

Determination of synthetic drugs as adulterant in herbal anti-diabetic medicines by HPLC

A Project submitted

by

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To my parents

Acknowledgement

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Certification Statement

This is to confirm that this undertaking titled "Determination of synthetic drugs as adulterant in herbal anti-diabetic medicines by HPLC" is submitted for the incomplete satisfaction of the necessities for the level of Bachelor of Pharmacy (Hons.) from the Department of Pharmacy, BRAC University. This project constitutes my own work under the supervision of Associate Professor Dr. Hasina Yasmin, Department of Pharmacy, BRAC University and this venture is the consequence of the creator's unique research and has not beforehand been submitted for a degree or certificate in any college. To the best of my insight and conviction, the undertaking contains no material beforehand distributed or composed by someone else with the exception of where due reference is made in the venture itself.

Signed,

Counter signed by the supervisor

Abstract

Herbal medicines are increasingly being used in both preventative and treatment-based medicines and tonics because consumers perceive herbal drugs as “natural” safe, harmless and free from adverse side effects. However, adulteration of herbal drugs is very common nowadays and counterfeit drug manufacturers thrive success by exploiting the trust of the patients on herbal medicine. As diabetes is one major chronic disease, patients with diabetes comply with herbal medicines almost all the time for overall well-being and better control of the disease. This makes anti-diabetic herbal drugs an easy target for the counterfeiters. What patients fail to understand is, herbs are filled with numerous bioactive molecules which can interact with the prescription oral hypoglycemic drugs and can lead to serious adverse effects. Furthermore, diabetic patients require multiple drug therapy, which put them at risk of frequent drug interactions between the herbal drugs and the synthetic medicines. In the worst-case scenario, taking herbal drugs that are adulterated with synthetic oral hypoglycemics along with prescription oral hypoglycemics can lead to overdose and acute hypoglycemia followed by death. Therefore, this experiment is aimed at determining the presence of synthetic oral hypoglycemics in herbal anti-diabetic formulations. Herbal anti-diabetic drugs available at the local market of Dhaka were collected as samples for the experiment. Metformin and Glimpiride were considered as reference standards for the experiment. Samples and standards were subjected to HPLC for analysis with acetonitrile: Phosphate buffer (pH 2.1) at a 70:30 ratio as the mobile phase. This mobile phase ratio provided effective elution of both of the standards at distant retention times, which helped with clear identification and identification of both of the standards. The devised method was also successful in clearly identifying and quantifying substances present at the samples (herbal anti-diabetic drugs). From 25 tested samples 16 samples confirmed the presence of adulterants which concluded that 64% of the samples were adulterated. This high rate of drug adulteration inside the capital gives an easy assumption of equal or higher rate of drug adulteration in the outskirts areas and these calls for more sensitive QC methods and stricter regulations to control such act.

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List of Acronyms

AUC- Area Under the Curve

DM- Diabetes Mellitus

EHTA- European Herbal and Traditional Medicine Practitioners Association

FDA- Food and Drug Administration

FPG- Fasting Plasma Glucose

GIT- Gastrointestinal Tract

GLP- Glimepiride

HbA1c- Hemoglobin A1c

HL- Hyperlipidemia

HPLC- High Performance Liquid Chromatography

HT- Hyper Tension

IFDA- Iran Food and Drug Administration

IPI- Intentional Intellectual Property Institute

IMPACT- International Medicinal Products Anti-Counterfeiting Taskforce

IR Spectroscopy- Infrared Spectroscopy

LADA- Latent Auto Immune Diabetes in Adults

MEP- Methyl Ephedrine

MET- Metformin HCL

MHRA- Medicines and Herbal Products Regulatory Agency

MRNA- Messenger Ribonucleic Acid

MS- Mass Spectrometry

NEP- Nor Ephedrine

NIMH- National Institute of Medical Herbalists

NMR- Nuclear Magnetic Resonance

NSAID- Non- Steroidal Anti- Inflammatory Drugs

OGTT- Oral Glucose Tolerance Test

OST- Opioid Substitution Treatment

OTC- Over The Counter

PDA- Photo Diode Array

PDE-5i- Phospho Diesterase Inhibitor-5i

PDMA- US Prescription Drug Marketing Act

PSE- Pseudoephedrine

RP-HPLC- Reverse Phase High Performance Liquid Chromatography

RT- Retention time

SARS- Severe Acute Respiratory Syndrome

SERS- Surface Enhanced Raman Spectroscopy

TLC- Thin Layer Chromatography

TNF- α - Tumor Necrosis Factor α

USP- United States Pharmacopoeia

UV-Vis – Ultra Violet and Visible

WHO- World Health Organization

WPRO- West-Pacific Regional Office

Chapter 1 Introduction

1.1 Herbal Drug

Herbal drugs are “Plant-derived materials or products with therapeutic or other health benefits which contain either raw or processed ingredients from one or more plants” (WHO, 1998). According to WHO, there are three types of herbal drugs that are generally common in practice which include crude plant materials, processed plant materials and medicinal herbal products.

Crude plant materials: These plant materials that are marketed in fresh or dry form as a whole or essentially cut into small pieces. These plant parts are consumed or applied in their original form without subjecting them to any further processes (Figure 1.1). Drugs in this category do not require any prescription prior administration.



Figure 1.1 Crude plant materials used in therapies

Processed plant materials: Plant materials that are subjected to customary processes for making them more effective, aiding clinical effectiveness, or for creating therapeutic preparations (WHO, 1998) e.g. infusing herbs into carrier oils etc. are known as processed plant materials (Figure 1.2).



Figure 1.2 Processed plant materials (herbs infused into carrier oils)

Medicinal herbal products: When crushed plant constituents, extracts, completely purified extracts, or partly purified active substances isolated from plant materials are converted to finished and labeled pharmaceutical products, they are known as medicinal herbal products (Figure 1.3). It is to be noted that, medicines that are prepared by combining plant material with chemically defined active substances, isolated plant constituents, are not regarded as herbal medicines (WHO, 1998). These medicines usually require prescription prior administration.



Figure 1.3 Medicinal herbal products

1.2 History

The heinous act of deceiving people by selling them synthetic drugs under the illusion of pure herbal drugs, has been going on in several countries for years now. What makes it so easy to defraud people in the name of herbal drugs is their strong faith in the system of herbal medicines. It's understandable because people have been turning to herbal resources for therapeutic purposes for a long time (from the ancient B.C period).

Ethnographic and archeological sources can be regarded as a trustworthy source of evidence regarding the history of herbs in medicine. According to those, the first use of plants for treatment purpose was recorded by Shen Nung in his book *Pen Ts'ao* in 2800 B.C. Sovereign Shen Nung made an assembly of barren restorative floras. He is still accredited with the disclosure of tea, also a large number of plants substances which are utilized today: ephedra, cinnamon, camphor, rhubarb, and also incredible yellow gentian (Cragg et al, 2013). Next up is the clay tables found in Mesopotamia in 2600 BC that both describes and shows the therapeutic application and galenical form. Commonly used herbs during that time were castor oil, poppy juice, coffee, cypress, licorice, myrrh (Tasic' et al, 2009). In 400 B.C the Greeks entered into the world of herbal medicine. Then in 50 A.D, the use of herbal medicines spread the Roman Empire, which also started the commerce of cultivating herbs for medicinal purposes. Later on, in 200 A.D the pairing of usual illnesses with their herbal remedies emerged for the first time which was done by a Greek physician named Galen (Figure 1.4). In addition, Hippocrates (460-377 BC) from Greece who is known as the father of medicine, published *Corpus Hyppocraticum* where he schematized the overall medical and pharmaceutical experience. The first botanical garden was established by botanist Theophrastus (371–286 BC) along with students in Athens. He also explained the therapeutic and toxic effects of more than 500 plants with medicinal properties e.g. monkshood, cardamom, false hellebore, cinnamon etc. (Ptrovska, 2012). After that the use herbal medicines increased greatly covering larger areas e.g. in treating sickness in most monasteries and infirmaries (800 A.D). The Arab world turned into a focal point of medicinal impact in 1100A.D when Physician Ibn Cenna composed the *Canon of Medicine*, which specifically acknowledged the significance of plant medicines (Philip, 1992). With this constant increment in the utilization of natural medications, botanists were advanced and bolstered by Henry VII of England and the Parliament in 1500A.D (Wichtl et al, 2004). Around 1700A.D, plant medicines got an additional

prestigious approval from reverend Charles Wesley. He promoted balanced diet, proper hygiene and herbal treatments for a healthy living.

In the progressing years, pharmaceuticals started becoming more popular which was a setback for the plant medicines. Anyway, as adverse effects from the medications started to be archived, home grown cures came into support once more. The National Alliance of Medicinal Herbalists was created, and retitled the National Institute of Medical Herbalists (NIMH.) in 1800 A.D in the future. Later on, during World War I in 1900 A.D. the utilization of herbal medicines escalated again due to the deficit of synthetic drugs. With the end of the war, creation of pharmaceuticals expanded and penicillin was found. Herbal medicine professionals had their rights to administer their medicines taken away and after that reestablished. The establishment British Herbal Medicine Association and creation of the British Herbal Pharmacopeia also occurred during that time. Individuals started to show the worry over expansive occurrences of adverse effects and ecological effect of medications from 1950s. Later on, in 2000 A.D.EU made a move on the regulation and quality control tests of herbal drugs like those utilized intended for pharmaceuticals. Accordingly, the herbal medicines came to work from old occasions and right up 'til today these medicines have picked up another force in the therapeutic field.

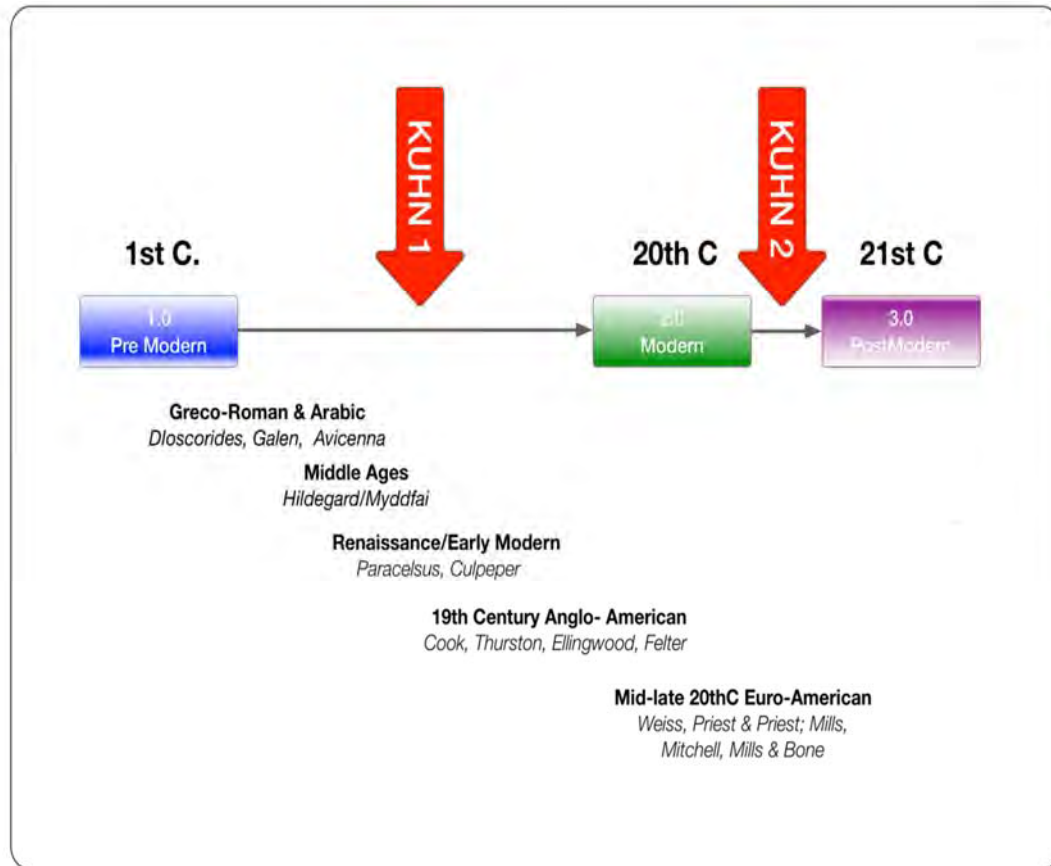


Figure 1.4 Journey of herbal medicines

Due to this long-term contribution of herbs in medicine people have placed their trust on herbal medicines as a safe means to treatment.

1.3 Herbal medicine in the treatment of chronic diseases

Due to pharmaceutical medications' damaging side effects and dependence they turned out to be less favored by people and they are inspired to seek substitutes for the contemporary therapeutic interventions. In USA, about 8% of total clinic admissions are contributed by the side or adverse effects of synthetic medication (Philomena,2011). About 100,000 patients die yearly as a result of drug toxicities. Which indicates that, number of deaths in U.S. by pharmaceuticals is thrice as more than number of deaths by drunk chauffeurs. Thousands of individuals also die every year from allegedly "safe" OTC drugs. Demises or hospital admissions caused by herbal drugs are too rare to find. Also, the category for adverse effects caused by herbs is nonexistent in the database of National Center of Poison Control, USA (Nasri, 2013). Thus, individuals consistently turn to natural medications since they trust that plant cures are free from unwanted reactions (Haq, 2004;

Nasri, 2012). So, at the point when numerous individuals look for alternative medications and start to look at customary and Eastern medicines, herbs are winding up to be more famous. For the same reason, physicians are also seeking newer and safer treatments for many common illnesses by revisiting the traditional remedies, which uses herbal medicines. Enhancing, and keeping up a healthy life style by natural means is turning into an exceptionally famous way to achieve overall fitness, making the herbal medicines a preferred choice of the patients (Sturluson, 2014). Also, many believe that therapy with herbs is a therapy that is holistic, which integrates mental, emotional, and spiritual levels. Utilizing herbs mostly does not include unfavorable impacts. Clearly, educational understanding of the impacts of restorative plants and additionally finishing a clinical trial to understand proper therapeutic effect is important.

Also, herbal medications generally coordinate their effects towards helping the body's own recuperating procedure. These drugs typically act tenderly, supporting the body's systems and processes that became inadequate or act to aid expel abundances that rendered prevalent. Side effect alleviation is only a segment of medicinal herbs' helpful techniques. Such as, arthritis is typically treated with steroidal anti-inflammatory medicines that possess far-reaching aggravating unfavorable effects. Treating these conditions with herbs results in saturating of dry synovia, stimulation of circulation at affected districts, assistance of excretion through kidneys and hepatic/biliary systems, changes in digestion and furthermore (Philomena, 2011; Haq, 2004; Kazemipoor, 2012). For this general recuperating procedure of plant prescriptions numerous individuals who are now under pharmaceutical treatments, additionally include natural meds in their treatment since they accept medicinal herbs have agonistic effects or trigger synergistic actions. Herbal medicine practitioners additionally trust a similar idea that the primary indications of diseases are not usually treated by herbal drugs but instead they are focused aiding other body systems that are accentuated by side effects. This way the body is enabled to recoup its condition and healing capability so that the body would be capable to harmonize such abilities for mending the displaying ailment (Tavafi, 2013; Mardani et al, 2013).

Thus, it's reasonable when patients with constant maladies (Arthritis, Diabetes, Asthma and so forth) requiring long haul treatment, are normally inclined to take herbal drugs since they can mend them comprehensively and have uncommon events of antagonistic impacts. Also, according to statistical studies, herbal medicines are found to be the most preferred form of complimentary treatment among patients (Figure 1.5).

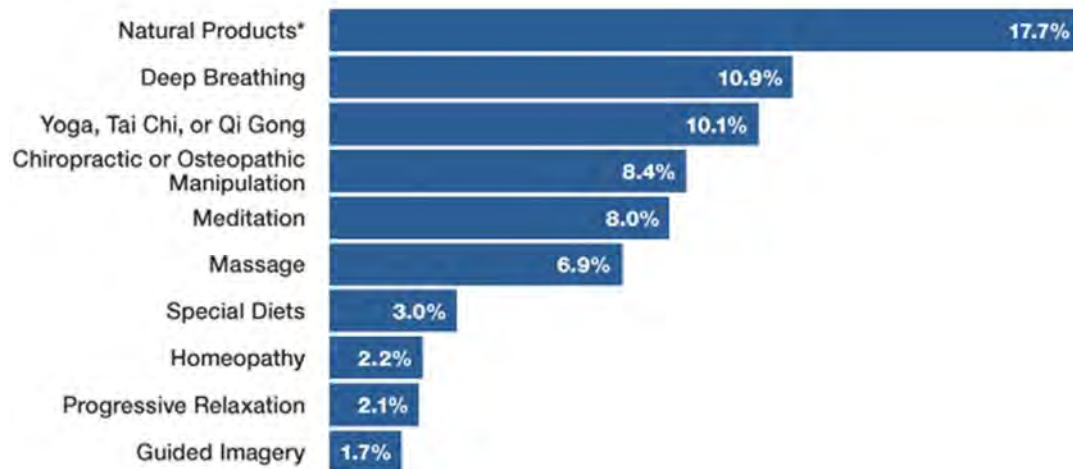


Figure 1.5 Herbal medicine as most preferred form of complimentary treatment (Clarke & Black, 2015)

As per a cross-sectional descriptive examination directed in Hasköy Outpatient Clinics of Family Medicine Department of Dışkapı Yıldırım Beyazıt Training and Research Hospital in Ankara, it has been demonstrated that use of homegrown medication was observed to be higher among patients who had been determined to have constant maladies (Tulunay et al, 2015). A sum of 217 patients having hypertension (HT), diabetes mellitus (DM), and hyperlipidemia (HL) were incorporated into this examination. An up close and personal questionnaire was directed to the members. Questionnaire used in the experiment comprised of data about chronic diseases, socio-demographic highlights, natural medicine and conventional medicines utilization. Consistence to traditional medication utilize was characterized as submission and non-submission whether the patients consistently had gotten their endorsed medicines as indicated by the prescribers' directions. Furthermore, information about the identity of the herbal medication, the reasons behind natural medicine utilization, conviction regarding effectiveness, learning of latent unfavorable impacts, and by whom (e.g., families or companions, media or medicinal services providers) its utilization was suggested, were gathered. Among the 55 males (25.4%) and 162 females (74.6%) patients, 35 patients (16.1%) had DM, 59 patients (27.2%) had HT, 7 patients (3.2%) who HL, and there were 116 patients (53.5%) who had in excess of one ailment. The quantity of homegrown drug clients was 63. They discovered that roughly 33% of the patients had utilized homegrown medication for the treatment of perpetual illnesses.

1.4. Herbal medicines in treating diabetes

1.4.1 Diabetes

Definition

“Diabetes mellitus is a general term for heterogeneous disturbances of metabolism for which the main finding is chronic hyperglycaemia. The cause is either impaired insulin secretion or impaired insulin action or both.” (Kerner & Brückel, 2014). In other words, diabetes is the abnormal homeostatic state of carbohydrates and fats caused by improper regulation of hormones (Gadapa & Tripathi, 2012).

Classification

I. Type 1 diabetes: This occurs when the β cells of our pancreas are destroyed by auto immune responses. As the β cells are being destroyed, it renders the patient completely deficient in insulin and hence insulin has to be provided to the patient from in vitro sources. For this reason, this type is also known as insulin dependent diabetes. Latent auto immune diabetes in adults (LADA) is also grouped as type I diabetes.

II. Type II diabetes: This is mainly caused by insulin resistance and also relative insulin deficiency. It is much of the time related with different issues of the metabolic disorders.

III. Diabetes triggered by disease conditions: There are several unfortunate disease conditions that can render a patient diabetic. For example, diseases related to exocrine pancreas (pan-creatitis, cystic fibrosis, hemochromatosis), Endocrinopathies (e. g. Cushing syndrome, acromegaly, pheochromocytoma), Drug induced (e.g .glucocorticoids, neuroleptics, alphasinterferons, pentamidine), Genetic defaults of the β -cell activity (e. g. MODY forms), Genetic defects of improper insulin action caused by genetic disorders (Kerner & Brückel, 2014).

IV. Gestational diabetes: Hyperglycemia that is observed during pregnancy.

Diagnosis

The methods that are standardized and quality guaranteed can only be applicable for estimation of venous plasma glucose and HbA1c. According to the current best quality method of diagnosis the glucose in venous plasma is measured for diagnosing diabetes -. This measurement will be exact just if glycolysis can be inhibited in the sample right after

the sample is taken. This should be possible in two different ways. Either the blood tube is put away on ice and the blood is centrifusion of blood is done amid 30minutes, or glycolysis in the tube is viably hindered by appropriate added substances (citrate in addition to fluoride; fluoride by itself isn't adequate). The glucose levels expressed in the practice guidelines is applicable to venous plasma. (These levels relate to the ones recommended by the Deutsche Gesellschaft für Klinische Chemie und Laboratoriumsmedizin (DGKL) and the Deutsche Diabetes Gesellschaft (DDG) (Kerner & Brückel, 2014). For more clear understanding, recommended guidelines for diagnosis are displayed in Figure 1.6.

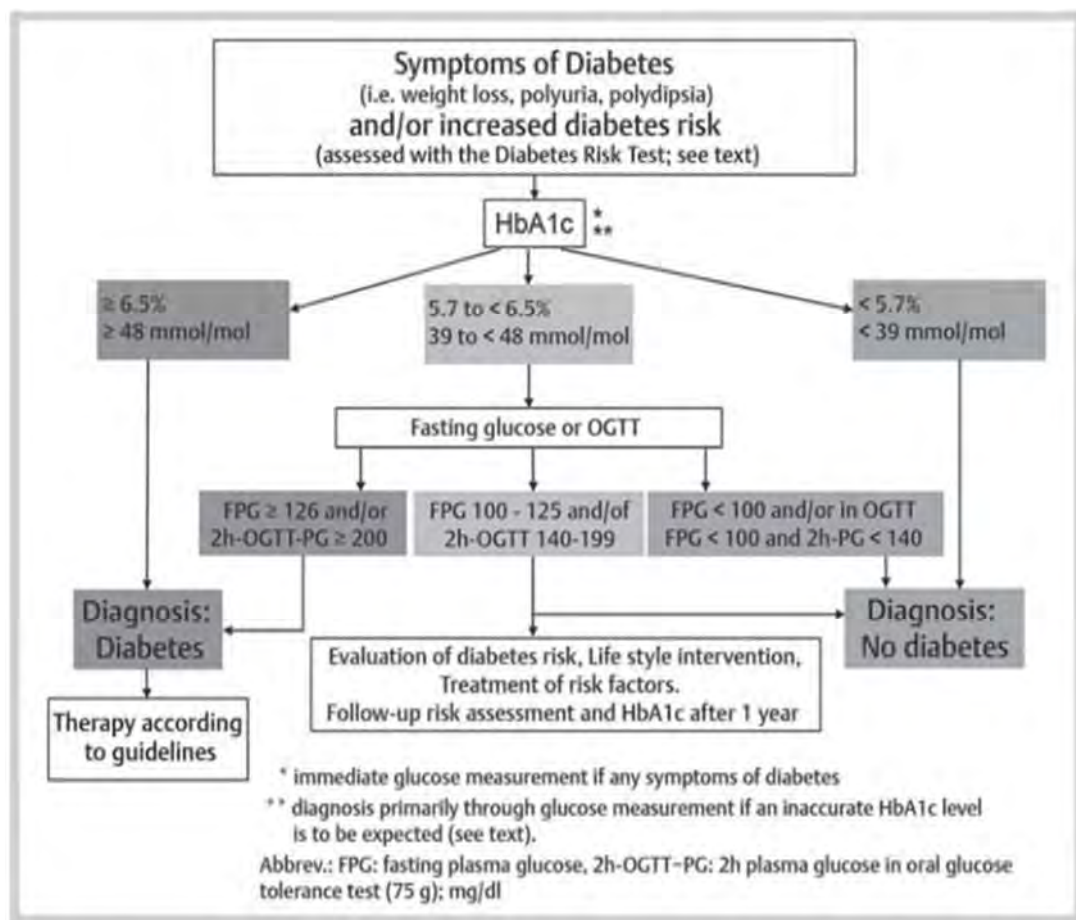


Figure 1.6 Diagnosis method according to guidelines (Kerner & Brückel, 2014)

1.4.2 Anti -diabetic herbal drugs

Allopathic medications utilized for the treatment of diabetes have their own reaction and unfavorable impact like hypoglycemia, queasiness, retching, hyponatremia, tooting, looseness of the bowels or blockage, liquor flush, cerebral pain, weight increase, lactic

acidosis, malignant frailty, dyspepsia, unsteadiness, joint torment. So rather than allopathic medications, homegrown medications are an appreciable decision, since they have less to no unfavorable impacts (Kokar & Mantha, 1998). Ethno plant data distinguished around 800 Indian plants which may have antidiabetic potential (Gupta et al, 1986). All the herbs detailing was acquired from nearby, legitimate herbs provider shops, had some expertise in deal of therapeutic plants and kept running by the Ayurvedic experts as OTC Ayurvedic medications. Despite the fact that correlative and elective prescriptions medicines are prevalent, logical proof help their application to diabetes mind is rare (Tripathi K.D, 2007). Past CAM diabetes studies has by and large centered on single modalities yet CAM professionals all the more normally endorsed complex, multi dietary mediation. Ayurvedic mediations may benefits patients with higher baseline HbA1c value, justifying further research (Yadav et al, 2002).

For these multiple benefits herbal anti diabetic drugs are a major part of the total diabetes management system and the market of these drugs are only growing with the inventions of newer plants having hypoglycemic properties.

1.5 Market size of herbal drugs

1.5.1 Global market size

The worldwide natural prescription market measure was esteemed to be 71.19 billion USD in the year of 2016 and is required to show beneficial development within the gauge time frame (Figure 1.7). The expansion is credited to the expanding inclination of shoppers to customary pharmaceuticals (Traditional Chinese, Ayurveda and Unani Medicine). Furthermore, escalating substantial exploration speculations and financing is hoped to strengthen the market development soon (Hexa Research's, 2017)

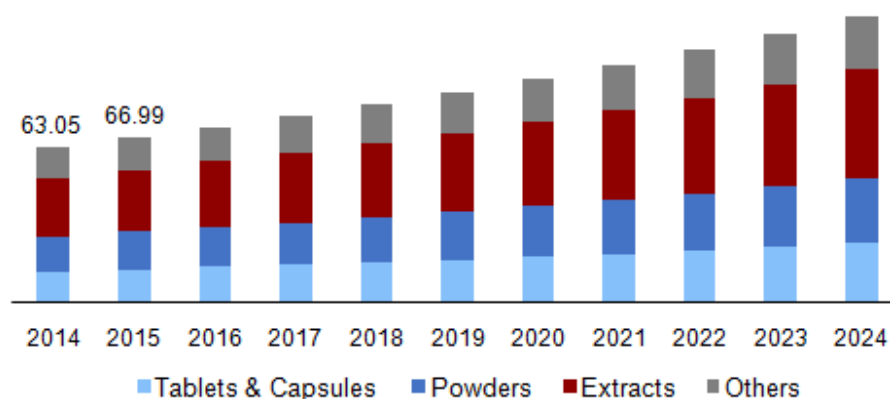


Figure 1.7 Worldwide herbal drug market income, by product, 2014 - 2024 (USD Billion) (Hexa Research's, 2017)

The worldwide plant items market is conjectured to rise over the anticipated period because of their cheaper price range in contrast to allopathic medicines. Such medicines are getting enormous thought at a universal level. Say, these drugs were applied to heal severe acute respiratory syndrome (SARS) in China. Escalating disposition for low budget treatment methods for different therapeutic states, for example, seasonal fever, kidney problems, stomach related issues and congestive chest, is foreseen to upgrade the further acknowledgment of these prescription structures around the world (Hexa Research's, 2017).

Like the allopathic medicines the herbal drugs are also marketed in various dosage forms e.g. tablets and capsules, powders, extracts, pastes, oils and gels. Capsules and tablets give measurements exactness compared to other dose frames. Subsequently, the section is required to record the speediest development over the conjecture time frame (Li, 2000). Poor administrative system over the globe and less number of establishments giving information of home grown therapeutics because of absence of important research data are required to thwart the market development.

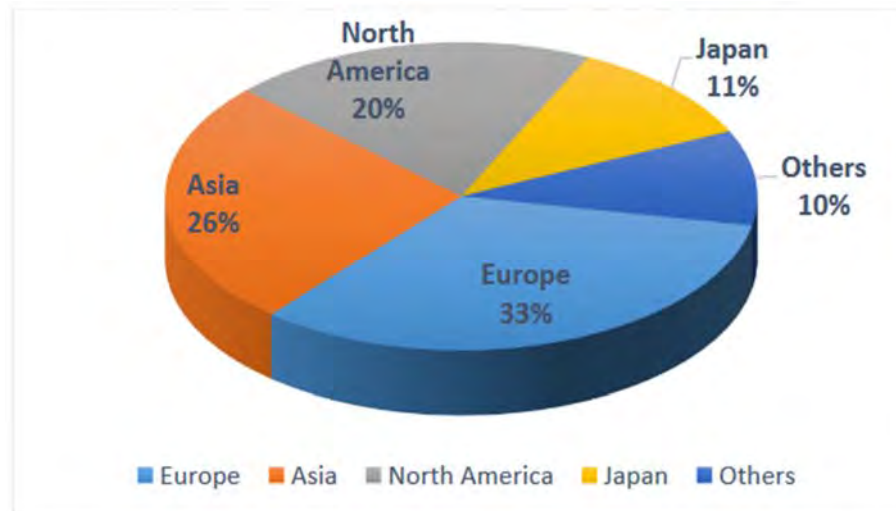


Figure 1.8 Herbal medicine market size in different countries (Nirmal et al, 2017)

In the recent years, Europe is believed to demonstrate the speediest development over the anticipated period because of expanding awareness regarding these items which incorporate medications, nutritional supplements, and skin care items (Figure 1.8). China and India being significant markets for home grown therapeutic items in the locale, these nations have a solid foundation. Also, concurring to World Health Organization (WHO) around 80% populace of most developing nations like Asia, Africa, Latin America and so forth still depend on customary natural solutions for their essential wellbeing (Bannerman, 1982). The Ayurveda framework from India has been giving treatment choices to different scatters identified with the respiratory framework and GIT since ages. Moreover, the continuous pattern of customary pharmaceutical utilization is believed to help the development of the market.

The worldwide market is exceedingly divided in nature because of the nearness of little players. Moreover, the utilization of restorative plants on a household level makes the business disorderly. Existence of numerous local players restricted to their district has an overwhelming portion. Furthermore, the significant partners in the business incorporate TSUMURA and CO., Dr. Willmar Schwabe India Pvt. Ltd., Blackmores and NATURE'S ANSWER. The makers are focusing on enhancing the effectiveness of home grown items. Besides, innovative progressions, for example, enhanced extraction procedures and gear conveyed by worldwide players and additionally local players to keep up the item quality in this way rendering market more stable (Shanmugakumar, Nakasthra, Mamatha, Yamuna, & Swamy, 2015).

1.5.2 Market size in Bangladesh

With growing appreciation of herbal medicines around the world along with increasing rate of consumption of these drugs, Bangladesh has also shown enthusiasm towards herbal medicines as a form of alternative therapy. Another main reason behind such enthusiasm is the extremely wealthy in Bio-assorted variety of the country. It has in excess of 500 therapeutic plants species (Yusuf et al., 1994) where a large portion of the basic therapeutic plants are available throughout the nation.

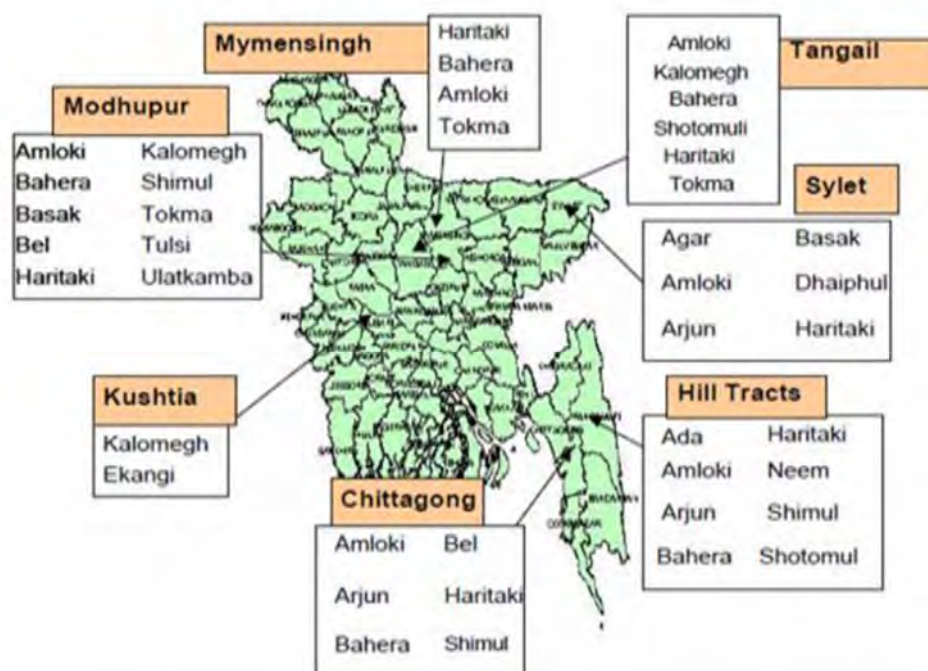


Figure 1.9 The major medicinal production zone in Bangladesh (BFTI, 2016)

About 30 kinds of restorative plants are accounted for to be developed in the Natore area, and among them Aloe Vera (Ghridakumari) is generally mainstream. Other plants, to be specific, Misridana, Bhuikumari, Shimul mul, Shotomul (*Asparaus racemosus*) Kalomegh (*Chiretta*), Nilkantha, Ashwagandha (Winter Cherry), and Rajkantha have been set up as profitable bonanzas. Around 65 sections of land are secured by 10 species (Shahidullah & Haque, 2010). Additionally, agriculturists create Tulshi (Sacred Basil), Basak (Malabarnut or Vasak) and so on. Moreover, these plants are also utilized as crude materials in getting ready pharmaceuticals and also shipped in the worldwide market. In expansion, ranchers are developing distinctive sorts of restorative herbs in Mymensingh, Tangail, Sylhet, Modhupur, Kushtia and Chittagong Hill Tracts. The diverse source of therapeutic herbs in

this country also inspires the existence of a lot of herbal drug manufacturers. An expansive number of home grown and restorative production lines have flourished with a yearly utilization of around 20,000 tons of therapeutic herbs. Till now one-fourth of synthetic medications are produced using restorative herbs. At present, our country has a market of approximately TK 300 crore value of natural or conventional items yearly (Jahan, 2016).

Currently there are 17 authorized herbal medication makers in the nation for delivering home grown meds. Square Herbal and Nutraceuticals, an undertaking of Square Group, is a forerunner in such segment. Other authorized organizations are Acme Laboratories, Modern Herbal Pharmaceuticals, Drug International and Hamdard Laboratories (Waqf) Bangladesh, Neptune Laboratories Ltd., Holy Food and Beverage Ltd., Sadhana Ayurvedic. The home grown pharmaceutical market is gigantic both locally and universally. The worldwide natural solution advertise is developing at 15-20 percent consistently - the most elevated development is in Germany & USA" said MM Asad Ullah, group product manager of Square. He additionally specified that, in the early ages they used to target least developed countries for exporting but they are trying to export to EU US market as well being inspired the growing interest of herbal drugs there. The business individuals said the synthetic pharmaceuticals advertise in Bangladesh is worth around Tk 4,000 crore, while the market measure for natural drugs along with unani and ayurvedic

Nation wise earning by the exportation of Bangladeshi therapeutic plants is presented in Table 1.1 and Figure 1.10

Table 1.1 Nation-wise export incomes of Bangladesh's curative herbs (BFTI, 2016)

Countries	Export value in 2013-2014	Export value in 2014-2015	Growth rates (%) During 2014-2105
Vietnam	413,975.05	317,578.89	-23.29
United Arab Emirates	155,767.61	30,894.49	-80.17
Myanmar	5988.91	22,409.94	274
Thailand	6552.72
Oman	6068.20
Romania	2019.83
Malaysia	510.62	1990.06	289

United Kingdom	57.61
Austria	2610.77
India	8116.39
Singapore	13857.44
Yemen	1634.55
Total	599850.57	390182.52	34.95

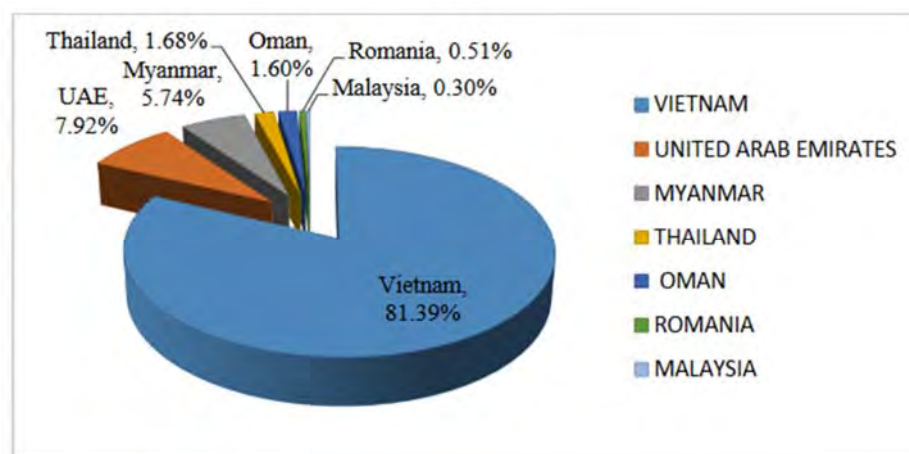


Figure 1.10 Major nation-wise fraction stocks in overall export worth of curative herbs (2014-2015) (BFTI, 2016).

Bangladesh being one of the countries with large numbers of patients diagnosed with diabetes mellitus, anti-diabetic herbal drugs has been a major focus of the herbal drug manufacturers for quite some time now. Herbal drug manufacturers of Bangladesh serve a large group of people in this country with their massive product lines and anti-diabetic preparations cover a major part of that. A few those ant diabetic products that are currently ruling the market are as follows.

- | | |
|--|----------------------|
| 1. Tablet DOLABI® Natural medicine for diabetes. | 11. Diacare |
| 2. Natrium Sulfuricum Capsule | 12. Diagym |
| 3. Mushroom Gynura Capsule | 13. Alisa |
| 4. Glucium | 14. Robi Cap |
| 5. Diarex | 15. Ziabit |
| 6. Diabigo | 16. Diacare |
| 7. R-Diatech | 17. Diagym |
| 8. Diabit | 18. Insu Control |
| 9. Chiratan | 19. Insu Tab |
| 10. Diavion | 20. Fenugreek powder |

The plants in Table 1.2 are the most commonly used plants for the production these drugs (Rafe, 2017).

Table 1.2 Commonly used medicinal plants in Bangladesh against diabetes

Species name	Family	Bengali name
A. paniculata	Acanthaceae	Kalomegh
A. conyzoides	Asteraceae	Oochant
S. chirata	Gentianaceae	Chirata
T. arjuna	Combretaceae	Arjun
A. indica	Meilaceae	Neem

1.6 Counterfeit drugs

The list of herbal medicines that are preferred and appreciated by the people goes a lot further giving us an idea of how large their market size in this country and how widely appreciated these are by the patients. Due to their enormous market size and wide acceptability among the people as natural remedies for ailments, the authenticity and integrity of the neutrality of these products are of great concern. According to WHO “*counterfeit medicines are those medicines that are described as deliberately and fraudulently mislabeled with respect to source and/or identity. Counterfeiting can apply to both generic and branded products. Counterfeit products may include: Products with the correct ingredients, products with the wrong ingredients, products without ingredients, products with incorrect quantities of active ingredients, or products with fake packaging.*”

1.6.1 Types of adulteration of drugs

The WHO has sorted fake medications into seven categories in general (Wright, 2006).

These incorporate-

- I) False packing, right amount of right compound (clone).
- II) False packing, incorrect compound.
- III) False packing, absence of active compound.
- IV) Counterfeit packing, off base amount of right compound.
- V) Proper packaging, incorrect compound.
- VI) Proper packaging, absence of active compound.
- VII) Certified packing, off base amount of right compound.

The following three scenarios are the most common ways of how adulteration occurs in herbal medicines-

I. Herbal medicines adulterated with undeclared synthetic pharmaceuticals

The basic synthetic adulterants in herbal drugs are steroids, drugs for erectile dysfunction anti-histamines, NSAIDs, thinning pills and anti-diabetic drugs.

II. Herbal medicine with undeclared heavy metals

According to Medicines and Herbal Products Regulatory Agency (MHRA), it has been proven that heavy metals like arsenic lead and mercury are added in unlicensed ayurvedic and customary Chinese medicines. In 2004, several reports from the US proposed that various patients endured lead harming after the utilization of a range of undefined Ayurvedic prescriptions. Organizations too gotten reports from Hong Kong in December 2004 that an item called Tik Dak Win of the Ng Chung Mark was found amid testing to contain abnormal states of lead (Deisingh, 2005).

III. Herbal medicines with wrong active ingredient

Less expensive and comparative incorrect plants are utilized and supplanted rather than the correct one according to the accompanying table. This prompts extreme lethal impacts (MHRA, 2014). Availability of the natural prescription can be sorted as home grown drug endorsed by authorized specialist in endorsed herbal clinics like Unani,

Ayurvedic and Chinese natural facilities and home-grown items accessible in all drug stores. Next class is herbal medicine endorsed by unlicensed specialists from unapproved home-grown centers and home grown pharmaceutical effortlessly accessible in grocery stores. The third available home-grown solution is by ad in commercials and web solution.

1.6.2 Degree of the problem

Around 10% of drugs vended globally are accepted to be fake according to an evaluation by the world health organization. It is evaluated that up to 25% of the drugs expended in poor nations are fake or substandard. These numbers give an idea of the incomes, earned by the selling of counterfeit drugs around the world which is more than 32 billion USD internationally. As said by WHO, wealthier nations have the most noteworthy utilization of natural medicines, for example, hormones, steroids and antihistamines, cholesterol-bringing down medications and anti-cancer. In developing nations, the medications that are mostly counterfeited are those utilized for hazardous conditions, for example, malaria, tuberculosis, and AIDS. In underdeveloped nations every costly medication is mostly falsified (WHO, 2003).

As indicated by the European Commission, falsifying drugs contributes to around 5-7% of world trade (Wright, 2006) this gauge shows the colossal extents of the issue and uncovers the sparing enthusiasm of bootleg market medicate makers. As much as 15% of the worldwide supply chain of medications could pose to be counterfeit. This rate is considerably higher in specific locales, especially less developed nations. According to the World Health Organization (WHO), various countries contribute their share to the total medicine supply e.g. 20% from Russia, 40% from Mexico and as much as 80% from Nigeria (Wright, 2006). Notwithstanding, the WHO has since turned out to be less authoritative in assessing the extent of the issue, as there is an innate trouble in measuring fake items, which have turned out to be progressively refined and hard to separate from legit prescriptions. In a 2016 proclamation, the WHO expressed that "there are numerous assessments of the degree and size of the market in fake restorative items, yet minimal approved proof to support those gauges." It is irrefutable that web deals, the most regularly known wellspring of fake medications, have contributed extraordinarily to their expansion on the world market lately. Be that as it may, illicit web exchange is just a single piece of the story. Falsified drugs are likewise made into the legal market in Europe. In spite of the

fact that an expansive offer of the fake medications in Europe are sold by means of the web and on the underground market, there is prove that falsifying is a part of the genuine supply network too. These incidents are intricate plans of the counterfeiters which is a reason for concern (Wright, 2006) According to the European Commission, somewhere in the range of 2001 and 2005, the EU experienced 27 occurrences including such fake drugs.¹⁰ In July 2005, counterfeit variants of Pfizer's cholesterol-reducing medication Lipitor (atorvastatin) were found on the standard market in the UK. Reacting to the discoveries, the UK controller, Medicines and Healthcare Items Regulatory Agency (MHRA) issued recalls of the drugs. More than half of the returned drugs were observed to be fake (Wright, 2006).

1.6.3 Impact of counterfeit drugs

Falsifying has major effect on wellbeing; social what's more, economy. From health perspective, it can hurt patients in two different ways-independently and at public level. It can hurt a person from startling unfriendly responses what's more, serious toxicities. Notwithstanding the intellectual property rights that forgers disregard, the items they make can put customers in peril. Licensed pharmaceuticals must pass lively measures and experience substantial, randomized preliminaries previously being formally endorsed for people in general. At that point, every single authorized drug is prepared under intensely observed conditions. Fake drugs sidestep every one of these protections (Hellstrom, 2011).

The normal utilization of fake medications prompts remedial disappointment or resistance of the drug: In numerous cases, it can prompt passing, dis-functionality or damage of organ and compounding of illness condition. In the previous two decades this developing business sector of fake drugs has brought about an expanding number of deaths. Most prominently, in Nigeria in 1990, in India the production of cough syrup with toxic solvent resulted in the passing of 100 youngsters and in 1998; diethylene glycol toxicity prompted the passing of 30 youngsters (Chiang, Yafi, Dorsey Jr, & Hellstrom, 2017). In people group level, it can likewise be perilous what's more; have caused death of patients everywhere throughout the world. In U.K., perilous impacts which have come about because of utilization of over the counter natural prescription items spoiled with anything from microbial contaminants. Heavy metals, chemical toxins are defiled with pharmaceutical medications.

Some tragic occurrences resulting from counterfeit drugs are-

I. In 1938, a tragedy occurred during the formulation of sulphanimide elixir due to the use of toxic ethylene glycol instead of the desired solvent (propylene glycol). 76 patients who were mostly children died as a result of such event. Such deaths resulted in the enactment of U.S Food, Drug and Cosmetic act 1938 which specifically ensured that all the new drugs meet specific requirements before entering the market. Later on, this event also inspired the establishment of U.S FDA.

II. Counterfeit anti-malarial caused the death of 30 people in Cambodia in 1999. This is happened when the drug was prepared from sulphadoxine and pyrimethamine (a less effective version of anti-malarial) and sold under the name of Artesunate (Aknyili, n.d.).

III. Fake vaccines resulted in the death of 2500 people in Niger during the meningities epidemic occurred amid 1995-1996.

IV. “Paracetamol syrup disaster” in 1990 caused the death of 109 children in Ibadan and Jos when counterfeit syrups were prepared by swapping poisonous ethylene glycol in propylene glycol’s place (Akinwande, 2013).

V. In 1989 substandard chloroquine syrup caused the death of 4 kids when it was administered by University of Nigeria teaching hospital without the knowledge of those being counterfeit (Akunyili, n.d.).

VI. A Chinese natural planning applied for weight loss caused extreme kidney harm in 105 Belgians. Same natural treatment also showed carcinogenic effects in 18 patients. Investigations verified that a specific element of the weight diminishing drug, *Stephania tetrandra*, was swapped with *Aristolochia fangchi*, a herb recognized as herbs otherwise called birthwort, snakeroot and Dutchman's pipe. According to animal studies, *Aristolochia fangchi* posed to be carcinogenic and causes kidney harm (Hussin, 2001).

Along with numerous health hazards counterfeit drugs have negative impact on finance too. Availability of Fake items causes a significant loss of profit of the legal manufacturers and in case of medicines; the amount is around a few billions of dollars for every year. Nobody has correct figures, but it can be assumed from the estimation provided by international customs organization that 5% of all world trade marked merchandise is fake,

and if the total yearly turnover of the pharmaceutical industry is evaluated to be \$US400 billion, it can be easily calculated that the industry would suffer a loss of \$US20 billion every year (Ham, 2003).

1.6.4 What makes the counterfeit drug market successful and growing?

The explanation behind why pharmaceuticals became a target of forgers has a considerable measure to do with the way that fake drugs would now be able to be made moderately simple. The benefit may accordingly be similar to opiates, while there is lower risk because of the absence of tenets, law implementation and worldwide cooperation. Indeed, even in industrialized nations, nonetheless, the implementation of law and punishments for duplicating are yet lacking compared to US. Also, it is generally simple to market these drugs on the web, or in under developed nations where the genuine medications are costly and large numbers of potential purchasers are present and there is constrained control and requirement. The manner in which medications are generally sold likewise adds to their capability to be focused by forgers. The end-client has practically zero learning of the item and in this manner totally confides in drug stores, manufacturers and hospitals (Wright, 2006)

The advancement of the social security system in the western countries is also responsible for the successful journey of counterfeit drug market. As states try to lessen the cost of arrangement of social security service, people especially those on lessened salary levels, can see themselves, confronting expanding drug bills for which they get not as much as sufficient help. It is relatively inescapable that they swing to other, clearly cheaper sources to satisfy their pharmaceutical needs. The way that the educated people might know about the dangers that such an approach presents does not, without satisfactory and suitable data, resolve the issue. People who must face the commitment to pay for their medications out of pocket regularly have no entrance to a credible conveyance framework. They cannot bear to pay the ordinary costs and they fall back on elective sources, for example, street markets.

To be specific, there are three reasons that contribute to the increase of counterfeit drugs. To begin with, conventional family units, little cabin enterprises, even sometimes underneath a tree, it is sufficient to complete the creation of fake items. Furthermore, it's a colossally profitable business because of appeal and little cost of generation. It's

additionally more profit generating as it has less risky. Lastly, nonappearance of stern enactment is a reason for expanding counterfeiting (FDA, 2011)

Basically, deceptive and non-deceptive are two ways of creating counterfeit drugs. In deceptive kind, shopper can't perceive the fake items by visual inspection. It's splendidly made as unique item. It requires assessment examination to distinguish such items. In a non-deceiving compose, shopper can undoubtedly perceive as these are having non-unvarying hues, poor pressure, and poor marking (Mullaicharam, 2011).

1.6.5 Drugs that are mostly counterfeited

Antibiotics are proved to be the most commonly falsified medications and record for 28% of medication duplicating as per a WHO report published somewhere in the range of 1999 and 2002 Fake drugs comprises of 5% of the considerable number of antibiotics sold overall. Each nation on the planet is worried by forging of antibiotics. By the by, forging of anti-infection agents remains uncommon in industrialized nations, since they are barely concerned by getting antibiotics at a low cost. In any case, the solid interest for anti-infection agents, which are fundamental needs medicate in under developed nations, is an extremely profitable market for forgers (Delepierre, Gayot, & Carpentier, 2012).

A hundred and sixty-three fake drugs were revealed up to 2009 as indicated by a report, 78% were manufactured in the South-Eastern part of Asia (Figure 1.11). The nations of Sub-Saharan Africa are very influenced by infectious maladies. Regardless of whether these nations represent just 12% of the total populace, they contribute to half of the global deaths because of various infections. The tall rate of deaths caused by infections is a factor actuating traffickers of fake medications to offer in these nations. The porosity of outskirts, their ineptness for globalization, the high rate of absence of education, and the plain low salary of their populace are risk factors for the dispersion of fake medications. In these nations, fake medications can be regarded as "blockbusters" for traffickers. In South-East Asia (counting India and China), the rate of infectious ailments is likewise tall and can be analyzed to that of nations of a Sub-Saharan Africa. Nonetheless, in India and particularly in China, extraordinary inconsistencies in social insurance amid the provincial and municipal regions have showed up after a fast-economic development.

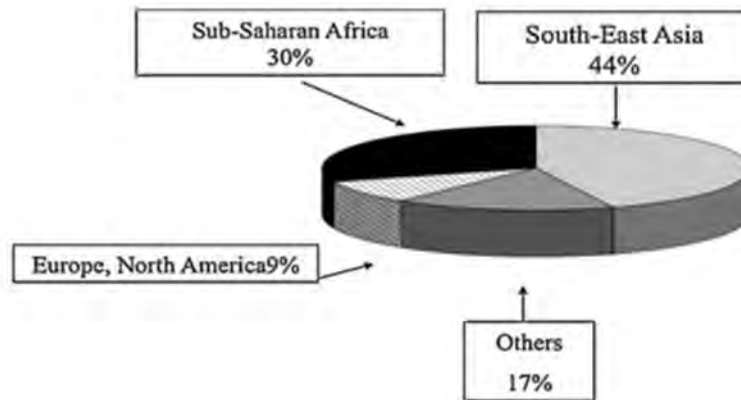


Figure 1.11 Target nations for the 163 counterfeit antibiotics spotted in the world until 2009 (Delepierre et al., 2012)

Although internationally, anti-biotic comprises of the biggest class of fake drugs, however in Europe, PDE-5i are the most regularly duplicated drugs. Truth be told, for PDE-5i, the unlawful market in industrialized nations approaches the span of that in under developed nations. Somewhere in the range of 2004 and 2008, 35.8 million fake sildenafil tablets were seized in Europe, which is 7 times more noteworthy than the measure of all other falsified Pfizer items consolidated. Two separate examinations in Europe evaluated that 0.6 to 2.5 million men are being presented to illegal sildenafil contrasted with roughly 2.5 million clients of lawful sildenafil. Be that as it may, the genuine extent of illegal PDE- 5i consumption is hard to evaluate. In one investigation endeavoring to evaluate unlawful sildenafil consumption, groupings of sildenafil and sildenafil metabolites were estimated in sewage treatment facilities in the Netherlands. The aggregate sewage stack was back ascertained to assess overall sildenafil utilization, and it was discovered that more than 60% of distinguished sildenafil was used without authorized prescriptions (Chiang et al., 2017).

1.6.6 Steps taken against the counterfeit medicines

Several actions are being exercised around the world for prevention of this hateful act-

I. Group IMPACT (WHO): The grouping Impact (International Medical Products Anti-Counterfeiting Taskforce) was made by the WHO in 2006. It is an association amid every single significant association included I the aversion of therapeutic item falsifying. It bunches between national associations, affiliations, administrative specialists, and gatherings of human services experts and patients (Delepierre et al., 2012). It has goal to keep the produce and the offer of fake medications, and to encourage correspondence and joint effort between every one of the accomplices to arrange the activities executed to screen and wipe out medication duplicating. Five work bunches were made in five fields for which measures are important to keep the duplicating of restorative items. One gathering took a shot at laws to draft a record "Standards and Elements to incorporate into national laws against the forging of medicinal items" to help the concerned nations execution or refresh national laws. Another aggregate is in charged with implementing the regulations. It offers exhortation to national experts to enhance controls, devices for national assessment, and models of methodology to manage fake medications.

II. The European Council established the worldwide convention, Medicrime allowed for nations who are members yet additionally to other countries (Deisingh, 2005). The adoption of this convention was by the European Council on December 8, 2010, and signing was done on October 28, 2011. It prosecutes the duplicating of therapeutic items and is in this manner the first compelling legal instrument in the space of reformatory law. It propels endorsers to recognize as violations the making of fake medications, provisions, offering to give, and managing fake medications, duplicating reports identified with restorative items, non-approved assembling or giving of therapeutic items, and advertising medicinal gadgets that are not in conformity with the specified requirements. It defines utilizing the Internet to offer fake medications as a contributing variable, for preventing the purchase of fake medications by means of the Internet (Delepierre et al., 2012).

III. Track and Trace project: The European pharmacopeia propelled a task named "Track and Trace" as a major aspect of its aversion plan against forging of medications. This venture, open to all makers showcasing crude materials in one of the European pharmacopeia member nations, has for goal to execute an arrangement of medication

traceability. It was planned to implement this task to the member nations amid 2012 (Delepierre et al., 2012).

IV. Increased communication: A few governments, mindful that the populace disregarded the issue of fake medications, have started activities to educate citizens about potential risks. Thus, Nigeria, Thailand, and Cambodia created web records on fake medications. Cambodia likewise started different refinement crusades utilizing publications, the radio, and TV. These activities intend to incite patients to purchase medicines just in approved drug stores and to check at every conveyance the nonattendance of suspicious things (Cockbrun et al., 2005).

V. Anti-counterfeit technologies: Fake medications can prompt medication reviews and obligation suits. Moreover, loyalty towards a brand is endangered as buyers see extra dangers when utilizing an organization's items. A powerful enemy of fake procedure stays away from this and guarantees understanding security. Standard methods for fighting forging include: lawful activities on illegal dealers, countermeasures utilizing advancements, buyer's knowledge, private examinations, and participation with agencies related to enforcement. The usage of anti-counterfeit technologies seems to be the conspicuous preventive measure (Sanjay & Sanjeev, 2009). Notwithstanding giving confirmation, they make the creation of a persuading duplicate regarding a medication more troublesome and expensive (Goldhammer & Scott, 2006). The administration experts, by utilizing these innovations, may guarantee that medications in the supply chain are legal. For instance, the US Prescription Drug Marketing Act of 1987 (PDMA), altered by the Prescription Drug Amendments of 1992 (PDA), made it compulsory for the wholesalers to provide a genealogy before the conveyance of professionally prescribed medications.

The desired anti counterfeit technology ought to have an extensive state of security which renders it impossible to clone, higher item application and validation speed, demonstrated guidelines, be hard to expel and reapply, simple to check, have programmed confirmation, easy to use by buyers, and must be lawfully consistent by the ventures. Be that as it may, the FDA suggests the utilization of various, occasionally changing, validation measures on an item particular premise (FDA, 2004).

1.7 Literature review: Adulterants in anti-diabetic herbal drugs

Adulteration of herbal drugs around the world inspired numerous researches on this topic in many countries. A lot of recent research works and investigations proved the presence of undeclared synthetic compounds, heavy metals and other toxic compounds in herbal drugs. FDA identified shrouded metformin furthermore, Sitagliptin in dietary enhancements and Ayurvedic medications which are unlawful. A few investigations proved to contain covered up manufactured medications, metals, or other poisonous substances in high fixations which may put the well-being of patients in danger. Examination of 2600 Chinese home-grown drugs found that 24% of the samples contained no less than one manufactured drug (Ernst, 2002). Also, meta-analysis demonstrated the presence of undeclared synthetic compounds in herbal drugs which are poisonous for the well-being of human. A study conducted by the toxicology & Pharmacology department of Islamic Azad university (IAUPS), Tehran, Iran for determining undeclared synthetic drug in herbal formulations used in opioid replacement therapy is used this technique for the analysis of herbal samples. The experiment was both qualitative and quantitative where 80 common home-grown prescriptions utilized as OST were taken as samples. The addresses of home grown shops were accumulated from electronic databases. There were 81, 162, 190, 130 and 243 natural pharmaceutical shops in five zones; north, south, east, and west and focal point of Tehran, Iran. Basic irregular examining was utilized and 10% of shops in every region were chosen for test accumulation. It ought to be noticed that home grown shops were not similarly conveyed between the five zones and none of the natural shops was enrolled with the Iran Food and Drug Administration (IFDA). Tests were partitioned into four noteworthy subgroups; tablets, containers, powders and fluid structures (syrup) (Foroughi, Akhgari, & Jokar, 2017). The instrument used was an Agilent model 7890A gas chromatograph which was provided with split/splitless injector.

Results demonstrated that all the samples were absent in batch number, standard logo, manufacturing date and date of expiry articulation, formulation and producer's name. Also, product lables did not claim the presence of any synthetic compound. Around 96% of the bundles had a name specifying 'Regular Product'. Capsules were the most abundant dosage form gathered (83.8%). It was revealed that 96.3 % of the sample contained one undeclared synthetic compound to the least and most commonly detected compounds were- tramadol and diphenoxylate (Foroughi et al., 2017).

Another study conducted by the Banaras Hindu University on 2011 revealed that Metformin Hydrochloride (chemical anti diabetic agent) was present in an herbal anti diabetic preparation (Kumar, Mandal, & Hemalatha, 2011). Herbal medications that provoked suspicion were taken as samples and collected from local health care providers. According to the label claims of the products they contained nothing but natural substances and most product were collected from rural areas of India for identification, the herbal pills were analyzed using UV-Vis spectroscopy. UV spectroscopy also provided a quantification of the metformin present in the herbal pill (93.1 mg of metformin base). When the herbal pills were subjected to dissolution study 85% of drug release occurred in 240 minutes and according to Indian Pharmacopeia 85% release of drugs occurs within 45 minutes in case of uncoated Metformin Hydrochloride (Kumar et al., 2011).

Recently a study was conducted for the onsite rapid identification of ephedrine and its analogues from thinning dietary supplements. In many countries herbal dietary supplements are adulterated with ephedrine and its various analogues e.g. pseudoephedrine (PSE), methyl ephedrine (MEP), and nor ephedrine (NEP) etc. This study combined the conventional TLC method with surface enhanced Raman spectroscopy (SERS). The experiment concluded the combined method to be an effective method for the specific ephedrine analogues used in the study.

1.8 Analysis of herbal drugs

Along with verifying quality control requirements, another common reason of analyzing herbal drugs is the identification of adulterants in their formulation. In the course of the most recent couple of decades there has been an increasing development in the field of home grown drug. It is getting promoted in developing and industrialized nations and has a functioning impact in individuals' wellbeing. These items are viewed by numerous as being safe as they are produced from natural origin and accommodating to the treatment of some ceaseless illnesses and to the upkeep of physical fitness. Nevertheless a few makers have included synthetic compounds in the detailing procedure of their items showcased as "home grown solutions" or "dietary supplements", to enhance the impacts of their items (Balayssac et al., 2009) This is one major reason why the analysis of herbal drugs has become extremely important and the common methods employed in such analysis are-

1.8.1. UV-Vis Spectroscopy

In UV spectroscopy the analyte molecule absorbs the light rays of ultra violet district (200-400 nm.) and gets excited. UV spectroscopy obeys the Beer-Lambert law, which states that: “when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.” According to this law the higher the number of light absorbing molecule the higher will be the extent of absorption and this is the basic principle of UV-Vis spectroscopy.

It is no surprise now that in many countries the herbal drugs are found to be adulterated. In U.K, USA, and in many other countries plant medications and supplements are reported to be contaminated with undeclared synthetic pharmaceuticals and UV- Vis spectroscopy is an important tool in the analysis of herbal drugs in this regard along with other types of analytical purposes as well.

1.8.2. Thin layer Chromatography (TLC)

Among numerous partition techniques, TLC is generally straightforward also, shoddy, and has for quite some time been utilized for essential screening of drugs originating from unrefined plant sources and pharmacologically dynamic segments of plants and their extracts (Dillon et al., 2012). It can be additionally utilized for testing the integrity of drugs during the primary identification process (Görög, 2012).

Recently a study was conducted for the onsite rapid identification of ephedrine and its analogues from thinning dietary supplements. In many countries herbal dietary supplements are adulterated with ephedrine and its various analogues e.g. pseudoephedrine (PSE), methylephedrine (MEP), and nor ephedrine (NEP) etc. This study combined the conventional TLC method with surface enhanced Raman spectroscopy (SERS). SERS method takes advantage of the specificity of vibrational spectroscopy with ultra-high affectability at an atomic level (Cialla et al., 2012). For this reason, SERS can be really effective when we are going for low concentration and nondestructive analytical methods specially for biotechnology products and drug substances. Moreover, this technique is good for qualitative measurements as well (Piergies et al., 2012).

The experiment was conducted in three steps (Figure 1.12) (Lv et al., 2015)-

- I. Four reference standards of ephedrine were eluted using silica 60-F₂₅₄ plate and an elution buffer prepared by CH₃Cl₂:CH₃OH:NH₄·H₂O with a ratio of 9:1:0.1.
- II. After the evaporation of the plate the distinguished spots were investigate and marked using a TLC scanner at 365 nm followed by the addition of silver colloid suspension (4 μL) to the spots.
- III. For recording of SERS spectra for all the spots a portable Raman spectrometer was used.

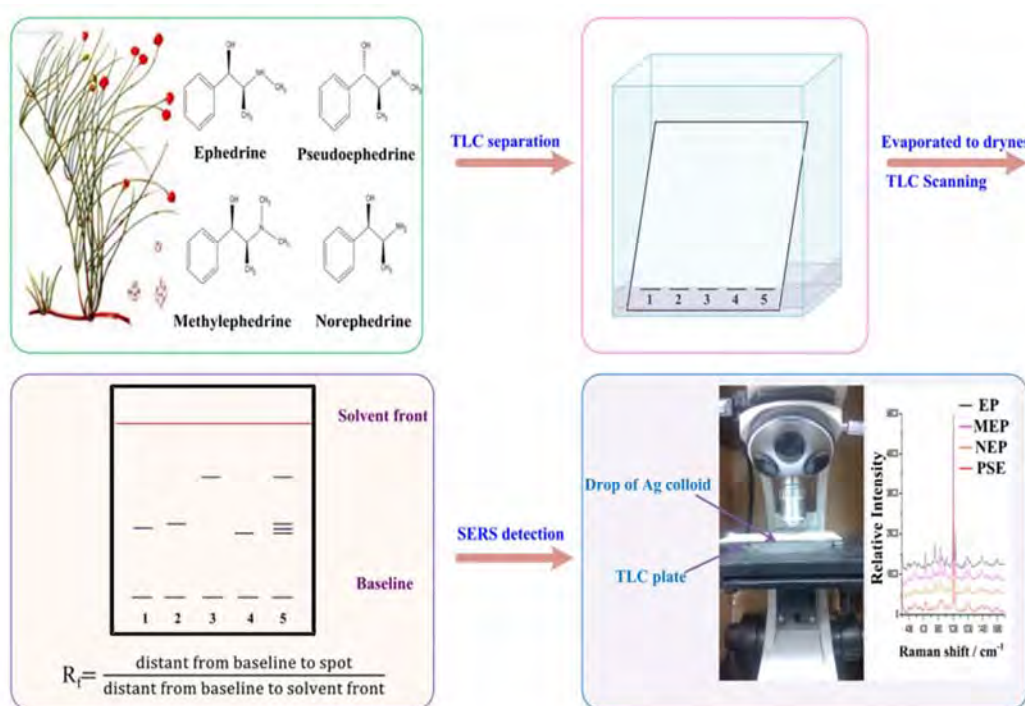


Figure 1.12 Identification of ephedrine and its analogues by TLC-SERS method (Lv et al., 2015)

The experiment concluded the combined method to be an effective method for the specific ephedrine analogues used in the study but the method does not guarantee the detection of other ephedrine analogues. It is because with the increase in various types of ephedrine analogues the summarization of common and specific peaks will also rise. Another limitation of the method was the specific silver colloid used in the experiment might not interact with all other ephedrine and which will require the use of other colloid suspensions (Lv et al., 2015).

1.8.3. High performance liquid Chromatography (HPLC)

Both analytical and preparative HPLC techniques are vastly used for the isolation and purification of herbal drugs. In the most basic manner, preparative HPLC can be separated into two categories based on the pressure at which the technique is being operated (Chimezie et al., 2008)-

- a. High pressure HPLC- operated under 5 bar
- b. Low pressure HPLC- operated above 20 bar

Significant operational parameters vary widely between the two HPLC methods i.e. in case of analytical HPLC fast analysis time and sensitivity are important parameters of consideration where as important parameters for preparative HPLC are- degree of purity of the solute and compound recovered per unit time (Rao & Anna, 2009).

There are countless records of utilization of HPLC in the analysis of herbal drugs – sometimes for identification active components, sometimes for detection of adulterants and also HPLC is used for the standardization of herbal drugs as well. HPLC was used for the estimation of the most bioactive alkaloid (Vascine) of *Adhatoda vasica* from two polyherbal drugs- Shereeshadi Kashaya and Yastyadivati. The amount of the compound was calculated to be 18.1 mg/100 g and 0.7 mg/100g respectively in the two drug formations (Anupam, Krishan & Handa, 1992).

1.3 Purpose of the study

Due to the long history of herbal drugs in therapeutics (from 2800 B.C), patients' trust in this category of drug is huge. This trust contributes to patients' preference of herbal drugs around the world at a growing rate. This is because herbal drugs claim to be prepared from completely natural sources and are free from undesired side effects. This long-term trust on herbal drugs and their wide acceptance by the patients around the world makes them an easy target of counterfeiters. In addition, in contrast to synthetic drugs herbal drugs do not have such strict regulation systems on them. The regulation systems of herbal drugs are rather lenient, which is another major reason that contributes to the adulteration of these drugs. Adulteration of herbal drugs is unfortunately a very common scenario in various countries of the world. The basic synthetic adulterants are anti-diabetic drugs, steroids, drugs for erectile dysfunction, anti-histamines, NSAIDs, and thinning pills. Several studies around the world proved that herbal drugs are sold with undeclared

pharmaceuticals in their formulation (Kumar, Mandal, & Hemalatha, 2011). Moreover, it has been also found that ayurvedic and customary Chinese medicines that are unlicensed, shows the presence of lead and mercury over the security level which prompts substantial heavy metal poisoning (Mullaicharam, 2011).

So, this research/study is specifically dedicated towards finding out pharmaceutical adulterants in herbal anti-diabetic preparations available in Bangladesh. A unique aspect of this study is that, there are no previous records of similar researches regarding the adulteration of herbal drugs in the country. So, the following study aims to be the first of its purpose. Since diabetes is a chronic disease and requires long-term intervention for treating symptoms, most people prefer natural remedies for long-term use as natural products have less side effects than the synthetic drugs. Moreover, Bangladesh is a country that has a noticeably high number of diabetic patients, 71 Lakhs to be exact according to a report by Bangladesh Diabetic Samity published in 2015 (UNB, 2015). Therefore, this research intends to aware the patients and the mass people about the actual condition of the herbal drugs in Bangladesh.

Chapter 2 Materials and Methods

2.1 Chemicals and reagents

Metformin (MET) and Gilmepride (GLP) standards were obtained from Eskayef Pharmaceuticals Limited, Bangladesh in the form of standard API powder which was used as standards in this study. HPLC grade Acetonitrile and 85 % ortho Phosphoric acid were purchased from Samchan lab, China and RCL Labscan, Asia respectively. Potassium dihydrogen phosphate was purchased from Active Fine Chemicals Ltd, Bangladesh. Samples of herbal oral hypoglycemics were purchased from local market of Dhaka city, Bangladesh.

2.2 Instrumentation: Instruments used in the overall experiment

1. Shimadzu HPLC LC-20 AT series binary gradient pump (Tokyo, Japan) with-
 - SPD-M20A PDA detector
 - Solvent delivery system (SIL-20AHT)
 - Auto injector
 - Online Line 5 Channel degasser
 - HPLC filtration unit 1000 ml-

(For qualification and quantification of adulterants in samples)



Figure 2.1 High Performance Liquid Chromatography (HPLC) machine, model: LC-20A, Shimadzu, Japan.



Figure 2.2 Vacuum filtration unit (HPLC)

2. UV-Vis spectrophotometer (UV 1800) (For initial identification of the standards - MET and GLP.)



Figure 2.3 UV-Vis spectrophotometer, model-UV-1800, Shimadzu, Japan.

3. Digital pH meter bench top (S220) (For adjusting pH of buffer solutions.)



Figure 2.4 Digital pH meter bench top, model: (S220).

4. Electronic balance (ATY 224) (For precise weighing of substances.)



Figure 2.5 Electronic Balance, Model: (ATY 224), Shimadzu, Japan.

5. Ultra-sonic sound bath (LUC 405) (For improved dissolving of materials and removal of bubbles from the final solutions and solvents.)



Figure 2.6 Ultra sonic sound bath, model: (LUC 405).

2.3 Preparation of standard stock solution

For the preparation of working standards at first a combined (MET + GLP) stock solution having concentration of 500 $\mu\text{g}/\text{mL}$ was prepared. Initially, to prepare the stock solution 10mg standard powders of MET and GLP were taken in separate 10 ml volumetric flasks and volume was made up to the mark with diluent (acetonitrile: water = 9:1) and two solutions having the concentration of 1000 $\mu\text{g}/\text{mL}$ were achieved. From each of these two solutions, 5 mL was withdrawn and mixed in a separate 10 mL volumetric flask and volume was made up to 10 mL with diluent (Acetonitrile: Water = 9:1) to achieve a combined solution of MET and GLP having the concentration of 500 $\mu\text{g}/\text{mL}$. From this stock solution, working standards were prepared by serial dilution in several concentrations for the preparation of standard curve later on. The concentrations-11.25 $\mu\text{g}/\text{mL}$, 22.5 $\mu\text{g}/\text{mL}$, 45 $\mu\text{g}/\text{mL}$, 90 $\mu\text{g}/\text{mL}$, 180 $\mu\text{g}/\text{mL}$ were selected for the convenience of the experiment. For preparing the working standards of said concentrations, at first 3.6 mL was withdrawn from stock solution and taken in a separate 10 mL volumetric flask and diluent was added up to the mark to obtain solution having concentration of 180 $\mu\text{g}/\text{mL}$. Later on, from this 180 $\mu\text{g}/\text{mL}$ solution 5 mL of solution was withdrawn and taken in a 10 mL volumetric flask and diluent was added up to the mark to obtain a solution having concentration of 90 $\mu\text{g}/\text{mL}$. Other concentrations were prepared following the same procedure. All the solutions were filtered (by .45 μ membrane filter) and sonicated for 3 minutes (at 28 ° C) prior insertion into the HPLC machine.

2.4 Preparation of sample solutions

The 25 different solutions of samples were prepared by addition of 10 mL of diluent (Acetonitrile: Water =9:1) to each 50 mg of sample powder obtained from either sample tablets or capsules. Since the concentration of adulterants in the samples was unknown 50 mg was decided to be a sufficient amount for the identification of adulterants considering that the usual approved dose of marketed MET and GLP drugs are 500 mg and 1-4 mg, respectively. The prepared solutions were filtered by 0.45 μ membrane filter and subjected to ultrasonic bath for 3 minutes prior testing at a steady temperature of 28°C.

2.5 Preparation of 500 mL of phosphate buffer (pH 2.1)

At first 0.5 g of monobasic sodium phosphate in 450 mL of water, then pH was adjusted with 85% ortho phosphoric acid and finally volume was made up to 500 mL with water.

2.6 Chromatographic conditions

- HPLC column: Phenomenex Luna C-18 (150× 4.6mm) (USA) packed with 5 μ m particles.
- Column temperature: 25 ± 2 ° C.
- Detector: SPD-M20A PDA detector.
- Mobile Phase: Acetonitrile: Phosphate buffer (pH 2.1) = 70:30.
- Flow rate: 1mL/min.
- Detection wavelength: 230 nm.
- Injection volume: 10 μ L for both standard and sample.
- Retention time: 2.2 min for MET, 5.7 min for GLP.

All the solutions were filtered by 0.45 μ membrane filter and sonicated for 3 minutes (at 28 ° C) prior insertion into the HPLC machine.

Chromatographic conditions applied in the experiment were followed from the assay method established in USP 2015 version. Slight modification to the mobile phase ratio was made due to the convenience of the experiment. Mobile phase ratio used for this study was Acetonitrile: Phosphate buffer = 70:30, instead of a 50:50 ratio between the two which is mentioned in the assay method of USP 2015.

Chapter 3 Results

3.1 Solubility study of the standard drugs

For finding out the optimum solvent for HPLC analysis, the solubility of both of the standard drugs in several solvent systems was studied following journals regarding similar studies. For both of the drugs solubility in methanol: water solvent system and dichloromethane: water solvent system was studied. Finally, the optimum mobile phase for the experiment was decided according to USP 2015 version with slight modification and the optimum mobile phase was acetonitrile: phosphate buffer (pH 2.1) in a ratio of 70:30.

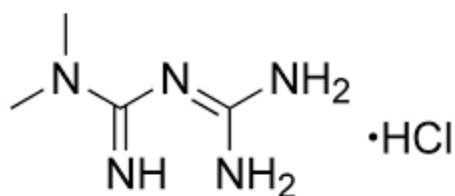


Figure 3.1 Chemical structure of MET

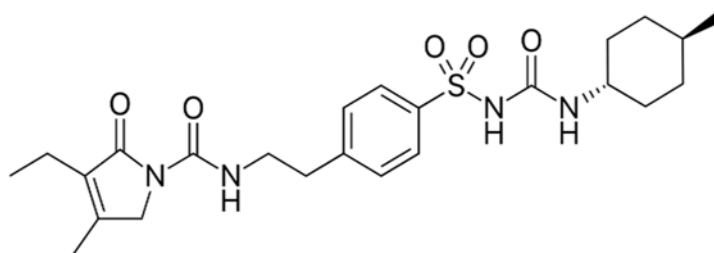


Figure 3.2 Chemical structure of GLP

Physical properties:

Molecular Weight 165.625 g/mol (MET) (Pubchem, 2018).

Molecular Weight 490.619 g/mol (GLP) (Pubchem, 2018).

3.2 Construction of calibration curves

Retention time (RT) and Area under the curve (AUC) were selected as primary form of data collection where RT was used for identification and AUC was used as a quantitative measure. Readings were taken in triplicates for solutions of each concentration which is shown in Table 3.1 and Table 3.2. These data were further used to create the calibration curves (average area vs. concentration) for both of the standards in order to determine the unknown concentrations of adulterant present in the samples. For the first two times the runtime was set at 30 minutes. Since the retention time of either of the standards was unknown, a longer runtime was considered to be safer as it creates a bigger window for identification. In the said chromatographic conditions, the RT for MET and GLP was found to be 2.2 minutes and 5.7 minutes, respectively. Although RT for both of standard compound, was found to be below 10 minutes, the retention time was again set to 30 minutes in the second time. This was done to ensure consistency of the result. MET being more polar than GLP, it should be eluted earlier than GLP if a RP –HPLC method is used. The fact that MET is eluted earlier (2.2 mins) than GLP (5.7 mins) according to the used method ensures the correctness of it as a RP-HPLC method. From the third time and onward, the runtime was fixed at 10 minutes since no peaks were observed after the eighth minute. The following chromatogram was achieved for the standards (Figure 3.3)

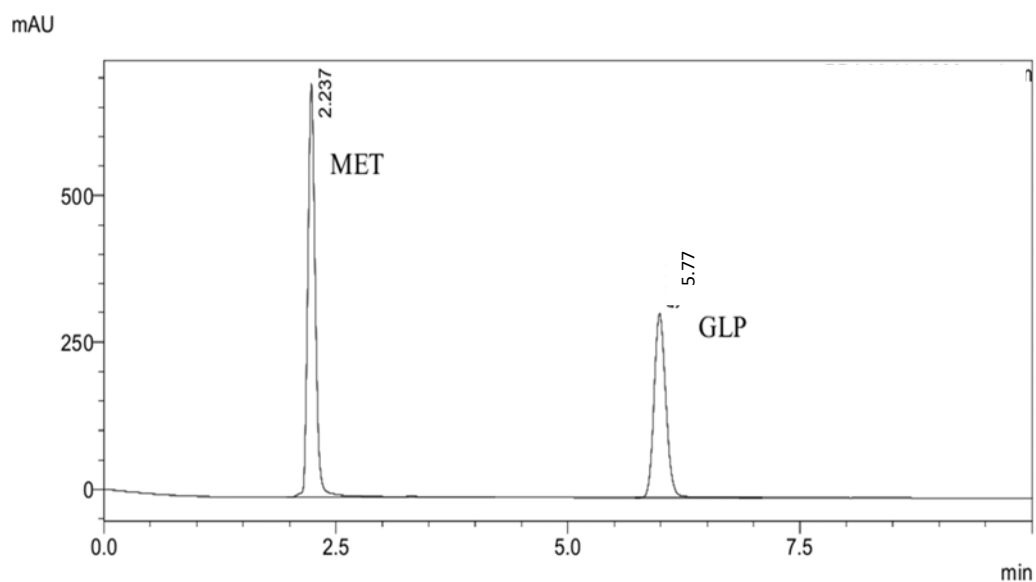


Figure 3.3 Chromatogram of standards MET and GLP

Table 3.1 Average AUC for different concentrations of MET.

Name of the standard	Concentration (µg/mL)	Area	Average	Standard deviation
Metformin	11.25	324216	323342.66	777.22
		322328		
		323484		
	22.5	699850	700970	1371.23
		700159		
		702901		
	45	1290414	1289382	9865.80
		1300916		
		1276816		
	90	4532892	4542739.33	46678.01
		4604192		
		4491134		
180	8722225	8900022	125770.12	
	8993205			
	8984636			

Table 3.2 Average AUC for each concentration of GLP.

Name of the standard	Concentration (µg/mL)	Area	Average	Standard Deviation
Glimepiride	11.25	158489	159045	427.67
		159529		
		159118		
	22.5	349846	349976	170.64
		350208		
		350208		
	45	647772	680968	49710.73
		751234		
		643898		
	90	2157042	2159147	1599.69
		2159482		
		2160917		
	180	3733496	3722226	10284.98
		3724555		
		3708628		

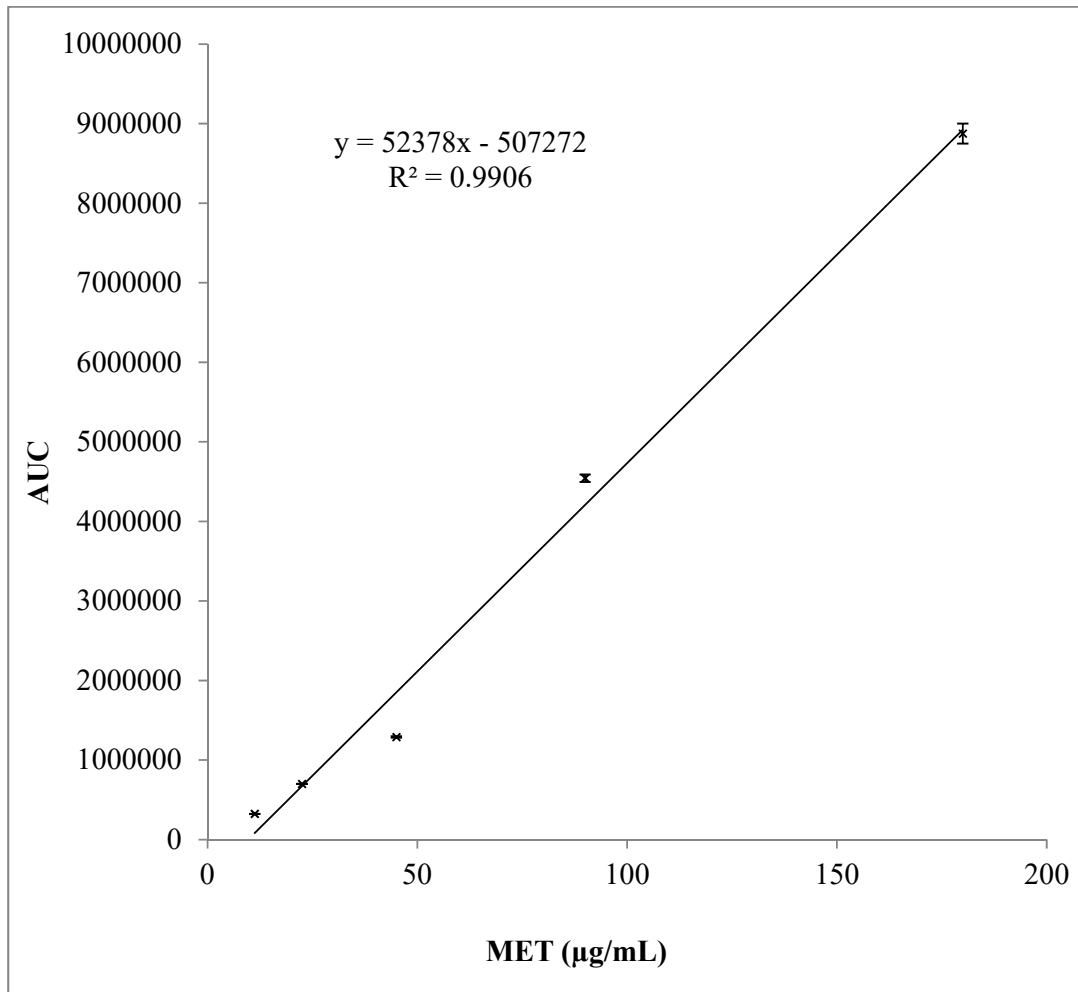


Figure 3.4 Calibration curve of MET. Each measurement was performed in triplicate.

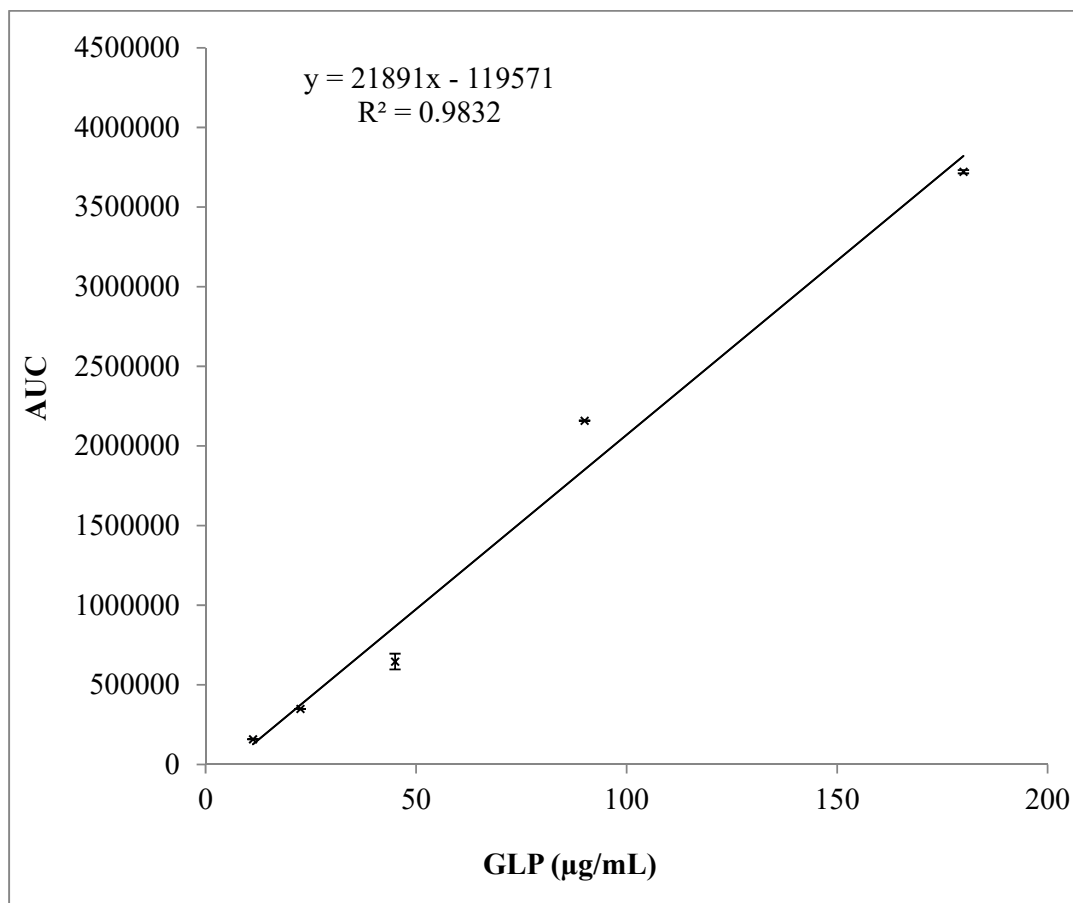


Figure 3.5 Calibration curve of GLP. Each measurement was performed in triplicate

3.3 Study of the physical properties of samples

The herbal anti-diabetic drugs available at the local market of Dhaka were collected as samples. This experiment was not confined to any specific type of dosage form of samples; rather samples having several types of dosage forms were analyzed. The physical properties of the samples are available in Table 3.3.

Table 3.3 Physical properties of the samples

Serial no.	Sample ID	Dosage form	Average weight (mg)	Color
1	Sample A	Hard gel capsule	388	Black and red
2	Sample B	Hard gel capsule	416	Olive
3	Sample C	Hard gel capsule	430	Green
4	Sample D	Tablet	706	Grey
5	Sample E	Hard gel capsule	387	White and Green
6	Sample F	Hard gel capsule	455	Green
7	Sample G	Hard gel capsule	336	Green and Yellow
8	Sample H	Tablet	563	Blue
9	Sample I	Tablet	648	Yellow
10	Sample J	Tablet	110	Brown
11	Sample K	Tablet	55	Orange
12	Sample L	Hard gel capsule	368	Blue and white
13	Sample M	Tablet	589	Dark brown
14	Sample N	Hard gel capsule	516	Light Blue
15	Sample O	Hard gel capsule	445	Green
16	Sample P	Tablet	398	Light brown
17	Sample Q	Powder	-	Dark brown
18	Sample R	Hard gel capsule	361	Black and Red
19	Sample S	Tablet	504	Mustard
20	Sample T	Hard gel capsule	374	Blue and white

21	Sample U	Tablet	472	Dark brown
22	Sample V	Powder	-	Light yellow
23	Sample W	Powder	-	Mustard
24	Sample X	Tablet	641	Yellow
25	Sample Y	Hard gel capsule	422	Blue and White

3.4 Analysis of samples

The HPLC chromatograms of the samples that showed the presence of adulterants are shown in Figure 3.6- 3.21. From the 25 tested samples, 16 samples showed the presence of the oral hypoglycemic drugs (MET & GLP) as adulterants. 9 samples contained only MET (Figure 3.6, 3.9, 3.12, 3.13, 3.15, 3.16, 3.18-3.20). 5 samples contained only GLP (Figure 3.7, 3.8, 3.10, 3.11, 3.17) and 2 samples contained both MET and GLP (Figure 3.14, 3.21). The amount of MET & GLP in different samples were found to vary from $7.297 \pm 0.073 \mu\text{g/mL}$ - $613.93 \pm 4.650 \mu\text{g/mL}$. The presence of MET and GLP in the samples were confirmed based on the retention time of standard MET and GLP. Respective AUC of the peaks were used for quantitative determination of MET & GLP using calibration curves. The results are shown in Table 3.4. Thus, the presence of adulterants in the samples was ensured and we can definitively infer that 64 % of the samples are adulterated.

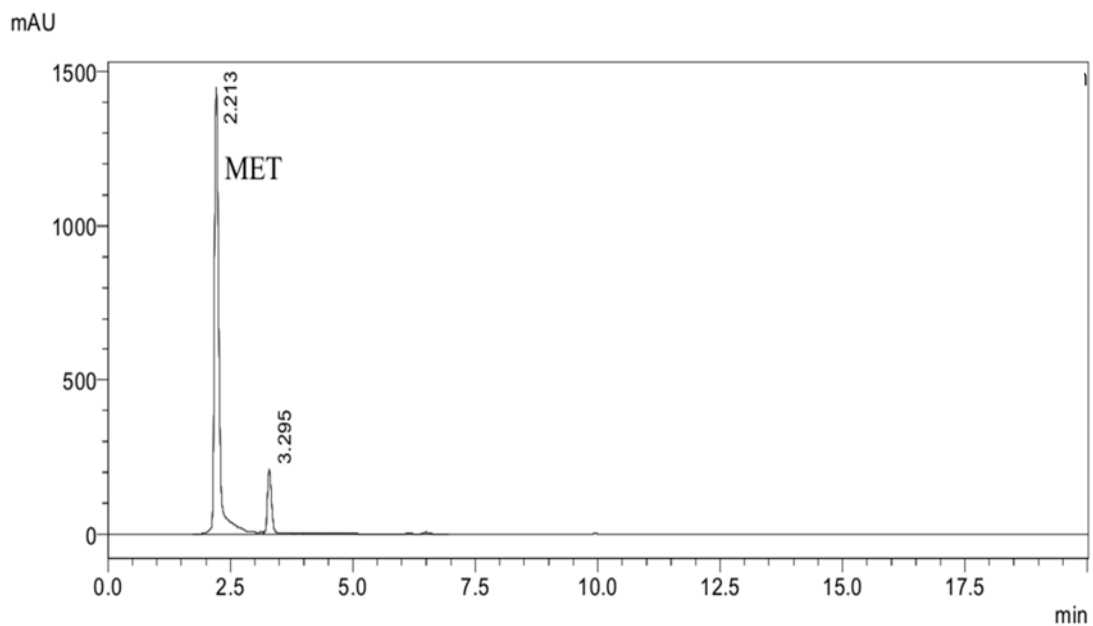


Figure 3.6 Chromatogram of sample A

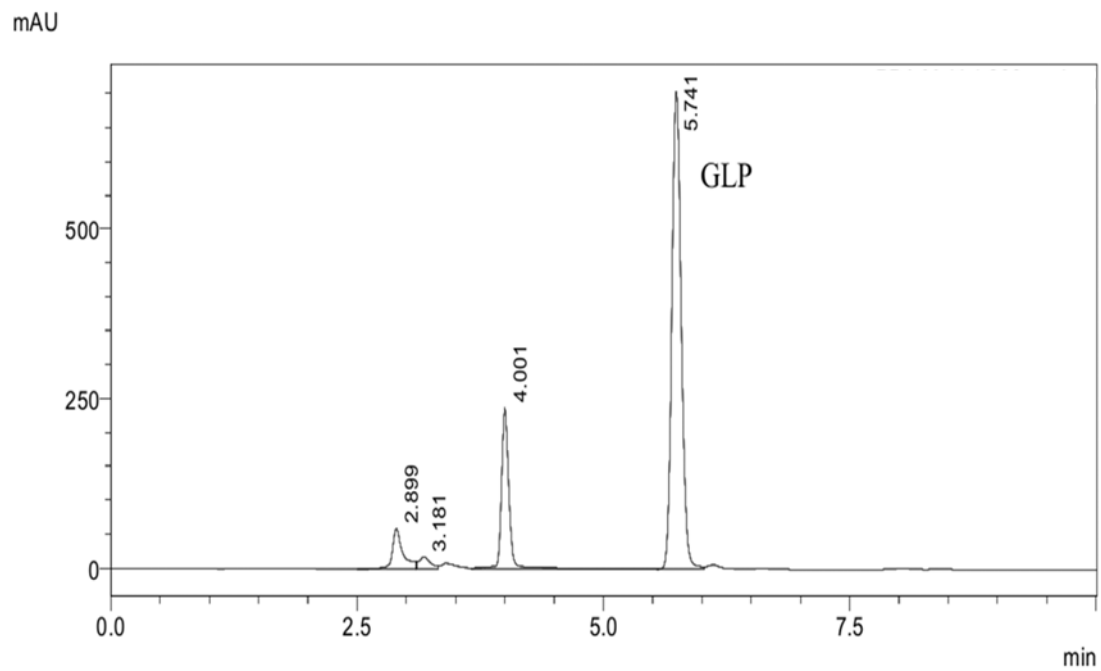


Figure 3.7 Chromatogram of sample C

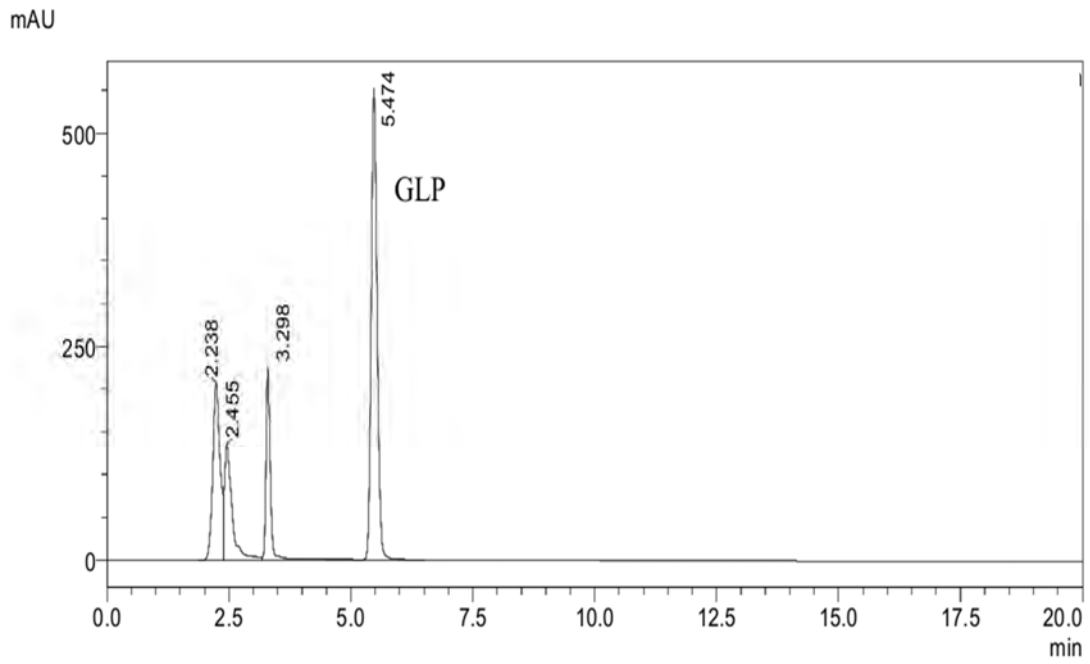


Figure 3.8 Chromatogram of Sample D

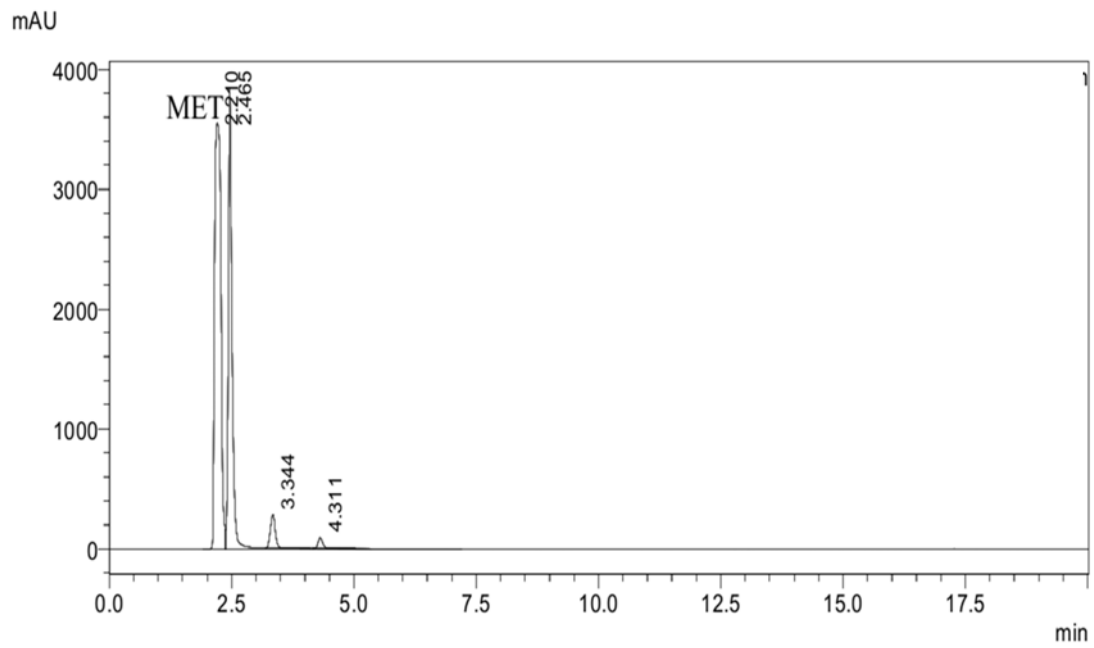


Figure 3.9 Chromatogram of Sample E

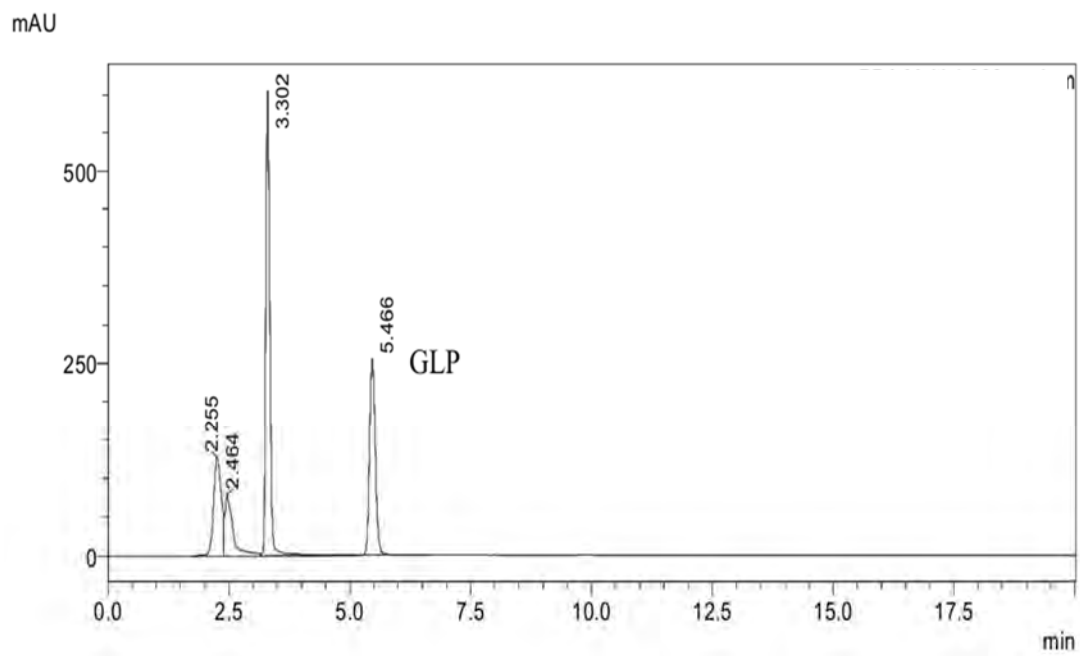


Figure 3.10 Chromatogram of Sample F

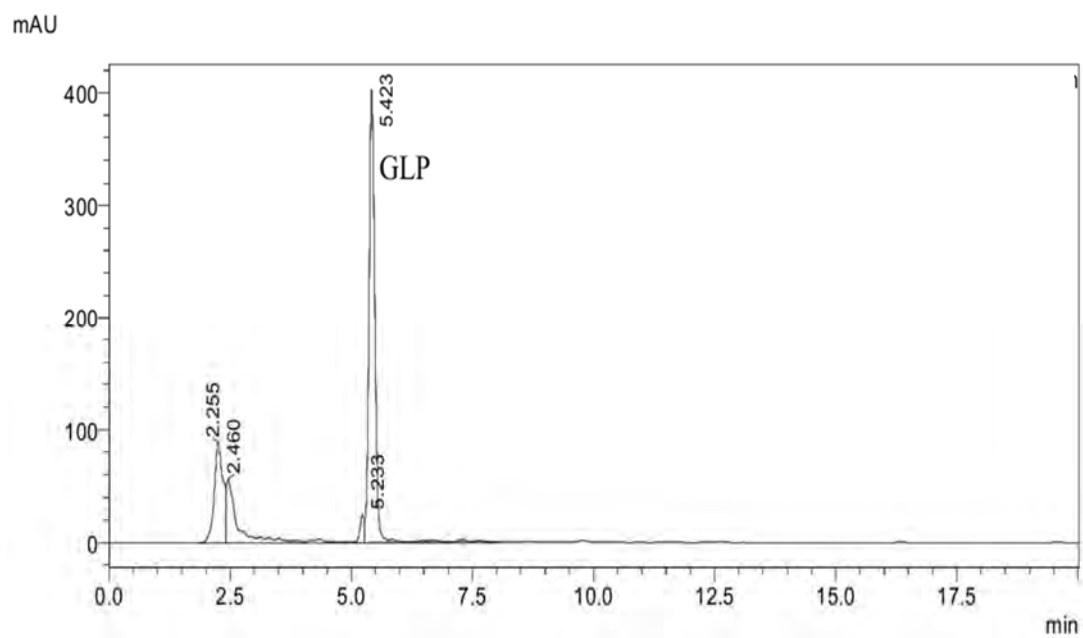


Figure 3.11 Chromatogram of Sample G

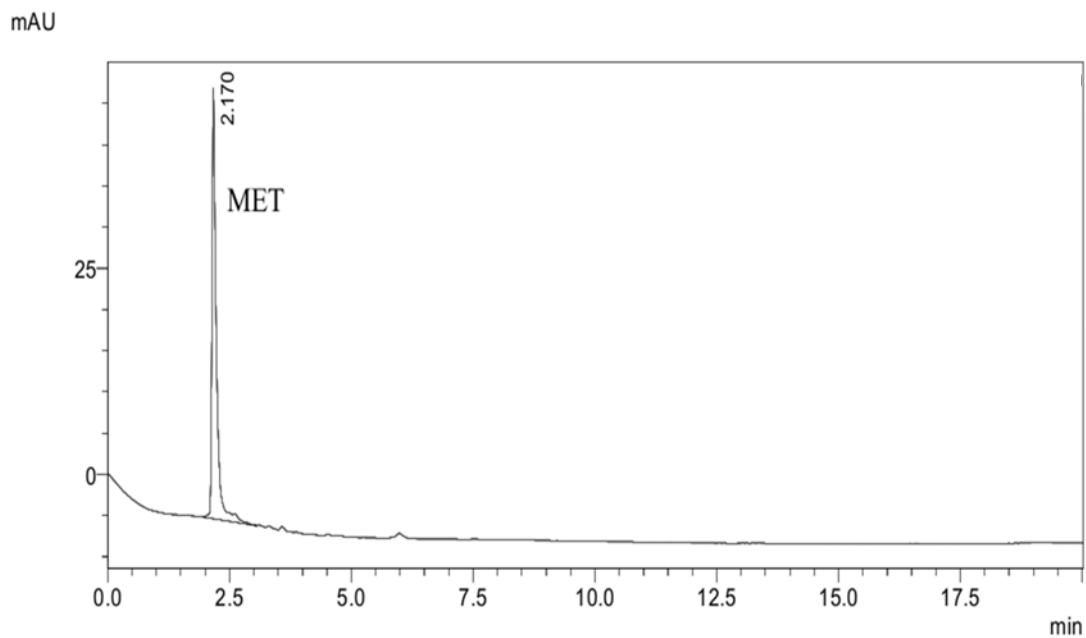


Figure 3.12 Chromatogram of Sample J

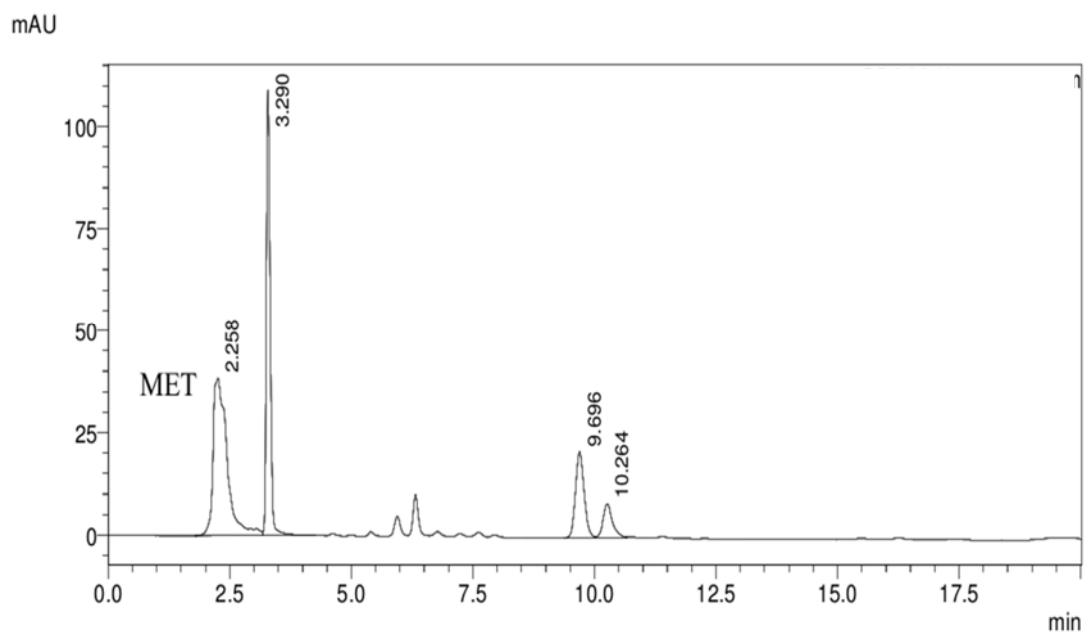


Figure 3.13 Chromatogram of Sample N

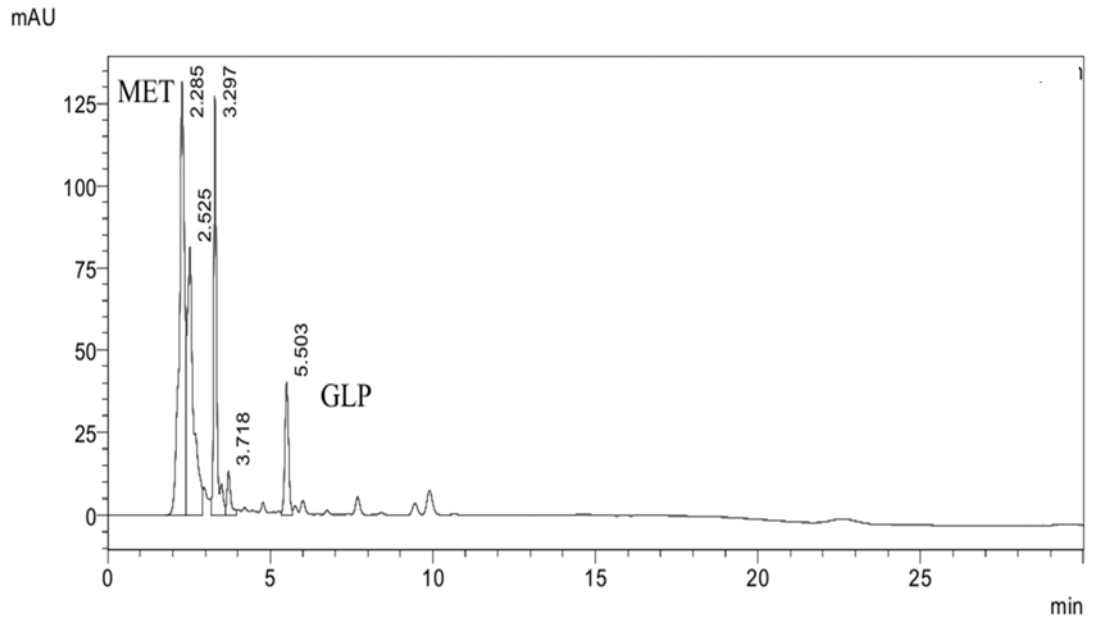


Figure 3.14 Chromatogram of Sample O

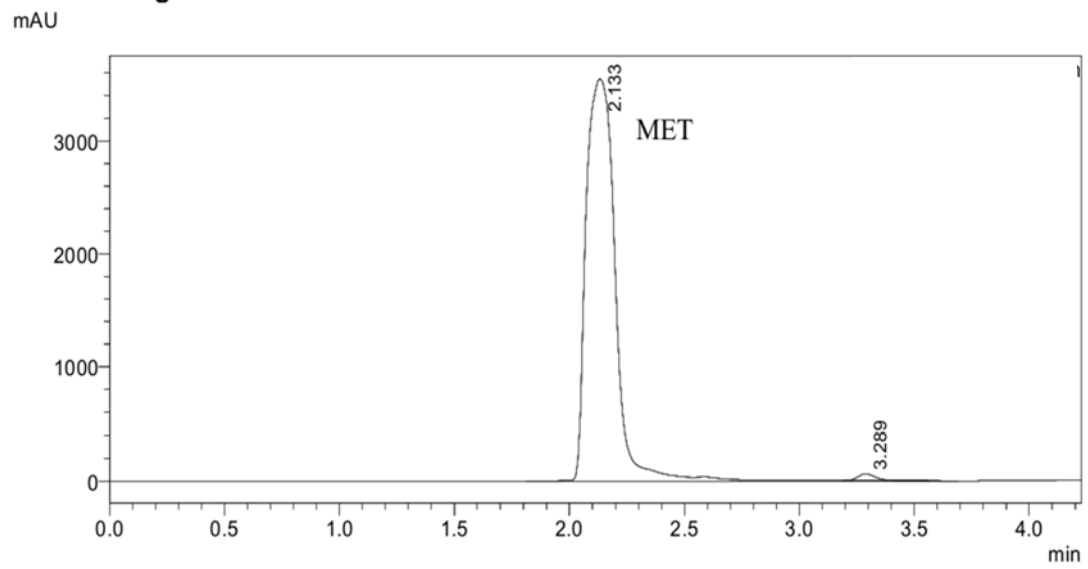


Figure 3.15 Chromatogram of Sample P

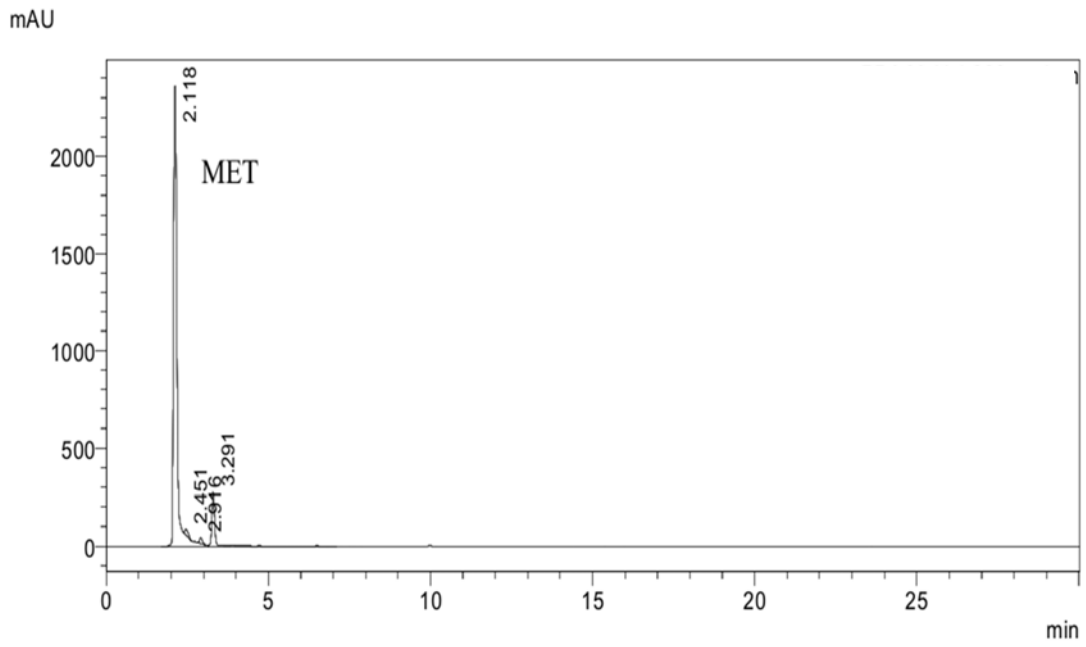


Figure 3.16 Chromatogram of Sample R

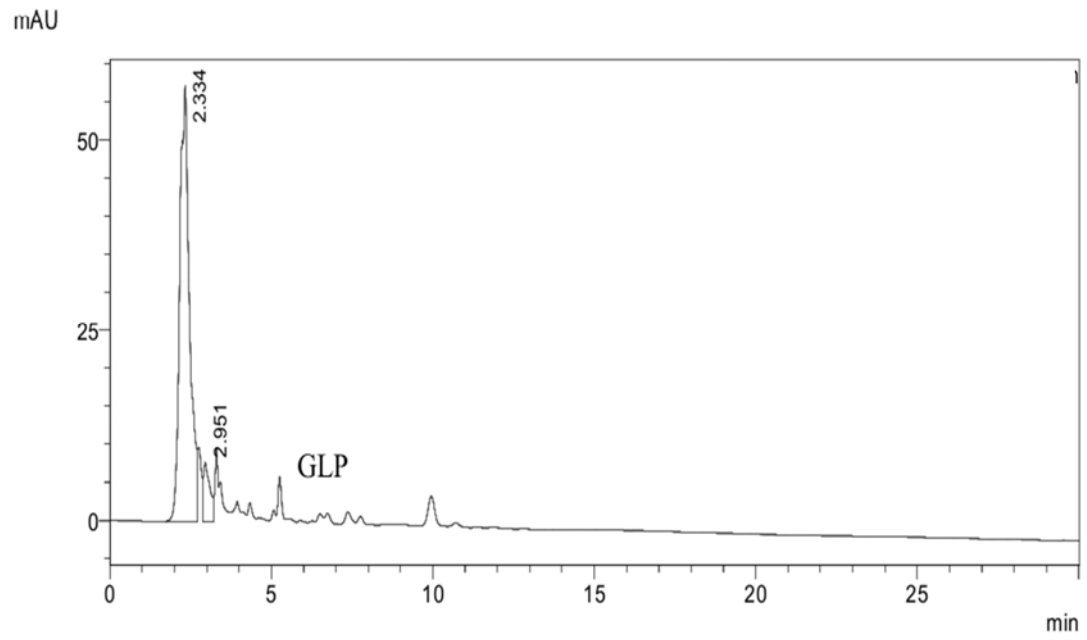


Figure 3.17 Chromatogram of Sample S

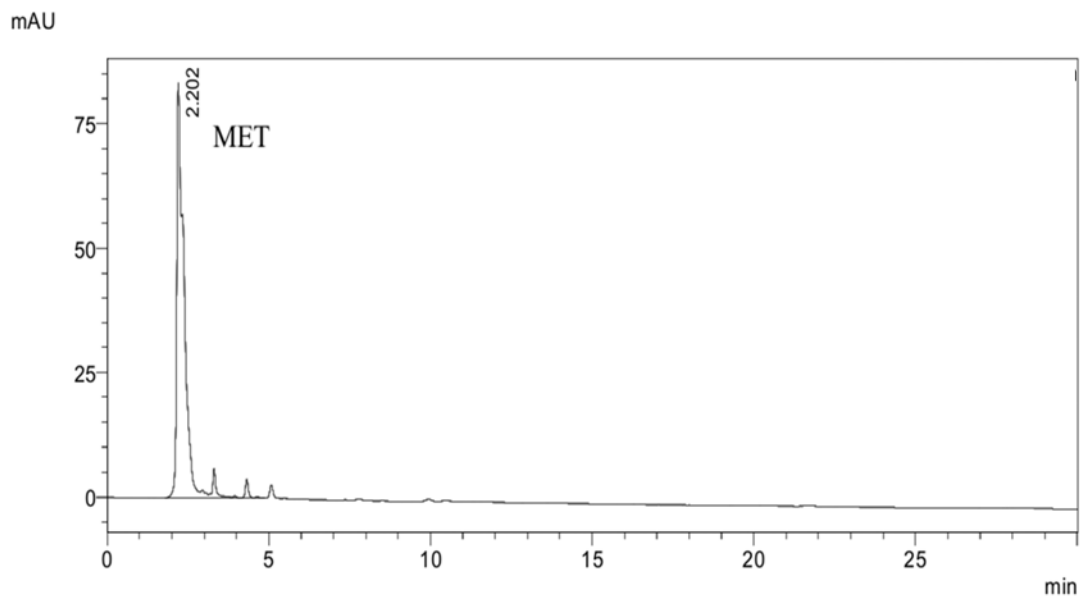


Figure 3.18 Chromatogram of Sample V

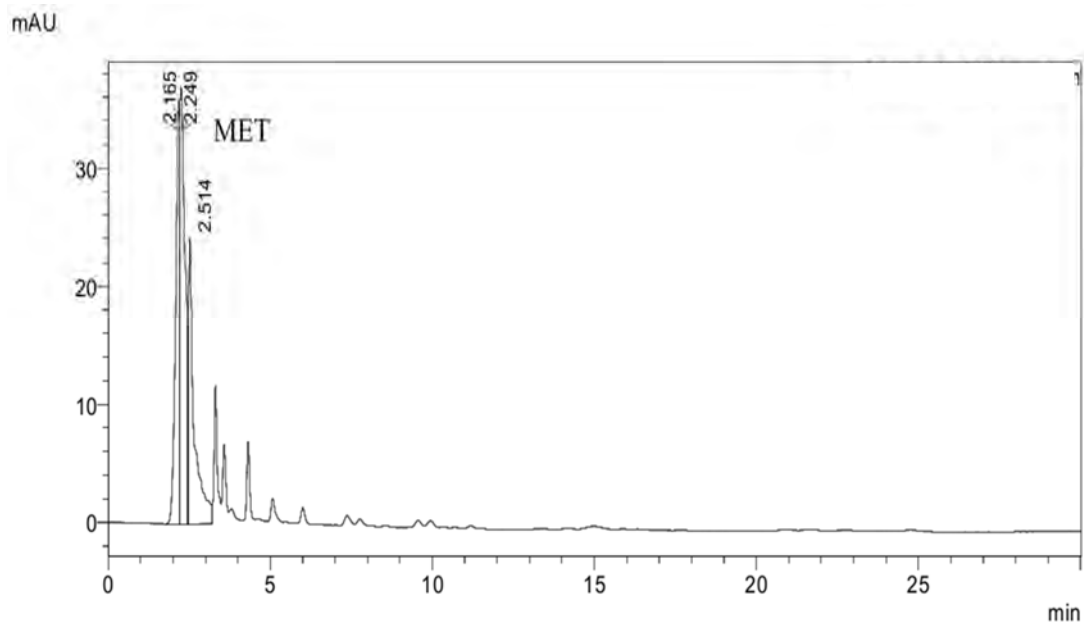


Figure 3.19 Chromatogram of Sample W

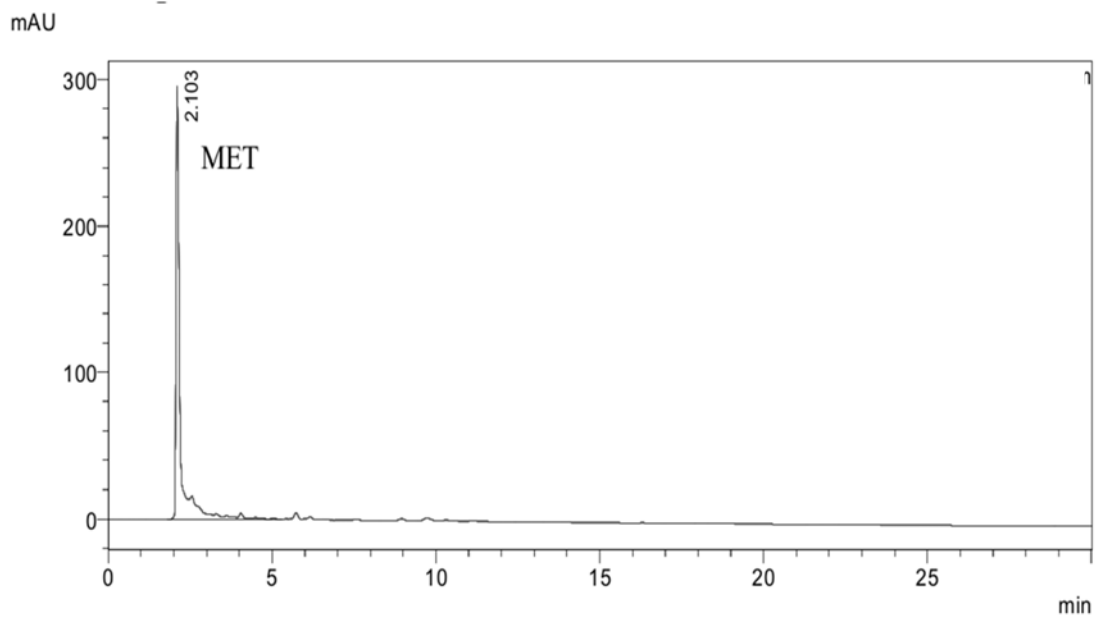


Figure 3.20 Chromatogram of Sample X

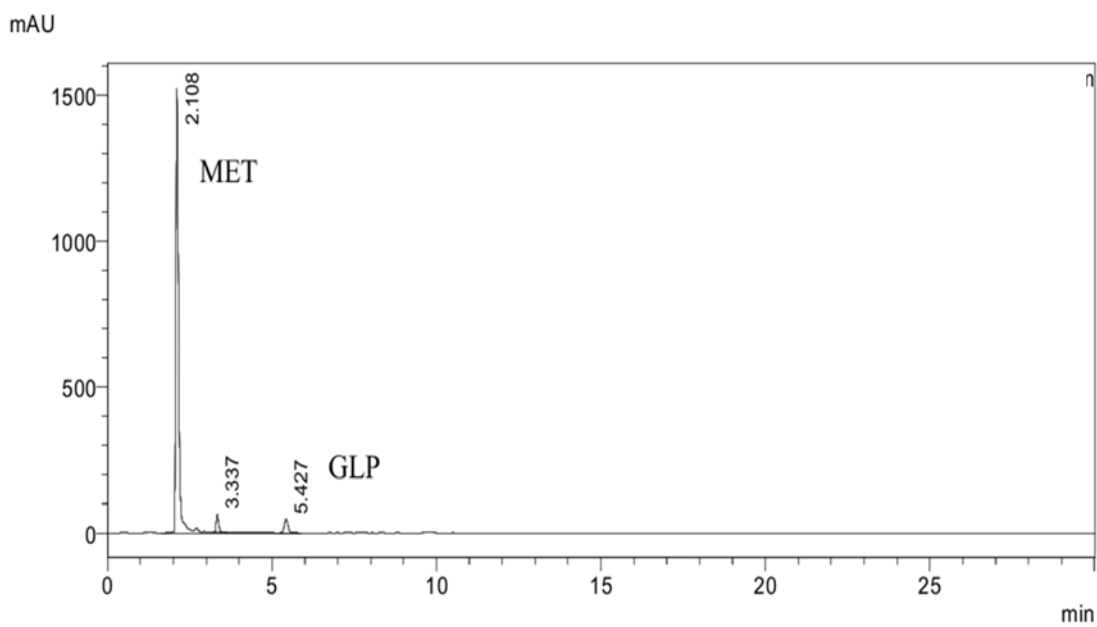


Figure 3.21 Chromatogram of Sample Y

Table 3.4 Concentrations of MET and GLP in herbal products \pm SD (n=3)

Compound type	Compound Name	Average Retention time (min)		Concentration ($\mu\text{g/mL}$) \pm SD	
		MET	GLP	MET	GLP
Samples	Sample A	2.2	-	186.191 \pm 0.533	-
	Sample C	-	5.7	-	39.7 \pm 88115
	Sample D	-	5.4	-	278.5 \pm 7343
	Sample E	2.210	5.8	613.93 \pm 4.650	102 \pm 9770
	Sample F	-	5.4	-	129 \pm 5278
	Sample G	-	5.4	-	201 \pm 54115
	Sample J	2.216	-	7.297 \pm 0.073	-
	Sample N	2.255	-	15.677 \pm 0.066	-
	Sample O	2.288	5.5	29.323 \pm 0.119	19 \pm 288.5
	Sample P	2.133	-	594.991 \pm 0.990	-
	Sample R	2.128	-	357.46 \pm 2.146	-
	Sample S	-	5.3	-	8 \pm 431
	Sample V	2.203	-	25.70 \pm 0.262	-
	Sample W	2.250	-	10.89 \pm 2.692	-
	Sample X	2.103	-	49.61 \pm 0.461	-
Sample Y	2.111	5.4	181.02 \pm 0.245	27 \pm 1275.5	

Chapter 4 Discussion

Herbal and synthetic drugs are effective at therapeutic concentrations and may become poisonous at higher concentrations. Co-administering herbs and pharmaceutical drugs can either increase or lessen the pharmacological or toxicological impacts of medications. Also, complications may arise during long term treatments due to synergistic effects obtained by the co-administration of herbal and synthetic drugs. Therefore, recommending these kinds of natural medications without knowing the history of a patient may prompt adverse reactions because of overdosing and (YR et al, 2003). Thus, it is never advisable to administer both herbal and pharmaceutical drugs without a fixed dose regimen.

A worse scenario than co-administration of these drugs without a fixed regimen is, when the herbal anti-diabetic drugs are adulterated with synthetic oral hypoglycemics (e.g. MET, GLP etc.) and hence co-administration occurs without the knowledge of both the patient and the prescriber. This means that even if the patient thinks that he/she is only taking herbal medicines, in reality the patient is actually taking both herbal and synthetic hypoglycemics due to the adulteration. This puts the patient at risk of undesired side effects like hypoglycemia since most of these herbal drugs are taken without any fixed regimen. Another case is when the patient knowingly co-administers herbal and synthetic hypoglycemics for a better control of blood glucose level but due to adulteration of the herbal drugs, overdosing occurs which leads acute hypoglycemia that is sometimes followed by death. As horrible as it sounds, the presence of undeclared synthetic oral hypoglycemics in herbal anti-diabetic drugs isn't uncommon around the globe. Unfortunately, several studies around the world proven that herbal drugs are commonly adulterated with synthetic oral hypoglycemics without any declaration of the compounds (Ernst, 2002) (Kumar, Mandal, & Hemalatha, 2011). Adulteration of herbal drugs is even more amidst Asia. According to California Department of Health 32 percent of Asian herbal drugs contain undeclared pharmaceuticals (Calahan, Howard, Almalki, & Gupta, 2016). A case report by the Banaras Hindu University, India revealed that a traditional herbal drug used in the country was adulterated with MET (Kumar et al, 2011). The case report also revealed that, after quantification by UV spectroscopy the 100 mg of the herbal

drug contained 93.1 mg of MET while 100 mg of MET tablet (synthetic drug) contained 97.1 mg of MET. Several other studies also suggested similar occurrences (Akinwande, 2013) (Hussin, 2001).

So, the objective of this experiment was to ensure the integrity of herbal anti-diabetic drugs in Bangladesh by finding out if they contain any undeclared synthetic oral hypoglycemic in their formulation and alert the mass people. The approach of this experiment was statistical as to figure out the percentage adulteration of the herbal drugs available at the local market. This experiment did not focus on quantifying the amount of adulteration present in a specific herbal drug or meet a certain adulteration level in a specific drug. Since the study focused on the overall percentage of adulteration in the drugs at the local market, all the available herbal drugs were subjected to this study and the smallest amount of adulteration was regarded as positive result. According to the obtained data, from 25 tested samples 16 of them confirmed the presence of adulterants, 9 contained MET, 5 contained GLP and 2 contained both the drugs, meaning that 64% of the samples were adulterated. From the data it can be seen that most samples contained MET. This is understandable since MET being the drug of choice for type II diabetes it also turned out to be most common adulterant used in herbal drugs. Although MET is the drug of choice for type II diabetes, taking it unknowingly without any specific dosage can turn out to be catastrophic. For example, MET might be contraindicated for a patient and uninformed ingestion of MET can pose to be injurious for the patient i.e. MET is contraindicated for patients with hepatic or renal impairment (serum creatinine levels ≥ 1.5 mg/dL for males, serum creatinine levels ≥ 1.4 mg/dL for females) (Kumar et al, 2011). Furthermore, uninformed administration of MET can also result in undesired drug interactions and harm the patient instead of healing e.g. severe interactions have been reported with MET and drugs like nifedipine that increases plasma MET Cmax level by 20 %, with drugs like thiazide diuretics MET decreases insulin sensitivity which leads to hyperglycemia and loss of diabetic control. On the other hand, taking GLP without knowing or without a fixed dose can be very dangerous since GLP is an insulin secretagogue. Being an insulin secretagogue GLP has a higher chance of causing hypoglycemia than MET and without a proper dosage regimen the result of taking GLP can be catastrophic.

From the final result it can also be said that more than half of the herbal drugs in the local market are adulterated with synthetic drugs. As the samples in this experiment were collected from the local markets of Dhaka it is safe to say that the results give a definitive idea about the integrity of the herbal drugs in the city. Therefore, it is logical to assume that the degree of adulteration might be similar or more (due to more liberal regulation systems) in case of the herbal drugs available at the local markets in remote areas. It is unfortunate that herbal anti-diabetic medications that promise control of blood glucose using all-natural plant extracts, are being adulterated with synthetic drugs. It is needless to say that this heinous act is puts a large number of patients at risk considering that the number of diabetic patients is significantly high. According to a report by Bangladesh Diabetic Samity, the number of diabetic patients in our country is 71 lakhs (UNB, 2016). It is imperative that the concerned authorities and the mass people are informed about this shameful act of adulteration. More importantly strict regulations should be ensured at all parts of the country if we want to save our patients from effects of this undesired and illegal act.

Chapter 5 Conclusion

5.1 Conclusion

The result of this investigation clearly concludes that the integrity of the available herbal anti-diabetic medications is impaired and 64% of the available drugs are adulterated with synthetic oral hypoglycemic metformin and glimepiride. This also calls for a thorough focus on making the regulation systems for these drugs stricter. The regulations related to licensing, labeling of drug product should be as strong as to ensure 100 % product integrity. More importantly intensive care should be given to quality control mechanisms that identify ingredients of the drug products. It is crucial that the patients are informed about this act and their long-term trust on these natural products is not exploited further.

5.2. Future directions

The study can be performed for the same category of herbal drugs (anti-diabetic) as done in this study, but using different analytical methods like NMR, MS for determination of structures of adulterants. Since this experiment was conducted only on the herbal products available at the local market of Dhaka, in future the study can be done at a larger scale by investigating herbal products from other parts of the country. In addition, different experiments can be designed to identify the presence of other synthetic oral hypoglycemics e.g. Glipizide, Repaglinide, Glyburide, Linagliptin etc. Furthermore, similar studies can be done for other categories of herbal drugs as well.

Chapter 6 Reference

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