QUALITY EVALUATION OF DIFFERENT BRANDS OF METFORMIN HYDROCHLORIDE 500 MG AND 850 MG TABLETS AVAILABLE IN MEKELLE CITY

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Quality Evaluation of Different Brands of Metformin Hydrochloride 500 Mg and 850 Mg Tablets Available in Mekelle City

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List of Abbreviations and Acronyms

API	Active Pharmaceutical Ingredient
ANOVA	Analysis of variance
BP	British Pharmacopeia
CI	Confidence Interval
DM	Diabetic Mellitus
EFDA	Ethiopian Food and Drug Authority
f1	Difference Factor
f2	Similar Factor
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
ICH	International Council on Harmonization
IDF	International Diabetes Federation
IVIVC	In Vitro In Vivo Correlations
KBr	Potassium Bromide
KGF	Kilo Gram Force
pH	Potential of Hydrogen
RSD	Relative Standard Deviation
SD	Standard Deviation
T1 DM	Type 1 Diabetes Mellitus
T2 DM	Type 2 Diabetes Mellitus
USP	United State Pharmacopeia
UV-Visible	Ultraviolet-Visible Spectroscopy
WHO	World Health Organization

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Abstract

Introduction: Metformin is the most commonly prescribed drug. The accessibility of substandard and falsified metformin products in the market lead to treatment failure, increased mortality, and morbidity. Ethiopia has a low port control and a weak regulatory system. Additionally, in Tigray, several brands of metformin tablets have been released into the market for the past three years without proper regulatory systems. To address this concern, this study was conducted.

Objective: This study aims to assess and compare the quality of different brands of metformin 500 mg and 850 mg tablets available in Mekelle City, Tigray, Ethiopia.

Method: Quality parameter tests such as physical characteristics, friability, dissolution, disintegration, weight variation, assay, and hardness tests were conducted as per British Pharmacopeia (BP), World Health Organization (WHO), and non-official standards. Data were analyzed using Origin Pro 2024 and one-way analysis of variance (ANOVA). To compare the dissolution profile of the tested product against the innovator, similarity factor (f2), and difference factor (f1) was used.

Result: Except for one brand, they all had online EFDA registration numbers. For metformin 500 mg brands: Weight variation ranges from -3.96 % to 2.22 %, assay value ranges from 95.2% to 102.1%, and dissolution value ranges from 90.4 % to 99.7 % at 45 minutes. For metformin 850 mg brands: Weight variation ranges from -2.1 % to 2.6 %, assay value ranges from 97.9% to 103.7%, and dissolution value ranges from 97.9 % to 101.6. % at 45 minutes.

Conclusion: Except for the hardness test, all the tested brands of metformin hydrochloride 500 mg and 850 mg tablets met the official BP and WHO specifications. This study could help to patients, EFDA, independent research groups, and other concerned bodies to get updated information on the quality status of metformin 500 mg and 850 mg tablets.

Keywords: Metformin Hydrochloride, Assay, Quality, Drug release.

1. Introduction

1.1. Background

Diabetes mellitus (DM) is a metabolic disorder characterized by persistently elevated blood glucose levels caused by disturbed insulin secretion, varying levels of insulin resistance, or a combination of both factors which leads to vascular complications. The inability of the cells to use the sugar in the blood increases the level of blood sugar as a result of either a small amount of insulin produced by the pancreas or inefficiency in cells responding to the insulin produced (WHO, 2016b, Schleicher *et al.,* 2022).

DM is divided into various subtypes, but the two primary forms are type 1 DM, which is an insulin-dependent form of the disease, and type 2 DM, which is a non-insulin-dependent form. Type 2 DM affects over 95% of diabetic individuals. Although it can happen at any age, this disease most commonly manifests in middle age and is primarily caused by unhealthy lifestyles including smoking tobacco, inadequate diet, and not exercising, which results in excess body weight or obesity (Petrovick, 2018, Ardoino *et al.,* 2023).

Globally, the prevalence of DM in adults aged 20–79-year-olds in 2021 was estimated to be 10.5% (536.6 million people), and by 2045, that number is expected to increase to 12.2% (783.2 million). DM prevalence was similar in men and women and was highest in those aged 75–79 years. Worldwide, 55% of diabetics aware that they have the disease (Sun *et al.,* 2022, WHO, 2023).

Currently, in Africa, the prevalence of DM in adults is 24 million people. By 2045, the number is expected to increase by 129% to 55 million. Some of the obstacles to DM testing in the African region include a lack of testing facilities and equipment, a shortage of medical professionals with the necessary training, limited access to healthcare facilities, and a lack of knowledge about DM. The prevalence of DM in Ethiopia ranges from 2.0% to 6.5%, with smaller rural areas having a low frequency of 2% (WHO, 2023, Bishu *et al.,* 2019).

For drug products to be considered pharmaceutically equivalent, they contain the same active pharmaceutical ingredient be in the same dosage form, and also meet the same compendia or other applicable standards for strength, quality, purity, and identity. Pharmaceutically equivalent products may vary in other characteristics such as shape, packaging, and excipients including

colors, flavors, and preservative agents, as well as any expiration date specified (Elghnimi *et al.*, 2019, Osman *et al.*, 2017).

Before a drug is absorbed into the bloodstream, it must first be dissolved. This means the drug dosage form must effectively release the drug in the gastrointestinal tract so that it can be properly absorbed. As a result, in vitro dissolution testing is a critical step for understanding how quickly and to what extent the drug is released within the body (AlBratty *et al.,* 2020).

The management of type 2 DM includes healthy eating, regular exercise, blood sugar monitoring, weight loss, DM medication or insulin therapy. Oral hypoglycaemic drugs are considered only after a regimen of dietary treatment combined with exercise has failed to achieve the therapy targets set. There are two major groups of oral hypoglycaemic drugs: sulphonylureas and biguanides. Sulphonylureas promotes the action of insulin through extrapancreatic mechanisms and by inducing the release of insulin from beta cells. The Sulphonylureas category includes tolbutamide and glibenclamide (Junior *et al.,* 2020, WHO, 1994).

The biguanides category includes metformin, which works by decreasing the absorption of glucose in the intestines, reducing glucose production in the liver, and enhancing insulin sensitivity. Metformin was discovered more than 100 years ago, and with 60 years of clinical use(Chan *et al.*, 2024). Metformin hydrochloride is an orally administered anti-diabetic medication that is primarily used for the treatment of type 2 DM mellitus. The chemical name for Metformin hydrochloride is N, N-dimethyl imido dicarbonimidic diamide hydrochloride (1,1-dimethylbiguanide hydrochloride). Metformin hydrochloride is freely soluble in water and practically insoluble in acetone and chloroform. The initial dose of metformin is generally 500 mg, two or three times daily, or 850 mg once or twice daily. Furthermore, it can be gradually increased if necessary, at intervals of at least 1 week, from 2 to 3 gram daily(Afifi and Ahmadeen, 2012, Mansour and Isbera, 2016, Alnedhary *et al.*, 2021, Chandrasekaran *et al.*, 2011, Petrovick, 2018, Tesfay *et al.*, 2019).

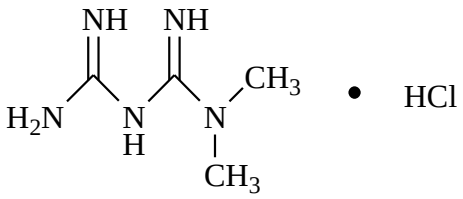

Figure 1: Chemical structure of metformin (Mansour and Isbera, 2016)

Variability in clinical response among different manufacturers version of the same drug, such as metformin hydrochloride has been reported. To ensure that generic drug products are therapeutically equivalent to their original innovator products, robust regulations and testing standards are essential. Establishing robust regulations can provide physicians and patients with greater confidence in substituting generics, ensuring therapeutic efficacy, and allowing them to benefit from low-cost options (Mekonnen *et al.,* 2021).

Ethiopia has a limited pharmaceutical industry, so the country primarily relies on imported drug products. Various multinational brands of metformin hydrochloride tablets are available in Ethiopia. Metformin hydrochloride tablets are the most commonly prescribed drugs. This has resulted in increased importation and manufacturing of various brands in Ethiopia (Abatea *et al.,* 2020).

Ethiopia has a low port control and a weak regulatory system. Additionally, in Tigray, several different brands of metformin hydrochloride have been released into the market for the past three years without proper regulatory and quality control systems. To address this concern, this study was conducted on the quality assessment of different brands of metformin hydrochloride 500mg and 850 mg available in Mekelle City, Tigray, Ethiopia.

1.2. Statement of the problem

One of the latest threats facing the global pharmaceutical industry and healthcare is the presence of fake and low-quality drugs in the market. The WHO estimates that 10% of the global market for pharmaceuticals consists of counterfeit medicines. This figure is significantly greater in developing countries, where counterfeit drugs can represent as much as 25% of the market, and in some cases can reach 50% or more. Ethiopia is a low-income country facing this challenge. In contrast, in the developed world, the incidence of counterfeit drugs is comparably low, around 1%. Detecting counterfeits is often difficult, because many of these goods pass through a long and complicated distribution network, thereby creating opportunities for counterfeits to enter the legitimate supply chain (Uddin *et al.,* 2017, AlBratty *et al.,* 2020, Blackstone *et al.,* 2014, Fahad Abdulaziz Alghannam *et al.,* 2014).

Interestingly, China and India rank highest among the world's counterfeit drug manufacturers (Khan and Ghilzai, 2007). Most of the active pharmaceutical ingredients (APIs), produced in China and India, are employed to manufacture fake or counterfeit medicines, which can be marketed worldwide. According to the WHO, these counterfeiting pharmaceutical products represent an approximate annual trade of 73 billion Euros (Khan and Ghilzai, 2007, AlBratty *et al.,* 2020).

Numerous generic medications have been withdrawn from the market because of numerous quality-related problems. These issues may include impurities, failure to meet dissolution testing requirements, or a potency not within the stated range. Medications that have been recalled are, for example, metformin 500 mg tablets, quinapril 20 mg tablets, hydrochlorothiazide 12.5 mg tablets, anagrelide 1 mg capsules, paliperidone 3 mg extended-release tablets, tetracycline HCl 250 mg capsules, lidocaine HCl topical solutions, and thyroid tablets (Arafat *et al.,* 2023, Keire *et al.,* 2022). Treatment failures and death have been reported in the use of substandard anti-diabetic drugs like glibenclamide in China and insulin in Nigeria (Eraga *et al.,* 2017).

Ethiopia currently depends almost entirely on imported medicine, with only a few local drug-manufacturing industries. Such reliance can become an issue, specifically when different manufacturers produce different brands of medicines, which elicits a variable clinical response. The reason for the emergence of such drugs in low and middle-income countries is largely linked to a lack of regulatory enforcement, unregulated informal populations, poor infrastructure,

unregulated pharmacies, poor port controls, lack of cooperation from various executives, and lack of resources in low and middle-income countries. The accessibility and utilization of substandard and counterfeit metformin products in the market may lead to the risk of treatment failure, increased mortality and morbidity, potential development of drug resistance, and an overall economic loss (Osman *et al.,* 2017, Mekonnen *et al.,* 2021, Newton *et al.,* 2009).

The emergence of generic medicines from various sources, in particular, in developing countries, has enhanced the availability and affordability of certain life-saving medications. This rationale is also applicable to sales of genetic drugs whose prices are considerably low, thus leading to lower costs in the provision of public health services. However, despite such benefits, there are still many areas where generic products have become a cause of variation from the original which puts the health of many customers in danger. The substitution of the generic formulation for the branded one is still controversially accepted (Mekonnen *et al.,* 2021).

A study conducted by Younes *et al.,* in Qatar on a total of 10 brands of metformin hydrochloride (nine metformin 500mg and one 850mg brand tablets) evaluated based on the United State Pharmacopeia (USP) guidelines. Of the ten brands tested, six met the Pharmacopeial specification. The rest four brands did not meet the content uniformity test criteria (Younes *et al.,* 2016).

A study conducted by Mansour *et al.,* to evaluate the physicochemical properties of five brands of 850mg metformin hydrochloride tablets marketed in Syria using both official and non-official testing standards including weight variation, friability, hardness, content uniformity, and dissolution rate. The results indicate that only two of the brands fulfilled the pharmacopoeial requirements and may be used interchangeably, while the other three brands did not meet the official standards and therefore cannot be used interchangeably (Mansour and Isbera, 2016).

A study conducted by Olusola *et al.,* assessed the chemical and biopharmaceutical equivalence of eight metformin hydrochloride generic brands available in the Nigerian market. The tested brands were evaluated using both official and non-official tests. From the tested brands in that study, four out of eight were regarded as being bio-pharmaceutically equivalent and therefore they can be used interchangeably in clinical practice (Olusola *et al.,* 2012). Oral metformin is widely used in Ethiopia, with several new brands of the drug introduced into the Ethiopian market in recent years. The variety of metformin brands in circulation often puts clinicians and pharmacists in a difficult

situation when it comes to choosing which brand to use and the potential interchangeability between brands (Kassahun *et al.,* 2019, Olusola *et al.,* 2012).

1.3. Significance of the study

This work will increase awareness among health practitioners, regulatory bodies, and independent research teams regarding the evaluation of the quality of different brands of metformin hydrochloride.

Ensuring the supply and availability of quality medicines for patients: Through the assessment of the quality of various generic metformin hydrochloride products, the study will offer some assurance that patients will have access to safe and effective generic medicines that can be used interchangeably with the brand products.

Identifying poor quality, falsified, or substandard metformin hydrochloride: The up-to-date information from this study will help to identify if there are poor quality, counterfeit, or substandard metformin hydrochloride products available to the public, and this information can be utilized to call attention to the public, or governmental bodies and to take appropriate actions.

Guiding decision-making and advancing regulatory improvements: The findings from this study may also inform regulatory bodies so they can act on time, by taking appropriate actions to comply with their policies and guidelines advancing their regulation of this population well in advance of other investigational studies.

Offering recommendations to manufacturers: The findings from the study may also offer manufacturers important recommendations, in the future, to improve their processes to produce a more scientifically reliable product and potentially higher quality of metformin hydrochloride.

For researchers, this study will form the foundation upon which other related and replicated studies can be based.

1.4. Conceptual frame work

The conceptual frame work which was used for comparative quality evaluation of different brands of metformin hydrochloride 500 mg and 850 mg tablets available in Mekelle City

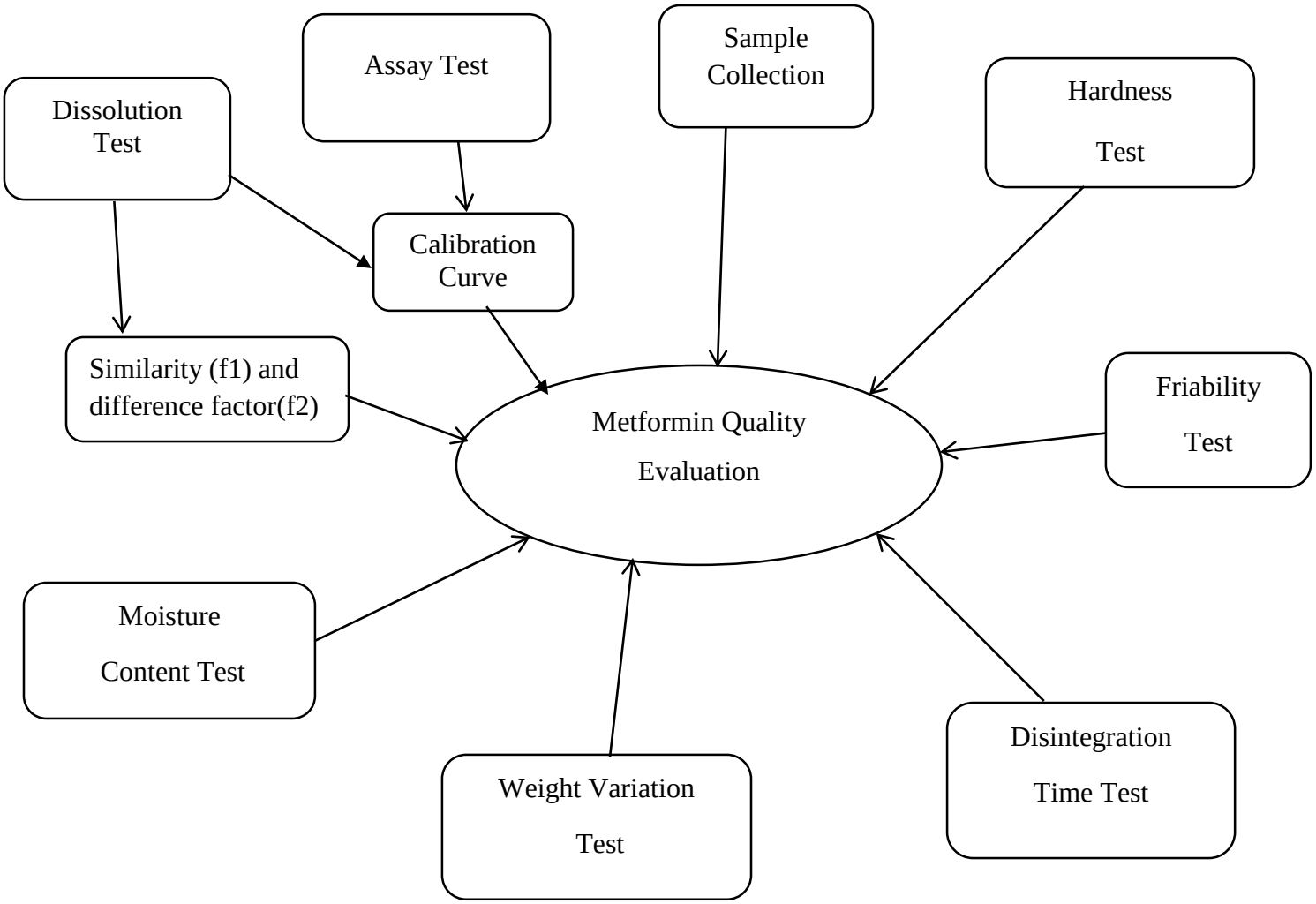

Figure 2: The conceptual frame work for quality evaluation of different brands of metformin hydrochloride 500 mg and 850 mg tablets.

2. Literature Review

2.1. Global burden of DM

Over the past thirty years, mortality and vascular complication rates in individuals with DM have significantly decreased by half in many cases. However, people with type 2 DM still face a twofold higher risk of mortality both overall and from cardiovascular causes despite advancements in prevention strategies. This increased risk is even more obvious in individuals with type 1 DM (Harding *et al.,* 2019, Rawshani *et al.,* 2017). According to estimates from the International Diabetes Federation (IDF), someone dies from DM or its complications every six seconds. Half of these deaths, around 4 million each year, occur in individuals under 60 years old (Standl *et al.,* 2019). According to Sharma *et al.,* global studies of Type 2 DM, more than 50% of deaths are due to cardiovascular causes, with sudden death being the most common cause, followed by deaths from a combination of myocardial infarction or stroke, and deaths from heart failure (Sharma *et al.,* 2017).

A study was conducted by Sun *et al.,* on prevalence and health expenditure estimates of DM at the global, regional, and country levels for 2021 and projections for 2045. A total of 219 data sources were identified that met quality criteria, from 215 countries and territories between 2005 and 2020. For countries with no quality data, estimates were derived from countries with similar economic, ethnic, geographic, and linguistic characteristics. Logistic regression was used to generate age-specific prevalence estimates of DM. The DM health expenditure was calculated using an attributable fraction method. 2021 prevalence was applied to the 2045 population to project future DM prevalence. He reported that in 2021, global DM prevalence among 20-79-year-olds was 10.5% (536.6 million people), estimated to increase to 12.2% (783.2 million) by 2045. Prevalence was similar for both sexes, peaking in the 75-79 age group. Urban areas had a higher prevalence (12.1%) than rural areas (8.3%) and high-income countries had a higher prevalence (11.1%) than low-income countries (5.5%). Middle-income countries will see the biggest increase in DM prevalence between 2021 and 2045 (21.1%) compared to 12.2% in high-income and 11.9% in low-income countries. He also reported that the global DM health expenditure was 966 billion USD in 2021 and is estimated to be 1,054 billion USD by 2045 (Sun *et al.,* 2022).

Heald *et al.,* conducted a comprehensive analysis utilizing data from the National DM Audit and the Office of National Statistics to assess the impact of type 1 DM and Type 2 DM on life expectancy in the United Kingdom. The study highlights significant mortality implications associated with DM diagnoses. Life years lost: The study estimates that the current United Kingdom population may collectively lose approximately 6.4 million future life years due to the higher mortality rates associated with type 1 DM and type 2 DM. Life Expectancy Comparisons: For individuals with Type 1 DM (average age 42.8 years), the life expectancy is 32.6 years, contrasted with 40.2 years for age-matched individuals without DM, resulting in an average loss of 7.6 years per person. For individuals with Type 2 DM (average age 65.4 years), the life expectancy is 18.6 years, compared to 20.3 years for non-diabetic counterparts, which translates to an average loss of 1.7 years per person. Glycaemic control and mortality: The research indicates that for both Type 1 DM and Type 2 DM, each year with an HbA1c level exceeding 58 mmol/mol is associated with a loss of approximately 100 life days. This connection provides the importance of glycaemic control in managing DM and its complications (Heald *et al.,* 2020).

Magliano *et al.,* conducted a systematic review to assess the trends in the incidence of diagnosed DM from 1980 to 2017. The researchers used multiple databases including Medline, Embase, along with reference lists of relevant publications. The eligibility criteria focused on studies involving open population-based cohorts, DM registries, and administrative databases that reported secular trends in DM incidence among adults. Findings: Out of 22,833 screened abstracts, 47 studies were included, representing 121 distinct populations categorized by sex and ethnicity. Particularly, 42 of these studies (approximately 89%) focused specifically on diagnosed DM. Trends from 1960-1989: Among the populations studied, 36% (8 out of 22) exhibited increasing trends in DM incidence, 55% (12 out of 22) showed stable trends, and 9% (2 out of 22) reported decreasing trends. Trends from 1990-2005: The incidence of DM increased by 66% (33 out of 50) of the population, remained stable at 32% (16 out of 50), and decreased by 2% (1 out of 50). Trends from 2006-2014: A significant shift was observed, with only 33% (11 out of 33) reporting increasing trends. Conversely, 30% (10 out of 33) had stable incidence rates, while 36% (12 out of 33) demonstrated declining trends (Magliano *et al.,* 2019).

2.2. Role of metformin in the management of DM

The systematic review conducted by Patel *et al.*, examined the effectiveness of metformin in preventing DM, synthesizing data from 17 studies with a combined sample size of 30,474 participants. The study focused on two primary analyses that evaluated metformin's impact on individuals at risk for DM. The first analysis involved a meta-analysis specifically assessing the effects of metformin on individuals with pre DM. The results indicated a significant reduction in the risk of progressing to type 2 DM mellitus. The pooled odds ratio was found to be 0.65, with a 95% confidence interval (CI) ranging from 0.53 to 0.80. This suggests that individuals with pre DM who received metformin interventions had a 35% lower odds of developing T2DM compared to those in control groups. In the second key analysis, the meta-analysis focused on the overall efficacy of metformin interventions in preventing the onset of type 2 DM (T2DM). This analysis yielded a pooled risk ratio of 0.58 (95% CI 0.44-0.77), indicating a 42% reduction in the risk of developing T2DM among individuals receiving metformin compared to those in non-metformin intervention groups. From this study, it is concluded that the effectiveness of metformin in preventing the progression from pre DM to T2DM and in reducing the overall incidence of the disease serves as a valuable tool in public health strategies aimed at mitigating the growing burden of DM mellitus (Patel *et al.*, 2023).

A study conducted by the Diabetes Prevention Program (DPP) Research Group investigated the long-term effects of lifestyle interventions and metformin on the development of DM and microvascular complications over a 15-year follow-up period. Of the original cohort, 2,776 participants (88%) were included in the DPP Outcomes Study (DPPOS), which ran from September 1, 2002, to January 2, 2014. The analysis was performed based on the initial treatment assignments, adhering to an intention-to-treat approach. The primary outcomes of interest were the incidence of DM and the prevalence of microvascular diseases, which were assessed using a composite measure that included nephropathy, retinopathy, and neuropathy. Over an average follow-up period of 15 years, the incidence of DM was reduced by 27% in the lifestyle intervention group and by 18% in the metformin group compared to the placebo group, although the differences between the groups diminished over time. By the end of the study period, the cumulative incidences of DM were 55% in the lifestyle group, 56% in the metformin group, and 62% in the placebo group. Notably, among women (n=1,887), the lifestyle intervention was linked to a

significantly lower prevalence of DM compared to both the placebo and metformin groups, with reductions of 21% and 22%, respectively. Additionally, participants who did not develop DM exhibited a 28% lower prevalence of microvascular complications compared to those who did (DPR, 2015).

Shurab *et al.,* conducted a comprehensive review to assess the therapeutic efficacy and adverse effects of metformin, focusing on its use in obesity. The researchers searched the literature data on PUBMED and identified relevant studies, including animal experiments, randomized controlled trials, and case reports involving obese patients with or without type 2 DM. The study indicated that although metformin may help in weight loss irrespective of DM status, it does not guarantee long-term safety. Particularly, metformin was associated with various complications beyond gastrointestinal issues, such as pancreatitis, hepatitis, vitamin B12 deficiencies, coagulation abnormalities, and reactive hypoglycemia. The findings emphasize the necessity for increased awareness among patients and healthcare practitioners regarding the potential long-term risks of metformin, particularly when prescribed only for weight loss (Shurrab and Arafa, 2020).

2.3. Quality concern of different brands of metformin tablets

Several studies have been conducted on the quality evaluation of different brands of metformin hydrochloride in a different country and the studies show different results.

A study was conducted by Younes *et al.,* to evaluate the in vitro bioequivalence and interchangeability of ten brands and generics of metformin multi-sourced tablets. The research aimed to assess these products per the USP guidelines. The study involved testing ten brands of metformin tablets (nine brands of metformin 500 mg and one brand of 850 mg) through content analysis and dissolution tests. For content analysis, twenty tablets from each brand were analyzed using Ultra-Violet (UV) spectrophotometry, while dissolution tests were performed on six tablets from each brand using a basket apparatus at 100 rpm in 1000 ml in a phosphate buffer potential of hydrogen (pH 6.8). The UV scanning and calibration were carefully performed to ensure the reliability of the results. The dissolution profiles of the tested products were compared to the reference product, Glucophage, using the f2 for evaluation. The findings demonstrated that products 2, 4, 5, 8, 9, and 10 showed assay percentages within the acceptable range of 95–105% for the content test as per USP standards. Additionally, all brands showed over 70% dissolution

within 45 minutes. The similarity factor analysis indicated that products 1, 3, 4, 5, 7, and 10 were bioequivalent to the reference, as they achieved f2 values of 50% or higher (Younes *et al.*, 2016).

In recent research, Akdag *et al.*, (2020) assessed the dissolution and permeability characteristics of immediate-release metformin hydrochloride tablets, focusing on five generic products obtained from the Turkish drug market and two reference products from both Turkish and European markets. Their study aimed to determine whether these formulations met the established criteria for rapid dissolution and if there were significant differences in permeability among the products. The methodology used for the metformin hydrochloride tablet monograph was USP, employing in vitro dissolution tests. The findings showed that most generic tablets, except for generic tablet B, did not achieve the criteria for very rapid dissolution. While the dissolution profiles of the reference products demonstrated similarity ($f2 > 50$), the generic formulations failed to show comparable profiles to either reference, indicating potential inconsistencies in formulation quality (Akdag *et al.*, 2020).

In 2020, a study was carried out in Brazil to investigate the quality of four brands of metformin hydrochloride 500 mg and four brands of metformin hydrochloride 850 mg tablets. The study was conducted per the requirements set out in the Brazilian Pharmacopoeia, including evaluation of tablet weight, hardness, disintegration, content uniformity, dissolution, and assay. Generally, one hundred percent (100%) of the metformin hydrochloride 850mg samples passed all quality-tested parameters. However, one brand of the four metformin hydrochloride 500 mg tablets failed to meet all criteria for testing and was hence not suitable for clinical interchangeable usage with the other marketed brands (Junior *et al.*, 2020).

Ozyilmaz *et al.*, conducted a study comparing the physicochemical properties and release profiles of eight different brands of metformin tablets available in the Northern Cyprus, Turkey pharmaceutical market, including an innovative product, Glucophage. The research involved purchasing seven brands of metformin from community pharmacies and evaluating them for impurities using Fourier transform infrared spectroscopy. The analysis was performed using UV Visible spectroscopy and the USP Pharmacopeia. An in vitro dissolution test was performed, and the dissolution data were analyzed by calculating the dissolution factor f1 and f2. The results indicated that all tablet brands met the official USP Pharmacopeia specifications for uniformity of weight, hardness, and disintegration, although brand MF failed the friability test with a result

of>1%. Additionally, brands MC, MF, and MG did not meet the content uniformity (assay) test, showing values below the required threshold of 95%. The dissolution profiles were evaluated in a pH 6.8 buffer medium, revealing that brand MB had an f1 value of 15.45, which exceeded 15, while f2 values for brands MB and MF were 48.57 and 47.13, respectively, both falling below the threshold of 50. This indicated that the dissolution profiles of brands MB and MF significantly differed from that of the innovative brand, Glucophage (Ozyilmaz, 2022).

A study conducted by Mansour *et al.*, to evaluate the physicochemical equivalence of five different brands of Metformin hydrochloride tablets (850 mg) available in the Syrian drug market by using BP and USP specifications was conducted. Weight variation and friability: All five brands met the BP official specifications for both weight variation and friability tests, indicating consistency in these critical quality parameters. Hardness: Only two brands (B and E) satisfied the non-official specification test for crushing strength/hardness. The remaining brands (A, C, and D) did not meet the required standards, raising concerns about their mechanical stability. Assay: three brands achieved content uniformity values within the acceptable range of 95-105% as specified by the USP. However, brands A and D failed this test, suggesting variability in the active ingredient distribution. Dissolution Rate: three tested brands released more than 75% of the drug within 30 minutes, per the USP acceptance criteria. Brands A and C did not meet this criterion, indicating slower drug release profiles. Generally, the study highlights significant variations in the quality attributes of metformin brands marketed in Syria. While all brands complied with weight variation and friability standards, notable discrepancies were found in hardness, content uniformity, and dissolution rates, particularly among brands A and C. This shows the importance of rigorous quality assessment to ensure therapeutic efficacy and safety in pharmaceutical products (Mansour and Isbera, 2016).

A research study was conducted in India in 2016 to examine, compare, and evaluate the quality standards of four different brands of Metformin tablets (500mg) using the official UV-Visible double beam spectrophotometer method available in the local market of Kanpur, India. The study was conducted following the requirements set out in the Indian Pharmacopoeia. The physicochemical equivalence of all the tablet brands was assessed by evaluating both official and non-official standards, such as uniformity of weight, friability, hardness, disintegration, assay, and dissolution rate. The disintegration time for all brands was within the 15-minute limit prescribed

by the official compendium. All four brands of Metformin hydrochloride tablets fulfilled the official in-vitro dissolution rate test specification, with more than 70% of the drug being released within 45 minutes. The findings suggest that almost all brands of Metformin Hydrochloride meet the quality control analysis specifications and can be used interchangeably (Sachan *et al.*, 2016).

Eraga *et al.*, conducted a study assessing the pharmaceutical quality of various brands of metformin hydrochloride tablets available in Abuja, Nigeria. The research involved the evaluation of ten different brands, which were purchased and subjected to several pharmaceutical quality tests, including friability, hardness, disintegration, and dissolution assessments. The active pharmaceutical ingredient (API) content was analyzed using spectrophotometric methods and reverse-phase high-performance liquid chromatography (HPLC), using the BP standards method. All brands were properly labeled, packaged, and within their shelf lives, except for one brand, all had registration numbers from the National Agency for Food and Drug Administration and Control. The results indicated that the weight uniformity, friability, hardness, and disintegration times for all brands confirmed acceptable limits. However, three brands failed the dissolution test, releasing less than 70% of their API within 45 minutes. A difference had occurred between the assay results obtained from UV spectrophotometry and HPLC; specifically, HPLC identified only one brand as failing, with 86% of the API, while UV analysis indicated that four brands did not meet the required standards. The findings suggest that despite the apparent compliance of most brands, four did not meet official specifications, emphasizing the need for ongoing surveillance of metformin tablets in the market. The study supports the use of HPLC for product analysis due to its superior sensitivity and accuracy (Eraga *et al.*, 2017).

The study was conducted by Elghnimi *et al.,* to evaluate and compare six different brands of Metformin 500 mg tablets that are available in the Libyan market. These brands were produced by different pharmaceutical companies under different trade names and were assessed for their physicochemical equivalence based on both official and non-official standards methods in the British Pharmacopoeia. The parameters evaluated included uniformity of weight, thickness, hardness, disintegration time, drug content, and dissolution rate. The result showed that all brands met acceptable external characteristics and uniformity in both diameter and thickness and, all brands complied with the official specifications for weight uniformity, with no tablet deviating by more than ±5%. Among the brands, B, C, and F were found to be harder than the others, which

affected their disintegration and dissolution properties, though still within acceptable limits Importantly, all six brands were showed bioequivalent, suggesting that they can be interchanged in clinical practice without compromising efficacy or safety. This study serves as a valuable indicator for assessing the quality of commercial pharmaceutical products (Elghnimi *et al.*, 2019).

Osman *et al.,* conducted a study to evaluate the pharmaceutical equivalence of five different brands of Metformin hydrochloride 500 mg tablets available in the Sudanese market. The brands were randomly purchased from registered pharmacies in Khartoum and coded as A, B, C, D, and E, with brand A being the innovator. The researchers used both official and non-official tests from the BP and the USP, assessing parameters such as assay of content, uniformity of weight, friability, hardness, thickness, disintegration time, and dissolution tests. The result showed that all five brands met the requirements for weight variation, thickness, friability, disintegration time, and dissolution tests. However, the assay test showed that, although the five brands passed with pharmacopoeial specifications, brand B did not pass. In the hardness test, four brands met the criteria, while brand D failed the non-official specification. Dissolution profiles were analyzed using f2 and f1, revealing good release profiles for all brands, with f2 values greater than 50 and f1 values lower than 15 when compared to the innovator drug A. From the study it is concluded that brands A, C, D, and E could be considered biopharmaceutical and chemically equivalent, suggesting they can be used interchangeably in clinical practice (Osman *et al.*, 2017).

A research study was conducted by Olusola *et al.,* in Nigeria in 2012 to assess the biopharmaceutical and chemical equivalence of eight different brands of Metformin tablets marketed in the Nigerian market. The researchers evaluated the physicochemical properties of these tablets, including their uniformity of weight, friability, hardness, disintegration, assay, and dissolution rate, based on BP and non-official standards. The results showed that all brands complied with official specifications for uniformity of weight, disintegration, and dissolution tests. However, brand B revealed the highest crushing strength, while brand C had the lowest. In the friability test, one of the eight brands failed to meet the BP specification. For the assay, seven brands passed the acceptable specification, but brand G did not pass the test. The study concluded that only four out of the eight brands tested were regarded as being bio pharmaceutically and chemically equivalent. This means that only four brands could be considered interchangeable and

used clinically in a substitutable manner. The other four brands did not meet the required standards and may not be suitable for direct substitution in clinical practice (Olusola *et al.,* 2012).

The study conducted in Ethiopia aimed to evaluate the quality and physicochemical bioequivalence of six different brands of metformin hydrochloride tablets marketed in Addis Ababa. The researchers used the methods outlined in the USP 2007 for their analysis. The study found that all six brands of metformin hydrochloride tablets met the official specifications for hardness, friability, assay, and disintegration. However, five of the six brands were found to comply with the USP's dissolution tolerance limits, while the brand "Metformin Denk" failed to release the stated amount of the active ingredient (Kassahun *et al.,* 2019).

A study was conducted by Mekonnen *et al.,* in Ethiopia to compare the quality of metformin hydrochloride 500 mg tablets available in Jimma town. Evaluation parameters including the physical characteristics, packaging, and labeling information of the samples were assessed according to WHO guidelines. In vitro tests, such as weight variation, friability, dissolution rate, and assay, were performed on six brands of Metformin hydrochloride tablets following methods outlined in the USP. For comparing the dissolution profiles of the generic products with the innovator product, a model-independent approach was employed, using the f2 and f1. The results showed that all tested brands complied with WHO specifications for physical characteristics, packaging, and labeling of pharmaceuticals. However, the brand insumet revealed a percent weight deviation greater than 5%, failing to meet the USP specification for uniformity of weight. Statistically, there was a significant difference in the mean weight variation among all brands ($P<0.001$). The assay results ranged from 95.21% to 99.61%, demonstrating that all brands met the USP requirements. Additionally, the single-point dissolution test results for the brands ranged from 85.4% to 96.7%, indicating compliance with USP specifications (Mekonnen *et al.,* 2021).

3. Objective

3.1. General objective

- To evaluate the quality of different brands of metformin Hcl 500 mg and 850 mg tablets available in the market of Mekelle City, Tigray, Ethiopia

3.2. Specific objective

- To assess and compare the physical characteristics, packaging, and labeling of different brands of metformin Hcl 500 mg and 850 mg tablets in Mekelle City, Tigray, Ethiopia
- To compare the dissolution profile of different brands of metformin Hcl 500 mg and 850 mg tablets in Mekelle City, Tigray, Ethiopia
- To compare the assay of different brands of metformin Hcl 500 mg and 850 mg tablets in Mekelle City, Tigray, Ethiopia

4. Materials and Methods

4.1. Study Setting and Period

The study was conducted on seven brands of metformin hydrochloride 500 mg and three brands of metformin hydrochloride 850 mg tablets available in Mekelle City, Tigray, Ethiopia, 783 km away from Addis Ababa, the capital of Ethiopia. The laboratory work was conducted in APF Adigrat branch, Tigray, Ethiopia (Annex7), in EFDA, Addis Ababa branch, Ethiopia. The time taken for this study was from September 2023 to October 2024.

4.2. Instruments and equipment

Analytical Balance (Sartorius, Germany), dissolution apparatus (Hainburg, Germany), UV–Vis Spectrophotometer (Agilent Technology, USA), Fourier Transform Infrard Spectroscopy (FTIR) (Bruker, Berlin, Germany) Friability tester (Hainburg, Germany), pH meter (Hainburg, Germany), disintegration tester (Hainburg, Germany), hardness tester (Berlin, Germany) and moisture Balance (Sartorius, Germany) was used for the study.

4.3. Chemicals and reagents

Sodium hydroxide (97 %, Nice chemicals LTD, India), absolute alcohol (99.8%, Fisher scientific, Belgium), potassium dihydrogen orthophosphate (Research-Lab fine chem., India, 98%) KBr pellet, distilled, metformin hydrochloride USP reference standard (99.7 %) donated by EFDA, different tested brands of metformin tablets were utilized.

4.4. Sampling technique and sample collection

Before the actual sample collection, information was gathered on available brands of both metformin 500 mg and metformin 850 mg tablets from pharmacy professionals in community pharmacies of Mekelle City, Tigray, Ethiopia. A total of 7 brands of metformin 500 mg tablets and 3 metformin 850 mg tablets were available in the market during the study period. Data was obtained from the EFDA Mekelle branch office and there were registered lists of 150 pharmacies and 160 drug stores during the study period. The lists were sorted alphabetically, numbered, and coded. To avoid repetition of drugs to be sampled, 10 community pharmacies of which 7 were pharmacies and 3 drug stores were selected by interval using the lottery method. Guidelines to conduct the survey of quality of medicines were used for the sampling strategy (Newton *et al.*, 2009). Based on a previously conducted study, for each brand of metformin tablet, a total of 120

tablets (120×10=1200 tablets) were purchased from the selected community pharmacies in Mekelle City, Tigray, Ethiopia (Teshome *et al.*, 2023). Pharmacy personnel who were trained and acted as simulated caregivers visited each of the selected community pharmacies with a prescription for a metformin tablet. The samples were kept in their original package, transported to the APF, and stored under storage conditions specified on the label of each product until the analysis was done. The collected sample was subjected to visual inspection, name of API, batch number, manufacturing date, expiry date, batch/lot number, country of origin, packing size, regulatory status, and number of sample units taken Annex 1 (WHO, 2005).

4.5. Quality assessment parameters

The following parameters were assessed during this study: Physical characteristics, packaging, and labeling, friability test, dissolution test, assay test, disintegration test, weight variation test, hardness test, moisture content test using official compendia: BP (2022), WHO guidelines, and non-official compendia.

4.5.1. Physical characteristics, packaging, and labeling

The physical characteristics, packaging, and labeling of the tablets were assessed according to the guidelines set by the WHO. All samples underwent a visual inspection to evaluate their physical characteristics, such as shape, color, breaks, cracks, and splits. Additionally, the packaging and labeling information was examined, including the name of the active pharmaceutical ingredient, the country of origin, the manufacturing company, the manufacturing date, the expiry date, the batch/lot number, the number of units per strip/package, and the labeled dosage (strength) of the active ingredient as shown in annex 2. This assessment was carried out using a modified WHO checklist, which is designed to help healthcare professionals visually inspect medicines for signs of counterfeiting and report any findings to the appropriate national authority or directly to the WHO (WHO, 2016a, WHO, 2013).

4.5.2. Identification test

For identification according to BP specification a quantity of the powdered tablets, containing 20 mg of metformin hydrochloride, was mixed with 20 ml of absolute ethanol (BP, 2022). The mixture was filtered, and the filtrate was evaporated to dryness. The residue was then dried at 105°C for one hour to prepare it for the identification test. The same procedure was followed for

the metformin reference standard. Finally, the test sample was scanned using the FTIR KBr plate method. The infrared absorption spectrum of the residue was found to be concordant with the reference spectrum of metformin hydrochloride.

4.5.3. Weight variation test

The weight variation test for different brands of metformin hydrochloride tablets was conducted according to the official method specified in the BP (BP, 2022). Twenty tablets from each brand were randomly selected and individually weighed using a calibrated analytical balance. The average weight for each brand was then determined. The percentage deviation from the average tablet weight was calculated using Equation 1 and compared against the BP limit. According to BP, the acceptable weight variation limit for tablets with an average weight of 250 mg or more is no more than two individual tablets should deviate by more than 5% of the average tablet weight, and none should deviate by more than 10% of the average tablet weight (Balamuralidhara, 2011, BP, 2022).

$$\%\ Deviation = \frac{Weight\ of\ individual\ tablet - Average\ weight\ of\ tablet}{Average\ weight\ of\ tablet} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots 1$$

4.5.4. Friability test

The friability test for metformin hydrochloride tablets was conducted following the official compendia method of BP (BP, 2022). Twenty tablets were randomly selected from each brand, weighed using an analytical balance, and then subjected to abrasion using a friability tester drum. The drum was rotated at 25 revolutions per minute for four minutes (100 times). After the tumbling process, the tablets were removed from the drum, de-dusted, and weighed again. The tablets' final weights were compared to their initial weights, and the percentage of weight loss, or friability, was calculated using Equation 2. According to the BP specification for compressed tablets, the acceptable percentage of weight loss is not more than 1%.

$$\%\ \text{Weight loss} = \frac{Initial\ weight - Weight\ after\ abbrasion}{Initial\ weight} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

4.5.5. Disintegration time test

The disintegration test was carried out by placing a randomly selected six tablets in a disintegration tester filled with distilled water at 37±2°C. The tablets were considered completely disintegrated

when all the particles were passed through the wire mesh and time was recorded. While the apparatus is running, the time taken for all six tablets of each brand to break up and for the primary particles to completely pass through the mesh of the disintegration basket was recorded and the mean disintegration time relative standard deviation was determined (Abatea *et al.,* 2020, BP, 2022).

4.5.6. Hardness test

The hardness of the tablets was measured by randomly selecting ten tablets and testing them using a hardness tester (Dulla *et al.,* 2018). Each tablet was placed between two anvils, and force was applied to the anvils. The crushing strength required to cause the tablet to break was recorded. The average crushing strength of the ten tablets was then reported.

4.5.7. Dissolution profile test

The dissolution test of the metformin hydrochloride tablet was conducted according to the official specification of BP (BP, 2022). Six tablets from each brand were placed in the dissolution basket and lowered into the dissolution vessel containing 900 ml of phosphate buffer (PH 6.8) maintained at a temperature of 37±0.5 ^{o}c. The dissolution basket was set to rotate at 100 revolutions per minute. Then, a sample of 10 mL was withdrawn from the medium at 10, 20, 30, 45, and 60 minutes and filtered. An equivalent amount of fresh 10 ml of the dissolution medium was replaced immediately to maintain the vessel volume constant throughout the analysis. Then, 10 mL of the filtrate was diluted to 100 mL with water and further diluted 10 mL of the resulting solution to 100 mL with water. After filtration and appropriate dilution, the corresponding absorbance readings of the withdrawn samples were determined at 233 nm using a UV-Vis spectrophotometer. Finally, the total content of metformin hydrochloride released, C4H11N5, HCl, in the medium was calculated at the maximum wavelength of 233 nm at each time stated using the following formula (Chandrasekaran *et al.,* 2011, Alnedhary *et al.,* 2021):

$$\% \text{ Drug released} = \frac{\text{Asam}}{\text{Astd}} \times \frac{\text{Wstd}}{100} \times \frac{10}{100} \times \frac{10}{100} \times \frac{900}{Lc} \times \frac{100}{10} \times \frac{100}{10} \times \frac{Pstd}{100} \times 100 \ldots\ldots\ldots\ldots\ldots\ldots 3$$

Where A_{sam} is the absorbance of the sample, A_{std} is the absorbance of the standard, W_{std} is the weight of standard taken in mg, Lc is label claim in mg and P_{std} is metformin HCl USP purity standard (99.7%).

4.5.7.1. Model independent approach using a similarity factor

A simple model-independent approach uses a f_1 and a f_2 to compare dissolution profiles (Food *et al.*, 1997).

The f_1 calculates the percentage difference between the two curves at each time point and is a measurement of relative error between the two curves.

$$f1 = (\langle \sum_{t=1} n \, |Rt - Tt \, | \rangle / [\sum_{t=1} n \, Rt]) \times 100 \text{----------4}$$

Where n is the number of time points, R_t is the dissolution value of the reference batch at time t, and T_t is the dissolution value of the test batch at time t.

The f_2 is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percent (%) dissolution between the two curves.

$$f2 = 50 \times \log. \left[\left[1 + \left(\frac{1}{n}\right) \sum_{t=1} n \, (Rt - Tt)^2\right)^{-0.5} \times 100\right] \text{----------5}$$

The dissolution profile of brands of metformin 850 mg tablets were compared by using a model-independent approach tested brands with innovator brands (Glucophage 850 mg). However, brands of metformin 500 mg tablets were not compared with the innovator because the innovator brand Glucophage 500 mg was not locally available in the market.

4.5.8. Assay test

The assay test of the metformin hydrochloride tablet was conducted according to the official specification of BP (BP, 2022).

Sample solution preparation: From randomly selected samples of metformin hydrochloride tablets from each brand, 20 tablets were weighed and then powdered using a mortar and pestle. A quantity of the powdered sample containing 0.1 g of metformin hydrochloride was shaken with 70 mL of water for 15 minutes, diluted to 100 mL with water, and filtered. The first 20 mL of the filtrate was discarded, and then 10 mL of the remaining filtrate was diluted to 100 mL with water. Finally, 10 mL of this resulting solution was diluted to 100 mL with water, and the absorbance of this final solution and a standard preparation was measured at a wavelength of 232 nm.

Standard preparation: A reference standard solution of metformin hydrochloride was prepared in water, with a known concentration of 10 micrograms per milliliter (μg/ml). This reference standard

solution was prepared similarly to the sample solution. Using UV-visible spectroscopy, the content of metformin hydrochloride in the sample was calculated at the maximum wavelength of 232 nm.

The content of metformin hydrochloride was calculated using (Dange *et al.,* 2017).

$$\text{Assay} = \frac{\text{Asam}}{\text{Astd}} \times \frac{\text{Wstd}}{100} \times \frac{10}{100} \times \frac{10}{100} \times \frac{100}{\text{Wsam}} \times \frac{100}{10} \times \frac{100}{10} \times \frac{Av.Wt}{Lc} \times \frac{Pstd}{100} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots 4$$

Where, A_{sam} is absorbance of sample, A_{std} is absorbance of standard, W_{std} is weight of standard taken in mg, W_{sam} is weight of sample taken in mg, $A_{v.}\,W_{t.}$ is average weight of 20 tablets taken in mg, P_{std} is purity of standard (99.7%), Lc is label claim in mg.

4.5.9. Moisture content test

The moisture content was determined by weighing and powdering ten randomly selected tablets from each brand. Then one gram of the powder was dried in a Sartarious moisture analyzer at a temperature of 100 ^{o}C to constant weight. Finally, the percentage of mass loss after drying was calculated with respect to the mass before drying (Haider *et al.,* 2011).

4.6. Calibration curve of metformin HCl USP standard in distilled water

A standard curve was created by dissolving 100 mg of USP metformin Hcl reference standard in 100 mL of distilled water to prepare a stock solution having a concentration of 1 mg/mL for the determination of assay test method for brands of metformin 500 mg and 850 mg tablets. From the stock solution, five concentration levels of 10, 9, 8, 7, and 6 µg/ml were prepared using water as diluent. Then, the absorbance of this concentration was determined spectrophotometrically at a wavelength of 232 nm and plotted against the five known concentrations to obtain the calibration curve.

4.7. Calibration curve for metformin Hcl USP standard in phosphate buffer

A standard curve was plotted in phosphate buffer at pH 6.8 and wavelength of 233 nm using pure USP metformin Hcl reference standard powder for dissolution test method.

From the stock solution five concentrations levels of 6.66, 6.11, 5.55, 4.99, and 4.44 µg/ml metformin USP standard for the determination of brands of metformin 500 mg tablets, and five concentrations levels of 11.33, 10.38, 9.44, 8.49 and 7.55 µg/ml for the determination of brands of metformin 850 mg tablets were prepared. Then, the absorbance of this concentration was

determined spectrophotometrically and plotted against the five known concentrations to obtain the calibration curve.

4.8. Calibration for disintegration apparatus

The disintegration time tester apparatus was calibrated as follows: Temperature; a calibrated thermometer was inserted into the immersion fluid in the beaker which is distilled water and the temperature was set at 37 C: Up/down cycle; the up/down cycle was set at 30 cycles per minute: Timer: the timer was set at 15 minutes and the stopwatch was stopped as soon as the timer reaches 15 minutes.

4.9. Data analysis

Data obtained was presented using ORIGINPRO 2024B graphing and scientific analysis software program, one-way ANOVA, Microsoft Excel 2013.

Statistical significant differences were considered when p-value <0.05, and one-way ANOVA was carried out for comparison of weight variation, assay, and dissolution profile study. All the data measured and reported are averages of a minimum of triplicate measurements and the values are expressed as mean ± relative standard deviation (RSD).

5. Result and Discussion

Quality is the performance of the product as per the commitment made by the producer to the consumer and it is the result of wise efforts (Tessema *et al.,* 2022). In the present study, ten brands of metformin hydrochloride tablets were purchased from retail pharmacies in Mekelle City, Tigray, Ethiopia. Seven of the brands had a label claim of 500 mg, while three brands had a label claim of 850 mg API. Nine of the brands were imported from foreign countries, while one was manufactured locally. All the brands of metformin tablets used in this study were within their shelf life at the time of study and were immediate release. All the brands were subjected to different quality control tests, including evaluating their physical characteristics, weight variation, friability test, dissolution profile test, assay test, hardness test, disintegration time test, and moisture content test. The samples were blindly coded as M1, M2, M3, M4, M5, M6, M7, M8, M9 and M10 for the analysis.

Product registration suggests primary investigation by the regulatory authorities. EFDA is the national regulatory body of Ethiopia which is under the ministry of health and it has the responsibility to ensure the quality, safety, and efficacy of medicines, food, cosmetics, and medical devices. It is empowered to investigate, register, and authorize the sales of drug products (Waktola, 2020). From all the brands studied, brand M8 had no EFDA online registration number which means that the drug may have undergone a process known as paralleling; where the distributor of the product bypassed the regulatory body by bringing in the drug into the country. Detailed information about the brands is shown in Table 1.

5.1. Calibration curve of metformin HCl USP standard in distilled water for assay

The calibration curve (linearity) was constructed for the applied UV-visible spectroscopic method using concentration and absorbance of metformin USP standard (Purity 99.7%) in the determination of assay of metformin HCl 500 and 850 mg tablets using distilled water as diluent at a wavelength of 232 nm (Table 2).

The calibration curve (graph) for the assay of tested brands of metformin 500 mg and 850 mg is the same because according to the BP specification, the weight of the reference standard used is the same for both strengths which is 100 mg.

Table 1: General information on all Metformin brands of this study

Code	Brand	Strength (mg)	Manufacturer	Country	Batch No.	Mfg. Date	Exp. Date	EFDA Online registration
M1	Metformin Tablet	500	Brawn Labs LTD	India	BNT0422042	04/22	03/25	30049090
M2	Nefort	500	SAGA	India	NEBO12217	03/22	02/25	30049090
M3	Metformin Denk	500	Denk Pharma	Germany	28369	05/23	05/26	30049090
M4	Metafin	500	Reyoung Pharma	China	223121062	05/22	04/25	30049090
M5	Brot	500	Medochemie LTD	Cyprus	A5G130	07/21	07/26	30049090
M6	Insumet	500	Cadila pharms	Ethiopia	D22017T212	09/22	08/25	30049090
M7	MF-Day	500	AUROBINDO pharma	India	MESA22004A	11/22	10/26	30049090
M8	Glucophage	850	MERCK	France	E205970	*	03/25	**
M9	Metformin Denk	850	Denk Pharma	Germany	25683	09/21	09/26	30049090
M10	Glifor	850	Bilim pharma	Turkey	23072006A	01/23	01/26	30049090

* The manufacturing date is not available

** Whose EFDA online registration is not available

5.2. Calibration curve of metformin HCl USP standard in phosphate buffer for dissolution

A calibration curve was developed for the applied UV-visible spectroscopic method using concentration and absorbance of metformin USP standard (Purity 99.7 %) in the determination of dissolution profile of metformin HCl 500 and 850 mg tablets in phosphate buffer at pH 6.8 and wavelength of 233 nm. (Table 2).

As shown in Figure 4 in the determination of brands of metformin 500 mg tablets, a strong linear relationship between the absorbance and concentration of metformin hydrochloride USP standard in the concentration range of 4.44 to 6.66 µg/mL is observed. The regression equation is Y = 0.0813X - 0.0047, where Y is the absorbance and X is the concentration in µg/mL, and the correlation coefficients (R^2) of the linear regression of the calibration curve is 0.999.

Table 2: Calibration curve of metformin standard for assay and dissolution

Calibration curve for assay in distilled water*		Calibration curve for dissolution in phosphate buffer			
		For metformin 500 mg		For metformin 850 mg	
Conc. (µg/mL)	Absorbance	Conc. (µg/mL)	Absorbance	Conc. (µg/mL)	Absorbance
6	0.5442	4.44	0.3562	7.55	0.6184
7	0.6192	4.99	0.3987	8.49	0.6789
8	0.7135	5.55	0.4482	9.44	0.7559
9	0.7938	6.11	0.4948	10.38	0.8364
10	0.8474	6.66	0.5341	11.33	0.9045

*: The calibration curve of assay in distilled water is for metformin 500 & 850 mg brands

Conc: Concentration µg/mL: microgram per milliliter

As shown in Figure 5 in the determination of brands of metformin 850 mg tablets, a strong linear relationship between the absorbance and concentration of metformin hydrochloride USP standard in the concentration range of 7.55 to 11.33 µg/mL is observed. The regression equation is Y = 0.0772X + 0.03, where Y is the absorbance and X is the concentration in µg/mL, and the correlation coefficients (R^2) of the linear regression of the calibration curve is 0.998.

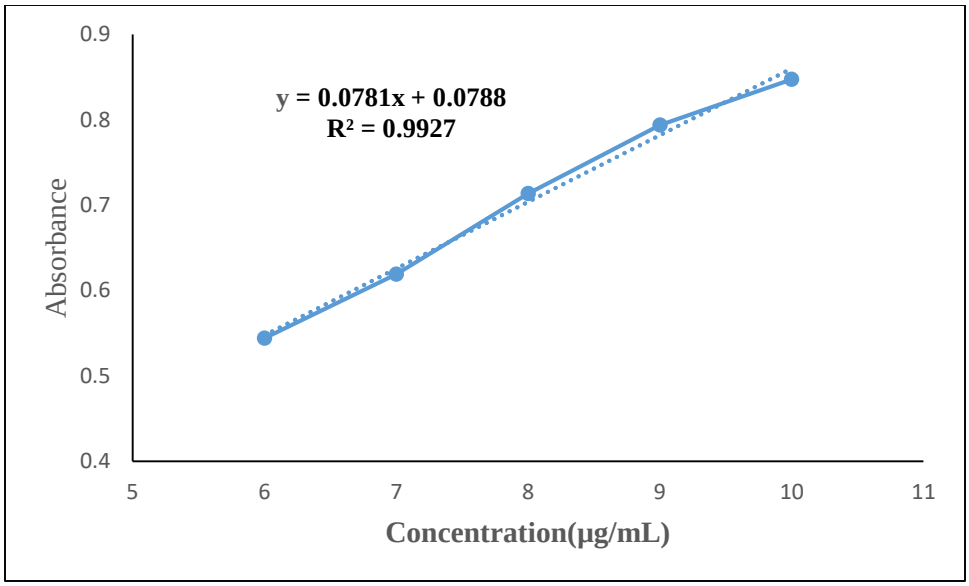

Figure 3: Calibration curve of metformin HCl reference standard in distilled water

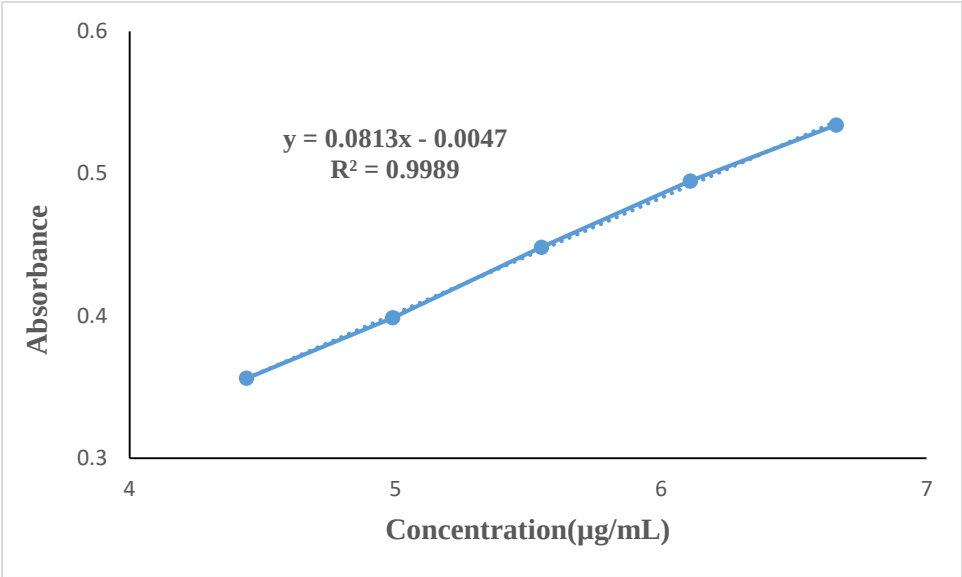

Figure 4: Calibration curve of metformin HCl reference standard in phosphate buffer for metformin 500 mg

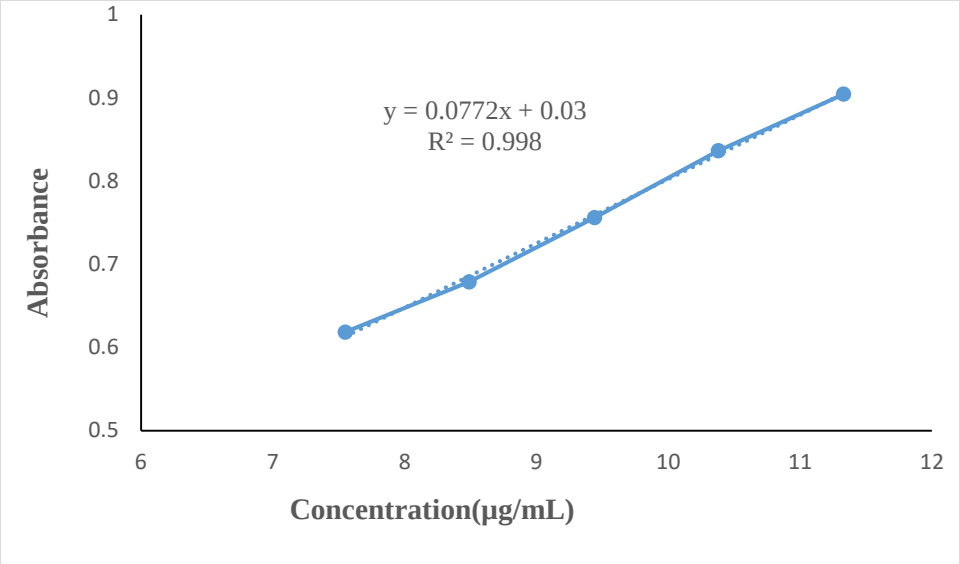

Figure 5: Calibration curve of metformin HCl reference standard in phosphate buffer for metformin 850 mg

5.3. Physical characteristics, packaging, and labeling of metformin brands

As shown in Table 1 all the brands except one brand (brand M8) were online registered by the EFDA. According to the present study, the physical characteristics of the studied metformin brands showed that all the tablets had a uniform white color, were undamaged, and had no odor. Except for Brand M4, Brand M6, and Metformin Brand M9, all the brands under the test had a circular shape, but Brand M4, Brand M6, and Brand M9 had an oval shape. The visual inspection of physical characteristics, packaging, and labeling did not reveal any indications of spurious, inaccurately labeled, falsified, or counterfeit products as defined by the WHO. The packaging and labeling of all the brands as shown in Annex 2, met the minimum requirements set by the WHO for packaging and labeling (WHO, 2013).

5.4. Identification test

The infrared spectrum gives information about functional groups present in the compound. The FTIR spectrum of pure metformin HCl shows a peak at wavenumber (cm^{-1}) which corresponds to the functional present in the structure of the drug (Kellner, 2018). As revealed in Table 3, the observed wave number 3360 cm^{-1} in the range of 3300–3500 cm^{-1} confirms the presence of strong N-H stretching primary amine functional group in metformin. The observed wave number 3240

cm^{-1} in the range of 3100–3400 cm^{-1} confirms the presence of N-H stretching secondary amine in metformin, supporting the compound's amine character. The C-H stretching vibration in the wavenumber of 2900 cm^{-1} indicate the presence of aliphatic group with in metformin structure. The C=N stretching in the wavenumber of 2650 cm^{-1} confirms the presence of imine functional group. The C-N stretching in the observed wave number of 1080 cm^{-1} indicates the presence of amide group in metformin structure.

Identification of active ingredients in generic products was established by comparing the FTIR spectrums with that of pure metformin HCl reference standards (99.7 %). The spectra of all tested brands were completely superimposed with that of pure metformin HCl reference standards, which confirms the presence of metformin as an active ingredient in all tested brands. FTIR spectra of pure metformin standard and the tested metformin brands are shown in annex 7.

Table 3: Metformin wavenumber values of the peak in the FTIR spectra

S/No.	Functional group	Observed wave number (cm^{-1})	Range wave number (cm^{-1})(Stuart, 2004)	Remark
1	N-H stretching 1^0 amine	3360	3300 – 3500	Complied
2	N-H stretching 2^0 amine	3240	3100 – 3400	Complied
3	C-H stretching	2900	2850 – 3000	Complied
4	C=N stretching	1650	1600 – 1700	Complied
5	C-N stretching	1080	1000 – 1300	Complied

5.5. Weight variation test

The uniformity of weight test result for the tested brands of metformin had shown in Table 4. Among brands of metformin 500mg tablets; Brand M4 had the highest average weight (0.8088 ±0.78gm) and M7 had the lowest average weight (0.531± 0.99gm). Among brands of metformin 850mg tablets; M10 had the highest average weight (1.003±0.65gm) and M8 had the lowest average weight (0.897±1.02 gm.). Statistical analyses were conducted for weight variation tests using one-way ANOVA at 95% CI and showed that there were statistically significant ($P<0.05$) in mean weight among sample mean weight of all brands of metformin 500mg and 850 mg tablets.

To reveal the source of difference in mean weight among the different brands, Tukey pairwise comparison with each other was conducted at a 95% confidence interval and showed that all the brands of metformin 500 mg (except brand M3 and M6) and metformin 850 mg have statistical significance mean weight from each other (Annex 3).

Weight variation determination is crucial for verifying the uniformity of units within the same batch, ensuring that the sampled tablets contain consistent content. This ensures that the drug substance in the batch falls within a narrow range around the label claim.

This may prevent the chance of receiving overdose or under-dose tablets, which could result in unpredictable therapeutic effects.

Table 4: Weight variation test result of different brands of metformin hydrochloride tablets

Sample Code	Weight variation(gm)* (Mean ±%RSD)	UL	LL	% Deviation	Remark	P – Value
M1	0.6343±1.6	0.6484	0.6092	-3.96 to 2.22	Passed	<0.05
M2	0.551.2±1.07	0.5613	0.5346	-3.02to 1.83	Passed	
M3	0.649.4±0.74	0.6606	0.6401	-1.43 to 1.73	Passed	
M4	0.8088±0.78	0.8265	0.8002	-1.07 to 2.19	Passed	
M5	0.679±0.6	0.6867	0.6698	-1.35 to 1.14	Passed	
M6	0.653±1.45	0.6654	0.6329	-3.09 to 1.88	Passed	
M7	0.531±0.99	0.5408	0.521	-1.9 to 1.83	Passed	
M8	0.897±1.02	0.921	0.8784	-2.1 to 2.65	Passed	<0.05
M9	0.944±0.58	0.9615	0.9365	-0.84 to 1.81	Passed	
M10	1.003±0.65	1.0161	0.9892	-1.37 to 1.32	Passed	

***:** 20 times replication for each brand, RSD: relative standard deviation, UL: upper limit, LL: lower limit

According to BP, the acceptable weight variation limit for tablets with an average weight of 250 mg or more is no more than two individual tablets should deviate by more than 5% of the average tablet weight, and none should deviate by more than 10% of the average tablet weight (BP, 2022).

According to this study, all the brands passed the weight variation test. As shown in Table 4 no individual brand weights deviated by ± 5% from the average tablet weight, the maximum positive % deviation was 2.22 and the maximum negative % deviation was -3.96 for brand M1. This report is similar to the study conducted by Junior *et al.* on the quality assessment of four different brands of metformin 500 mg and four brands of 850 mg tablets commercially available in Brazil, and they also found satisfactory results on weight determination test (Junior *et al.,* 2020). This weight variation test report also agrees with the study conducted in Nigeria (Olusola *et al.,* 2012), Libya (Elghnimi *et al.,* 2019), and India (Mate *et al.,* 2020) conducted on quality evaluation of different brands of metformin 500 mg tablets. The same report was found in the study conducted by Mansou*r et al.,* which evaluated the physicochemical properties of metformin 850 mg tablets marketed in Syria, and all the brands complied with the official specification for weight variation test (Mansour and Isbera, 2016).

5.6. Friability and moisture content test

The result of the tablet friability test showed that all the brands tested had impressive friability values ranging from 0 to 0.062% w/w. As shown in the friability test result in Table 5, brand M1, brand M5, and brand M10 did not lose their content (0 %) after the friability performed.

Moisture content: For brands of metformin 500 mg: Brand M5 had the highest moisture content (2.55 %) and brand M2 had the lowest moisture content (1.27 %). For brands of metformin 850 mg: Brand M10 had the highest moisture content (2.23 %) and brand M8 had the lowest moisture content (1.03 %).

A friability test was performed to determine a tablet's ability to withstand shock and abrasion during packaging, handling, and transportation. If a tablet exhibits friability beyond acceptable limits, it can lead to weight loss, negatively impacting the drug's therapeutic effectiveness. Therefore, measuring loss due to abrasion or friability can be a crucial indicator of how a tablet will behave during these processes (Tessema *et al.,* 2022, Ozyilmaz and ÇOMOĞLU, 2022).

According to this study, the friability test of all the tested brands revealed good friability values ranging from 0 % (M1, M5, M10) to 0.062% w/w (M3 and M4). All the tested brands met the BP pharmacopeia specification (BP, 2022). The percentage weight loss of ≤ 1%w/w is considered an acceptable limit for the tablet friability test

Table 5: Friability and moisture content test results of different brands of metformin hydrochloride

Sample Code	Weight (gm)*		Friability % w/w	Remark	Moisture content (%)*	Remark
	Initial wt.	Final wt.				
M1	12.68	12.68	0	Passed	2.12	Passed
M2	10.964	10.96	0.036	Passed	1.27	Passed
M3	12.968	12.96	0.062	Passed	1.94	Passed
M4	16.2	16.19	0.062	Passed	1.56	Passed
M5	13.54	13.54	0	Passed	2.55	Passed
M6	13.083	13.08	0.023	Passed	1.87	Passed
M7	10.583	10.58	0.028	Passed	1.36	Passed
M8	17.924	17.92	0.022	Passed	1.03	Passed
M9	18.926	18.918	0.042	Passed	1.74	Passed
M10	19.988	19.988	0	Passed	2.23	Passed

*: 20 times replication for each brand, **:10 times replication for each brand

A study conducted in India to evaluate the quality of four different brands of metformin hydrochloride tablets found that all the tablets met the requirements for weight variation and friability test (Sachan *et al.,* 2016). A similar study in Saudi Arabia also reported that all of the tablets passed the friability (Afifi and Ahmadeen, 2012).

Moisture content is one of the quality criteria for finished tablets that takes into account mechanical strength, solubility, and overall shelf-life stability. When mixing and granulating raw materials, such as APIs and excipients, moisture content is a crucial consideration. The consistency of blended powders and overall flow characteristics are influenced by moisture content (Lad *et al.,* 2022). According to the non-official specification for moisture content, which specifies not more than 3 %, all the tested brands of metformin 500 mg and 850 mg passed the moisture content test (Haider *et al.*, 2011).

5.7. Disintegration time test

As shown in the disintegration time test result in Table 6, among metformin 500mg brands: brand M7 had the highest disintegration time (17.5 minutes) and brand M5 had the lowest disintegration time (6.4 minutes), and among metformin 850mg brands: brand M9 had the highest disintegration time (17.1) and brand M8 had the lowest disintegration time (9.3).

Table 6: Disintegration time test results of different brands of metformin hydrochloride tablets

	Sample cod									
Tablets	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Tablet 1	10	9.8	14.5	11	6.16	7	14.3	9	16.5	13.8
Tablet 2	10	10	14.5	11	6.16	7	14.5	9	16.5	14
Tablet 3	10	10	14.5	11.55	6.25	7.1	16	9.33	16.5	15
Tablet 4	10	10	14.5	11.5	6.33	7.16	19	9.33	16.66	15.25
Tablet 5	10	10	14.5	11.67	6.5	7.16	20	9.5	18	16
Tablet6	11	10	14.5	11.67	7	7.16	21	9.5	18.5	17
Mean	10.17	9.97	14.5	11.4	6.4	7.1	17.5	9.3	17.11	15.17
SD	0.41	0.082	0	0.32	0.32	0.08	2.91	0.23	0.82	1.21
RSD	4.0	0.82	0	2.77	5.0	1.1	16.64	2.45	5.25	7.97
Remark	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed

SD: Standard Deviation, RSD: Relative Standard Deviation

Tablet disintegration is a pre-request to dissolution and subsequent absorption of a drug from dosage form. For the medicinal agent in a tablet to become fully available for absorption, the tablet must first disintegrate and discharge the drug to the body fluids for dissolution (Allen and Ansel, 2013).

The nature of formulation excipients and manufacturing techniques employed by various manufacturers affect the disintegration time of tablets, which in turn affects the drug's bioavailability (Eraga *et al.,* 2017). According to this study report all the brands passed the disintegration time test which was specified by the British official pharmacopeia, the specification limit was less than 30 minutes for film-coated tablets (BP, 2022) As shown in Table 5, the

disintegration time for metformin hydrochloride 500 mg and 850 mg tablets had fallen within the acceptance range with brand M7 (7.1 ±1.1 minute) and brand M9 (17.5±16.64 minute) had low disintegration time values possibly attributed to the presence of a large number of disintegrants.

5.8. Hardness test

As shown in the hardness test result in Table 7, among metformin 500mg brands: brand M3 had the highest hardness mean value (33.3±1.9kg) and brand M5 had the lowest hardness mean value (13.8±7.9kg). Among metformin 850mg brands: brand M10 had the highest hardness mean value (33.9±3.7 kg) and brand M8 had the lowest hardness mean value (29.4±3.2 kg).

Hardness is the capability of a tablet to survive mechanical shocks during handling, production, packaging, and delivery. Tablets need to have a specific degree of hardness (Uddin *et al.*, 2017, Khar, 2013). The hardness of a tablet is proportional to compressional force and it is inversely proportional to its porosity (Junior *et al.*, 2020). Consequently, this parameter may influence the disintegration time of solid dosage forms. According to nonofficial specifications, typically for oral tablets the acceptance limit for the hardness of a tablet is 5 to 8 kg. However, a force ranging from 4-10 kg is also considered satisfactory (Karmakar and Kibria, 2012, Khar, 2013, Hani *et al.*, 2020, Dulla *et al.*, 2018). In this study the hardness of seven brands of metformin hydrochloride 500 mg and three brands of metformin 850 mg tablets was determined and, all of the tested brands failed to meet the nonofficial specification. All the tested brands had hardness values>10 kg. This may be due to the compression force, the nature, and the amount of binder used in tablet manufacturing. Excessive pressure applied during compression and higher concentration of binder used leads to higher tablet hardness value (Aulton and Taylor, 2013).

Table 7: Hardness test results of different brands of metformin hydrochloride tablets

	Sample code									
Tablets	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Tablet 1	16.4	32.5	34.8	14.3	14.2	30.5	15.5	30.6	32	34.6
Tablet 2	16.6	28.5	33	15.2	13.1	27.2	16.7	29.4	34.6	34.2
Tablet 3	15	29	32.9	14.5	14.5	28.5	18.4	27.3	30.2	35.5
Tablet 4	17	29	32.8	15.5	12.2	30.8	17.3	29.1	33.6	34.5
Tablet 5	16.8	28	33.6	15.3	12.9	29.5	12.6	29.8	32	34.8
Tablet 6	16.7	25	33.7	15.4	16.3	27.6	17.3	30.5	32	35.2
Tablet 7	15.3	27	32.5	15.4	13.2	27.8	17.4	28.5	31.4	32.4
Tablet 8	16.2	28.5	32.6	15.2	14.1	28.6	18.4	29.2	32.2	33.5
Tablet 9	16.5	28.2	33.2	15	13.5	27.6	16.5	29.8	32.5	31.2
Tablet 10	15.8	28.7	33.5	15.4	14.7	29.2	17.5	30.2	31.5	33.5
Mean	16.2	28.4	33.3	15.1	13.8	28.73	16.76	29.4	32.2	33.9
SD	0.63	1.7	0.65	0.4	1.1	1.2	1.6	0.9	1.1	1.3
RSD	3.8	6.2	1.9	2.5	7.9	4.1	9.6	3.2	3.5	3.7
Remark	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed	Failed

SD: Standard Deviation, RSD: Relative Standard Deviation

A similar finding was reported in the study conducted by Flatie *et al.,* on the evaluation of seven different brands of metformin hydrochloride tablets available in the market in Gondar City, Ethiopia (Flatie Alemu *et al.,* 2024). All the seven tested brands failed to meet the hardness test specification. This report had some resemblance to the study conducted by Olusola *et al.,* in Nigeria in 2012, where five brands out of eight tested metformin hydrochloride brands did not pass the hardness test (Olusola *et al.,* 2012). A similar result was also reported in the study conducted by Albratty *et al.,* on the assessment of physicochemical properties and comparison of the

dissolution profile of metformin hydrochloride tablets in Saudi Arabia; one out of ten brands passed the hardness test limit (AlBratty *et al.*, 2020).

5.9. Assay test

The absorbance reading for metformin USP standard in the determination of assay for brands of metformin 500 mg and 850 mg tablet: standard one is 0.8355, standard two is 0.8349, and standard three is 0.8356 and the mean absorbance of the standard is 0.8353. As shown in the assay test result in Table 8, among brands of metformin hydrochloride 500 mg tablets: brand M7 had the highest assay value (102.1±0.9%) and brand M1 had the lowest assay value (95.2±0.12%). Among brands of metformin hydrochloride 850 mg tablets: brand M8 had the highest assay value (103.7±0.1%) and brand M9 had the lowest assay value (97.9±0.9%). According to this study, all the brands of metformin hydrochloride 500 mg and 850 mg tablets were within the specification of BP 2022 for percentage purity. The assay specification for metformin hydrochloride is 95% to 105 % of the label claim (BP, 2022).

Statistical analyses were conducted for the assay test using one-way ANOVA at a 95% confidence CI and showed that there was statistically significant ($P < 0.05$) in the mean assay of all brands of metformin 500mg and 850 mg tablets from each other (Table 8).

To identify the differences in the mean assay among different brands, a Tukey pairwise comparison was performed at a 95% confidence level. The analysis revealed that, except for the pairs brand M1 and M6 among the 500 mg metformin brands, and brands M9 and M10 among the 850 mg metformin brands, all other brands showed statistical significance in their mean assay results from one another (Annex 4).

Analysis of the drug assay is very important to determine the presence, absence, or quantify the amount of one or more active pharmaceutical ingredients in the dosage form and the amount of this active ingredient present in the drug product affects the quality of the product and will have an impact on therapeutic effect (Uddin *et al.*, 2016). So, no matter how a drug product is in physical parameters, failure to meet the standard in the content of active ingredients (assay) will result in poor quality with adverse consequences (Ogah and Kadejo, 2013).

Table 8: Assay test results of different brands of metformin hydrochloride tablets

Sample Code	Average wt.(mg)	Equiv. wt.(mg)	Absorbance Reading				Mean Assay*± RSD%	Remark	P-value
			Sample			Mean STD			
			1st	2nd	3rd				
M1	634.3	126.86	0.7966	0.7979	0.7985	0.8353	95.2±0.12	Passed	
M2	550.2	110.04	0.846	0.8438	0.8445	0.8353	100.8±0.13	Passed	
M3	649.4	129.88	0.8639	0.8661	0.8656	0.8353	103.3±0.13	Passed	
M4	808.9	161.78	0.8315	0.8318	0.8356	0.8353	99.4±0.27	Passed	
M5	679	135.8	0.8149	0.8175	0.8189	0.8353	97.5±0.25	Passed	
M6	653.1	130.62	0.7995	0.7989	0.8012	0.8353	95.5±0.15	Passed	
M7	531.1	106.22	0.855	0.8562	0.8549	0.8353	102.1±0.9	Passed	<0.05
M8	897.2	105.5	0.8696	0.8678	0.8689	0.8353	103.7±0.1	Passed	
M9	944.4	111.1	0.8155	0.8165	0.8285	0.8353	97.9±0.9	Passed	
M10	1003	118	0.8225	0.8228	0.8285	0.8353	98.4±0.4	Passed	<0.05

*: 3 times replication for each brand, Equiv. Wt.: Equivalent Weight, STD: Standard, mg: milligram

This finding was similar to the study conducted by Afifi *et al.,* which evaluated six different brands of metformin 500 mg tablets marketed in Saudi Arabia (Afifi and Ahmadeen, 2012), Jain *et al.,* which evaluated four different brands of metformin 500 mg tablets marketed in Ambala region, Haryana, India (Jain *et al.,* 2019), Elghnimi *et al.,* which evaluated six different brands of metformin 500 mg tablets marketed in Tripoli, Libya (Elghnimi *et al.,* 2019), Kassahun *et al.* which evaluated six different brands of metformin 500 mg tablets marketed in Addis Ababa, Ethiopia (Kassahun *et al.,* 2019).

5.10. Dissolution profile of different brands of metformin hydrochloride

The dissolution profile test results of seven brands of metformin hydrochloride 500 mg and three brands of 850 mg tablets are illustrated in Table 9. From the present dissolution profile study, among brands of metformin 500mg tablets; brand M2 had the highest mean percent of drug released (99.75±1.77 %) and brand M3 had the lowest mean percent of drug released (90.4±2.7

%) at 45 minutes. Among brands of metformin 850mg tablets; brand M10 had the highest mean percent of drug released (101.6±2.77%) and brand M8 had the lowest mean percent of drug released (97.95±1.48 %) at 45 minutes.

According to the BP 2022 specifications, the dissolution test mandates that a minimum of 80% of the active ingredient should dissolve within the 45-minute time frame (BP, 2022). All the brands of metformin hydrochloride 500 mg (figure 6) and 850 mg tablets (figure 7) studied released the needed amount of active pharmaceutical ingredients at the specified time of 45 minutes. Statistical analysis was conducted for the dissolution profile test in 30 minutes using one-way ANOVA at a 95% CI and showed that there was a significant difference ($P<0.05$) among samples of all brands of metformin 500mg from each other, and there was no statistical significance ($P>0.05$) in mean percent of drug release among sample of all brands of metformin 850mg tablets from each other (Table 8).

To identify the difference in the mean percent of drug release among the brands, a Tukey pairwise comparison was performed at a 95% confidence level. The results showed that, except for the pair brands M4 and M1, M4 and M3, M6 and M2, M6 and M4, and M7 and M4, all brands of metformin 500 mg, as well as all brands of metformin 850 mg, did not show statistical significance mean percent of drug release difference from each other (Annex 5).

Table 9: Dissolution profile test results of different brands of metformin hydrochloride tablets

Sample Code	Mean % of drug released ±RSD %					Remark	P-Value
	10 min*	20 min*	30 min*	45 min*	60 min*		
M1	49.8±3.8	95.32±2.59	92.31±2.7	92.69±3.05	91.95±2.95	Passed	
M2	79.17±3.44	95.04±2	95.53±2.69	99.75±1.77	92.37±2.67	Passed	
M3	46.44±4.29	75.12±0.42	92.91±1.65	90.4±2.7	89.8±0.183	Passed	
M4	50.71±3.74	79.64±3.52	96.37±0.16	94.36±4.39	92.26±3.25	Passed	
M5	77.45±1.76	94.57±2.58	93.54±0.17	93.05±2.8	92.44±3.12	Passed	
M6	63.61±2.26	93.57±2.48	91.38±2.59	89.89±1.66	88±2.65	Passed	
M7	83.92±1.73	92.64±2.98	92.58±1.36	90.69±3.24	90.17±3.46	Passed	<0.05
M8	57..22±7.81	85.42±4.23	99±1.59	97.95±1.48	94.31±2.66	Passed	
M9	61.56±4.04	94.88±1.13	99.79±0.84	98.78±1.14	97.06±0.73	Passed	
M10	59.16±1.54	92.8±2.02	99.84±0.27	101.6±2.77	99.08±0.86	Passed	>0.05

***:** 6 times replication for each brand, RSD: Relative Standard Deviation, Min: minute

Dissolution tests are used to guide the development of new formulations, monitor the quality of drug products, assess the potential impact of post-approval changes on product performance, and, in some cases, predict the in vivo performance of the drug product. In vitro dissolution as an important element in drug development, under certain conditions can be used as a surrogate for the assessment of bioequivalence. Expensive in vivo bioequivalence testing can be waived if dissolution profile similarity is demonstrated between different strengths of the medicinal product (Mitrevska *et al.,* 2020). A dissolution profile reflects the cumulative amount of drug substance dissolved at any time point.

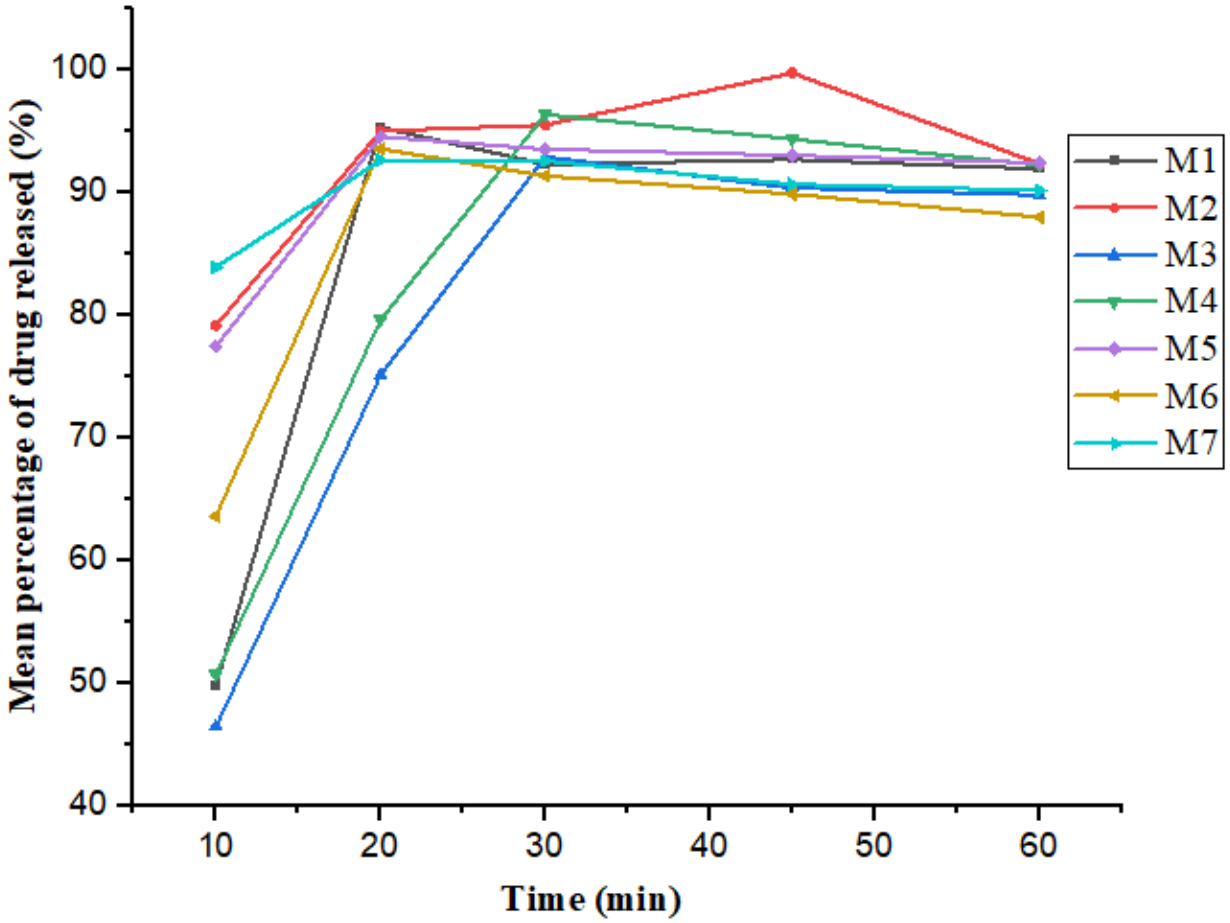

Figure 6: Dissolution Profiles for seven brands of metformin HCl 500 mg tablets

In the pharmaceutical industry, drug dissolution testing is routinely used to deliver essential in vitro drug release data for quality control, ensuring batch-to-batch uniformity of solid oral dosage forms like tablets, and for drug development to forecast in vivo drug release patterns. The in vitro drug dissolution data obtained from these tests can be linked-to in vivo pharmacokinetic data through in vitro-in vivo correlations (IVIVC) (Eraga *et al.*, 2017, Troja *et al.,* 2015). As per BP 2020 specification, all the brands passed the dissolution time test.

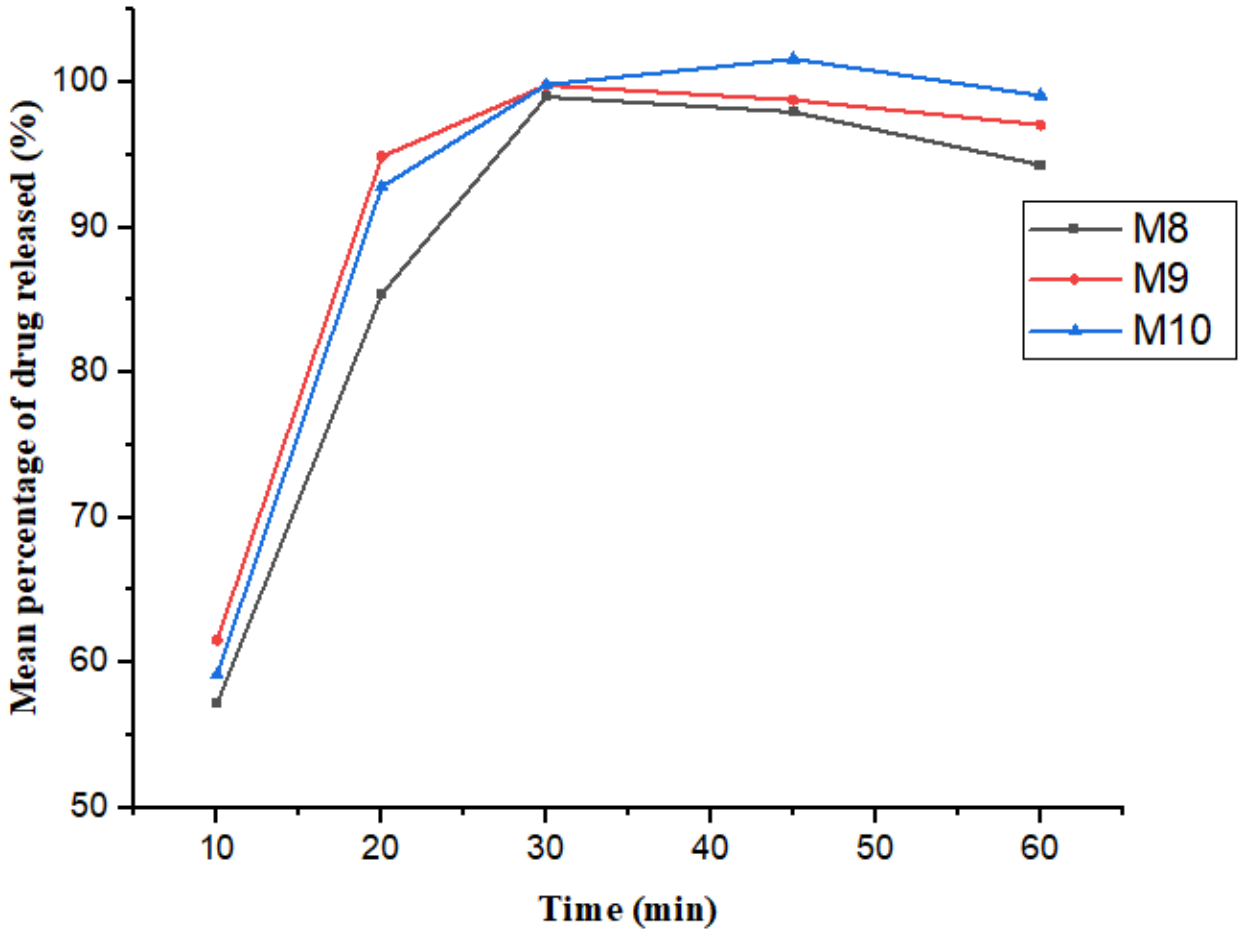

Figure 7: Dissolution Profiles for three brands of metformin HCl 850 mg tablets

5.10.1. Comparison of dissolution profile

The result of statistical analysis for the dissolution profile test in 30 minutes using one-way ANOVA at a 95% CI showed that there was statistical significance ($P<0.05$) among samples of all brands of metformin 500mg among each other, and there was no significant difference ($P >0.05$) in mean percent of drug release among sample of all brands of metformin 850mg tablets among each other (Table 10).

To compare the dissolution profiles of the innovator Glucophage and test products, a model-independent approach of f1 and f2 was used. For two dissolution profiles to be considered interchangeable or similar, the f2 should be 50 to 100, and the f1 should be 1 to 15 (Food *et al.*, 1997).

Table 10: Model-independent f1 and f2 values of the tested brands

Tested Brands	Model-independent approach		Remark
	f1	f2	
M8 (Innovator)	-	-	-
M9	4.2	65.3	Complied
M10	4.3	67.5	Complied

As shown in Table 10, the f2 value for the studied brands of metformin 850 mg tablets was found 65.3 for brand M9 and 67.5 for brand M10, and the f1 value was 4.2 for brand M9 and 4.3 for brand M10 compared to the innovator brand (brand M8). Thus brand M9 and brand M10 can be considered to be pharmaceutically equivalent (interchangeable) to the innovator product (brand M8).

6. Limitations of the study

This study has the limitation of assessment of brands of metformin 500 mg tablet interchangeability (f1 and f2 factors). Because the innovator Glucophage 500 mg product is not available in the local market. This limitation should be considered when interpreting the result. Further research is needed to address this limitation and gain a comprehensive understanding of the pharmaceutical quality of metformin hydrochloride tablets in the market.

7. Conclusion

Commercially available seven brands of metformin hydrochloride 500 mg and three 850 mg tablets available in Mekelle City, Tigray, Ethiopia market were subjected to several quality control tests. All the tested brands were in line with the WHO for evaluation of packaging and labeling of pharmaceuticals. All selected products were found to comply with BP 2022 specifications concerning weight variations, disintegration time, friability, assay, and dissolution profile. However, they had not passed the hardness test. The model-independent dissolution profile comparison for metformin 850 mg formulations has established the interchangeability of the tested products with the comparator product.

8. Recommendations

Based on the result of the current study:

- EFDA should focus on the post-marketing evaluation of metformin hydrochloride circulating in the market which is sourced from different manufacturers.
- Researchers should conduct future study for evaluation of metformin hydrochloride 500 mg tablets using f1 and f2 factors.
- The pharmaceutical industry should invest more resources in quality improvements to ensure the standard of pharmaceutical products, ultimately benefiting the country's health care system.

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Annexes

Annex 1: WHO Sample collection Form(WHO, 2005)

Sample code: _____

1. Area/Region/Country:_____
2. Name of location/place where sample was taken:_____
3. Address (with telephone, fax number and email address)_____
4. Names of people who collected the sample:_____
5. Product name of the sample:_____
6. Name of active pharmaceutical ingredient(s) (INN) with strength:_____
7. Dosage form (tablet, injection, powder for injection, etc.):_____
8. Package size, type and packaging material of the container:_____
9. Batch/lot number: _____
10. Date of manufacture: _____ Expiry date:_____
11. Regulatory status in the country, registration number, if applicable:_____
12. Name and address of the manufacturer: _____
13. Number of sample unit taken (tablet, capsule, etc):_____
14. Brief physical/ visual description of sample_____

    _____

    _____

Annex 2: All finished drug products should be identified by labeling, as required by the national legislation , bearing at least the following information(WHO, 2013)

S/N	WHO requirements for Packaging & labeling	Brands									
		M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
1.	Name of the Drug product	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2.	A list of the active ingredients showing the amount of each present	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3.	Batch number assigned by the manufacturer	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4.	The expiry date in an un coded form	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
5.	Any special storage conditions	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6.	Directions for use, and warnings and precautions that may be necessary	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
7.	Name and address of the manufacturer or the company	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Annex 3: Tukey Simultaneous tests for weight means difference of the tested brands from each other

Tested Brands	Mean Difference	SEM	Adjusted P-value	Sig	95% Confidence interval	
					Lower Bound	Upper Bound
Brands of metformin 500 mg tablets						
M2 - M1	-0.08309	0.00219	<0.0001	1	-0.08966	-0.07651
M3 - M1	0.01508	0.00219	<0.0001	1	0.00851	0.02166
M3 - M2	0.09817	0.00219	<0.0001	1	0.0916	0.10474
M4 - M1	0.17452	0.00219	<0.0001	1	0.16795	0.18109
M4 - M2	0.25761	0.00219	<0.0001	1	0.25103	0.26418
M4 - M3	0.15944	0.00219	<0.0001	1	0.15286	0.16601
M5 - M1	0.04466	0.00219	<0.0001	1	0.03809	0.05123
M5 - M2	0.12774	0.00219	<0.0001	1	0.12117	0.13432
M5 - M3	0.02957	0.00219	<0.0001	1	0.023	0.03615
M5 - M4	-0.12986	0.00219	<0.0001	1	-0.13643	-0.12329
M6 - M1	0.01878	0.00219	<0.0001	1	0.01221	0.02535
M6 - M2	0.10187	0.00219	<0.0001	1	0.09529	0.10844
M6 - M3	0.0037	0.00219	0.62815	0	-0.00288	0.01027
M6 - M4	-0.15574	0.00219	<0.0001	1	-0.16231	-0.14917
M6 - M5	-0.02588	0.00219	<0.0001	1	-0.03245	-0.01931
M7 - M1	-0.10322	0.00219	<0.0001	1	-0.10979	-0.09665
M7 - M2	-0.02014	0.00219	<0.0001	1	-0.02671	-0.01356
M7 - M3	-0.11831	0.00219	<0.0001	1	-0.12488	-0.11173
M7 - M4	-0.27774	0.00219	<0.0001	1	-0.28431	-0.27117
M7 - M5	-0.14788	0.00219	<0.0001	1	-0.15445	-0.14131
M7 - M6	-0.122	0.00219	<0.0001	1	-0.12857	-0.11543

Brands of metformin 850 mg tablets						
M9 - M8	0.0472	0.00229	<0.0001	1	0.04169	0.05272
M10 - M8	0.10569	0.00229	<0.0001	1	0.10017	0.11121
M10 - M9	0.05848	0.00229	<0.0001	1	0.05297	0.064

SEM: Standard Error of the Mean
Sig: significance
Sig equals 1 indicates that the difference of the means is significant at the 0.05 level
Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level.

Annex 4: Tukey Simultaneous tests for assay means difference of the tested brands from each other

Tested Brands	Mean Difference	SEM	Adjusted P-value	Sig	95% Confidence interval	
					Lower Bound	Upper Bound
Brands of metformin 500 mg tablets						
M2 - M1	5.63333	0.13333	<0.0001	1	5.17806	6.08861
M3 - M1	8.06667	0.13333	<0.0001	1	7.61139	8.52194
M3- M2	2.43333	0.13333	<0.0001	1	1.97806	2.88861
M4 - M1	4.2	0.13333	<0.0001	1	3.74472	4.65528
M4 - M2	-1.43333	0.13333	<0.0001	1	-1.88861	-0.97806
M4 - M3	-3.86667	0.13333	<0.0001	1	-4.32194	-3.41139
M5 - M1	2.33333	0.13333	<0.0001	1	1.87806	2.78861
M5 - M2	-3.3	0.13333	<0.0001	1	-3.75528	-2.84472
M5 - M3	-5.73333	0.13333	<0.0001	1	-6.18861	-5.27806
M5 - M4	-1.86667	0.13333	<0.0001	1	-2.32194	-1.41139
M6 - M1	0.26667	0.13333	0.45526	0	-0.18861	0.72194
M6 - M2	-5.36667	0.13333	<0.0001	1	-5.82194	-4.91139
M6 - M3	-7.8	0.13333	<0.0001	1	-8.25528	-7.34472
M6 - M4	-3.93333	0.13333	<0.0001	1	-4.38861	-3.47806
M6 - M5	-2.06667	0.13333	<0.0001	1	-2.52194	-1.61139
M7 - M1	6.86667	0.13333	<0.0001	1	6.41139	7.32194
M7 - M2	1.23333	0.13333	<0.0001	1	0.77806	1.68861
M7 - M3	-1.2	0.13333	<0.0001	1	-1.65528	-0.74472
M7 - M4	2.66667	0.13333	<0.0001	1	2.21139	3.12194
M7 - M5	4.53333	0.13333	<0.0001	1	4.07806	4.98861

M7 - M6	6.6	0.13333	<0.0001	1	6.14472	7.05528
Brands of metformin 850 mg tablets						
M9 - M8	-5.83333	0.43119	<0.0001	1	-7.15638	-4.51029
M10 - M8	-5.26667	0.43119	<0.0001	1	-6.58971	-3.94362
M10 - M9	0.56667	0.43119	0.43851	0	-0.75638	1.88971

SEM: Standard Error of the Mean
Sig: Significance
Sig equals 1 indicates that the difference of the means is significant at the 0.05 level
Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level

Annex 5: Tukey Simultaneous tests for percentage drug release means difference of the tested brands from each other

Tested Brands	Mean Difference	SEM	Adjusted P-value	Sig	95% Confidence interval	
					Lower Bound	Upper Bound
Brands of metformin 500 mg tablets						
M2 - M1	3.21295	1.03386	0.05218	0	-0.01882	6.44473
M3 - M1	0.59281	1.03386	0.9972	0	-2.63897	3.82459
M3- M2	-2.62015	1.03386	0.17832	0	-5.85193	0.61163
M4 - M1	4.05468	1.03386	0.00653	1	0.8229	7.28646
M4 - M2	0.84172	1.03386	0.98189	0	-2.39006	4.0735
M4 - M3	3.46187	1.03386	0.02918	1	0.23009	6.69365
M5 - M1	1.22819	1.03386	0.89399	0	-2.00359	4.45997
M5 - M2	-1.98476	1.03386	0.48144	0	-5.21654	1.24702
M5 - M3	0.63539	1.03386	0.9959	0	-2.59639	3.86716
M5 - M4	-2.82648	1.03386	0.11959	0	-6.05826	0.4053
M6 - M1	-0.93015	1.03386	0.9702	0	-4.16193	2.30163
M6 - M2	-4.14311	1.03386	0.00516	1	-7.37489	-0.91133
M6 - M3	-1.52296	1.03386	0.75826	0	-4.75474	1.70882
M6 - M4	-4.98483	1.03386	5.05E-04	1	-8.21661	-1.75305
M6 - M5	-2.15835	1.03386	0.38179	0	-5.39013	1.07343
M7 - M1	0.26529	1.03386	0.99997	0	-2.96649	3.49707
M7 - M2	-2.94766	1.03386	0.0932	0	-6.17944	0.28411
M7 - M3	-0.32752	1.03386	0.99991	0	-3.5593	2.90426

M7 - M4	-3.78939	1.03386	0.01298	1	-7.02117	-0.55761
M7 - M5	-0.9629	1.03386	0.96475	0	-4.19468	2.26888
M7 - M6	1.19544	1.03386	0.90545	0	-2.03634	4.42722
Brands of metformin 850 mg tablets						
M9 - M8	0.79062	0.60174	0.40942	0	-0.77238	2.35362
M10 - M8	0.83801	0.60174	0.36939	0	-0.72499	2.40101
M10 - M9	0.04739	0.60174	0.99659	0	-1.51561	1.61039

SEM: Standard Error of the Mean
Sig: Significance
Sig equals 1 indicates that the difference of the means is significant at the 0.05 level
Sig equals 0 indicates that the difference of the means is not significant at the 0.05 level

Annex 6: Assay test result of different brands of metformin hydrochloride tablets

Sample Code	Average wt.(mg)	Equiv. wt.(mg)	Absorbance Reading			Mean STD	Assay (%)			Mean Assay*± RSD%	P-value
			Sample								
			1st	2nd	3rd		1st	2nd	3rd		
M1	634.3	126.86	0.7966	0.7979	0.7985	0.8353	95.1	95.2	95.3	95.2±0.12	
M2	550.2	110.04	0.846	0.8438	0.8445	0.8353	100.9	100.7	100.8	100.8±0.13	
M3	649.4	129.88	0.8639	0.8661	0.8656	0.8353	103.1	103.4	103.3	103.3±0.13	
M4	808.9	161.78	0.8315	0.8318	0.8356	0.8353	99.2	99.3	99.7	99.4±0.27	
M5	679	135.8	0.8149	0.8175	0.8189	0.8353	97.3	97.6	97.7	97.5±0.25	
M6	653.1	130.62	0.7995	0.7989	0.8012	0.8353	95.4	95.4	95.6	95.5±0.15	
M7	531.1	106.22	0.855	0.8562	0.8549	0.8353	102.1	102.2	102.1	102.1±0.9	<0.05
M8	897.2	105.5	0.8696	0.8678	0.8689	0.8353	103.8	103.6	103.7	103.7±0.1	
M9	944.4	111.1	0.8155	0.8165	0.8285	0.8353	97.3	97.5	98.9	97.9±0.9	
M10	1003	118	0.8225	0.8228	0.8285	0.8353	98.2	98.2	98.9	98.4±0.4	<0.05

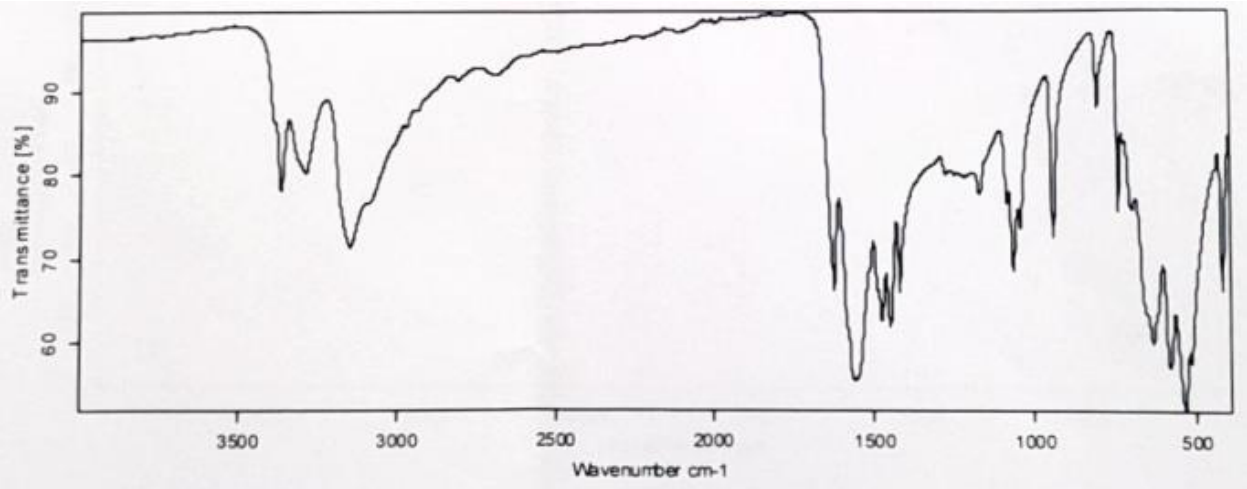
*: 3 times replication for each brand

Equiv.Wt.: Equivalent Weight

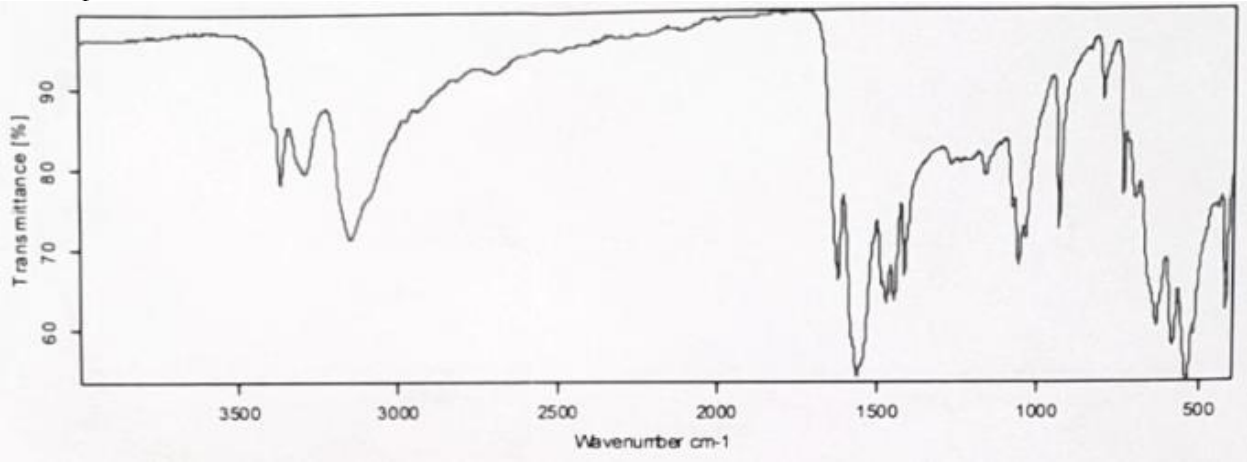
STD: Standard

mg: miligram

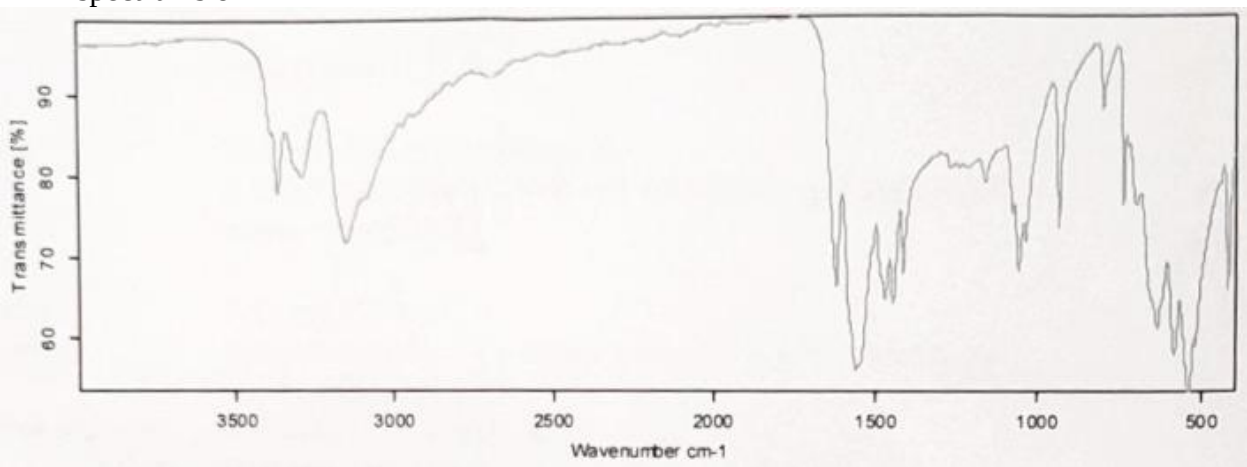
Annex 7: FTIR spectrums of metformin HCl USP standard and tested brands of metformin HCl tablets



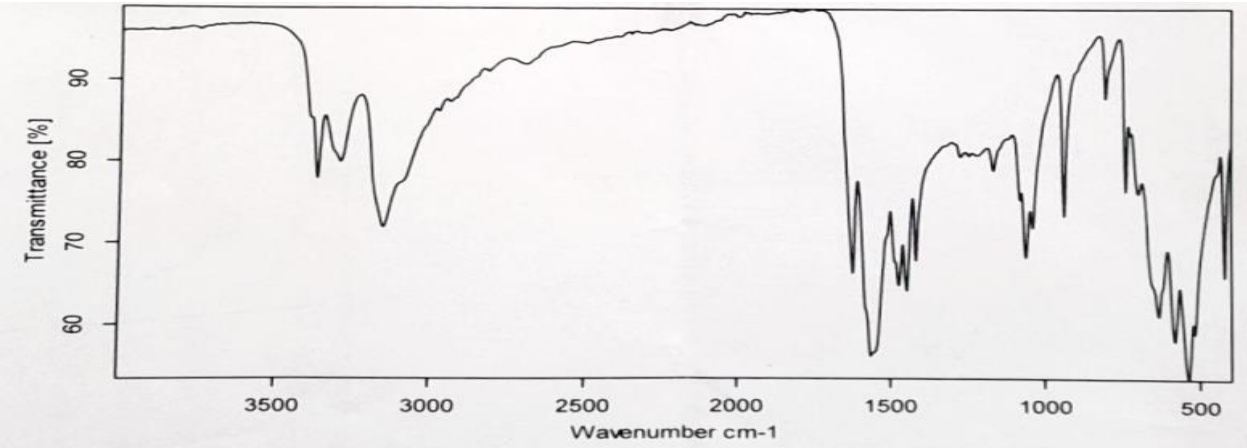
FTIR spectra of metformin HCl USP reference standard



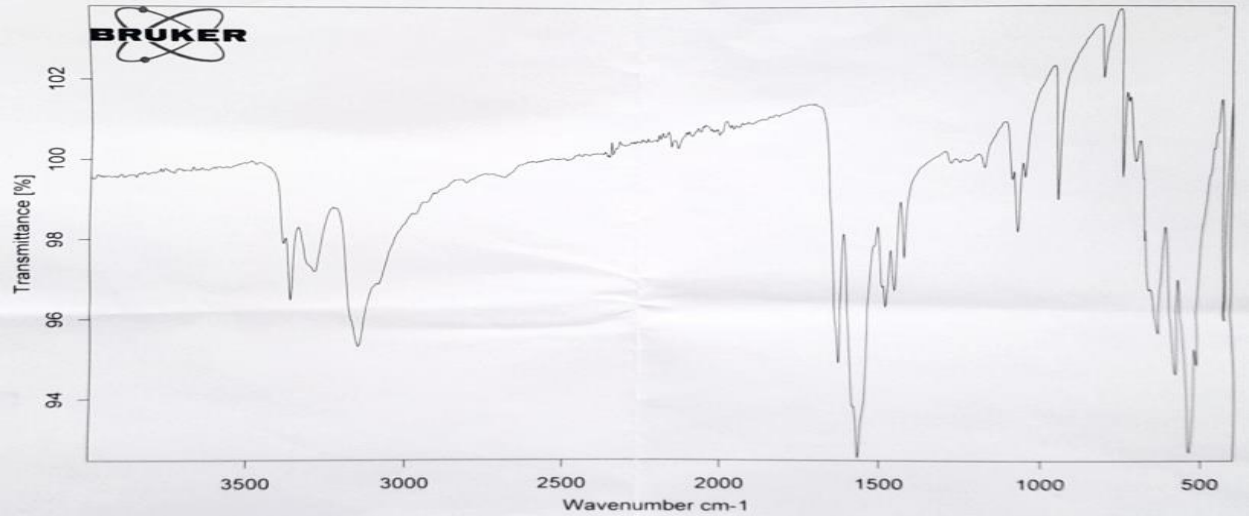
FTIR spectrums of M1



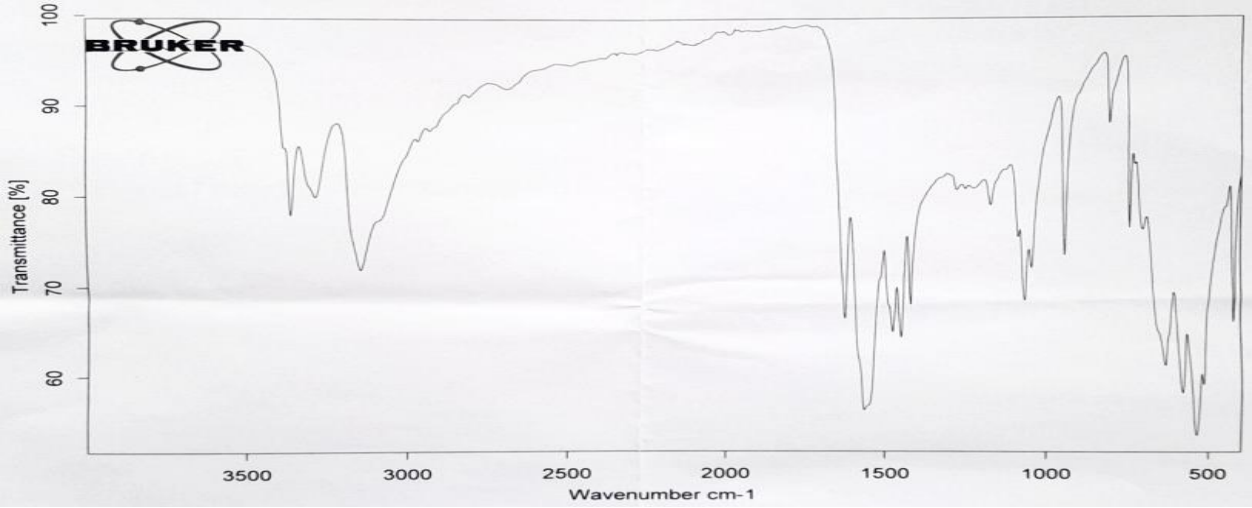
FTIR spectrums of M2



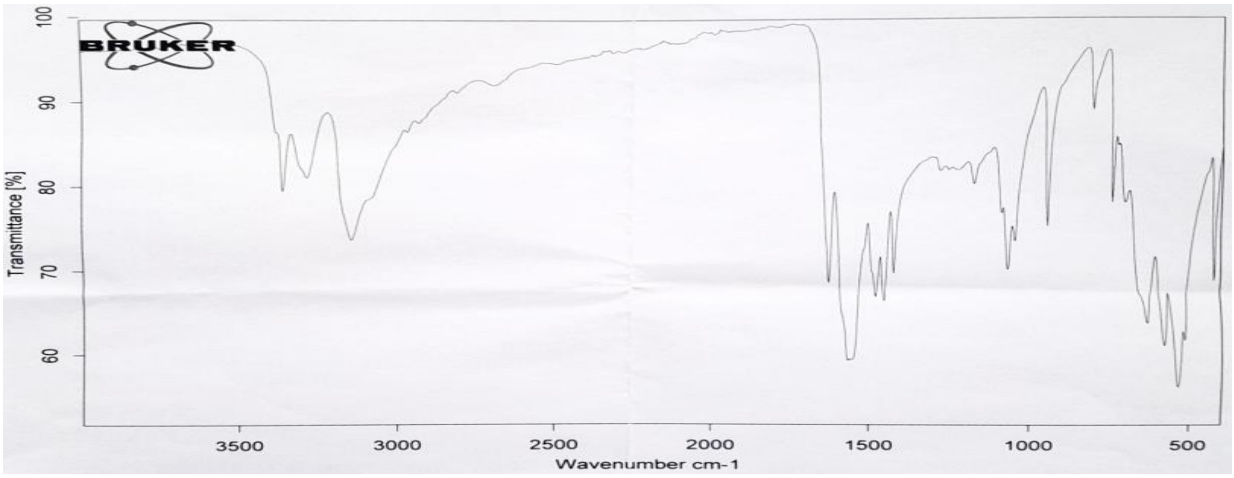
FTIR spectrums of M3



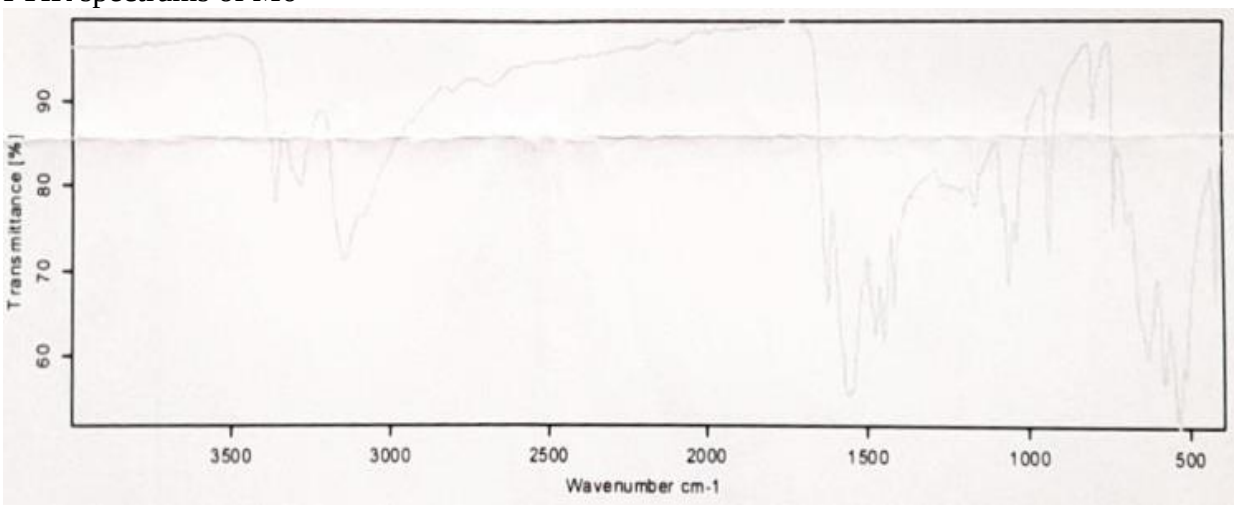
FTIR spectrums of M4



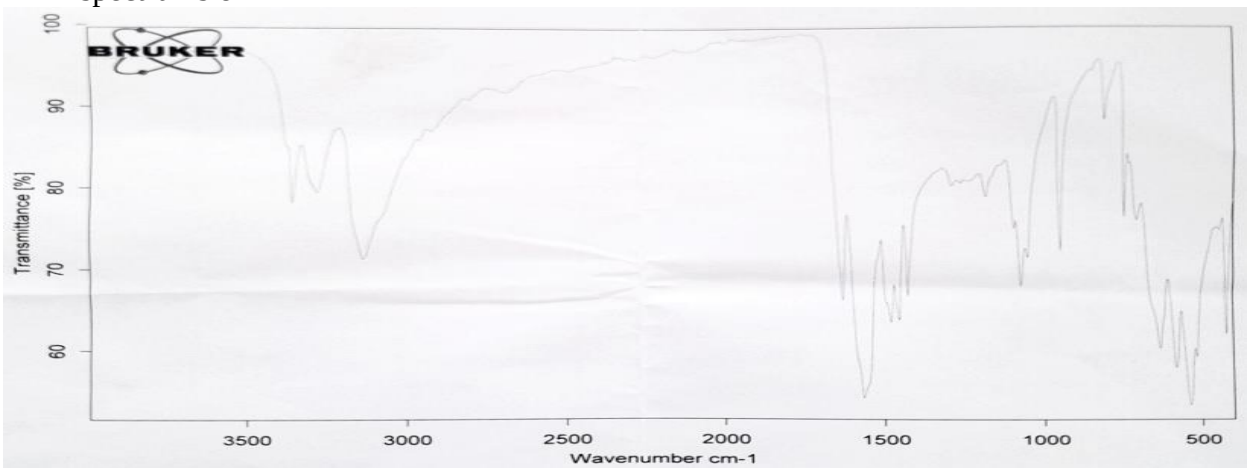
FTIR spectrums of M5



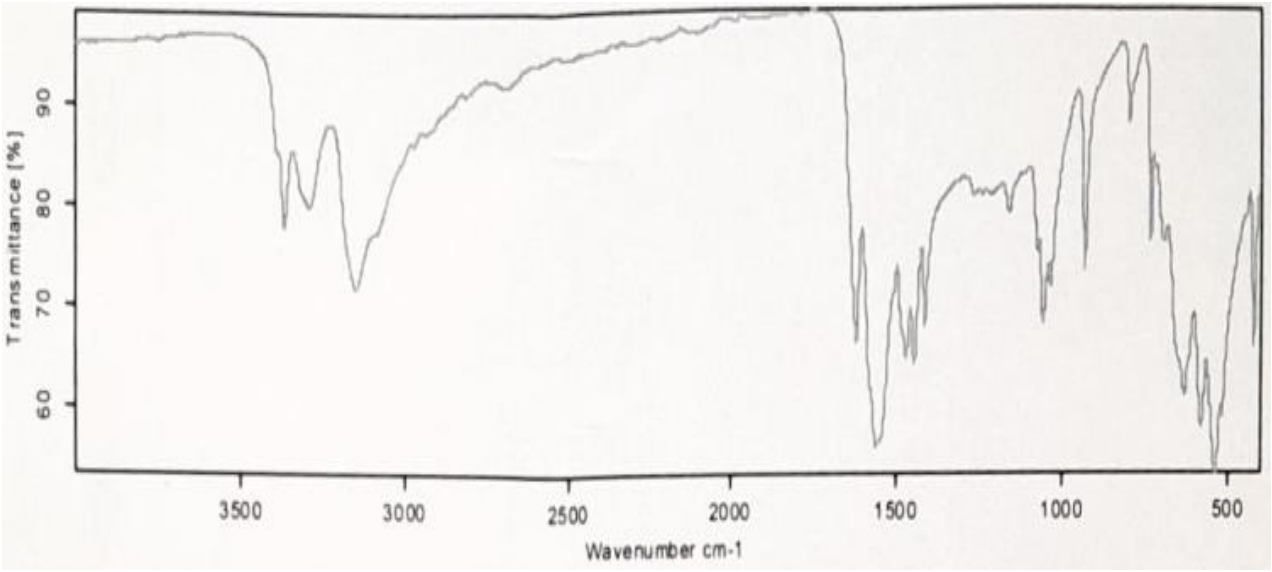
FTIR spectrums of M6



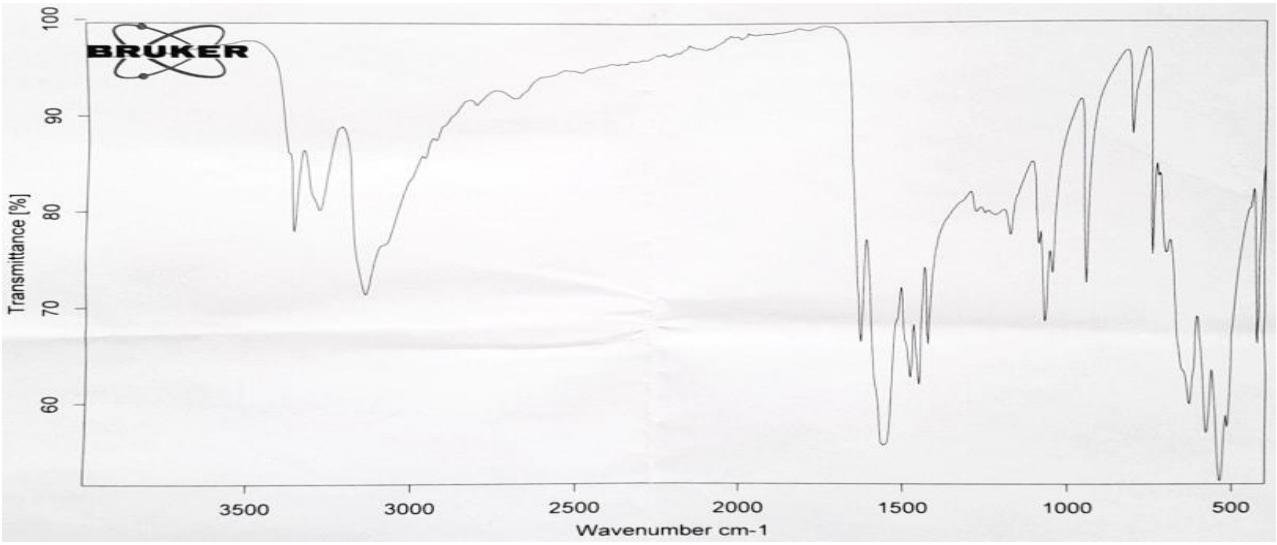
FTIR spectrums of M7



FTIR spectrums of M8



FTIR spectrums of M9



FTIR spectrums of M10

Annex 8: Image of the Study area, Addis Pharmaceutical Factory, Adigrat branch, Tigray, Ethiopia

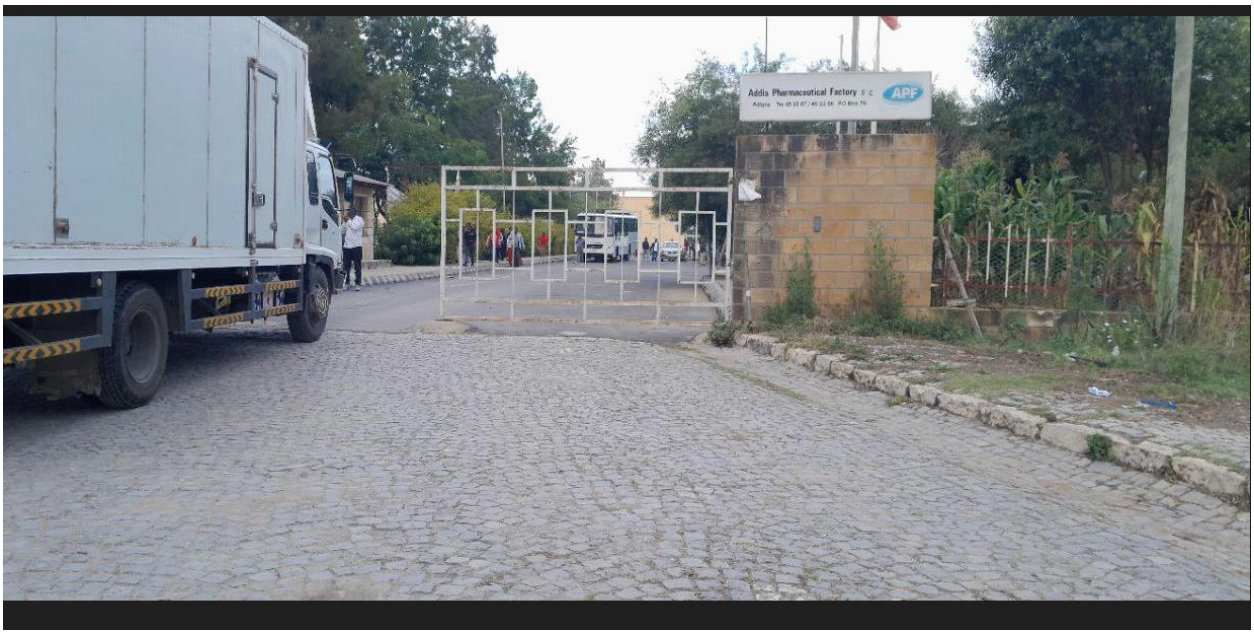
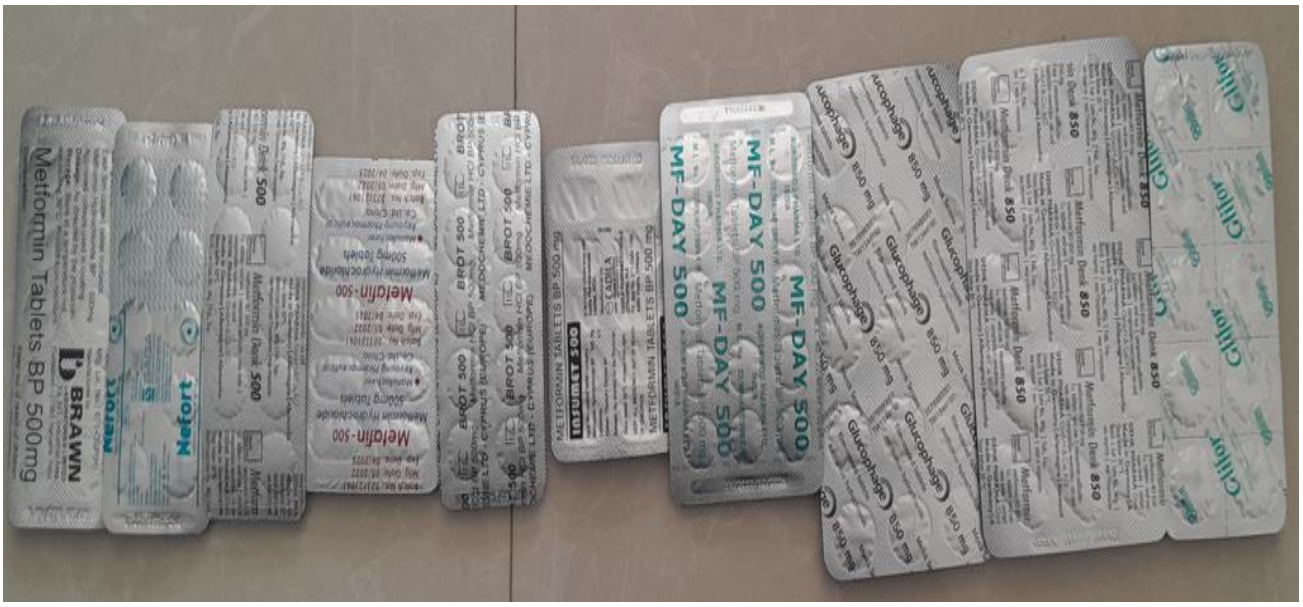


Image of Metformin HCl tablets collected sample

Annex 9: Picture of ten brands of metformin HCl tablet collected front view M1 to M10 from left to right

Picture of ten brands of metformin HCl tablet collected Back view M1 to M10 from left to right.



Annex 10: Image of metformin HCl USP standard (purity 99.7 %)

Image captured when performing Disintegration time and Friability Test in APF

Annex 11: Image when performing Dissolution profile of different brands of Metformin tablets