overlapping signals of methylene protons. This was further confirmed by ^{13}C and DEPT NMR.

^{13}C and DEPT NMR data showed the signals of four methylene, eight methine and six quaternary carbon atoms. The DEPT signals at 53.1 ppm and 48.6 ppm have intensities which were double of those from the rest of the methylene carbon

signals, suggesting two methylene carbon atoms each contributing to the signal at 53.1 ppm and 48.6 ppm. HMQC results further indicated that these two methylene carbon signals at 53.1 ppm and 48.6 ppm were correlated to the protons signal at 2.73 ppm and 4.08 ppm respectively, which corresponded to four protons each. The finding confirmed overlapping methylene carbon signals (at 53.1 ppm and 48.6 ppm) and methylene proton signals (at 2.73 ppm and 4.08 ppm). Hence, the unknown compound has six methylene carbon atoms with a total of twelve methylene protons. The chemical shifts of the twelve methylene protons suggested that they were attached to relatively electronegative atoms. It was speculated that the six methylene groups were attached to the nitrogen atoms and the electron withdrawing effect of these electronegative nitrogen atoms resulted in the deshielding of the protons. HMBC and COSY correlations were used to assign the rest of the protons.

The ^{13}C NMR data (Table 5.1) showed that there were two quaternary carbon at 155.6 ppm and 151.3 ppm. The carbon with chemical shift 155.6 ppm was C-2. In the structure of imidazolone, carbonyl carbon C-2 was attached to two nitrogen atoms which helped to withdraw electrons from oxygen to C-2. Hence, C-2 was less deshielded as compared to a normal carbonyl carbon which has chemical shift above 170 ppm. Eight methine carbons and two quaternary carbons with chemical shifts above 108 ppm suggested the presence of two aromatic rings. The quaternary carbon with chemical shift 125.4 ppm was C-22 which was attached to

three fluorine atoms. Due to the strong electron withdrawing effect of the fluorine atoms, C-22 was highly deshielded and had a high chemical shift.

The ^{1}H and ^{13}C NMR , DEPT, HMQC, HMBC and ^{1}H-^{1}H COSY spectra are shown in Figure 5.7 to 5.12.

The NMR results confirmed the unknown compound to be 1-[2-[4-(3-trifluoromethyl phenyl) piperazin-1-yl] ethyl] benzimidazol-[1H]-2-one, which is also known as flibanserin. The chemical structure of flibanserin is the proposed structure in Figure 5.5.

The IR spectrum of the isolated compound showed absorption bands of amide ($\nu_{C=O}$ 1685 cm^{-1}, $\nu_{N-H\ (stretch)}$ 3180 cm^{-1}, $\nu_{N-H\ (bending)}$ 1610 cm^{-1}), alkyl fluoride (ν_{C-F} 1077 cm^{-1}, 1112 cm^{-1}, 1158 cm^{-1}), aromatic ring (ν_{Ar-H} 3028 cm^{-1}, 3078 cm^{-1} and $\nu_{C=C}$ 1401 cm^{-1}, 1446 cm^{-1}, 1453 cm^{-1}, 1468 cm^{-1}, 1487 cm^{-1}) and alkane (ν_{C-H} 2891 cm^{-1}, 2930 cm^{-1} 2948 cm^{-1}). The IR spectrum supported the structure of flibanserin.

Flibanserin, a serotonin-1A (5-HT (1A)) receptor agonist and the serotonin-2A (5-HT (2A)) receptor antagonist was developed as a novel, non-hormonal treatment for pre-menopausal women with HSDD (Borsini et al. 2002, Invernizzi et al. 2003). However, the FDA Advisory Committee for Reproductive Health Drugs rejected the New Drug application as the efficacy was deemed not sufficiently

robust to justify the risks and there were concerns over the safety signals and potential drug interactions involving flibanserin (US FDA 2010e). Data on long term use of flibanserin and further documentation of efficacy will be needed for FDA's reconsideration.

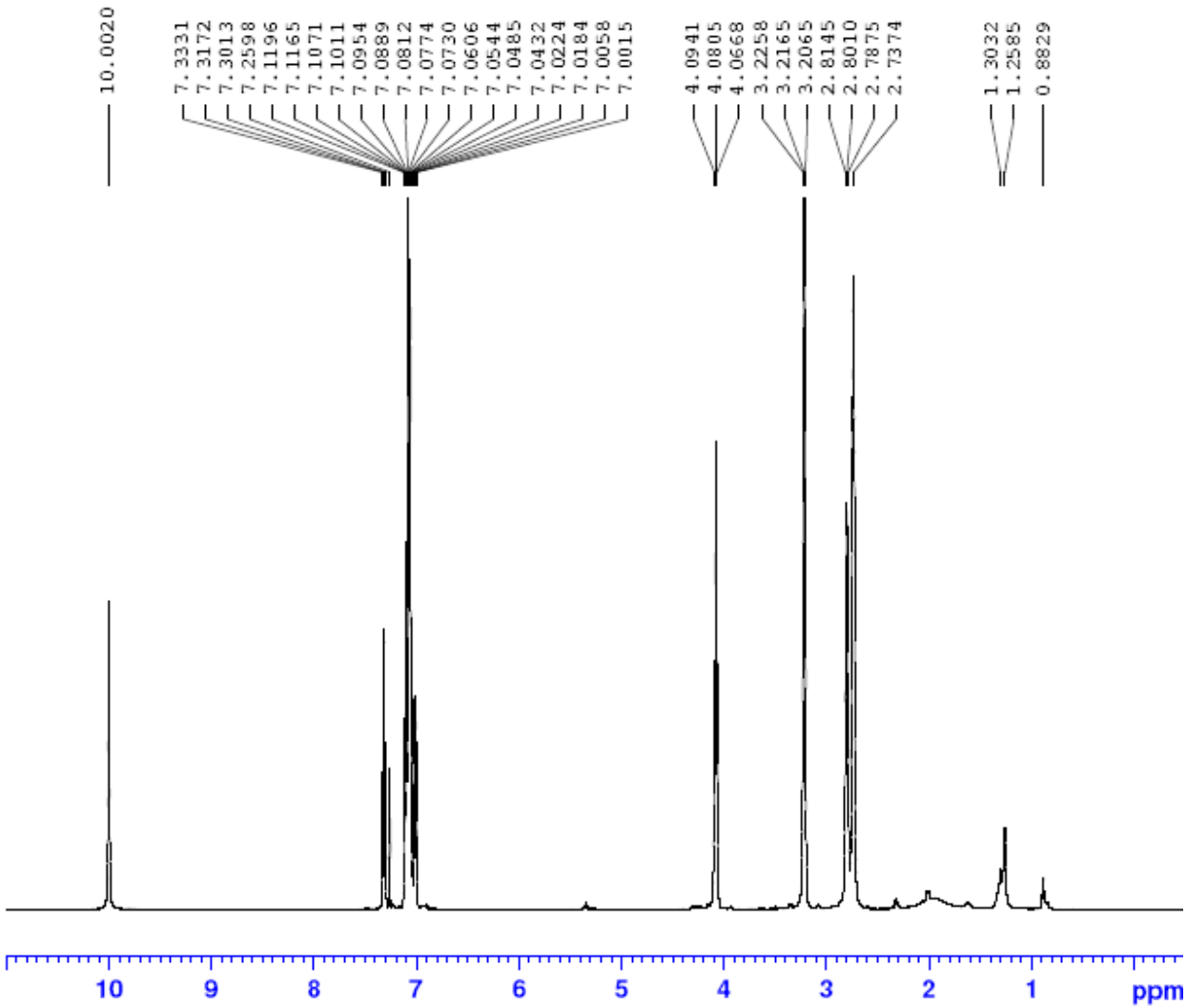


Figure 5.7 ^{1}H-NMR spectrum of unknown compound in deuterated chloroform

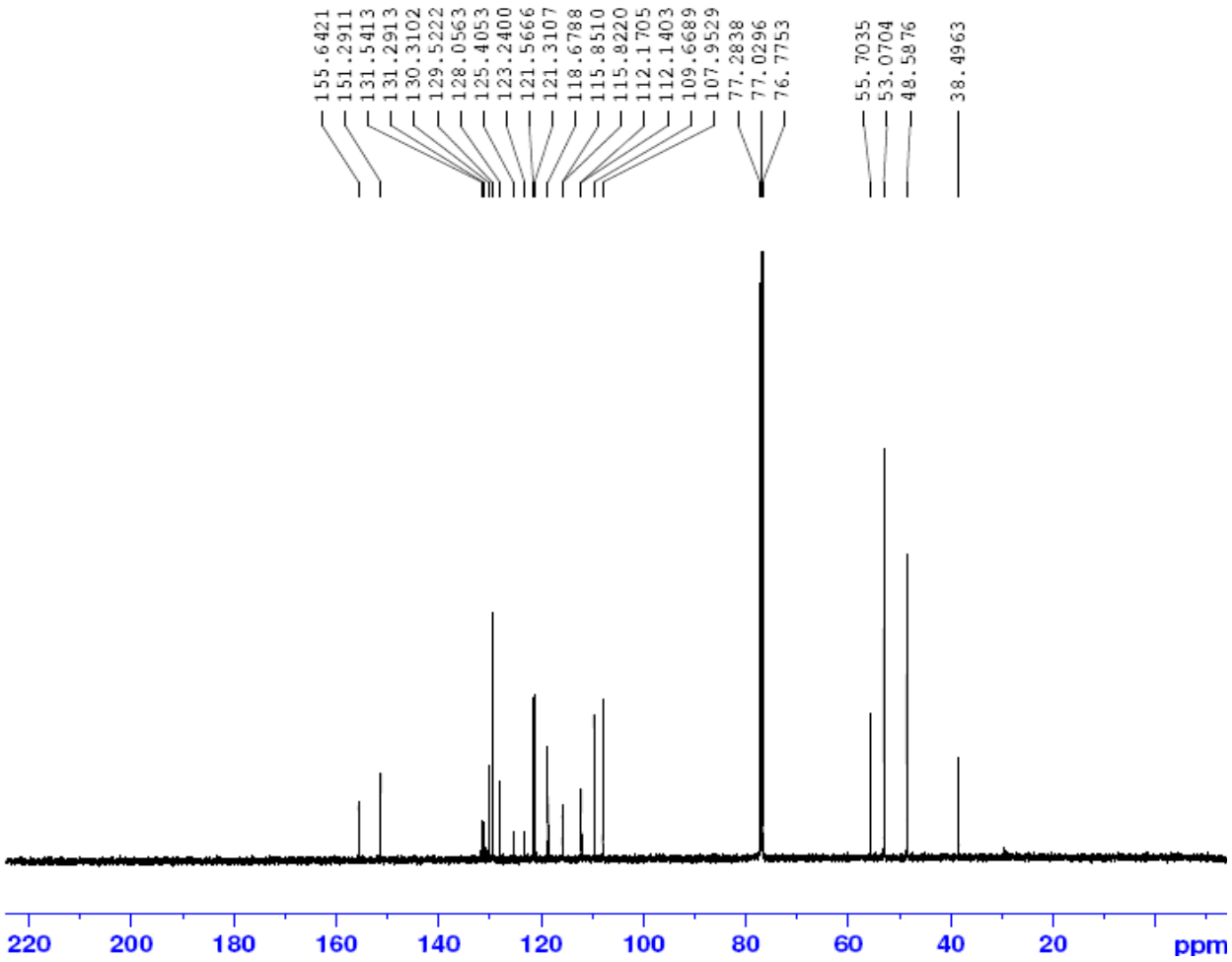


Figure 5.8 ^{13}C-NMR spectrum of unknown compound in deuterated chloroform

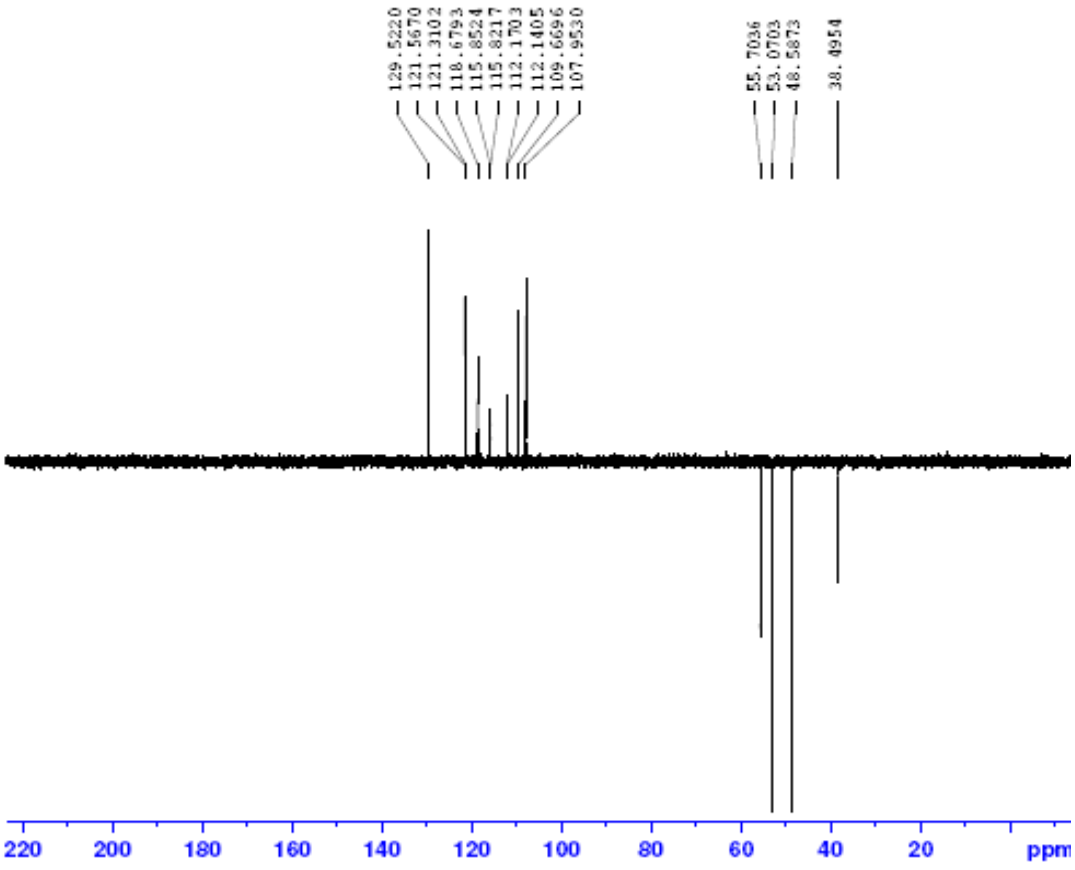


Figure 5.9 DEPT spectrum of unknown compound in deuterated chloroform

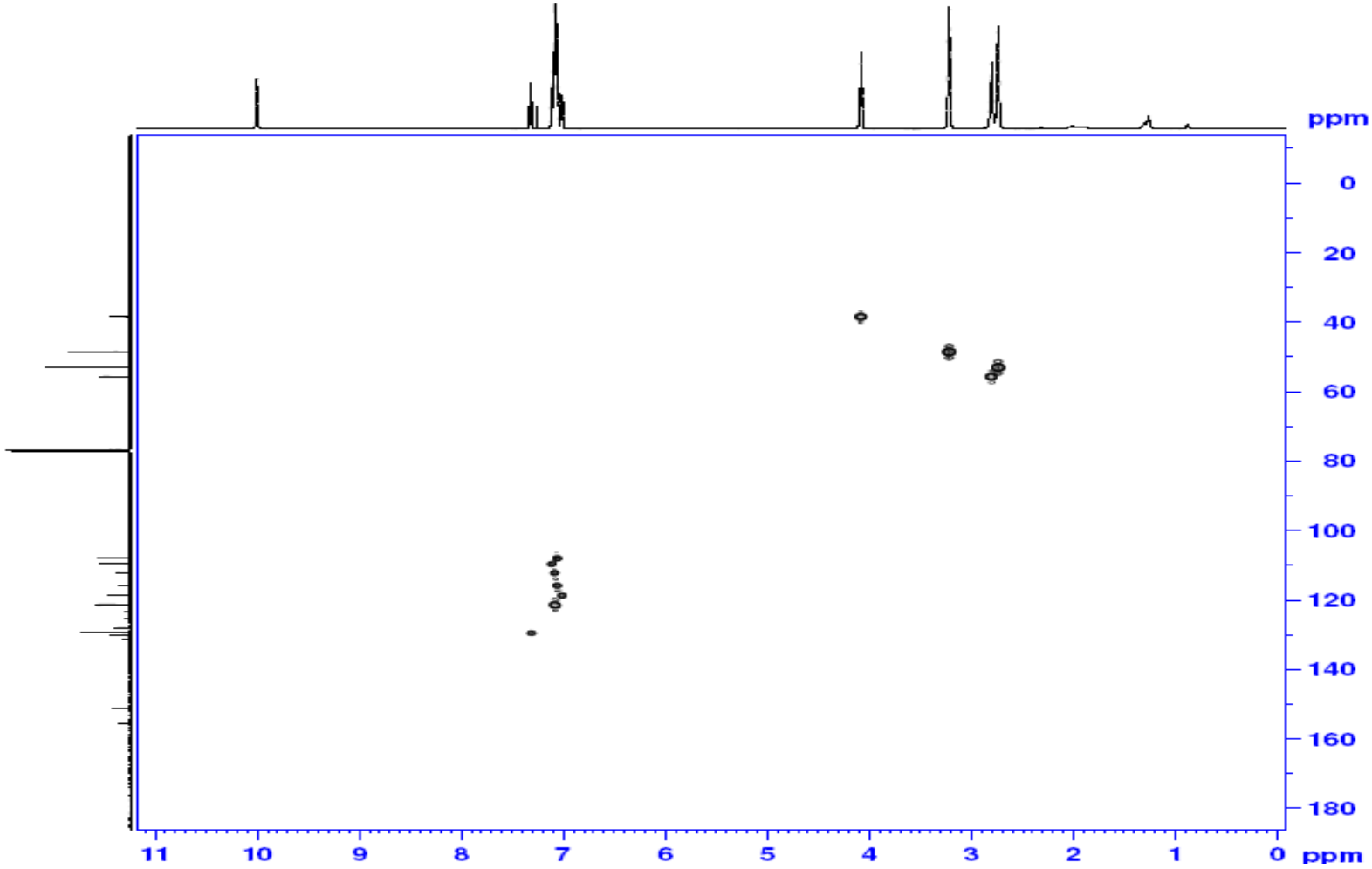


Figure 5.10 HMQC spectrum of unknown compound in deuterated chloroform

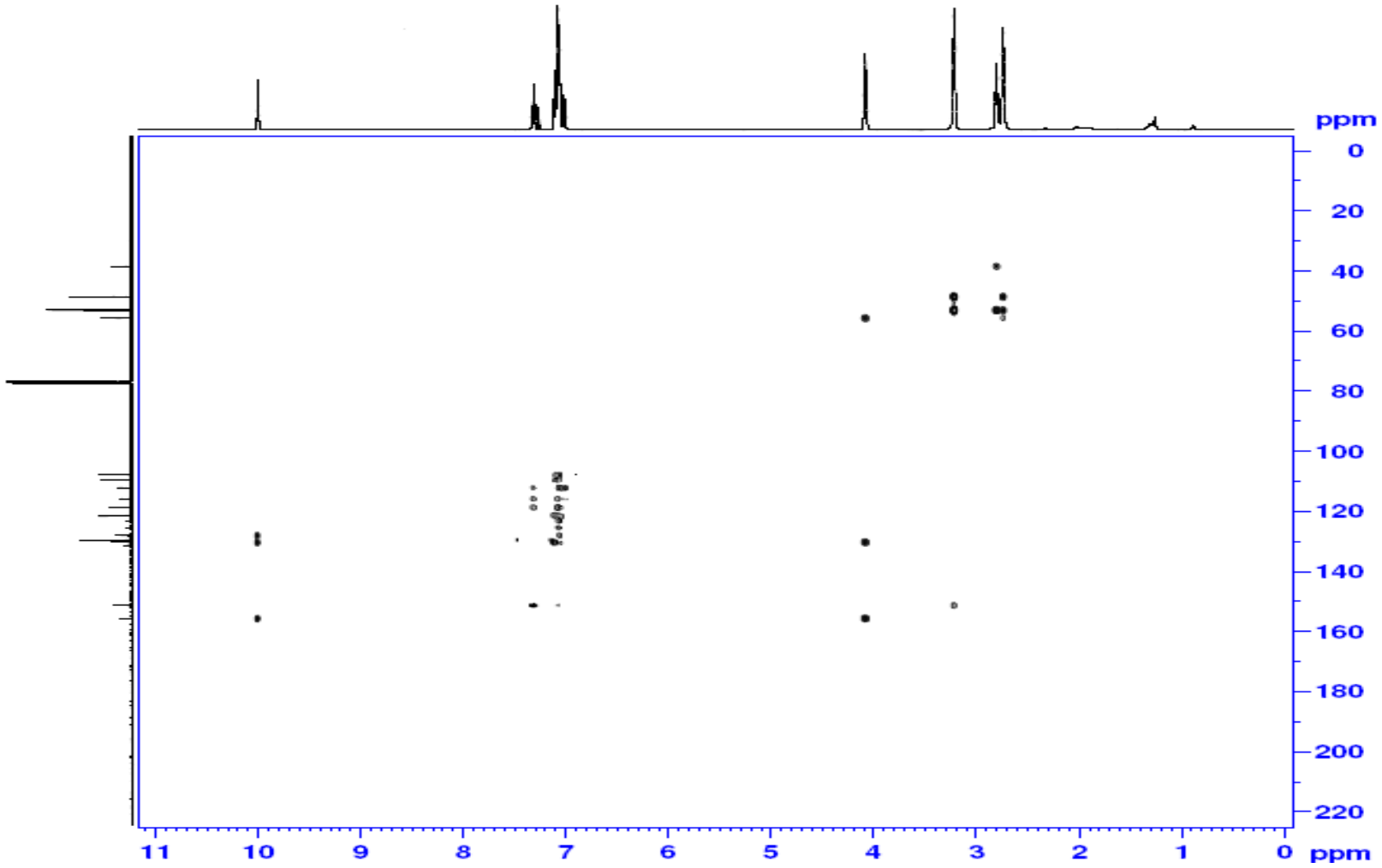


Figure 5.11 HMBC spectrum of unknown compound in deuterated chloroform

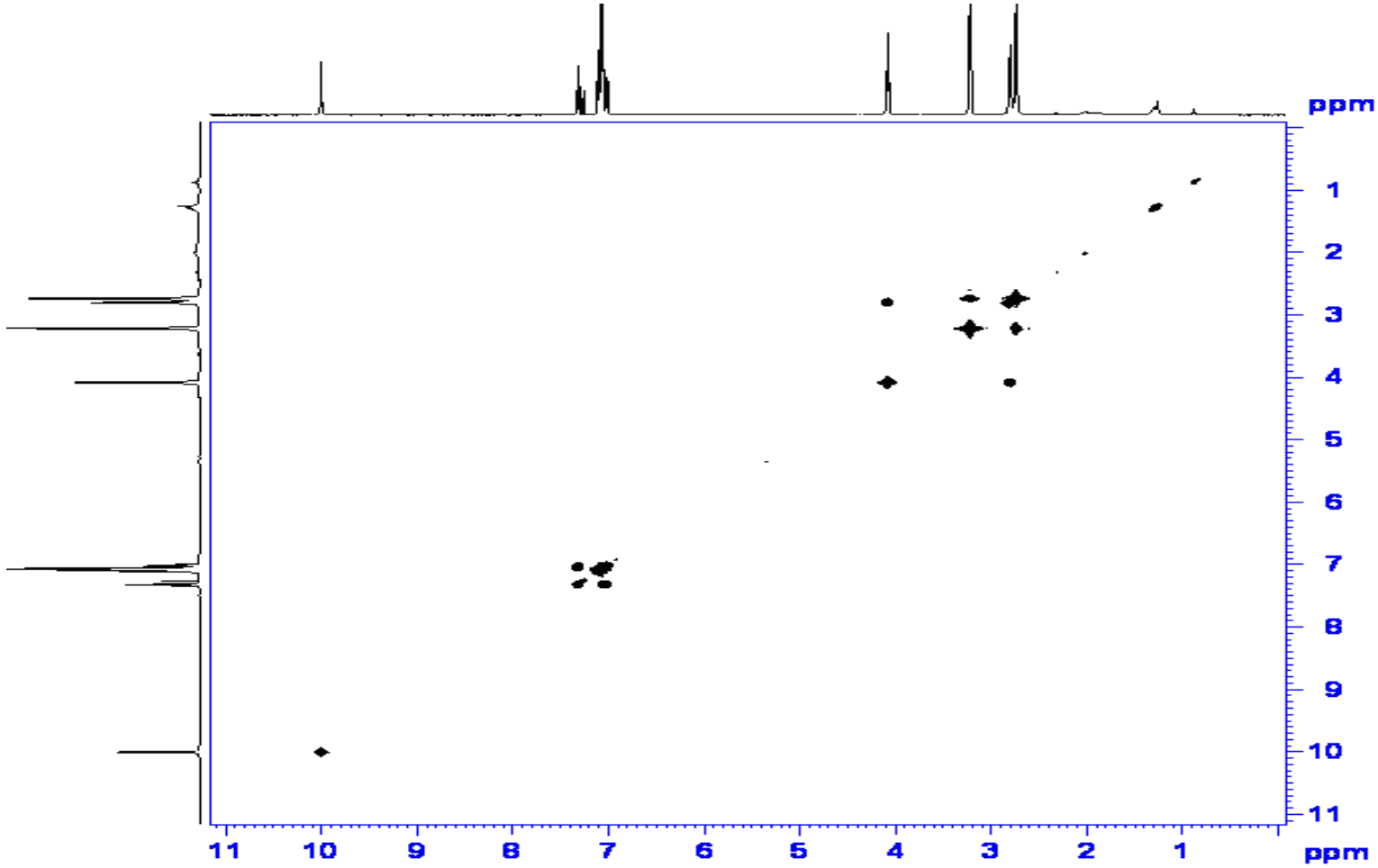


Figure 5.12 ^{1}H-^{1}H COSY spectrum of unknown compound in deuterated chloroform

Table 5.1 NMR data for unknown compound

No.	^{1}H (δ_H)	^{13}C (δ_C)	DEPT**	COSY	HMBC
2	–	155.6	0	–	–
3	10.00 (1H, s)	–	–	–	C-2, C-4, C-9
4	–	128.1	0	–	–
5	7.06 (1H, m)	108.0	1	–	C-4
6*	7.10 (1H, m)	121.3	1	–	***
7*	7.10 (1H, m)	121.6	1	–	***
8	7.11 (1H, dd, *J* = 6.3, 1.6)	110.0	1	–	C-6, C-7, C-9
9	–	130.3	0	–	–
10	4.08 (2H, t, *J* = 6.8)	38.5	2	H-11	C-2, C-9, C-11
11	2.80 (2H, t, *J* = 6.8)	55.7	2	H-10	C-10, C-12, C-14
12, 14	2.74 (4H, t, *J* = 4.5)	53.1	2	H-13, H-15	C-11, C-13, C-15, C-12/C-14,
13, 15	3.22 (4H, brs)	48.6	2	H-12, H-14	C-12, C-14, C-13/C-15, C-16
16	–	151.3	0	–	–
17	7.09 (1H, m)	112.2	1	–	***
18*	–	123.2	0	–	–
19	7.01 (1H, dd, *J* = 8.0, 2.0)	115.8	1	H-20	C-17
20	7.31 (1H, t, *J* = 8.0)	129.5	1	H-21/H-19	C-16, C-19, C-21
21	7.07 (1H, m)	118.7	1	H-20	***
22*	–	125.4	0	–	–

δppm in $CDCl_3$, *J* in Hz.

* Overlapping signals
** Number of attached protons
*** Correlation cannot be accurately assigned due to overlapping signals

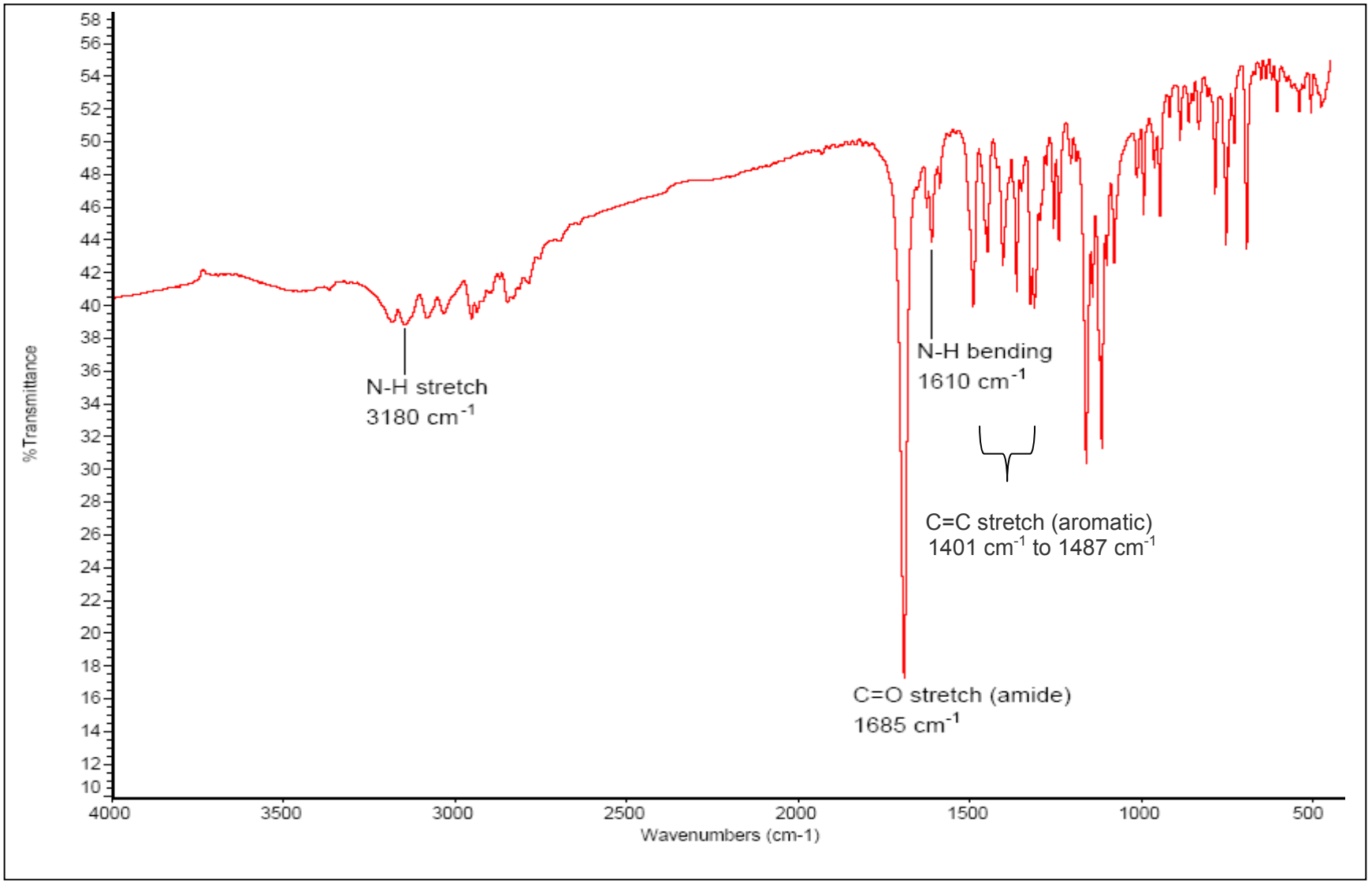


Figure 5.13 FTIR spectrum of unknown compound

5.5 Conclusion

In this study, a serotonin-1A (5-HT (1A)) receptor agonist and serotonin-2A (5-HT (2A)) receptor antagonist, flibanserin, was isolated from a health supplement and its chemical structure was elucidated using NMR, IR, high-resolution ESI-MS and MS/MS. The presence of flibanserin in the health supplement is dangerous for consumers as it is not an approved drug. This is the first report of flibanserin as an adulterant in a health supplement used for female sexual performance enhancement. Due to the limited amount of sample available, quantitative analysis was not performed in this study.

CHAPTER 6

CONCLUSION

There is a global upsurge in the use of herbal medicines in both developed and developing countries over the years. As the regulatory control for herbal medicines is less stringent than that for the conventional pharmaceuticals, the safety and quality of these products pose a concern to the consumers. The adulteration of herbal sexual enhancement health products with unlabeled synthetic therapeutic substances such as PDE-5 inhibitors and their related analogues is hazardous to public health and severe adverse effects may develop with the consumption of such adulterated products.

The study on the safety and quality assessment of the 247 illegal sexual enhancement herbal products seized by HSA from the red light districts and at Singapore Customs revealed that a high proportion of these products (199, 77%) were adulterated with western drugs and their analogues. The extent of the adulteration may in part explain the effectiveness and the high demand for such products despite the many adverse drug reactions reported. As there are currently no registration requirements for herbal medicines except for a listing system for CPM products in Singapore, adulterated sexual enhancement herbal supplements can escape from the listing requirement and pre-marketing safety assessment, which include the screening for adulteration. The study reveals the pressing need for more stringent regulatory efforts to deter such unethical and illegal practices

and to curb the demand of such products through public education. The study also provides a good reference for public education, creating greater public awareness on the danger of consuming illegal health supplements and dispelling the myth that “natural” means “safe”. Consumers are often reluctant to reveal to physicians and health authorities the nature of the products they have taken, the source of supply and identities of the distributors or peddlers, due to the sensitivity of the matter. Physicians should be encouraged to discuss with their patients about the consumption of herbal medicines and supplements and be aware of potential adverse events associated with the consumption of herbal medicines. Both the physicians and consumers should keep a lookout for such adverse events and should be encouraged to make an adverse drug reaction report to alert the authorities and manufacturers. This will help to prevent under reporting of adverse events associated with herbal products.

With the emerging threat from adulteration of sexual enhancement health products, it is important for regulatory laboratories to develop a rapid and sensitive method to simultaneously detect PDE-5 inhibitors and their related analogues. This will enable the authorities to effectively control the sexual enhancement health products in the market and investigate the adverse reaction reports associated with the consumption of such products. The LC-Hybrid Tandem MS Screening System developed in this study for rapid and simultaneous detection of the PDE-5 inhibitors and their analogues in sexual enhancement health products is able to detect the 3 PDE-5 inhibitors (sildenafil, tadalafil and

vardenafil) and their 22 related analogues in a single run of 20 min. The developed method will be adopted by HSA to complement its current screening methodologies for sexual enhancement health products.

In recent years, health products for treating female sexual dysfunction have also become increasingly popular as there are no approved hypoactive sexual desire disorder (HSDD) therapies available to women. Instead of seeking help from their physicians, such patients may turn to natural health products for help. In this study, a serotonin-1A (5-HT (1A)) receptor agonist and the serotonin-2A (5-HT (2A)) receptor antagonist, flibanserin, was isolated from a health supplement claimed to enhance female sexual performance. Its chemical structure was elucidated using NMR, IR, high-resolution ESI-MS and MS/MS. The findings allow HSA to follow up the case with the supplier and stop the product from entry to the local market. This is also the first report of flibanserin as an adulterant in a sexual performance enhancement health supplement.

Herbal medicine is a complex mixture of biological origin. Effective control of the quality and safety remains challenging and requires regulatory control, scientific methodologies and data, post-marketing quality surveillance, pharmacovigilance, availability of reliable information and education of all stakeholders (industry players, healthcare professionals and consumers). It is important for the health authorities to recognize the urgent need for imposing effective statutory regulations to ensure the quality, safety, efficacy and commercial distribution of herbal health products. Health authorities should also

encourage and support research development in the quality, safety and efficacy of herbal medicines, which includes the development of testing methodologies for the quality and safety control of herbal health products. The work presented in this thesis is useful to health authorities, healthcare professionals and consumers to ensure a safe integration of good quality herbal medicine into conventional medical practice.

For future work, toxicity studies and pharmacokinetic investigations of the PDE-5 inhibitor analogues reported will be useful to confirm the health risks involved in consuming such adulterated health products.

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APPENDIX I

S/N	Product name/ description	Batch Number	Manufacture	Dosage Form	Ingredients	Adulterant Detected	Content
1	18 Spring 十八春 30 ml	-	深圳康妮尔保健品有限公司	Pink tube with transparent cream	-	No common poison detected	
2	Africa Black Ant 非洲黑蚁王	-	-	White capsule with " 金聖力" imprint	非洲黑蚂蚁，高丽参，人参，肉苁蓉，淫羊藿，藏红花，枸杞子等。	Sildenafil	114.69 mg/cap
3	Africa Black Ant 黑蚁王【非洲】	-	-	White capsule with " 金聖力" imprint	非洲黑蚂蚁，高丽参，人参，肉苁蓉，淫羊藿，藏红花，枸杞子等	Sildenafil	205.80 mg/cap
4	Ai Jia Ai 爱加爱	-	香港大力神生物医药技术中心	Red and black capsule	冬虫夏草，人参，鹿鞭，海狗肾	Sildenafil	210.28 mg/cap
5	Ali Baba	-	-	Transparent vial containing brown liquid	-	No common poison detected	
6	America Brother 美国威哥王	-	-	Blue tablet with "VAG" engraver	牦牛鞭，冬虫夏草，海狗肾，人参，天山雪莲，枸杞子，肉苁蓉， 淫羊藿等。	Sildenafil	29.76 mg/tab
7	America Cowboy 美国牛仔	-	香港华建国际贸易有限公司	Green capsule	-	Sildenafil	133.94 mg/cap
8	American Moxman 美国魔根 2500 mg	-	-	Red and white capsule	-	Sildenafil	139.72 mg/cap
						Sibutramine	0.011 mg/cap
9	American Moxman 美国魔根	-	-	Red and white capsule	东北人参，淫羊藿，银杏叶，亚洲红参，鹿鞭，藏红花，天山雪莲，山楂果等	Sildenafil	136.49 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
10	America Viagia 美国战神	-	海南新贝乐生物科技有限公司	Brown tablet	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子等。	Sildenafil	12.31 mg/tab
						Vardenafil	25.40 mg/tab
						Piperidenafil	Not determined
11	America Viagra	-	-	Yellow tablet with "HF" engraver	-	Sildenafil	156.91 mg/tab
						Sibutramine	< 0.003 mg/tab
12	Bai Wei 百威	-	-	Black tablet	-	Sildenafil	66.93 mg/tab
13	Bai Wei USA 百威	-	-	Black tablet with "HF" engraver	-	Sildenafil	129.64 mg/tab
14	Black King 黑金刚	Q/LXB010-2005	西藏升陽保健品有限公司	White capsule	-	Sildenafil	145.05 mg/cap
15	Black King Kong 黑金刚	-	-	Yellowish orange capsule	-	Sildenafil	200.56 mg/cap
16	Blue Fairy U.S.A 蓝精灵		Bolin Merker Health Products Co. Ltd	Transparent and white bottle	-	No common poison detected	
17	Buda 强神粒	-	拉萨市力达保健品有限公司	Brown tablet	Sildenafil	Sildenafil	81.35 mg/tab
18	Buda 金槍不倒	Q/HS02-2000	拉萨市力达保健品有限公司	Red tablet	-	Sildenafil	92.33 mg/tab
19	Buda 金槍不倒	Q/HS02-2000	拉萨市力达保健品有限公司	Red tablet with "800mg" engraver	-	Sildenafil	84.15mg/tab
20	Bu Shen Zhuang Yang	-	-	Pink capsule	-	Sildenafil	158.39 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
21	Cang Ying Fen 苍蝇粉	-	日本红丸珠式会社	Sachet containing white powder	淫性草， 红参，鹿茸等。	No common poison detected	
22	Cang Ying Shui 苍蝇水	-	中国深圳祥瑞生物工程有限公司	White tube	-	No common poison detected	
23	Cattle Capsules 牛宝胶囊	-	安徽三体医药保健品公司	Blue and white capsule with "牛宝 CATTLE" imprint	黄牛鞭，鹿茸，人参，五味子，枸杞子，菟丝子，淫羊藿，龙眼肉等	Sildenafil	36.13 mg/cap
24	Cattle Capsule 牛寶膠囊	-	安徽三體醫藥保健品公司	Blue and white capsule with "牛宝 CATTLE" imprint	Pizzie, antlar, ginseng, Schisandra fruit, lycium fruit, Cuscuta barren wort, Arillus Longan, astragalus root.	Sildenafil	36.42 mg/cap
25	Caverta 100 mg	5024541	Ranbaxy	Blue tablet with "USA VGR" engraver	Sildenafil, titanium dioxide, iron oxide red, lake of indigo carmine and lake of ponceau 4R	Sildenafil	71.36 mg/tab
26	Caverta 100 mg (Ranbaxy)	1661385	Ranbaxy	Brown tablet with "100" engraver	Sildenafil, titanium dioxide, iron oxide red, lake of indigo carmine and lake of ponceau 4R	Sample A:Sildenafil	30.33 mg/tab
						Sample A:Chloramphenicol	0.44 mg/tab
						Sample B:Sildenafil	115.68 mg/tab
27	Champ - Gentlemen Only	-	-	Yellow and transparent capsule	-	Lignocaine	0.105 mg/cap
28	China Vigers 中华伟哥 2500 mg	-	中国人民财产保险股份有限公司	Green capsule with "金聖力" imprint	黄牛鞭，海狗鞭，黑蚂蚁，鹿鞭，肉苁蓉，高丽参等 50 多种名贵药材。	Sildenafil	145.74 mg/cap

S/N	Product name/description	Batch Number	Manufacture	Dosage Form	Ingredients	Adulterant Detected	Content
29	China Vigers 中華偉哥	-	中国人民财产保险股份有限公司	Green capsule with "金聖力" imprint	黑蚂蚁，海狗鞭，鹿鞭，黄牛鞭，肉苁蓉，高丽参等	Sildenafil	134.00 mg/cap
30	Chinese Magic Stimulator 中华魔棒	-	西藏金盛利保健品有限公司	Yellowish orange capsule with "金盛利" imprint	Rich highly purified composite oceanic life extract with supreme permeability, Sky Fruit, active compound combines various elements in natural herbal extract. Rich in various amino acids. Proteins, cellulose, VA, VB, VB2, VC, and DHA.	Sildenafil	154.94 mg/cap
						Acetil acid	Not determined
31	Chong Cao Bu Jing Wan 虫草生精丸	-	上海双龙生物有限公司	Black pill	OPP+, L-精氨酸，秘鲁人参 高丽参，可乐子，燕麦秆，尼克酸。	Sildenafil	76.09 mg/pill
32	Chong Cao Duo Bian Wan 虫草多鞭丸	Q/CFS-068-2006	海南長青保健品有限公司	Black pill	鹿鞭，牛睾丸，雄蚕蛾，肉苁蓉，淫羊藿，杜仲，锁阳，龟板，人参，虫草	Sildenafil	137.72 mg/pill
33	Chong Cao Lu Bian Wan 虫草鹿鞭丸 9 g	-	香港金龙生物有限公司	Black pill	冬虫夏草，鹿鞭，人参，枸杞子，肉苁蓉，淫羊藿等	Sildenafil	160.48 mg/pill
34	Chun Qing Xue Sheng Mei 純情學生妹	-	-	Sachet containing white powder	-	No common poison detected	

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
35	Cialis 20 mg	A051038	Eli Lilly and Company Limited	Yellow tablet with “C20” engraver	Tadalafil-20mg,Croscarmellose sodium, Hydroxypropylcellulose, Hypromellose, Iron oxide yellow C177492, Lactose, Magnesium stearate, Cellulose-microcrystalline, Sodium lauryl sulfate, Talc-purified, Titanium dioxide, Glycerol triacetate	Sildenafil	12.69 mg/tab
						Tadalafil	19.59 mg/tab
36	Cialis Tadalafil 80 mg	-	Eli Lilly and Company Limited	Yellow tablet with “C80” engraver	Tadalafil, lactose monohydrate, croscarmellose sodium, hydroxypropylcellulose, microcrystalline cellulose, sodium lauryl sulfate, magnesium stearate, hypromellse, triacetin, titanium dioxide (E171), iron oxide yellow (E172), talc	Sildenafil	80.18 mg/tab

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
37	Cialis 20 mg (bottle)	66018	Eli Llilly Australia Pty Limited	Yellow tablet with "C20" engraver	20mg tablet-tadalafil 20mg, 10mg tablet-tadalafil 10mg, croscarmellose sodium, hydroxypropylcelluose, hypromellose, iron oxide yellow C177492, lactose, magnesium stearate, cellulose-microcry stalline, sodium lauryl sulfate, talc-purified, titanium dioxide, glycerol triacetate	Sildenafil	90.29 mg/tab
38	Da Di Yong Shi 大地勇士	-	-	Brown tablet wth "TL" engraver	-	Sildenafil	114.44 mg/tab
39	Dancing Lady	-	-	Transparent cream	-	No common poison detected	
40	Darling Peculiar Hormone Cream 愛人	-	香港製药有限公司	Transparent cream	-	No common posion detected	
41	De Guo Hei Jin Gang 德国黑金刚	-	西藏拉萨市力龙生物制品有限公司	Black tablet with "C200" engraver	虎鞭，海狗鞭，牛鞭，人参，淫羊藿。	Sildenafil	107.93 mg/tab
42	De Guo Jin Gang Pen 德国金刚喷	-	西藏梅龙保健品有限公司	White and brown bottle	-	Lignocaine	59.0 mg/ml
43	Delay Spray 日本 M88	-	Doc Johnson Enterprises	Black spray bottle containing transparent liquid	Lignocaine based USP	Lignocaine	99.94 mg/ml
44	Doc Johnson Delay Spray 日本 M88	-	Doc Johnson Enterprises	Black bottle	Lignocaine Base USP	Lignocaine	127.0 mg/ml
45	Di Liu Dai Yi Pao Dao Tian Liang 第六代一炮到天亮	QXM06-2003	贵州宏源医药保健品公司	Black and red capsule	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马等	Sildenafil	114.55 mg/cap
						Tadalafil	0.809 mg/cap

S/N	Product name/description	Batch Number	Manufacture	Dosage Form	Ingredients	Adulterant Detected	Content
46	Ding Chuan Tian 顶穿天	-	台湾神龙生物保健品开发有限公司	Blue and white capsule	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马等。	Sibutramine	0.029 mg/cap
						Sildenafil	160.01 mg/cap
47	Dong Fang Bu Bai 东方不败强神粒	-	拉萨市力达保健品有限公司	Brown tablet with "800mg" engraver	-	Sildenafil	76.24 mg/tab
48	Dong Fang Bu Bai 東方不敗	Q/HS02-2000	拉萨市力达保健品有限公司	Red tablet with "800mg" engraver	-	Sildenafil	86.68 mg/tab
49	Eros Delay Spray For Men	-	Savoy Laboratories (International) Ltd	Yellowish orange and black bottle	-	Lignocaine	25.33%
50	Eros Delay Spray For Men	-	Savoy Laboratories (International) Ltd England	Black spray bottle containing transparent liquid	Lignocaine BP 10% w/v	Lignocaine	52.86 mg/ml
51	Exceed Viagra and Cialis 宏圣生物	-	-	Green capsule	-	Sildenafil	25.50 mg/cap
52	F + F Super Enforcement capsule 力加力	-	-	Yellowish orange capsule	-	Sildenafil	127.08 mg/cap
53	Ferity Lily 野百合	-	西藏金聖力保健品有限公司	Transparent bottle with pink cap containing transparent liquid	-	No common posion detected	
54	Fly D5 Yuan Ye 苍蝇水	-	西藏拉萨力发保健食品有限公司	Yellow and transparent bottle containing transparent liquid	-	No common poison detected	
55	French Silver Fox 法国银狐 2800 mg	-	-	Sachet containing white powder	-	No common poison detected	
56	Formula For Man 壮阳极品	-	-	Green capsule with "金聖利" imprint	-	Sildenafil	136.79 mg/cap
						Sibutramine	< 0.003 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
57	Germany Sex Drops	-	Germany-Allemagne Deatschland Posttach	Grey and yellow bottle containing transparent liquid	Water, fructose, flaror, etc.	No common poison detected	
58	Gong Ting Sheng Bao 宫廷圣宝	-	香港天龙生物	Light and dark blue capsule with “天龙制造” imprint	藏红花，冬虫夏草，雪莲花，雪鹿鞭，藏牦牛睾丸，海马等	Sildenafil	81.48 mg/cap
59	Hao Gong Fu 好功夫	-	香港力龙生物技术有限公司	Yellow tablet with “力龙” engraver	蜂房, 淫阳藿等。	Sildenafil	116.93 mg/tab
60	Hei Gua Fu Cui Qing Fen 黑寡妇催情粉 2000 mg	-	-	Sachet	日本 inverma 原粉，淫性草，红参，鹿茸等	No common poison detected	
61	Hei Ma Yi Wang 黑蚂蚁王	-	西藏拉萨市力龙生物技术有限公司	Red and white capsule with “力龙生物” imprint	虎鞭，海狗鞭，牛鞭，人参，淫羊藿等	Sildenafil	101.43 mg/cap
62	Hong Zhi Zhu 红蜘蛛	-	中国深圳祥瑞生物工程有限公司	White and pink bottle	-	No common poison detected	
63	Hua Hu Die 花蝴蝶	-	西藏拉萨情力保健食品有限公司	Red and transparent bottle containing white powder	-	No common poison detected	
				Yellow and transparent bottle containing transparent liquid	-	No common poison detected	
64	Hua Tuo Shen Dan 华陀神丹	-	西藏健民保健品有限公司	Red capsule	鹿鞭，牛鞭，海狗肾，枸杞子，冬虫夏草，肉苁蓉，淫羊藿，巴戟天，秘鲁玛咖等名贵中草药精制而成。	Sildenafil	167.43 mg/cap
65	Huang Di Sheng Dan 黄帝圣丹	-	-	Black pill	-	Sildenafil	49.87 mg/pill

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
66	Huang Wei Lu Rong 皇威鹿茸	-	秦皇岛皇威制药厂	Red capsule	鹿茸	Sildenafil	73.65 mg/cap
						Sibutramine	< 0.003 mg/cap
67	India Doc Johnson Delay Spray	-	Doc Johnson Enterprises	Black bottle	Lignocaine Base USP	Lignocaine	178.0 mg/ml
68	India External Lotion 印度久战油	-	海南平安生物制剂有限公司	Blue bottle	-	No common poison detected	
69	Indian God Lotion 印度神油	-	海南平安生物制剂有限公司	Pink bottle with transparent liquid	-	No common poison detected	
70	Indian Godly Oil 印度神油	-	香港宏发生物保健品有限公司	Brown liquid	冬虫夏草，鹿鞭，人参，牦牛睾丸，雄蚕蛾，肉苁蓉，淫羊藿，杜仲，锁阳，龟板等	No common poison detected	
71	Yin Du Jiu Zhan You 印度久战油	-	鑫威生物制剂有限公司	Purple bottle containing transparent liquid	-	No common poison detected	
72	Ji Pin Lang Yi Hao 极品狼 1 号	Q/LZB-002-2000	黑龙江省佳木斯市高新制药总厂	Blue tablet with “DL” engraver	鹿鞭，牛鞭，人参，海马，海蛇，海星等	Sildenafil	89.28 mg/tab
73	Ji Pin Zhong Hua Wei Ge 极品中华伟哥	-	西藏拉萨市力龙生物制品公司	Green capsule with “力龙生物” imprint	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子，肉桂	Sildenafil	127.79 mg/cap
74	Jin Ba Men 劲霸男人	-	-	Yellowish orange capsule with “KANGLI 速效壮阳” imprint	-	Pill: Naproxen	0.02 mg/cap
						Pill: Lignocaine	0.44 mg/cap
						Capsule: Sildenafil	68.75 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
75	Jin Gang Shen Dan 金刚神丹	-	-	Green capsule with “力龙生物” imprint	-	Sildenafil	158.54 mg/cap
76	Jin Mao Shi Wang 金毛狮王	-	青海格尔木天马保健品有限公司	Brown tablet with “SV” engraver	益智子，阳起石，鹿筋，鹿茸，龟板, 胡桃肉，海马,肉巫蓉等。	Sildenafil	110.91 mg/tab
77	Jing Ba 劲霸	-	中国 香港华人生物工程有限公司	White capsule	人参，鹿茸，海狗鞭，五味子等	Sildenafil	107.15 mg/cap
78	Jiu Chong Tian 九重天	-	九重天生物科技有限公司	Red and beige capsule	-	Sildenafil	117.80 mg/cap
79	Jue Dui Gao Chao Cui Qing Shui 绝对高潮催情水	-	-	Transparent and red bottle containing transparent liquid	-	No common poison detected	
80	K Fen K 粉	-	中国 香港百家乐医药国际集团	Sachet	Brazil hackmatack, betelnut, millet pox powder, peru panax, ethanol etc.	No common poison detected	
81	Kama Sutra	-	USA Standard New York	Transparent vial containing brown liquid	-	Lignocaine	0.12%
82	Kang Li 壮阳极品	-	-	Yellowish orange capsule with “金盛利” imprint	-	Sildenafil	110.03 mg/cap
						Sibutramine	< 0.003 mg/cap
83	Kang Xi Da Di 康熙大帝	-	青海金聖力保健品有限公司	Green capsule with “金聖力” imprint	海狗肾，牦牛鞭，鹿鞭，肉苁蓉，冬虫草，枸杞子，人参，牡蛎等	Sildenafil	88.26 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
84	Kang Xi Da Di 康熙大帝	-	青海金聖力保健品有限公司	Green capsule with "金聖力" imprint	海狗肾，牦牛鞭，鹿鞭，肉苁蓉，冬虫草，枸杞子，人参，牡蛎等。	Sildenafil	126.21 mg/cap
						Tadalafil	169.87 mg/cap
85	King of Postponing 延时王	-	石家庄正天科技开发研究所	Yellow bottle containing transparent liquid	蜂房, 淫阳藿等。	Lignocaine	32.0 mg/ml
86	King Oil 皇帝油	-	-	-	-	Lignocaine	< 0.24 mg/ml
87	Lang Shen Yi Hao 800mg 狼神 1 号	-	西藏拉萨康威保健品有限公司	Brown tablet with "800" engraver	牦牛鞭，鹿鞭，海狗鞭，海马，高丽参，枸杞，淫羊藿等。	Sildenafil	105.77 mg/tab
88	Lang Shen Yi Hao [Jin Wei Ge] 狼神一號 【金威哥】	Q/1LS-049-2001	西藏拉萨康威保健品有限公司	Red tablet with "力达" engraver	牦牛鞭，鹿鞭，海狗鞭，海马，高丽参，枸杞，羊淫藿等	Sildenafil	84.52 mg/tab
89	Lang Wang 狼王	-	香港仁和堂生物研发有限公司	Blue tablet with "DL" engraver	鹿鞭， 藏牦牛睾丸，海马, 生精果，西洋参等。	Sildenafil	77.10 mg/cap
90	Lang Yi Hao 极品狼 1 号 145 mg	-	黑龙江省佳木斯市高新制药总厂	Blue tablet with "DL" engraver	鹿鞭， 牛鞭，人参，海马，海蛇，海星等。	Sildenafil	107.57 mg/tab
91	Lemak Lintah Leech Fat	-	-	Transparent bottle with green liquid	Essence of leeches, leech fats, eeches oil, hirudo medicinalis, cocos nucifera oil, aleurites maluccana .	No common poison detected	

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
92	Lemak Lintah Plus Linang Jantan	-	Fatimah Trading Entreprises	Black bottle containing yellow liquid	Leech fat, anguila mauritiana, essence of leech, nigella sativa, kaempferia galanga, cocs nucifera oil	No common posion detected	
93	Levitra 20	0026K69	-	Orange tablet with "100" engraver	-	Sildenafil	135.96 mg/tab
						Tadalafil	0.30 mg/tab
94	Levitra 20	BXBLJJ1	-	orange tablet with 20 marking		Sildenafil	61.82 mg/tablet
95	Li Jia Li 力加力	-	-	Green capsule	-	Sildenafil	95.48 mg/cap
96	Li Jia Li Chao Qiang Sheng Li Jiao Nang 力加力超强生力胶囊	-	-	Yellowish orange capsule	-	Sildenafil	123.59 mg/cap
97	Li Wei Ruan Jiao Nang 立威软胶囊	-	-	Brown pill	-	Sildenafil	118.31 mg/pill
98	Longer Vaigra 长效伟哥	-	深圳博尔康生物科技有限公司	Blue and white capsule	秘鲁人参，L-精氨酸，高丽参，尼克酸等	Sildenafil	129.86 mg/cap
						Sibutramine	0.061 mg/cap
99	Louis 16	-	美国 Doc Johnson 公司	Black bottle containing transparent liquid	Lignocaine based USP	Lignocaine	83.17 mg/ml
100	Louis 16 Relax Spray	-	-	Black bottle containing transparent liquid	Lignocaine based USP	No common poison detected	
101	Love You Power 老虎油	-	郑州市金阳保健品有限公司	Silver and Yellowish orange bottle containing transparent liquid	海螵蛸，龙骨，五味子，细辛等	No common poison detected	

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
102	Love You 全新 2 代老虎油	-	郑州市金阳保健品有限公司	Silver and Yellowish orange bottle containing transparent liquid	海螵蛸，龙骨，五味子，细辛等	No common poison detected	
103	Lu Quan 鹿泉	-	-	Red and yellow capsule	-	Sildenafil	43.65 mg/cap
104	Magic Power Antiseptic Wet Tissue	-	-	Sachet containing wet tissue	Purified water, ethyl alcohol, fragrance, other active ingredients	Lignocaine	86.06 mg/piece
105	Magic Power Cream		USA Standard New York	White tube containing colorless cream	-	No common poison detected	
106	Magic Power Tissue	-	USA Standard New York	Sachet containing wet tissue	-	Lignocaine	51.45 mg/piece
107	Mei Guo Lan Jing Ling 美国蓝精灵 (bottle)	-	香港艾得生物技术有限公司	Green capsule	Lignocaine (USP) 9.6%	Sildenafil	79.37 mg/cap
						Sibutramine	0.015 mg/cap
108	Mei Guo Mo Bang 美国魔棒 2500 mg	-	香港力龙生物技术有限公司	Green capsule	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子，肉桂等	Sildenafil	90.44 mg/cap
109	Mei Guo Zhan Shen 美国战神	-	香港科龙科技有限公司	Brown tablet	-	Sildenafil	3.64 mg/tab
						Vardenafil	21.19 mg/tab
110	Mei Guo Mo Bang 美国魔棒	z/LZB003-2004	西藏拉萨市力龙生物技术有限公司	Green capsule with “力龙生物” imprint	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子，肉桂等	Sildenafil	130.23 mg/cap
111	Mei Guo Zhan Shen 美国战神	-	西藏美龙保健品有限公司	White, transparent and brown bottle	-	Lignocaine	58.0 mg/ml

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
112	Mei Guo Zhan Shen 美国战神	-	西藏拉萨市力龙生物技术有限公司	Red and yellow capsule	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子，肉桂等	Sildenafil	77.65 mg/cap
						Tadalafil	0.055 mg/cap
113	Mei Guo Zhan Shen 美国战神	50212	香港太和堂生物工程有限公司	Brown tablet with "Viger" engraver	牛，羊睾丸，雄蛾，雪虫草，锁阳，海马，及 Amglish	Sildenafil	146.05 mg/tab
114	Mei Guo Zhan Shen 美国战神	Z/LZB003-2004	香港力能生物技术有限公司	Red and yellow capsule	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子，肉桂等	Sildenafil	78.49 mg/cap
						Tadalafil	0.31 mg/cap
115	Meng Hu Bei Zeng 猛虎倍增	-	-	Red capsule	-	Sildenafil	70.14 mg/cap
116	Midnight Express	-	-	Transparent vial containing brown liquid	Natural herbal extract	Lignocaine	<0.24 mg/ml
117	Migino	-	-	Transparent and green capsule	-	Lignocaine	5.14 mg/cap
118	Minyak Lintah Tapa Plus 60 ml (bottle)	-	Art Soulist SDN BHD	Black bottle containing transparent liquid	-	No common poison detected	
119	Minyak Linteh Power 1	-	PT.MUTIARAEMAS SEMARAUG-Indonesia	Black bottle containing yellow liquid	Hiruda medicinal, leech oil, essence of leech, cocos nuchifera.	No common poison detected	
120	Minyak Lintah Power-Plus	-	Toko Nabila	Black bottle containing transparent liquid	Hiruda medicinal rhizoma smilax, essence of leech, cocos nucifera	No common poison detected	
121	Miraculous Evil Root 魔根	-	-	Yellow tablet with "C120" engraver	-	Sildenafil	120.39 mg/tab

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
122	Mo General 猛男魔根	-	西藏拉萨市多得生物制品公司	Yellow tablet with "C50" engraver	狗鞭，鹿鞭，牦牛鞭，雪山虫草， 藏红花，枸杞子，肉苁蓉，锁阳，玄参，淫羊藿	Sildenafil	136.88 mg/tab
123	Nan Gen Jing Hua Su 男根精华素	-	香港金黄金科技开发有限公司	Blue and white capsule	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马，莲子，肉桂	Sildenafil	104.96 mg/cap
						Sulfosildenafil	-
						Sulfohomosildenafil	-
124	Nan Shi Zhuan Yong 男士专用	-	-	Green capsule	-	Sildenafil	191.43 mg/cap
125	Niu Bian Da Bu Wan 牛鞭大补丸	Q/JDS060-2007	延吉康力保健品有限公司	Opaque ball containing black pill	虫草，牛鞭，黄精，锁阳，龟板，当归，海马等	Sildenafil	135.94 mg/pill
						Tadalafil	161.42 mg/pill
126	Old Captain Sex Drops	-	臺灣梁氏醫藥集團	Transparent bottle containing transparent liquid	L- arginine, aloe vera.	No common posion detected	
127	Ottofol	-	-	Transparent tube containing dark blue cream	-	No common poison detected	
128	Pan Jin Lian You Huo Fen 潘金莲诱惑粉	-	USA 瑞精生物工程有限公司	Sachet containing white powder	-	No common poison detected	
129	Payaraat Spray	PYR-C003	-	Liquid	-	No common poison detected	

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
130	Pfizer Viagra 100 mg	314833021	Pfizer Inc	Blue tablet with “Pfizer” engraver on one side and “VGR 100” engraver on the other	-	Sildenafil	65.40 mg/tab
						Tadalafil	1.04 mg/tab
131	Pfizer Viagra 100 mg (bottle)	04R006A	Pfizer Inc	Blue tablet with “Pfizer” engraver on one side and “VGR 100” engraver on the other	Sildenafil Citrate-100mg, Microcrystalline Cellulose, Anhydrous Dibadic Calcium Phosphate, Croscarmellose Sodium, Magnesium Stearate, Hydroxypropyl Methylcellulose, Titanium Dioxide, Lactose, Triacetin	Sildenafil	62.23 mg/tab
						Tadalafil	0.07 mg/tab
132	Playboy Cream	-	Union & Son Limited PO. Box 432	Orange and white tube containing transparent cream	-	No common oison detected	
133	Posh for Men Only	-	-	Transparent and yellow capsule	-	Lignocaine	18.58 mg/cap
134	Power 1	-	-	Transparent vial containing brown liquid	-	No common poison detected	
135	Power 1 Walnut 动力一号核桃素片	20080106	广州新快力企业有限公司	Pink tablet with “PIS-100” engraver	-	Glibenclamide	98.85 mg/tab
						Sildenafil	1.56 mg/tab
136	Power 1 Walnut 动力一号核桃素片	20070918	广州新快力企业有限公司	Pink tablet with “PIS-100” engraver	-	Sildenafil	68.45 mg/tab
137	Procomil Cream	-	Walter Ritter	Transparent cream	-	No common poison detected	

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
138	PT. Timur SujinTong Wan 舒经通	-	Pi Timur	Red and yellow capsule	-	No common poison detected	
139	Qiang Gen Bao Jiao Nang 强根宝胶囊	-	-	Green capsule	-	Sildenafil	76.43 mg/cap
140	Qiang Jin Shen Qi Yi Li OK 强劲神奇 一粒 OK	-	-	Blue tablet with “VAG” engraver	-	Sildenafil	46.50 mg/tab
		-		Blue tablet with “800mg” engraver	-	Sildenafil	48.46 mg/tab
141	Qiang Jin Wei Ge Wang 500 mg 强劲威哥王	-	-	Blue tablet with “500mg” engraver	海狗鞭，水牛鞭，广狗鞭，羊鞭，鹿鞭，人参，枸杞子。	Sildenafil	34.23 mg/tab
142	Qiang Jin Wei Ge Wang 800 mg 强劲威哥王	-	-	Blue tablet with “800mg” engraver	海狗鞭，水牛鞭，广狗鞭，羊鞭，鹿鞭，人参，枸杞子。	Sildenafil	15.66 mg/tab
						Tadalafil	0.020 mg/tab
143	Qiang Jin Wei Ge Wang 1000 mg 强劲威哥王	-	-	Blue tablet with “1000mg” engraver	海狗鞭，水牛鞭，广狗鞭，羊鞭，鹿鞭，人参，枸杞子。	Sildenafil	29.07 mg/tab
						Tadalafil	0.016 mg/tab
144	Qiang Jin Wei Ge Wang (Chao Nong Suo) 强劲威哥王(超浓缩) 200 mg	-	-	Blue tablet with “200mg” engraver	-	Sildenafil	39.73 mg/tab
145	Qiang Li Wei Ge Wang 强力威哥王	-	-	Blue tablet with “800mg” engraver	-	Sildenafil	33.34 mg/tab
						Tadalafil	13.60 mg/tab

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
146	Qiang Shen Xiao Jin Wan 3 g 强肾小金丸	-	西藏升阳保健品有限公司	Black pill	淫羊藿，枸杞子，东北人参，藏红花，天山雪莲，山楂果，菟丝子。	Sildenafil	105.39 mg/pill
147	Raging Fire Girl	100311	Doc Johnson Enterprises	Transparent bottle containing transparent liquid	L-arginine, aloe vera	No common poison detected	
148	Real Man	-	-	Yellowish orange tablet with “DL” engraver	-	Sildenafil	121.65 mg/tab
149	Reman’s Delay Spray D00Z® 14000 SPRAY	-	Reman Enterprises	Blue spray bottle containing transparent liquid	-	Lignocaine	87.13 mg/ml
150	Rogen Cream	-	Sankyo Zoki Co. Ltd	Black and yellow tube containing transparent cream	-	No common oison detected	
151	Samsu Cream	-	PD. Samsu Jakarta-Indonesia	Transparent bottle containing grey cream	-	No common oison detected	
152	Samsu Super Oil	-	PD. Samsu Jakarta-Indonesia	Transparent bottle containing brown liquid	-	No common poison detected	
153	San Ti Niu Bian 三體牛鞭	Q/WS-9-95	安徽三体医药保健品公司	Blue and white capsule with “勃动力” imprint	黄牛鞭，鹿茸，人参，五味子，枸杞子，菟丝子，淫羊藿，肉苁蓉，龙眼肉等	Sildenafil;	28.02 mg/cap
154	San Ti Niu Bian Bo Dong Li 三體牛鞭勃动力	Q/WS9-95	安徽三体医药保健品公司	Blue and white capsule with “勃动力” imprint	黄牛鞭，鹿茸，人参，五味子，枸杞子，菟丝子，淫羊藿，肉苁蓉，龙眼肉等	Sildenafil	33.2 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
155	Santi Scalper Penis Erection capsules 三体牛鞭	-	安徽三体医药保健品公司	Blue and white capsule with "勃动力" imprint	黄牛鞭，鹿茸，人参，五味子，枸杞子，菟丝子，淫羊藿，肉苁蓉，龙眼肉等	Sildenafil	28.66 mg/cap
156	Sex Fairy 性精灵	-	-	Sachet containing white powder	-	No common poison detected	
157	Shen Bai Jin 肾白金	-	西藏拉萨市力龙生物制品公司	Blue and white capsule with "力龙生物" imprint	-	Sildenafil	189.23 mg/cap
158	Shen Bai Jin Sheng Li Jiao Nang Di San Dai 肾白金生力膠囊第三代	HO5023	西藏拉萨市力龙生物制品公司	Blue and white capsule with "力龙生物" imprint	虎鞭，海狗鞭，牛鞭，人参，淫羊藿等	Sidenafil	137.72 mg/cap
159	Shen Long Jiu Bian 神龍九鞭	SH/02LW006-1997	-	Green capsule	枸杞子，冬虫草，海狗肾，肉苁蓉，淫羊藿，人参，天山雪莲，牦牛鞭，鹿鞭等	Sildenafil	90.59 mg/cap
160	Sheng Jing Jiao Nang 生精胶囊	-	香港天龙生物	Green and light green capsule with "天龙制造" imprint	-	Sildenafil	130.91 mg/cap
						Tadalafil	0.19 mg/cap
161	Sheng Jing Pian 生精片	-	香港天龙生物科技（国际）集团公司	Black tablet with "TL" engraver	-	Sildenafil	123.68 mg/tab
162	Sheng Yang Sheng Wu Yi Li OK 升阳生物一粒 OK	-	-	Yellowish orange capsule with "升阳胶囊" imprint	-	Sildenafil	121.34 mg/cap
163	Sex Power Happy Factor	-	HongKong Jin Sheng Li Health Products Co. Ltd	Blue tablet with"SP150" engraver	-	Sildenafil	91.68 mg/tab

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
164	Shi Bian Wan 十鞭王 1000 mg	-	西藏金圣力保健品有限公司	Blue tablet with "VAG" engraver	牦牛鞭，冬虫夏草，海狗肾，人参，天山雪莲，枸杞子，肉苁蓉，淫羊藿等。	Sildenafil	21.19 mg/tab
165	Shi Wei Hei Bing Pian Wan 十味黑冰片丸	-	西藏聂拉木藏药厂	Black pill	-	No common poison detected	
166	Spanish Fly	-	-	Green bottle containing transparent liquid	-	No common poison detected	
167	Spanische Fliege	-	-	Green bottle containing transparent liquid	-	No common poison detected	
168	Stud 100	-	Stud Holdings Limited	White and Yellowish orange bottle containing transparent liquid	Lignocaine (USP) 9.6%	Lignocaine	252.0 mg/ml
169	Super Cialis 超级西力士	-	-	Yellow tablet	-	Sildenafil	18.18 mg/tab
170	Super is Fierce Male 超级猛男	-	香港天雄医药生物技术有限公司	White capsule with "天雄制造" imprint	Ginseng, Lilac glucoside, Garlic, Lily, Chinese Angelica, Fruit of the Chinese wolfberry, aweto, epimedium leaf, merinda officinalis.	Sildenafil	141.40 mg/cap
171	Super Penis 超级男根 1800 mg	-	西藏升阳保健品有限公司	Yellowish orange capsule	-	Sildenafil	145.09 mg/cap
172	Super Power Pills 1800 mg 力加力	-	Tibet Lhasa Kangwei Biology Corporation	Brown tablet with "SUPER" engraver	-	Sildenafil	147.34 mg/tab

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
173	Super Power Pills 力加力	-	西藏拉萨康威保健品有限公司	Red tablet with “800mg” engraver	牦牛鞭，牦牛精莘，雪莲花精莘，阳起石精莘，淫羊藿精莘等	Sildenafil	151.97 mg/tab
174	Super Stud 007	-	Stud Imperial Ltd	White tube containing transparent cream	Lignocaine (USP) 8.2%	Lignocaine	0.83 mg/g
175	Supra Ginseng	-	Royale Pharmacy New York U.S.A	Orange tablet	American Ginseng, Vitamin A, D, B1, B2, B6, B12, C, E. Nicotinamide. Choline bitartrate, biotin, lysine, methioinine, lecithin, linoleic acid.	No common poison detected	
176	Te Zhi Wu Ye Shen 特制五夜神	-	香港天龙生物科技（国际）集团公司	Yellow tablet with “TL” engraver	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马等	Sildenafil	107.73 mg/tab
177	Tian Di Ying Xiong 天地英雄 2000 mg	-	西藏拉萨神威生物工程有限公司	Pink tablet	Tibetan goat penis, Tibetan ox penis, Tibetan horse penis, deer penis, sea dog penis, Tibetan donkey penis, actinolite, cynomorium.	Sildenafil	149.88 mg/tab
178	Tian Di Ying Xiong 天地英雄	-	天龙生物	Orange and white capsule	-	Sildenafil	112.70 mg/cap
179	Tian Long 天龙	-	天龙生物	Orange and white capsule	-	Sildenafil	109.87 mg/cap
180	Tian Xiong 天雄霸王片	-	-	White capsule with “天雄制造” imprint	-	Sildenafil	56.466 mg/cap
						Sibutramine	< 0.003 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
181	Tian Xiong 天雄保健	-	-	White capsule	-	Sildenafil	127.90 mg/cap
						Tadalafil	6.19 mg/cap
182	Tian Xiong Wei Meng Nan Ren 天雄威猛男人	-	香港天雄医药生物技术有限公司	White capsule with “天雄制造” imprint	-	Sildenafil	126.98 mg/cap
183	Tiang Xiong Zhuang Yang Wang 天雄壮阳王 3000 mg	-	香港天雄医药生物技术有限公司	White capsule with “天雄制造” imprint	鹿鞭，牦牛睾丸, 黄精，锁阳，龟板，当归，海马等	Sildenafil	149.69 mg/cap
						Tadalafil	< 0.003 mg/cap
184	Tongkat Ajimat Madura 快活棒	-	Persatuan Jamu Madura Asli	White herbal stick	-	No common oison detected	
185	Tongkat Ali Ginseng Habbatus Sauda	-	RFL Jamu Corner	Transparent capsule with brownish green powder	Eurycoma longifolia 40%, radix ginseng 30%, nigella sativa 30%	No common poison detected	
186	Tongkat Ali Ginseng Ubi-Jaga	-	Perusahaan Jamu Salina	Transparent capsule with brownish green powder	Eurycoma longifolia 40%, radix ginseng 30%, nigella sativa 30%	No common poison detected	
187	Tosan Splay Daito	-	Daito Medical Co. Osakan JAPAN	Transparent vial containing yellow liquid	-	No common poison detected	
188	Tra Palung Rad	PLR-G012, PLR-G013	V. C. Group	Green bottle containing green liquid	-	No common poison detected	
189	Tuo Yi Nv Lang 脱衣女郎	-	-	Sachet containing white powder	-	No common poison detected	
190	USA Zhan Shen America Vigier 美国战神	QB/MNY011-02-02	内蒙鄂尔多斯市康得保健品厂	Brown capsule	西洋参，田七，雄蛾，牛鞭，狗鞭，蜻蜓，植物花粉等	Sildenafil	147.86 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
191	V6 Tian	-	HONGKONG TIAN XIONG YI YAO SHENG WU KE JI YOU XIAN GONG SI	Green tablet with “TX” engraver	-	Sildenafil	134.12 mg/tab
192	Viagra	04R006A	Pfizer Laboratories	Blue tablet with “Pfizer” and “VGR 100” engraver	-	Sildenafil	125.65 mg/tab
						Tadalafil	1.80 mg/tab
193	Viagra Doc Johnson Delay Spray 伟哥神油	-	美国 Doc Johnson 公司	Black spray bottle containing transparent liquid	-	Lignocaine	87.77 mg/ml
194	Vimax 增大丸 700 mg	-	-	Blue and white capsule	海狗性腺提取物	Sildenafil	127.89 mg/cap
195	Vitgo Oil	-	-	Transparent vial containing brown liquid	-	No common poison detected	
196	Wake 金刚一号	-	-	Yellowish orange bottle	-	Lignocaine	17.000 mg/ml
197	Wei Ba Wang 800 mg 威霸王	-	海口宏大医药保健品公司	Blue tablet with “800mg” engraver	黄牛鞭，海狗鞭，人参，海龙，肉苁蓉。	Sildenafil	40.898 mg/tab
198	Wei Ge 99 偉哥 99	Q/LZB-003-2003	西藏拉萨市力龙生物制品公司	Blue and white capsule with “力龙生物” imprint	虎鞭，海狗鞭，牛鞭，人参，淫羊藿等	Sildenafil	133.13 mg/cap
199	Wei Ge Xing Ba Wang	-	-	Green capsule with “力龙生物” imprint	-	Sildenafil	88.39 mg/cap
200	Wei Meng 威の猛	-	天津市纪元保健用品厂	Silver and black bottle	-	Lignocaine	38.0 mg/ml
201	Wolf 极品狼	-	西藏健民保健品有限公司	Red capsule	秘鲁玛咖，鹿鞭，牛鞭，海狗肾，枸杞子，冬虫夏草，肉苁蓉，淫羊霍，巴戟天等	Sildenafil	166.11 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
202	Wu Di Wei Ge 无敌伟哥	60708	-	Blue and white capsule with “力龙生物” imprint	-	Sildenafil	123.14 mg/cap
203	Wu Ye Shen 五夜神	-	香港天龙生物科技（国际）集团公司	Yellow tablet with “TL” engraver	鹿鞭， 牦牛睾丸，黄精，锁阳， 龟板， 当归， 海马等。	Sildenafil	123.25 mg/tab
204	Wu Ye Shen 五夜神	-	青海格尔木市雪峰生物工程有限公司	Light green and dark green capsule	西洋参，田七，雄蛾，牛鞭，狗鞭，蜻蜓, 狗脊，植物花粉等	Sildenafil	105.61 mg/cap
205	Xi Men Qing 西門慶	Q/LZS-026-2001	西藏拉萨力达保健食品有限公司	Red tablet with “LD” engraver	鹿鞭，狗鞭，海狗鞭等	Sildenafil	85.22 mg/tab
206	Xi Yu Shen Bao 西域肾宝	-	-	-	-	Sildenafil	107.37 mg/tab
207	Xi Zang La Sa 西藏拉萨 500 mg	-	西藏拉萨藏大保健食品公司	Blue tablet with “500mg” engraver	-	Sildenafil	97.29 mg/tab
208	Xin Yi Dai Hei Ma Yi Wang 新一代黑螞蟻王	-	西藏拉萨市力龙生物制品公司	Red and white capsule with “力龙生物” imprint	虎鞭，海狗鞭，牛鞭， 人参，淫羊藿等	Sildenafil	200.02 mg/cap
209	Xin Yi Dai San Ti Niu Bian 新一代三体牛鞭	-	-	Blue and white capsule with “勃动力” imprint	-	Sildenafil	23.04 mg/cap
210	Xing Ba Wang 性霸王	-	-	Green capsule	-	Sildenafil	110.50 mg/cap
211	Ya Run 雅润人体润滑剂 20 g	-	重庆市海洁消毒卫生用品有限负责公司	Blue and transparent tube containing yellow liquid	-	No common poison detected	
212	Ye Lang Shen 夜狼神	-	-	Brown capsule	-	Sildenafil	36.87 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
213	Ye Tai Yang Jiao Nang 野太阳胶囊	-	西藏九重天生物科技有限公司	Red and beige capsule	牦牛鞭，羚羊鞭，海狗肾，藏红花，天山雪莲，枸杞子等。	Sildenafil	41.45 mg/cap
214	Ye Ye Bu Dao 夜夜不倒	-	-	Yellow tablet with “C100” engraver	-	Sildenafil	102.71 mg/tab
215	Ye Lai Xiang 夜来香	-	深圳康妮尔保健品有限公司	Blue tube containing transparent cream	-	Lignocaine	< 0.24 mg/ml
216	Ye Tai Yang Jiao Nang 野太陽胶囊	-	西藏九重天生物科技有限公司	Red and beige capsule	牦牛鞭，羚羊鞭，海狗肾，藏红花， 天山雪莲，枸杞子等	Sildenafil	139.66 mg/cap
						Sibutramine	0.44 mg/cap
217	Yeilus 一粒神	-	香港天龙生物科技(国际)集团公司	Red capsule with “天龙制造” imprint	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马等。	Tadalafil	12.97 mg/cap
						Sildenafil	91.72 mg/cap
218	Yi Li OK 一粒 OK	-	升阳生物	Bright yellow capsule with “升阳胶囊” imprint	-	Sildenafil	119.49 mg/cap
		-		Yellow capsule	-	Sildenafil	126.20 mg/cap
219	Yi Li Shen 蚁力神	-	西藏拉萨罗马保健品公司	Yellow tablet	黑蚂蚁，淫羊藿，人参，冬虫夏草，海马，当归，枸杞子，山羊睾丸，肉苁蓉，胡桃肉，冬从夏草，锁阳等名贵药材。	Sildenafil	117.02 mg/tab
220	Yi Li Wang 蚁力王	-	西藏拉萨神威生物工程有限公司	Orange tablet with “Black God” engraver	黑蚂蚁，鹿鞭，海马，人参，鹿茸等	Sildenafil	83.14 mg/tab

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
221	Yi Li Ting 一粒挺	Q/JH01-2004	青海西宁宏达保健品有限公司	Blue and white capsule	牦牛鞭，鹿鞭，海马，锁阳等	Sildenafil	110.42 mg/cap
222	Yi Pao Dao Tian Liang 一炮到天亮	20070505	贵州宏源医药研究中心	Red and black capsule with "宏远制造" imprint	鹿鞭， 牦牛睾丸，黄精，锁阳， 龟板， 当归， 海马等。	Sildenafil	105.43 mg/cap
223	Yin De Kuai 硬得快	-	西藏拉萨市神力保健品有限公司	Red and yellow capsule	龙眼肉，枸杞子，肉桂，茯苓，冬虫夏草，人参，淫羊藿等。	Sildenafil	14.84 mg/cap
224	Yin Du Shen You 印度神油	-	海南平安生物制剂有限公司	Brown solution	-	No common poison detected	
225	Ying Da Wang 硬大王	-	西藏升阳保健品有限公司	Yellow tablet with "C80" engraver	锁阳，仙矛，牛膝，当归，枸杞子，菟丝子，补骨脂等	Sildenafil	131.41 mg/tab
226	Ying Xiong Sheng Li Pian 英雄生力片	Q/HS 02-2004	拉萨市力达保健品有限公司	Blue tablet	-	Sildenafil	94.75 mg/tab
227	Zang Mi Shen Wei 藏秘神威	-	西藏金源生物工程有限公司	Green capsule	藏羊鞭，藏牛鞭，藏马鞭鹿鞭，海狗鞭， 藏驴鞭，阳起石，锁阳，藏红花，枸杞子， 肉苁蓉，熟地黄等	Sildenafil	151.84 mg/cap
228	Zang Niu Bian 藏牛鞭	Q/WS-9-95	西藏圣源生物保健品公司	Brown capsule	牦牛鞭，鹿茸，人参，淫羊藿，肉苁蓉，狗肾，海狗鞭，海马鞭等	Sildenafil	138.39 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
229	Zang Wang Xiong Ying 藏王雄鹰	-	中国 西藏宇拓健康品有限公司	Blue capsule	Cordyceps 5%, Radix Ginseng 15%, Rhizoma Rhodiola 45%, Stigma Croc 15%, Rhizoa Gymnadenia 20%	Sildenafil	86.94 mg/cap
230	Zang Wang Xiong Ying	-	-	Blue capsule	-	Sildenafil	93.31 mg/cap
231	Zeng Chu Zeng Da 增粗增大 延时片	-	西藏拉萨市藏大保健品有限公司	Blue tablet with "500mg" engraver	-	Sildenafil	98.87 mg/tab
232	Zeng Cu Zeng Da 增粗增大	Q/320583XOS001-2000	西藏拉萨市藏大保健品有限公司	Blue tablet with "500mg" engraver	马苁蓉，肉苁蓉，人参，鹿茸，牛鞭，羊鞭 等	Sildenafil	89.86 mg/tab
233	Zhi Zhu Wang 蜘蛛王	-	西藏升阳保健品有限公司	Pink and transparent bottle containing transparent liquid	-	No common posion detected	
234	Zhuang Gen Jing Hua Su 壮根精华素	-	香港天龙生物科技（国际）集团公司	Red capsule with " 天龙制造" imprint	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马等。	Tadalafil	14.95 mg/cap
						Sildenafil	112.08 mg/cap
235	Zhuang Gen Jing Hua Su 壮根精华素	20070302	香港天龙生物科技（国际）集团公司	Red capsule with " 天龙制造" imprint	鹿鞭， 牦牛睾丸，黄精，锁阳， 龟板， 当归， 海马等。	Sildenafil	107.38 mg/cap
236	Zhuang Gen Jing Hua Su 壮根精华素	-	香港天龙生物科技（国际）集团公司	Red capsule with " 天龙制造" imprint	鹿鞭，牦牛睾丸，黄精，锁阳，龟板，当归，海马等	Sildenafil	102.87 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
237	Zhuang Gen Ying Da Wang 壮根硬大王	-	西藏升阳保健品有限公司	Yellow tablet with “C80” engraver	锁阳，仙矛，牛膝，当归，枸杞子，菟丝子，补骨脂等	Sildenafil	117.45 mg/tab
238	Zhong Han Sheng Wu 中韓生物	-	-	Red tablet with “SUPER” engraver	-	Sildenafil	140.54 mg/tab
239	Zhong Hua Niu Bao 中华牛宝 1800 mg	-	西藏金盛利保健品有限公司	Black tablet	牛鞭，牛肾，人参，淫羊藿，红花，枸杞等。	Sildenafil	115.55 mg/tab
						Tadalafil	0.018 mg/tab
240	Zhong Hua Niu Bian 中华牛鞭	-	中国深圳市康力生物科技开发有限公司	Orange and grey capsule	Collagen, active peptide, mucase, vitamins, multiple acid And over ten micro elements such as calcium, iron, phosphor, strontium, manganese, copper.	Tadalafil	0.072 mg/cap
						Sildenafil	87.11 mg/cap
241	Zhong Hua Niu Bian 中华牛鞭	-	青海省康力生物科技开发有限公司	Grey and orange capsule	黄牛鞭，鹿茸，人参，五味子，枸杞子，菟丝子，淫羊藿，肉苁蓉，龙眼肉等	Sildenafil	117.31 mg/cap
						Tadalafil	0.09 mg/cap
242	Zhong Hua Niu Bian 中华牛鞭 800 mg	-	西藏升阳保健品有限公司	Yellow tablet with “C80” engraver	锁阳，仙矛，牛膝，当归，枸杞子，菟丝子，补骨脂等	Sildenafil	92.73 mg/tab
243	Zhong Hua Niu Bian 中华牛鞭 1500 mg	-	青海省康力生物科技开发有限公司	Grey and orange capsule	黄牛鞭，鹿茸，人参，五味子，枸杞子，菟丝子，淫羊藿，肉苁蓉，龙眼肉等	Sildenafil	97.47 mg/cap

S/N	Product name/description	Batch Number	Manufacturer	Dosage Form	Ingredients	Adulterant Detected	Content
244	Zhong Hua Niu Bian 中华牛鞭	-	青海省康力生物科技开发有限公司	Grey and orange capsule	-	Glibenclamide	13.06 mg/cap
						Sildenafil	93.38 mg/cap
245	Zhong Hua Niu Bian 中华牛鞭	-	青海省康力生物科技开发有限公司	Grey and orange capsule	黄牛鞭，鹿茸，人参，五味子，枸杞子，菟丝子，淫羊藿，肉苁蓉，龙眼肉等	Sildenafil	102.84 mg/cap
						Tadalafil	0.87 mg/cap
						Sibutramine	0.25 mg/cap
246	Zhuang Yang Dan 壮陽丹	-	-	Brown pill	淫羊藿，金樱子，菟丝子， 虎鞭等	Vardenafil	0.10 mg/pill
247	Zhuang Yang Dan 壮陽丹	K/PB/08-2002	-	Brown pill	淫羊藿, 金樱子，菟丝子，虎鞭 等	Sildenafil	1.95 mg/pill