COMPARATIVE *IN-VITRO* EQUIVALENCE STUDIES OF SOME LOCALLY MANUFACTURED AND IMPORTED GENERIC BCS CLASS III DRUG PRODUCTS AGAINST THEIR COMPARATOR COUNTER PRODUCTS

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Comparative *in-vitro* equivalence studies of some locally manufactured and imported generic BCS Class III drug products against their comparator counter products

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This is to certify that the thesis prepared by Tsegaye Nigussie, entitled: Comparative in-vitro equivalence studies of some locally manufactured and imported generic BCS Class III drug products against their comparator counter products, and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmaceutics, complies with University regulations and meets the accepted standards for originality and quality.

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ABSTRACT

According to the Biopharmaceutical classification system (BCS), active pharmaceutical ingratiate (API) classified into four classes (BCS Class I-IV) based on aqueous solubility and intestinal permeability. BCS Class III medications are highly soluble and poorly permeable, and they are among the drug products that require documented evidence of bioequivalence study. In addition to the permeability problems, there may be differences between generic and innovator products in terms of the type, source, and quantity of ingredients, manufacturing method and process, and machinery used for production, and these factors can bring major differences in dissolution and bioavailability. The objective of this study was therefore to compare the *in vitro* equivalence of four locally manufactured and imported BCS Class III drug products, namely, Metoclopramide hydrochloride 10 mg tablets, Cloxacillin sodium 500 mg capsules, Metformin hydrochloride 500 mg tablets, and Enalapril maleate 5 mg tablets against their comparator counter products (MTF_3, CLX_3, MCP3, and ENA3), respectively. The selected drug products and their comparator counter products were purchased from retail pharmacies found in Addis Ababa, Ethiopia. For each drug product, physical properties such as weight uniformity, hardness, thickness, diameter, and friability were determined using appropriate equipment as per the USP monograph. The assay content and content uniformity for each drug product were also determined as per their respective procedures in the USP monograph and validated methods. *In vitro* dissolution profiles were performed according to monograph methods and validated methods, and *in vitro* drug release tests were also done to assess equivalence of the products investigated. The results of weight variation content uniformity, and assay content indicated that all the investigated drug products were within the pharmacopeia requirements. Similarly, all products investigated complied disintegration time tests (i.e., ≤ 30 min) of immediate-release drug products. Except for two generic products of metformin hydrochloride tablets MTF_2 (62.66%±9.74), and MTF_4 (78.71%±6.86), all the drug products included in this study fulfilled the acceptance criteria for dissolution (i.e., Q ≥ 80% at 30 min). The *in-vitro* equivalence of these products was assessed by statistical, model-independent, and mode-dependent methods. One generic products of metformin hydrochloride (MTF2) and all generic products of metoclopramide hydrochloride showed significant difference ($p < 0.05$) in dissolution profiles with comparator

products. Of the five generic products of metformin hydrochloride film coated tablets [MTF_2 (32.88), MTF_4 (39.80) and MTF_6 (49.94)] and generic products of metoclopramide hydrochloride [MCP1 (37.04) and MCP2 (41.56)] failed to meet the f2 acceptance criteria. This means that these products are not interchangeable. However, cloxacillin sodium capsules and enalapril maleate tablets complied with the f2 > 50 acceptance criteria. In conclusion, this study showed that all the generic products of metformin hydrochloride tablets, metoclopramide hydrochloride tablets, enalapril maleate tablets, and cloxacillin sodium capsules complied the quality specifications of weight uniformity, hardness, friability, disintegration, and assay. Three generic products brands of metformin hydrochloride MTF2, MTF4, and MTF6, and both generic products brands of metoclopramide hydrochloride (MCP1 and MCP2) did not show *in-vitro* equivalence with their comparator products. All generic products of cloxacillin sodium capsules and enalapril maleate tablets included in this study met model independent fitting factor specifications, were statistically insignificant (P>0.05) in dissolution profiles and can be considered equivalent to their comparator counter product.

Key words: BCS Class III drugs, Generic products, Comparator pharmaceutical products, *In-vitro* dissolution, *In-vitro* equivalence, Bioequivalence

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ABBREVIATION /ACRONYMS

API:	Active pharmaceutical ingredient
ANOVA:	Analysis of variance
ACE-Is:	Angiotensin –Converting Enzyme Inhibitors
ARBs:	Angiotensin II receptor Blockers
AHTDs:	Antihypertensive Drugs
AMR:	Antimicrobial Resistance
AUC:	Area under plasma concentration vs time curve
BA	Bioavailability
BE:	Bioequivalence
BCS:	Biopharmaceutical classification system
BP:	British Pharmacopeia
CVDs:	Cardiovascular disease
CTZ:	Chemoreceptive Trigger Zone
CINV:	Chemotherapy-induced nausea and vomiting
CGMP:	Current Good Manufacturing Practice
EMA:	European Medicine Agency
FDA:	Food and Drug Administration
FTIR	Fourier-transform infrared spectroscopy
GS:	Generic substitution
GMP:	Good manufacturing practice
IRP:	Immediate release products
IVIVC:	*In vitro- In vivo* Correlation
$\mathbf{C_{max}}$	Maximum conc.
PK:	Pharmacokinetics
PD:	Pharmacodynamics
QC:	Quality control
RS:	Reference standard
WHO:	World Health Organization
USP:	United States Pharmacopoeia

1. INTRODUCTION

A wide variety of drugs manufactured by different pharmaceutical industries are available on the global market (Barends *et al*., 2005, Karalis *et al*., 2010, Reddy *et al*., 2014). These products can be administered to the body in a variety of ways, including oral, intravenous, topical, rectal, and vaginal administration, among others. The oral route of administration as solid dosage a form is the most often used and preferred mode of drug delivery. This route is preferred over other delivery systems due to its high patient compliance, easy to provide and plug (economical), accurate dose, and has good physical and chemical stability. Due to these reasons, this delivery system is widely prescribed in clinical practice. However, due to absorption process, this delivery system poses bioavailability problems (Fernandes *et al*., 2006, Ferraz *et al*., 2007, Chen *et al*., 2011, Kassaye and Genete, 2013).

Oral drug products are marketed worldwide as brand names or generics. Typically, when a new drug is developed, the funding company usually obtains a drug patent. A patent is granted because pharmaceutical companies invest a lot of money and resources in developing and discovering a new drug substance, and they are granted exclusive rights to manufacture and distribute the medicine for a set period of time (DiMasi *et al*., 1991, DiMasi *et al*., 2003, Bunnage, 2011, Taylor, 2015). Because of this, they are expensive and not easily accessible, especially in developing countries (Reddy *et al*., 2014).

When an innovator drug product's legal rights expire, it eligible to be made into generic product (Fahmy and Abu-Gharbieh, 2014, Prithi *et al*., 2018, Garza-Ocañas *et al*., 2019). The generic product is a copy of the innovator product containing the same amount of the active ingredient, in the same dosage form and route of administration, and meets the standard of the strength, purity, quality, and identity standards as the innovator drug product (Akpabio *et al*., 2011, Kassaye and Genete, 2013, Rodriguez *et al*., 2015). In developing countries, expenditure on medicines accounts for a significant proportion of health costs, and access to treatment is dependent on the availability of affordable generic medicines (Taylor *et al*., 2001, Rodriguez *et al*., 2015). Generic drug products are an important asset to national projects since they are less expensive (Genazzani and Pattarino, 2008, Hanafy, 2016, Nigatu *et al*., 2019).

The World Health Organization (WHO) encourages the use of generic medicines to make more accessible, particularly to developing countries, and/or interchangeable for cost-effective treatment. The substitution of generic products is considered if they are bioequivalent with their innovator or branded product (Food and Drug Administration., 1997). Ethiopian industrial policy focuses on promoting self-reliance through the local production of generic drugs to ensure affordable domestic availability of essential medicines (Gebre-Mariam *et al*., 2016). As a result, there are various generic products in the market. However, pharmaceutical quality is a worldwide challenge; substandard and counterfeit medicines are being identified (Saurabh *et al*., 2008, Bano *et al*., 2011, Sahle *et al*., 2012). The bioequivalence of various generic products on the market is critical to ensuring that generic products and innovator product can be used interchangeably (Fahmy and Abu-Gharbieh, 2014).

In early 1995, BCS was introduced by Amidon and his colleagues (Amidon *et al*., 1995), active pharmaceutical ingredients can be classified into four based on aqueous solubility and intestinal permeability. The BCS classification system considers three important variables into account when determining the bioavailability of IR solid oral dosage forms: dissolution, solubility, and intestinal permeability (Amidon *et al*., 1995, WHO, 2009, Rawat and Pandey, 2015, Mehta *et al.*, 2017).

According to BCs, drugs are classified as follows:

Class 1: High Solubility – High Permeability
Class 2: Low Solubility – High Permeability
Class 3: High Solubility - Low Permeability
Class 4: Low Solubility – Low Permeability

The solubility classification of an immediate release product is based on its highest dose strength. a drugs substance can be considered very soluble if its highest strength is soluble in 250 mL or less of aqueous media with a pH range of 1.0-7.5 (FDA, 2000, Polli *et al*., 2002). The classification of drugs based on permeability is directly related to the degree of a drug substance's intestinal absorption in humans or indirectly to the assessment of the rate of mass transfer across the human intestinal membrane (FDA, 2000, Benet *et al*., 2008). The BCS is important not only for granting waivers of in-vivo bioequivalence studies, but also for the discovery and prediction of new drug

formulation development (Amidon *et al*., 1995, Ku, 2008). However, BCS is only applicable to active pharmaceutical ingredients (API) which are absorbed from the small intestine (WHO, 2009).

BCS Class III drug products are highly soluble and poorly permeable (Barends *et al*., 2005, Parr *et al*., 2016). In addition to their identified permeability problems, products may differ in terms of composition (type, source, and quantity of chemicals), manufacturing process, and machinery used for production (Parr *et al*., 2016, Metry and Polli, 2022). According to FDA, BCS guidance in 2000 authorizes biowaivers for BCS Class I medications based on *in vitro* dissolution of the drug product for immediate-release (IR) solid oral dosage form (FDA, 2000, Shah and Amidon, 2014, Tsume *et al*., 2014, Cook, 2015). However, FDA in 2017 and EMA in 2020 were extended BCS based biowaiver for Class III drugs (FDA, 2017, EMA, 2020).

Due to their low permeability, BCS class III medication must contain excipients that are qualitatively similar and quantitatively same to the reference product for biowaiver to be scientifically justified, as excipients can have a significant impact on Class III drug absorption (FDA, 2017, EMA, 2020). These factors can ultimately bring major differences in the rate of dissolution and bioavailability of generic products belongs to this class (Farah *et al*., 2020). It has been demonstrated that including osmotically active excipients in the formulation affects the bioavailability of this class of medicines. For example, Sorbitol reduces ranitidine bioavailability by increasing intestinal fluid volume, gastrointestinal motility, and decreasing ranitidine intestinal transit time. Similarly, mannitol decreases cimetidine bioavailability, and PEG 400 decreases ranitidine absorption (Basit *et al*., 2001, Schulze *et al*., 2003, Ashiru *et al*., 2008). According to limited human data, some excipients may influence the BA of this class of medicines (Basit *et al*., 2001, Ashiru *et al*., 2008). Therefore, BCS Class III drug products are among those medicinal products that are legally required to have documented equivalence data compared to their innovative products.

Hence, the equivalence of generic drug products to their innovator product should be demonstrated prior to market authorization. However, this does not imply that all products necessitate a bioequivalence study. Despite this, such study appears to be missing in resource-constrained countries for a variety of reasons. *In vitro* equivalency testing of generic products against innovator products/comparator counter products could

therefore be critical in guaranteeing generic product quality, safety, efficacy, and interchangeability.

1.1. Physical properties of drug products

1.1.1 Hardness and friability

Hardness measures a tablet's ability to withstand mechanical shocks during handling, manufacturing, packing, transportation, and storage for patient use (USP, 2021). Hardness evaluates the tablet’s strength and resistance to friability. Adequate tablet hardness and friability are necessary for customer acceptance and drug product quality (Getie and Gebre-Mariam, 1998, Dires, 2005, Mosharraf and Islam, 2012, Tuli, 2014). There are several factors that can affect tablet hardness and friability. These include variation in particle size, distribution in granulation mixes, amount and duration of mixing with lubricants and, etc (Faqih, 2007, Allen, 2013, Birhanu, 2013). Large, low-density particles generate softer tablets, while fine, high-density granular particles produce hard tablets. Tablet manufacturing variables such as comprehension force and comprehension rate have a substantial impact on hardness and friability (Lieberman, 1989, Shipar, 2014)

1.1.2. Uniformity of dosage units

Uniformity of dosage units can be demonstrated through one of two methods: weight variation or content uniformity, which is one of the tablet quality control parameters. The weight variation test is a process test parameter that assures the dosage unit's consistency during compression. The weight variation test is applied when the product contains 50mg or 50% of an active ingredients or more by weight of the dosage form unit, and otherwise content uniformity test is applied (USP, 2021f). Tablet weight should be frequently tested to guarantee proper medication content and batch consistency in the appropriate size range (Banker, 1974, Odeniyi *et al*., 2003, Abatea *et al*., 2020). High variability of dosage units may cause ineffective therapeutic drug levels and toxicity (Remington, 2000, Akarawut *et al*., 2002, Rahman *et al*., 2019). According to some official monograph weight variation test should be within acceptance limit as illustrated in Table 1.

Table 1: Some official monographs set weight variation limits for tablets.

Limit (%)	**Ph. Int/BP**	**USP**
±10	80mg or less	130mg or less
±7.5	More than 80mg or less than 250mg	130mg to 324mg
±5	250mg or more	More than 324mg

BP: British Pharmacopeia; Ph. Int; International Pharmacopeia; USP; United States Pharmacopeia

1.1.3. Disintegration

Drug particles gain increased surface area when the tablets breakdown, allowing for localized activity in the gastrointestinal tract. The disintegration process must break down a tablet into smaller particles or granules. Complete tablet disintegration is the state in which any residue of the tablet is a soft mass having no palpably firm core (Uddin *et al*., 2011, Rajbhar, 2020). The disintegration test is more applicable when a relationship to solubility has been established and is a limiting factor for dissolution, especially for poorly water soluble drugs. The tablets must be disintegrated in order to overcome the cohesive force brought into the mass by compression and any binders present, which is achieved by introducing disintegrants. Disintegration can be affected by formulation variables like properties of excipient and its conc. as well as processing variables. The type and amount of disintegrants employed in formulation may contribute to the rapid disintegration of products (Esezobo and Pilpel, 1977, Lieberman, 1989, Kassahun, 2019, Nigatu *et al*., 2019).

1.1.4. Assay of the active ingredients

Assay is an important control parameter used to determine the strength or the amount of the active ingredient in the dosage form. Significant variation in the content of dosage unite leads to ineffective therapeutic drug levels or toxicity (Akarawut *et al*., 2002, Rahman et al., 2019). Quantitative determination of the content of dosage is preferentially determined by physicochemical methods. There are various analytical techniques used for the evaluation of drugs in raw materials and dosage. This technique

must provide the specificity, precision, accuracy, sensitivity and cost effectiveness of the specific ingredients of interest in the dosage form (Wilson *et al*., 2002, Lee *et al*., 2003, Dange *et al*., 2017, Abdelrahman *et al*., 2020).

1.1.5. Dissolution and dissolution tests

1.1.5.1 Dissolution

Dissolution is the process by which solid substances dissolved when they enter a solvent (Fig. 1). To bring tablets into solution, the tablets should disintegrate into smaller particles and provide a greater surface area to the dissolving media (USP, 2021c)

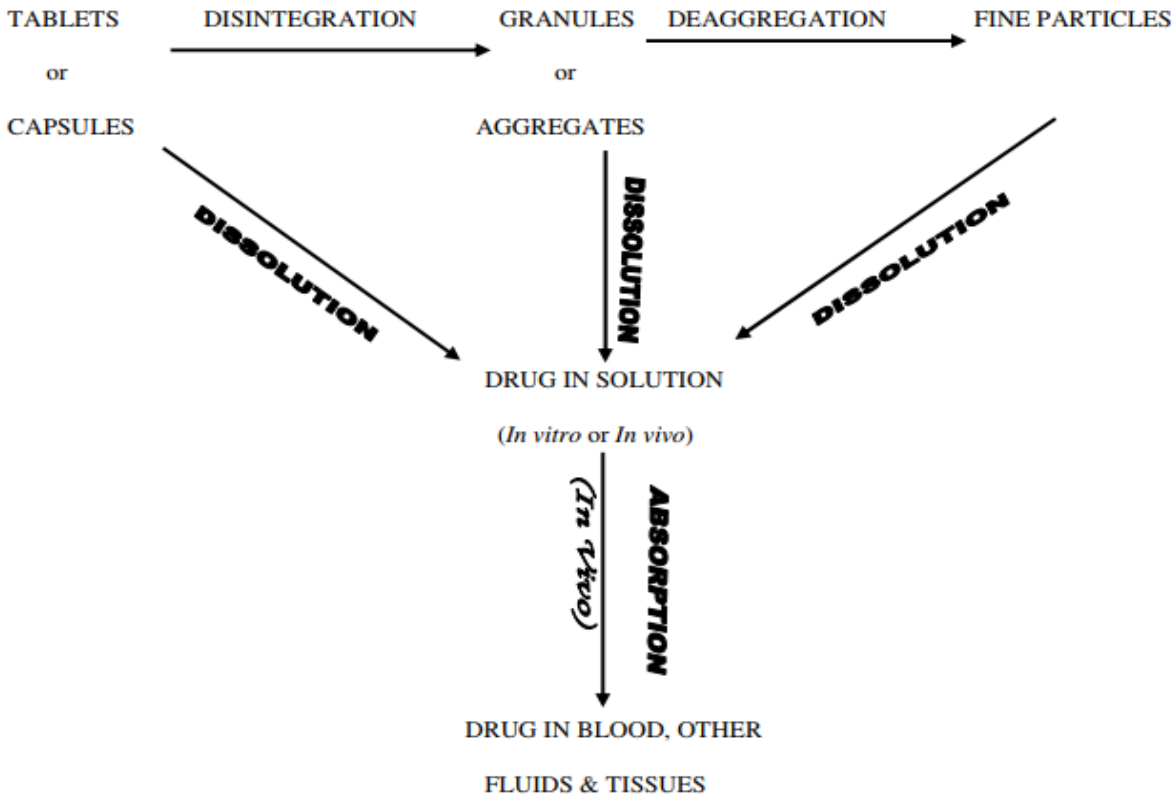

Figure 1: Schematic illustration of solid dosage form dissolution process (Aulton, 2004).

The dissolution test serves as a surrogate marker for the bioequivalence test. *In vitro* dissolution testing of solid oral dosage forms is a critical method in drug development for selecting and optimising formulations, studying drug release mechanisms, ensuring batch-to-batch consistency, monitoring stability, and establishing bioequivalence (*in-vivo* performance) (FDA, 1995, FDA, 1997, Shah, 2001, Uddin *et al*., 2011). Because drug

dissolution is a crucial step in the absorption process, the availability of active ingredients in dosage form for absorption is determined by the drug's dissolution in GI fluids. Under specified conditions, the rate of dissolution is the amount of drug substance that dissolves per unit of time (Shah, 2001, Juveria *et al*., 2018, Rajbhar, 2020).

1.1.5.2 Factors affecting tablet dissolution.

Appropriate and rapid *in-vitro* release specifications require high drug solubility and high permeability. Drug dissolution rate is affected by formulation and processing variables. Formulation variables, include the nature of diluents, mixing process, granule size and distribution, nature and amount of disintegrants, nature and conc. of lubricants, presence and absence of surface active agents, physical properties of the drugs, flow of granules through hoper and dies. Processing variables that affect the rate of dissolution, such as compression force, granulation methods etc. (FDA, 1997). The effect of processing variables on drug *in-vitro* dissolution characteristics demonstrated that a change in the manufacturing process could result in significantly different dissolution profiles for the same formulation *(*Santos *et al.*, 2010, Al Ameri *et al.*, 2012, Singhvi *et al*., 2014). In addition, the granulation method influences the dissolution rate, and formulation variables such as polymer type, polymer grade, drug-polymer ratio, drug stability, drug, and polymer particle size all have varying degrees of influence on drug release rate (Adeleye *et al*., 2014).

The dissolution rate of medicinal products is influenced by a variety of intrinsic characteristics. The drug's wetting, swelling, solubility, and diffusion from a solid pharmaceutical dosage form effect tablet disintegration and content exposure to the medium, allowing drug release. The surface area of a solid determines the fluid flow parameters during the dissolution process. Fluid flow is determined by particle size, shape, and density. Dissolution rate often increases as particle size decreases. The hydrophobic characteristics of the liquid-solid interface, as well as mutual interference in the specific motion, have significant effects on the dissolution of smaller particles, which may result in a slower dissolution rate. Moisture content in granulation techniques (Bansal *et al*., 1994) and the type and conc. of lubricant significantly affects the rate of drug release (Gordon, 1994).

The conc. and prolonged mixing time with hydrophobic lubricants leads to a reduced dissolution rate due to the formation of a hydrophobic coating on the surface of granules. This reduces water penetration and thus reduces the rate of dissolution, while water-soluble lubricants such as sodium lauryl sulphate significantly increase the rate of dissolution (Levy and Gumtow, 1963, Shah and Mlodozeniec, 1977).

Dissolution rate are also affected by the type and conc. of binder used in the formulation. In general, increasing the binder conc. resulted in a decrease in dissolution rates of the tablets (Chalmers and Elworthy, 1976, Esezobo and Pilpel, 1976). However, if the amount of binder is constant, granule size does not significantly affect dissolution time(Chalmers and Elworthy, 1976, Agrawalx and Prakasam, 1988) and also type and mode of incorporation of disintegrants have a pronounced effect on dissolution rates (Levy, 1963, Underwood and Cadwallader, 1972, Khan and Rooke, 1976). Type, conc., and mode of incorporation significantly influence disintegration and dissolution time but crushing strength is not affected by the mode of incorporation and conc. of disintegrants (Khattab *et al*., 1993).

In scale up or process transfer, when the tableting process transferred from one tablet press to another, the tablet hardness, friability, disintegration, dissolution and other properties can be changed. Tablet qualities have been demonstrated to be affected by granulation properties, drying times, drying modes, compressor speeds, and other changes that occur during scale-up (Armstrong and Palfrey, 1987, Anthony Armstrong and Palfrey, 1989, Cook and Summers, 1990).

1.1.5.3 Dissolution test as quality control parameter

The rate at which drug release is determine by the dissolution rate. In the manufacturing of drug products, several formulation and possessing factors are involved, which have significant effects on the dissolution and release characteristics of the products. These parameters may differ in condition and situation from manufacturer to manufacturer, resulting in differences in drugs product release and dissolution behaviours (Gordon, 1994, Chen *et al*., 2002, Leane *et al*., 2003, FDA, 2017, Zaborenko *et al*., 2019).

Dissolution testing is an important parameter in the pharmaceutical industry for drug development and quality control (Anand *et al*., 2011, Maddineni *et al*., 2012). It also serves to grant biowaivers for lesser strengths of a given manufacturer's product after the

greater strength has been approved based on the appropriate bioavailability/ bioequivalence test procedure (Shah *et al*., 1989, Siewert *et al*., 2003).

In vitro dissolution tests are applied to ensure quality consistency across lots and these values are used to determine the change of components and composition, manufacturing site, manufacturing process, and equipment used (FDA, 1997). *In vitro* dissolution test is used to ensure product consistency through profile comparisons between products before and after modification using appropriate tests and profile comparisons (Van Buskirk *et al*., 1997).

1.2. Bioavailability and Bioequivalence

1.2.1. Bioavailability

Bioavailability is defined as the rate and extent to which an active ingredient or active moiety from a dosage form is absorbed and becomes available at the site of action, and it can be determined by measuring the area under the plasma conc. time curve (AUC) and the maximum conc.(C_{max}) (Chow and Liu, 2008, Chow, 2014, Currie, 2018). Bioavailability refers to how quickly and how much of a medicine appears in the blood when a specific dose is administered. The bioavailability of a drug product frequently determines its therapeutic efficacy since it influences the onset, intensity, and duration of therapeutic response (Banakar, 1991).

1.2.2 Bioequivalence

Bioequivalence is defined as the absence of a significant difference in the rate and extent of absorption of API or its metabolite(s) at the site of action when administered the same molar dose under similar conditions (Chow and Liu, 2008).

Bioequivalence determination is crucial for therapeutic equivalence and is the most difficult component of generic drug product development. There is universal agreement that bioequivalence testing should use the most accurate, sensitive, and reproducible approach available for the therapeutic product under investigation. The following methods have been recommended for bioequivalence studies: (a) comparative pharmacokinetic studies in humans, (b) comparative pharmacodynamics studies in humans, (c) comparative clinical trials, and (d) comparative *in vitro* dissolution tests (Chen *et al*., 2011).

Comparative pharmacokinetic approaches are commonly used for bioequivalence studies to evaluate drug conc. in blood/plasma vs time profiles. Pharmacodynamics and comparative clinical equivalence studies are used only when appropriate pharmacokinetic studies are not feasible (e.g., bio-analytical methods are either unavailable or insufficiently sensitive for measurements of parent drugs or their metabolites inaccessible in biological fluids), and drug products that act on cutaneous structures may not be extrapolated from plasma conc. because (a) topically applied doses are less defined, and (b) plasma conc. do not reflect at the site of action, and (c) topical formulation bioavailability is known to be affected by multiple factors, including cutaneous attributes, active ingredient solubility and conc., vehicle characteristics, and disease states (Chen *et al*., 2011). Comparative *in vitro* dissolution studies may be employed for particular drugs that meet the BCS based biowaiver requirements (Chen *et al*., 2011, FDA, 2003).

1.3 Assessment of bioavailability

Evaluating a drug's bioavailability in any dosage form provides a direct evidence of the dosage form's efficiency in fulfilling its intended therapeutic function or a method of forecasting a drug's clinical efficacy. The bioavailability of active ingredients incorporated into pharmaceutical dosage form is a primary goal of formulation design and is critical to the clinical efficacy of the medication. One way of demonstrating a drug's therapeutic efficacy is by determining its bioavailability by assessing the rate and extent of absorption (Chereson, 2009).

Drug manufacturers conduct bioavailability studies to guarantee that a given drug product is delivering the drug to the site of action in the appropriate conc. at the desired time. Bioavailability studies are also conducted to compare the availability of a drug in different dosage form or the same dosage form manufactured by different manufacturers (McGilveray, 1991). Some bioavailability studies revealed that tablets with the same active ingredients and drug content did not provide comparable therapeutic responses. Excipients used in dosage form, physical properties of the drugs, and the processing variables all contribute to differences in performance and therapeutic effects (Rani *et al*., 2012). Bioavailability or Bioequivalence studies includes both *in vivo* and *in vivo* studies (Chereson, 1996).

1.3.1 *In-vivo* methods

In vivo bioavailability study in humans is considered to be the "gold standard" for determining product bioequivalence (BE) of immediate-release (IR) solid oral dosage forms (Polli, 2008). However, such studies are complex, expensive, time-consuming, and necessitate a sensitive and quantitative measurement of the desired response. In addition, responses are often highly variable, requiring large test populations and performed in normal adult populations under standardized conditions (McGilveray, 1991). The critically ill patient shall not be included under *in vivo* bioavailability evaluation unless the attending physician determines potential benefit to the patient (FDA, 2020). Because of these limitations, alternative *in vitro* approaches to predict therapeutic equivalency of drug products were developed. *In vitro* studies are advantageous because they (a) lower expenses, (b) more directly measure of drug performance, and (c) provide benefits in terms of ethical consideration (Löbenberg and Amidon, 2000, Gupta *et al*., 2006, Polli, 2008).

1.3.2 *In-vitro* methods

In vitro dissolution may serve as a surrogate for *in vivo* bioequivalence studies under certain condition. Bioequivalence studies are performed on the highest dose strength, and the sponsor may request a biowaiver for lower strength. In this case, the drugs is in the same dosage form but at different strengths, and its active and inactive ingredients are identical to the strength at which the bioequivalence study was conducted (usually the highest strength). *In vivo* bioequivalence studies of lower strengths may be waived based on *in vitro* dissolution studies (Verbeeck and Musuamba, 2012, WHO, 2014, FDA, 2017).

Regulatory standards for BCS-based biowaivers may differ between countries and/or regions. According to the FDA, the necessity for *in vivo* bioequivalence studies is waived for BCS Class 1 and III drugs that are very rapidly dissolving (85% within 15 minutes) or Class I drugs that are rapidly dissolving (85% within 30 minutes) (FDA, 2017). In addition, European Medicine Agency (EMA) guidelines include BCS Class 3 drugs (EMA, 2010). Similarly, the WHO proposal for a BCS-based bio-waiver is similar to the EMA proposal for BCS Class 1 and 3 drugs. Additional bio-waiver for BCS Class 2 weak acids if (a) the API has a dose/solubility ratio of 250 mL or less at pH 6.8, (b) the

multisource product dissolves rapidly (85% in 30 minutes at pH 6.8), and (c) the dissolution profile is similar to that of the comparator product under defined dissolution conditions at pH 1.2, 4.5, and 6.8 (WHO, 2006).

For many years, pharmaceutical scientists have attempted to establish a link between the physicochemical properties of a dosage form and the biological availability of a drug from that dosage form. The term "*in vivo-in vitro* correlation" is commonly used to describe the relationship between *in vitro* drug dissolution and *in vivo* performance. It is believed that if such a correlation can be established, *in vitro* data can be used to predict a drug's *in vivo* bioavailability. This significantly decreases or completely eliminates the necessity for bioavailability testing. When considering the expense and period of time required for bioavailability studies, as well as the safety concerns associated in providing drugs to healthy volunteers or patients, the necessity of *in vitro* dissolution studies becomes evident. It would certainly be preferable if *in vitro* dissolution test could substitute *in vivo* bioavailability studies. This would be feasible if *in vitro* tests were able to accurately predict the absorption of drugs and reflect a drug's *in vivo* performance in humans (Banakar, 1991).

1. 3.2.1 Disintegration test

The disintegration test is the first attempt to establish a drug bioavailability indicator, focusing on disintegration as the most important quality parameters. In 1950, the United States Pharmacopoeia (USP) published the first official disintegration test. While it is true that a solid dosage form must disintegrate before dissolution and absorption can occur, meeting the disintegration test standards only ensures that the dosage form (tablet) will disintegrate into smaller particles within the time frame specified. The rate of dissolution is insufficient to provide the required conc. of drugs in the systemic circulation. Therefore, tablet disintegration test is important for quality control in production and drug bioavailability (Hartley *et al.*, 1991, Al-Gousous and Langguth, 2015).

1. 3.2.2 Dissolution tests

Dissolution is the process of determining the amount of substance dissolves per unit of time under specified conditions such as the liquid/solid interface, solvent composition, and temperature. Dissolution is an *in vitro* test used in the pharmaceutical industry to

evaluate bioavailability and product quality (Ferraz *et al*., 2007, Menegola *et al*., 2007). Solid oral dosage form dissolution testing is used in drug development to select and optimise formulations, evaluate drug release processes, guarantee batch-to-batch consistency, monitor stability, and ensure bioequivalence (Cheng *et al*., 2004, Menegola *et al.*, 2007, Nainar *et al*., 2012). Therefore, the significance of the dissolution profile in determining bioequivalence must be emphasized (Hailu *et al*., 2013). Instead of time-consuming and expensive *in vivo* bioequivalence studies, the dissolution test could be applied as a surrogate basis for evaluating the equivalence of two drug products (WHO, 2009).

1. 3.2.2.1 Official dissolution tests

Dissolution testing is a key approach of guaranteeing drug product quality. Formulation factors such as varying particle sizes and excessive amounts of lubricants or coatings generate differences between products. These factors are related to dissolving testing. Therefore, dissolution testing is very important in determining batch-to-batch and intra-batch pharmaceutical equivalence of drug formulations. Therefore, *in vitro* dissolution testing can be a quick way to determine *in vivo* performance (Rabbani *et al*., 1996). According to USP, there are seven possible dissolution apparatuses that can be employed to establish a suitable dissolution method based on the characteristics of the drug product. However, two compendial dissolution procedures, apparatus 1 (rotatory basket) and apparatus 2 (rotating paddle) are frequently employed for evaluation IR drug products (USP, 2021c, USP, 2021d).

1. 3.2.2.2 Limitations of dissolution tests

Dissolution testing limitations could present challenges to consider, making it difficult to correlate with *in vivo* performance. The first is associated with instrumental variability and a lack of standard methods. The method used for dissolution testing, changes in devices, and dissolution media can all have significant effects on dissolution rate (Banakar, 1999, Chereson, 2009).

1.3.3 *In-vitro- In-vivo* Correlation (IVIVC)

In vitro-in vivo correlation (IVIVC) is defined by the FDA as a "predictive mathematical model that describes the relationship between an *in vitro* dissolution profile of a dosage

form and *in vivo* performance" (Rudman *et al*., 1996). IVIVC is critical in the product development and optimisation process, which is costly, and time-consuming (Murthy *et al*., 2007, Sakore and Chakraborty, 2011, Chavda *et al*., 2016).

IVIVC not only save money and time on *in vivo* studies, but it is also suggested for regulatory purposes. The successful correlation can be used as a surrogate for bioequivalence and biowaiver studies (Uppoor, 2001). IVIVC is also competent for rationalizing formulation therapeutically relevant drug release requirements (Bendas, 2009). However, developing an IVIVC for an oral immediate release dosage form can be difficult because physiological digestive processes (such as gastric emptying, hydrodynamics, and pH fluctuations along the GIT, etc.) have a significant impact on the rate of dissolution of the immediate release dosage form as well as absorption parameters. IVIVCs for immediate release dosage forms can be achieved when the *in vivo* drug dissolution process is the rate-limiting step in the absorption process (Emami, 2006, Ghosh and Choudhury, 2009).

1.4. BCS Class III drug products investigated.

1.4.1 Metformin hydrochloride

Metformin (MET) is a drug that belongs to the 'biguanide' class and is used to treat type II diabetes (Ramalingam *et al.*, 2014, Varillas *et al*., 2018, Prithi *et al.*, 2018). Metformin hydrochloride (MTF), chemically as N, N-dimethyl imido dicarbionimidic diamide hydrochloride (1, 1- diamethylbiguanide hydrochloride), has a molecular mass of 165.6 g/mol and a melting point range of 223-226 ^{o}C. MET is readily soluble in water (0.1 g/mL), mildly soluble in alcohol, and practically insoluble in acetone and methylene chloride (Najib *et al*., 2002, da Trindade *et al*., 2018).

MET has a logarithmic partition coefficient (P) of -0.5 and a dissociation constant (pKa) of 12.4 (Kasim *et al.*, 2004, Mady *et al*., 2019). Metformin hydrochloride reduces intestinal glucose absorption, suppresses hepatic gluconeogenesis and enhance peripheral tissue insulin sensitivity by increasing the uptake of glucose and utilisation (Poretsky, 2010). Metformin is poorly absorbed from the GIT in humans following oral administration, with a 40-60% absolute bioavailability and shows a short elimination half-life (1.5–1.6 h) (Martínez-Gómez *et al.*, 2017). Therefore, metformin belongs to the drugs of BCS class III. Using the rationale of the BCS, it can be claimed that bio-

waivers for metformin immediate-release formulations based on *in vitro* dissolution profiles (Cheng *et al*., 2004, WHO, 2009, Mokhtare *et al*., 2017, Varillas *et al*., 2018).

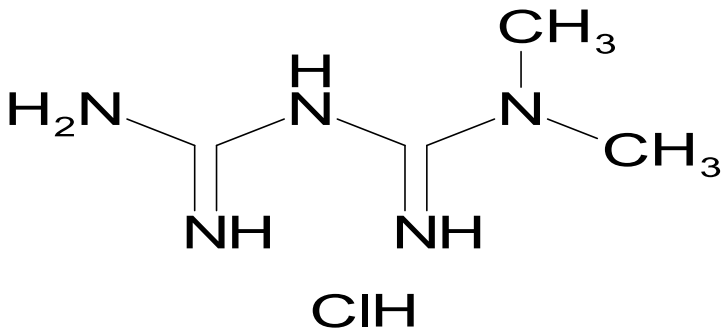

Figure 2: Chemical structure of metformin hydrochloride

1.4.2 Cloxacillin sodium

Cloxacillin (CLX) is semi-synthetic beta-lactamase-resistant penicillin antibiotics (Fig. 3) with antibacterial activity. Cloxacillin binds to and inactivates penicillin-binding proteins (PBPs) present on the bacterial cell wall's inner membrane. This prevents cross-linking peptidoglycan, an essential component of the bacterial cell wall. This causes the bacterial cell wall to disruption, resulting in bacterial cell lysis (Katzung, 2011). Cloxacillin Sodium, chemically known as monosodium (2S, 5R, 6R)-6-[3-(o-chlorophenyl) -5-methyl-4-isoxazolecarboxyamido]-3, 3 – dimethyl – 7 – oxo – 4 – thia -1- azabicyclo [3.2.0] heptane-2-carboxylate monohydrate. Its molecular weight is 475.88 g/mol and one part of cloxacillin soluble in 2.5 parts water, and a 10% aqueous solution has a pH of 5 to 7 (USP, 2021g).

It is white, hygroscopic, crystalline power, and soluble in methanol, and freely soluble in water (EuropeanPharmacopoeia, 2013). Cloxacillin is partially absorbed from the GIT following a 500 mg oral dose the maximum plasma concentration in fasted subjects reaches 7-15 g/ml after 1-2 hours. With intramuscular injection, absorption is more complete, and a maximum plasma concentration of roughly 15 g/ml is within 30 min after a 500 mg dose and their plasma half-life is 0.5 to 1h (Sweetman, 2005).

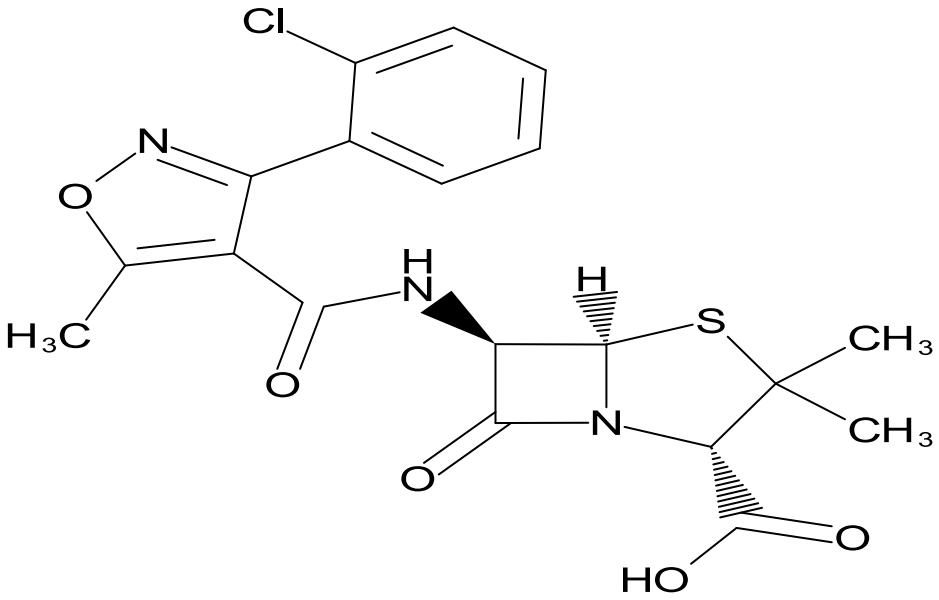

Figure 3: Chemical structure of cloxacillin

1.4.3. Metoclopramide hydrochloride

Metoclopramide (MCP) is antiemetic and gastroprokinetic agents (MCP). Chemically, [4-amino-5-chloro-N-(2-diethyl amino ethyl-1-methoxybezamide) hydrochloride monohydrate] (Fig. 4) is a dopamine receptor antagonist with a molecular weight of 299.8g/mol & melting point 147 ^{o}C (Bateman et al., 1980, Rao and Camilleri, 2010). Metoclopramide is freely soluble in water (200mg/l) at 25^{o}C. Metoclopramide is readily absorbed from the GI tract after oral administration. Their absolute oral bioavailability is approximately 80% and reaches peak plasma conc. in 1 to 2 hr. It has a half-life of 4 to 6 hr and is mainly excreted in the urine (Beckett *et al*., 1987).

Metoclopramide inhibits dopamine D2 receptors in the medulla's chemoreceptor trigger zone (CTZ), and oral tablet formulation are effective treatments for nausea and vomiting (Mukhopadhyay *et al*., 2018, Adhikari *et al*., 2019). Metoclopramide hydrochloride is classified as BCS Class III since it is "highly soluble" in water at room temp (Stosik *et al*., 2008, Varillas *et al*., 2018).

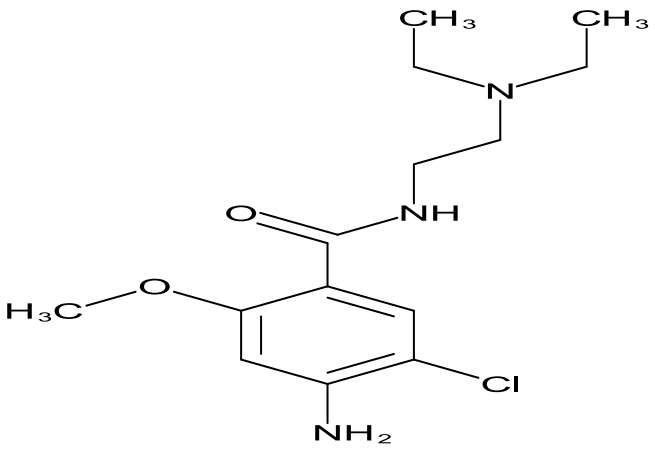

Figure 4: Chemical structure of metoclopramide hydrochloride

1.4.4. Enalapril maleate

Enalapril (ENP) maleate is a prodrug that, after biotransformation to its active form (enalaprilat), competitively binds to and inhibits angiotensin converting , hence preventing angiotensin I to II conversion. Therefore, prevents the powerful vasoconstrictive effects of angiotensin II and lead to vasodilation (McMurray, 2010). Enalapril reduce angiotensin II induced aldosterone secretion by the adrenal cortex, increase sodium secretion and subsequently increased water outflow whole degree of essential hypertension, especially in patients with diabetes and chronic kidney disease like glomerulosclerosis (Oates and Brown, 2008).

Enalapril maleate is chemically known as N-(1-ethoxy-1-oxo-4-phenyl-2-butanyl)-L-alanyl-L-proline (2Z)-2-butenedioate (1:1) (Fig. 5), with the molecular weight of 492.53g/mol. It is sparingly soluble in water, soluble in ethanol, and freely soluble in methanol (EuropeanPharmacopoeia, 2013). Its aqueous solubility increase from approximately 25 mg/ml at pH 3.5 to 200 mg/ml at pH 7.0. It is classified as BCS class 3 with high solubility but low permeability properties (Verbeeck *et al.*, 2017, Yasin *et al*., 2021). Solubility tests at Goethe University at 37°C revealed that the solubility is 5mg/ml across the pH range of interest (pH 1.2 to 6.8) and at the isoelectric point (Al-Omari *et al*., 2001).

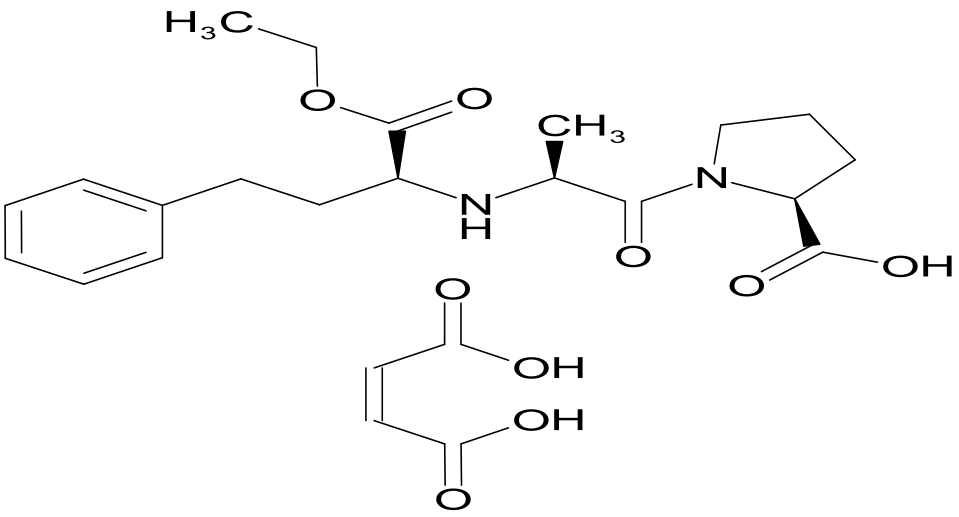

Figure 5: Chemical structure of enalapril maleate

1.5. Rational of the study

In-vitro dissolution test is employed to reduce *in vivo* bioequivalence requirement and it can be adopted as the surrogate basis to judge whether two pharmaceutical products are equivalent or not (WHO, 2009). The drug products (metformin tablets, cloxacillin capsules, metoclopramide tablets, and enalapril maleate tablets) that are selected for this *in-vitro* study are frequently prescribed, life-saving, and taken on a long-term basis. Generic drugs are expected to provide patients with as effective but cheaper medical treatment as brand-name products. The substitution of generic products considered when they are pharmaceutically equivalent, therapeutically equivalent, and meets standards for strength, purity, quality, and identity of a reference product. Therefore, generic substitution (GS) has become important in promoting the employment of more cost-effective medicines. Thus, the current study offers necessary information about the physical properties, dissolution profiles, drug release profiles and content uniformity of locally manufactured generic drug products (cloxacillin sodium 500 mg capsules, metformin hydrochloride 500 mg tablets, metoclopramide hydrochloride 10mg tablets and enalapril maleate 5 mg tablets) as compared to their comparator counter products.

The findings of this study might help local regulatory authorities to establish requirements for evidence of quality, content uniformity, and interchangeability of products.

2. COMPREHENSIVE REVIEW ON BCS CLASS III DRUG PRODUCTS

In early 1995, BCS was introduced by Amidon and its colloquies (Amidon et al., 1995), classified active pharmaceutical ingredient into 4 groups namely: Class I (Highly solubility/high permeability), Class II (low solubility/high permeability), Class III (high solubility/low permeability) and Class IV(low solubility/low permeability) based on aqueous solubility and intestinal membrane permeability. According to WHO list of essential medicines, 61 of the 130 orally administered drug substances could be reliability assigned. Twenty-one (34%) are Class I, 10 (17%) Class II, 24 (39%) Class III, and 6 (10%) Class IV (WHO, 2006). In WHO list of essential medicine, some BCS class (I-IV) drugs are listed in Table 1.

Table 2: Some examples of orally administered drugs on the WHO model list of essential drugs according to the BCS (WHO, 2006).

BCS class	**Drugs**
Class I	Metoprolol, amlodipine, allopurinol, verapamil, propranolol, acetylsalicylic acid, etc.
Class II	Ibuprofen, naproxen, ketoprofen, ezetimibe, glibenclamide carbamazepine, etc.
Class III	Enalapril. captopril cimetidine, ranitidine, cloxacillin sodium, vancomycin, chloramphenicol, metformin, metoclopramide, acyclovir, abacavir, atenolol, etc
Class IV	Hydrochlorothiazide, furosemide, indinavir, ritonavir nelfinavir, aluminium hydroxide, etc.

2.1. BCS class III drugs

BCS Class III drugs are highly soluble and poorly permeable, and they are also considered to be more susceptible to the excipient effects (FDA, 2017). Therefore, all excipients should be qualitatively similar and quantitatively same, with the exception of film-coated excipients or capsule shell excipients. The amount of excipients should be quantitatively similar and within a cumulative difference of no more than 10% (EMA, 2020). The rate and extent of absorption may vary in the case of rapid dissolution

due to GI transit time and content, as well as membrane permeability (Luque and Barril, 2012). Even though BCS class III drugs have good pharmacological activity, they pose a challenge when formulated as a solid oral dosage form due to permeability issues (He, 2009). Because of this problem, many formulation approaches were developed with the aim of addressing the problem of low permeability without compromising solubility properties (Amidon, 2006, Miller, 2009, Miller *et al*., 2010, Gupta *et al*., 2013).

2.2. Formulation strategies

Various formulation strategies are available for enhancing the oral BA of this class of medicines, including imparting lipophilic characteristics to the hydrophilic drugs and increasing their retention time, as well as using permeation enhancers (Hedaya, 2012).

2.2.1. Formulation imparting lipophilic character to drugs

2.2.1.1. Prodrugs

Prodrugs are drug formulation approaches as derivatives of active drug molecules that undergo *in vivo* enzymatic or other biochemical transformation to release their parent pharmacological active drug molecules Prodrug approaches offer opportunities to overcome various obstacles in formulation and drug delivery, such as solubility, physicochemical instability, oral drug permeability, presystemic metabolism, and insufficient tissue permeability (Stella, 2007). Therefore, prodrug approaches improve the absorption of poorly permeable medications by increasing their lipophilicity (log P) (Beaumont *et al*., 2003). For example, the esterification of enalapril (ethyl ester), ampicillin (pivaloyloxymethyl ester, adefovir dipivoxil (a diester) and famiciclovir (a prodrug of penciclovir) (Stella, 2007).

The esterification of adefovir by pivaloyloxymethyl moieties to mask polar phosphonic acid groups gives adefovir dipivoxil prodrug, which increases adefovir lipophilicity Therefore, prodrugs radially cross cell membrane by passive diffusion (Cundy et al., 1994). A study conducted by Dando and plosker, revealed prodrug significantly higher BA in human (59%) as compared to the parent adefovir drug (Dando and Plosker, 2003). In addition, the prodrug formulation of the parent molecule of acyclovir (valacyclovir) was found to increase BA by 3- to 10-fold compared to the parent molecules (Beauchamp *et al*., 1992).

2.2.1.2. Double emulsions

Formulation strategies that confer lipophilic character on hydrophilic drugs include double emulsions (Garti, 1997, Okochi and Nakano, 2000, Koga *et al*., 2010). Multilayer emulsion (double emulsion) approaches improve permeability of BCS Class III drugs. In this approaches, drugs present in the inner hydrophilic core, which provide a safe environment for drugs and a storage cavity, are easily absorbed as oil droplets from GIT. They revealed that the w/o/w emission system had a significantly increases intestinal of calcein in rats than calcein control (Koga *et al*., 2010). However, their industrial application is limited due to their instability during shelf life (Chakraborty *et al*., 2016). Similarly, the niosomal dispersion of acyclovir improved BA more than twice as much as the free drug solution because it is osmotically active and chemically stable (Attia *et al*., 2007).

2.2.1.3. Self-emulsifying drug delivery system

Self-emulsifying drug delivery systems (SEDDS) are important and promising techniques for increasing the oral bioavailability of poorly soluble drugs (Julianto *et al*., 2000, Setthacheewakul *et al*., 2010). However, SDEDDS use a mixture of hydrophilic surfactant and W/O) emulsion that is stable through formulation optimization. According to an *in vivo* study, plasma conc. time profiles in mice reveal enhanced absorption of pidotimod-loaded SDEDDS compared to its free solution (Qi *et al*., 2011).

2.2.1.4. Liposomes

Liposomes are synthetic vehicles made up of one or more phospholipid bilayers that are capable of holding water-soluble compounds. They are used as a transport system for drugs, genes and vaccine in therapy (Shashi *et al*., 2012). Liposomes are an approach to improve the permeability of the formulation. *In vivo* oral bioavailability on metformin-loaded liposome coated with chitosan and cross-linked with the biocompatible β-glycerol phosphate demonstrated that oral bioavailability was improved (Manconi *et al*., 2013).

2.2.1.5. Ion paring

The ion paring method essentially works by complexing the molecule of interest with oppositely charged ionic species, results a neutral ion pair that enhances the molecule's lipophilicity, membrane permeability, and absorbance (Barry, 2001, Wang *et al.*, 2008). Among commonly used counter-ions, 1-hydroxy-2-naphtholic acid (HNAP) is a

frequently employed counter-ion. Because of its high lipophilicity, relatively strong binding constant, and history with drugs like salmeterol, zanamivir, and oseltamivir this counter-ion is an appropriate means of balancing the high polarity of various BCS class III drugs (Miller, 2009, Miller *et al*., 2010). HNAP counter-ion used to prepare ion-paired complexes of zanamivir hipetyl ester and guanidine oseltamivir oral formulation, showed a significantly increases up to 3.7 fold in lipophilicity of drugs (Miller *et al*., 2010).

2.2.1.6. Nanotechnology-based approaches

Nanoparticle-based approaches work by encapsulating drugs in polymeric nanoparticles to address challenges related to solubility, permeability, degradation and drug toxicity (Vrignaud *et al.*, 2011). The pharmacokinetics of doxorubicin were studied in laboratory animals by incorporating ion pairs into solid lipid nanoparticles (SLN), which revealed that the concentration of doxorubicin in blood, lung, spleen, and brain was much higher with the commercial solution (Zara *et al*., 1999). Furthermore, as compared to conventional medications, SLN enhanced the area under the curve (AUC) of doxorubicin. Likewise, the preparation and pharmacokinetic evaluation of doxorubicin loaded poly-(lactic-co-glycolic acid) (PLGA) nanoparticle, showed that the BA of doxorubicin loaded nanoparticles was significantly higher (363%) and there was 6 fold of its T_{max} (Kalaria *et al*., 2009).

Solid lipid nanoparticles were also used to impart a lipophilic character to drugs. In the solid dispersion of drug-phosphatidylcholine, the percentage of permeation increases compared to its pure drugs, and the permeability of medicines increases with the amount of phospholipid increases. This approach has the ability to penetrate various anatomical boundaries, content release in a controlled manner and stabilize them at the nanometer size. *In vitro* permeability study on atenolol solid dispersion with fatty excipients significantly increased intestinal permeability compared to its pure drug (El-Leithy *et al*., 2012).

2.2.2. Formulation strategies that increase gastric residence time

Gastroretentive (GR) drug delivery systems have the ability to retain medications in the stomach for extended periods of time. Approaches such as sinking systems in the stomach floor, buoyant systems that produce buoyancy in gastric fluid, and

mucoadhesive systems that act via bioadhesion to the gastric mucosa were employed to design and develop GR systems (Amit *et al*., 2010). Based on swelling and mucoadhesive mechanisms, in *in vivo* studies of the GR formulation of acyclovir showed longer retention in the upper gastrointestinal system, sustained *in vitro* drug release, prolonged *in vivo* absorption, and higher bioavailability than the IR formulation (Sankar and Jain, 2013).

Metformin hydrochloride has a narrow absorption window and high aqueous solubility; it is preferable to keep the drug in the stomach for a longer period of time for optimal absorption and bioavailability. A study on gastro-retentive tablet formulations using sodium alginate and sodium carboxymethyl cellulose as polymers was employed to successfully deliver a once-daily controlled-release oral formulation of metformin hydrochloride (Boldhane and Kuchekar, 2009). Similarly, gastro-retentive floating tablet formulation of atenolol revealed that increase gastric retention, prolonged release and increase bioavailability of drugs (Pawar *et al*., 2013).

Permeation enhancers (PE) are chemical components added to pharmaceutical formulations to enhance drug absorption. PE affects the absorption of the drugs by various mechanisms, such as: B. Modification of mucus rheological properties, alteration of GI membrane fluidity, inhibition of enzyme and efflux pumps (Ganem-Quintanar *et al*., 1997). Ionic surfactant like sodium dodecyl sulphate (SDS) significantly increase the permeability of fexofenadine hydrochloride using CACO-2 cell (Gundogdu *et al*., 2011). Ibandronate-DCK complex significantly increases C_{max} and AUC values by 2.8 to 4.3 times when compared to pure ibandronate (Park *et al*., 2013).

2.3. Bioequivalence studies of BCS Class III drug products

It is critical to assess the bioequivalence of the various generics on the market in order to ensure interchangeably with their innovator products (Fahmy and Gharbieh, 2014). Quality testing and BE studies were required to assess whether a generic products matched the performance of the reference product. In different countries around the world, various quality control studies and BE studies of BCS Class III drug products were conducted. There are various drug products belongs to BCS Class III, including antidiabetics; antihypertensive drugs; antibiotics, antiemetic's, and antiviral agents, etc.

2.3.1. Anti-diabetic agents

Diabetes mellitus (DM) is a metabolic diseases characterised by hyperglycemia that is associated with serious complications such as diabetic neuropathy, retinopathy, nephropathy, and cardiovascular disease. It is also a primary cause of death and disability globally (Bastaki, 2005). Type 2 diabetes is the most common, accounting for 90-95% of all diabetes cases (Boruah *et al*., 2017, Prithi *et al*., 2018, Luo *et al*., 2019, Saeedi *et al*., 2019). It is the second most common noncommunicable disease in Ethiopia and 34,262 death were reported in 2014 (Atlas, 2015). Metformin is a biguanide-class oral antidiabetic medication that is considered to be first-line for the treatment of type 2 diabetes, especially in people who are overweight or obese and have normal kidney function (Prithi *et al*., 2018).

After the innovator product's patent expires, several generic metformin hydrochloride tablets are available in the worldwide drug supply system. Because of the variations in clinical responses from similar medicines observed by different manufacturers, there should be a way to assure that generic drug products are therapeutically equivalent and interchangeable with their innovator product (Adegbolagun *et al*., 2007, Eraga *et al*., 2017).

Various reports of *in vitro* and *in vivo* BE comparative studies of generic metformin hydrochloride in different countries have been conducted to compare therapeutic equivalence and interchangeability between generics and their innovator counter products. *In vitro* equivalence studies conducted in Iran (Zakeri-Milani et al., 2012), Beirut (H AL Arwadi *et al*., 2020), Qatar (Younes *et al*., 2023), Saud Arabiya (Afifi and Ahmadeen, 2012, AlBratty *et al.*, 2020), Nigeria (Eraga *et al*., 2017, Olusola *et al.*, 2012), Ghana (Sougi *et al*., 2016), Sudan (Osman *et al.*, 2017), and Addis Ababa Ethiopia (Kassahun *et al*., 2019), revealed that some generic metformin hydrochloride products did not meet the pharmacopeial specification for quality parameters and the FDA BE requirements $f1 < 15$ and $f2 > 50$ and also significant difference ($p < 0.05$) in the comparison to innovator products. In addition, a similar study conducted in Libyan (Abozaid, 2020) and Nigeria (Akinleye *et al.*, 2012**)** found that all generic products assessed did not meet FDA bioequivalence requirements and are therefore not used interchangeably with innovative products in clinical practice.

On the other hand, in a similar study in Egypt, three generic metformin hydrochloride products were bioequivalent with the innovator products (Hashem *et al.*, 2019). Also, *in vitro* equivalence studies conducted in different countries found that generic metformin hydrochloride products are therapeutically equivalent and interchangeable with innovative products (Hettiarachchi *et al.*, 2015, Tesfay *et al*., 2019, Jain *et al*., 2019, Elghnimi *et al*., 2019, Abatea *et al*., 2020, Arora *et al*., 2021). Moreover, *in vivo* BE studies conducted in China (Sun *et al.*, 2022) and Nigeria (Olusola *et al*., 2012, Adegbola *et al*., 2017) showed that the bioavailability of the generic products were non-inferior to that of the innovator product.

2.3.2. Antihypertensive agents

Cardiovascular disease (CVDs) are a major cause of death globally (Naghavi *et al*., 2017), with hypertension being the primary risk factor, which predispose to heart attacks and stroke (Kjeldsen, 2018, Stanaway *et al*., 2018, Mills *et al*., 2020). Antihypertensive drugs (AHTDs) are among the most commonly prescribed medication worldwide. Treatment of hypertension based on blood pressure and overall cardiovascular risk. AHTDs that are frequently prescribed include diuretics, β-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors (ACE-Is), and angiotensin II receptor blockers (ARBs), that significantly lower blood pressure while reducing the risk of cardiovascular morbidity and mortality (Law *et al.*, 2009, Turnbull, 2003). Antihypertensive drugs are among the drugs categories that are widely available on the market from a variety of sources, with significant price differences (Kumar *et al*., 2015). Therefore, evaluating and comparing the bioequivalence of such products is critical. In vitro studies have been performed in several countries to evaluate the dissolution profiles and bioequivalence of generic BCS class III hypertensive drug products.

According to a quality control studies conducted in 10 African countries, around a quarter of antihypertensive drugs were a poor quality (Macquart de Terline *et al*., 2018). In a study conducted on enalapril maleate in Brazil (Lima *et al*., 2008) and India (Kumar *et al*., 2018), most generic products showed that the drug release profiles in terms of bioavailability were significantly lower than that of that of the reference product and also showed marked differences in release profile. Similarly, *in vitro* comparative study of three generic products of enalapril maleate tablets available in Guatemala revealed that

all except one generic products were bioequivalent with the innovator product (Mendoza-Prillwitz *et al*., 2020).

In addition, an *in vivo* BE study on two generic products available in Greek showed that they were bioequivalent to the innovator product in terms of C_{max} and AUC. Therefore, two products can be used interchangeable in clinical practice (Niopas *et al*., 2003). A study of six generic Captopril products available in Pakistan revealed that all of them fulfilled the quality parameter requirements and also met the limit test of not <80% dissolving within 20 minutes to be used interchangeably (Shaikh *et al*., 2020). Likewise, in a comparative study in Romania, all captopril brands dissolved no less than 80% of the claimed amount within 20 minutes and met the requirement of f2 > 50 (Abdalrb *et al*., 2017)**.**

A biowaiver study of three generic products of the atenolol dissolution test in three dissolution media in the United Arab Emirates found that two generic products showed a fast and good dissolution profile at pH 1.2, but one generic product failed to meet the similarity factors. Except for one product at pH 6.8, all brands met the bio-waiver criterion at pH 4.5 and 6.8. This could be due to differences in the manufacturing method (Usman *et al*., 2014). However, a comparable study conducted in Bangladesh (Begum *et al*., 2019), Sir Lanka (de Silva *et al*., 2020), and Libya (Mohamed M. Siaan, 2017) found that all generic products of atenolol met pharmacopeial quality parameter and also met FDA requirements of f2 > 50 and f1 < 15. In addition, an *in vivo* pharmacokinetic and bioequivalence study of generic atenolol 50 mg compared to innovator products was conducted in South Korea and found out that they were bioequivalent and can be prescribed interchangeably (Chang and Shin, 2012)**.**

In quality control study conducted on three generic products available in Bangladesh only one generic product met quality parameters (Hossain et al., 2021). The evaluation four generic losartan potassium film-coated 50mg tablets available in Turkey showed that all products met dissolution tolerance limit of 85% of the labelled claim dissolved in 30 minutes and met FDA requirements of f2 > 50 and f1 < 15 (Gundogan, 2008). In addition, three generics of losartan potassium 25 mg tablets available in Bangladesh were evaluated for *in vitro* bioequivalence and all tested generics were found to meet the USP dissolution test specification (Sarker, 2014).

2.3.3. Antibiotics

According to the WHO, antimicrobial resistance (AMR) is a major global public health challenge (Leung *et al*., 2011), particularly in low and middle income countries with increased use of antimicrobial (Xiao *et al*., 2011, Kosikowska *et al.*, 2016, Sakeena *et al.*, 2019, Assen Seid and Seid Hussen, 2021). Irrational use, high levels of infectious disease, poor infection prevention and control, substandard medicines, low AMR awareness, wrong diagnoses, and the lack of a drug susceptibility test all contribute to AMR. Therefore, in order to address the AMR problem, an international approach is required (Vila and Pal, 2010, García *et al.*, 2011, Assen Seid and Seid Hussen, 2021). More than 10 million people died from tuberculosis, malaria, cholera, diarrhea, and pneumonia in only one year. The most commonly counterfeited and adulterated antibiotics have been supplied on global markets (Ejikeme and Ademola, 2010). Slight changes in the API contents of antibiotics can have an effect on their efficacy. Therefore, measuring the active pharmaceutical ingredient in the antibiotic formulation becomes essential in determining the pharmaceutical equivalence of generics products with their innovator counter product (Hewitt, 2012, Dafale *et al*., 2015).

Various reports have been published on the *in vitro* and *in vivo* equivalence of different antibiotic products in different countries. The evaluation on ten generic ciprofloxacin products available in Bangladesh revealed that six out of ten generic ciprofloxacin failed the BP specification of 80% dissolution in 30 minutes and four of the products failed the USP specification (Uddin *et al*., 2017). Likewise, similar study conducted in Kenya showed that only 10 of the 19 generic products of ciprofloxacin were considered to be pharmaceutically equivalent to their innovator product (Minyeto *et al.*, 2015). Similar, study conducted in Kenya at pH 1.2 and 4.5; all tested ciprofloxacin released 85% within 30 minutes and met the requirements of $f1 < 15$ and $f2 > 50$. However, at pH 6.8, all samples did not meet the above specification (Minyeto, 2014).

Likewise, in Mongolia, only 40% (Ganbat *et al*., 2020) and Nigeria, 75% (Olayemi *et al.*, 2023, Shaibu and Audu, 2018) of the marketed generic ciprofloxacin tablets had a similar dissolution profile to a comparator product and were used interchangeably. Similarly, bioequivalence study of four generic ciprofloxacin available in Nigeria market revelled that none of the generic products can be used to replace their innovator products in staphylococcal infection (Osonwa Uduma *et al*., 2011). The evaluation of three

generic ciprofloxacin 500mg tablets available in Iraq also showed that with the exception of one all generic products complied with the USP 85% dissolution tolerance limit until 60 min and FDA bioequivalence requirement of f2 > 50 (Alzubaidi, 2022).

In vitro comparative study conducted on generic products of the ciprofloxacin available in Ethiopia (Kahsay *et al*., 2007, Fereja and Tufa, 2015) and found that except one all the generic products met both the BP and USP dissolution tolerance limits. However, all generic products showed a significant difference ($p < 0.05$) in *in vitro* drug dissolution. Furthermore, in an *in vitro* quality study performed by (Desta and Teklehaimanot, 2020), it was observed that all generic ciprofloxacin tablets evaluated were significantly different ($p < 0.05$) in terms of in drug dissolution profiles while comparing the t50% and t90% value of generic and innovator products. Likewise, an *in vitro* efficacy study of ciprofloxacin performed in Bishoftu, Ethiopia revealed that significant difference ($p<0.05$) in the mean diameter of the zone of inhibition (ZI) between the generic sample and the ciprofloxacin standard (Geleta and Tufa, 2023).

In contrast to the above, some studies conducted in India (Nayak and Pal, 2010), Bangladeshi (Rahman *et al*., 2019) and United Arab emirate (Fahmy and Gharbieh, 2014) showed that all generic ciprofloxacin hydrochloride products tested were bioequivalent and can be used interchangeably with their innovator products. In some *in vivo* BE studies conducted in Pakistan and Nigeria (Khan *et al*., 2009, Nduka *et al*., 2022), all generic products ciprofloxacin compared in terms of bioavailability, i.e., T_{max}, C_{max}, AUC, and absorption rate constant (Ka), dissolution rates at T30%, T50%, T90% with aims as *in vivo* bioequivalence waiver. In this study, generic products showed no significant ($p<0.05$) difference to innovator product.

In a comparative dissolution studies of ten generic chloramphenicol capsules marketed in Indonesia, it was found that out of the ten generic products examined, four generic products did not meet pharmacopeial specifications and had significant differences (f2> 50) in dissolution profiles and were not comparable to innovative products (Lucida. H, 2019). Likewise, a BE study of flucloxacillin capsules available in Ghana found that all generic brands were bioequivalent in all pharmacokinetic parameters and showed no inter-subject statistical variability. Therefore, generic products are used interchangeably with innovator products (Ayensu *et al*., 2016).

In a BE study of four generic products of vancomycin available in Colombia, all of the generic products failed to meet the expected bactericidal efficacy of the innovator product (Vesga *et al*., 2010). In addition, an *in vivo* pharmacokinetic and pharmacodynamics study comparing five generic products of vancomycin available in Korea, revealed that some of the marketed generic products had inferior PK and PD profiles, especially in mice infected with vancomycin resistant staphylococcus aureus (Kim *et al*., 2020). On the other hand, another microbiological study conducted in Colombia in 2011 and 2022 found that both generic and reference brands of vancomycin had no significant difference ($p<0.05$) in antimicrobial activity and are pharmaceutically equivalent (Diaz *et al*., 2011, Castaño-Rendón *et al*., 2022).

2.3.4. Anti-ulcerative agents

Gastric ulcers are a type of chronic ulcerative disease that mostly affects gastric mucosa and duodenum. H2 blockers, such as cimetidine, ranitidine, and famotidine, are used to reduce the amount of gastric acid secretion. Proton pump inhibitor (PPIs), such as omeprazole, lansoprazole, pantoprazole, rabeprazole, and esomeprazole are a groups of drugs that results significant and long lasting reduction in gastric acid. PPIs are chemically stable, fat-soluble weak bases with not much antacid activity at neutral pH. PPIs are administered in an inactive form that is neutrally charged or lipophilic and cross the cell membrane easily into acidic intracellular compartments. The inactive medication is protonated and transformed to its active form in an acidic environment (Hoogerwerf and Pasricha, 2006).

Equivalency studies of ten generic products of ranitidine 150 mg tablets marketed in Russia revealed that all generic ranitidine tablets were studied in different pH media. The similarity coefficients for the dissolution profiles of some generic products were not bioequivalent to the innovator product (Smekhova *et al.*, 2009). In addition, Pavel and his colleagues evaluated six generic products of ranitidine available in Bangladesh and it was showed that some of the generic products had significant differences ($P < 0.05$) in *in vitro* drug release profiles when compared to innovator product (Pavel, 2016). However, the evaluation of four generic ranitidine hydrochloride 150mg tablets available in Karachi, Pakistan revelled that all products meet USP specifications for quality control parameters and are interchangeable (Naveed *et al*., 2014**).**

2.3.5. Antiemetic drugs

Chemotherapy-induced nausea and vomiting (CINV) is caused by neurotransmitter and substances that stimulate the receptor in the vomiting center (chemoreceptor trigger zone), such as dopamine, serotonin, histamine, acetylcholine, and substance p (NK-1) (Leslie, 1985, Bountra et al., 1996, Hesketh, 2008). These relevant receptor sites are targeted by antiemetics. Phenothiazine and metoclopramide drugs, widely used since the 1980s, inhibit the effects of dopamine (Saller and Hellenbrecht, 1986). The first-generation serotonergic receptor antagonists (5HT3 inhibitors) significantly suppressed acute CINV (Hesketh and Gandara, 1991). The quality of each formulation is depends on processing variables, GMP, and cGMP practiced during production (Weise *et al*., 2009). To evaluate the quality and equivalency of tablets and capsules, content and dissolution tests are employed (Yogananda *et al*., 2009).

Three generic products of metoclopramide hydrochloride tablets available in Argentina were studied for pharmaceutical equivalency and similarity and revealed that all generic products complied with the requirements for 'very rapidly dissolving' and they are described as essentially similar (Varillas *et al*., 2018). In addition, in an *in vitro* study of four generic products of metoclopramide hydrochloride 10mg tablets marketed in Saud Arabiya, showed that all generic products were pharmaceutically equivalent as they met critical quality parameters (Hani *et al*., 2020). In this study, all except one product fulfilled 'very rapid dissolution' test.

Likewise, equivalency studies of six generic products of metoclopramide hydrochloride tablets marketed in Pakistan revealed that all generic products fulfilled USP dissolution tolerance limit and also complied with the FDA equivalence requirement ($f2 > 50$) (Zeb-un-Nisa *et al*., 2021). In addition, *in vivo* BE study conducted in Mexico and Pakistan showed that all generic products of metoclopramide hydrochloride were similar in the dissolution profile with their innovator product (Alonso-Campero et al., 2011, Ahmad *et al*., 2015).

2.3.6. Antiviral agents

The *in vivo* BE studies of two acyclovir products available in Iran revealed that generic products of acyclovir were not bioequivalent to their innovator product and did not complied with US FDAs BE acceptance range (80-120%). In contrast, an *in vivo* BE

study conducted in united Arab emirates between locally manufactured generic acyclovir tablet and innovator product, revealed that generic product was bioequivalent in terms rate and extent of absorption with their innovator product (Najib *et al*., 2005). In a *in vivo* BE study on abacavir available in India containing abacavir and lamivudine found that both products were comparable in terms of AUC_{0-t} and relative BA and concluded that generic products are bioequivalent to reference products (Dan *et al*., 2015).

3. OBJECTIVES

3.1 General objective

- ✓ To assess *in vitro* equivalence of some locally manufactured and imported BCS Class III drug products against their comparator counter pharmaceutical drug products.

3.2 Specific objectives

- ✓ To undertake a comprehensive literature survey on BE studies on BCS Class III drug products
- ✓ To evaluate physical properties of generic drug products (metformin tablets, cloxacillin capsules, metoclopramide tablets and enalapril maleate tablets) as compared with their comparator pharmaceutical drug products.
- ✓ To compare the assay contents of each tablet using official methods.
- ✓ To compare the *in vitro* dissolution profiles of the generic products and comparator products.
- ✓ To compare the *in vitro* drug release profiles of the generic products against their working reference standard.

4. EXPERIMENTAL

4.1 Materials and methods

4.1.1 Reagents and materials

USP reference standards of Metformin hydrochloride and working reference standards of Enalapril maleate (100.33%) were obtained from Ethiopian Food and Drug Authority (EFDA), Cloxacillin sodium (100.33%) from Ethiopian Pharmaceutical Manufacturing company Sh. Co (EPHARM) and Metoclopramide hydrochloride (99.44%) were kindly provided by Cadila Pharmaceuticals (Ethiopia) PLC.

All chemicals used were of analytical grade. Sodium hydroxide 97 % (Oxford Lab Chem LLP; India), Potassium dihydrogen phosphate-99.5% (Blulux Laboratories Pvt Ltd; India), Hydrochloric acid 35.4% (Loba Chemie Pvt. Ltd; India), Sodium dihydrogen phosphate 98-100.5% (Trust Chemical Laboratory, UK), and distilled water were used as received.

4.1.2 Sample collection method

Market samples of locally manufactured and imported drugs of one comparator product for each class of drugs and five generic metformin hydrochloride film-coated tablets (500 mg), two generic products of; cloxacillin sodium capsules (500 mg), and metoclopramide hydrochloride tablets (10 mg), and enalapril maleate tablets (5 mg) were purchased from retail pharmacies found in Addis Ababa, Ethiopia. These products were selected based on Ethiopian Essential Medicines List, 2020, market availability in the same dosage form and strength in the local market. At the time of the evaluation, all products used were within their shelf lives, and their details are shown in Table 3.

Table 3: Detail information of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate brands investigated.

Product	**Brand**	**Manufacturer**	**Country of origin**	**Batch number**	**Mfg. date**	**Exp. Date**
Metformin hydrochloride 500mg film coated tablets						
MTF_1	Metfor-SSP	Sanshin Pharmaceutical (Ethiopia) PLC	Ethiopia	01822040100	04/2022	03/2024
MTF_2	Insumet	Cadila	Ethiopia	020031T212	11/2020	10/2023

		Pharmaceuticals (Ethiopia) PLC				
MTF_3*	Glyformin	Remedica, Limassol industrial state	Cyprus	J69688	05/2020	05/2025
MTF_4	Metformin -denk	Denk Pharma GMBH & CO.KG.	Germany	9xu	01/2020	01/2025
MTF_5	Glucomet	Y.S.P industrial (M) SDN. BHD	Malaysia	EI006	10/2020	10/2023
MTF_6	Metformin -sandoz	Lek SA. Strkow	Poland	LN6498	07/2021	06/2024
	Cloxacillin sodium 500mg capsule					
CLX_1	cloxa	Ethiopian Pharmaceutical Manufacturing Company Sh. Co	Ethiopia	2150016	12/2021	11//2024
CLX_2	Clox	Addis Pharmaceutical Factory S.C	Ethiopia	32247		09/2023
CLX_3*	m-clox	Jackson Laboratories Pvt. Ltd.	India	21500018	12/2021	11/2024
	Metoclopramide hydrochloride10mg tablets					
MCP_1	Cloperan	Ethiopian Pharmaceutical Manufacturing Company Sh. Co	Ethiopia	0090143	09/20	09/23
MCP_2	Premosan	Gulf Pharmaceutical Industries, Ras Al Khaimah	U.A. E	0128	03/21	03/26
MCP_3*	Metopharm	Remedica Ltd, Limassol Industrial State	Cyprus	91125	11/20	11/25
	Enalapril maleate 5mg tablets					
ENA_1	Envas	Cadila Pharmaceuticals (Ethiopia), PLC	Ethiopia	D21006BX52	04/2021	Jan/23
ENA_2	ANA – enalapril	Humanwell Pharmaceuticals	Ethiopia	57220204	02/22	02/24

	maleat	Ethiopia PLC				
ENA_3*	Korandil	Remedica, Limassol Industrial State	Cyprus	90786	11/2020	11/23

*Comparator products

4.2 Methods

4.2.1. Identification test

Identification test was performed according to (USP, 2021a) as follows. Equivalent to 20 mg of powdered metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate were dispersed separately in 20ml of ethanol, followed by shaking and filtering the mixture. After the filtrate was evaporated to dryness, the residue was dried at 105°C in a water bath for 2hr before used in the identification test. The same method was used for metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate reference standards. After that, the test sample and the reference standard were scanned in the FTIR (Shimadzu, Japan) utilizing the KBR plate method in the 4000 to 400 cm^{-1} range. Finally, the spectra of the test sample and the reference standard were compared.

4.2.2. Physical properties

4.2.2.1. Weight variation

Twenty tablets and capsules of each product included in this study were randomly selected and weighed individually on an electronic balance (AAA 160L, Wagtech international Ltd, England). The % weight variation for each drug product is calculated based on Eq.1 (Gupta *et al*., 2014, USP, 2021e).

$$\%\text{Weighet variation} = \frac{\text{Individual weight} - \text{Average weight}}{\text{Average weight}} x100 \ldots Eq.1$$

4.2.2.2. Hardness, diameter test and thickness

The thickness of ten tablets of each product was measured with slider calliper (Nippon Sokutei, Japan). The hardness and diameter of the tablets were measured with a hardness tester (CALEVA, THT-2 series, Germany). Ten tablets of each products were placed between the spindles of the hardness tester until the tablets was broken. The results were expressed as mean and standard deviation.

4.2.2.3. Friability test

Ten intact tablets from each product was weighed and subjected for abrasion in a Roche Friabilator at 25 rpm per min for 4 min (ERWEKA, TAR 20; Germany). The tablets were dedusted and weighed again, and then the percent of weight loss was recorded, the friability of the tablets was calculated as per *Eq*.2 (Gupta *et al.*, 2014, Kassahun *et al.*, 2018, More *et al.*, 2022).

$$\% \text{ F} = \frac{\text{wi} - \text{wf}}{\text{wi}} \times 100 \ldots\ldots\ldots\ldots\ldots Eq.2$$

Where, Wi is initial weight of tablets before friability testing, Wf is the weight of tablets after the testing.

4.2.2.4. Disintegration time

The disintegration time of tablets was evaluated using the USP monograph (USP, 2021b). Six tablets from each product were tested using ERWAKA disintegration tester (Heusenstamm, Germany). Distilled water at temperature of 37 ± 0.5 °C was used as media for disintegration test of metformin hydrochloride, metoclopramide hydrochloride tablets, cloxacillin sodium capsules and enalapril maleate tablets. The disintegration time is the duration of time it takes for no tablet fragments remained on the sieve.

4.2.3. Assay tests

Assay of Metformin hydrochloride 500mg tablets

Calibration curve

A stock solution was prepared by dissolving 10mg of metformin hydrochloride reference standard in 100ml of distilled water. Using the same solvent, six serial conc. levels (4, 6, 8, 10, and 12 µg/ml) were prepared from the stock solution. The absorbance was measured by UV spectrophotometer (SOLAR Spectrophotometry, CM2203, Belarus) at the maximum wavelength of 232 nm. Conc. vs. absorbance was plotted to generate the calibration curve.

Assay

The assay test was conducted according to the USP 2021 in order to determine the content of active ingredient in each tablet product in comparison to the claimed amount on the label. Twenty tablets of each product was weighed and crushed separately,

equivalent to 10mg of metformin hydrochloride was transferred to a 100ml of volumetric flask. Seventy millilitre of distilled water was added and dissolved using a magnetic stirrer for 15 min. Finally, it was diluted to 100ml with same solvent and filtered with Whatman #0.45µm filter paper. The first 5ml of filtrate was discarded. Finally, 2ml of filtrate was taken and diluted to 25ml with distilled water. The absorbances of the reference and test preparations were determined using UV/Vis spectrophotometry (1 cm cell) at 232 nm. Distilled water was used as a blank sample (USP, 2021j).

Assay of Cloxacillin sodium 500mg capsules

Calibration curve

A stock solution was prepared by dissolving 10mg of cloxacillin sodium working reference standard in 100ml of methanol to obtain a conc. of 100µg/ml. For the calibration curve, serial dilutions (5, 10, 15, 20, 25, 30 g/ml) were prepared from stock solution.

Assay

Twenty capsules of each product were randomly selected. The cap of the capsules was carefully opened, and contents were poured into glass mortar, and finely powdered. The equivalent of 10mg of cloxacillin sodium powder was dissolved in 100ml of methanol, and stirred using a magnetic stirrer for 10min. The sample solution was filtered by Whatman filter paper #0.45µm and the first 5ml of filtrate was discarded. The absorbance of sample solution was measured at maximum wavelength of 220 nm using UV/vis spectrophotometry. Methanol was used as a blank (Jadhav and Kohale, 2020)

Assay of Metoclopramide hydrochloride 10mg tablets

Calibration curve

Ten milligrams of the working reference standard of metoclopramide hydrochloride was transferred to a 100ml of volumetric flask. 75ml of 0.1NHCl was added and stirred for 1 hr. Then the same solvent was added to exactly 100 ml and centrifuged for 10 min. 2ml of supernatant solution was taken and diluted to a conc. of 20 g/ml with 0.1N HCl. The 10 µg/ml solution was scanned in the UV/Vis spectrophotometer from range 200-800 nm using 0.1N HCl as a blank. At the maximum wavelength of 309 nm, a range of 10-30µ g/mL conc. vs. absorbance is plotted to create a calibration curve (JP, 2021).

Assay

Twenty tablets were randomly selected, weighed and finely powdered. Equivalent of 10mg powder were taken and dissolved in 75ml of 0.1N HCl and stirred for 1hr. Then the same solvent was added to 100 ml and centrifuged for 10 min. 2ml of supernatant solution was taken and diluted with 0.1N HCl to provide a conc. of 20µg/ml. The absorbance of sample solution was measured by UV / Vis spectrophotometry at maximum wavelength of 309 nm (JP, 2021).

Assay of Enalapril maleate 5mg tablets

Calibration curve

A stock solution was prepared by dissolving 10mg of enalapril maleate working reference standard in a 100ml of phosphate buffer (pH =4). Phosphate buffer was prepared by dissolving 3.58gm of disodium hydrogen phosphate in distilled water and diluted to 500ml with same solvent. The pH was adjusted with phosphoric acid. The 10µg/ml solution was scanned in the UV/Vis spectrophotometry from a range of 200-400 nm, and the absorption maximum of enalapril maleate was found to be 208 nm. A serial dilution (4, 6, 8, 10, 12, and 14µg/mL) of the stock solution were prepared and their absorption was taken at 208 nm and calibration curve of enalapril maleate was constructed.

Assay

Twenty tablets of enalapril maleate from each product were randomly selected, weighed and then crushed. Equivalent of powdered 10 mg tablet was transferred to a 100ml volumetric flask and 100ml of phosphate buffer (pH = 4) was added. The sample was filtered by Whatman #42 filter paper, and the first 5ml of the filtrate was discarded. Then 1ml of filtrate was diluted in 10-ml of phosphate buffer (pH = 4) and analysed by UV/visible spectrophotometry at maximum wavelength 208nm (Gherman *et al*., 2015).

The percentage of the labelled amount of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride and enalapril maleate in each product was calculated using Eq. 3 (USP, 2021j).

$$\text{Assay}(\%) = \left(\frac{\text{Ru}}{\text{Rs}}\right) \text{ x } \left(\frac{\text{Cs}}{\text{Cu}}\right) \text{x} 100 \ \ldots\ldots\ldots\ldots Eq.3$$

Where, **Ru:** Absorbance of sample solution, **Rs:** Absorbance of the standard solution **Cs:** Conc. of standard solution, **Cu:** Conc. of sample solution.

4.2.7. Content uniformity test

The content uniformity of the metformin hydrochloride 500mg tablets, cloxacillin sodium 500mg capsule, metoclopramide hydrochloride 10mg tablets and enalapril maleate 5mg tablets was examined. Ten tablets of each comparator and generic products were individually weighed accurately and finely powdered separately. The content of active ingredient in each of the 10 tablets is calculated as producer directed in assay.

4.2.4. Dissolution test

In vitro dissolution studies of the BCS Class III drug products were performed according to the USP monograph (2021), JP (2021) and IP (2018), and other validated methods. Each procedure was done as indicated below

Dissolution profile of Metformin hydrochloride 500mg tablets

Ten mg of metformin hydrochloride reference standard was dissolved in 100ml of phosphate buffer (pH=6.8) and adjusted using a pH meter (Kedide ®, CT -6021A) to prepare a stock solution of 100µg/ml. Using phosphate buffer, six serial conc. (4, 6, 8, 10 and 12g/ml) were prepared from the stock solution. The absorbance was then measured using UV/Vis spectrophotometry at the maximum wavelength of 232 nm and plotted against the six serial conc. to construct the calibration curve (USP, 2021j).

Dissolution studies of metformin hydrochloride tablets were performed using 1000ml of phosphate buffer (pH = 6.8) as a dissolution medium. The dissolution test was performed on a USP apparatus type II (ERWEKA, DT600, Germany) at 50 rpm. The medium's temperature was kept constant at 37 ± 0.5°C.Twelve tablets of from each brand were randomly selected and placed in separate dissolution vessels. Twelve tablets from each product was selected randomly and placed in separate dissolution vessels. The dissolution test was performed for a total of 1hr. Samples of 10 ml were withdrawn at 5, 10, 15, 30, 45, and 60 min, each withdrawn sample was replaced with 10ml of fresh dissolution medium to maintain sink condition. The filtered samples were diluted appropriately (100-fold), and absorbance values were measured by UV/visible

spectrophotometer at maximum wavelength 232 nm. Phosphate buffer was used as a blank (USP, 2021i).

Dissolution of cloxacillin sodium 500 mg capsules

To prepare stock solution, 10mg of cloxacillin sodium working standard was dissolved in 100ml of distilled water, which has a concentration of 100 µg/ml. Six serial concentration levels (10-30 µg/ml) were prepared from stack solution. A UV/Vis spectrophotometer with a maximum wavelength of 220 nm was used to measure absorbance (IP, 2018).

Dissolution of cloxacillin sodium was performed using 900ml distilled water as dissolution medium. Dissolution test was performed using a USP type 1 apparatus (ERWEKA, DT600; Germany) at 100rpm and 37±0.5°C. Twelve capsules of each product were randomly selected and placed in a separate dissolution vessel. The dissolution process was performed for 1hr. At 5, 10, 15, 30, 45, and 60 min, 10ml of samples were withdrawn, and the same amount of fresh dissolution medium was replaced to maintain sink condition. The absorbance of cloxacillin sodium dissolved was determined by UV/Vis spectroscopy at a wavelength of 220nm (IP, 2018).

Dissolution of Metoclopramide hydrochloride 10 mg tablets

Ten mg of metoclopramide hydrochloride as a working standard was dissolved in 100 ml of distilled water to give obtain a known concentration of 100 µg/ml stock solution. Six serial concentrations (10, 14, 18, 22, 26, 30 µg/ml) were prepared to obtain calibration conc. from the stack solution. The absorbance of solutions were determined using UV/Vis spectrophotometer at maximum wavelength 309nm (USP, 2021h).

Dissolution of metoclopramide hydrochloride was performed using 900 ml distilled water as dissolution medium, and the tests were performed using USP apparatus 2 at 37±0.5°C and 50 rpm. At 5, 10, 15, 30, 45, and 60 min, 10ml of sample was withdrawn and the same fresh dissolution medium was replaced to maintain the sink conditions. The filtered samples were diluted, and their absorbance was measured with a UV/VIS spectrophotometer at maximum wavelength 309 nm. Distilled water was used as a blank (USP, 2021h).

Dissolution of enalapril maleate 5 mg tablets

Ten mg of enalapril maleate working reference standard was transferred into a 100ml volumetric flask, and 75 ml of phosphate buffer (pH= 6.8) was added and stirred for 1hr by magnetic stirrer. Then the same solvent was added to exactly 100 ml. Six serial dilutions (2, 4, 6, 8, 10 and 12µg/ml) ware made to obtain calibration conc. from stock solution. The absorbance of solutions were determined using UV/Vis spectrophotometer at maximum wavelength 208 nm (Kumar *et al.*, 2018).

Dissolution test of enalapril maleate tablets were determined using phosphate buffer (pH= 6.8) as the dissolution medium. The test was performed using USP-2 dissolution apparatus at 37± 0.5 °C and 50rpm. At 5, 10, 15, 30, 45, and 60 min, 10 ml of samples were withdrawn and the same amount of fresh dissolution medium was replaced to maintain sink condition. The samples were filtered by Whatman #0.45µm filter paper (Kumar *et al*., 2018). The absorbance of enalapril maleate dissolved was measured using UV-visible spectrophotometer at maximum wavelength 208 nm (Sowjanya *et al*., 2012).

The calibration curve was used to estimate the concentration of each sample, and the percentage of drug release at each time can be calculated using the following relationships (Chandrasekaran *et al*., 2011).

$$Y=mx\pm b$$

Where, m is the slope m and b is the intercept

$$\text{Amount of drug dissolved} = \frac{\text{Conc. x volume of medium}}{\text{CF}} x \text{ DF} \ldots\ldots\ldots\ldots\ldots Eq.4$$

Where DF is dilution factor, CF is conversion factor 0.001 mg/µg.

$$\text{Drug released}(\%) = \frac{\text{Amount of drug dissolved at time t}}{\text{Label claim}} \text{x } 100 \ldots\ldots\ldots\ldots\ldots\ldots Eq.5$$

$$\text{Commulative drug released } (\%) = \frac{\text{Volume of sample withdrawn(ml)}}{\text{Bath volume(v)}} \text{x p(t} - 1) + \text{pt.} Eq.6$$

Where Pt = Percentage release at time t, P (t – 1) = Percentage release before t

4.2.5. *In vitro* drug release studies

In vitro drug release studies of Metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride and enalapril were performed by dialysis bag technique.

Ten mg of accurately weighted powder metformin hydrochloride and enalapril maleate dispersed in 100ml of phosphate buffer (pH=6.8) and cloxacillin sodium and metoclopramide hydrochloride dispersed in 100ml of distilled water of each product were dispersed in 100 to obtain stock solutions. For the diffusion tests, serial dilutions from stock solution were made to construct a calibration curve conc. against absorbance.

A high grade regenerated cellulose dialysis membrane (with a flat width of 25mm or 1inch and a MWCO 12KDa) was cut into 7 cm lengths and kept overnight in a medium to ensure wetting of the membrane and opening of the hollow tube. The ends of the dialysis bag were then tightly closed with short piece around 1 cm from one end of the membrane.

Metformin hydrochloride 500mg tablets

A measured quantity (50 mg) of metformin hydrochloride power of each product was dispersed in 5ml of phosphate buffer (pH=6.8) and transferred into the dialysis bag (donor compartment) (Sigma-Aldrich, CHEMIE, GmbH, USA) and sealed (Paswan and Saini, 2021). Special precautions were made to avoid air bubbles inside a bag and any leakage of the solution. The dialysis bag was then immersed and freely rotated in the dissolution vessel of a USP 24 type II dissolution test apparatus (ERWEKA, DT600; Germany) containing 900 ml phosphate buffer (pH 6.8) at 37 0.5 °C and stirred at 100 rpm. At 5, 10 15, 20, 30, 45, 60, 90 and 120 min, 5 ml of sample was withdrawn with replacement of equal volume of fresh medium. The samples were diluted appropriately and analysed with UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) at 232 nm (Oishi *et al*., 2011).

Cloxacillin sodium 500gm capsules

A measured quantity (50 mg) of cloxacillin sodium power of each product was dispersed in 5ml phosphate buffer (pH=6.8) and transferred into the dialysis bag (donor compartment)(Sigma-Aldrich, CHEMIE, GmbH, USA) and sealed (Paswan and Saini, 2021). Special precaution was made to avoid air bubbles and any leakage of the solution. Then dialysis bag was immersed and allowed to rotate freely in the dissolution vessel of a USP 24 type II dissolution apparatus (ERWEKA, DT600; Germany) that contained 900ml of phosphate buffer (pH 6.8) at 37 ± 0.5 °C and100 rpm. At 5, 10, 15, 20, 25, 30, 45, 60, 90 and 120min, 5 mL of sample was withdrawn with the replacement of equal

volume of fresh medium to maintain sink condition. The samples were properly diluted before analysed with a UV/Vis spectrophotometer (UV-1700, Shimadzu, Japan) at 220 nm (Oishi *et al*., 2011).

Metoclopramide hydrochloride 10 mg tablets

Accurately weighed 10 mg of metoclopramide hydrochloride tablet powder was dispersed in 3ml of distilled water and transferred into prewashed dialysis membrane and tightly closed. Special precaution was made to avoid air bubbles inside the dialysis bag and solution leakage. The dialysis membrane was floated in the glass beaker containing 200ml of distilled water. The acceptor medium was agitated by magnetic stirring at 100rpm and 37±0.5^{o}C. Five ml of sample was taken at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120min with the replacement of equal volume of fresh dissolution medium to maintain sink condition. After diluted appropriately, the samples were examined with a UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) at 309nm (Ribeiro *et al*., 2015).

Enalapril maleate 5mg tablets

Five mg of enalapril maleate powder was dispersed in 3ml of phosphate buffer (pH=6.8) and transferred into prewashed dialysis membrane and tightly closed. Special precaution was made to avid air bubbles in side dialysis bag and solution leakage. The dialysis membrane was floated in the glass beaker containing 200ml of phosphate buffer (pH=6.8). The acceptor medium was agitated by magnetic stirring at 100rpm and 37±0.5^{o}C. At 5, 10, 15, 20, 25, 30, 45, 60, 90, 120min, 5ml of samples were withdrawn with the replacement of equal volume of fresh medium to maintain sink condition. After appropriate dilution, the samples were analysed with a UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) at 208nm (Ribeiro *et al*., 2015, Oishi *et al.*, 2011).

4.2.6. Data analysis

Microsoft Excel 2016, SPSS version 26 and OriginPro 8.5.1(2018) software, as well as Kinetic DS3 software K (OriginLab Corporation, MA, and USA), were used for statistical and graphical analysis of the experimental data. All measured and reported data are averages of at least triplicate measurements, with values provided as mean and standard deviation. One way analysis of variance (ANOVA) was used to compare dissolution profiles of locally manufactured and imported product with their comparator products; $P<0.05$ was considered as statistically significant.

A model-independent mathematical approach, dissimilarity factor (f1) (Eq. 6) and similarity factor (f2) (Eq. 7), dissolution efficiency (DE) (Eq.8), mean dissolution time (MDT) (Eq.12), and t50% and t90% were employed to compare dissolution profiles of generic products with comparator product. For two dissolution profiles to be regarded bioequivalent or similar, the similarity factor (f2) should be within 50 to 100 (FDA, 1997, Karmoker *et al*., 2016, Kassahun, 2019).

$$f1 = \frac{\sum_{i=1}^{n}[Rt - Tt]}{\sum_{i=1}^{n} Rt} \dots\dots\dots\dots\dots\dots\dots\dots Eq.7$$

$$f2 = 50log\left[\frac{1}{\sqrt{1 + (\frac{1}{n})\sum_{i=1}^{n}(Rt - Tt)^2}}\right] * 100 \dots\dots\dots\dots\dots\dots\dots Eq.8$$

Where, **n** is the number of time point, $\mathbf{R_t}$ is the dissolution value of comparator product at time t and **Tt** is the dissolution value of the tests at time t.

Dissolution efficiency (DE)

DE is define as the area under dissolution time curve up to certain time (*t*), expressed as a percentage of the area of the rectangle described by 100% dissolution at the same time point (Khan and Rhodes, 1972, Nayak and Pal, 2010, Oishi *et al*., 2011)

$$\text{DE}(\%) = \frac{\int_{t1}^{t2} ydt}{y100(t2 - t1)} * 100 \dots\dots\dots\dots\dots Eq.9$$

Where, y is the percentage of drug dissolved. The trapezoidal method was used to determine the integral of the numerator (AUC) (Ashraful-Islam *et al*., 2012).

$$\text{AUC} = \sum_{i=0}^{n} \frac{(t1 - ti - 1)(yi - 1 + y1}{2} \dots\dots\dots\dots\dots Eq.10$$

Where, t_i is the i^{th} time point and y_i is the percentage of drug dissolved at time t_i

Mean dissolution time (MDT)

At the same sample time, compare the mean dissolution times of the test products with their comparator product (Khan and Rhodes, 1972). It is calculated by.

$$\mathrm{MDT} = \frac{\sum_{i=0}^{n} \bar{t}\mathrm{i}\Delta\mathrm{Mi}}{\sum_{i=o}^{n} \Delta\mathrm{Mi}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.11$$

Where, *i* is sample number, n is the number of dissolution sample times, $\bar{t} = (t_{i-1}+t_i)/2$ is the time at midpoint between t_{i-1} and t_i, and ΔM_i *is* the additional amount of drug dissolved between t_{i-1} and t_i. More precisely rearranged to.

$$\mathrm{MDT} = \frac{\sum \mathrm{ti}\ \Delta\mathrm{Mi}}{M\ \infty} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.12$$

Where, t_i is intermediate time of the interval of sampling time, ΔM_i is the amount of API dissolved in every interval of t and M∞ is maximum of API dissolved.

The mean dissolution time and t50% t80% and t90% of each product was calculated using OriginPro 2018 software. Each product's dissolution profiles and sampling points were entered into the software separately.

Drug release kinetics

To explain the kinetics and mechanism of drug release from tablets. Model-dependent approaches were employed such as zero-order; first-order, Hixson–Crowell, Weibull and Michaels menten with lag. KineticDS3 software was used to calculate the drug release kinetics of each product (Mendyk and Jachowicz, 2007).

The dissolution profiles of the each product, as well as their sampling time were separately entered into the software (Gibaldi and Feldman, 1967, Wagner, 1969, Cornish-Bowden, 2013).

$$\text{zero order kinetics: } \mathrm{Q_t} = \mathrm{Q_0} + \mathrm{K_o t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 13}$$

$$\text{First order kinetics: } \log\mathrm{Q_t} = \log\mathrm{Q_0} + \frac{\mathrm{K_1 t}}{2.303} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 14}$$

$$\text{Hixson} - \text{crowell kinetics: } \sqrt[3]{\mathrm{W_0}} - \sqrt[3]{\mathrm{W_t}} = \mathrm{k_t t} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 15}$$

$$\text{Weibull kinetics: } \mathrm{M} = \mathrm{M_0}(1 - \mathrm{e} - \frac{(\mathrm{t} - \mathrm{Ti})\mathrm{b}}{\mathrm{a}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 16}$$

$$\text{Michaels menten: } v = \frac{V * a}{Km + a} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.17$$

Where, Q_t is amount of drug dissolved at time t, Q_0 is initial amount of drug in solution and K_0 is the zero order release constant. K_1 is the first order release constant; M is

accumulate fraction of drug, b is shape of parameter, T_i is location parameter, a is scale parameter. W0 is initial amount of drug in the dosage form; Wt is the remaining amount of drug in the dosage form at time t, Ks is a constant involving the surface-volume relationships. *V* is rate of drug dissolution, *a* is cumulative percentage of drug released and K_M is Michaels menten constant.

5. RESULTS AND DISCUSSION

The assessment of equivalence of locally manufactured and imported medicines available in the local market is critical to lower the risk of substandard medicines in the supply chain and guarantees interchangeability. This study investigated the equivalence of selected brands of BCS Class III drugs, namely, metformin hydrochloride 500mg tablets, cloxacillin sodium 500mg capsules, metoclopramide hydrochloride 10mg tablets, and enalapril maleate 5mg tablets available in Addis Ababa, Ethiopia. Five generic and comparator products of metformin hydrochloride tablets, two generic and comparator products of cloxacillin sodium capsules, metoclopramide tablets, and enalapril maleate tablets were included in this study.

5.1. Identification

One of the most significant tests that must be done in order to uniquely identify the API is the identification test. Identity testing of generic products of metformin hydrochloride, cloxacillin sodium, metoclopramide and enalapril maleate, and reference standards were determined prior to further experimental work.

The FTIR spectrum of working standard of metformin hydrochloride showed absorption bands at the following wave number: N-H absorption band are assigned to wave number 736 and 937cm^{-1}; C-N stretching at 1060 and 1080 cm^{-1} and C=N stretch vibration accounting for absorption band at 1583 and 1625 cm^{-1}. The IR spectrum of five generic products of metformin hydrochloride and their comparator products obtained absorption band at following wave number 736, 937, 1060, 1080, 1583 and 1625 cm^{-1}. Therefore, the results of the identification tests of metformin hydrochloride as shown in Annex (1 and 2), indicated that all products contained metformin hydrochloride as active pharmaceutical ingredients.

In the FTIR spectrum of pure cloxacillin sodium standard showed absorption band at following wave number, C-H stretching at 1456.16, 1375.15 and 1338.51cm^{-1}, C-N stretching at 1099.35 and 1247.86cm^{-1}, C=N vibration stretching1766.69cm^{-1} , N-O stretching at 1496.66cm^{-1}, C-Cl stretching at 846.69cm^{-1}. The IR spectrum of three generic products of cloxacillin sodium obtained absorption band at aformed wavenumber as showed in Annex 3.

The FTIR spectrum of pure metoclopramide hydrochloride showed major peaks at the following wavenumber; 3369.41cm^{-1}, 3394.48cm^{-1}, 1595.02cm^{-1}, 721.33cm^{-1} corresponding to the –NH stretching, -OH stretching C=O and C-Cl stretching respectively. The FTIR spectrum of all generic products metoclopramide hydrochloride showed an absorption band at the indicated wavenumber as shown in Appendix 4.

The FTIR spectrum of enalapril maleate showed a prominent absorption peak at 667.32cm^{-1} due to the C=C bend in the alkene, at 875.62 cm^{-1} due to the strong C-H bend and at 1645.17cm^{-1} due to C=O bending in amide. The FTIR spectrum of generic enalapril maleate products demonstrated an absorption peak at the appropriate wavenumber, as shown in Annex 5.

5.2. Physical properties

The USP specification specifies two approaches for determining dosage unit uniformity: weight variation and content uniformity. According to this standard, weight variation test for drugs with a strength greater than 324 mg, a tablet is suitable if the individual weight does not exceed 5% of the average weight and none of the drugs differ by 10%; For tablets with a strength of less than 130 mg, the individual weight must not differ by 10% from the average weight and for tablets with an average weight between 130mg and 324mg, it must not differ by 7.5%. The average weights of the tested products from all brands, apart from the products MTF1 (6.10%) and MTF2 (13.11%), were within the stated limits as illustrated in Table 4. However, there were differences between brands. This could be due to a variety of factors, including the die fill of the tablet press, which is influenced by factors such as compression machine tooling, spindle pressure, machine speed, and flow properties of the powder, powder or granule density, particle size distribution, and compression rate. Friability of tablets is another major source of variations in weight during compression (Rahman *et al.*, 2019, Othman *et al*., 2021). Variations in capsule weight are caused by non-GMP formulation practises such as capsule filling machine vibration, filling speed, particle size, air permeability, powder density, and environmental condition (Llusa *et al*., 2013). Variations in excipients used in formulation, such as diluents, binders, disintegrants, lubricants, glidants, and others, can also affect weight uniformity.

Table 4: Weight variation results of different products of metformin hydrochloride 500mg tablets, cloxacillin sodium 500mg capsules, metoclopramide hydrochloride 10mg tablets and enalapril maleate 5mg tablets.

Tablet name		**Mean weight (mg)±SD**	**Minimum weight deviation %**	**Maximum weight deviation %**	**Number of tablets out of specification**
Metformin hydrochloride	MTF1	649.62±20.68	0.06	6.01	2
	MTF2	633.00±22.96	0.47	13.11	2
	MTF3	655.56±8.87	0.69	2.36	None
	MTF4	637.50±18.32	0.39	3.53	None
	MTF5	560.09±9.12	0.18	1.60	None
	MTF6	522.01±11.05	0.38	4.21	None
Cloxacillin sodium	CLX1	643.93±14.33	0.07	2.64	None
	CLX2	679.87±18.52	0.40	2.76	None
	CLX3	672.23±20.95	0.40	4.70	None
Metoclopramide hydrochloride	MCP1	125.48±4.98	0.78	7.67	1
	MCP2	125.95±2.36	0.16	1.76	None
	MCP3	118.40±3.18	0.08	7.09	1
Enalapril maleate	ENA1	198.78±10.01	0.01	2.32	None
	ENA2	126.01±2.39	0.004	3.49	None
	ENA3	199.84±10.00	0.02	1.22	None

The hardness of a tablet determines its ability to withstand mechanical shock during handling, manufacture, packaging, transportation, and storage until patient use. The results of tablet hardness are shown in Table 5. There were large differences in hardness values between brands. Product MTF6 had the highest crushing strength (279.6 N) and product MTF2 had the minimum (161.5 N). The mean tablet hardness of metoclopramide hydrochloride tablets was found to be between 32.4N and 70.31N and that of enalapril maleate were between 58.8N and 60.7N. A force of 40N is the minimum requirements for satisfactory tablets hardness (Allen and Ansel, 2013).

All generic and comparator products of metformin hydrochloride tablets were found to have hardness values above 100N and only one product from metoclopramide tablets was found to have hardness value below 40N. However, all generic and comparator products of enalapril maleate tablets were found to comply with the requirement for satisfactory tablet hardness. In general, tablets should be firm enough not to break during normal handling, yet soft enough to disintegrate properly and release its active ingredient within a certain period of time (Allen and Ansel, 2013). The possible reason for the difference in hardness between the generic and comparator products may be related to the drug manufacturer's methods, such as change in the speed of device, particle size in granules mixture, and type and amount of lubricant (Birhanu *et al*., 2013, Allen and Ansel, 2013).

Table 5: Summary of hardness, friability, thickness, and diameter of the metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate.

Tablet name		**Hardness (N) ±SD**	**Friability %**	**Mean thickness ±SD**	**Mean diameter (mm) ±SD**	**Mean disintegration time(min) ± SD**
MTF	MTF1	239.70±17.92	0.11	4.66±0.09	17.89±0.06	7:42±0.12
	MTF2	163.40±10.86	0.13	5.21±0.03	16.41±0.03	11:24±0.88
	MTF3*	173.20±6.86	0.04	5.88±0.04	12.53±0.09	6:80±1.12
	MTF4	208.30±14.24	0.07	6.08±0.04	12.88±0.34	7:08±0.04
	MTF5	161.50±20.09	0.59	5.00±0.01	11.31±0.01	10:56±0.85
	MTF6	279.60±47.95	0.14	5.82±0.06	11.04±0.02	6:31±0.05
CLX	CLX1	-	-	-	-	6.96±0.45
	CLX2	-	-	-	-	5.86±0.34
	CLX3*	-	-	-	-	5.93±0.21
MCP	MCP1	70.31±16.13	0.54	3.38±0.11	6.28±0.024	10:80±0.71
	MCP2	60.70±5.79	0.06	3.18±0.21	7.10±0.026	3:63±0.52
	MCP3*	**32.40±5.42**	0.13	2.33±0.05	6.92±0.009	2:52±0.32
ENA	ENA1	60.70±9.84	0.13	3.01±0.03	7.85±0.014	5.56±0.24
	ENA2	72.50±6.95	0.37	3.63±0.08	6.93±0.014	3.04±0.08

	ENA3*	58.80±10.21	0.56	3.48±0.04	7.21±0.018	1.40±0.08

*comparative products

Tablet hardness and friability, which also contribute to tablet weight variations and content uniformity and also necessary for tablet appearance and consumer acceptance. As tablet hardness increases, the percentage of friability in the formulation decreases. The harder the tablets, the lower the percentage friability and vice versa (Mosharraf, 2012). The friability test results of products investigated ranged from 0.11% to 0.59%, 0.132% to 0.542%, and 0.130 to 0.546% for MTF, MCP, and ENA generic and comparator products tested, respectively as shown in Table 5. According to USP specifications for friability, a maximum weight loss of 1% or less of tablet weight is generally considered acceptable. All products tested were found to comply with this specification.

As showed thickness measurement results in Table 5. Accordingly, the tablet thickness range values for metformin hydrochloride, metoclopramide hydrochloride, and enalapril maleate generic and comparator products tested were found to be from 4.66 to 6.08 mm, 2.33 to 3.38 mm, and 3.01 to 3.48 mm, respectively. The uniformity of tablet thickness is required not only for consumer requirements but also for packaging ± 5% variation is permissible (Battah, 2021). Differences in tablet size (i.e., weight, diameter, and thickness) may have negative impact on clinician and patients because they could raise doubt regarding interchangeability of the generic and comparator products (Okoye and Iwuagwu, 2010).

Disintegration is the breaking up of tablets into small particles when they come into contact with digestive fluids, which resulted by the breaking of the inter-particle bond that hold the particle together (Okor *et al*., 1998). This is a critical step because the rate of disintegration determines dissolution, which influences drug therapeutic efficacy (Sultana *et al*., 2017). All generic and comparator products tested were found to comply with the USP, 2021 specifications for mean disintegration times of less than 15 minutes for immediate release tablets and less than 30min for film coated tablets as shown in Table 5. The MTF6 product had a fast disintegration time (6.31 min), whereas the MTF2 product had a slow mean disintegration time (11.24 min). Disintegration time variations may be caused by formulation parameters such as the type and amount of disintegrants,

binders, and lubricants, as well as processing variables such as granulation method and compression force (Nigatu *et al*., 2019).

5.3. Assay and content uniformity

The assay and content of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate were determined based on the calibration curve generated (Fig. 6-9) respectively.

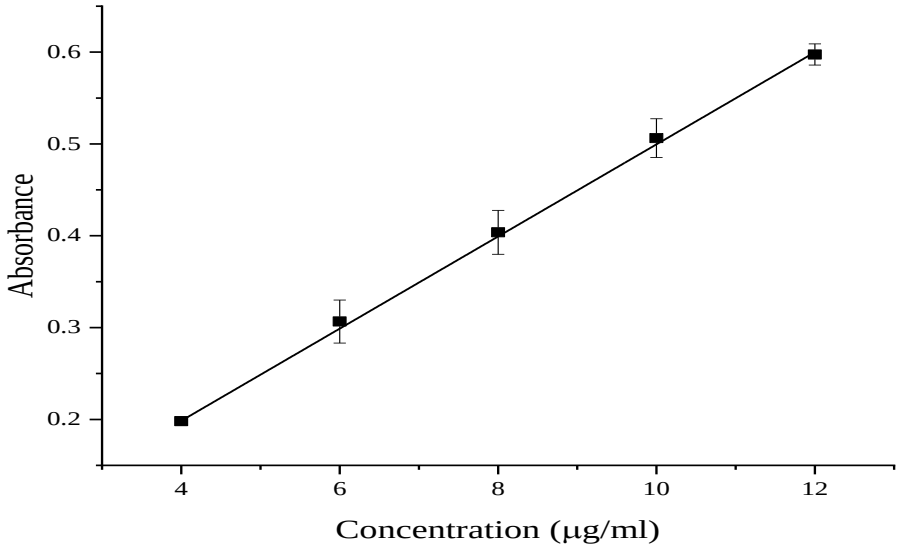

Figure 6: Beer-Lambert calibration curve of metformin hydrochloride in distilled water at maximum wavelength 232nm over the range of 4 to 12µg/ml.

Fig. 6 depicted the derived linear regression equations. The regression equation was y=0.0502x-0.0022, where, y is absorbance and x is where y is the absorbance and x is concentration in µg/ml. The correlation coefficient of R^2=0.9997 indicated a good linear correlation between the concentration of test sample and absorbance.

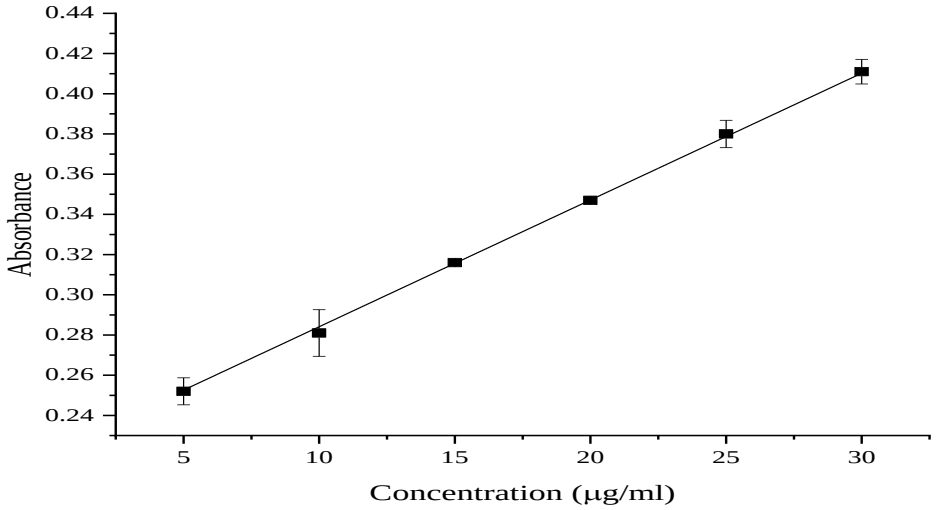

Figure 7: Beer-Lambert calibration curve of cloxacillin sodium in methanol at maximum wavelength 222nm over the range of 5 to 30µg/ml.

Fig. 7 depicted the derived linear regression equations. The regression equation y=0.016x +0.045, where, y is the absorbance and x is the conc. in µg/ml. The value of correlation coefficient R^2=0.9996 indicated a linear correlation between concertation of test sample and absorbance.

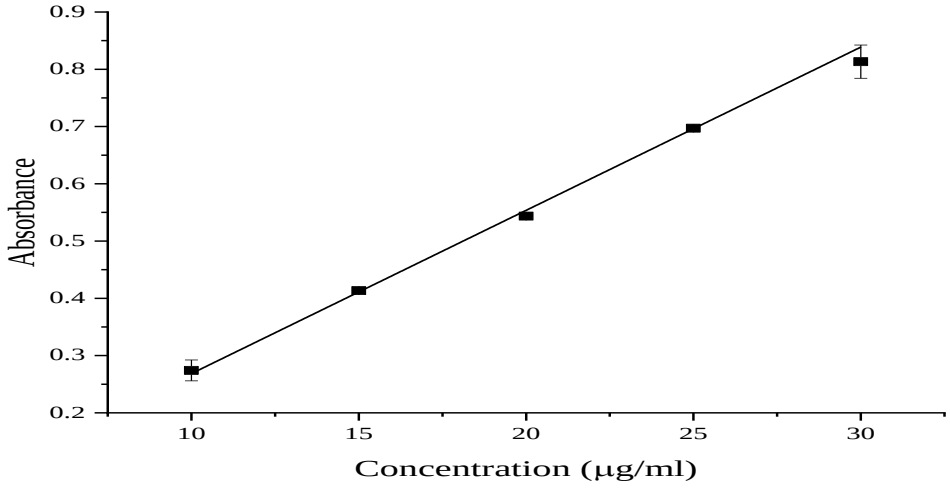

Figure 8: Beer-Lambert calibration curve of metoclopramide hydrochloride in 0.1N hydrochloride at maximum wavelength 309nm over the range of 5 to 30µg/ml.

Fig. 8 depicted the derived linear regression equations. The regression equation was y=0.0285x-0.0169, where, y is the absorbance and x is the conc. in µg/ml. The value of correlation coefficient R^2=0.999 indicated a good linear correlation the concentration test sample and absorbance.

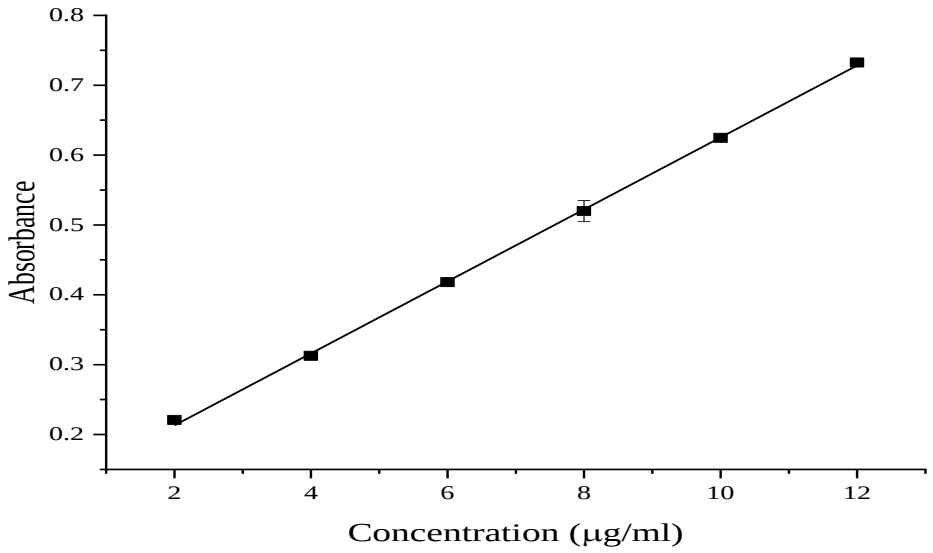

Figure 9: Beer-Lambert calibration curve of enalapril maleate in HCl sodium phosphate buffer (PH=4) at maximum wavelength 208nm over the range of 2 to 12µg/ml.

Fig. 9 depicted the derived linear regression equations. The regression equation was y=0.051x+0.011, where, y is the absorbance and x is the conc. in µg/ml. The value of correlation coefficients R^2=0.9998 indicated a good linear correlation between conc. of test sample and absorbance.

The result obtained from the assay and content uniformity study for the generic and comparator products of six metformin hydrochloride tablets, three for each generic and comparator products of; cloxacillin sodium capsules, metoclopramide hydrochloride tablets, and enalapril maleate tablets is indicated in Table 6.

Table 6: Percentage of drug assay and content (mean ± SD) of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride and enalapril maleate and their comparator counter products (n=15).

Tablet name	**Code**	**Assay (%±SD)**	**Content uniformity (%±SD)**
Metformin hydrochloride	MTF1	99.73±1.50	99.73±0.03
	MTF2	99.74±0.78	99.85±0.06
	MTF3*	99.63±1.70	99.75±0.03
	MTF4	99.80±2.10	99.85±0.07
	MTF5	99.66±3.20	99.86±0.09
	MTF6	99.66±0.19	99.80±0.04
Cloxacillin sodium	CLX1	96.18±2.20	93.74±1.52
	CLX2	96.29±0.17	106.06±1.82
	CLX3*	94.21±0.85	91.52±0.61
Metoclopramide hydrochloride	MCP1	100.30±0.07	99.93±0.09
	MCP2	101.61±0.02	99.67±0.04
	MCP3*	99.95±0.02	99.48±0.11
Enalapril maleate	ENA1	100.38±0.18	99.92±0.13
	ENA2	97.03 ±1.08	100.32±0.76
	ENA3*	100.42±0.23	99.72±0.06

*Comparator product

According to USP specification, BCS class III drugs investigated in this study; content should not be less than 90% and not more than 110% of the labelled claim of metformin hydrochloride, enalapril maleate tablets, and metoclopramide hydrochloride. The USP specification for cloxacillin sodium capsules ranged from 90 to 120%. Therefore, all brands demonstrated assay and content consistency results to be within this specification.

5.4. Dissolution profiles

Dissolution profile of solid oral dosage forms such as tablets and capsules is an important *in vitro* quality control standard for drug development, identifying key manufacturing variables, and monitoring product quality It is also prerequisite for drug bioavailability study and an alternative approaches for predicting bioequivalence

between pharmaceutically equivalent drug products (Anand *et al*., 2011, Morais and Lobato, 2010). To determine whether the differences in dissolution profiles were significant, the dissolution profiles of the generic products were compared with that of the comparator product.

Metformin hydrochloride 500 mg tablets

Calibration curve was plotted (Fig. 10) using the value of absorbance against corresponding conc.

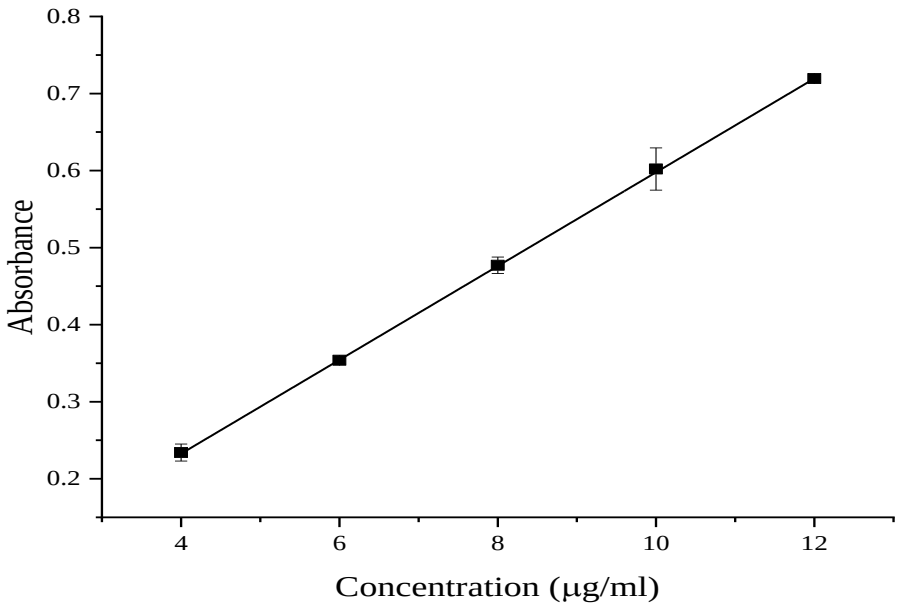

Figure 10: Beer-Lambert calibration curve of metformin hydrochloride in phosphate buffer (PH=6.8) at maximum wavelength 232nm over the range of 4 to 12µg/ml.

Fig. 10. Depicted the derived linear regression equations. The regression equation was y=0.061x-0.011, where, y is the absorbance and x is the conc. µg/ml. The value of correlation coefficient R^2=0.9999 indicated a good linear correlation between the conc. of the test sample and absorbance.

According to (USP, 2021j) specifications, metformin hydrochloride must release “not less than 80% of the labile claim within 30min”. The results of tablet dissolution tests can show how formulation ingredients affect a drug's *in vivo* performance. Quick content

release from the dosage form is required for metformin hydrochloride to lower glucose levels in the short term. In this study, out of six generic and comparator products of metformin hydrochloride tested four passed the specifications of the single-point dissolution test as indicated below in Fig. 11 and Annex 6. Of the products tested, all brands except MTF2 (14.53%) were fast (> 20%) in releasing content within 5 min. At the end of 30 min, MTF5 released maximum drug content (91.46%). However, MTF2 and MTF4 exhibited lower release of 62.66% and 78.71%, respectively and failed to meet dissolution test specifications. MTF2 did not release 80% of the specified amount until 60 min. A medication having a poor dissolution profile may not be completely available in the body to achieve the desired therapeutic effect (Moore and Flanner, 1996). All brands of metformin had high compressive strengths (> 100 N), but their disintegration times were less than 10 min, except for MTF2 (11.24 minutes) and MTF5 (10.56 min). From MTF products investigated except MTF2 and MTF4, the rest of all MTF brands showed the expected dissolution characteristics. All of these characteristics are a consequence of the manufacturing process, formulation variables, and the properties of all of the ingredients in the formulation.

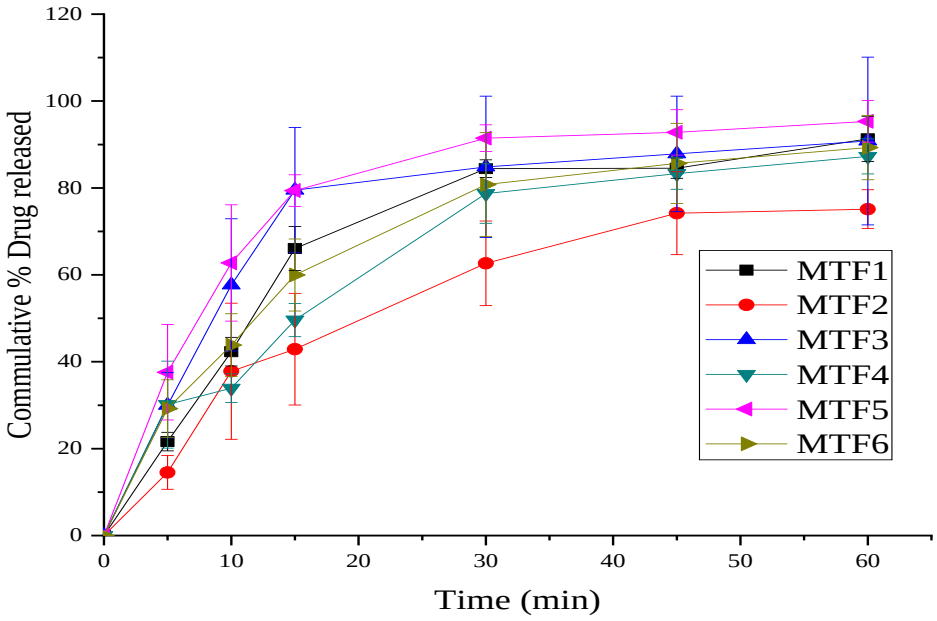

Figure 11: Dissolution profiles of five generic products of metformin hydrochloride tablets and comparator product (MTF3) in phosphate buffer pH=6.8 at maximum wavelength 232nm and 37±0.5^{o}c

Dissolution of cloxacillin sodium 500 mg capsules

Fig. 12 shows the calibration curve of cloxacillin sodium conc. against absorbance.

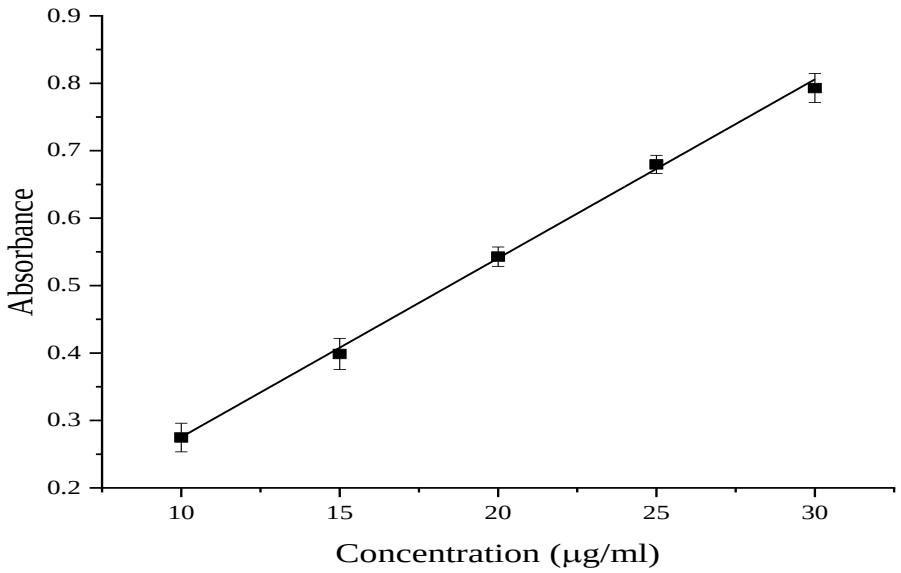

Figure 12: Beer-Lambert calibration curve of cloxacillin sodium in distilled water at maximum wavelength 220nm over the range of 10 to 30µg/ml.

Fig. 12 depicted the derived linear regression equations. The regression equation was y=0.026x+0.0099, where, y is the absorbance and x is the conc. µg/ml. The value of correlation coefficient R^2=0.998 indicated a good linear correlation between the conc. of the test sample and absorbance.

The dissolution profiles of different generic and comparator products of cloxacillin sodium capsules are shown in Fig. 13 and Annex 7. All tested products release over 40% of their content within 5 minutes. After 30 minutes, all generic and comparator products released over 85% and meet dissolution specification. Similarly, all generic and comparator products of cloxacillin sodium capsules included in this study (CLX1, CLX2, and CLX3) exhibited faster disintegration times (<7 minutes), exhibited faster dissolution rates, and complied with USP dissolution specification. All brands investigated in this study showed a nearly similar dissolution profile. Therefore, they can be used interchangeably.

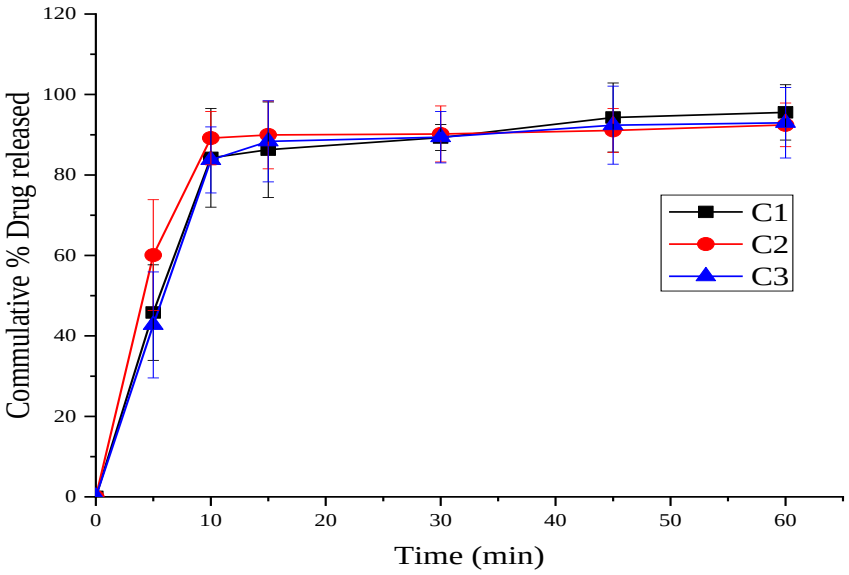

Figure 13: Dissolution profiles of two generic product of cloxacillin sodium capsules and comparator product (CLX3) in distilled water at maximum wavelength 220nm and 37±0.5°c.

Metoclopramide hydrochloride 10 mg tablets

The calibration curve of Metoclopramide hydrochloride is displayed in Fig. 14.

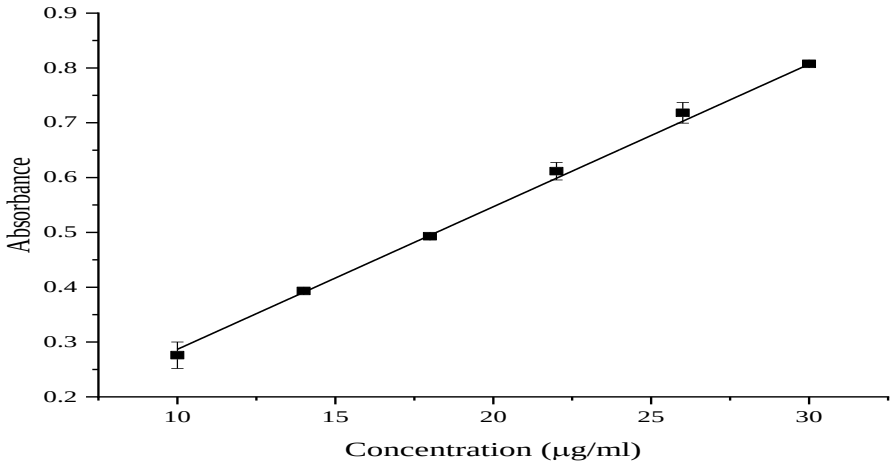

Figure 14: Beer-Lambert calibration curve of metoclopramide hydrochloride in in distilled water at maximum wavelength 309nm over the range of 5 to 30µg/ml.

Fig. 14 depicted the derived linear regression equations. The regression equation was y=0.027+0.014, where, y is the absorbance and x is the conc. µg/ml. The value of correlation coefficient R^2=0.9993 indicated a good linear correlation between the conc. of the test sample and absorbance.

According to USP specification, the release of metoclopramide hydrochloride from the tablet formulation should not less than 75% of label claim within 30min. All generic and comparator products of metoclopramide hydrochloride products under investigation complied with the USP dissolution tolerance limit.

The results in Table 6, Figure 15, and Annex 8 revealed a significant variation in the disintegration time and dissolution profile of generic and comparator products. Among tested products MCP3 showed relatively faster release (68.31%) of their drug content within 5min. it might be associated with tablet hardiness (<40N), and fast disintegration time (2.52min). Drug dissolution can be influenced by the physicochemical properties of the API, dosage form design, manufacturing process, formulation variables, and testing conditions (Kassahun *et al*., 2019)**.**

MCP2 and MCP3 exhibited faster disintegration times (<4 minutes), whereas MCP1 exhibited longer disintegration times (10.80 minutes). All brand release >80% of drug content at the end of 30min. However, all products investigated showed significant dissolution profile difference $p < 0.05$ and their f_2 value less than 50 and didn't release claimed amount until the end of 60 min.

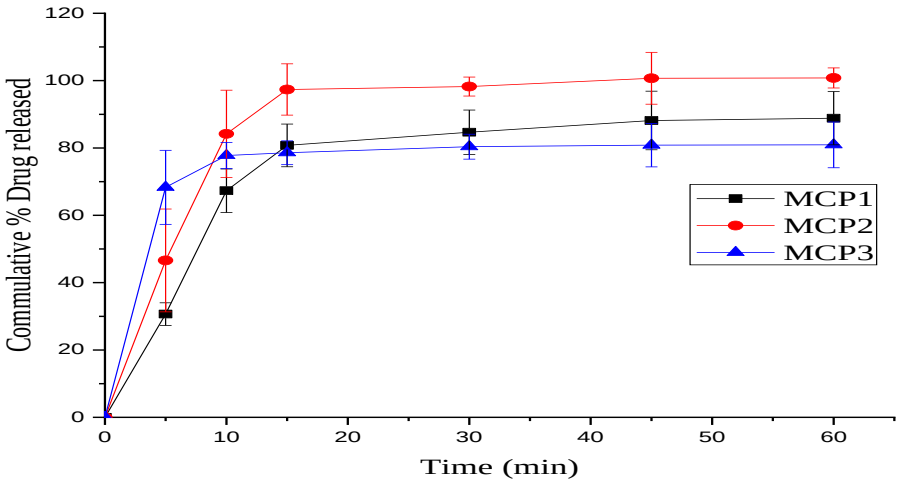

Figure 15: Dissolution profiles of two generic products of metoclopramide hydrochloride tablets and comparator product (MCP3) in distilled water at maximum wavelength 309nm and 37±0.5^{o}c.

Enalapril maleate 5mg tablets

The calibration curve of Enalapril maleate conc. against absorbance is shown in Fig. 16.

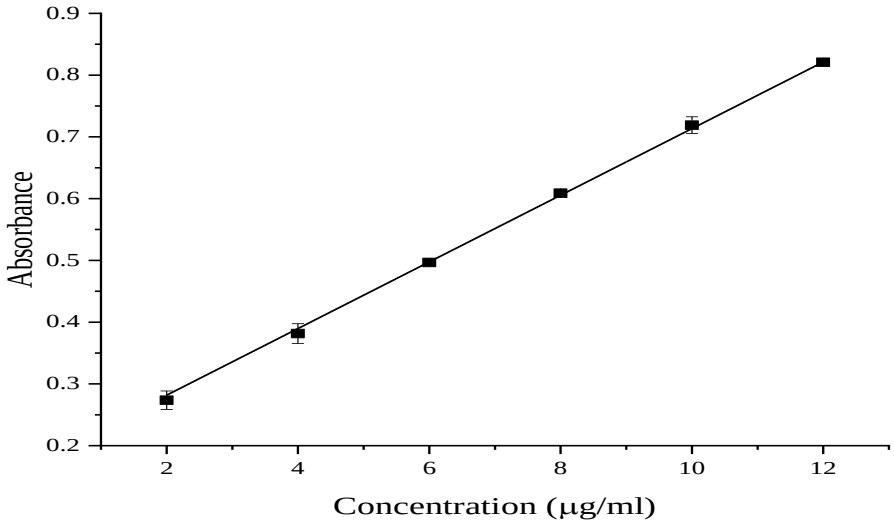

Figure 16: Beer-Lambert calibration curve of enalapril maleate in phosphate buffer (PH 6.8) at maximum wavelength 208nm over the range of 2 to 12µg/ml.

Fig. 16 depicted the derived linear regression equations. The regression equation was y=0.054x+0.174, where, y is the absorbance and x is the conc. µg/ml. The value of correlation coefficient R^2=0.9998 indicated a good linear correlation between the conc. of the test sample and absorbance.

According to USP specifications, the release of enalapril maleate tablet contents should not less than 80% of the labelled claim within 30min. Therefore, all generic products and their comparator product of enalapril maleate tablets included in this study complied USP dissolution limits. In this study, ENA2 and ENA3 relatively had the lowest disintegration times (3.04 and 1.40 min respectively) and faster dissolution profiles as compared to ENA1 disintegration time (5.56 min). However, the dissolution profiles of enalapril maleate tablets are shown in Fig. 17 and Annex 9, all products had similar dissolution profiles, and which are statistically insignificant (p > 0.05).

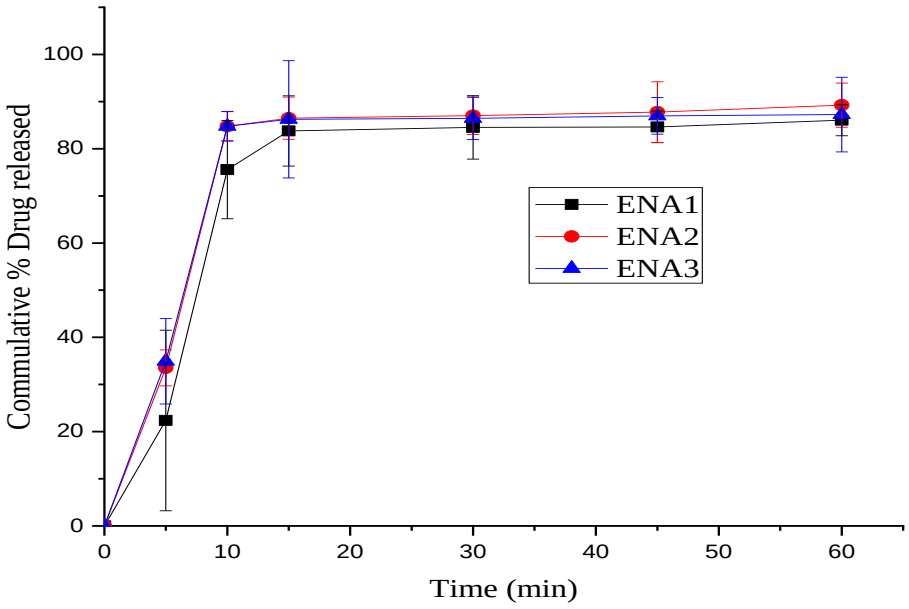

Figure 17: Dissolution profiles of two generic products of enalapril maleate tablets and comparator product (ENA3) in phosphate buffer pH=6.8 at maximum wavelength 208nm and 37±0.5^{o}c.

5.5. *In vitro* drug release

In vitro drug release studies of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate were performed using a dialysis bag technique based on established calibration curves. The calibration curve regression equations were Y=0.077x+0.00413, R^2=0.9998, Y=0.0423x-0.0463, R^2=0.9989, y=0.0342x+0.0261, R^2=0.9994 and Y=0.055x+0.015. R^2=0.99937, respectively.

Reports on bioequivalence between water-soluble solid formulations delivered orally can be important since they indicate that absorption limitation is caused by differences in the formulation approaches of immediate release dosage forms (EMA, 2010). If a biowaiver is employed for BCS class III drug substance, the excipients must be qualitatively very similar and quantitatively same to the reference product. Excipients like sorbitol, mannitol, sodium lauryl sulphate, and other surfactants can reduce bioavailability (Wu and Benet, 2005, Verbeeck and Musuamba, 2012). Proof of complete absorption in the human body is desirable for the application of BCS based biowaiver. However, well-conducted in vitro permeability studies, including a reference standard, can be utilised to support in vivo performance dat.

In vitro, drug release assays for metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate were measured using the diffusion-in-dialysis bag (cellulose membrane) technique. The absorbance of samples taken from the receiving compartment as a function of time was measured using distilled water and phosphate buffer (pH = 6.8). Release of the drug through the membrane (dialysis bag) into the receiving compartment was measured within 2 h in sink condition. A calibration curve, absorbance against time, was used to calculate the conc. of the diffusing drugs.

In vitro, drug release profile of metformin hydrochloride is depicted in Fig. 18 and Annex 10. Products with higher dissolution profiles (MTF 3 and MTF 5) resulted in higher membrane diffusion profiles. This indicates that drugs with higher dissolution profiles also have higher membrane diffusion due to longer membrane contact times. However, brands of MTF4 and MTF5 showed significant differences from the reference standard ($p<0.05$, 0.024, and 0.046, respectively).

Generic and comparator products of CLX with a fast dissolution profile exhibits a higher membrane diffusion profile as shown in Fig. 19 and Annex 11. Apart from CLX1, both CLX2 and CLX3 showed a significant difference in membrane diffusion from their reference standard $p < 0.05$.

All MCP generic and comparator products described in Table 9 showed significant differences in dissolution profile ($P < 0.05$). Similarly, all MCP generic and comparator products except MCP1 ($p = 0.064$) showed significant differences in the drugs' membrane diffusion profiles compared to their reference standard ($P < 0.05$), as shown in Fig. 20 and Annex 14.

All generic and comparator products of ENA tested revealed similar dissolution profiles. However, significant differences ($p<0.05$) in *in vitro* drug release profiles were observed. It is well-known that the partition coefficient (log P value) influences drug permeability through cell membranes, in addition to other parameters such as molecular size and conc. gradient (Chakrapani *et al.*, 2008, Rautio *et al.*, 2008). There are several strategies are available to overcome permeability problems, including traditional methods like prodrugs, permeation enhancer, and ion paring, as well as novel techniques such as Nano encapsulation and Nano sizing to enhance permeability of BCS C lass drug (Dave *et al.*, 2017). Enalapril is a pro-drug that exhibits a higher drug diffusion profile as described in Fig. 21 and Annex 13.

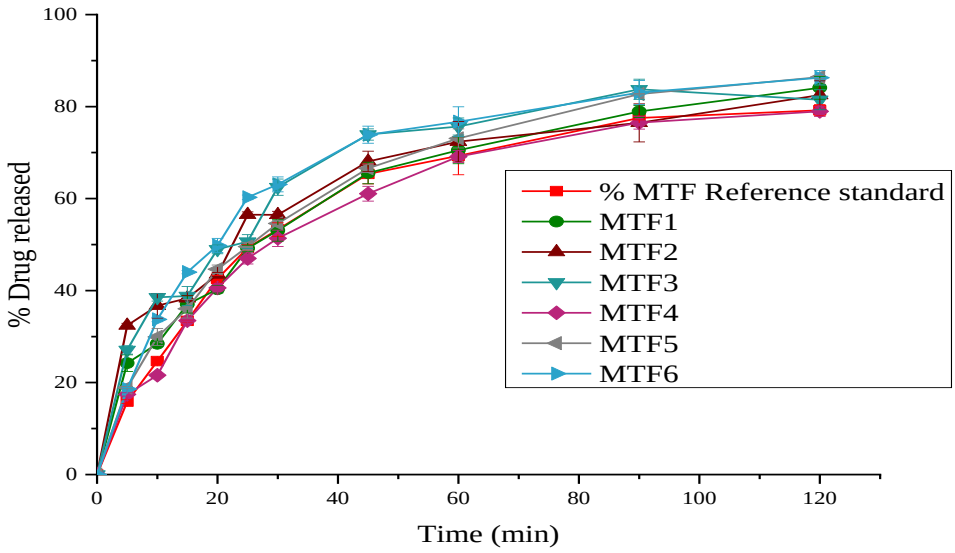

Figure 18: Drug release profiles of six products of metformin hydrochloride 500mg tablets and reference standard powder in phosphate buffer (pH=6.8) at maximum wavelength 232nm and 37±0.5°C.

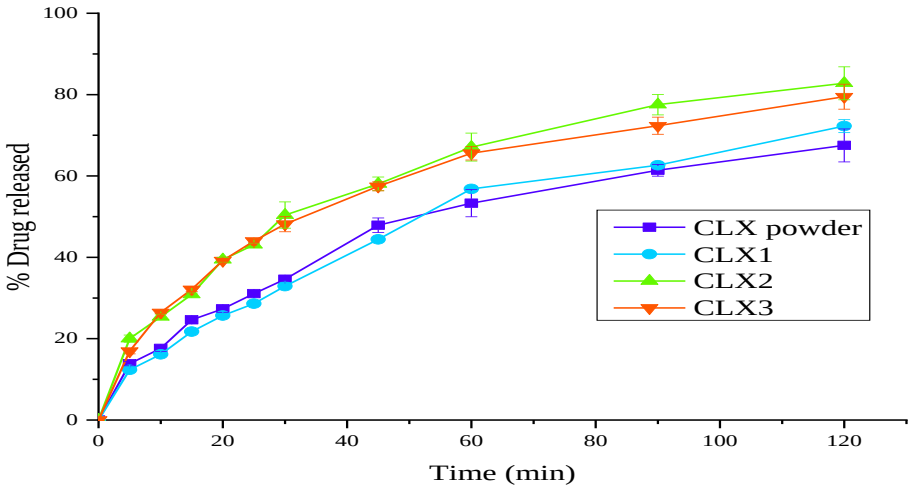

Figure 19: Drug release profiles of three products of cloxacillin sodium 500mg capsule and working reference standard powder in phosphate buffer (pH=6.8) at maximum wavelength 220nm and 37±0.5°C.

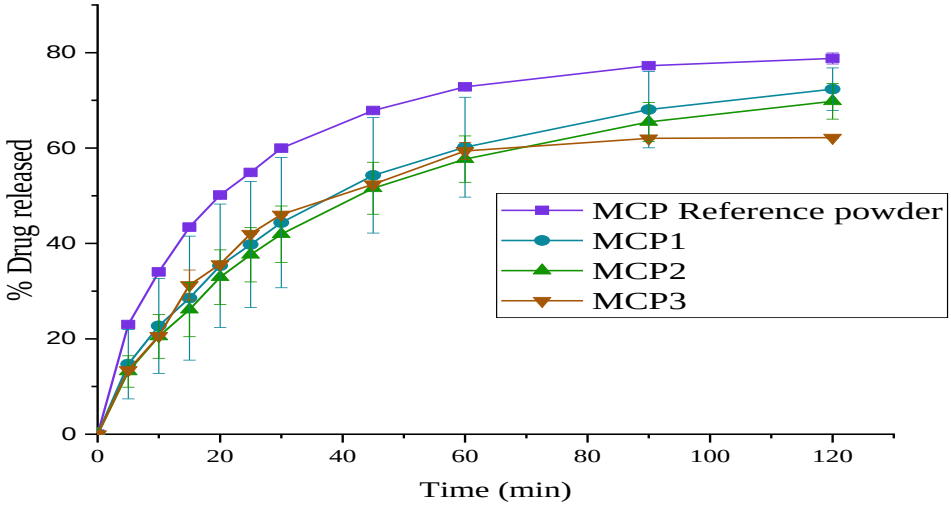

Figure 20: Drug release profiles of three products of metoclopramide 10mg tablets and working reference standard powder in distilled water at maximum wavelength 309nm and 37±0.5°C.

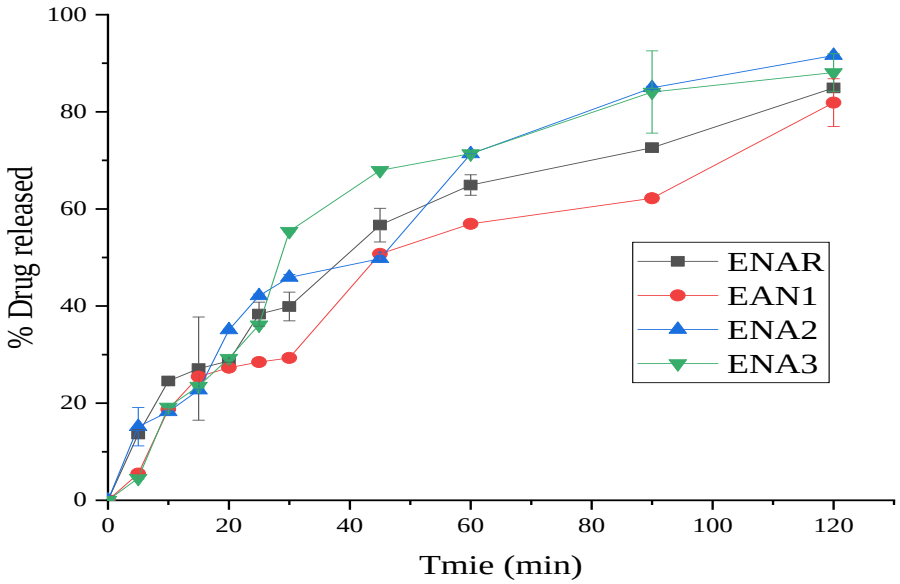

Figure 21: Drug release profile of three products of enalapril maleate 5mg tablets and working reference standard powder in phosphate buffer (pH=6.8) at maximum wavelength 208nm and 37±0.5^{o}C.

Dissolution profile comparison

To compare *in vitro* dissolution profiles, three mathematical approaches are available: model independent, statistical, and model dependent (Food and Drug Administration., 1997) and (EMA, 2010). To compare the dissolution profiles and determine equivalence, model independent, statistical, and model dependent approaches were employed in this study.

If the dissolution profile has been successfully characterised using enough sampling time points, the dissolution profile similarity testing can be considered as acceptable. For BCS Class I drug substance, the dissolution profile can be assumed similar without further calculation for products that dissolve rapidly (greater than 85% of the labile amount is dissolved within 15 minutes). If the requirements are not fulfilled, the similarity factor f2 is the parameter of choice by both the FDA and the EMA due to its simplicity. For BCS Class III drug substance, both reference and test products should display vary rapidly dissolving (≥85% within ≤15 minutes) *in vitro* dissolution characteristics under the defined conditions. According to this guideline, f2 is more accurate for determining

sample similarities; an f2 value in the range of 50-100 indicates that the dissolution profiles are similar. However, as per f2, dissolution profiles are considered dissimilar if the mean percentage of the released drugs at each time point differs by 10% between the test (T) and reference (R) products. In this case, f2 is always less than 50, while f1 describes the dissolution profile difference based on sample times. To be considered for similar dissolution behaviour, the f1 values must be in the range of 0-15 (FDA, 1997).

The results of the model-independent evaluation of products included under investigation are presented in Table 7. Apart from two brand of metformin hydrochloride, MTF1 = 51.90 and MTF5 = 63.18, all brands of metformin hydrochloride and metoclopramide hydrochloride brands investigated didn`t meet the above specification. However, all brands of cloxacillin sodium capsules and enalapril maleate tablets included in this study were specification-compliant and can be used interchangeably. From the forgoing, it is apparent brands of the same drug exhibited different drug release profiles, and this can make a difference in drug bioavailability and therapeutic efficacy. As stated earlier, the difference in drug dissolution profiles is due to difference in formulation variables such as type of diluent, conc. of binder, size of granules, distribution and conc. of lubricant, physical properties of the drugs, etc) and processing variables like mixing time, granulation methods and compression force (Esezobo and Pilpel, 1976, Chalmers and Elworthy, 1976).

Dissolution efficiency up to 60 min was also calculated from dissolution profiles of all products to ensure interchangeability between generic and comparator products. As reported, the reference/comparator and test products can be considered equivalent if the difference in dissolution efficiency is less than 10% (Anderson et al., 1998). Based on this requirement, all products investigated are equivalent to their comparator product as the differences (test product – reference product) were < 10%.

Furthermore, dissolution data corresponding to sampling times such as t50% and t90% were also employed to compare dissolution profiles of the products investigated. t50% and t90% describe the time required for 50% and 90% of the drugs to be released, respectively (Kassahun, 2019).

The t50% and t90% of the metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate are given in Table 8. Accordingly,

MTF5 (t50% = 6.62 min, t90% = 25.98 min) and all brands of CLX, MCP, and ENA released their content rapidly. Therefore, it can be expected that these products would be absorbed and become available rapidly. MTF2 and MTF4 products had relatively a longer time (t50% = 20.20 min and t90% = 96.62 min) and (t50% = 14.3 0min and t90% = 59.61min), respectively. Hence these products may have lower bioavailability in the body. Even though MCP3 showed fast release of its contents at (t50%=0.06 min), it takes longer time to reach t90% = 452.08 min.

Table 7: Model-independent approaches f1, f2 and DE of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate tablets investigated.

BCS class III drugs		**f1**	**f2**	**DE%**	**Difference % DE**	**Remarks**
Metformin hydrochloride	MTF_1	6.71	51.90	76.79	5.89	Similar
	MTF_2	28.66	32.88	73.37	9.31	Dissimilar
	MTF_3	*R_b	*R_b	82.68	-	*R_b
	MTF_4	15.79	39.80	74.49	8.19	Dissimilar
	MTF_5	4.94	63.18	83.47	0.79	Similar
	MTF_6	9.55	49.94	77.11	5.57	Dissimilar
Cloxacillin sodium	CLX_1	1.47	*82.45*	86.93	1.62	Similar
	CLX_2	1.97	*56.13*	91.18	2.63	Similar
	CLX_3	*R_b	*R_b	88.55	-	*R_b
Metoclopramide hydrochloride	MCP_1	19.80	*37.04*	85.47	3.28	Dissimilar
	MCP_2	13.55	*41.56*	88.75	4.77	Dissimilar
	MCP_3	*Rb	*Rb	93.52	-	*Rb
Enalapril maleate	ENA_1	3.67	58.89	87.36	2.73	Similar
	ENA_2	1.07	91.67	88.66	1.43	Similar
	ENA_3	*R_b	*R_b	90.09	-	*R_b

*R_b: Comparator products

Table 8: Dissolution parameter (t50% MDT, t80% and t90%) of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride and enalapril maleate and their comparator products

BCS class III drugs		**Time**			
		t50% (min)	**MDT (min)**	**t80% (min)**	**t90% (min)**
Metformin hydrochloride	MTF_1	11.78	17.44	29.03	42.61
	MTF_2	20.20	32.58	**60.58**	96.62
	MTF_3*	7.98	12.58	22.73	35.45
	MTF_4	14.30	22.12	**38.95**	**59.61**
	MTF_5	6.62	10.36	17.05	25.98
	MTF_6	11.50	18.22	**33.09**	51.84
Cloxacillin sodium	CLX_1	5.15	7.08	10.70	14.60
	CLX_2	1.87	3.93	10.34	21.43
	CLX_3*	5.60	7.29	10.25	13.26
Metoclopramide hydrochloride	MCP_1	6.91	11.26	21.19	34.11
	MCP_2	5.33	6.74	9.13	11.47
	MCP_3*	**0.06**	**0.93**	**31.96**	**452.08**
Enalapril maleate	ENA_1	7.65	9.28	11.94	14.42
	ENA_2	6.38	7.93	10.20	12.45
	ENA_3*	6.27	7.77	10.27	12.67

*comparator products

To evaluate statistical differences between generic and comparator product dissolution profiles, one way analysis of variance (ANOVA) was employed. Dennett's test was employed to determine the differences. The dissolution profiles of metformin hydrochloride (MTF2) and metoclopramide hydrochloride (MCP1 and MCP2) differed significantly (95% CI, $P<0.05$) to the comparators at 30 and 60 minutes. However, at 30 and 60 minutes, cloxacillin sodium or enalapril maleate products differ significantly (95% CI, P 0.05) from their respective comparator products.

Table 9: Dennett's test results of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride and enalapril maleate and their comparator products at 0.05 levels and 95% confidence interval.

BCS class III drugs	Time (min)	Pair comparison	Mean difference (I-J)	Std. Error	Sig.
Metformin hydrochloride	30	MTF_1 Vs MTF_3	-0.38608	3.95480	1.000
		MTF_2 Vs MTF_3	-22.1771*	3.95480	**0.000**
		MTF_4 Vs MTF_3	-6.13433	3.95480	0.396
		MTF_5 Vs MTF_3	6.61792	3.95480	0.325
		MTF_6 Vs MTF_3	-4.06108	3.95480	0.756
	60	MTF_1 Vs MTF_3	0.47525	3.77563	1.000
		MTF_2 Vs MTF_3	-15.6887*	3.77563	**0.000**
		MTF_4 Vs MTF_3	-3.52650	3.77563	0.816
		MTF_5 Vs MTF_3	4.52042	3.77563	0.637
		MTF_6 Vs MTF_3	-1.51867	3.77563	0.993
Cloxacillin sodium	30	CLX_1 vs CLX_3	2.56071	2.35218	0.454
		CLX_2 vs CLX_3	-0.52571	2.35218	0.964
	60	CLX_1 vs CLX_3	1.90242	2.91960	0.741
		CLX_2 vs CLX_3	-2.41271	2.91960	0.624
Metoclopramide hydrochloride	30	MCP_1 vs MCP_3	-17.8455*	1.89933	**0.000**
		MCP_2 vs MCP_3	-9.38202*	1.89933	**0.000**
	60	MCP_1 vs MCP_3	-19.8265*	2.55525	**0.000**
		MCP_2 vs MCP_3	-16.0927*	2.55525	**0.000**
Enalapril maleate	30	ENA_1 vs ENA_3	-0.39818	2.12753	0.975
		ENA_2 vs ENA_3	0.57000	2.12753	0.949
	60	ENA_1 vs ENA_3	-2.62364	2.30081	0.423
		ENA_2 vs ENA_3	1.98818	2.30081	0.598

*The mean difference is significant at the $P < 0.05$ level.

a. Dennett t-tests treat one group as a control and compare all other groups against it.

Different kinetic models were fitted to the dissolution data of locally manufactured products and comparator product to explain the overall release mechanism of the drug

from the dosage forms. The model with the highest correlation coefficient (r2) value after fitting to specified units of dissolution data is considered to be the best fit for the release data (Shah *et al*., 1997).

As shown in Tablet 10, the Weibull model gave the best fit to the dissolution data of Metformin hydrochloride tablets of the five models fitted. Therefore, it can be declared that all products of metformin hydrochloride tablets under study revealed the same release mechanism. As shown in Table 10, Michaels Menten with lag exhibited the best fit to the dissolution data of all cloxacillin sodium capsules, metoclopramide hydrochloride, and enalapril maleate tablets products.

Table 10: Determination of correlation coefficient of different release kinetics model of six products of metformin hydrochloride tablets, three products of cloxacillin sodium capsules, metoclopramide hydrochloride tablets and enalapril maleate tablets and their comparator brands.

Products	**Zero order R^2**	**First order R^2**	**Hixson-Crowell R^2**	**Weibull R^2**	**Michaelis Menten with lag R^2**
MTF_1	0.852	0.677	0.744	**0.982**	0.936
MTF_2	0.754	0.634	0.772	**0.966**	0.883
MTF_3	0.626	0.531	0.563	**0.939**	0.929
MTF_4	0.872	0.824	0.842	**0.958**	0.473
MTF_5	0.679	0.591	0.621	**0.949**	0.894
MTF_6	0.836	0.748	0.780	**0.985**	0.764
CLX_1	0.484	0.422	0.442	0.814	**0.985**
CLX_2	0.340	0.325	0.329	0.623	**0.997**
CLX_3	0.406	0.363	0.376	0.720	**0.993**
MCP_1	0.492	0.477	0.482	0.769	**0.946**
MCP_2	0.533	0.446	0.473	0.810	**0.975**
MCP_3	0.440	0.396	0.410	0.913	**0.976**
ENA_1	0.292	0.281	0.284	0.551	**0.999**
ENA_2	0.358	0.316	0.328	0.623	**0.998**
ENA_3	0.358	0.297	0.303	0.584	**0.999**

R^2: Correlation coefficient

6. CONCLUSION

In this study, a comparative *in vit*ro equivalence assessment of BCS class III drugs, namely: metformin hydrochloride 500mg tablets, metoclopramide hydrochloride 10mg tablets, cloxacillin sodium 500 mg capsules and enalapril maleate 5 mg tablets marketed commercially in Ethiopia was made. The quality control physical parameters for these products such as weight variation, hardness, and friability, disintegration time, assay test, dissolution time and *in vitro* release were determined according to the specifications of USP, 2021 and validated methods in various studies.

All BCS Class III drugs investigated in this study fulfilled USP thickness, hardness, friability, weight uniformity, and disintegration time specifications. There was no direct association between tablet hardness, disintegration time, and dissolution time. Although the crushing strength of metformin hydrochloride tablets was over 100 N, they disintegrated completely within 15 min, whereas a product of metoclopramide hydrochloride had a crushing strength of less than 40 N but friability of less than 1%.

With the exception of MTF2 and MTF4, all other metformin hydrochloride products, as well as all metoclopramide hydrochloride tablets, cloxacillin sodium capsules, and enalapril maleate tablets, met the USP dissolution test limit (greater than 80% dissolved within 30min). Except one product of metformin hydrochloride, all products didn't showed statistically significant difference ($P<0.05$) in dissolution profile. However, all metoclopramide products revealed a statistically significant difference ($P<0.05$). All generic cloxacillin sodium capsules and enalapril maleate tablets demonstrated comparable dissolution profile to their comparator counter product. Therefore, all except five of the 15 generic BCS Class III drugs products investigated met USP dissolution test specifications, met FDA requirements of $f1 < 15$ and $f2 > 50$, were statistically insignificant ($P>0.05$), and can be considered as equivalent.

Suggestion for the further work

The *in vivo* bioavailability of the investigated products should be studied to obtain their complete picture on their bioequivalence and clinical efficacy.

The *in vivo* permeability of investigated products should be demonstrated to be able to obtain permeability profiles for these products.

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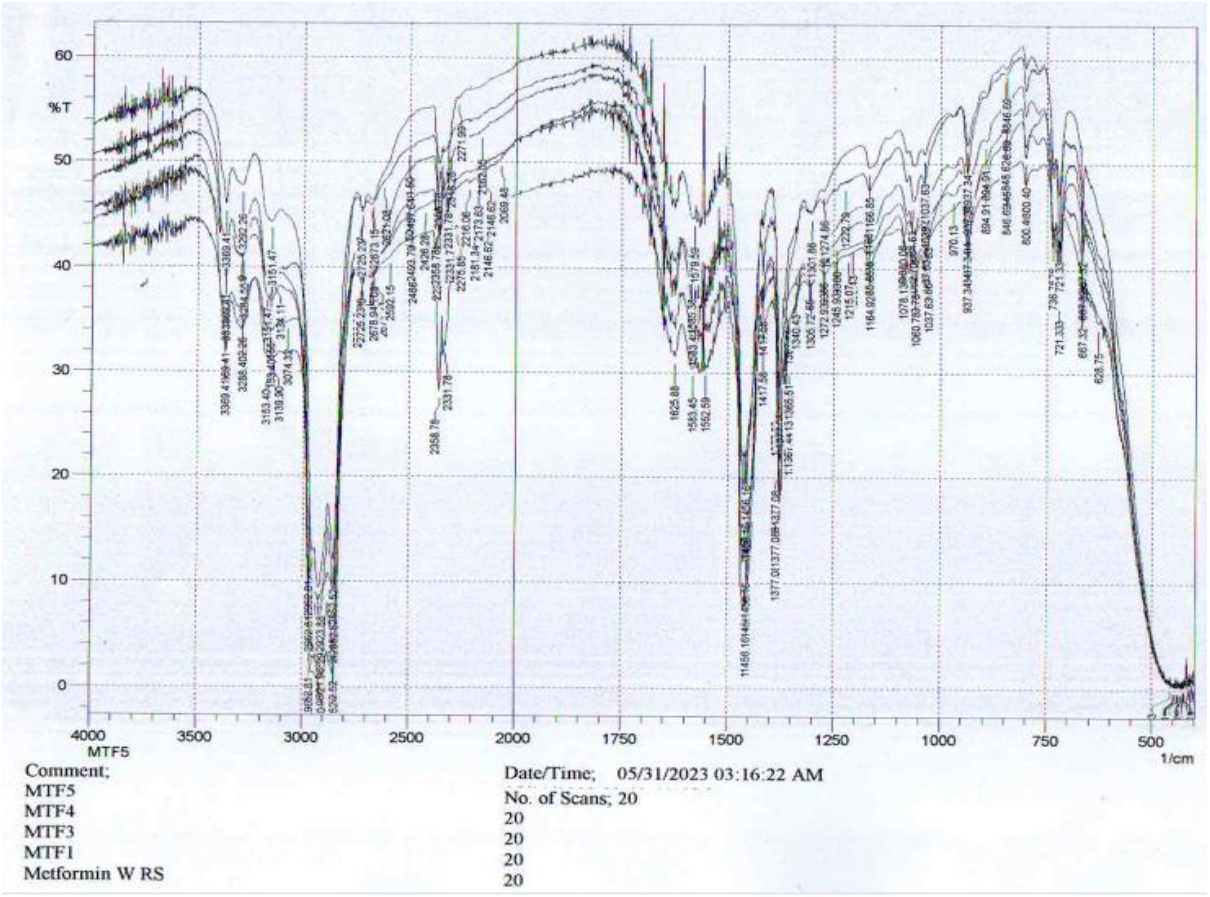
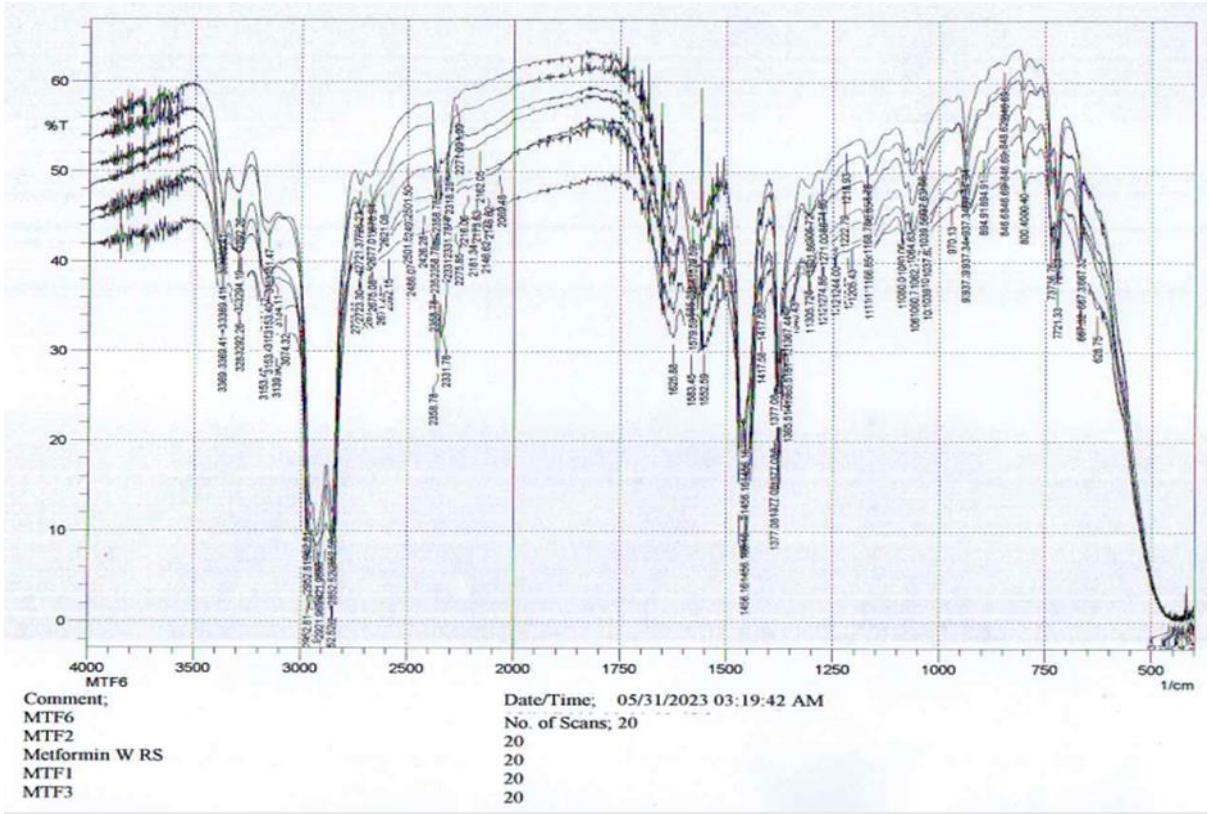
ANNEX'S

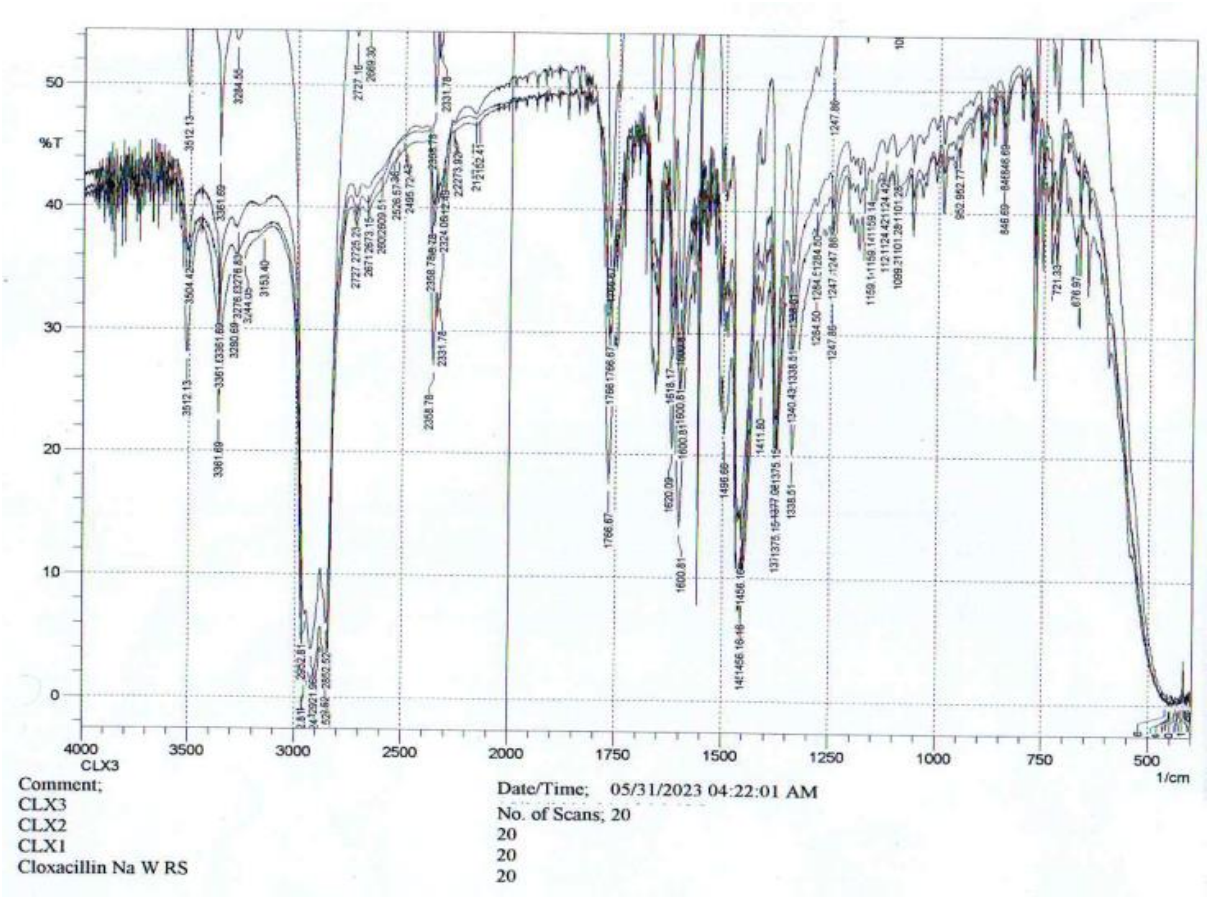
FTIR scanning spectrum overlap results of reference and sample products tested**.**



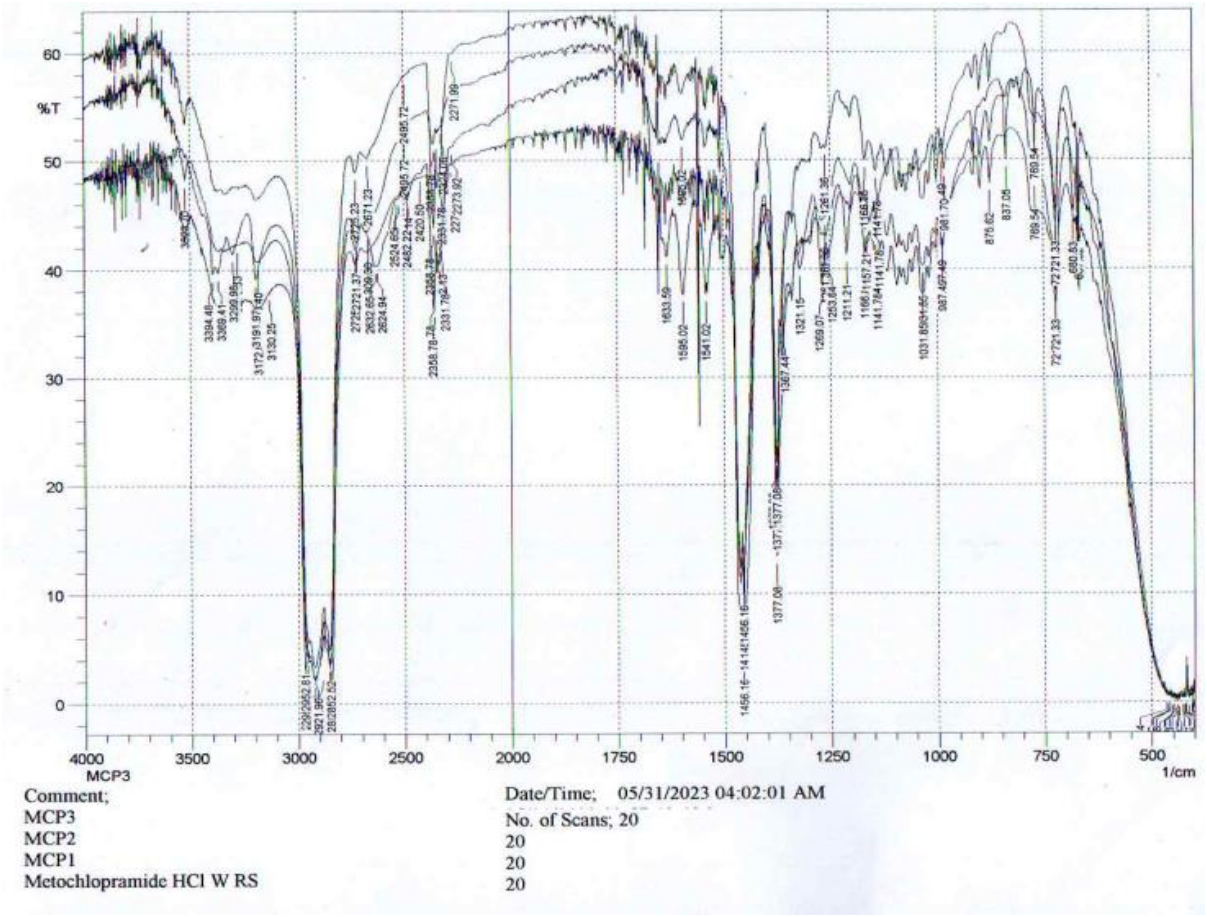
Annex 1: FTIR overlap scanning spectrum of four products of metformin hydrochloride (MTF1, MTF3, MTF4 and MTF5) and their working reference standard**.**



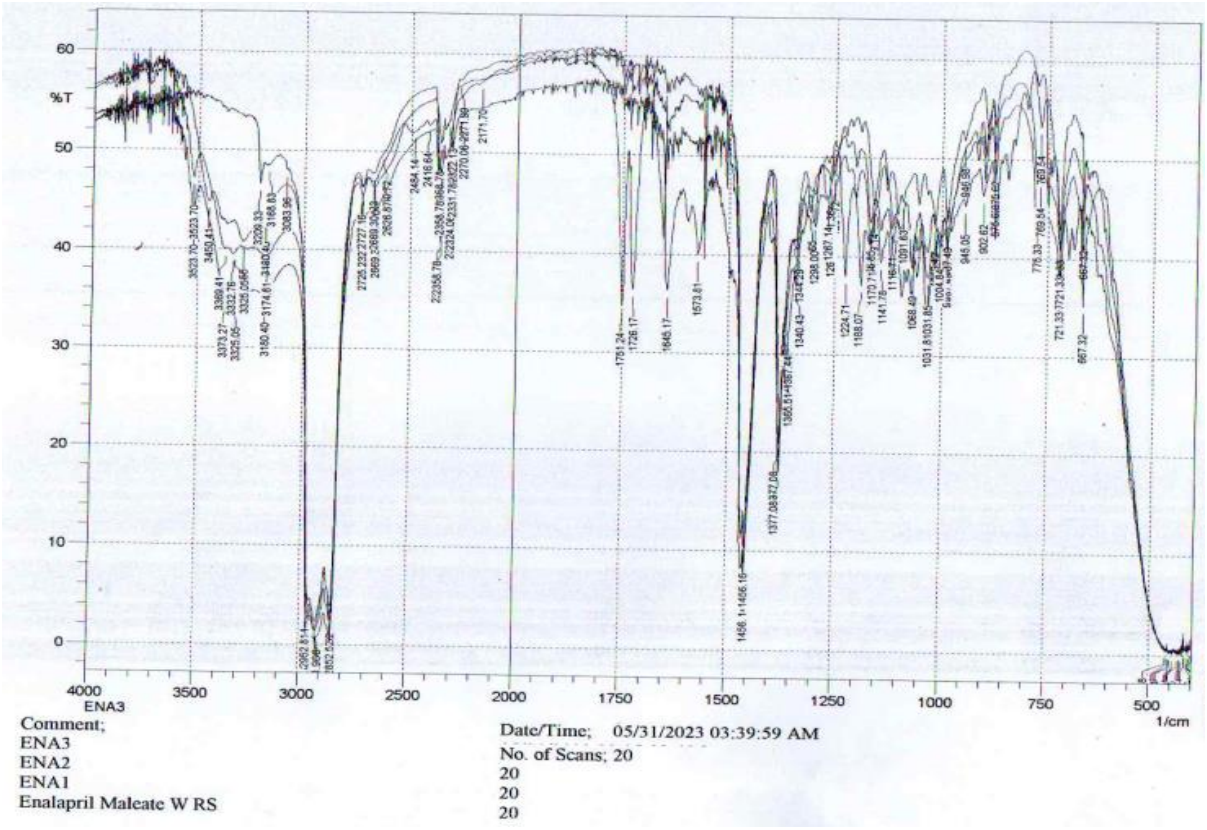
Annex 2: FTIR overlap scanning spectrum of four brands (MTF1, MTF2, MTF3 and MTF6) of metformin hydrochloride and their working reference standard.



Annex 3: FTIR overlap scanning spectrum of three products of cloxacillin sodium and their working reference standard.



Annex 4: FTIR overlap scanning spectrum of three products of metoclopramide hydrochloride and their working reference standard.



Annex 5: FTIR overlap scanning spectrum of three products of enalapril maleate and their working reference standard.

Dissolution profiles test results of locally manufacture and imported drug product products included in this study.

Annex 6: Cumulative percentage of dissolution profiles of five products of metformin hydrochloride 500mg tablets, and comparator counter products (MTF3) in phosphate buffer pH=6.8 at maximum wavelength 232nm and 37± 0.5^{o}C

Time(min)	**Brands investigated**					
	MTF1	**MTF2**	**MTF3**	**MTF4**	**MTF5**	**MTF6**
5	21.62±2.13	14.53±3.88	29.99±7.49	30.14±10.01	37.59±10.99	29.22±6.62
10	42.32±3.28	37.81±15.67	57.68±15.22	33.93±3.29	62.74±13.38	43.84±7.20
15	66.06±5.07	42.89±12.85	79.50±14.42	49.60±3.82	79.40±3.65	59.97±8.29
30	84.45±2.03	**62.66±9.74**	84.84±16.27	**78.71±6.86**	91.46±3.09	80.78±11.94
45	84.56±2.35	74.20±9.54	87.84±13.29	83.29±3.61	92.79±5.23	85.65±9.22
60	91.28±5.21	**75.12±4.46**	90.80±19.29	87.28±4.06	95.33±4.79	89.29±7.41

Annex 7: Cumulative percentage of dissolution profiles of two products of cloxacillin sodium 500mg capsules and comparator counter products (CLX3) in distilled water at maximum wavelength 220nm and 37± 0.5^{o}C.

Time(min)	**% Cumulative drug release**		
	CLX1	**CLX2**	**CLX3**
5	45.8±11.87	60.08±13.79	42.74±13.18
10	84.26±12.28	89.16±6.64	83.74±8.21
15	86.27±11.87	89.95±8.42	88.35±10.08
30	89.30±3.23	90.19±6.96	89.41±6.39
45	94.28±8.59	91.06±5.45	92.36±9.70
60	95.54±6.88	92.45±5.42	92.98±8.76

Annex 8: Cumulative percentage of dissolution profiles of two products of metoclopramide hydrochloride 10mg tablets and comparator counter products (MCP3) distilled water at maximum wavelength 309nm and 37± 0.5^{o}C.

Time(min)	**Cumulative drug release (%)**		
	MCP1	**MCP2**	**MCP3**
5	30.66±3.36	46.60±15.28	68.31±10.99
10	67.3±6.47	84.19±12.96	77.75±3.90
15	80.76±6.34	97.36±7.62	78.58±3.55
30	84.67±6.58	98.23±2.82	80.38±3.69
45	88.16±8.71	100.70±7.71	80.83±6.45
60	88.85±7.90	100.8±2.98	80.93±6.80

Annex 9: Cumulative percentage of dissolution profiles of two products of enalapril maleate 5mg tablets and comparator counter products (ENA3) in phosphate buffer pH=6.8 at maximum wavelength 208nm and 37± 0.5^{o}C.

Time(min)	**Cumulative drug release (%) ±SD**		
	ENA1	**ENA2**	**ENA3**
5	22.37±19.15	33.52±3.82	34.92±9.05
10	75.58±10.42	84.78±3.08	84.77±3.16
15	83.77±7.45	86.47±4.48	86.24±12.43
30	84.53±6.73	87.01±3.99	86.44±4.50
45	84.63±3.34	87.76±6.44	86.97±3.89
60	86.05±3.28	89.24±4.68	87.25±7.91

Annex 10: Percentage of drug release profile of six metformin hydrochloride 500mg tablets and working reference standard of metformin hydrochloride in phosphate buffer (pH=6.8) at maximum wavelength 232nm and 370.5^{o}C.

Time	**% Drug released**						
Min	**MTFR**	**MTF1**	**MTF2**	**MTF3**	**MTF4**	**MTF5**	**MTF6**
5	15.86±0.39	24.23±1.82	32.42±0.46	27.08±0.91	17.43±0.37	18.62±0.24	18.91±0.22
10	24.68±0.46	28.44±0.55	36.71±2.66	38.54±0.83	21.59±0.36	33.73±0.47	29.92±1.85
15	33.41±0.23	37.02±1.90	38.32±0.56	38.83±2.06	33.47±0.22	43.99±0.35	36.05±2.02

20	42.57±0.73	40.30±0.83	43.50±0.44	48.97±0.96	40.59±0.66	49.79±1.54	44.65±0.90
25	49.31±0.66	49.18±0.67	56.49±0.61	50.65±1.50	46.98±1.18	60.27±0.51	49.57±0.91
30	53.26±1.49	53.06±2.33	56.48±0.76	62.48±1.77	51.36±1.78	63.09±1.63	54.60±0.61
45	65.36±2.15	65.56±2.34	68.09±2.22	73.93±1.22	61.06±1.61	73.88±1.86	66.59±0.92
60	69.32±4.13	70.51±2.92	72.37±4.39	75.67±1.86	69.14±1.30	76.71±3.25	73.08±2.08
90	77.52±0.41	78.93±1.32	76.46±4.15	83.77±2.19	76.49±1.39	83.04±2.61	82.72±0.15
120	79.21±1.20	84.08±2.48	82.50±3.09	81.49±2.19	78.96±1.09	86.28±1.58	86.42±0.36

Annex 11: Percentage of drug release profiles of three products of cloxacillin sodium 500mg capsules and their working reference standard in phosphate buffer pH=6.8 at maximum wavelength 220nm and 37±0.5^{o}C.

Time	**% Drug released**			
Min	**CLXR**	**CLX1**	**CLX2**	**CLX3**
5	13.85±0.52	12.35±0.42	20.01±0.86	16.93±0.67
10	17.61±0.72	16.14±0.20	25.37±0.68	26.46±0.32
15	24.65±0.37	21.74±0.46	30.86±0.36	32.13±0.50
20	27.33±0.84	25.69±0.30	39.40±0.61	39.21±0.36
25	31.07±0.51	28.60±0.42	43.09±0.39	43.99±0.42
30	34.60±0.57	32.92±0.27	50.39±3.23	48.14±1.85
45	47.89±1.78	44.41±0.39	58.06±1.67	57.46±1.11
60	53.31±3.32	56.81±0.52	67.10±3.43	65.59±1.66
90	61.40±1.45	62.63±0.71	77.52±2.50	72.35±2.11
120	67.52±4.04	72.36±0.42	82.79±4.06	79.49±3.08

Annex 12: Percentage of drug release profiles of three products of metoclopramide hydrochloride 10mg tablets and their working reference standard in distilled water at maximum wavelength 309nm and 370.5^{o}C.

Time (min)	**% Drug released**			
	MCPR	**MCP1**	**MCP2**	**MCP3**
5	23.01±0.12	14.69±7.28	13.16±3.30	13.43±0.18
10	34.03±0.18	22.72±9.96	20.49±4.59	20.60±0.23
15	43.43±0.14	28.54±13.00	26.12±5.67	31.28±3.15

20	50.15±0.11	35.33±12.94	32.92±5.73	35.65±0.06
25	54.89±0.14	39.78±13.20	37.63±5.70	42.05±0.18
30	59.97±0.18	44.36±13.65	41.94±5.90	46.06±0.14
45	67.88±0.33	54.27±12.11	51.58±5.45	52.42±0.24
60	72.84±0.57	60.18±10.47	57.68±4.86	59.39±1.75
90	77.26±0.15	68.07±8.01	65.47±4.12	62.03±0.18
120	78.78±1.16	72.35±4.45	69.79±3.72	62.18±0.20

Annex 13: Percentage of drug release profile of three brands enalapril maleate 5mg tablets and their working reference standard in phosphate buffer (pH=6.8) at maximum wavelength 208nm and 37±0.5^{o}C.

Time (min)	**% Drug released**			
	ENAR	**ENA1**	**ENA2**	**ENA3**
5	13.65±0.89	5.36±0.19	14.41±3.51	4.56±0.19
10	24.56±0.89	18.86±0.29	17.47±1.36	19.23±0.11
15	27.12±10.62	25.48±0.04	22.41±0.56	23.52±0.22
20	28.71±0.27	26.94±0.61	34.78±0.58	29.26±0.27
25	38.34±2.96	28.51±0.11	41.79±0.77	36.12±0.19
30	39.91±2.46	56.85±0.22	46.92±2.21	55.39±0.14
45	56.67±3.47	58.55±1.60	49.18±1.18	67.93±0.11
60	64.92±2.11	62.11±0.11	86.67±2.90	71.39±0.15
90	72.59±0.45	81.77±0.17	88.67±1.26	84.08±8.47
120	84.92±0.27	29.21±0.13	91.03±1.01	88.10±3.86

Annex 14: ANOVA statistical analysis of drug release profiles of metformin hydrochloride, cloxacillin sodium, metoclopramide hydrochloride, and enalapril maleate at 95% Confidence interval and $P < 0.05$.

Generic brands	**(I) brand**	**(J) brand**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
						Lower Bound	**Upper Bound**
Metformin hydrochloride	MTF1	MTFP	1.19805	2.50277	0.992	-6.0911	8.4872
	MTF2	MTFP	3.05844	2.50277	0.678	-4.2307	10.3476
	MTF3	MTFP	6.35065	2.50277	0.099	-.9385	13.6398
	MTF4	MTFP	-8.25974*	2.50277	**0.024**	-15.549	-0.9706
	MTF5	MTFP	7.39286*	2.50277	**0.046**	0.1037	14.6820
	MTF6	MTFP	3.76948	2.50277	0.493	-3.5197	11.0587
Cloxacillin Sodium	CLX1	CLXP	13.78776*	2.07559	**0.000**	7.8108	19.7648
	CLX2	CLXP	3.50407	2.07559	0.283	-2.4729	9.4811
	CLX3	CLXP	12.27738*	2.07559	**0.001**	6.3004	18.2544
Metoclopramide hydrochloride	MCP1	MCPP	-12.66476	4.66779	**0.064**	-26.106	0.7769
	MCP2	MCPP	-14.4706*	4.66779	**0.036**	-27.912	-1.0290
	MCP3	MCPP	-13.4479*	4.66779	**0.050**	-26.889	-0.0063
Enalapril Maleate	ENA1	ENAP	-8.07273*	1.00810	**0.000**	-10.975	-5.1697
	ENA2	ENAP	23.74545*	1.00810	**0.000**	20.842	26.6485
	ENA3	ENAP	3.00606*	1.00810	**0.043**	0.1031	5.9091