Comparative Quality Evaluation of Different Brands of Metformin Hydrochloride 500 mg Tablets Marketed in Jimma Town.

BY:-

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Comparative Quality Evaluation of Different Brands of Metformin Hydrochloride 500 mg Tablets Marketed in Jimma Town.

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Abstract

Introduction: - Metformin hydrochloride, classified under the class of biguanide is an oral anti-hyperglycemic agent and it is a mechanism of action that is sensitizing insulin. Metformin has hydrophilic properties and a low diffusion rate through the cell membrane.

Objective: - The aim of the study is to compare the quality of different brands of metformin hydrochloride 500 mg tablets.

Method: - The study was conducted in Jimma town. Different In-vitro tests such as weight variation, friability, dissolution rate, and assay were performed. Data were analyzed using mini tab 19 and one-way ANOVA was used for comparing dissolution profile, assay and weight variation of drugs with the comparator. Similarity factor (f_2), difference factor (f_1) and dissolution efficiency were also used for comparative study and KinetDS software was used for pharmacokinetic study.

Result: - Insumet was failed weight variation test. None of the tested brands were failed friability test. All brands have a statistically significant difference in mean weight from the comparator ($P<0.001$). Glyformin had the lowest assay value of all brands (84.96%). There was a statistically significant mean difference ($P=0.029$) in the drug content among different brands. Glucomet and glyformin release 61.7% and 75.9% of active ingredient at the specified time of 30 minutes respectively. Statistically, no significant mean difference in the dissolution profile was observed ($P=0.929$). Three of the brands had a similarity factor of (f_2=50-100) and five brands had a difference in dissolution efficiency of ±10%. The drug release model showed that only etform follow the Weibull model ($r^2=0.9887$).

Conclusion: - Comparatively, the quality of different brands of metformin hydrochloride included in the study was good. Insumet, metformin denk, brot, etform, and metformin can be used interchangeably with comparator drug Glucophage. Except for etform all of the tested brands follow Michaelis–Menten with Lag model for the release drug of drug substance.

Key words: - Physicochemical property, Quality, Metformin hydrochloride, Jimma, official test.

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Abbreviations and Acronyms

- ✓ ANOVA: - Analysis of Variance
- ✓ BCS: - Biopharmaceutical Classification System.
- ✓ BP: - British Pharmacopeia
- ✓ Da: - Dalton
- ✓ DE:- Dissolution Efficiency
- ✓ EFDA:- Ethiopian Food and Drug Authority
- ✓ $f_{1:-}$ Similarity factor
- ✓ f_2:- Difference factor
- ✓ h: - Hour
- ✓ HCL: - Hydrochloride/hydrochloric acid
- ✓ HIV/AIDS:- Human Immunodeficiency Virus, Acquired Immunodeficiency Syndrome
- ✓ HPLC: - High Performance Liquid Chromatography
- ✓ IDF:- International Diabetes Federation
- ✓ IP: - International Pharmacopeia
- ✓ L: - Litter
- ✓ LL:- Lower Limit
- ✓ LMICs:- Low and Middle income countries
- ✓ N: - Normality
- ✓ Ng/mL: - Nano gram per milliliter
- ✓ Plc: - Private limited company
- ✓ RS:- Reference Standard
- ✓ T1DM:- Type 1 diabetes mellitus
- ✓ $t_{1/2}$:- The elimination half-life of the drug
- ✓ TLC: - Tin Layer Chromatography
- ✓ T_{max}: - Maximum Time required for maximum drug concentration
- ✓ UL:- Upper Limit
- ✓ USD: - United State Dollar
- ✓ USP:- United State Pharmacopeia
- ✓ UV: - Ultraviolet light
- ✓ WHO: - World Health Organization

1. Introduction

1.1 Background

Metformin hydrochloride, an oral antidiabetic drug, is a commonly prescribed drug for the management of type 2 Diabetes mellitus by lowering both basal and postprandial plasma glucose. Other antidiabetic agent such as sulfonylurea which is used for the management of diabetes mellitus may induce hypoglycemia but metformin HCL does not cause hypoglycemia at any reasonable dose and usually called anti-hyperglycemic rather than hypoglycemic drug. Metformin HCL work as an insulin sensitizer; allowing the body to use insulin in normal way and it is classified under the class of biguanide [1, 2, and 3].

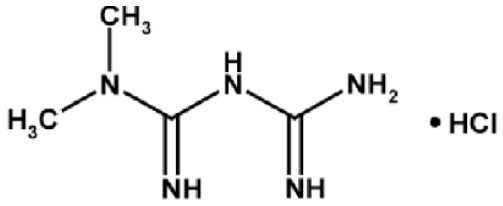

Figure 1:- Chemical structure of metformin hydrochloride

Metformin hydrochloride (N, N-dimethyl-imido-dicarbonimidic diamide hydrochloride) is small basic compound which has molecular weight of 129 Da and ionized at physiological PH [4]. Even though metformin is hydrophilic base chemically, it's usually present in an oral dosage forms in its hydrochloride salt form. This chemical property shows that metformin has low lipophilic property and therefore, the diffusion of metformin through cell membrane is low.

According to WHO 2019 report, diabetes mellitus is commonly classified as Type 1 and type 2 diabetes, with type 2 diabetes accounting for the majority (>85%) of total diabetes prevalence. Diabetes is found in every population in the world and in all regions, including rural parts of low and middle income countries. The number of people with diabetes is steadily rising, with WHO estimating there were 422 million adults with diabetes worldwide in 2014. The age-adjusted prevalence in adults rose from 4.7% in 1980 to 8.5% in 2014, with the greatest rise in low and middle income countries compared to high-income countries. In addition, the International Diabetes Federation (IDF) estimates that 1.1 million children and adolescents aged 14–19 years have type 1 diabetes mellitus (T1DM). Without interventions to halt the increase in diabetes, there will be at least 629 million people living with diabetes by 2045. High blood glucose causes

almost 4 million deaths each year, and the international diabetes federation (IDF) estimates that the annual global health care spending on diabetes among adults was US$ 850 billion in 2017. The effects of diabetes extend beyond the individual to affect their families and whole societies. It has broad socio-economic consequences and threatens national productivity and economies, especially in low and middle income countries where diabetes is often accompanied by other diseases [5, 6, 7].

According to sixth edition of International Diabetes Federation 175 million of cases were yet undiagnosed. Each year more than 231,000 people in the United States and more than 396 million people worldwide die from diabetes and its complications.

According to Biopharmaceutics Classification System (BCS), metformin is classified under class III [8]. For such drugs permeability is the rate controlling step in drug absorption. Rapid dissolution is particularly desirable in order to maximize the contact time between the dissolved drug and absorption mucosa. Therefore, the duration of dissolution should be stringent for class III drugs. As drug permeation is rate controlling, no *in-vivo-in-vitro* (IVIV) correlation is expected. The main objective of an IVIVC is to serve as a surrogate for *in-vivo* bioavailability and to support bio-waivers. IVIVCs could also be employed to establish dissolution specifications and to support and/or validate the use of dissolution methods [9, 10].

Poor-quality and especially counterfeit medicines can seriously harm patients. An insufficient dosage of an anti-infective drug can lead to bacterial resistance on the one hand and therapeutic failure on the other hand. Using ineffective therapy leads to longer hospital stay for patients, which can also leads to mortality. The longer hospital stay of the patient also leads to loss of income, productivity, and national prosperity. Toxic impurities, which are more common in counterfeit than in substandard drugs, can poison the patient and lead to persistent health problems or even death [11].

1.2 Statement of the problem

For better health of the public, supplying good quality medicine is mandatory but it is often missing in developing country like Ethiopia which has week regulatory system for pharmaceuticals. Now days, the quality of pharmaceuticals are getting world attention due to the increase in existence of poor quality drug in the world and the burden is high in developing country like Ethiopia. The use of this poor quality drug may leads to therapeutic failure,

increased morbidity and mortality, erosion of public confidence in health care, unexpected side effects, and resistance [12].

World Health Organization (WHO) has estimated that about 30% of the medicines on sale on Africa countries and parts of Asia and Latin America are counterfeit. Bangladesh's mammoth Pharmaceutical industry exports drugs to as many as 52 countries worldwide and in 2013 the Public Health and Drug Testing Laboratory (PHDTL) tests 5000 drug samples from this pharmaceutical industry and reported that 300 drugs are either counterfeit or of very poor quality [5, 13].

Estimated global health care expenditures to treat and prevent diabetes and its complications are 376 billion US Dollars (USD) in 2010. By 2030, this number is projected to exceed some 490 billion USD. Diabetes in Africa is a serious, chronic and costly disease that is estimated to have 23.9 million cases by 2030.

Even though there are so many brands of drugs incorporated in to the world pharmaceutical market to improve public heath outcome, the proportion of poor quality drugs are increasing proportionally. The study done in Europe (Albania) to evaluate the interchangeability of three different brands of metformin hydrochloride indicate that only two of the brands are used interchangeably [14]. Other study done in Asia (Qatar) to assess bioequivalence and interchangeability of multisource marketed metformin hydrochloride tablets by using ten different brands revealed that only six of the brands can be used interchangeably with the innovator drug (Glucophage) and the other four brands did not used interchangeably [15].

Similar with other part of the world, Africa continent as a whole is also facing great challenge on quality of medicines. The study done in Nigeria for Comparative Evaluation of Physicochemical Properties of Some Commercially Available Brands of Metformin Hcl Tablets on eight different brands showed that only four of the brands are bioequivalent and can be used interchangeably [16]

Generally, medicine used for the public health need certain types of standards to have quality, safety and efficacy. So, monitoring drug quality through *in-vitro* test is mandatory to safe guard the health of the public as a whole and to reduce development of drug resistance [17].

1.3. Significance of the study

Accurate and up-to-date information on the burden of poor quality drugs are necessary for the development of effective as well as efficient regulatory system in LMICs including Ethiopia. The study will give an evidence for governmental and non-governmental organizations which work in the area of drug regulation and manufacturing by providing basic information on burden of poor quality metformin hydrochloride and would allow closer follow-up and more targeted interventions on identified poor quality drugs that ultimately reduces mortality and morbidity of patients caused due to use of those poor quality drugs.

Therefore, creating awareness for regulatory body and manufacturers by identifying the availability of poor quality metformin hydrochloride with in sighted information in poor settings will be a stepping stone for us. Moreover, the finding may call for attention of concerned bodies to make decision and take measure in the spirit of improving their regulatory system for regulatory bodies and improving the manufacturing process for manufacturers by forwarding necessary recommendations for possible change and to scale up current regulatory programs and would allow closer follow-up and more targeted interventions.

2. Literature review

Several studies are published on *in-vitro* comparative quality evaluation of different brands of metformin hydrochloride in a different country and the studies show different results about the quality of the drug present on the drug market of different countries. In Asia, so many studies were conducted and showed variable results. The study done in Bangladesh (2012) to evaluate the *in-vitro* dissolution profile of ten different brands of metformin hydrochloride sustained release tablet in simulated intestinal medium (pH 6.8 ± 0.1) for 10 hours' time period using USP reference dissolution apparatus showed that all of the brands except two of them meet the specification [18]. After a year another study was conducted in Bangladesh on seven different brands of metformin hydrochloride by using USP and BP and the result of the study showed that six of the brands meet specification (USP) for weight uniformity and seven of the brands passed the test for hardness. Friability test was also performed for all of the brands according to USP and all of the tablets passed the test and all of the tablets also meet the specification for the disintegration test. The dissolution rate is also done according to BP and USP for all of the tablets and all of the tablets were passed the test for dissolution and can be used interchangeably (f_2> 50%) [19]. Then, in 2015 the same study was done in the same country on three different brands of metformin hydrochloride (two brands and one generic) by using IP specification as a reference; The result showed that three of the brands were passed the test for hardness, disintegration time and drug content. The study also tested the dissolution rate of the three brands in phosphate buffer of PH 6.8 as prescribed on IP and all of them were passed the test and there were no statistically significant differences in the dissolution characteristics of the three products tested (P > 0.05) [20]. Three years later in 2018 three different brands of metformin hydrochloride were tested *in-vitrocally* in Bangladesh by performing test for weight variation, thickness, diameter, hardness, friability; dissolution and the result indicated that all of the brands included in the study were within specification set by USP [21].

In similar continent, another comparative study was done by using six different brands of metformin hydrochloride 500 mg tablets marketed on Saudi Arabia market (2012) by using the innovator drug Glucophage® as a comparator and the result of the study showed that all of the brands were within specification limit for weight variation, friability, disintegration and the percentage content of active ingredient of six brands of Metformin hydrochloride tablets showed

values within the monograph specifications (95-105%). The dissolution profile study of all brands indicated that all of the brands were identical except for Glucare® which was inequivalent with the innovator drug and therefore, all of the brands can be substituted with the innovator drug except for Glucare® [22].

In Syria, in the year 2016 *in-vitro* test was done for the evaluation of the physicochemical property of metformin by using USP and BP specification as a reference standard. Five different brands of metformin hydrochloride tablets were included in the study. The study reported that only two of the brands were passed the non-official test for hardness and all of the brands were meet the specification for friability as stated on the BP. The result also showed that all of the tablets were passed the test for weight uniformity and only three of the brands meet the specification for content uniformity. The dissolution profile study of the five brands was also performed and three of the brands have complied with the specification for dissolution profile [23].

In the year 2012 other in-vitro test was done in India to evaluate the pharmaceutical equivalence of nine different brands of metformin hydrochloride and the result showed that all of tested brands meet the specification for weight variation (IP), hardness, friability (BP: < 1%) and assay (IP: 95% - 105%) and the dissolution test was performed for only six brands and all brands were passed the test (IP: ≥75%) [24]. Four years later comparative study was done in India on four different brands of metformin hydrochloride available on the drug market of India and revealed that all of the brands can be used interchangeably [25].

In Africa *in-vitro* test on different brands of metformin hydrochloride to evaluate its quality was also performed and reported variable results. In Ghana, seven different brands (2013) of metformin hydrochloride were tested to assess the potency of the brands according to BP and the study reported that all of the brands were passed the test for assay, dissolution rate and weight uniformity [26]. Other studies done in similar country (2016) on fifteen different brands of metformin hydrochloride to evaluate the physicochemical property of the tablets from which twelve of them were film-coated and the rest are uncoated tablets and the result revealed that all of the fifteen tablets had metformin hydrochloride as an active ingredient which is identified by identification test done by employing Thin Layer Chromatography (TLC). The tablets were passed the test for uniformity of weight and friability except for one tablet which is failed the

friability test. All of the twelve film-coated tablets meet the specification for disintegration time and one of the uncoated tablets did not pass the disintegration time. From the fifteen tablets, twelve of the tablets were passed the assay test and all of them passed the dissolution test for immediate release tablets [27].

A year later, the same study was performed on ten different brands of metformin hydrochloride marketed in Abuja; from these ten brands, one brand was sustained release formulation with label strength of 1000 mg and the remaining were immediate-release formulations with label strength of 500 mg. The weight uniformity test indicated that there was no statistically significant difference ($P > 0.05$) in the weight of tablets from different brands and conformed to BP specification. From the tested brands, only five of them have complied with specifications on the hardness of the tablets and one of the tablets did not meet the specification for friability. Except for one tablet, all of the tested tablets meet the specification for disintegration time and the sustained release tablet did not disintegrate within an hour. From the nine immediate-release tablets four of them did not meet the specification for the dissolution test (BP: - within 45 min. 70% of drug content should be released) but the dissolution test for sustained release tablet meets the specification. The result of assay of chemical content using HPLC and UV analysis showed that four of the brands were failed the test using UV analysis and except one brand all brands were passed the test using HPLC assay [28]

In sub-Saharan African country there was limited literature on the quality of different brands of metformin hydrochloride tablets. The study done in Sudan (2017) on five different brands of metformin hydrochloride showed that four of the tablets were passed the assay test (NLT 95% and NMT 105% according to BP) which was done by using UV spectrophotometry and all brands meet the test for weight uniformity (no tablet deviate by 5% and 10%). The study also indicated that all of the tablets were passed friability test (0-0.05%) and except for the uncoated tablet, all of the tablets were passed the specification for the non-official test of Hardness. All of the coated tablets meet BP specification for the disintegration time (6.07-9.3 min) and the uncoated tablet also meet BP specification (13.32 min). The dissolution test of the five brands was also performed and all the brands were passed the test according to BP ($f_2 > 50$ and $f_1 < 15$) [29]. The study was done in Ethiopia (Addis Ababa) on six different tablets of metformin hydrochloride by using USP 2007 method reported that all the brands were complied with the

official specification for hardness, friability, disintegration and assay. Five brands of metformin hydrochloride have complied with the USP dissolution tolerance limit. But, Metformin Denk was failed to release the stated amount and Statistical comparison for *in vitro* drug release revealed that some of the products of metformin hydrochloride tablets showed statistically significant difference ($P<0.05$) [30]. The other study conducted in Western and North Western Tigray on seven different brands of metformin hydrochloride reported that all of the brands were passed the test for assay, dissolution, weight variation, friability, hardness test and moisture content [31].

2.1. Conceptual frame work

The conceptual frame work used for comparative quality evaluation of different brands of metformin hydrochloride 500 mg tablets.

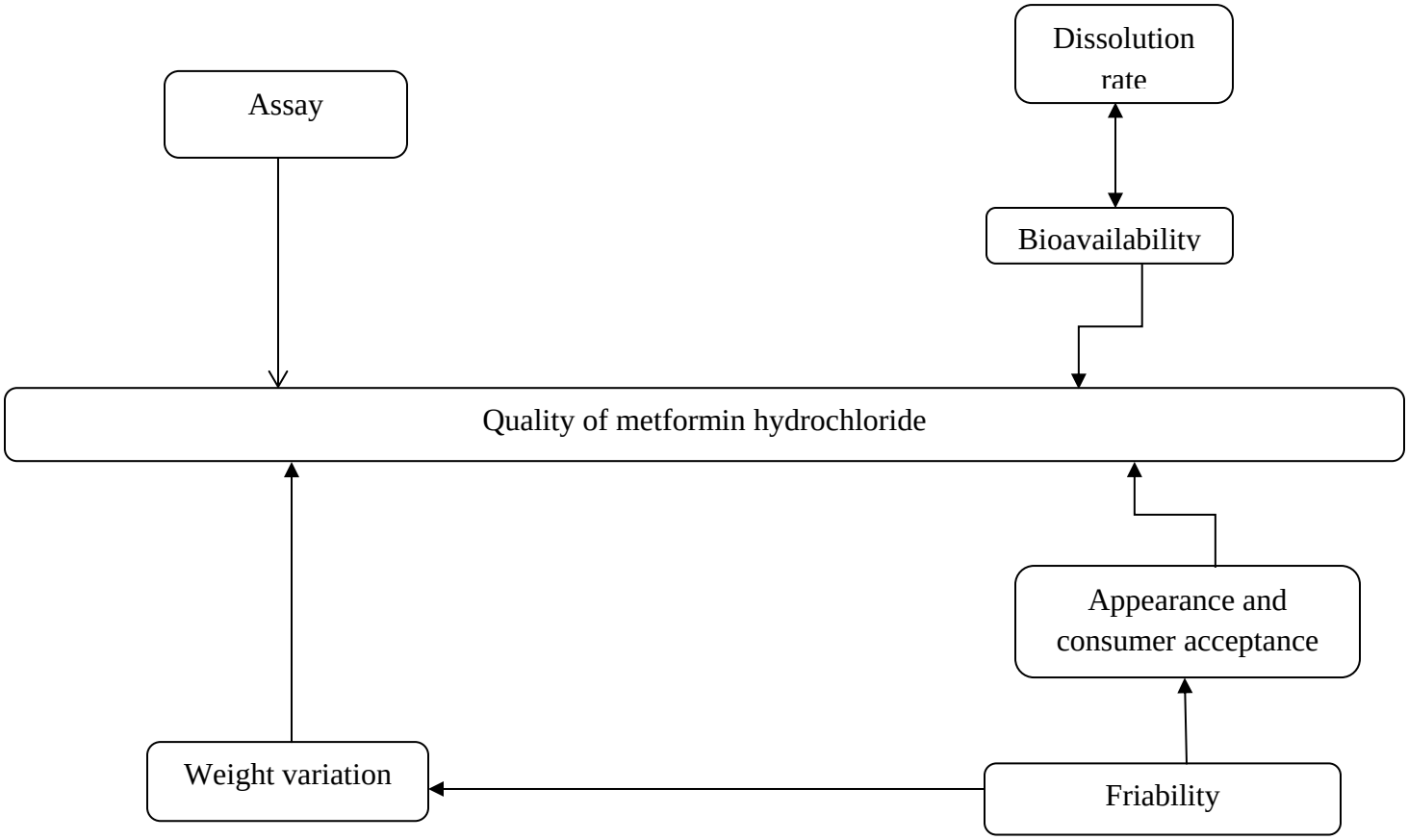

Figure 2:-Conceptual frame work for comparative quality evaluation of different brands of metformin hydrochloride 500 mg tablets.

3. Objectives

3.1. General objective

To compare the quality of different bands of metformin hydrochloride 500 mg tablets available in Jimma Town.

3.2. Specific objectives

3.2.1. To compare the physical property of different brands of metformin hydrochloride 500 mg tablets.

3.2.2. To compare the dissolution profiles of different brands of metformin hydrochloride 500 mg tablets.

3.2.3. To compare the percentage content of active pharmaceutical ingredient for different brands of metformin hydrochloride 500 mg tablets.

3.2.4. To identify the drug release model of different brands.

4. Material and methods

4.1. Study setting and area

The study was conducted in Jimma town. Jimma is located 357 Km southwest of Addis Ababa. The town is divided into 17 kebeles with a population of 120,960 and 32,192 households [32]. The laboratory work was conducted in Jimma University Laboratory of Drug Quality (JuLaDQ) on metformin hydrochloride 500 mg tablets.

4.2. Instruments

Analytical Balance (Mettler Toledo, Greifensee, Switzerland), RC-6D Dissolution Apparatus (Apparatus 2; Tian Jin Optical Instruments, Tianjin, China), UV–Vis Spectrophotometer (Cecil Instruments, Cambridge, United Kingdom), Friability Tester (Pharma Test), PH meter (AD 1020 PH /MV/ISE) and Water Purification System (Thermo Scientific, Model-7143, Waltham, MA, USA) were used for the study.

4.3. Chemical and reagents

Distilled water, sodium hydroxide (BDG Laboratory Supplies, Purity= 97.5%), potassium dihydrogen orthophosphate (Techno Pharm Chem, Bahadurgarh, Purity= 99-101%, India) were used and the reference standard of metformin hydrochloride was donated by EFDA.

Table 1:-General information for all brands included in the study

Code	Brands	Manufacturer	Country	Batch number	Man. Date	Exp. Date	Online registration status in Ethiopia (MA number)
008	Glucophage	Merch Serono	France	F0582	10/2018	09/2023	6315/REN/2018
004	Metformin denk	Denk pharma	Germany	9N6	07/2018	06/2013	3357/REN/2017
001	Insumet	Cadila Pharmaceuticals PLC	Ethiopia	D18076T212	12/2018	09/2021	*
007	Brot	Medochemie Ltd; Limassol,	Cyprus	A1H115	08/2018	08/2022	5514/REN/2017
005	Etform	Lek SA, Styrykow,; Sandoz anovartis company	Poland	JC2413	06/2018	06/2021	*
003	Glucomet	Y.S.P. Industries (M) SDN,BHD,	Malaysia	BL007	12/2017	12/2020	2323/NMR/LD
006	Metformin	Ningho Shuangwei Pharm.co.Ltd.	China	180719	07/2018	07/2021	*
002	Glyformin	Limassol Industrial Estate, Aharnon street.	Cyprus	76858	06/2018	06/2023	2482/REN/2016

*:- Online registration number not available

4.4. Sampling technique and sample collection

4.4.1. Sampling technique

All available brands of metformin HCL tablets, each with a label claim of 500 mg were purchased from drug retail outlets and hospital pharmacy that are located in Jimma town. Regarding sampling strategies, WHO Guidelines to Conduct of Surveys of the Quality of Medicines were used [33]. Accordingly, a convenience sampling technique was used for sample collection sites.

4.5. Quality assessment parameters

The quality of different brands of metformin hydrochloride was evaluated according to USP 2015 guideline.

4.5.1. Physical characteristics, packaging and labeling

The physical characteristics of the tablets were determined by physical inspection of shape, color and the presence or absence of odor. They should be undamaged, smooth, and usually of uniform color.

The packaging and labelling of the tablets were determined according to WHO guideline for packaging and labelling of pharmaceuticals (annex 2)

4.5.2. Weight variation

Randomly selected twenty tablets from each brand were weighed individually with an electrical analytical weighing balance and their average weight was determined; then, the percentage deviation was calculated from average weight.

As stated on USP 2015, the tablet pass the test if not more than two of the individual weights deviate from the average weight by ± 5% and none deviates by ± 10 %.

4.5.3. Friability Test

Randomly selected twenty tablets of each brand were weighed and subjected to abrasion using drum of friability tester at 100 revolutions for 4 min and then tablets were removed from the drum and dedusted and weighed again. Then, percent of weight loss was recorded. Then, the friability of the tablets was calculated using the following formula.

Friability (%) = (Initial weight- Final weight/ initial weight) * 100 (1)

According to USP, the Compress tablets that lose not more than 1.0 % of the Tablet weight are considered to be acceptable and if obviously cracked, cleaved, or broken tablets are present in the tablet sample after tumbling, the sample also fails the test.

4.5.4. Dissolution test

The dissolution test was conducted according to the USP monograph on six tablets of each brand using USP Apparatus 2 operated at 50 rpm. The dissolution medium was 1000 ml phosphate buffer (pH 6.8) maintained at 37°C±0.5°C.

USP 2015 specifies at single time of 30 mins at least 80% of the drug needs to be dissolved. However, 10 ml of sample was drawn at 5, 15, 30 and 45 mins to study the dissolution profile of the drug and fresh 10 ml dissolution medium was used to replace the withdrawn sample after each sampling. Each of the withdrawn samples was filtered. After filtration and appropriate dilution, the corresponding absorbance readings of diluted filtrates were taken by UV-Vis spectrophotometer at a wavelength of 233 nm. Then, the concentration was determined from the calibration curve of standard solution having a known concentration of Metformin hydrochloride RS in the same medium and the percentage drug release was calculated at each time.

4.5.4.1. Calibration curve for dissolution test method

A stock solution was prepared by dissolving 50 mg of metformin hydrochloride USP RS in 100 ml of phosphate buffer, pH 6.8. Five concentration levels of 500, 250, 125, 62.5 and 31.125 µg/ml were prepared with phosphate buffer. Their absorbance was determined spectrophotometrically. Then, concentrations of metformin hydrochloride against absorbance were plotted to obtain the calibration curves.

As revealed on the calibration curve, a linear regression equation is $Y=0.0052x+0.1076$, where Y is the absorbance and X is the concentration in µg/ml. This curve showed that three is a strong linear relationship between the concentration of the tested samples and the absorbance values over the concentration range of 31.125–250µg/ml ($r^2=0.999$) and by using the equation obtained from the calibration curve, the percentage release values of samples taken at times 5, 15, 30 and 45 minutes were calculated.

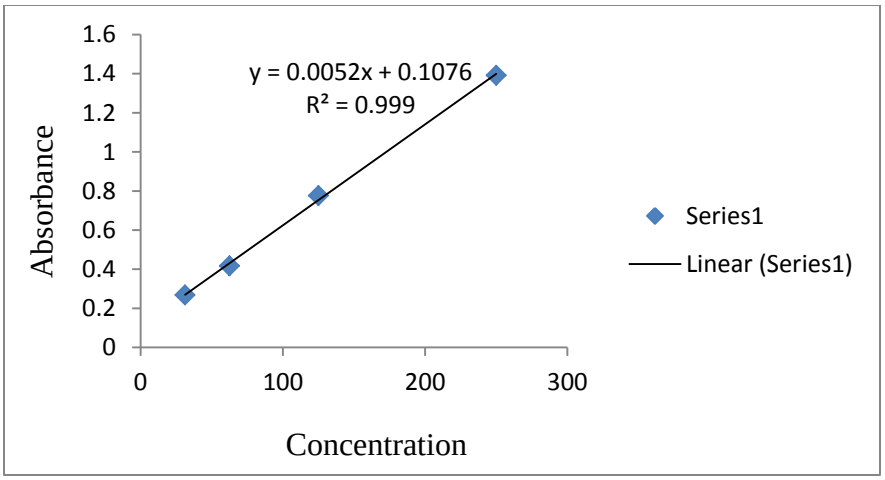

Figure 3:-Calibration curve for UV- spectroscopy for dissolution study

4.5.5. Assay

4.5.5.1. API content of metformin hydrochloride

Sample preparation: - From each brands randomly selected twenty tablets of metformin hydrochloride were weighed and then powdered using mortar and pestle. A quantity of the powder equivalent to 0.1g of metformin hydrochloride was transferred to 100 ml volumetric flask and then 70 ml of water was added. The resulting solution was shaken by mechanical means for 15mins and then diluted with water to volume and filtered. 20 ml of the first filtrate was discarded and then, 10 ml of the filtrate was diluted with water to 100ml. Finally, 10ml of the resulting solution was diluted with water to 100 ml and the absorbance of the resulting solution and standard preparation was measured at wavelength of 232 nm using water as a blank.

Standard preparation: - A solution of USP Metformin Hydrochloride RS having a known concentration of 10 µg/ml in water was prepared in similar manner with the preparation of sample.

The content of metformin hydrochloride was then calculated using USP 2015.

$$\text{Result} = A_u/A_s * (C_s/C_u) * 100 \quad (2)$$

Where,

A_u= Absorbance of sample

A_s= Absorbance of standard

C_s= Concentration of metformin hydrochloride reference standard (µg/ml)

C_u= Concentration of metformin hydrochloride in sample solution (µg/ml)

4.6. Data analysis

Microsoft Excel 2010 and mini tab version 19 software programs were used for statistical and graphical evaluations of analytical data obtained from the experimental part of the investigation. Statistically significant differences were considered when $P<0.05$, and one-way ANOVA was also carried out for comparison of weight variation, assay and dissolution profile study.

The dissolution profiles of various brands of metformin hydrochloride were also compared by using model independent approach, and dissolution efficiency (DE).

Model independent methods involve comparison of the two profiles only at the pharmacopeially observed time point (30min). This approach includes difference factor (f_1 factor) and similarity factor (f_2 factor). The difference factor (f_1) calculates the percentage difference between the two curves (comparator and test drug) at each time point and is a measurement of the relative error between the two curves. The similarity factor (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.

$$f_1 = \{[\sum_{t=1}^{n} | R_t - T_t |]/[\sum_{t=1}^{n} R_t]\} \bullet 100 \quad (3)$$

$$f_2 = 50 \bullet \log \{[1+(1/n)\sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \bullet 100\} \quad (4)$$

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.

DE is the area under the dissolution curve within a time range.

To explain the kinetics and mechanism of drug release from the tablets, KinetDS software was also employed.

5. Result

Among eight brands of metformin hydrochloride tablets included in this study, seven brands were imported from foreign countries while one was manufactured locally. All metformin HCL brands were subjected to different quality control tests in order to assess their dissolution profile, weight variation, friability and assay

5.1. Physicochemical property of drugs

The physical characteristics result of the studied brands showed that all of the tablets have uniform white color, undamaged and did not have any odor. Except insumet all metformin hydrochloride tablets under the test had circular shape. But, insumet had oval shape.

The packaging and labelling of all brands meet the minimum requirement required by World Health Organization for packaging and labelling (annex 2).

5.2. Weight variation and friability

Brot and Etform had highest and lowest mean weight respectively. Metformin denk and etform did not lose their content after friability test. Except for Insumet all brands of metformin hydrochloride were passed weight variation test. Statistical analysis conducted using one-way ANOVA at 95% confidence interval (CI) revealed that there were significant differences ($P<0.001$) in mean weight among sample mean weight of all brands. All of the brands were passed friability test (Table 2 below).

Table 2:-Weight variation and friability test result.

Code	Brands	Weight variation (mean± SD)	UL	LL	P-value	Friability (%)
008	Glucophage(comparator)	532.08±4.07	544.29	519.87	P<0.001	0.014
004	Metformin denk	656.87 ±5.31	672.8	640.94		-
001	Insumet	660.72±6.10	679.02	642.42		-0.0197
007	Brot	677.14±3.91	688.87	665.41		$7.37*10^{-4}$
005	Etform	524.51±3.66	535.49	513.53		-
003	Glucomet	563.65±4.25	576.40	550.90		0.4
006	Metformin	554.26±15.85	601.81	506.71		0.055
002	Glyformin	667.82±6.41	687.05	648.59		0.0023

SD: - Standard Deviation; LL: - Lower Limit; UL: - Upper Limit

To reveal the source of difference between the comparator and different brands, Dunnet multiple comparisons with the comparator brands and Tukey pairwise comparison with each other was conducted at 95% confidence interval and revealed that all of the brands have statistically significant mean weight difference from the comparator brand Glucophage. Brot and glyformin did not have statistically significant mean weight difference from each other (Table 3 and 4 respectively)

Table 3:-Dunnett Simultaneous Tests for Level Mean vs Comparator Mean

Difference of Levels	Difference of Means	SE of Difference	95% CI	T-Value	Adjusted P-Value
Brand 1 - brand 8	133.79	2.86	(126.22, 141.36)	46.72	<0.001
Brand 2 – brand 8	135.74	2.86	(128.17, 143.30)	47.40	<0.001
Brand 3 – brand 8	31.57	2.86	(24.00, 39.14)	11.02	<0.001
Brand 4 – brand 8	124.68	2.86	(117.11, 132.25)	43.54	<0.001
Brand 5 – brand 8	-7.57	2.86	(-15.14, -0.00)	-2.64	0.050
Brand 6 – brand 8	19.46	2.86	(11.89, 27.02)	6.79	<0.001
Brand 7 – brand 8	144.13	2.86	(136.56, 151.69)	50.33	<0.001

Key: - Brand 1:- Insumet, Brand 2:- Glyformin, Brand 3:- Glucomet, Brand 4:- Metformin denk, Brand 5:- Etform, Brand 6:- Metformin, Brand 7:- Brot, Brand 8:- Glucophage (comparator)

Table 4:-Tukey Simultaneous Tests for Differences of Means from each other

Difference of Levels	Adjusted P-Value
Brand 2 – brand 1	**0.997**
Brand 3 – brand 1	<0.001
Brand 4 – brand 1	0.037
Brand 5 – brand 1	<0.001
Brand 6 – brand 1	<0.001
Brand 7 – brand 1	0.010
Brand 3 – brand 2	<0.001
Brand 4 – brand 2	0.004
Brand 5 – brand 2	<0.001
Brand 6 – brand 2	<0.001
Brand 7 – brand 2	0.074
Brand 4 – brand 3	<0.001
Brand 5 – brand 3	<0.001
Brand 6 – brand 3	0.001
Brand 7 – brand 3	<0.001
Brand 5 – brand 4	<0.001
Brand 6 – brand 4	<0.001
Brand 7 – brand 4	<0.001
Brand 6 – brand 5	<0.001
Brand 7 – brand 5	<0.001
Brand 7 – brand 6	<0.001

Key: - Brand 1:- Insumet, Brand 2:- Glyformin, Brand 3:- Glucomet, Brand 4:- Metformin denk, Brand 5:- Etform, Brand 6:- Metformin, Brand 7:- Brot.

5.3. Assay

Insumet and Glyformin had highest and lowest assay value with 99.61% and 84.96% respectively. Glyformin was failed to comply with USP specification for assay. One way ANOVA conducted at 95% confidence interval for mean difference of drug content revealed that

there were statistically significant mean difference (P=0.029) in the drug content among different brands of metformin hydrochloride (Table 5 below).

Table 5:- Assay result for different brands of metformin hydrochloride.

Code	Brands	Assay	P-value
001	Insumet	99.61	
002	**Glyformin**	**84.96**	
003	Glucomet	97.9	P=0.029
004	Metformin denk	95.21	
005	Etform	98.28	
006	Metformin	96.12	
007	Brot	95.41	
008	Glucophage(comparator)	96.46	

To augment the one way ANOVA test dunnett multiple comparisons with comparator was performed and except for glyformin all of the brands did not have statistically significant mean difference for content of API with the comparator drug glucophage (Table 6 and 7 below).

Table 6:-Grouping Information of assay result using the Dunnett Method

Brands	Grouping
brand8 (comparator)	A
brand1	A
brand5	A
brand3	A
brand6	A
brand7	A
brand4	A
brand2	

Means not labeled with the letter A are significantly different from the comparator level mean

Table 7:-Dunnett Simultaneous Pairwise Tests for all brands - comparator

Difference of Levels	P-Value
Brand1-brand8	0.966
brand2-brand8	**0.026**
brand3-brand8	1.000
brand4-brand8	0.997
brand5-brand8	0.998
brand6-brand8	1.000
brand7-brand8	1.000

Key: - Brand 1:- Insumet, Brand 2:- Glyformin, Brand 3:- Glucomet, Brand 4:- Metformin denk, Brand 5:- Etform, Brand 6:- Metformin, Brand 7:- Brot, Brand 8:- Glucophage (comparator)

5.4. Dissolution study at specified time of 30 minutes

Except for Glyformin and Glucomet all of the tested brands released the necessary amount of API at specified time of 30 minutes. Model independent approach of difference (f_1) and similarity (f_2) factor was used to study the bioequivalence of different brand with comparator drug glucophage. To ensure similarity and bioequivalence of two dissolution profiles, f_1 should be between 0-15 whereas f_2 should be 50-100 [37]. Accordingly, metformin denk, insumet and brot were bioequivalent with the comparator drug glucophage and can be used interchangeably (Table 8 below).

On the other hand f_1 value of etform and metformin justify the interchangeability of those brands with the comparator. To ascertain the interchangeability of all of the products, the release profile was also compared by dissolution efficiency (DE). To be bioequivalent, the difference in dissolution efficiency of the comparator and tested drugs should be within approximate limit of (±10%) (Table 9). Based on this, etform and metformin can be used interchangeably with the comparator. Therefore, metformin denk, insumet, brot, etform and metformin are bioequivalent with the comparator.

Table 8:-Dissolution study report at USP specified time of 30 minutes

Code	Brands	Dissolution (%) at 30 minutes	Model independent approach	
			f_1	f_2
008	Glucophage(comparator)	94.9	-	-
004	**Metformin denk**	85.7	**9.19**	**84.50**
001	**Insumet**	87.1	**7.85**	**61.56**
007	**Brot**	87.8	**7.08**	**50.08**
005	Etform	96.7	1.77	3.13
003	**Glucomet**	**69.4**	33.23	652.21
006	Metformin	89.4	5.54	30.67
002	**Glyformin**	**75.9**	19.00	361.00

5.5. Comparison of dissolution profile

In order to observe if there was statistically significant difference in the release profile, one-way ANOVA followed by Dunnett's test was undertaken between the comparator drug glucophage and other brands at 95% CI, from this result it was shown that there were no statistically significant difference in release profile between comparator drug glucophage and other brands (Table 9 and fig 4 below).

Table 9:-Comparative dissolution study report for different brands

Code	Brands	Dissolution (%)				DE (%)	Difference of dissolution efficiency (%)	P-value
		5 min	15 min	30 min	45 min			
008	Glucophage(comparator)	14	79.0	94.9	96.3	71.96	0	
004	**Metformin denk**	4.8	36.9	85.7	91.1	65.80	**-6.16**	
001	**Insumet**	1.3	69.2	87.1	99.6	65.07	**-6.83**	
007	**Brot**	25.5	87.1	87.8	93.6	73.31	**1.35**	**0.929**
005	**Etform**	36.3	76.5	96.7	98.2	75.90	**3.94**	
003	Glucomet	5.5	29.8	69.4	94.2	43.03	-28.93	
006	**Metformin**	53.8	88.6	89.4	92.5	78.79	**6.83**	
002	Glyformin	14.0	71.9	75.9	77.1	58.54	-13.42	

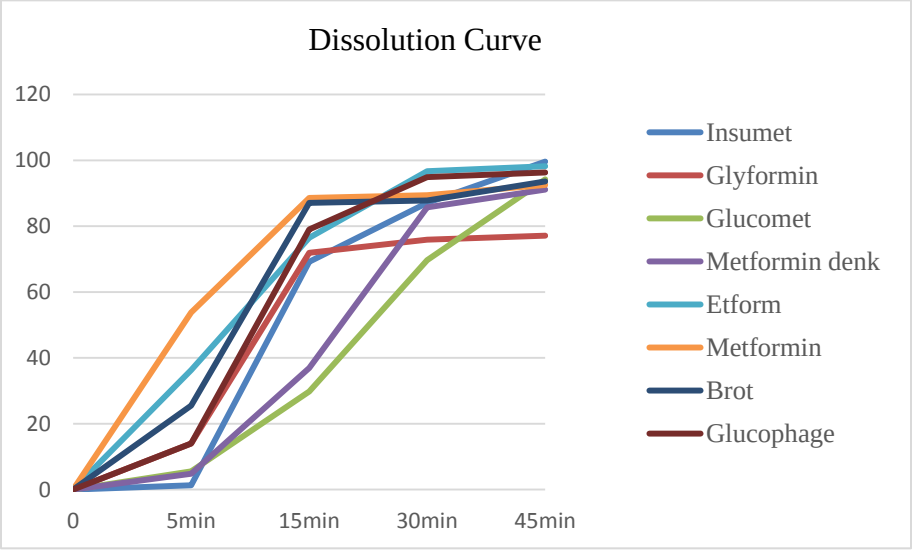

Figure 4:-Dissolution profile of different brands

Table 10:-Grouping Information of dissolution profile result using the Dunnett Method

Brands	Grouping
brand8 (comparator)	A
brand6	A
brand7	A
brand5	A
brand4	A
brand1	A
brand2	A
brand3	A

Means not labeled with the letter A are significantly different from the comparator level mean

To order the dissolution profile of the brands mean dissolution time was calculated and metformin had fast dissolution profile and glucomet require long time to dissociate.

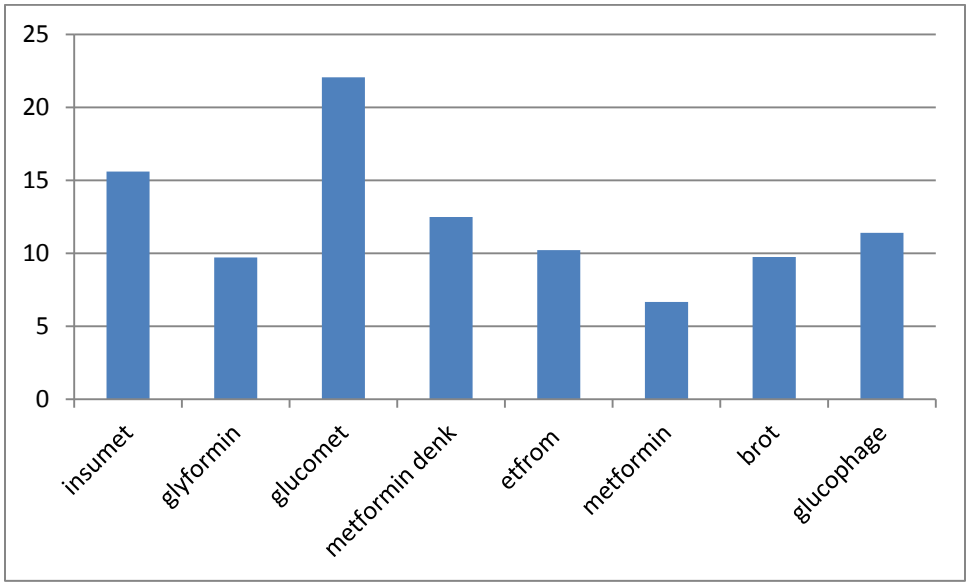

Figure 5:-Mean dissolution time for all brands

5.6. Drug release kinetics

Model dependent approach was used to study the API release model by using kinetDS software and all of the brands follow Michaelis–Menten with Lag model except etform (Table 12 below).

Table 11:- Model dependent approach for studying drug release model

Drug release model	**Brands**							
	001	002	003	004	005	006	007	008
Zero order	R^2=0.7796	R^2=0.5791	R^2=0.9901	R^2=0.6558	R^2=0.7849	R2=0.5964	R2=0.5916	R2=0.6837
1st Order	R^2=0.5809	R^2=0.5441	R^2=0.8528	R^2=0.5598	R^2=0.7144	R2=0.5776	R2=0.5566	R2=0.5953
2nd Order	R2=0.5150	R^2=0.5234	R^2=0.6235	R^2=0.5189	R^2=0.6476	R2=0.5617	R2=0.5333	R2=0.5408
Weibull	R2=0.9282	R^2=0.8031	R^2=0.9906	R^2=0.8646	**R2=0.9887**	R2=0.8656	R2=0.8554	R2=0.9280
Korsmeyer-Peppas	R2=0.8343	R^2=0.8060	R^2=0.9897	R^2=0.8180	R2=0.9303	R2=0.8273	R2=0.8118	R2=0.8483
Michaelis–Menten With Lag	**R^2=0.99998**	**R^2=0.9998**	**R^2=0.9850**	**R^2=0.9941**	R^2=0.9745	**R^2=0.9971**	**R^2=9994**	**R^2=0.9988**

6. Discussion

6.1. Physicochemical property

Weight variation test is performed to check that the sampled tablets have a uniform content. So, to ensure the consistency of dosage units, a weight variation test is necessary. So that batch should have drug substance contained within a narrow range around the label claim. Dosage units are defined as dosage forms containing a single dose or a part of a dose of drug substance in each unit.

According to the speci-fication described in USP-2015, the test for weight variation where the strength is >324 mg, the tablet passes the test if not more than two of the individual weights deviate from the average weight by ±5% and none deviated by ±10%. According to this study etform and brot have the smallest and highest mean weight respectively and only insumet was failed the weight variation test by deviating by more than 10%. Weight variation test failure of insumet may cause the unavailability of the necessary amount of active pharmaceutical ingredient required for therapeutic effect in the unit dose. This might intern leads to a reduction in the therapeutic activity of the drug and cause a reduced patient outcome. Furthermore, the statistical analysis conducted using one-way ANOVA at 95% confidence interval shows that there were significant differences ($P< 0.001$) between the sample mean weights of all brands. In addition to one way ANOVA Dunnett test is also performed at 95% confidence interval to check the source of difference between the comparator drug Glucophage and other brands and revealed that all of the brands have different mean tablet weights when compared with comparator drug Glucophage. This weight variation might be due to different types of excipients like diluent, disintegrant, lubricant, and glidants used during the manufacturing process. This statistical mean weight difference between the comparator and all of the brands might also be due to differences in the manufacturing process. The study conduct in India on four different brands of metformin hydrochloride reported that the entire tablets were passed the test for weight variation and friability [25]. A similar study in Sudan also reported that all of the tablets were passed the test for weight variation and friability [29]. From eight different brands of metformin hydrochloride included in the study in Nigeria, all of the brands were also passed the test for weight variation and one of the brands was failed to pass the test for friability test [16]. A similar report was also

reported from India in 2012 and according to this report from nine different brands included in the study all of them were passed the non-official test for weight variation and friability [24]. However, according to this study insumet failed the non-official test weight variation. This difference might be due to a difference in the types of brands included in the study. The non-official test failure of insumet might bring dose variation at the single tablet level and the dose variation of this single tablet might bring clinical failure on the patients and this failure might be due to manufacturing process-related problems occurred during manufacturing.

6.2. Assay

According to this study, all except glyformin meet the specification for percentage purity as specified on US Pharmacopeia 2015 (95-105%). The assay test is an important test used to quantify the amount of active ingredients present in the product and the amount of active ingredient present in one product affects the quality of the product and will have an impact on the therapeutic effect. The product which does not have the required active pharmaceutical ingredient does not produce the required therapeutic effect and might leads to treatment failure, morbidity, and mortality to patients. This finding was similar to the report from Nigeria [16], but different from Tigray [31], Sri Lanka [34] and India [35] in which all of the tested drugs were passed the assay test. This difference might be due to the difference in the drug regulation system of different countries and the difference in types of brands included in the study.

6.3. Dissolution profile

While the ultimate objective of dissolution testing is to ensure adequate and reproducible bioavailability, the objective of the dissolution tests prescribed in the individual monographs of The International Pharmacopoeia is to obtain information about the drug-release characteristics of a particular formulation or batch of a product under standardized test conditions. Compliance with the test provides an assurance that most of the active pharmaceutical ingredient will be dissolved in an aqueous medium within a reasonable amount of time when the preparation is subject to a mild agitation. Compliance with the dissolution test does not by itself guarantee bioavailability. Dissolution is a test used throughout the life cycle of a pharmaceutical product to evaluate the rate of release of a drug substance from the dosage form. Generally, active pharmaceutical ingredients (API) are mixed with inactive excipient materials and pressed into a tablet or filled into a capsule. In the body, a pharmaceutically active ingredient must be "in

solution" before it can be absorbed by the blood and ultimately carried to the receptor site to render a therapeutic effect. Dissolution is the process by which that active ingredient enters into a solvent to yield a solution. Therefore, if the drug is unable to release the necessary amount at a specified time, the drug is unable to dissolve and it would have lower bioavailability. Bioavailability has a direct relationship with the amount of drugs absorbed into systemic circulation. If the amount of drug absorbed into the systemic circulation is reduced, it would have an ultimate effect on the patient outcome by increasing morbidity and may cause mortality in which interns have an economic impact on the patients and country.

As per USP specification, metformin hydrochloride should release at least 80% of the labeled amount within 30 minutes using USP apparatus II. This study revealed that except glucomet and glyformin the entire tested drug passed the single point dissolution test specification of USP 2015. The failure of the two brands may lead to the lower absorption of the drugs into system circulation. The lower systemic absorption property of the drug positively affects the bioavailability and which intern may reduce the pharmacological activity of the drug. This difference in dissolution property of the drug at a single point of 30 minutes might be due to the difference in excipients used for manufacturing the drugs and also other manufacturing-related processes might also cause this difference. This finding was similar to the report from Addis Ababa [30] and different from that of the report from India [24].

6.4. Comparison of dissolution profile result

The result of one-way ANOVA analysis at 95% confidence interval (CI) at the specified time of 30 minutes found that there were no significant differences in the release pattern of different brands of metformin hydrochloride ($p>0.05$). To support the one-way ANOVA Dunnet test was also performed between the comparator and different brands. From this comparative study, there was no statistically significant difference in the dissolution profile of different brands from the comparator drug Glucophage at the pharmacopeially specified time of 30 minutes. Additionally, to demonstrate the interchangeability of different brands with the comparator Glucophage, the model-independent approach of similarity factor and difference factor was used. For the drugs to be used interchangeably similarity factor (*f2*) should be 50-100 and the difference factor (*f1*) should be 1-15 and according to the above acceptance criteria insumet, metformin denk and brot can be used interchangeably. Besides, etform and metformin have difference factor (*f1*) of < 15

and so can be used interchangeably. To ascertain the interchangeability of those drugs with the comparator Glucophage, the release profile was compared by calculating the difference of dissolution efficiency. The test product is to be used interchangeably with the comparator if the difference between their dissolution efficiency (test drug - comparator) should be within ± 10%. Accordingly, etform and metformin can be used interchangeably with the comparator Glucophage.

To explain the kinetics and mechanism of drug release from the tablets, kinetDS was employed. After fitting the individual value of dissolution profile data, the model that gives the highest correlation coefficient (r^2) value was considered as the best fit for the drug release. It was claimed that the absorption rate of metformin hydrochloride decreases as the dose increases, suggesting some form of saturable absorption or permeability/transit time-limited absorption [4]. Among the different models tested Michaelis–Menten with Lag model was the best fit model for seven of brands and Weibull was the best model for etform. Where dose-dependent data are available for classic synthetic drugs, it is now evident that there are several situations in which plasma concentrations achieve levels appreciably less than expected as the dosage is increased. [36].

7. Conclusion and recommendation

7.1. Conclusion

The physicochemical evaluation showed that all of the brands have complied with USP quality specifications for friability and except insumet the entire brands were passed the test for weight variation. Statistically, all the brands had a significant difference in mean weight from the comparator ($p<0.05$). From the tested brands, seven of them have complied with USP specification for assay and except for glyformin the rest of the brands have statistically similar mean value for assay with the comparator ($p>0.05$). Among eight of metformin hydrochloride evaluated, one-fourth of them did not fulfill single-point specifications for dissolution study and statistically all of the tested brands have similar dissolution profiles with the comparator drug ($p>0.05$). However, from the tested brands, only five of them can be used interchangeably with the comparator drug. The model-dependent approach showed that all most all of the brands follow the Michaelis–Menten model for the release of active pharmaceutical ingredient which is the claimed drug substance release model for metformin hydrochloride.

7.2. Recommendation

EFDA

- ✓ Ethiopian Food and Drug Authority should focus on the post-marketing evaluation of metformin hydrochloride circulating in the drug markets which are originated from different manufacturers.

Jimma University

- ✓ Jimma University Laboratory of Drug Quality should have to perform periodic survey to assess the quality of highly useable drug in Jimma Zone as a whole.

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Annex 1

Sample collection form

Code: _____

1. Name of location/place where sample was taken:----------
2. Address (with telephone and fax number, if applicable):----------
3. Date of sampling: ----------
4. Name of people who took samples:

   1:----------
5. Product name of the sample:----------
6. Name of (active) starting material (INN, generic or scientific name) with dosage strength:----------
7. Dosage form (tablet, capsule, etc.): ----------
8. Batch number: ----------
9. Date of manufacture:---------- Expiry date:----------
10. Registration or license number (if applicable): ----------
11. Name of the manufacturer:----------
12. Number of sample unit taken (tablet, capsule, etc.: at least 20 but not more than 30 units):----------
13. Brief physical/visual description of sample:----------

Signature of person taking samples

1:----------

Annex 2

All finished drug products should be identified by labelling, as required by the national legislation, bearing at least the following information.

WHO Requirements for labelling	Brands								
	001	002	003	004	005	006	007	008	009
Batch number assigned by manufacturer	✓	✓	✓	✓	✓	✓	✓	✓	✓
Expiry date	✓	✓	✓	✓	✓	✓	✓	✓	✓
Special storage condition	✓	✓	✓	✓	✓	✓	✓	✓	✓
Direction for use, warning and precaution	✓	✓	✓	✓	✓	✓	✓	✓	✓
Name and address of manufacturer	✓	✓	✓	✓	✓	✓	✓	✓	✓
Name of drug product	✓	✓	✓	✓	✓	✓	✓	✓	✓
Active ingredient name	✓	✓	✓	✓	✓	✓	✓	✓	✓