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SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACEUTICS AND SOCIAL PHARMACY

COMPARATIVE IN VITRO AVAILABILITY OF SOME LOCALLY MANUFACTURED AND IMPORTED BCS CLASS II DRUG PRODUCTS AGAINST THEIR INNOVATOR/COUNTER REFERENCE PRODUCTS.

HABTAMU BULBULA (B. PHARM)

Addis Ababa, Ethiopia

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COMPARATIVE *IN VITRO* AVAILABILITY OF SOME LOCALLY MANUFACTURED AND IMPORTED BCS CLASS II DRUG PRODUCTS AGAINST THEIR INNOVATOR/COUNTER REFERENCE PRODUCTS.

A research thesis submitted to the Department of Pharmaceutics and Social Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University in partial fulfilment of the requirements for the Degree of Master of Science in Pharmaceutics**.**

By: Habtamu Bulbula

Advisor: Professor Tsige Gebre-Mariam

Addis Ababa, Ethiopia

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Addis Ababa University

School of Pharmacy

This is to certify that this thesis by Habtamu Bulbula, entitled: COMPARATIVE *IN VITRO* AVAILABILITY OF SOME LOCALLY MANUFACTURED AND IMPORTED BCS CLASS II DRUG PRODUCTS AGAINST THEIR INNOVATOR/COUNTER REFERENCE PRODUCTS submitted in partial fulfilment of the requirements for the Degree of Master of Science in Pharmaceutics.

By Habtamu Bulbula

Approved by:

Name	Signature	Date
Prof. Tsige Gebre-Mariam	_____	_____
(Advisor)		
_____	_____	_____
(Examiner)		
_____	_____	_____
(Examiner)		

_____

Dean, School of Pharmacy, AAU

ABSTRACT

According to the Biopharmaceutical classification system (BCS), active pharmaceutical ingredients (APIs) are classified into four (BCS Class I-IV) based on aqueous solubility and intestinal permeability. BCS Class II drug products are medicines which have low solubility in body fluids and high permeability through membranes and they are among the drug products that require documented evidence of bioequivalence study. In addition to the solubility problems, there may be differences between generic and innovator products in terms of the type, source, and quantity of ingredients, manufacturing methods and processes, and machineries used in the production, and these factors can bring major differences in dissolution and bioavailability. The objective of this study is therefore to compare *in vitro* availability of four types of locally manufactured and imported BCS Class II drug products namely two types of macrolide antibiotics, Azithromycin (AZM) 500mg and clarithromycin (CLM) 500mg tablets, atorvastatin (ATO) 40mg tablets (anti-hyperlipidemia) and glibenclamide (GLB) 5mg tablets (oral hypoglycaemic agent) against their innovator/counter comparator products. A total of 21 selected drug products and their comparator counter products were collected from different drug retail outlets in Addis Ababa. For each drug product, quality control parameters such as thickness, and diameter, were determined in house pre-determined standards (Manufacturer's specifications), and the hardness, weight variation, friability and disintegration time were determined according to USP monograph using appropriate analytical equipment. The assay and dissolution profile of each drug product were also determined as per their respective procedures in the official monograph using high-performance liquid chromatogram (HPLC) and UV/Vis spectrophotometer. All quality control parameters were assessed by a one-way analysis of variance (ANOVA). Statistically, a significant difference was considered when $P<0.05$. The dissolution profiles of the various brands were statistically assessed by utilizing the post-hoc Dunnett test, model-independent and model-dependent methodologies. It was observed that all tested pharmaceutical products met the stipulated criteria for thickness and diameter, as outlined within the manufacturer's specifications (±5%). The results of hardness, friability, weight variation, identification and assay showed that all the investigated drug products were within the pharmacopeial requirements. Similarly, all products investigated complied with disintegration time tests (i.e., ≤ 30 min) of immediate-release drug products.

Except for one generic drug product of atorvastatin (ATO4) and two generic drug products of clarithromycin (CLM2 and CLM3), all the drug products included in this study fulfilled the single point dissolution test specification as per USP 44 /NF39.

The one-way ANOVA results of the dissolution profile of all studied drug products showed that one generic product of atorvastatin calcium (ATO4), all generic products of azithromycin and two generic products of clarithromycin (CLM 2 and CLM3) showed significant difference ($p < 0.05$) in dissolution profiles with comparator products. The similarity and difference factors (f2 and f1) and Dissolution efficiency were calculated to ensure whether the *in vitro* results are equivalent or not. The study indicated that samples ATO4 and CLM3 were found to be inequivalent with their respective comparator products in relation to dissolution profile studies as they failed to meet the f2 acceptance criteria. The Weibull model was the best fit to the dissolution data of all drug products since this model demonstrated the highest value of coefficient, lowest Akaike Information Criteria (AIC) and highest Model Selection Criteria (MSC) values, Therefore, it can be deduced that all of the samples follow similar drug release mechanism. In conclusion, this study showed that all the tested drug products complied with the quality specifications of weight uniformity, hardness, friability, disintegration, and assay. Except for sample ATO4 from atorvastatin and sample CLM3 from clarithromycin drug products, all the generic products included in this study met model-independent fitting factor specifications and can be used interchangeably with their respective comparator products.

Keywords: Biopharmaceutical Classification System, bioequivalence, biopharmaceutics, generic drugs, *in vitro* dissolution,

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Acronyms

AIC:	Akaike Information Criteria
API:	Active pharmaceutical ingredient
ANOVA:	Analysis of variance
AMR:	Antimicrobial resistance
AUC:	Area under (plasma concentration vs time) curve
BCS:	Biopharmaceutical classification system
BP:	British Pharmacopoeia
Cmax:	Maximum concentration
DE:	Dissolution efficiency
EMA:	European Medicine Agency
GMP:	Good manufacturing practice
HPLC:	High-performance liquid chromatography
IVIVC:	In vitro In vivo correlation
LMICs	Low- and-Middle-Income Countries
MDT:	Mean dissolution time
MSC:	Model Selection Criteria
NLT:	Not less than
NMT:	Not more than
US FDA:	United States Food and Drug Administration
USP:	United States Pharmacopoeia
WHO:	World Health Organization

1 Introduction

Access to healthcare, which includes access to effective, safe and quality medicines, is a prerequisite for realizing health. If available, affordable, of good quality and properly used, medicines can offer a simple, cost-effective answer to many health problems. In many countries costs of medicines accounts for a large share of the total health budget. Despite the obvious medical and economic importance of medicines there are still widespread problems with lack of access, poor quality, irrational use and waste (Stevens et al., 2017; Abbas et al., 2020). A national policy is needed to control and monitor the various factors that have an impact on peoples' access to medicine. Among them are pressure-free systems for selecting effective medications, public funding of long-term drug market regulation, management of overtaxes and other price-influencing factors, effective distribution methods, and policies that encourage competition through generic policies, generic substitution, and ethical purchasing procedures. A generic medication policy's primary objective is to lower pharmaceutical care cost with generic medicines while maintaining quality of healthcare in developed and developing countries (Gray, 2004; Seiter, 2010; WHO, 2011; Attridge et al., 2015). Generic medicines are those where the original patent has expired which may now produce by manufacturer other than the original innovator (patent holding) company. The term ‘generic medicine’ is commonly understood, as defined by the World Health Organization (WHO), to mean a pharmaceutical product which is usually intended to be interchangeable with an innovator product, is manufactured without a license from the innovator company, and is marketed after the expiry date of the patent or other exclusive rights (WHO, 2006). According to the US Food and Drug Administration (US FDA), a generic drug must be

- pharmaceutically equivalent to an approved safe and effective reference product in that it has identical amounts of the same active drug ingredient in the same dosage form and route of administration and meets compendial or other applicable standards of strength, quality, purity, and identity;
- bioequivalent to the reference product in that it does not present a known or potential bioequivalence problem and it meets an acceptable in vitro standard (usually dissolution testing) or if it does present such a known or potential problem, it is shown to meet an

appropriate bioequivalence standard and adequately labelled and manufactured in compliance with current Good Manufacturing Practice (GMP) regulation (FDA, 2017).

The World Health Organization (WHO) advocates for the promotion of generic drug utilization as a means to reduce healthcare expenditures and enhance the efficiency of healthcare delivery mechanisms.(WHO, 2006).Because of generic manufacturers do not conduct costly clinical trials to ensure the safety and effectiveness of a generic version of a drug, it is important that generic substitutes are analyzed for their chemical and biopharmaceutical equivalence, strength, quality, purity, and releasing profile of active ingredient together with their possible interchangeability in comparison to the innovator drug. This is particularly important for developing countries where drug distribution and supply is known to be erratic and the prevalence of substandard/counterfeit medicines is significantly higher, and as such, it is difficult for effective monitoring of the quality of marketed generic drug products (Alfonso-Cristancho *et al.*, 2015; Hambisa *et al*., 2019). Comparison of bioequivalence study between the generic products versus the innovator products is one of the major challenges and prime factors for a generic marketing authorization and clinician also face a challenge due to the wide choice of generic products, especially in African countries. The efficacy of pharmaceutical dosage forms depends on their formulation properties, and manufacturing methods, hence it is likely that the quality and bioavailability of dosage form may vary (Oishi *et al*., 2011; Reddy *et al.*, 2014). 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 on the market. The bioequivalence of various generic products on the market is critical to ensuring that generic products and innovator product can be used interchangeably(Fahmy *et al.*, 2014; Glass, 2014). Hence, the interchangeability of generic drug products should be proved against their innovator products before they are distributed in the market. Despite this fact, such studies don't seem to be conducted in resource constraint countries because of many reasons. Therefore, conducting in vitro equivalence evaluation of generic products against innovator products/comparator counter products could play a vital role in ensuring generic product quality, safety, and efficacy. Hence, the current study undertakes an in vitro equivalence of some generic BCS Class II drug products against their comparative counter product.

The rate and extent of drug absorption from the gastrointestinal (GI) tract is very complex and is affected by many factors. These include physicochemical factors (e.g., pKa, solubility, stability, diffusivity, lipophilicity, polar–nonpolar surface area, presence of hydrogen bonding functionalities, particle size, and crystal form), physiological factors (e.g., GI pH, GI blood flow, gastric emptying, small intestinal transit time, colonic transit time, and absorption mechanisms), and factors related to the dosage form (e.g., tablet, capsule, solution, suspension, emulsion, and gel) (Abuhelwa *et al.*, 2016; Chillistone *et al.*, 2017). Despite this complexity, the work of (Amidon *et al.* (1995) revealed that the fundamental events controlling oral drug absorption are the permeability of the drug through the GI membrane and the solubility/dissolution of the drug dose in the GI milieu. These key parameters are characterized in the Biopharmaceutical Classification System (BCS) by three dimensionless numbers: absorption number (An), dissolution number (Dn), and dose number (D0). These numbers consider both physicochemical and physiological parameters and are fundamental to the oral absorption process. The BCS is a scientific framework for classifying drug substances according to their solubility in aqueous environments and ability to permeate the intestinal barrier. One important outcome of the provisional classification is that the clinical performance of the majority of approved drug products essential for human health can be assured with an in vitro dissolution test, rather than empirical in vivo human studies (Amidon et al., 1995; Charalabidis et al., 2019; Shah & Amidon, 2014). 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

BCS involves mathematical analysis to experimentally determined solubility and permeability of drugs under specified conditions. According to the US Food and Drug Administration, a drug is considered to be highly soluble when its highest clinical dose strength is soluble in 250 mL of aqueous media over a pH range of 1-7.5 at 37.5C, and it is considered to be highly permeable if the absorption of an orally administered dose in humans is > 90% when determined using mass balance or in comparison to an intravenous reference dose.

A biowaiver (permission to skip in vivo bioequivalence studies) may be applied for certain drugs that pass specific in vitro solubility and permeability requirements A drug product is eligible for a BCS-based biowaiver provided that the drug substance(s) satisfy the criteria regarding solubility and permeability, the drug product is an immediate-release oral dosage form with systemic action, and the drug product is the same dosage form and strength as the reference product. (Amidon et al., 1995; FDA, 2017).

BCS Class II drug products have low solubility and high permeability. The dissolution rate of poorly water-soluble medicines is often a rate-limiting step in their absorption from the GI tract. Such medicines suffer limited oral bioavailability and are often associated with high intra subject and inter subject variability (Charalabidis et al., 2019; Li et al., 2021). Therefore, constant surveillance on the marketed poorly water-soluble medicines by the regulatory body, manufactures and independent research groups is essential to ensure availability of quality medicines.

1.1 BCS Class II drug products investigated.

1.1.1 Atorvastatin calcium

Atorvastatin Calcium, [(3R,5R)-7-[3-(Phenyl carbamoyl)-5-(4-fluorophenyl)-2-isopropyl-4 phenyl-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid, calcium salt], (Fig. 1) is a selective, competitive second-generation statins that inhibits hydroxy methyl glutaryl coenzyme A (HMG-CoA) reductase and impedes the formation of mevalonic acid, which is the rate-limiting step in the biosynthesis of cholesterol (Sonje *et al.*, 2010; Kogawa *et al.*, 2019).Atorvastatin is a BCS class II drug that reduces levels of total cholesterol, low-density lipoprotein (LDL)-cholesterol, triglyceride and very low-density lipoprotein (VLDL)-cholesterol and increases high-density lipoprotein (HDL)-cholesterol in patients with dyslipidemias. The greater efficacy of atorvastatin than other currently available HMG-CoA reductase inhibitors in reducing total cholesterol and LDL-cholesterol levels is believed to result from a prolonged duration of HMG-CoA reductase inhibition rather than the degree of inhibition. Atorvastatin is well tolerated and adverse events are usually mild and transient and it is the most efficient and frequently prescribed drug for the treatment of hypercholesterolemia(Malhotra & Goa, 2001; Murrow et al., 2012).

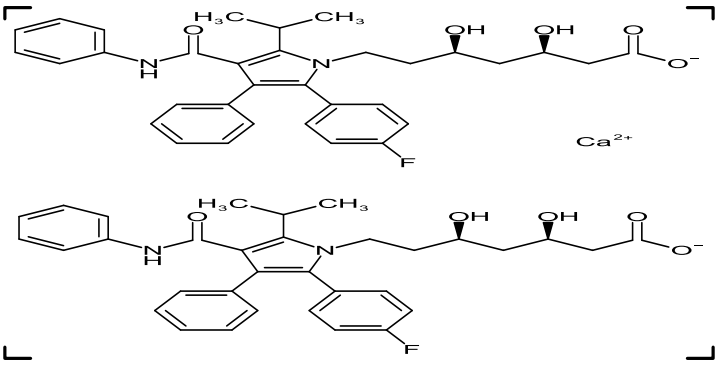

Figure 1: Chemical structure of atorvastatin calcium (USP, 2021c).

Among the statins, atorvastatin exhibits a number of pharmacokinetic characteristics that are different to the other members of the class. For example, it is the only statin has an ability to inhibit HMG-CoA reductase equivalent to that of the parent drug. The bioavailability of atorvastatin is only ~14% because of extensive pre-systemic extraction in the gastrointestinal tract and/or liver. This drug is ≥ 98% protein-bound in plasma(Poli, 2007; Saha, 2017).

1.1.2 Azithromycin

Azithromycin is a semi-synthetic macrolide antibiotic with a 15-membered ring, containing two deoxy sugars. It is derived from erythromycin by introducing a methyl-substituted nitrogen atom in the lactone ring. Its chemical name is 9-deoxy-9a-azo-9a-methyl-9ahomoerythromycin A, with molecular weight 748.88 and chemical formula C38H72N2O12. Its chemical structure is shown in a Fig.3 (Adebayo *et al.,* 2014). Azithromycin is used for treatment of ear infections, tonsillitis, laryngitis, throat infections, bronchitis, and pneumonia. It is a bacteriostatic agent that inhibits protein synthesis by binding reversibly to the 50s ribosomal subunits of sensitive organisms. (Adeli, 2014; Bakheit, *et al.*, 2014).

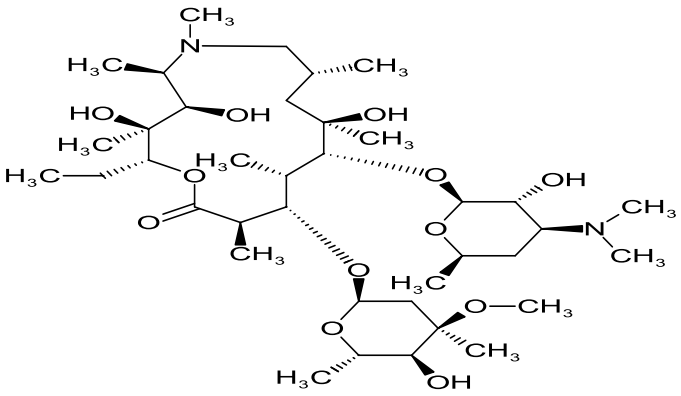

Figure 2: Chemical structure of Azithromycin (USP, 2021d).

Azithromycin is a broad-spectrum antimicrobial agent with oral bioavailability about 37%. The drug has pKa values around 8.74 and is sparingly soluble in water (~1.8 μg/mL). According to the biopharmaceutical classification system, azithromycin can be classified as a Class II drug; therefore, the drug dissolution is a rate-limiting step in the drug absorption process (Wadhwa, *et al.,* 2017).

1.1.3 Clarithromycin

Clarithromycin, 6-O methyl erythromycin, (Fig. 3) is a semi synthetic 14-membered lactone ring macrolide antibiotic that produce its antibacterial activity by interfering with the synthesis of bacterial protein, where it binds to the 50S ribosomal subunit of susceptible bacteria and therefore hinder translocation of aminoacyl transfer RNA and thereby inhibit protein synthesis (Abdulbaqi *et al.,* 2019; Alkhalidi *et al.,* 2019). Clarithromycin is used in the treatment of bacterial infections in respiratory tract, skin soft tissue, acute maxillary sinusitis, tonsillitis, pharyngitis, chronic bronchitis, pneumonia, as well as in Chlamydia and Helicobacter pylori infections. Clarithromycin has poor aqueous and pH-dependent solubility with dissolution rate-limited absorption corresponding to Bio pharmaceutics Classification System (BCS) Class II).After ingestion, clarithromycin has to be solubilized to allow its diffusion into the gastric mucus, a complex mixture of water (main composition), glycoproteins, proteins, and lipids (Manani *et al.*, 2017; Alkhalidi *et al.,* 2019; Labachevski *et al.,* 2019).

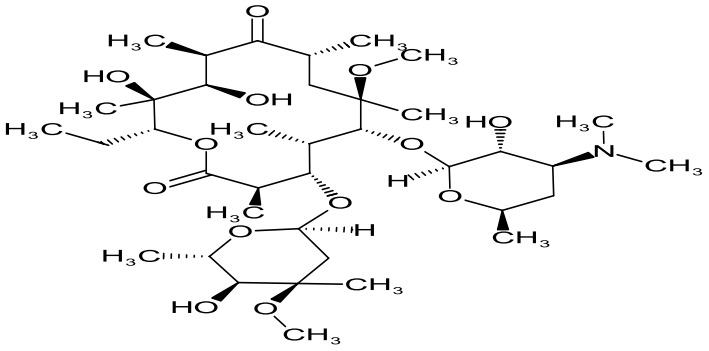

Figure 3. Chemical structure of Clarithromycin (USP, 2021e).

1.1.4 Glibenclamide

Glibenclamide, [4-(2-nitrophenyl)-2, 6-dimethyl-3,5-dicarbomethyl-1,4-dihydropyridine] (Fig 4), also known as glyburide an oral hypoglycemic agent belonging to the sulfonylurea class, is used for the treatment of noninsulin dependent diabetes mellitus (DM). Its hypoglycaemic effect

is mainly due to stimulation of insulin release from pancreatic beta cells and sensitization of the peripheral tissues to insulin (El-Sabawi, 2013; Elhamili et al., 2014).

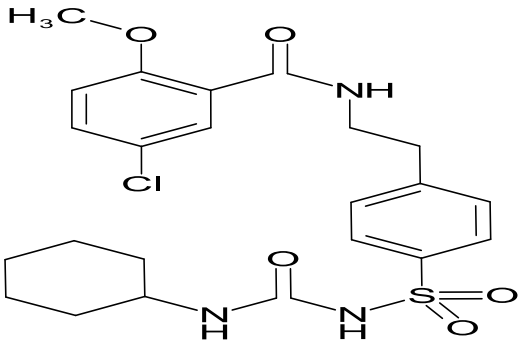

Figure 4: Chemical structure of Glibenclamide (USP, 2021f).

It has a history of low bioavailability, which is attributed to its poor dissolution properties. As a weak acid with a pKa of 5.3, its solubility strongly depends on the pH of the test medium and particle size. Because of its low aqueous solubility and high permeability, Glibenclamide is therefore a Class II drug based on BCS (Elhamili *et al.*, 2014; Rambiritch *et al.*, 2014).

1.2 Quality control parameters and properties of tablet dosage forms

Quality control parameters are variables through which the quality of a product or dosage form is assessed or determined. The analysis of quality, safety, and effectiveness is a crucial element of evaluating pharmaceutical products, relying on quality control assessments. The assessment of pharmaceutical quality relies on a variety of quality control examinations. Pharmaceutical tests (based on physical methods), tests based on chemical analysis (chemical tests) and biological methods are integral parts of quality control of pharmaceutical products. The tests are usually based on precisely defined and accepted specifications for critical quality attributes of the product. Pharmaceutical testing based on physical methods is a highly important part of the quality control tests usually intended to check the technical quality of the product. The objective of quality tests based on chemical methods is to assess the chemical composition of the finished product. The products are expected to comply with the accepted quality standards for the intended market throughout their shelf life. Thus, physicochemical quality analysis of drug products provides important quality control data on which decisions can be established (WHO, 1997). In general, quality control parameters commonly tested for oral solid dosage forms mainly include uniformity of dosage units, assay, disintegration and dissolution tests, and

identification tests for APIs. In addition, tablet friability, hardness, thickness, and diameter tests are used for tablet quality assessment (Chavan et al., 2018; Ghimire Prakash et al., 2020).

1.2.1 Identification test

The aim of the identification test is to authenticate the identity of the active pharmaceutical ingredient (API) and differentiate the compound(s) that share a close structural resemblance with compounds that may exist in pharmaceutical tablets. This test enables discrimination among compounds with similar structures that might be present(Chavan et al., 2018; USP, 2021a).

1.2.2 Assay of the active ingredients

This tests also known as a content test, serves to assess the potency or composition of the APIs found in the tablets. This approach is both precise and quantitative in nature, enabling the identification of chemical alterations throughout a period, hence regarded as a stability-indicating assay. Assay of drug content is a universal requirement and sample of tablets are typically evaluated based on the specified monograph and a percentage of the labelled amount of the active ingredient is compared with the defined specifications (Chavan *et al.*, 2018; Ghimire Prakash *et al.*, 2020).

1.2.3 Uniformity of dosage unit

The concept of "uniformity of dosage unit" refers to the extent of consistency in the quantity of the medicinal substance across various dosage units. This consistency can be established through either content uniformity or weight variation, both of which serve to regulate the uniform distribution of the active ingredient among tablets. (USP, 2021b). Table 1 presents the requirement to conduct the two tests.

1.2.3.1 Weight variation

Any weight variation in oral solid dosage formulations can indicate variations in the content of the API. The test is performed to oral solid dosage forms such as uncoated or film-coated tablets and hard gelatin capsules that contain 25 mg of an active drug substance constituting 25% or more by mass of the tablet or, in the instance of hard gelatin capsules, the capsule contents (USP 44-NF 39, 2021).

1.2.3.2 Content uniformity test

The purpose of testing for content uniformity is to verify that the active drug component is evenly distributed throughout the dosage units of a batch within a defined range around the label claim. The test involves the analysis of individual dosage units of a prescribed number for possible content variation (USP 44-NF 39, 2021).

Table 1: USP 44/NF 39 requirements to conduct content uniformity (CU) and weight variation (WV)

Dosage form	Type	Sub type	Dose and ratio of drug substances	
			≥ 25 mg and ≥ 25%	≤25 mg and ≤ 25%
Tablets	Uncoated		WV	CU
	Coated	Film	WV	CU
		Others	CU	CU

1.2.4 Hardness and friability tests

Tablets need to endure the challenges of handling and transportation encountered during the manufacturing process, in the drug supply chain, and in the hands of the end users (patients/consumers). The mechanical durability of tablets is of great significance and is regularly assessed(H. Chavan et al., 2018; Ghimire Prakash et al., 2020). Tablet strength is a crucial factor in both product development and quality control specifications. The breaking force of tablets, commonly called "hardness" is the force required to produce failure (i.e., breakage) in a specific plane and used to measure the mechanical integrity of tablets (USP, 2021h). Friability, commonly employed test of the ability of tablets to withstand mechanical stresses determines their resistance to chipping and surface abrasion by tumbling them in a rotating cylinder. The percentage weight loss after tumbling is referred to as the friability of the tablet. Friability is the tendency of tablets to powder, chip, or fragment, and this can affect the tablet's elegance and appearance, decrease consumer acceptance, and also add to the tablet's weight variation or content uniformity problems. The compendial specification for friability is not more than 1%. Usually harder the tablets less will be the percentage friability and vice versa (USP, 2021g).

1.2.5 Disintegration test

For drug substance in a tablet or capsule dosage form to be absorbed adequately, the dosage form must first be broken down into granules or primary powder particles, which may then be dissolved in body fluids, allowing the drug substance to be released for absorption. As a result, disintegration is required for dissolution and can be the rate-limiting step in drug absorption and dissolution, thereby influencing therapeutic outcome (Quodbch et al.,, 2016; Nickerson *et al.*, 2018). The disintegration process may be influenced by various formulation factors, properties, and the concentration of excipients,, in particular (Markl & Zeitler, 2017; Quodbach & Kleinebudde, 2016). According to the USP the disintegration test is provided to determine whether tablets or capsules disintegrate within the specified time when placed in an immersion medium under defined experimental conditions (USP, 2021i).

1.2.6 Dissolution test

Dissolution is defined as a process by which a solid substance is solubilized into the solvent to yield a solution. This process is fundamentally controlled by the affinity between the solid substance and the solvent and consists of two consecutive steps. The first step involves the liberation of molecules from the solid phase to the liquid layer near the solid surface (an interfacial reaction between the solid surface and the solvent). It is followed by the transport of solutes from the solid–liquid interface into the bulk solution (Anand et al., 2011; Bidkar et al., 2019). The process involved in the dissolution of solid dosage forms is shown in Fig 5. The scheme illustrates that the dissolution process for a solid dosage form (or a drug product) in solution starts with the wetting and the penetration of the dissolution medium into the solid formulation, followed by disintegration and/or deaggregation into granules or fine particles. This results in an increased surface area available for the dissolving media to facilitate the dissolution of tablets into solution. Consequently, the solution is absorbed into the bloodstream, that dissolution must be related to the availability of the drug to the body (Dressman *et al.*, 2005). A variety of factors can affect the in vitro rate of dissolution considerably and a great part of literature is concerned with identification and evaluation of these factors. The phenomenon of solubilization of a bioactive component from solid pharmaceutical formulations, specifically tablets, encompasses numerous intermediary physicochemical processes of the therapeutic agent which impact the manner in which the tablets fragment to unveil the drug constituents to the surrounding medium, thereby enabling in vitro liberation. (Ghayas *et al.*, 2013; Hasan, *et al* .,

2017). The fluid flow properties during the dissolution process are affected by the surface area of solids. Particle size, shape, and density play a significant role in influencing these fluid flow properties. It is commonly observed that a decrease in particle size leads to an increase in the dissolution rate. Nonetheless, the hydrophobic characteristics of the liquid-solid interface and the mutual interference in particulate motion can hinder the dissolution process, resulting in smaller particles exhibiting slower dissolution rates(Chu et al., 2012; Friedrich et al., 2006). Various interacting formulation and processing variables are involved in the production of tablet formulations for drugs, impacting the dissolution and release profiles of the products. These variables can differ among manufacturers, leading to variations in the release and dissolution profiles of products. Factors such as the type of diluents, mixing process, granule size and distribution, disintegrants nature, lubricants nature and concentration, presence or absence of surface-active agents, drug physical properties, granulation flow through hopper and dies, and compressional force during production all play a role in influencing dissolution (Ghayas et al., 2013; Yu, 2008).

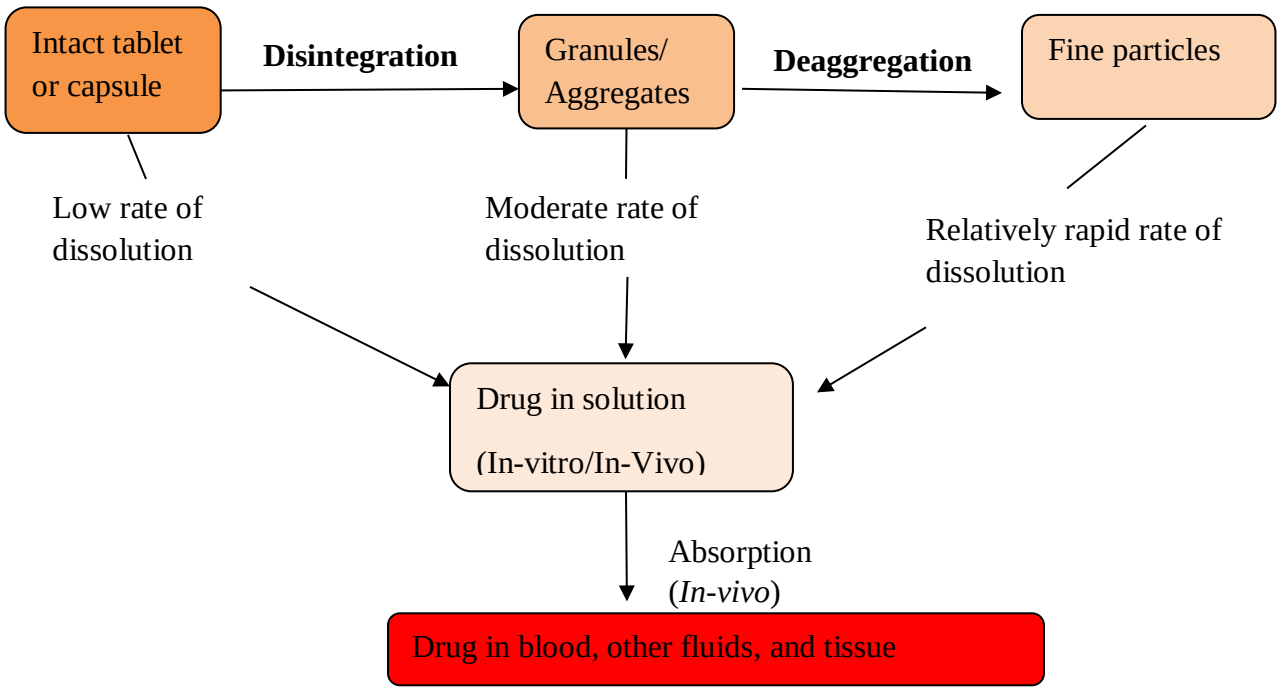

Figure 5: Schematic illustration of a dissolution process of a solid dosage form (Dressman et al, 2005).

Various scientific studies have explored the impact of processing and formulation variables on dissolution. Research findings have demonstrated that hydrophobic lubricants such as magnesium stearate hindered dissolution, whereas water-soluble lubricants like sodium lauryl sulfate accelerated dissolution rates (Best et al., 2004). It has been documented that the composition and concentration of binders utilized in the formulation play a significant role in influencing dissolution rates. A higher concentration of binders has been shown to correlate with a reduction in the dissolution rates of tablets. Furthermore, the type and method of integration of disintegrants have been highlighted as factors that greatly affect dissolution rates. Investigations into the influence of manufacturing process variables on the in vitro dissolution characteristics of drugs have revealed the impact of various process variables on drug dissolution (Bidkar et al., 2019; Dickinson et al., 2008; Hasan et al., 2017; A. Khan, 2021).

1.3 Bioavailability and bioequivalence

The FDA ensures the safety, efficacy, and compliance with all relevant standards of drug products intended for marketing. To achieve this goal, the FDA mandates bioavailability/pharmacokinetic studies and bioequivalence studies for all pharmaceuticals. According to the FDA, bioavailability is defined as "the speed and degree to which the active drug component or therapeutic moiety is absorbed from a pharmaceutical product and reaches the intended site of action." Due to the practical challenges in directly measuring drug concentrations at the target site, bioavailability is commonly described as "the speed and degree of absorption of the active drug from a specific dosage form and its availability in the systemic circulation" (FDA, 2017). Bioavailability studies play a crucial role in the initial phases of developing a suitable dosage form for a novel drug entity, assessing the impact of excipients and patient-specific factors, as well as potential interactions with other medications on absorption efficiency. These studies are also essential for creating new formulations of existing drugs, ensuring the quality control of pharmaceutical products during the early stages of market introduction to evaluate the effects of processing conditions, storage, and stability on drug absorption (Davit et al., 2016; X. Zhang et al., 2013). Bioequivalence (BE) refers to the absence of a substantial disparity in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study (FDA, 2017).

The significance of BE studies has been notable in the realm of drug development as well as during the post-approval period for both pioneer and generic drug products. These studies serve as bridging studies in the presence of formulation or manufacturing changes to provide supportive evidence for safety and efficacy of a drug product and they can be utilized to assure product quality and performance throughout the lifetime of a drug product. As there is an increase in production and consumption of generic drugs, the need for bioequivalence study is also rising. Generic drug products should conform to the same standards of quality, safety, and efficacy required as those of the reference product, and must be interchangeable. Since the generic drugs would be interchanged with innovator drugs in the market, it is therefore required to demonstrate that the safety and efficacy of both the innovator and generic drugs are comparable. Assessment of this "interchangeability" between the generic and innovator drug product is carried out by demonstrating bioequivalence studies (Karalis *et al.*, 2008; Jalali *et al.*, 2017).

1.3.1 Bioavailability assessment and in vitro/ in vivo correlations

According to the statutory delineation of Bioequivalence (BE), a variety of in vivo and in vitro techniques may be utilized to ascertain BE. The subsequent techniques have been suggested for the substantiation of BE. (Karalis *et al.*, 2008; Galgatte *et al.*, 2014; Jalali *et al.*, 2017) .

A. Comparative pharmacokinetic studies –are the most widely preferred method to assess BE of systemically absorbed drug products. The principles and methodology involved in assessing bioequivalence of drug products involve the measurement of the concentration of the active ingredient in the systemic circulation, either directly in blood or indirectly through urinary excretion studies.BA and BE use pharmacokinetic measures such as AUC to assess the extent of systemic exposure and Cmax and tmax to assess the rate of systemic absorption. This approach is based on the understanding that some predetermined relationship exists between the drug concentrations at the site of action and that in the systemic circulation (FDA, 2017; Jalali and Rasaily, 2017).
B. Comparative pharmacodynamics studies - The use of pharmacodynamics or clinical endpoints for BE demonstration is recommended where a pharmacokinetic approach is not possible. This method is mostly used for locally acting drug products and some systemically acting drug products for which drug levels are too low to be measured in biological fluid or there is a safety concern for using the pharmacokinetic approach to assess BE. An essential

component of a BA or BE study based on a PD response is documentation of a dose-response relationship. pharmacodynamics evaluation measures the effect on a pathophysiological process after administration of two different products to serve as a basis for BE assessment. (Faiyazuddin et al., 2010; Jalali et al., 2017b).

C. Comparative clinical studies – Clinical responses are frequently situated near or at the plateau of the dose-response curve, thereby rendering them unresponsive to distinguishing the therapeutic distinction between a test and reference formulation (FDA, 2013). Consequently, a substantial number of patients are necessary to be enrolled in these studies for BE assessment in order to identify formulation variances. The exhibition of dose-response connections is not obligatory for clinical BE investigations as they are designed solely to validate the absence of significant clinical distinctions between products being compared. The utilization of clinical endpoints in BE studies will be entertained only in scenarios where both pharmacokinetic and pharmacodynamic methodologies are infeasible for BE determination due to the aforementioned justifications.
D. Comparative in vitro studies - scientific evidence has shown that in vitro test data are correlated with in vivo results. Over the decades, however, the evolution in pharmaceutical science and technology may have provided opportunities for relying more on in vitro tests to support BE demonstration. Indeed, this can be exemplified by the recent application of a BCS that classifies drugs based on their biopharmaceutical attributes and predicts the BA/BE of the drug products.

1.3.1.1 In vitro dissolution/Release testing

The evaluation of BE through in vitro dissolution/release testing is a widely used method. Dissolution testing has been utilized for the past five decades to maintain quality control procedures in research and development, identify the impact of critical manufacturing variables, and establish *in vitro in-vivo* correlation (IVIVC) in comparative studies. This method can identify potential bioequivalence issues in formulations, making it an essential tool in predicting in-vivo bioavailability and, in some cases, replacing clinical studies to determine bioequivalence. In-vitro dissolution testing plays a crucial role as it pertains to the liberation of therapeutic agents from solid pharmaceutical formulations subsequent to their oral intake, a process fundamental for drug absorption and bioavailability. This examination stands as a pivotal assessment,

recognized as the rate-determining stage within the succession of actions facilitating drug absorption into the systemic circulation. The absorption process involves the conveyance of drug entities from the gastrointestinal lumen into the systemic circulation. While dissolution does not serve as a direct indicator of therapeutic efficacy, it functions as both a qualitative and quantitative instrument, furnishing valuable insights into the pharmacological availability of a drug and ensuring consistency from batch to batch. Notably, dissolution testing is deemed a cornerstone among the array of quality assessment procedures applied to pharmaceutical dosage forms, with the validation of dissolution methodologies constituting an integral component of sound manufacturing practices. Given the contemporary technological landscape and the evolving landscape of drug delivery research, coupled with an increased emphasis on the in-vivo predictability of therapeutic outcomes through in-vitro assays, dissolution evaluations have garnered escalating attention. It is imperative to uphold stringent standards regarding drug dissolution each time a novel solid dosage form is designed or manufactured. The overarching goal of these evaluations remains the anticipation of drug release extent and the establishment of in vitro-in vivo correlations *(IVIVC).* An *in vitro* drug dissolution profile comparison can be used to assess the *in vitro* equivalence or interchangeability of multisource generic products. The methods for the comparison of in vitro dissolution profiles can be classified as the methods based on analysis of variance (ANOVA), model-dependent methods and model-independent methods.

Model independent methods of comparison: The most frequently used statistical model-independent procedures, the difference factor (f_1) and similarity factor (f_2), were used to compare the dissolution profiles. The difference factor (f_1) is defined by the US FDA in terms of the observed percentage difference between two profiles at each time point. This factor essentially measures the relative error between the two profiles and calculated as in Eq. (1):

$$f_1 = \{[\sum_{t=1^n}|R_t - T_t|]/[\sum_{t=1^n} R_t] * 100\} \quad \text{Eq. 1}$$

Where n is the number of sampling time points, R_t and T_t are the amount of drug dissolved at time t of reference and test, respectively. The acceptance criterion for two profiles to be not distinctly different is when f_1 value is between 0 and 15. According to the US FDA, the similarity factor is a measurement of the similarity in the percentage dissolution between two profiles. This factor is just the logarithmic reciprocal square root transformation of the squared error sum, calculated by Eq. (2):

$$f_2 = 50\ x\ log\left\{\left[\left[1 + \left({}^{1}/_{n}\right)\sum_{t=1}^{n}(R_t - T_t)^2\right]\right]^{-0.5} x\ 100\right\} \quad \text{Eq. 2}$$

Where n is the number of sampling time points, R_t and T_t are the amount of drug dissolved at time t of reference and test, respectively. The acceptance criterion for two profiles to be markedly similar is when $100 \leq f_2 \geq 50$.

Model-dependent methods of comparison: A comparison of the drug dissolution profiles can be done using different model-dependent methods.

Zero order kinetics: The drug dissolution in pharmaceutical dosage forms that do not disaggregate and release the API slowly can be represented by the following Eq. (3):

$$Q_T = Q_0 + K_0 t \quad \text{Eq. 3}$$

Where Q_0 is the initial amount of drug in the pharmaceutical dosage form, Q_t is the amount of drug in the pharmaceutical dosage form at time t and K_0 is the proportionality constant.

First order kinetics: In this case, the amount of API released from pharmaceutical dosage forms is delivered at a rate comparable to the amount of API remaining within, resulting in a decrease in the amount of API released per unit of time. First-order dissolution can be represented by Eq. (4):

$$ln\, Q_t = ln\, Q_0 + K_0 t \quad \text{Eq. 4}$$

Where Q_0 is the initial amount of API in the pharmaceutical dosage form, Q_t is the amount of API in the pharmaceutical dosage form at time t and K_0 is the proportionality constant.

Hixson-Crowell model: According to the Hixson-Crowell model, a particle's normal area is equivalent to the cube root of its volume. The Hixson-Crowell model dissolution profile can be represented by Eq. (5):

$$Q_0^{1/3} + Q_t^{1/3} = K_d t \quad \text{Eq. 5}$$

Where Q_0 is the initial amount of API in the pharmaceutical dosage form, Q_t is the amount of API in the pharmaceutical dosage form at time t and K_d is the dissolution constant.

Higuchi model: The Higuchi equation uses a release kinetics known as the "square root of time." When used with modified liberation systems or semisolid dosage forms, this kinetic model provides strong experimental fit data for API dissolution processes. This model can be represented by Eq. (6):

$$Q = K_d\sqrt{t - t_0} \qquad \text{Eq. 6}$$

Where K_d is the dissolution constant.

Weibull model: The dissolution rate can be investigated using the Weibull function, a mathematical model without a physicochemical basis. The following Eq. (7): represents the model:

$$ln\left(ln\frac{Q_\infty}{Q_{\infty-Q}}\right) = \beta\, ln(t - t_0) - \beta\, ln\, t_d \qquad \text{Eq. 7}$$

Where Q is the amount of API dissolved at time t, Q_∞ is the amount of API dissolved at time infinite, also called ''total dissolution", t_0 is the lag time and t_d are the time interval necessary to dissolve or release 63.2% of the API present in the pharmaceutical dosage form.

1.3.1.2 In vitro/ In vivo correlations

IVIVC is a general term that refers to the establishment of a relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical characteristic of the same dosage form produced by a dosage form (Emami, 2006; Qiu *et al.*, 2017). Establishment of an IVIVC could facilitate drug development by reducing the number of in vivo studies required for confirming either the safety or the efficacy of a drug product or the bioequivalence of products containing the same drug. For drug products intended for systemic activity, the biological property produced by the dosage form is usually assumed to be related to the presence of the drug in the systemic circulation, i.e., the pharmacokinetic profile.

On the in vitro side, dissolution or release of this process are the most frequently in vitro variables used to generate an IVIVC (Dressman & Krämer, 2005; FDA, 2017; Jalali & Rasaily, 2017; Qiu & Duan, 2017).

1.4 Rationale of the study

According to the Federal Ministry of Health of Ethiopia, communicable and non-communicable diseases accounted for most of the top ten causes of illness and death in 2016 (Central Statistical Agency [Ethiopia] and ICF International, 2016). This indicates that there is a considerable need for high-quality medicinal products. However, there are reports showing suboptimal access to affordable, safe, and quality essential drug products in Ethiopia as a whole (Boche *et al*., 2020). 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 (FDA, 2017).The drug products (Atorvastatin, azithromycin clarithromycin and glibenclamide tablets) that are selected for this in-vitro study are the most frequently prescribed, life-saving, and taken on a long-term basis, BCS Class II drug products. Routine laboratory testing of drugs in the market is crucial to protect public health especially in developing countries where counterfeit and substandard drugs have become a major challenge to healthcare services (Ngwuluka et al., 2009). For generic products, the quality of excipients and the manufacturing process must allow full and timely release of the drug in the same way as the reference product does at predefined conditions.

An adequate release evaluation of generic drugs promotes Interchangeability of these products, to ensure the same pharmacological effect, with the benefit of a lower cost to the patient (Pettarin et al., 2020). There are many factors that affect the in vivo performance of an oral dosage form, and it is crucial to develop drug products in order to accurately predict this in vivo performance. The main aim is to reduce the costly and ethically controversial animal and human experiments by various approaches and to form biowaiver evaluations with these approaches (Yilmaz Usta et al., 2018). Thus, the present study is carried out to evaluate the *in vitro* availability of locally manufactured and imported Atorvastatin 40mg Azithromycin 500mg, clarithromycin 500mg and glibenclamide 5mg tablets against their innovator/counter comparator Products marketed in Ethiopia; in order to ensure that all generic drug products, conform to the same standards of quality and efficacy, required of innovator drug 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 II DRUG PRODUCTS

Oral drug delivery is the most commonly used route due to its ease of administration, high patient compliance, cost effectiveness, reduced sterility constraints, and flexibility of dosage form design (Homayun et al., 2019; Shreya et al., 2019). When a drug is administered orally, it has to cross certain checkpoints (varies from drug to drug) within the biological system including dissolution in gastrointestinal fluids, permeation across the gut membrane, and first pass metabolism to finally reach its site of action via systemic circulation. Every checkpoint presents a potential bottleneck, of which dissolution in gastric fluid is of prime importance. Indeed, for most drugs, it is the main requirement to enable systemic circulation which determines the bioavailability (Baghel et al., 2016; Hua, 2020). Considering the conceivable rate-constraining steps, Amidon and his colleagues developed a BCS, that classifies drug substances into four based on aqueous solubility and intestinal permeability (Amidon et al., 1995). According to WHO list of essential medicines, of 61 of the 130 orally administered drugs classified reliably. Twenty-one of these 61 (34%) were classified as class I drugs, 10 as class II drugs (17%), 24 as class III drugs (39%) and 6 as class IV drugs (10%) (Lindenberg et al., 2004). Classification of some orally administered drugs on the WHO model list of Essential Medicines according to the BCS is listed in Table (2).

2.1 BCS class II drug products

Generally, the bioavailability of BCS class II drug is rate-limited by its dissolution, so that even a small increase in dissolution rate sometimes results in a large increase in bioavailability (Amidon et al., 1995; Lindenberg et al., 2004).Therefore, an enhancement of the dissolution rate of the drug is thought to be a key factor for improving the bioavailability of BCS class II drug products. Therefore, several strategies are there for the solubility improvement of poorly water-soluble drugs. These techniques are preferred based on specific features such as the characteristics of the drug under consideration, intended dosage form types, and chosen excipient characteristics (Kasimedua et al., 2015; Rajpoot et al., 2020).

2.1.1 Techniques for solubility enhancement

2.1.1.1 Conventional approaches

Conventional approaches have been in use for decades for the enhancement of the aqueous solubility of poorly soluble drugs. Micronization, solid dispersion, prodrug, cyclodextrin

inclusion complexes, supercritical fluid technology, and cryogenic technology are strategies that fall under the category of conventional approaches (Kasimedua et al., 2015; Rajpoot et al., 2020;Kumari et al., 2023).

Table 2: Classification of some orally administered drugs on the WHO model list of Essential Medicines according to the BCS (Amidon et al., 1995; Lindenberg et al., 2004)

BCS class	Criteria	Example
Class I	Rapidly dissolve with good absorption. The rate-determining steps are a dissolution or gastric emptying. The release is not related with dissolution so Precise dissolution studies must be performed to confirm bioavailability	Metoprolol, amlodipine, allopurinol, verapamil, propranolol etc.
Class II	Drugs are absorbed well (lower than class I) but inconsistent due to formulation and *in-vivo* factors. Dissolution is rate-dependent, and it must have biorelevant dissolution media to mimic the *in-vivo* environment. Predictable *in-vivo in-vitro* relationship	Azithromycin, clarithromycin, atorvastatin glibenclamide, carbamazepine, etc.
Class III	The rate-determining step is permeability. Drug absorption could be non-uniform. Precise dissolution profile, partial or unpredictable *in vitro-in vivo* correlation	Cimetidine, ranitidine, acyclovir, abacavir, atenolol
Class IV	Unreliable bioavailability and must require an alternate route of administration other than oral. Complications with drug delivery and un-predictable *in-vitro-in-vivo* association.	Hydrochlorothiazide, furosemide, indinavir, nelfinavir, ritonavir, aluminium hydroxide

A. Particle size reduction

Particle size reduction approach is widely used to increase dissolution rate. The dissolution rate of a drug proportionally increases with increasing surface area of drug particles. When the particle size is decreased, the larger surface area of the drug allows the increase in the surface area to volume ratio thus increasing the surface area available for solvation. Particle size reduction technologies are therefore routinely used to increase the bioavailability of poorly

soluble drugs. Particle size reduction can be achieved micronazation and nanosuspension. Each technique utilizes different equipment for reduction of the particle size (Khadka et al., 2014; Bhalani et al., 2022; Kumar et al., 2023). The process of micronization was applied to carvedilol phosphate, by air jet mills, revealed that improved the dissolution rate of carvedilol phosphate by comparing the in vitro drug release profiles where the formulation of matrix tablet containing 40 mg equivalent micronized carvedilol phosphate showed 99% drug release after 24h while the same formulation without micronization of the drug showed 75% drug release (Chatterjee & Pal, 2010).

B. Complexation

Complexation is the association between two or more molecules to form a non-bonded entity with a well-defined stoichiometry. Complexation relies on relatively weak forces such as London forces, hydrogen bonding, and hydrophobic interactions. Stanching complexation and inclusion complexation are major types of complexation (K. N. Chavan et al., 2023; Freitas et al., 2012). Inclusion complexes are formed by inserting a non-polar molecule (guest molecule) into the cavity of another molecule or group of molecules (host molecule). The inclusion complex creation approach has been used to increase the aqueous solubility, dissolution rate, and bioavailability of the drug products (Deshmukh et al., 2017; Rajpoot et al., 2020). Cyclodextrin (CD) is the most frequently used host molecule which form a group of non-hygroscopic crystalline substances composed of cyclic oligosaccharides formed by 6 (αCD), 7 (βCD) or 8 (scud) (α-1,4)-linked D-glucopyranose units. Since each glucopyranose unit has a chair structure, the CD molecule has a cone-shaped structure with the hydroxy groups extending from the outside, making CD a very hydrophilic molecule externally but hydrophobic internally (Ainurofiq & Putro, 2021; Kumari et al., 2023; Rajpoot et al., 2020). CD has a number of benefits, including greater solubility, bioavailability, stability, and prevention of incompatibility. Rivaroxaban (RIV), an oral anticoagulant, is a poorly soluble drug and formulated rivaroxaban-loaded β-Cyclodextrin-based inclusion complexes via the kneading method, spray drying, and physical mixing, which revealed an increased solubility in water by 3.36-, 2.34-, and 4.02-fold; increments of 1.88-, 3.68-, and 1.78-fold were obtained in the acetate buffer (Sherje et al, (2018). Furthermore, carvedilol formed as an inclusion complex with CD using microwave irradiation showed that the solubility of poor-soluble carvedilol can be increased many folds by successfully constructing inclusion complexes with beta CD (Wen et al. 2004).

C. Solid dispersions

Solid dispersions (SD) have been a good technique for enhancing drug solubility, absorption, and therapeutic efficacy. SD is a group of solid materials with at least two distinct components: a hydrophilic matrix and a hydrophobic drug. The molecular dispersion of one or more hydrophobic drugs in a hydrophilic carrier matrix is referred to as solid dispersion(Iswarya Sridhar et al, 2013; Kasimedua et al., 2015). Formulating solid dispersions is a method of choice within pharmaceutical industries for improving drug solubility in the dosage form. Solid dispersion of repaglinide (RPG) through solvent evaporation method using polyethylene glycol 4000 (PEG 4000) as a hydrophilic carrier in three-drug to PEG 4000 ratios (1:1, 1:3 and 1:5). The aqueous solubility of RPG was increased with increased PEG 4000 conc. and maximum solubility was attained at 1:5 drug: PEG 4000 ratio with an enhancement in the solubility of pure RPG in distilled water was observed from 22.5 ± 5.0 to 235.5 ± 5.0 µg/ml at 37 °c. Based on the in vitro release study carried out in phosphate buffer (pH 7.4), a burst release of between 80% and 86% from the solid dispersion preparations was observed in the first fifteen minutes (Yang et al. 2016).

D. Solubilization by surfactants

Reducing the interfacial tension between the surfaces of the solvent and the poorly soluble substance's surface will improve wetting and salvation interaction. Surface active agents enhance the solubility of poorly water-soluble drugs due to the formation of micelles. This phenomenon is known as micellar solubilization (Rajpoot et al., 2020; Vinarov et al., 2018).

E. Prodrug

A prodrug is an inactive, chemically modified parent drug that has enhanced aqueous solubility and can be converted into the active parent drug via rapid biotransformation. Undesirable properties, including poor aqueous solubility, chemical instability, insufficient oral or local absorption, fast pre-systemic metabolism, low half-life, toxicity and local irritation are commonly resolved using the prodrug approach. In addition, problems related to drug formulation and delivery can also be overcome by using this strategy (Jornada et al., 2016; Kumari et al., 2023).

F. Modification of the crystal habit

The formation of crystal offers a promising technique to enhance solubility, dissolution rate, and bioavailability of the lipophilic agents by the polymorphs, hydrates/solvates process (Kumar et al., 2016).

i. Hydrates/solvates

When water is used as solvent and drug is associated with this, then this solvate is called as hydrate. These hydrates require less energy to break up the crystal than anhydrous forms. In a study, the development of salt hydrates of norfloxacin with three other drugs, that is, diclofenac, diflunisal, and indomethacin showed significant improvement in the solubility of the drug for buffer solution (at pH 7.4) (Bhattacharya et al., 2018). Moreover, Romdhan, et al. revealed preferentially solvation of drug sulfacetamide by employing water in both water and ethanol-rich mixtures (Romdhani et al., 2019).

ii. Polymorphs

The majority of drugs show polymorphism, which can be explained as the capability of the active molecules to occur in different crystalline forms. Further, these drugs with different crystalline forms may exhibit many physicochemical aspects and biological effects, for instance, solubilizing ability, half-life, melting temperature, morphological properties, and bioavailability(Rajpoot et al., 2020; Ritika et al., 2012). In a study, a new kind of anhydrous polymorph using a free base of the sildenafil and two solvates (acetonitrile and propane nitrile) revealed that desolvate prepared from acetonitrile solvate was regarded most stable form-I. However, the metastable form of the drug is associated with increased energy and surface area, resulting in increased solubility (Barbas et al., 2018).

G. pH Modification

This plays a critical role in drug solubility. It can influence the aqueous solubility of drugs. By varying the solution pH, one can alter the charge state of the drug molecules. If the pH of the solution is such that a particular molecule carries no net electric charge, the solute often has minimal solubility and precipitates out of the solution. The pH at which the net charge is neutral is called the isoelectric point (Bhalani et al., 2022; Rajpoot et al., 2020).

H. Co-Crystallization

Co-crystals are the complexes of non-ionic supramolecular materials. They can be utilized to address issues regarding physical properties, i.e., drug solubility, bioavailability, and stability, without affecting the chemical structure of APIs. Co-crystals are prepared using two or more different molecular units, in which the weak forces are intermolecular interactions such as π–π stacking and hydrogen bond interactions. The composition and molecular interaction of pharmaceutical compounds will be changed by co-crystallization, and it is accepted as a good option to optimize the drug characteristics. Co-crystals will offer various routes, where any APIs can be crystallized regardless of belonging to acidic, basic or ionizable groups. This can be helpful for compounds with low pharmaceutical profiles due to their nonionizable functional groups(Chatterjee et al, 2010; Patole et al, 2014; Shreya et al., 2019).

2.1.1.2 Nano technological approaches for solubility enhancement

Nanotechnology is applicable to promote the solubility and oral bioavailability of drugs with poor aqueous solubility. Nanotechnology involves extensive investigation and usage of structures and materials at the level of nanoscale, which is up to 100 nm (Kumari et al., 2023; Paroha et al., 2018; Rajpoot et al., 2020). Some nanocarriers used for the solubility enhancement of poorly soluble drugs are briefly described below

a) Liposomes

Liposomes are vesicles surrounded by phospholipid bilayers that can solubilize drugs that are insoluble in water in the lipid domain of the liposomal membrane. Because liposomes may solubilize weakly water-soluble pharmaceuticals, safeguard the drug from GI tract degradation, and improve permeability across the epithelial cell membrane (boosting oral bioavailability), liposomal administration appears promising for the oral delivery of hydrophobic drugs (Kumari et al., 2023). The liposomal drug delivery system for the enhancement of the solubility and bioavailability of Efavirenz. It has been found in their studies that there is an improvement in the solubility of Efavirenz with increasing concentration of soya lecithin in the liposomal formulation (i.e., after the addition of 900 mg of soya lecithin) along with the drug and water; solubility went up to 27.82 ± 2.55 µg/ml *(*Rao *et al* (2018).

b) Nanoparticles and nanostructured lipid carriers

Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs) are two lipid-based nano-systems that have sparked a great deal of interest for a method of orally delivering hydrophobic drugs with low bioavailability. These lipid nanocarriers offer several advantages including biocompatibility, simplicity of scale-up, enhanced lymphatic transport, and, hence, lower first-pass metabolism. several investigations have looked into SLN or NLC formulations (Jaiswal et al., 2016; Naseri et al., 2015). In a study, SLNs were designed to improve the oral bioavailability of all-trans retinoic acid (ATRA), a poorly soluble drug, and the results showed that adding ATRA into SLN formulations considerably improved ATRA absorption (Hu et al. 2010). Furthermore, they evaluated the potential of NLCs for improving the solubility and bioavailability of tacrolimus (TL), a tacrolimus-loaded nanostructured lipid carrier for this purpose, and discovered that it increased the relative bioavailability of TL-NLC by 7.2 times as compared to TL suspension (Khan et al.2016).

c) Dendrimers

Dendrimers, a new class of polymers, possess excellent potential for drug solubility enhancement. Dendrimers containing poly (amidoamine) (PAMAM) are the most extensively studied dendrimers as drug delivery systems. They consist of an ethylenediamine core and branched units made of methyl acrylate and ethylenediamine. The capacity of these hyper-branched, mono-dispersed molecules to covalently bind drug molecules to their peripheral branches and encapsulate them within the dendritic structure is unique (Choudhary et al., 2017; Kumari et al., 2023). Various studies have successfully employed dendrimers to improve the solubility of poorly soluble drugs. Dendrimers can increase the solubility of hydrophobic substances by physical encapsulation or covalent conjugation. A study investigated the effect of a full-generation PAMAM (G4) dendrimer on the solubility of candesartan cilexetil (a lipophilic calcium channel blocker). The maximum solubility of candesartan cilexetil increased approximately 373-fold at a PAMAM concentration of 10 mg/mL, and it has been shown that the improvement in solubility is dependent on the concentration of the dendrimers (Guatam et al. 2012).

2.2 Bioequivalence studies of BCS Class II drug products

Good-quality drug products that fulfil the regulatory parameters and are produced per the current good manufacturing practice (cGMP) standards are very critical for the best therapeutic outcomes. Therefore, bioequivalence studies become important to assist in the substitution of branded (innovator products) with generics for affordability while maintaining therapeutic efficacy, which is essential to ensure the absence of any significant difference in the rate and extent to which the active ingredients become available at the site of drug action when administered under similar routes and conditions (Gouveia et al., 2015; Hassali et al., 2012). Several BE studies of BCS class II drug products have been conducted in different countries around the world. There are various drug products belongs to BCS Class II, including antibiotics, antidiabetics; antihypertensive antiepileptics, antihyperlipidemic drug products, etc.

2.2.1 Antibiotics

Antibiotics, from natural or synthetic sources, inhibit the growth of microorganisms or kill the bacteria. Bacterial resistance is one of the most clinical and public health challenges facing the healthcare system. Many reasons have been implicated for bacterial resistance associated with administering antibiotics, ranging from natural biological processes to misuse and inadequate diagnosis and treatment. The frequent prescription of broad-spectrum antibiotics rather than prescribing more precise antibiotics and the inadequate dose and duration of treatment has been particularly embroiled. The sub inhibitory concentrations resulting from the later effect can modulate the bacterial virulence (Al-tabakha et al., 2017; Cope et al., 2014; Sandoval-Motta et al., 2016). A source of sub inhibitory levels of antibiotic can be from the often-neglected factor that generic drugs may not be in fact therapeutically equivalent to the branded antibiotic. Administering a generic antibiotic with poor drug release or subtherapeutic dose is not only ineffective, but can have the potential to expand bacterial resistance (Al-tabakha et al., 2017). Various reports have been published on the *in vitro* and *in vivo* equivalence of different antibiotic products in different countries. In a comparative *in vitro* dissolution profile studies of commercial available azithromycin dehydrate 500 mg tablets in Philippines, revealed that 2 of the 3 generic products are equivalent to the innovator product in terms of multi-point *in vitro* dissolution profile (Guzman et al., 2015). Similarly, a study conducted in Ethiopia, revealed that all of the generic products of azithromycin passed a single-point dissolution test and met USP specifications (releasing >80% within 30min). However, 2 generic products didn't meet USFDA

f1, and f2 accepted limit. Therefore, can't be considered as interchangeable with the comparator product (Mekasha et al., 2023). In a BE study conducted in Nigeria on three generic products of azithromycin and four generic products of clarithromycin with their respective innovator product revealed that, all the brands of azithromycin and clarithromycin were considered bioequivalent with the innovator brand (Umeh et al., 2018). On the other hand, a pharmaceutical equivalence study conducted in Kenya on clarithromycin oral dosage forms showed that only 4 (25%) out of the 16 products tested met the acceptance criteria for similarity factor, (f2) relative to innovator products (Manani et al., 2017). In another *in-vitro* bioequivalence study conducted in Saudi Arabia indicated that one generic ciprofloxacin hydrochloride tablets is not bioequivalent to the innovator drug product (Hanafy, 2016). 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). Therefore, health professionals should avoid assuming that some generic antibiotics are therapeutically similar, even if they are labelled with the same pharmaceutical active ingredient and strength.

2.2.2 Anti-diabetic agents

Diabetes mellitus (DM) is a chronic and complex metabolic disorder that is recognized as a serious public health concern with a considerable impact on human life and health expenditures in particular for poorly developed nations. Diabetes affects individuals' functional capacities and quality of life, leading to significant morbidity and premature mortality). Glibenclamide (GLB), an oral hypoglycemic agent categorized within the sulphonyl urea group, extensively utilized in the management of type II diabetes mellitus. Its mechanism of action involves the reduction of blood glucose levels through the stimulation of insulin secretion from the pancreatic beta cells (Khan et al., 2021; Standl et al., 2019). Following the expiration of the patent on the original brand, a variety of generic GLB tablets have become available worldwide within the pharmaceutical market. In Ethiopia, both locally produced and imported GLB tablet varieties can be found (Kassahun et al., 2018). Numerous studies have been conducted in different regions to assess the therapeutic equivalence and interchangeability of generic GLB medications with their innovator counterparts through comparative in vitro quality evaluation and bioequivalence studies. For instance, El-Sabawi et al. (2013) scrutinized five generic GLB tablet formulations in the Jordanian market, revealing distinct dissolution profiles among them and in comparison, to

the original Daonil. Furthermore, Elhamili et al. (2014) examined three different brands of GLB tablets available in the Libyan market, noting that all products met the specifications outlined in the British Pharmacopoeia. Likewise similar study conducted in Peru (Alvarado, Muñoz, Bendezú, García, et al., 2021),Mexico (Sánchez-Nava et al., 2019), Saudi Arabia (Shazly et al.,2014) revealed that some generic GLB products did not meet the pharmacopeial specification for quality control parameters and the FDA requirements $f1 < 15$ and $f2 > 50$ and also showed a statistically significant difference ($p < 0.05$) compared to innovator products.

2.2.3 Antiepileptic drugs

Epilepsy is a persistent neurological condition impacting a global population of 65–70 million individuals. It affects individuals across diverse demographics such as age, race, social status, and geographic regions. The primary approach to treatment lies in pharmaceutical intervention, with an approximate 70% of patients exhibiting response to prescribed medications, while the remainder may necessitate surgical procedures and alternative therapies to attain seizure management. Undesired reactions play a significant role in compromising medication adherence, with rates reaching 30–50% among adult epilepsy patients, leading to diminished quality of life and discontinuation of drug regimens (Beghi, 2020; Olusanya et al., 2017). Similar to other medical fields, the utilization of generic versions of antiepileptic drugs (AEDs) is common practice in epilepsy treatment following the expiration of patent protection on branded AED formulations; presently, the majority of new AEDs are accessible in generic form. The influx of generic pharmaceuticals from various origins into the healthcare systems of numerous developing nations has brought about an array of challenges, with the most pressing issue being the widespread circulation of counterfeit and substandard drug products. (Asrade et al., 2023; Saxena et al., 2006). 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 II anti-epileptic drug products. According to the study conducted on *in vitro* biopharmaceutical equivalence of carbamazepine sodium tablets in Peru the evaluated samples were not bioequivalent with the innovator brand based on the dissolution profiles ($f2 < 50$) (Alvarado, et al., 2021). Another quality control and *in vitro* BE study conducted in Nigeria on carbamazepine tablets, revealed that only one brand out of four tested brands has f1 value between 0 and 15 and f2 value between 50 and 100 (Uzor et al., 2018). In addition a study conducted in Sudan revealed that only two carbamazepine brands could be

used interchangeably with the originator brand based on the dissolution profile (Abdelrahman et al., 2023). Nonetheless, an investigation carried out in Ethiopia unveiled that every variation of carbamazepine tablet adhered to the standards of quality control outlined in pharmacopeial specifications. Consequently, it was determined that each brand could be utilized interchangeably in order to attain the intended therapeutic outcome (Asrade et al., 2023).

2.2.4 Anti-hyperlipidemia agents

Hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels and is considered as one of the major risk factors causing cardiovascular diseases (CVDs) (Hill et al 2021; Nouh et al., 2019). Antihyperlipidemic drugs target the problem of elevated serum lipids with complementary strategies (Last et al., 2011). Because of its lower costs, use of generic drugs is supported by healthcare systems by recommending physicians to prefer them whenever available over brand-name drugs. However, whether generic drugs are as therapeutically effective as their brand-name counterparts is still a matter of debate (Corrao et al., 2014). To ensure that generic anti-hyperlipidaemic medications are therapeutically similar to and interchangeable with the products of their innovators, number of studies that deal with comparative *in vitro* quality evaluation and BE having been conducted in various countries. A comparative *in vitro* bioequivalence study on atorvastatin tablets conducted in Bangladesh revealed that only 4 of the 10 generic products may be used as interchangeable with the innovator drug product (Oishi et al., 2011). Likewise, a study conducted in Pakistan revealed that all generic brands of atorvastatin are not equivalent in terms of *in vitro* release behaviour (Tariq et al., 2014). A bioequivalence study of atorvastatin tablets conducted in Pakistan revealed that the test and the reference products exhibited comparable values of pharmacokinetic parameters. Therefore, concluded that the evaluated samples are bioequivalent since there was no significant difference between the rate and extent of absorption of the drug from the test and the reference formulations (Mohammad et al., 2015). However, study conducted in Nigeria on *in vitro* and *in vivo* BE studies of film coated atorvastatin showed dissolution profiles inter brand variability. Four brands attained 70% dissolution within 45 minutes and did not meet the pharmacopeial specifications. While the *in vivo* bioavailability study showed that three out of the four brands

were bioequivalent to the innovator brand and can be substituted for each other in their prescription (Okorie et al., 2016).

2.2.5 Antihypertensive agents

Hypertension is a key risk factor for cardiovascular disease worldwide. The primary goal of antihypertensive therapy is to control blood pressure and reduce the long-term risk of cardiovascular morbidity and mortality (Kitt et al., 2019; Kjeldsen et al., 2018). Different classes of medication are available for the management of hypertension including diuretics, β-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors (ACE-Is), and angiotensin II receptor blockers (ARBs) (Jm et al., 2018). The marketing of multisource drug products registered by national drug agencies in developing countries, with the view of improving healthcare delivery through competitive pricing, has an attendant problem of ascertaining their quality and interchangeability. As a result, health-care professionals sometimes pose questions whether these generics are equivalent to their original counterparts and whether patients are put at risk (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 II hypertensive drug products. Pharmaceutical equivalence and comparative dissolution profile studies for coated tablets containing Verapamil hydrochloride conducted in Brazil revealed that none of the generic medications presented dissolution profiles similar to the reference medication, which may indirectly lead to the unsuitable bioavailability of the drug and therapeutical inefficacy (Marques de Andrade et al., 2018). Another comparative *in vitro* study on Valsartan tablets was conducted in Pakistan and showed that f1 and f2 for all brands were within the specified limits of 0–15 for f1 and 50–100 for f2. Mean dissolution efficiency and 95% confidence intervals were within the acceptable range of ± 10% for all brands. Therefore, the generic drug products can be prescribed in place of the reference drug products, which will be more cost-effective for the patients (Ansari et al., 2023). Likewise, similar study conducted in Kumasi Metropolis, Ghana (Osei-Asare et al., 2015) and Addis Ababa, Ethiopia (Agune et al., 2018) revealed that all the studied brands meet pharmacopeial specification for most of the physicochemical quality parameters but most of them failed to meet the *in vitro* dissolution tolerance limits which may affect the in vivo performance of these drugs.

3 OBJECTIVES OF THE STUDY

3.1 General objective

- To compare in vitro availability of some locally manufactured and imported BCS Class II drugs against their innovator/counter reference products.

3.2 Specific objectives

- To undertake a comprehensive literature survey on BE studies on BCS Class II drug products
- To evaluate physical properties of generic drug products (Atorvastatin 40 mg, Azithromycin 500 mg, Clarithromycin, 500 mg, and Glibenclamide 5mg tablets) as compared with their comparator drug products.
- To assess the drug contents of each tablet product using official methods.
- To determine the *in vitro* dissolution profile and compare each generic product against its innovator/counter reference product.

4 EXPERIMENTAL

4.1 Materials and Methods

4.1.1 Materials

Scientific articles from the last five years were selected from academic search engines such as PubMed, Google Scholar and NCBI following the keywords: “BCS class II drugs”, “Solubility enhancement strategies was used for compressive review of BCS class II drug products.

4.1.1.1 Chemicals and reagents

The following chemicals and reagents, were used in this study: working standards of atorvastatin calcium (Assay: 100.14%, Potency: 95.62, LOD: 4.52%) supplied from East Africa Pharmaceuticals PLC, USP Atorvastatin related compound H RS (Lot:R108G0,India), USP Azithromycin RS (Lot: R103C0, China), USP Clarithromycin RS (Lot: R07811,Israel) and Clarithromycin related compound A (Lot: R114R0, United States) obtained from EFDA QC directorate and Glibenclamide working RS (Assay: 98.76%, Lot:R022S0) obtained from Julphar pharmaceutical PLC were reference standards used for pharmacopoeial tests. Locally manufactured and innovator/counter reference products of Atorvastatin calcium 40 mg tablets, Azithromycin 500mg tablets, Clarithromycin 500mg and Glibenclamide 5mg tablets were collected from various drug retail outlets in Addis Ababa Ethiopia. Detailed description of the medicines used in the study is presented in Annex I.

HPLC grade acetonitrile (Fisher Scientific, U.K) and methanol (Fisher Scientific, U.K), Distilled water, Sodium hydroxide pellets (or pearls) 97% Extra Pure (Batch No. L235411704, Loba Chemie Pvt. Ltd. Mumbai, India), anhydrous citric acid (Batch No. L170142453, Loba Chemie Pvt. Ltd. Mumbai, India), Tetrahydrofuran (Batch No. KI8A/03182411/53, Sd fine chem Limited Mumbai, India), Glacial acetic acid (Central Drug House (P) Ltd. Batch No.481220 Delhi, India), Phosphoric acid 85% (Batch No.L324862001, Loba Chemie Pvt. Ltd. Mumbai, India), Monobasic potassium phosphate (KH2PO40, Batch No. L331682003 Loba Chemie Pvt. Ltd. Mumbai, India), Monobasic sodium phosphate (NaH2PO4.2H2O, Batch No.SP368, ALPHA CHEMIKA, India),and Hydrochloric acid (Batch No.L340342008, Loba Chemie Pvt. Ltd, Mumbai, India) were water were reagents and solvents used as received.

4.1.2 Equipment and instruments

A High-performance liquid chromatography (HPLC) system equipped with UV detector (Model: SPD-20A, Shimadzu, Japan), an ultrasonicator (Mumbai, India), analytical electronic micro balance (Metler Toledo, USA), ERWEKA dissolution test apparatus (Type: DT 820, Germany), UV-Visible spectrophotometer (Model: UV-1900, Shimadzu, Japan), CALEVA disintegration test apparatus (Type: ZT 304, Germany), CALEVA hardness, thickness and diameter tester (Type: TBH220, Germany), Friability tester (ERWEKA) were major equipment's and instruments employed for the study. Analytical stainless-steel columns of Waters Spherisorb ODS2 C-18 column 5 µm, 4.6 x 250 mm; Agilent's C-18 column 5 µm, 4.6 x 250 mm; and ACE C-18, 5 µm, 3.9 x 150 mm columns were used for HPLC analysis.

4.2 Methods

4.2.1 Identification tests

Identification of the active pharmaceutical ingredient (s) was performed with reference to USP 44/NF 39 and BP 2021 by comparing the retention times of the major peaks of the sample and standard solutions in the assay test for the respective drugs(USP, 2021a).

4.2.2 Assay

4.2.2.1 Assay of Atorvastatin calcium tablet

The contents of the API of Atorvastatin calcium tablet were determined according to USP monograph by using HPLC equipped with a 244-nm UV detector. A Phenomenex C-18 column with 5 µm particle size, 250 mm length, and 4.6 mm internal diameter was used. The system was run at a flow rate of 1ml/min of the mobile phase with an injection volume of 20 µl (USP, 2021c).

Preparation of the mobile phase: The mobile phase for the analysis of atorvastatin calcium tablet consisted of acetonitrile, stabilizer-free tetrahydrofuran, and citrate buffer. The buffer was prepared by dissolving 9.62 g of anhydrous citric acid in 950 ml of water and adjusted with ammonium hydroxide to a pH of 4.0 then diluted with water to 1000 ml. The mobile phase was then prepared by mixing 27:20:53 v/v of acetonitrile stabilizer-free tetrahydrofuran and buffer. This solution was filtered in *vacuo* through a 0.45 µm nylon membrane filter and sonicated for 20 minutes.

Standard solution system suitability solution preparation: Accurately weighed 10 mg Atorvastatin calcium RS was dissolved in 100 ml of diluent (A 1:1 v/v mixture of acetonitrile and 0.05 M ammonium citrate buffer pH 7.4) to obtain 100 μg/ml of stock solution. A 1:1 v/v solution of 0.01 mg/ml of USP Atorvastatin Related Compound H RS and the standard solution was used to check the suitability of the system. The requirements are the resolution between atorvastatin and atorvastatin related compound H should be not less than (NLT) 5.0, tailing factor should not be more than (NMT) 1.5, and relative standard deviation should be NMT 1%.

Sample solution preparation: Ten tablets of atorvastatin calcium from each brand were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 100 mg of atorvastatin was transferred to a 100ml volumetric flask and diluent was added to about 50% of the final volume of the flask and the mixture was mechanically shacked until it dissolved then the solution was diluted to volume and centrifuged. finally, 1ml of the solution taken to 10ml of volumetric flask to obtain 0.1mg/ml of sample solution. This solution was then transferred to 1.5 ml amber-coloured HPLC vials after being filtered through a 0.45 μm polytetrafluoroethylene (PTFE) syringe filter.

The percentage of the labelled amount of atorvastatin (C33H35FN2O5) in the portion of tablets taken was calculated by using Eq.8.

$$\text{Assay} = (rU/rS) \times (CS/CU) \times [M \times (Mr1/Mr2)] \times 100 \ldots\ldots\ldots\ldots \quad \text{Eq. 8}$$

Where: rU = peak response of atorvastatin from the Sample solution

rS = peak response of atorvastatin from the Standard solution

CS = concentration of USP Atorvastatin Calcium RS in the Standard solution (0.1mg/mL)

CU = nominal concentration of atorvastatin in the Sample solution (0.1mg/mL)

M = number of moles of atorvastatin per mole of atorvastatin calcium, 2

Mr1 = molecular weight of atorvastatin, 558.64

Mr2 = molecular weight of atorvastatin calcium, 1155.34

Acceptance criteria: 94.5%–105.0%

4.2.2.2 Assay of Azithromycin tablets

The contents of the API of Azithromycin tablet were determined using HPLC according to USP specification by using HPLC equipped with UV-Vis detector adjusted at the wavelength of 210-nm, column 4.6-mm × 25-cm;5-µm packing made up of C18 (L1). The flow rate was 1.5ml/min and injection volume were 50µl (USP, 2021d).

Mobile phase preparation: The mobile phase for the assay study of azithromycin tablet consisted of a phosphate buffer of pH 7.5 and acetonitrile. The buffer was prepared by dissolving 4.6g of monobasic potassium phosphate anhydrous in 900 mL of water and adjusted with 1 N sodium hydroxide to a pH of 7.5, and diluted with water to 1000 ml. Then a 35:65 v/v composition was prepared by mixing buffer with acetonitrile. The resultant solution was then filtered in vacuum pump through a 0.45 µm nylon membrane filter and sonicated for about 20 minutes.

Standard solution preparation:1mg/ml of standard solution was prepared by taking 100 mg of azithromycin RS and transferred 100 ml volumetric flask and dissolved by the mobile phase. The solution was sonicated and shacked to facilitate the dissolution. This solution was used for system suitability test and the tailing factor should be NMT 2 for each analyte peak, and the relative standard deviation should be NMT 2.0%.

Sample solution preparation: Twenty tablets of azithromycin from each brand were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 100 mg of atorvastatin was transferred to a 100ml volumetric flask to obtain 1mg/ml of sample solution. This solution was then transferred to 1.5 ml amber-coloured HPLC vials after being filtered through a 0.45 µm PTFE syringe filter. The percentage of the labelled amount of azithromycin ($C_{38}H_{72}N_2O_{12}$) in the portion of tablets taken is calculated by using Eq.9.

$$\text{Assay} = (rU/rS) \times (CS/CU) \times P \times F \times 100 \qquad \text{Eq. 9}$$

Where; rU = peak response of azithromycin from the Sample solution. rS = peak response of azithromycin from the Standard solution. CS = concentration of USP Azithromycin RS in the Standard solution (mg/mL). CU = nominal concentration of azithromycin in the Sample solution (mg/mL). P = potency of USP Azithromycin RS (µg/mg). F = conversion factor, 0.001 mg/µg.

Acceptance criteria: 90.0%–110.0%

4.2.2.3 Assay of Clarithromycin tablets

Assay of clarithromycin was done by HPLC equipped with UV/VIS detector, adjusted at the wave length of 210nm, Phenomenex column 150 x 4.6mm, 5 µm packing made up of C8 (L7), column temperature maintained at 50°c, flow rate 1.ml/min and injection volume of 50 µl. Five replicate of system suitability solution (A 1:1 v/v of 125 µg/ml of USP clarithromycin related compound A RS and the standard solution) were used to check the suitability of the system.

The requirements are the resolution between the clarithromycin and clarithromycin related compound A should be NLT 2, tailing factor should be between 0.9–1.5 and relative standard deviation should be NMT 2%(USP, 2021e).

Mobile phase preparation: The mobile phase for the assay study of clarithromycin tablet consisted of a 0.067 M monobasic potassium phosphate solution and methanol. 0.067 M monobasic potassium phosphate solution was prepared by dissolving 9.118g of monobasic potassium phosphate in 1000ml of water. Then a 13:7 v/v composition was prepared by mixing methanol with monobasic potassium phosphate solution, the pH was adjusted with phosphoric acid to 4.0, The resultant solution was then filtered *in vacuo* through a 0.45 µm nylon membrane filter and sonicated for about 20 minutes.

Standard solution preparation: 625µg/ml of standard stock solution was prepared by dissolving 31.25 mg of clarithromycin working standard in 50 ml methanol. The solution was shacked and sonicated for 15minute to facilitate the dissolution. From the resulting solution 125 µg/ml of standard solution was prepared by diluting 2ml standard stock solution in 10ml of mobile phase.

Sample solution preparation: Ten tablets of clarithromycin from each brand were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to 31.25 mg of clarithromycin was transferred to 50ml volumetric flask and dissolved with methanol in order to obtain 625µg/ml of sample stock solution. Finally, 2ml of the solution was taken and diluted with mobile phase in 10ml volumetric flask to obtain 125 µg/ml of sample solution. The percentage of the labelled amount of Clarithromycin ($C_{38}H_{69}NO_{13}$) in the portion of tablets taken is calculated by using Eq.10.

$$\text{Assay} = (rU/rS) \times (CS/CU) \times 100 \qquad \text{Eq. 10}$$

Where: rU = peak response from the Sample solution

rS = peak response from the Standard solution

CS = concentration of clarithromycin in the Standard solution (µg/mL)

CU = nominal concentration of the Sample solution (µg/mL)

Acceptance criteria: 90.0%–110.0%.

4.2.2.4 Assay of Glibenclamide tablets

Assay of Glibenclamide was done by HPLC equipped with UV/Vis detector, adjusted at the wavelength of 254 nm, column 150 x 4.6mm, 5 µm packing, flow rate 1.ml/min and injection volume of 50 µl. The glibenclamide standard solutions were injected into the HPLC system and the chromatograms were recorded to evaluate the system suitability parameters like tailing factor (NMT 2), theoretical plate number (NLT 2000 for and % RSD (NMT 2) (USP 2021).

Standard Preparation: Glibenclamide working standard (50 mg) was added in 100ml of volumetric flask and dissolved in 10 ml of acetonitrile with the aid of ultrasonic bath for 20 min, sufficient acetonitrile was added to volume produce 0.50 mg/ml of stock solution and finally 20 ml of water was added and mixed.

Sample Preparation: Twenty tablets from each sample were weighed and powdered. A quantity of the powdered tablets containing equivalent to 50 mg of glibenclamide was dispersed and wetted in 20ml of water then 100ml of acetonitrile was added and shacked for 30 min. Finally, the mixture was centrifuged and the clear supernatant was used for HPLC analysis.

The percentage of the labelled amount of glibenclamide (C23H28ClN3O5S) in the portion of tablets taken is calculated by using Eq.11.

$$\% \text{ Content} = \frac{PA\ of\ sample}{PA\ of\ standard} * potencey\ of\ reference\ standard \quad \text{..... Eq. 11}$$

Where PA =Peak area

Acceptance criteria; 90.0 to·110.0%.

4.2.3 Mass uniformity of tested products

The weight variation assessment for all pharmaceutical products was conducted in accordance with the guidelines outlined in USP 2021, which involved the individual weighing of 20 tablets followed by the calculation of the mean weight and its comparison with each individual tablet. The weight variation evaluation is quantified in terms of percentage. Compliance with the weight

variation assessment criteria is achieved if the weight deviation of no more than two tablets exceeds the percentage indicated in table 3, and none of the tablets exhibit a weight variation exceeding double the specified percentage.

$$\%\, weight\ variation = \frac{Individual\ tablet\ weight - average\ weight\ of\ 20\ tablet}{Average\ weight\ of\ 20\ tablet} * 100 \quad \ldots\ldots \text{Eq. 12}$$

Table 3: Weight variation tolerance for uncoated and film-coated tablets as per IP, BP and USP

IP/BP	Percentage deviation (%)	USP
80 mg or less	10	130 mg or less
More than 80 mg and less than 250mg	7.5	More than 130 mg and less than 325 mg
250mg or more	5	325 mg or more

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

4.2.4 Hardness test

The determination of the crushing strength of individual tablets was conducted in adherence to the guidelines outlined in USP44/NF39, utilizing a tablet hardness tester. To ascertain the hardness of each tablet, a selection of ten tablets from each specific brand was made in a random manner. Subsequently, each tablet was positioned between two anvils, followed by the application of force onto the anvils until the tablet fractured. The resulting data was presented in terms of the average value and standard deviation of the measured forces.(USP, 2021h).

4.2.5 Friability test

Friability tests of the studied products were conducted according to USP monograph by using Friabilator at 25 rpm for 4 min. For tablets weighing less than 0.65g each, (Atorvastatin and Glibenclamide) 20 tablets and for tablets weighing more than 0.65 g each, (Azithromycin and Clarithromycin) 10 tablets were weighed and subject to abrasion. The tablets were dusted and weighed again, and then the percent weight loss was recorded. Then the friability of the tablet is calculated using the following formula(USP, 2021g).

$$\%Friability = \frac{Initial\ weight - final\ weight}{Initial\ weight} * 100 \qquad \text{Eq. 13}$$

4.2.6 Disintegration time

The disintegration time test was conducted according to USP 2021 monograph. Six dosage units of each sample were placed in each of the six tubes on the basket rack. The apparatus was then run with 900 ml of water kept at 37 ± 2 ^{o}C in a 1000 mL vessel as the medium, so that the dosage units moved downward and upward while remaining 2.5 cm above the bottom of the vessel and below the medium's surface. The time taken for all six dosage units to completely go into solution through the sieve with no solid particles in the basket was recorded as the disintegration time.

4.2.7 Dissolution

The I*n* vitro dissolution studies of the BCS Class II drug products were performed according to the USP monograph, 2021.

4.2.7.1 Dissolution profile test for Atorvastatin calcium tablets

The dissolution of Atorvastatin calcium tablets was done according to the specification of USP/NF 44/39; 2021 by using 900ml of 0.05M phosphate buffer at pH 6.8 as dissolution medium and type II (paddle apparatus) dissolution apparatus with the rate of 75 rpm at 37±0.5∘C on 12 tablets of each brand (USP, 2021c).From the dissolution media 10 ml sample was withdrawn at 5 10, 15, 30 and 45 min and an equivalent amount of fresh dissolution medium was replaced. The sample were immediately filtered using Whatman filter paper and the absorbance of each sample was determined at 244nm wavelength by UV/Visible spectrophotometer.

Calibration standard preparation: Fifty mg of Atorvastatin calcium working standard was dissolved in 50 ml of diluent (A 1:1 v/v of acetonitrile and water) to prepare 1000 µg/ml of stock solution.40 ml of the resulting solution was diluted with a phosphate buffer pH 6.8 to 900 ml to obtain 44.44 µg/ml of standard solution. Finally, from the resulting solution, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 ml were pipetted out separately into 10 ml volumetric flask and were made to volumes by phosphate buffer to get a concentration range of 3.56, 5.33, 7.11, 8.89, 10.67 and 12.44 µg/ml, respectively. The absorbances of these solutions were measured at 244 nm to generate a calibration curve. The concentration of each sample was determined from calibration curve and the percentage release of the labelled amount of atorvastatin (C33H35FN2O5) was estimated from the following relationship;

Dissolved labelled amount (%) $= (AU/AS) \times CS \times V \times D \times [M \times (Mr1/Mr2)] \times (1/L) \times 100$ Eq. 14

AU = absorbance of the Sample solution. AS = absorbance of the Standard solution.

CS = concentration of USP Atorvastatin Calcium RS in the Standard solution (mg/mL).

V = volume of Medium, 900 mL. D = dilution factor for the Sample solution, if applicable

M = number of moles of atorvastatin per mole of atorvastatin calcium, 2

Mr1 = molecular weight of atorvastatin, 558.64

Mr2 = molecular weight of atorvastatin calcium, 1155.34 L = label claim (mg/Tablet)

According to USP 44, the tolerance limit for Atorvastatin calcium not less than 80% (Q) of the labelled amount of should be dissolved at 15 minutes at the specified medium (USP, 2022).

4.2.7.2 Dissolution profile test of Azithromycin tablets

The dissolution of Azithromycin tablets was done according to the specification of USP/NF 44/39; 2021 by using 900ml of 0.05M phosphate buffer at pH 6.0 as dissolution medium and type II (paddle) dissolution apparatus with the rate of 75 rpm at 37±0.5∘C on 12 tablets of each brand. From the dissolution media 5 ml sample was withdrawn at 5 10, 15, 30, 45 and 60 minute and an equivalent amount of fresh dissolution medium was replaced. From the withdrawn sample 4.5 ml was taken and diluted to 10ml with the diluent (A 20:80 v/v mixture of 17.5 mg/ml of dibasic potassium phosphate and acetonitrile). This freshly prepared solution was then filtered through 0.45 µm filters and placed in amber-coloured HPLC vials for analysis.

System suitability test and preparation of calibration standards: A standard solution was used for system suitability test. A 100 µg/ml of stock standard solution of azithromycin was prepared by accurately weighing 10mg of reference standard and dissolved in 100ml of medium. The solution was shacked and sonicated for 15minute to facilitate the dissolution. From the resulting solution 250 µg/ml of standard solution was prepared by diluting 2ml standard stock solution in 10ml of mobile phase. Six serial concentrations (1.25-7.5 µg/ml) ware prepared from the stock solution to obtain calibration concentration from stock solution. The percentage release of the labelled amount of azithromycin (C38H72N2O12) was estimated from the following relationship;

Dissolved labelled amount (%) = (rU/rS) × (CS/L) × V × D × 100……. Eq. 15

rU = peak response of azithromycin from the Sample solution

rS = peak response of azithromycin from the Standard solution

CS = concentration of USP Azithromycin RS in the Standard solution (mg/mL)

L = label claim (mg/Tablet), V = volume of Medium, 900 mL and

D = dilution factor for the Sample solution,

According to USP 44, the tolerance limit for Azithromycin tablet not less than 80% (Q) of the labelled amount of should be dissolved at 30 minutes at the specified medium (USP, 2022).

4.2.7.3 Dissolution test for clarithromycin tablets

The dissolution of clarithromycin tablets was done according to the specification of USP/NF 44/39; 2021 (USP, 2021e) by using 900ml of pH 5 Acetate buffer as dissolution medium and Type II (paddle apparatus) dissolution apparatus with the rate of 50 rpm at 37±0.5∘C on twelve tablets of each brand. From the dissolution media 10 ml sample was withdrawn at 5 10, 15, 30, 45, and 60 min and an equivalent amount of fresh dissolution medium was replaced. From the withdrawn sample 4.5 ml was taken and diluted with 20ml of mobile phase (Proceed as directed in the *Assay*) to obtain 125 µg/mL of clarithromycin sample solution then the solution was filtered appropriately by using 0.45µm filter before assay by HPLC at the wavelength of 210nm.

Calibration Standard preparation: 625µg/ml of standard stock solution was prepared by dissolving 31.25 mg of clarithromycin working standard in 50 ml of buffer solution. The solution was shacked and sonicated for 15minute to facilitate the dissolution. From the resulting solution 125 µg/ml of standard solution was prepared by diluting 2ml standard stock solution in 10ml of mobile phase. Six serial concentrations (1.25-7.5 µg/ml) ware prepared from the stock solution to obtain calibration concentration from stock solution. The percent of drug release at each time was calculated by using the following relationships.

$$\text{Percentage of the labelled amount dissolved} = \left(\frac{rU}{rS}\right) * \left(\frac{Cs}{Cu}\right) * 100 \qquad \text{Eq. 16}$$

Where rU = peak area from the Sample solution

rS = peak area from the Standard solution

CS = concentration of the Standard solution (µg/mL)

CU = nominal concentration of the Sample solution (µg/mL)

Tolerances: NLT 80% (Q) of the labelled amount of clarithromycin (C38H69NO13) is dissolved with in 30min.

4.2.7.4 Dissolution profile test of Glibenclamide tablets

A dissolution test of glibenclamide tablets was performed according to the method prescribed in the USP monograph using dissolution test equipment with a rotating paddle (USP apparatus 2) at 50 rpm, 900 ml of 0.05 M phosphate buffer, pH 7.5 at 37 ^{o}C (USP, 2021b). Then, 5 mL of sample withdrawn at 10, 15, 30, 45, and 60 minutes, were replaced with an equal volume.The samples were filtered through 0.45 µm syringe filters and analyzed using HPLC equipped with a UV detector at the wavelength of 254 nm.

Calibration Standard Preparation: A 100 µg/ml of stock solution was prepared by dissolving 10 mg of glibenclamide working standard in 100 ml of diluent (A 5:1 v/v mixture of acetonitrile and water). From this stock solution, a series of six concentration levels (1.11-6.67µg/ml) were prepared. All the above solutions were filtered through 0.45 µm syringe filters and placed in amber-colored HPLC vials for analysis.

The percentage of the labeled amount of glibenclamide (C23H28ClN3O5S) dissolved was calculated from the following Eq.

$$\% \text{ drug dissolved} = \frac{rU}{rS} * \frac{CS}{L} * V * 100 \qquad \text{Eq. 17}$$

Where: rU = peak response from the Sample solution. rS = peak response from the Standard solution. CS = concentration of the Standard solution (mg/mL). L = label claim (mg/Tablet). V = volume of Medium, 900 ml.

Comparison of dissolution profiles

Dissolution profiles of all products were compared using ANOVA, model-dependent and model-independent methods commonly recommended by the WHO and US FDA (US FDA, 1997; WHO, 2016).

Model-independent methods of comparison: Efforts were made in order to make a reasonable comparison of the test and reference products. One of the recommended mediums was pharmacopeial QC media; hence, six dosage units of the reference and test products were tested in pharmacopeial QC media. The selection of sampling time points was based on the recommendations that there be at least three sampling time points in the ascending portion of the profile and two time points in the latter phase. The mean value of the 6 tests is calculated for each sampling time point, and all observed points were included to calculate f_1 and f_2 values (US-FDA, 1997).

Dissolution efficiency: Dissolution efficiency is the area under the dissolution curve within a time range (t1–t2). DE was calculated by using the following relationships.

$$DE = \int_{t1}^{t2} \frac{y.dt.}{y100*100} \qquad Eq.\ 18$$

Where; y = is the percentage dissolved at time t. The integral of the numerator which is the area under the curve was calculated using the following relationships.

$$AUC = \sum_{i=1}^{n} \frac{(t1-ti-1)(yi-1-yi)}{2} \qquad Eq.\ 19$$

Where; ti = is the ith time point, yi is the % of dissolved product at time t.

The Mean dissolution time, t50% and t90% were also considered in the study to characterize the drug release rate from the dosage form and the retarding efficiency of the polymer. The mean dissolution time and t50% and t90% of each of the products were calculated using OriginPro 2024 software. The dissolution profiles of each product were individually entered into the software along with their respective sampling points.

Model-dependent methods of comparison: A comparison of the drug dissolution profiles was done using different model-dependent methods. The zero-order, first-order, Hixson-Crowell, Higuchi, and Weibull models were among the model-dependent methods employed for profile comparison. The dissolution data were analyzed using the DDSolver® (China Pharmaceutical

University, China) software (Y. Zhang et al., 2010), and the release mechanisms were established through model fitting using various built-in models. The mean cumulative dissolution data at each sampling point of each sample and reference product were used for model fitting. In order to select the best-fit model for the release, the Akaike information criterion (AIC), the adjusted coefficient of determination (R^2), and the model selection criterion (MSC) were all utilized. To choose the best model, the goodness of fit criteria of the lowest AIC, highest R^2, and highest MSC were applied (Usta & Incecayir, 2022).

Selection of comparator product

Since the innovator product's quality, safety, and efficacy should have been thoroughly evaluated in pre- and post- marketing studies, and since the data on its safety and efficacy are typically linked to those of a pharmaceutical product with defined specifications for quality and performance, the innovator product is typically the most logical comparator product. However, in the absence of innovative products in the local market, the WHO established selection criteria (WHO, 2015a) can be used to select another one than the innovator product. Therefore, due to the absence of the innovator product in the Ethiopian market,

The comparator product is a drug product used to assess the interchangeability of multisource products in a clinical setting. Multisource products should, as a general rule, comply with the same requirements of efficacy, safety, and quality as applicable to the relevant comparator product. Additionally, the quality characteristics of a multisource product has to be assessed in comparison to the comparator product that it ought to be interchangeable(WHO, 2006a).

Since the innovator product's quality, safety, and efficacy should have been thoroughly evaluated in pre- and post-marketing studies, and since the data on its safety and efficacy are typically linked to those of a pharmaceutical product with defined specifications for quality and performance, the innovator product is typically the most logical comparator product. However, in the absence of innovative products in the local market, the WHO established selection criteria (WHO, 2006b) can be used to select another one than the innovator product. Therefore, due to only one innovator product (Daonil 5mg) was present in the Ethiopian market, ATEROZ 40mg (ATO 5), Zithrotel 500mg, and Claricide 500 mg, were selected as a comparator product for respective multisource products based on the selection criteria.

4.3 Data analysis

The Microsoft Excel-2019, the Statistical Package for Social Sciences version-20 (IBM-SPSS, Chicago, USA), DDSolver® (China Pharmaceutical University, China) software and the Origin pro2024 software program were used for statistical and graphical analyses of analytical data obtained from the experimental part of the study.

5 RESULTS AND DISCUSSION

For many developing countries, bioequivalence studies may not economically viable. Hence, neighbouring countries or sub-regions may form a consortium to pool resources, human and material, or establish public private partnerships to initiate viable bioequivalence canters. Until such establishments prevail and become operational, comparative *in vitro* availability testing should be carried out to show that generic drug products are *in vitro* equivalent and interchangeable with their innovator counter products (WHO, 2006; Adegbolagun *et al.*, 2007). 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. In this study, the *in vitro* availability of locally manufactured and imported brands of atorvastatin 40 mg, azithromycin 500 mg clarithromycin 500 mg and glibenclamide 5 mg tablets collected from hospitals and privately-owned drug retail outlets found in Addis Ababa, Ethiopia was evaluated. A total of 21 samples composed of four product types were collected, from all of the products (branded or generic) available per facility, was purchased covertly from the visited facility.

5.1 Identification

Authenticating pharmaceuticals containing the anticipated active components necessitates an identification test on the API in the formulation. Incorrect active component of medicines may result in drug resistance, treatment failure or death, as well as loss of faith in health systems, especially in low and middle-income countries (LMICs), and therefore a reliable supply of good-quality medicine is essential for public health (Rahman et al. 2018). The result for identity test using HPLC showed that none of the samples failed to comply with the requirement for identity. This may be attributed to the sound scientific measurement being followed at every stage of the formulation process.

5.1.1 Identification of atorvastatin calcium

The chromatographic retention time of atorvastatin calcium in the sample solution was compared with the retention time for atorvastatin calcium in the reference standard solution as carried out in the assay procedure, to identify the API. The HPLC chromatogram obtained with the sample solution shows a peak with a similar retention time (11.600 min) as the major peak in the chromatogram obtained with the standard solution (11.537 min), as shown in Fig. 6 (A) and (B).

All the samples of atorvastatin in this study were identified to contain the required active pharmaceutical ingredient using the procedure described.

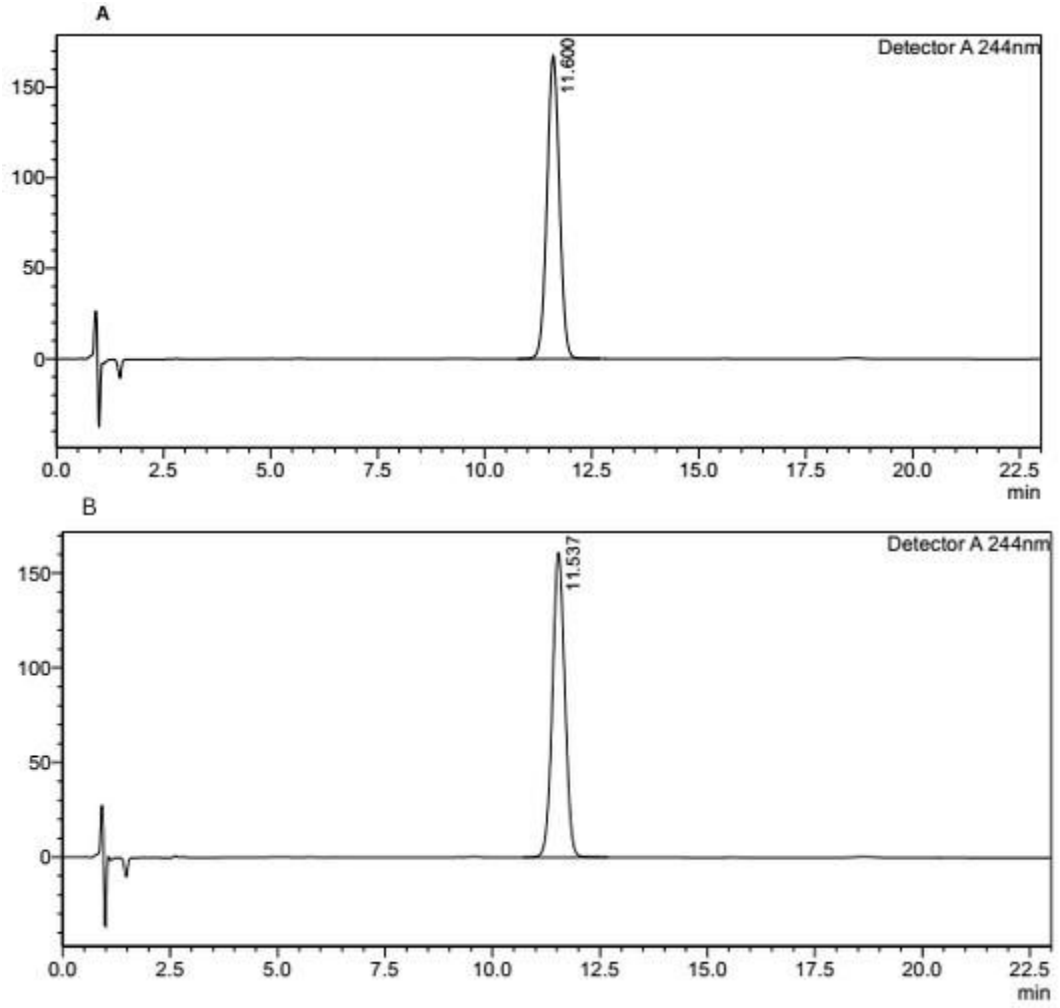

Figure 6: Chromatograms of test sample of atorvastatin calcium (A) and atorvastatin calcium reference standard (B) by using HPLC equipped with 244nm UV detector and C-18 column at,30^{o}c column temperature,1ml/min flow rate and 20 µL injection volume.

5.1.2 Identification of azithromycin

The retention time of *azithromycin in* the sample solution was compared with the retention time for azithromycin in the reference standard solution as carried out in the assay procedure, to identify the API. The HPLC chromatogram obtained with the sample solution shows a peak with a similar retention time (8.955 min) as the major peak in the chromatogram obtained with the standard solution (9.08 min), as shown in Fig. 7 (A) and (B). All the samples of azithromycin in

this study were identified to contain the required active pharmaceutical ingredient using the procedure described.

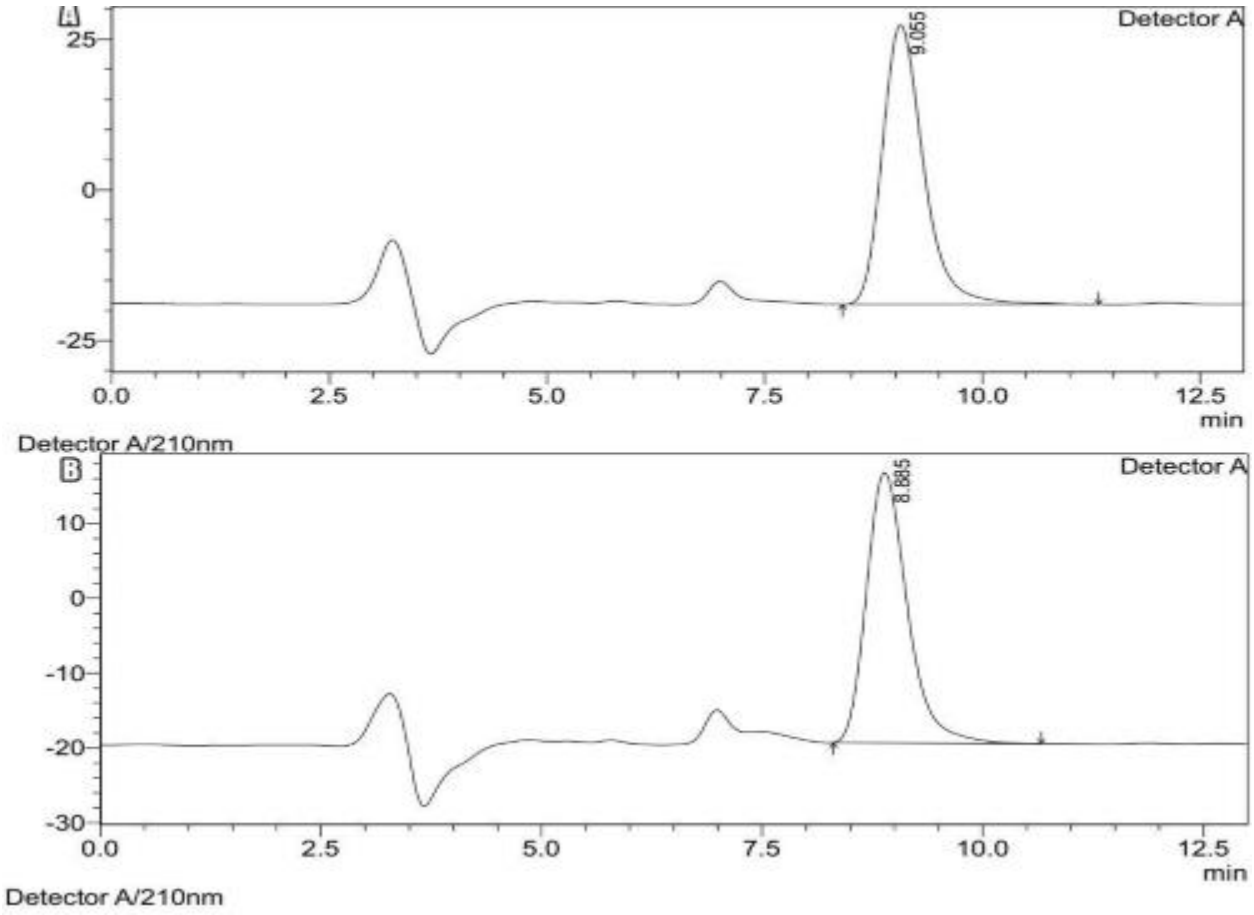

Figure 7: Chromatograms of test sample of azithromycin (A) and azithromycin reference standard (B) by using HPLC equipped with 210 nm UV detector and C-18 column at,50^{o}c column temperature,1.5 ml/min flow rate and 50 µL injection volume.

5.1.3 Identification of clarithromycin tablets

Similarly, the retention times of peaks obtained in the chromatograms of standard and sample solutions as obtained in the assay procedure were compared for the identification of clarithromycin. All the clarithromycin samples had similar retention times as the reference standard solutions. Fig. 8 (A) and (B) show the chromatogram of clarithromycin sample and reference standard with retention times of 5.194 and 5.204 min.

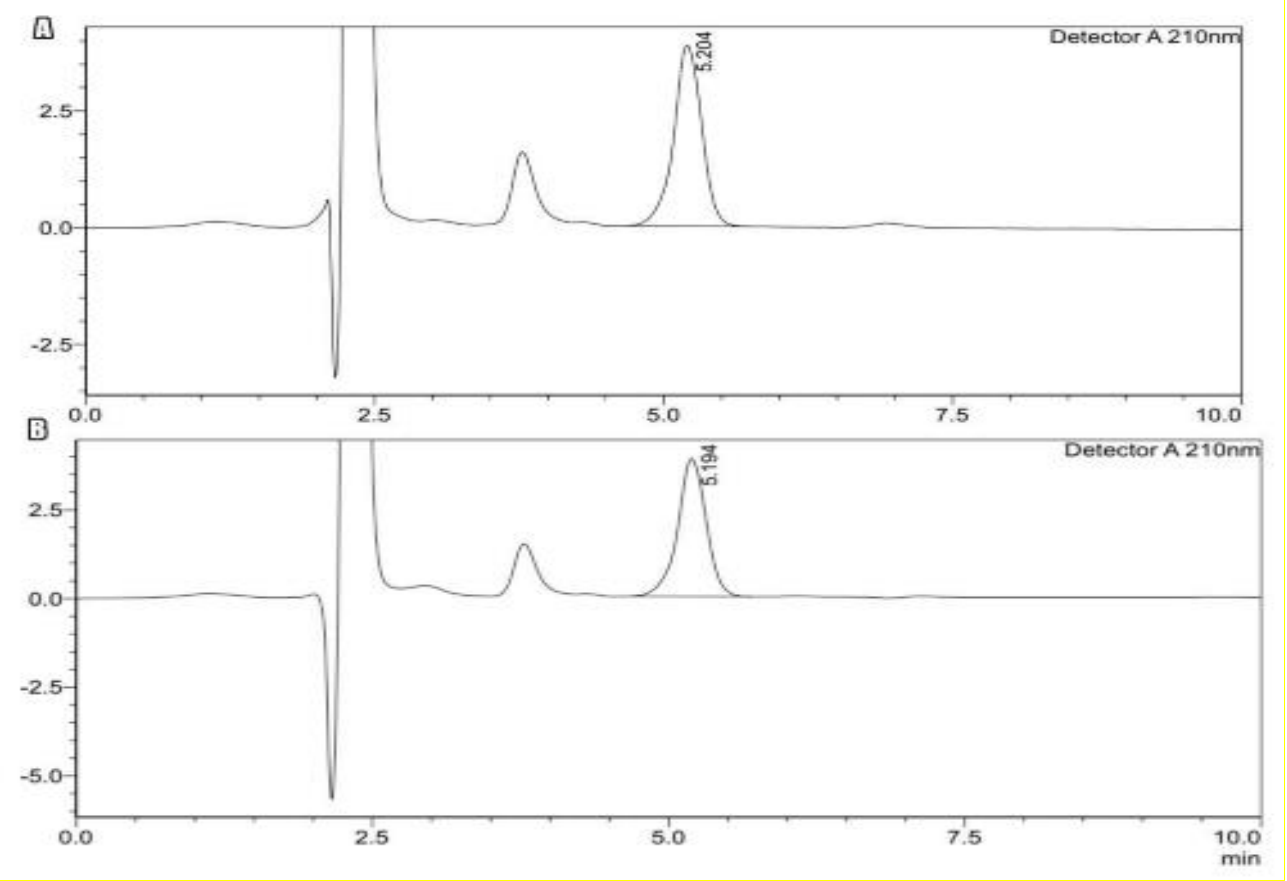

Figure 8: Chromatograms of clarithromycin reference standard and test. sample of clarithromycin. by using HPLC equipped with 210 nm UV detector and C-18 column at,50^{o}c column temperature,1ml/min flow rate and 50 µL injection volume.

5.1.4 Identification of glibenclamide tablets

The test for identification of *glibenclamide* was based on a comparison of the retention times of standard and sample solutions as described in the BP monograph for the assay of *glibenclamide* tablets (BP, 2021). The typical chromatograms obtained with sample and standard solutions are depicted in Figure 9 (A) and (B), having retention times of 4.564 and 4.569, respectively. Based on this procedure, all the glibenclamide samples tested were identified as containing the required glibenclamide active ingredient.

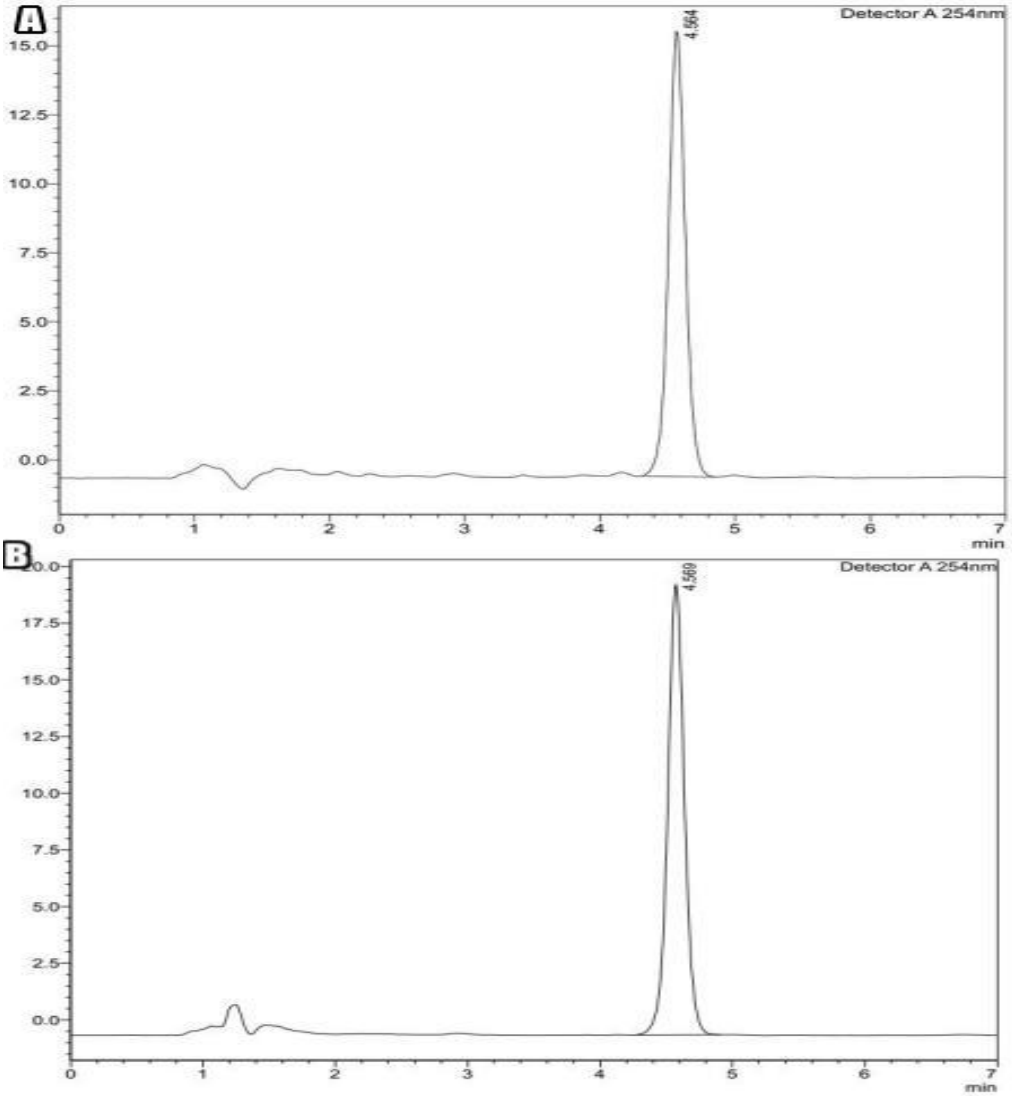

Figure 9: The HPLC chromatogram of test sample of glibenclamide (A) and reference standard of glibenclamide (B)by using HPLC equipped with 254 nm UV detector and C-18 column at 1ml/min flow rate and 50 µL injection volume.

5.2 Uniformity of dosage units

The weight variation test plays a pivotal role in the quality control of tablets during production, providing an accurate depiction of the variation present in the medication. Uniformity of weight serves as a monitor to GMP maintained by the manufacturer as well as amount of the API contained in the formulation (Chavan et al., 2018; Ghimire Prakash et al., 2020). The weight variation test for all drug products was performed according to USP 2021, The application of the USP weight variation procedure involved the assessment of 20 dosage units from each sample

through the utilization of the mean weight. This process holds significant importance in the evaluation of tablet quality during production, providing a precise depiction of the variation present in the medication. It is imperative to exercise strict control over this procedure in manufacturing operations to guarantee consistent distribution of the active pharmaceutical ingredient among the dosage units within a given batch (USP 44-NF 39, 2021).

A presentation of the weight fluctuation examination outcomes for each examined pharmaceutical item is displayed in Table 4. Illustrated in Table 4 is the average weight of atorvastatin calcium, azithromycin, and clarithromycin tablets, ranging from 431.5mg to 629.4mg, 754.79 mg to 942.5mg, and 699.41mg to 1062.34mg, respectively. These tablets are considered to meet the standard if not more than two individual weights deviate from the mean weight by over 5%, and none by over 10%, in accordance with the permissible weight fluctuation range for tablets with an average weight of 324 mg or more as specified by the United States Pharmacopeia. In the case of Glibenclamide tablets, with an average weight ranging from 93.33 mg to 160.53 mg, it is acceptable for no more than 2 tablets to exhibit a difference of more than 7.5% from the average weight, and none should deviate by 15% from the average weight. The research findings indicate that the weight consistency across all tested products complied with the standards outlined in the Pharmacopoeia.

The average weight of all brands was also compared statistically through a one-way ANOVA with a 95% confidence interval. A significant variation was noted in the average weight of the brands across the various samples ($P<0.05$). The discrepancy among samples could potentially be attributed to the formulation conditions, such as mixing techniques, the granulation process, the utilization of mass production machinery, and the quantity of excipients employed. Variability in active components may lead to potential toxicity and inefficacy, while excipients have the capability to modify the release profiles of medications. Moreover, a modification in tablet weight might suggest a shift in the API concentration within the pharmaceutical formulations(Ciavarella *et al.*, 2016;; Zaman *et al.*, 2020).

Table 4: Weight variation, friability and disintegration times of different products of atorvastatin calcium 40 mg, azithromycin 500 mg, clarithromycin 500 mg and glibenclamide 5 mg tablets.

Drug products	Weight (mg) mean ± SD	Lower limit	Upper limit	P value	No. of tablet out of specification	Friability (%)	Disintegration time (min) mean ± SD	Remark
ATO1	431.5 ± 11.02	410.41	453.61	0.000	None	0.53	3.40 ± 0.21	Passed
ATO2	510.31 ± 22.6	484.79	535.82		None	0.38	4.50 ± 0.25	Passed
ATO3	572.5 ± 6.77	543.875	601.125		None	0.89	4.70 ± 0.26	Passed
ATO4	629.4±9.38	597.93	660.87		None	0.68	5.90 ± 0.24	Passed
ATO5*	616.74 ± 8.07	585.9	647.57		None	0.44	4.30 ± 0.30	Passed
AZM1	859.89±1.8	886.053	893.74	0.000	None	0.21	9.08 ± 0.3	Passed
AZM2	605.44 ± 5.71	595.9	627.7		None	0.52	5.30 ± 0.30	Passed
AZM3	901.3±1.7	888.53	914.003		None	0.04	6.30 ± 0.45	Passed
AZM4	754.79±1.8	751.03	758.54		None	0.12	4.80 ± 0.3	Passed
AZM5	922.95±0.9	921.06	924.8		None	0.02	4.0 ± 0.5	Passed
AZM6*	942.5±0.5	941.4	943.61		None	0.05	3.6 ± 0.32	Passed
CLM1	682.32 ± 4.14	648.204	716.436	0.000	None	0.39	7.0 ± 0.14	Passed
CLM2	1032.57 ± 8.27	980.94	1084.19		None	0.29	2.3 ± 0.13	Passed
CLM3	699.41± 9.09	664.43	734.3805		None	0.58	5.6 ± 0.16	Passed
CLM4	869.13 ± 6.79	825.6735	912.5865		None	0.43	3.1 ± 0.14	Passed
CLM 5	1062.34±24.05	1009.23	1115.46		None	0.49	3.3±0.15	Passed
CLM6*	1046.45 ± 4.34	994.13	1098.77		None	0.31	4.1± 0.16	Passed
GLB1	93.33 ± 1.82	86.33	100.33	0.000	None	0.23	1.65 ± 0.23	Passed
GLB2	158.14 ± 0.65	146.28	170.01		None	0.26	2.39 ± 0.17	Passed
GLB3	146.34 ± 4.16	135.36	157.31		None	0.19	5.77 ± 0.52	Passed
GLB4*	160.53 ± 0.76	148.49	172.56		None	0.11	8.89 ±0.27	Passed

*Comparator drug products

5.3 Hardness, thickness and diameter of the tablets

By measuring the force required to crush the tablets, the test for hardness aims to determine their resistance to being broken under specific conditions. Conventional compressed tablets that have a crushing strength greater than 40 newtons (N) are generally considered acceptable. The USFDA recommends that the tablet's diameter be 8 mm or less, and that it not exceed 22 mm (US FDA, 2015), and if the batch's standard deviation of thickness is less than 5%, it may be considered acceptable (USP 44-NF 39, 2021).

To endure the mechanical shocks of handling during manufacture, packaging, and transportation without affecting the disintegration limit, tablets unquestionably need to have a certain level of hardness. If the tablet is too hard, it may not disintegrate in the needed period, and if it is too soft, it may not withstand the mechanical shocks of manufacture, packaging, and shipping. Pharmacopoeias specify a minimum acceptable hardness of 40 N but do not specify the maximum limit for hardness (USP 44-NF 39, 2021). Common industrial practices show immediate-release disintegrating oral tablets to have a hardness of 40 to 100 N. However, if the tablet's hardness is too high, its disintegration is examined before being rejected; if the disintegration falls within acceptable range, the product is also acceptable (Pharma-Education, 2021).

As shown in Table 5, all the products tested for hardness fulfilled the minimum requirement for hardness, while the majority of the products have a hardness value above 100 N, with the highest observed values of 249.3N for sample ATO2 of atorvastatin. 281 N for the sample AZM 3 of azithromycin, 234.4 N for the sample CLM5 of clarithromycin, and 186 N for the sample GLB2 of glibenclamide. Even though the tablets are too hard, all of the analyzed products disintegrated within an acceptable range.

For an acceptable tablet hardness test, a minimum force of approximately 40N is satisfactory. Therefore, all drug products that were assessed in accordance with the hardness test specification. Conversely, one-way ANOVA (95%) statistical analysis revealed a significant difference ($p<0.05$) between the sample mean of hardness of all medicines in the study. This may be the type of binder employed, the particle size. Distribution, moisture content of granules, and compression force used in the formulation Potentially affect tablet hardness(Gad, 2007). Uniform tablet thickness and diameter are required for consumer requirements and for the

packaging of tablets. The thickness and diameter of all tested drug products were determined to be within their allowed limits (±5%). As showed in Table 8 the thickness and diameter of atorvastatin calcium, azithromycin, clarithromycin and glibenclamide tablets ranged from 5.15 ± 0.05mm to 6.85 ± 0.05mm and 15.17 ± 0.01mm to 16.99 ± 0.04mm, 6.20±0.02mm to 6.89±0.03mm and 16.45±0.017mm to 20.10 ± 0.012mm, 5.68±0.087mm to 7.86±0.048mm and 17.93±0.013mm to 21.72±0.021mm,3.18±0.21mm to 3.63±0.08mm and5.92±0.009mm to 7.21±0.018mm respectively .As a result, all of the tested brands had thickness and diameter values that were deemed to be satisfactory as they were within the acceptable range.

However, statistical analysis of the one-way ANOVA results on thickness and diameter at 95% confidence interval revealed a significant difference ($p<0.05$) between the sample means of all brands. This may be due to granulation characteristics such as particle size, and particle dispersion, as well as variations in compressive force, die fill, and a lack of strict monitoring of the physical properties of the raw materials and continuous standardization of the length of the upper and lower punches (State et al., 2023). 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 (Oishi, *et al.*, 2011).

5.4 Friability of the tablets

The crushing strength of the tablets is not only determined by their hardness. Therefore, the friability test is crucial to ensure that the tablet can withstand attrition in the packaging container, which might result in partial powdering, chipping, or fragmentation of the tablets during handling and transportation. According to USP44-NF39, a weight loss of less than 1.0% is acceptable for tablet dosage form. In this study, the weight loss values for atorvastatin calcium, azithromycin, clarithromycin and glibenclamide tablets ranged from 0.38% to 0.89%, 0.04% to 0.21%, 0.29% to 0.58% and 0.11% to 0.26% respectively. As result, all tested brands met the requirements for hardness and friability specification. It can be concluded that the studied drug products are mechanically safe and comply with the quality control limit of pharmacopeia. This is the stringent approach that manufacturing companies take to in-process quality control to ensure consistency in the establishment of product quality (WHO, 2014).

Table 5: Hardness, thickness and diameter test result of atorvastatin calcium 40 mg, azithromycin 500 mg, clarithromycin 500mg and glibenclamide 5 mg tablets

Sample code	Hardness(N) (Mean ± SD)	P value	Thickness(mm) (Mean ± SD)	P value	Diameter (min) (Mean ± SD)	P value
ATO1	155.3 ± 6.9	0.004	5.15 ± 0.05	0.008	15.17 ± 0.01	0.001
ATO2	249.3 ± 13.2		6.85 ± 0.05		16.99 ± 0.04	
ATO3	135.4 ± 7.14		5.56 ± 0.054		15.70 ± 0.07	
ATO4	137.1 ± 4.39		5.86 ± 0.048		15.67 ± 0.09	
ATO5*	188.4 ± 14.47		5.64 ± 0.066		15.75 ± 0.02	
AZM1	124.6±9.40	0.000	6.42±0.04	0.004	19.04±0.008	0.000
AZM2	142.6±6.40		6.4±0.09		19.7±0.023	
AZM3	159.1± 8.92		6.24±0.07		16.45±0.017	
AZM4	281±11.87		6.58±0.03		16.67±0.017	
AZM5	277.5±7.18		6.89±0.03		19.37±0.023	
AZM6*	157.5±7.04		6.20±0.02		20.10 ± 0.012	
CLM1	149.4 ± 4.20	0.000	6.28±0.093	0.000	17.93±0.013	0.000
CLM2	158.3 ± 7.93		5.86±0.048		17.24±0.115	
CLM3	135.5 ± 5.9		6.48±0.087		21.72±0.021	
CLM4	205.4±7.29		5.68±0.087		19.05±0.02	
CLM5	234.4 ± 17.85		7.86±0.048		18.95±0.012	
CLM6*	182.3±14.9		7.42±0.087		19.64±0.016	
GLB1	163.39±11.71	0.007	3.38±0.11	0.001	6.28±0.024	0.001
GLB2	186.11±8.84		3.18±0.21		5.92±0.009	
GLB3	175.41±6.79		3.63±0.08		6.93±0.014	
GLB4*	159.34±11.1		3.48±0.04		7.21±0.018	

*Comparator drug products

5.5 Disintegration times of the tablets

Complete disintegration is described as the condition where the only remnants of the unit left on the test device' screen are soft masses without a discernibly firm core, such as pieces of insoluble coating or capsule shell. The USP and BP both specify that tablets and capsules should

disintegrate after a specific period of time, i.e., 15 minutes for uncoated tablets and 30 minutes for film coated tablets and hard gelatin capsules in water at 37 ± 2 ^{o}C as a disintegration medium (USP 44-NF 39, 2021). The results of disintegration test for atorvastatin, azithromycin clarithromycin and glibenclamide is presented in Table 5. As can be seen from the tables, all the samples comply with the requirement for disintegration time. The disintegration time for atorvastatin, azithromycin clarithromycin and glibenclamide ranged from 3.40 to 5.90 min, 3.6 to 9.08 min, 2.3 to 7.0min and 1.65 to 5.77 min, respectively. The lowest disintegration times observed were 1.65 min, for glibenclamide tablet, while the longest disintegration time recorded was 9.08 min, for azithromycin tablet. Statistically, one-way ANOVA with a 95% confidence interval showed a significant difference ($p<0.05$) between the tested brands. These difference in disintegration time may be due to differences in the quality and quantity of tablet excipients used (binder, disintegrant, and lubricant), as well as, the compaction pressure used in tablet production, which further influences the disintegration of the tablets (Kassahun, *et al.*, 2019).

5.6 Assay of the tablets

The main objective of assay test is to confirm the presence of the required quantity of the active component within the compendial specification. Significant differences may result in subtherapeutic drug levels that are ineffective and in case of antimicrobial drugs may lead to emergence of antimicrobial resistance or overdoses that may be toxic. official techniques described in the relevant monographs are employed to examine the chemical assay of each drug products.

5.6.1 Assay of atorvastatin calcium tablets

Atorvastatin was examined using the method outlined in the USP, and the monograph specifies that atorvastatin calcium tablets should contain not less than 94.5% and not more than 105% of the labelled amount (USP, 2021a).

System suitability test result: The system is suitable for assay of atorvastatin calcium tablets states the resolution between the peaks of atorvastatin and atorvastatin related compound H is not less than 5, the tailing factor of atorvastatin is not more than 1.5 and relative standard deviation of five injections of standard solution is not more than 1%. As presented in Table 6, the tailing factor was 1.19, %RSD of five injections was 0.0.96%. and the resolution between the peaks of atorvastatin and atorvastatin related compound H was 8.4, as shown in Fig. 10.

Table 6: System suitability test for assay of atorvastatin by using HPLC equipped with 244nm UV detector and C-18 column at,30°c column temperature,1ml/min flow rate and 20 µL injection volume.

	System suitability parameters of atorvastatin			
Standard injection	Retention time	Peak area	Tailing factor	Theoretical plate(N)
Reference std 1st injection	11.48	447098	1.20	6759
Reference std 2nd injection	11.50	437171	1.19	6647
Reference std 3rd injection	11.51	440943	1.20	6499
Reference std 4th injection	11.49	440241	1.19	6657
Reference std 5th injection	11.51	439333	1.19	6674
Mean	11.498	440957.2	1.194	6647.2
%RSD	-	0.96	-	-
Limit		NMT 1	NMT 1.5	NLT 2000

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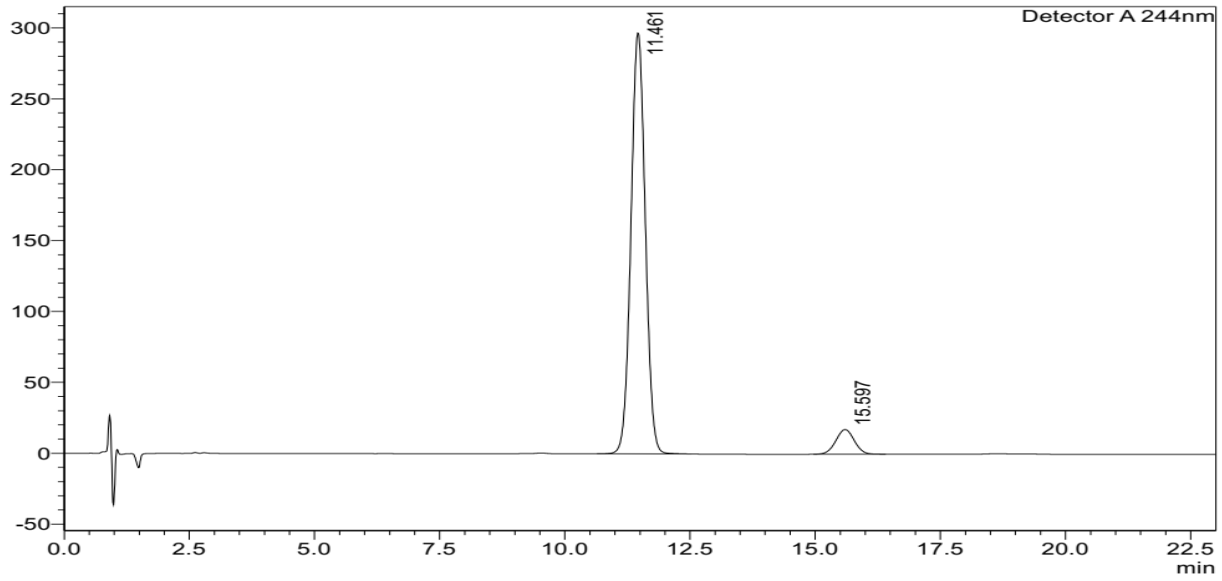

Figure 10: The resolution between the peaks of atorvastatin and atorvastatin related compound H by using HPLC equipped with 244nm UV detector and C-18 column at,30°c column temperature,1ml/min flow rate and 20 µL injection volume.

All the 5 samples of atorvastatin tablets were analyzed for percentage content of atorvastatin according to the method outlined in the USP. atorvastatin tablets should contain between 94.5 and 105% of the specified quantity. All the samples of atorvastatin tablets analyzed were within the USP limit, and the data is presented in Table 7. The minimum percentage content was observed for sample ATO3 (96.24%), and the maximum was observed for sample ATO4 (98.41%).

Table 7: Percentage content of API for (mean ± SD) of atorvastatin calcium, azithromycin, clarithromycin and glibenclamide tablet

Sample code	Assay value	USP acceptance limit	Conclusion	P Value
ATO1	96.28±1.47	94.5%–105.0%	Passed	0.001
ATO2	97.36±2,36		Passed	
ATO3	96.24±2.54		Passed	
ATO4	98.41±1.98		Passed	
ATO5*	96.37±0.76		Passed	
AZM1	100.8 ± 0.8	90.0%–110.0%	Passed	0.000
AZM2	101.5 ± 0.9		Passed	
AZM3	100.1 ± 1.3		Passed	
AZM4	99.4 ± 0.6		Passed	
AZM5	100.4± 0.7		Passed	
AZM6*	101.9± 1.2	90.0%–110.0%	Passed	
CLM1	98.8 ± 1.8		Passed	0.007
CLM2	104.5 ± 2.9		Passed	
CLM3	104.1 ± 1.7		Passed	
CLM4	100.4 ± 1.6		Passed	
CLM5	102.5± 1.7		Passed	
CLM6*	105.9± 1.2		passed	
GLB1	98.482 ±1.9	90.0%–110.0%	Passed	0.000
GLB2	99.032±2.9		Passed	
GLB3	99.398±1.8		Passed	
GLB4	100.800±2.7		Passed	

5.6.2 Assay of azithromycin tablets

All the 6 samples of azithromycin tablets were analyzed for percentage content of azithromycin according to the method outlined in the USP. Azithromycin tablets should contain between 90 and 110% of the specified quantity.

All the samples of azithromycin tablets analyzed were within the USP limit, and the data is presented in Table 7, The minimum percentage content was observed for sample AZM4 (99.4%), and the maximum was observed for sample AZM6 (101.9%).

***System suitability test*:** The system suitability test results for assay of azithromycin are provided in Table 8 with their respective specifications. According to the USP 2021, the tailing factor of the peak of azithromycin standard solution should not be more than 2 and percentage relative standard deviation of five replicate injections of the standard solution should not be more than 2.0%. As shown in table 8, the tailing factor obtained was 1.6, the relative standard deviation was 1.15, and number of theoretical plates was 2487.8, which satisfies the system suitability requirement.

Table 8: System suitability test for the assay of azithromycin by using HPLC equipped with 210 nm UV detector and C-18 column at,50^{o}c column temperature,1.5 ml/min flow rate and 50 µL injection volume.

System suitability parameters of azithromycin				
Standard injection	Retention time	Peak area	Tailing factor	Theoretical plate
Reference std 1st injection	7.5	4213509	1.6	2579
Reference std 2nd injection	7.4	4207843	1.6	2490
Reference std 3rd injection	7.9	4210210	1.6	2847
Reference std 4th injection	7.4	4301391	1.6	2461
Reference std 5th injection	7.4	4317672	1.6	2462
Mean	7.5	4250125	1.6	2487.8
%RSD	-	1.28		
LIMIT		NMT 2%RSD	NMT2	NLT 2000

5.6.3 Assay of clarithromycin tablets

Quantitative determination of the content of tablet formulations claiming to contain clarithromycin 500 mg tablet was conducted according to the method in the USP monograph for the specified medicine (USP, 2021b). According to the USP 2021, the tailing factor of the peak of clarithromycin should be between 0.9 and 1.5, percentage relative standard deviation of five replicate injections of the standard solution should not be more than 2.0% and the resolution between clarithromycin and clarithromycin related compound should not be less than 2. The results of system suitability requirements are shown Table 9. The resolution between clarithromycin and clarithromycin related compound was 4.4 (Fig. 11).

Table 9: System suitability test of clarithromycin compound by using HPLC equipped with 210 nm UV detector and C-18 column at,50^{o}c column temperature,1ml/min flow rate and 50 µL injection volume.

	System suitability parameters of clarithromycin			
	Retention time	Peak area	Tailing factor	Theoretical plate(N)
Reference std 1st injection	5.19	69865	1.04	2673
Reference std 2nd injection	5.18	69930	1.02	2577
Reference std 3rd injection	5.20	70359	1.02	2649
Reference std 4th injection	5.19	70469	1.06	2583
Reference std 5th injection	5.19	70407	1.06	2572
Mean	5.18	70206	1.04	2610.8
%RSD	-	0.41	-	-
limit		NMT 2	-	NLT 2000

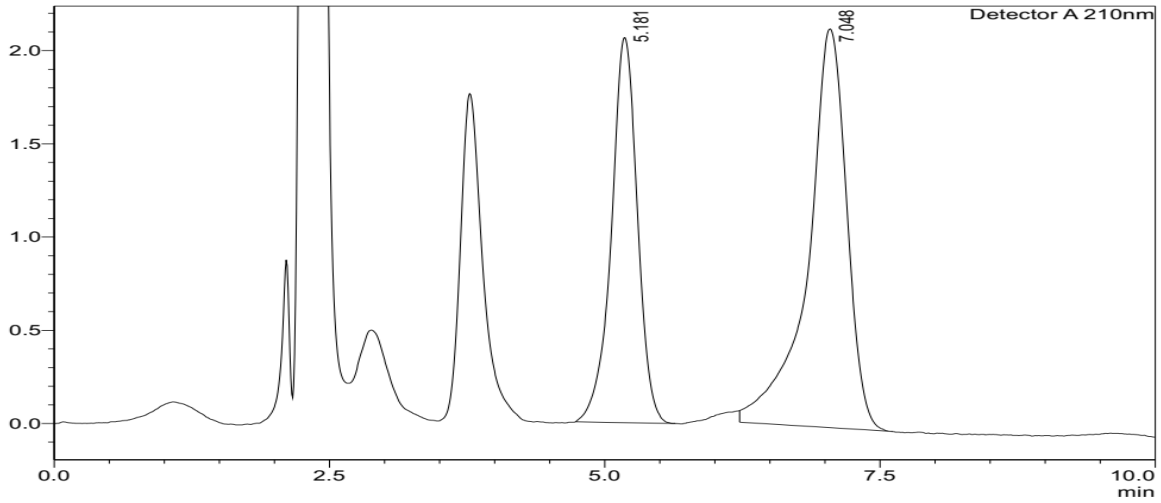

Figure 11: The resolution between clarithromycin and clarithromycin related compound by using HPLC equipped with 210 nm UV detector and C-18 column at,50^{o}c column temperature,1ml/min flow rate and 50 µL injection volume.

The monograph specifies that the tablets should contain an equivalent of NLT 90% and NMT 110% of clarithromycin. A total of 5 samples of clarithromycin products and one comparator product were analyzed. As shown in Table 7, all the samples met the requirement for percentage content for active ingredients.

5.6.4 Assay of glibenclamide tablets

Four samples of *glibenclamide tablets* were analyzed according to the method outlined in the USP44/NF39, 2021. The USP states that *glibenclamide tablets* should contain between 90.0 and 110.0 percent of the label claimed amount. All the samples of *glibenclamide tablets* analyzed were within the USP limit, and the data is presented in Table 7. The minimum percentage content was observed for sample GLB1 (98.48%), and the maximum was observed for samples GLB4 (100.8%).

System suitability test result: The requirement for system suitability is met if the symmetry factor of the principal peak of the standard solution of glibenclamide is less than 2.0 and the relative standard deviation of five injections of the standard solution is not more than 2.0%. As shown in Table 10, the symmetry factor obtained was 1.19 and relative standard deviation of the five injections was 0.92, satisfying the system suitability requirement.

Table 10: System suitability result of Glibenclamide by using HPLC equipped with 254 nm UV detector and C-18 column at 1ml/min flow rate and 50 µL injection volume.

System suitability parameters of Glibenclamide				
Standard injection	Retention time	Peak area	Tailing factor	Theoretical plate
Reference std 1st injection	4.91	166747	1.19	5215
Reference std 2nd injection	4.90	166936	1.19	5220
Reference std 3rd injection	4.90	166996	1.19	5294
Reference std 4th injection	4.91	166680	1.18	5203
Reference std 5th injection	4.93	163411	1.23	5207
Mean	4.91	166154	1.193	5227.8
%RSD	-	0.92	-	-
limit		NMT 2%	NMT 2	NLT 2000

Therefore, all brands demonstrated assay results to be within this specification. The results of one-way ANOVA statistical analysis showed that there was a significant difference ($P<0.05$) in the drug content of all the tested drug products. This can be explained by the fact that these samples were from varied manufacturers who may have carried out different manufacturing processes.

5.7 Dissolution profiles

Dissolution testing has emerged as a very important tool in the generic pharmaceutical industry. It widely used in formulation development, in monitoring the manufacturing process as a quality control test and also used to predict the in vivo performance and bioavailability of drug products(FDA, 2017). Dissolution profiles can be obtained by plotting the mean cumulative percentage of the API released versus each sampling time point. The claimed dosage strength was utilized to calculate the cumulative percentages of APIs released from the formulation at each sampling time point (Banakar, 2022). In this study, 21 samples of four product types were examined for dissolution profiles according to the methods described in the USP and BP.

5.7.1 Dissolution of Atorvastatin calcium tablets

A total of 5 different Atorvastatin calcium tablets were tested for dissolution using 900 mL of pH 6.8, 0.05M phosphate buffer at 37°C while the paddle rotated at 75 rpm (USP 44/NF 39, 2021). A six-point calibration curve was first generated and used to determine the percentage amount released at each time point. The calibration curve (Fig. 12) for atorvastatin demonstrated linearity in the concentration range of 3.56-12.45µg/ml with the linear regression equation y = 0.0579x+0.0057, where Y is the absorbance and X is the concentration in µg/ml. The value of correlation coefficient R^2=0.9997 indicated a good linear relationship between concentration of the tested samples and the absorbance.

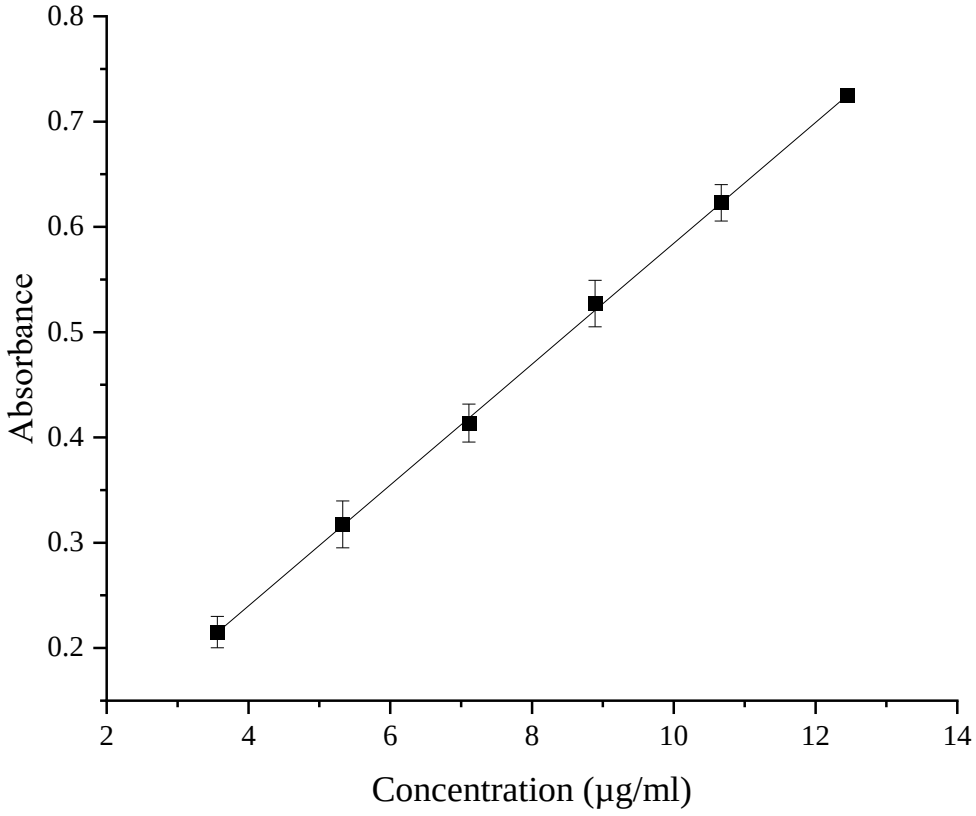

Figure 12: Standard calibration curve of atorvastatin calcium in phosphate buffer (pH=6.8) media at λmax 244nm over the concentration range of 3.56-12.45µg/ml.

The USP/NF specifies that the amount of atorvastatin released after 15 minutes should not be less than 80% of the stated amount. From the Table 11 and Fig 13, except sample ATO4 all tested brands of atorvastatin tablets passed the single point dissolution test specification as per USP 44 /NF39. The Percentage drug dissolved for atorvastatin samples at 15 minutes ranged from 78.210±1.008% to 92.095 ±1.051 % for ATO4 and ATO5, respectively. Sample ATO5 had the highest percentage of drug release, while brand ATO4 had the least amount of drug release at a single time point. Despite having moderate hardness (137.1 N) compared to the other atorvastatin samples, sample ATO4 was observed to have the longest disintegration time (9.73 min, as shown in Table 4. This could be one of the reasons for the delayed drug dissolution rate observed with this product (Gupta *et al*., 2009). However, sample ATO 3, having a hardness of 135.8 N, has been observed to have a disintegration time of 5 minutes. Although this sample showed the second-slowest drug dissolution rate, this product has met the pharmacopeial specification for dissolution. The amount of disintegrating agent used in the formulations, in addition to variations in the amount of binders and fillers, could be the cause of variation in disintegration time and thus dissolution rate for these products.

Table 11: Cumulative percentage of dissolution profiles of atorvastatin calcium tablets, in phosphate buffer pH=6.8 media at maximum wavelength 244nm and 37± 0.5ºC and one way ANOVA-post-hoc Dennett's test results.

Sample code	**Sampling time**					**ANOVA -post-hoc Dennett's test**			
	5	10	15	30	45	Mean Difference (I-J)	95% CI		P value
	Mean Cumulative Percentage Drug Release (±%RSD) (n = 5)						Lower bound	Upper bound	
ATO1	55.37±1.04	72.89±1.30	85.11±1.18	98.86±1.20	99.30±0.67	-6.93333	-16.91	2.99	.199
ATO2	53.12±1.29	70.45±1.55	84.13±1.07	94.29±0.51	98.11±1.36	-7.96333	-17.89	2.01	.128
ATO3	46.02±1.00	73.88±1.0	82.88±0.96	92.22±1.09	97.98±1.25	-9.18333	-19.14	.76	.072
ATO4	53.55±1.16	61.68±0.98	78.21±1.00	87.77±0.92	93.40±0.88	-11.89333	-21.81	-1.90	.002
ATO5*	68.14±1.14	77.47±1.64	92.09±1.05	98.80±0.74	99.64±0.73	-	-	-	-

*, J Comparator drug product, I Test product, a. Dennett t-tests treat one group as a control and compare all other groups against it.

In this study, the time dependent dissolution profiles of five distinct brands were assessed to offer insights into bioavailability.

As illustrated in table 11 and Fig.13, various atorvastatin calcium tablets showed different drug release pattern at different time points. To ascertain the presence of a statistical discrepancy between the tested samples and reference products, a one-way analysis of variance (ANOVA) with a significance level of $p<0.05$ was conducted, followed by the application of Dennett's post-hoc test. The results revealed that sample ATO4 showed a statistically significant difference from the reference, with a P value of 0.02, demonstrating the slowest dissolution rate (61.68 ± 0.98%) among the samples, whereas the reference product (ATO 5) released the highest amount of drug (77.47 ± 1.64%) within the initial 10 minutes. The variations in drug release profiles could be primarily attributed to differences in the composition and quantities of excipients utilized, as well as variations in processing and formulation parameters.

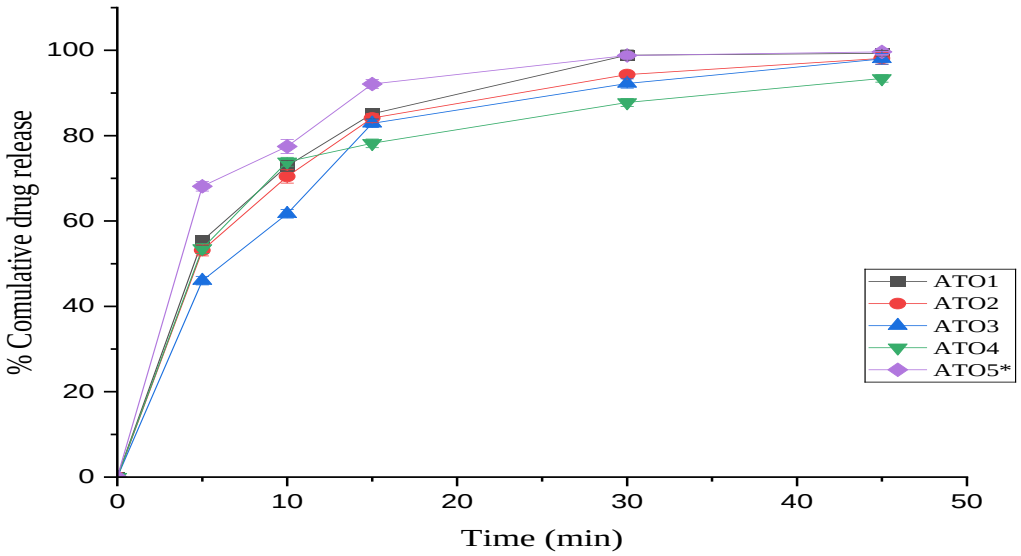

Figure 13: Time dependent dissolution profiles of five different brands of Atorvastatin calcium in phosphate buffer pH=6.8 media at λmax of 244 nm and 37±0.5.

The dissolution profiles of atorvastatin samples were also assessed for similarity with the comparator product using similarity (f_2) and difference (f_1) factors. The values of f_2 and f_1 were calculated using the sample ATO5 as a comparator product, selected based on the WHO recommendation due to the absence of reference product in the local market. As shown in Table (12), With regard to the value of difference factor, all of the samples were found to have acceptable similarity with the comparator product and may be equivalent to the comparator. According to the study, except sample ATO4 all other samples were similar to the comparator

products as their dissolution efficiency difference was less than 10% between the test sample and the comparator drug product (Table 15). The study findings meet the dissolution efficiency guideline's acceptance limit for therapeutic interchangeability if the difference in dissolution efficiency between the comparator and test products is less than 10%. In the meantime, mean dissolution time and other dissolution parameters like t50% and t90% were also considered to analyze dissolution profile of different pharmaceutical products and are essential for determining the amount of drug ingredient released from the dosage form and the polymer's retarding performance. The t50% is the time required for release of 50% of the contents of the tablets analyzed and t90% is the time required for the release of 90% of the contents. Very long t50% and t90% values of pharmaceutical products dissolution profile indicate that the product may manifest a lower rate and extent of bioavailability in the body.

Table 12. Model-independent approaches (f1, f2 and DE) and dissolution parameter (t50% MDT and t90%) of atorvastatin calcium tablets

Sample code	f1	f2	Dissolution Efficiency (DE)	Difference in DE	t50 %	MDT	t90%	Remark
ATO1	5.67	59.99	83.25	7.24	4.32	6.76	18.79	Similar
ATO2	8.25	55.35	81.75	8.74	4.52	7.28	21.6	Similar
ATO3	12.68	55.89	82.17	8.32	5.97	9.14	24.03	Similar
ATO4	12.32	45.19	79.05	11.44	3.73	7.06	30.11	Dissimilar
ATO5*	-	-	90.49	-	4.32	6.76	15.45	-

The dissolution data was also further characterized by fitting into different drug release kinetic models. Zero-order kinetics, first-order kinetics, Higuchi, Hickson Crowell and the Weibull models were employed to fit the dissolution data of atorvastatin tablet samples. The model selection parameters for all samples with their respective values are presented in Table 13. Among the models, the Weibull model can properly explain the release kinetics of all samples of atorvastatin based on the value of the highest value of R^2, lowest AIC and highest MSC values. Therefore, it can be deduced that all the samples follow similar drug release mechanism. The predicted and observed dissolution profiles of reference and test products of atorvastatin for Weibull model are given in AnnexII.

Table 13: Modelling parameters for the data on dissolution of Atorvastatin calcium tablets

Sample code	Kinetic model														
	Zero order			First order			Hickson Crowell			Higuchi			Weibull model		
	Parameters			Parameters			Parameters			Parameters			Parameters		
	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC
ATO1	0.085	54.610	-1.349	0.994	23.898	3.768	0.954	36.596	1.652	0.832	44.445	0.344	0.998	19.85	4.44
ATO2	0.110	54.159	-1.306	0.992	25.74	3.429	0.956	36.002	1.719	0.841	43.827	0.415	0.999	16.60	4.95
ATO3	0.304	52.707	-0.882	0.959	35.685	1.772	0.976	32.469	2.49	0.899	41.092	1.053	0.993	28.599	3.13
ATO4	-0.019	54.247	-1.558	0.978	31.10	2.29	0.901	40.204	0.781	0.789	44.782	0.018	0.999	16.538	4.726
ATO5	-0.212	56.370	-1.884	0.982	30.814	2.375	0.871	42.914	0.358	0.705	47.885	-0.47	0.995	27.302	2.96

5.7.2 Dissolution of Azithromycin tablets

The dissolution test for azithromycin tablets was carried out according to the in the USP monograph by employing 900 mL pH 6.0 of phosphate buffer at 37^{o}C with a paddle operating at 75 rpm (USP 44-NF 39, 2021 The calibration curve for azithromycin reference standard was plotted (Fig. 14) using the value of peak area against corresponding concentration. The curve showes linearity in the concentration range of 1.25-7.50µg/ml with the linear regression equation y = 11257.14x + 1969.6 and a correlation coefficient (r^2) of 0.997.

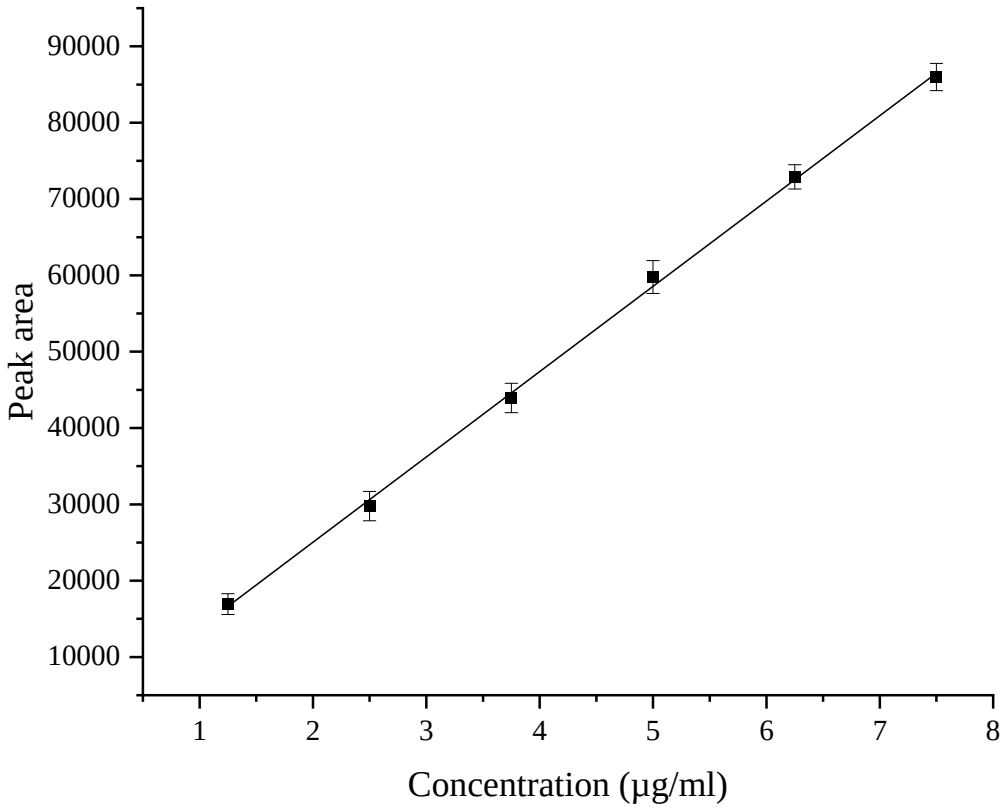

Figure 14: Standard calibration curve of azithromycin in 0.05M of phosphate buffer (pH=6.0) at λmax 210 nm over the concentration range of 1.25-7.5 µg/ml.

According to the finding, (Table 14 and Fig.15) all the brands passed a single-point dissolution test and met USP 44/NF 39 specifications by releasing more than 80% of the product in less than 30 minutes. The release rate of the drug at a single time point ranged from 80.54% to 93.72 % for AZM1 and AZM6, respectively. As compared to other samples, sample AZM1 (80.54%) and AZM2 (84.82%) had the least dissolution profile release.

Table 14: Cumulative percentage of dissolution profiles of azithromycin tablet, in phosphate buffer pH=6.0 media at maximum wavelength 210 nm and 37± 0.5ºC and one-way ANOVA -post-hoc Dennett's test results

Sample code	**Sampling time (min)**						**ANOVA -post-hoc Dennett's test**			
	5	10	15	30	45	60	Mean Difference (I-J)	95% CI		P value
								Lower bound	Upper bound	
	Mean Cumulative Percentage Drug Release (±%RSD) (n = 6)									
AZM1	48.82(2.03)	57.62(3.31)	67.38(4.7)	80.54(2.73)	86.67(2.88)	96.94(4.29)	-13.18	-15.54	-10.81	.000
AZM2	45.35(1.53)	65.98(1.85)	77.99(1.3)	84.82(0.74)	94.47(2.4)	97.2(1.15)	-8.90	-11.26	-6.53	.000
AZM3	50.37(1.22)	59.98(0.84)	68.82(1.7)	86.23(1.23)	96.45(2.12)	98.67(1.52)	-7.49	-9.85	-5.12	.000
AZM4	52.25(1.61	58.89(1.25)	68.31(1.1)	88.93(1.83)	93.43(1.65)	95.27(2.05)	-3.79	-6.15	-1.42	.002
AZM5	57.53(1.94)	64.49(2.11)	78.56(1.9)	89.14(1.35)	94.18(1.52)	94.95(1.24)	-4.58	-6.94	-2.21	.000
AZM6*	59.53(1.82)	68.2(1.05)	80.2(2.2)	93.72(1.33)	95.93(1.63)	96.74(2.51)	-	-	-	-

*J comparator drug product, I test sample

Drug products with a poor dissolution profile may not be available in sufficient quantity or early enough in the plasma to have a therapeutic effect on the target organ or tissue. Any factor that influences dissolution rate may also influence the rate and extent of drug absorption. Because of the low release profiles of AZM1 and AZM2 are local sources may need to improve some practices, and regulatory authorities may need to increase monitoring of the quality of pharmaceutical products manufactured by local industries.

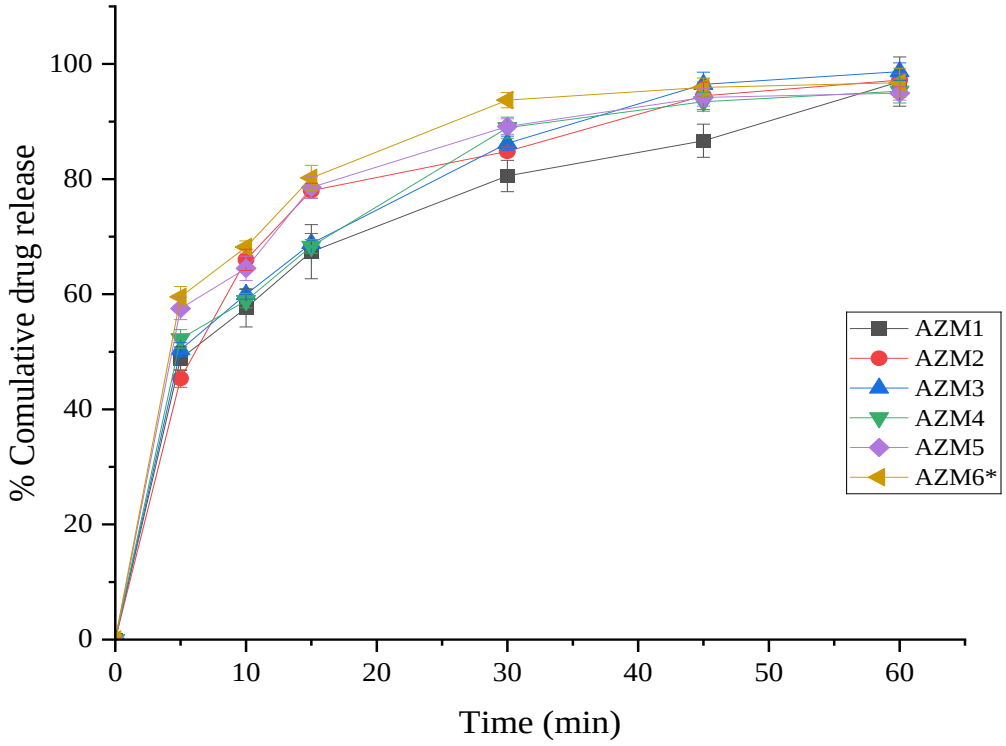

Figure 15: Time dependent dissolution profiles of different brands of Azithromycin in phosphate buffer (pH=6.0) media at λmax 210 nm over the concentration range of 1.25-7.50µg/ml.

Valid statistical analysis of the relevant data is clearly a pivotal part of dissolution profile comparisons. The dissolution profiles of different brands were compared by one-way ANOVA (95%CI) using Dunnett's test (Table 14). At different time intervals, the post hoc Dunnett test result revealed that there is a significant difference between the tested samples and the comparator product ($p<0.05$), This may be the variation of manufacturing process and the dissolution test parameters (Parameters like pH, viscosity, temperature, components, volume, and nature of the dissolution medium may have considerable effects on the dissolution profile of a drug). The interchangeability of various brands is not guaranteed by statistically equivalent medicinal products. For this fact, model independent approaches should be required to ascertain the exchange of different brands to the comparator. As revealed in Table (15) The dissolution

profile data shown that all of the tested Azithromycin brands were considered as equivalent to the comparator as f1 values were (<15), and f2 values were (>50).

Table 15: Model-independent approaches (f1, f2 and DE) and Dissolution parameter (t50% MDT and t90%) of azithromycin tablets

Sample code	f1	f2	DE	Difference in DE	t50%	MDT	t90%	Remark
AZM1	11.48	50.68	83.25	3.24	6.15	11.74	51.60	Similar
AZM2	5.95	59.21	81.75	4.74	4.52	7.28	34.61	Similar
AZM3	7.82	57.58	79.05	7.44	5.89	10.26	37.20	Similar
AZM4	7.53	58.51	82.17	4.32	5.56	10.12	39.6	Similar
AZM5	3.22	77.69	86.49	-	3.81	7.45	34.6	Similar
AZM6	-	-			3.53	6.60	27.60	

In the meantime, DE, t50%, MDT and t90% time ware considered to analyze dissolution profile of azithromycin tablets. The results revealed that all samples of azithromycin exhibited similarity to the corresponding comparator product, as their dissolution efficiency was below 10%, and all variations of azithromycin promptly released their contents. It is plausible to anticipate that these products would be swiftly absorbed and bioavailable (Table 15). Moreover, the dissolution profiles of the tested samples underwent model-dependent methodologies to determine the drug release kinetics from the dosage form. Within this research, the Weibull kinetic model was identified as the most suitable for describing the release of drug substances from the dosage forms (Table 16). For this reason, it can be declared that all brands under the investigation showed the same release mechanism. The predicted and observed dissolution profiles of reference and test products of azithromycin for Weibull model are given in Annex III.

Table 16: Modelling parameters for the data on dissolution of Azithromycin tablets

Sample code	Kinetic model														
	Zero order			First order			Hickson Crowell			Higuchi			Weibull model		
	Parameters			Parameters			Parameters			Parameters			Parameters		
	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC
AZM1	0.153	61.998	-1.106	0.931	44.452	1.399	0.861	49.333	0.702	0.862	49.268	0.711	0.993	32.262	3.14
AZM2	0.051	63.664	-1.218	0.914	46.828	1.186	0.911	47.039	1.156	0.818	52.103	0.432	0.961	44.497	1.393
AZM3	0.180	62.634	-1.039	0.960	41.399	1.994	0.914	46.828	1.218	0.933	45.023	1.444	0.992	34.110	3.035
AZM4	-0.086	63.071	-1.198	0.948	42.924	1.679	0.894	47.950	0.961	0.836	51.034	0.521	0.991	35.055	2.803
AZM5	-1.846	64.870	-1.648	0.951	42.520	1.544	0.844	50.672	0.379	0.728	54.566	-0.176	0.995	30.185	3.306
AZM6*	-0.237	65.528	-1.722	0.967	40.114	1.908	0.842	51.108	0.337	0.705	55.479	-0.286	0.996	29.159	3.473

5.7.3 Dissolution of Clarithromycin tablet

The dissolution test for clarithromycin tablet was carried out according to the LC method in USP/NF 44/39 monograph by using 900ml of pH 5.0 Acetate buffer as dissolution medium at 37^{o}C with the paddle operating at 50 rpm. The calibration curve for clarithromycin reference standard was plotted using the value of peak area against corresponding concentration. (Fig. 16) shows linearity in the concentration range of 1.25 -7.50µg/ml with the linear regression equation y = 11454.05x – 2009.33 and a correlation coefficient (R^2) of 0.997.

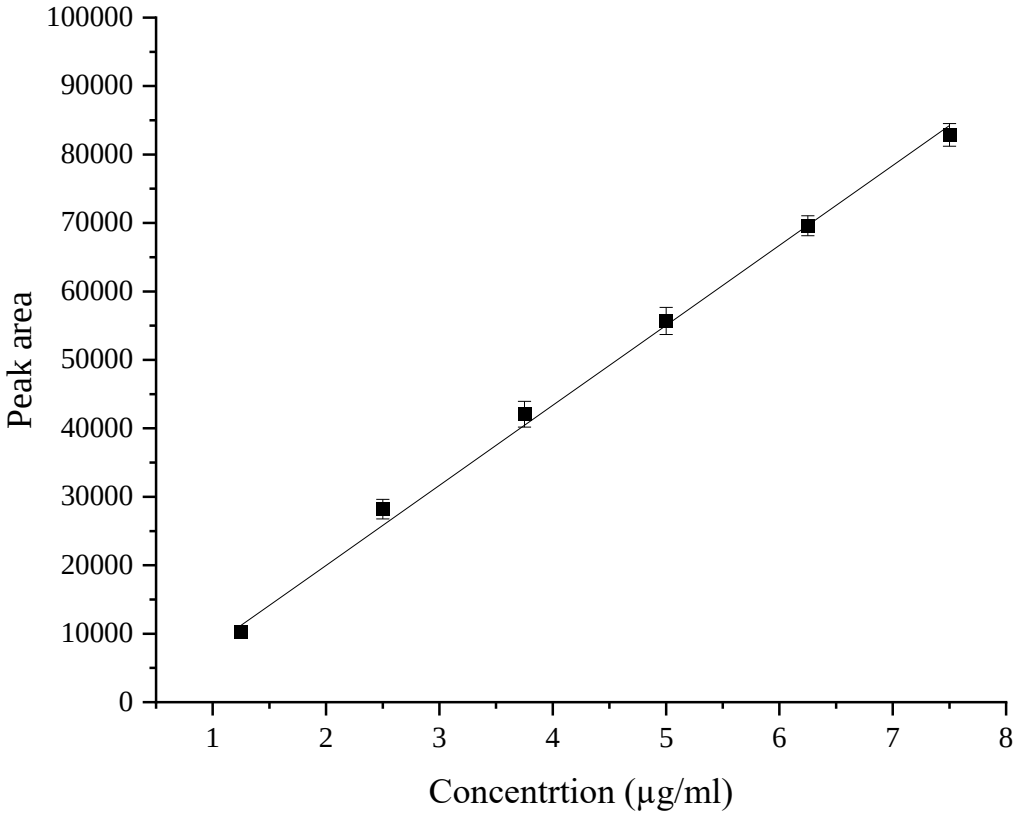

Figure 16: Standard calibration curve of clarithromycin in acetate buffer (pH=5.0) media at λmax 210 nm over the concentration range of 1.25-7.5 µg/ml.

According to the USP, clarithromycin tablets should release NLT 80% (Q) of the labelled amount of clarithromycin after 30 minutes for single point dissolution test. A summary of the dissolution data for 5 samples and 1 comparator product is presented in Table 17, and Fig. 17.

Table 17: Cumulative percentage of dissolution profiles of clarithromycin tablet in acetate buffer pH=5 media at maximum wavelength 210 nm and 37± 0.5 ºC and ANOVA -post-hoc Dennett's test result.

Sample code	**Sampling time (min)**						**ANOVA -post-hoc Dennett's test**			
	5	10	15	30	45	60	Mean Difference (I-J)	95% CI		P value
								Lower bound	Upper bound	
	Mean Cumulative Percentage Drug Release (±%RSD) (n = 6)									
CLM1	42.35(1.63)	55.95(1.29)	69.65(1.09)	80.80(1.64)	83.12(1.01)	89.70(1.32)	-6.010	-15.68	3.66	.303
CLM2	47.02(3.05)	53.0(1.66)	70.39(3.49)	75.32(1.29)	82.99(2.38)	97.3(1.41)	-11.490	-21.16	-1.819	**.019**
CLM3	39.47(2.08)	50.78(2.96)	58.55(1.10)	72.15(0.98)	81.12(1.12)	89.18(0.99)	-14.660	-24.33	-4.989	**.004**
CLM4	47.96(1.62)	55.85(1.38)	69.92(1.61)	82.07(1.15)	89.35(1.71)	98.35(2.36)	-4.740	-14.41	4.930	.505
CLM5	49.35(1.53)	59.07(1.85)	63.99(1.31)	80.14(0.74)	82.66(2.42)	97.2(1.15)	-5.990	-15.66	3.680	.306
CLM6*	53.97(3.96)	66.62(1.13)	75.32(1.29	86.81(2.26)	93.05(1.3)	99.93(0.63)	-	-	-	-

According to the finding, Except CLM2 (75.32%) and CLM3 (72.15%) all of the brands of clarithromycin had passed a single-point dissolution test and met USP specifications by releasing more than 80% of the product at 30 minutes. The release rate of the drug at a single time point ranged from 72.152 to 86.81 % for CLM3 and CLM6, respectively.

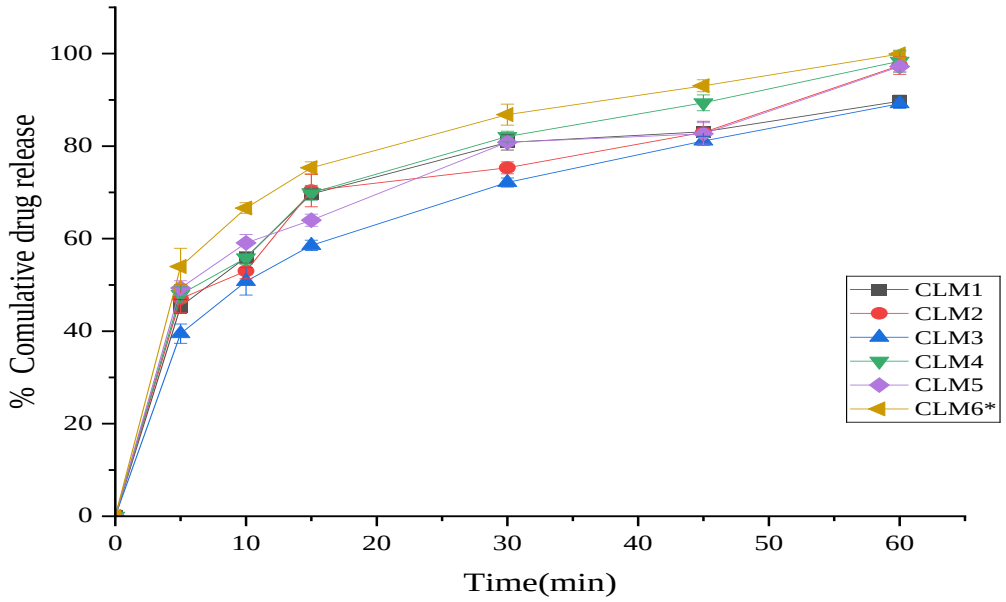

Figure 17: Time dependent dissolution profiles of different brands of Clarithromycin tablet in 0.05M of acetate buffer (pH=5.0) media at λmax 210 nm

The study has shown that distinct drug release patterns were observed at different time points among the tested drug products. To confirm whether there is a statistical difference or not between test samples and comparator products one-way ANOVA, ($p<0.05$) using post-hoc Dennett's test was applied. The result showed that two samples of clarithromycin (CLM 2 and CLM3) had significant difference with the comparator drug product. (Table 17). Similarity of the dissolution profiles of the samples to the comparator product was further assessed using similarity and difference factors. The values for f_2 and f_1 were calculated using all observed points and the results are presented in (Table18) Only one sample (CLM3) was found to be different to comparator product for it didn't meet the requirement for f_1 (below 15) and f_2 (above 50).

Table 18: Model-independent approaches (f1, f2 and DE) and dissolution parameter (t50% MDT and t90%) of clarithromycin tablets.

Sample code	f1	f2	Dissolution Efficiency (DE)	Difference in DE	t50%	MDT	t90%	Remark
CLM1	11.17	53.43	82.83	-2.34	6.43	12.37	61.60	Similar
CLM2	12.15	52.89	78.89	1.6	6.75	13.04	58.8	Similar
CLM3	15.55	45.02	74.72	5.77	5.89	17.98	67.20	Dissimilar
CLM4	7.26	62.24	77.58	2.91	6.41	11.57	44.4	Similar
CLM5	9.79	55.59	80.49	-0.34	6.03	12.00	57.6	Similar
CLM6*			80.15	-	4.38	8.19	34.8	

The dissolution data of clarithromycin tablets were also further characterized by fitting the data into different kinetic models. Zero-order kinetics, first-order kinetics, Higuchi, Hixson-Crowell, and the Weibull are among the models used to fit the dissolution data of clarithromycin samples and the comparator product. The model parameters used for the selection of best kinetic model for the dissolution data are presented in Table 19.

Table 19: Model selection parameters of clarithromycin tablets

Sample code	Kinetic model														
	Zero order			First order			Hickson Crowell			Higuchi			Weibull model		
	Parameters			Parameters			Parameters			Parameters			Parameters		
	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC
CLM1	0.048	62.277	-1.251	0.905	46.119	1.057	0.835	50.000	0.502	0.821	49.268	0.711	0.995	28.476	3.577
CLM2	0.197	61.454	-1.005	0.894	47.261	1.022	0.826	50.720	0.528	0.865	48.940	0.782	0.978	39.988	2.061
CLM3	0.326	59.439	-0.745	0.889	46.138	1.154	0.817	50.303	0.559	0.933	44.450	1.395	0.997	24.148	4.295
CLM4	0.205	61.951	-0.995	0.945	43.189	1.688	0.896	47.695	1.044	0.880	48.713	0.889	0.992	33.501	3.072
CLM5	0.132	61.944	-1.149	0.894	47.214	0.955	0.827	50.635	0.466	0.849	49.685	0.602	0.986	36.870	2.433
CLM6*	-0.034	64.089	-1.432	0.957	41.790	1.752	0.877	49.162	0.699	0.793	52.821	0.176	0.997	27.166	3.841

The model fitting revealed that the Weibull kinetic model was found to be the best fit for the dissolution data of the reference and all of the samples, indicating that the products follow similar drug release mechanism. The predicted and observed dissolution profiles of reference and test products of clarithromycin for Weibull model are given in Annex IV.

5.7.4 Dissolution of glibenclamide tablets

The dissolution test for glibenclamide tablet was carried out according to the HPLC method in USP/NF 44/39 monograph by using 900ml of pH 7.50 phosphate buffer as dissolution medium at 37ºC with the paddle operating at 50 rpm. The calibration curve for glibenclamide reference standard was plotted using the value of peak area against corresponding concentration. (Fig. 18) showes linearity in the concentration range of 1.11 -6.67 µg/ml with the linear regression equation y = 31728.3x – 2890. and a correlation coefficient (R^2) of 0.997.

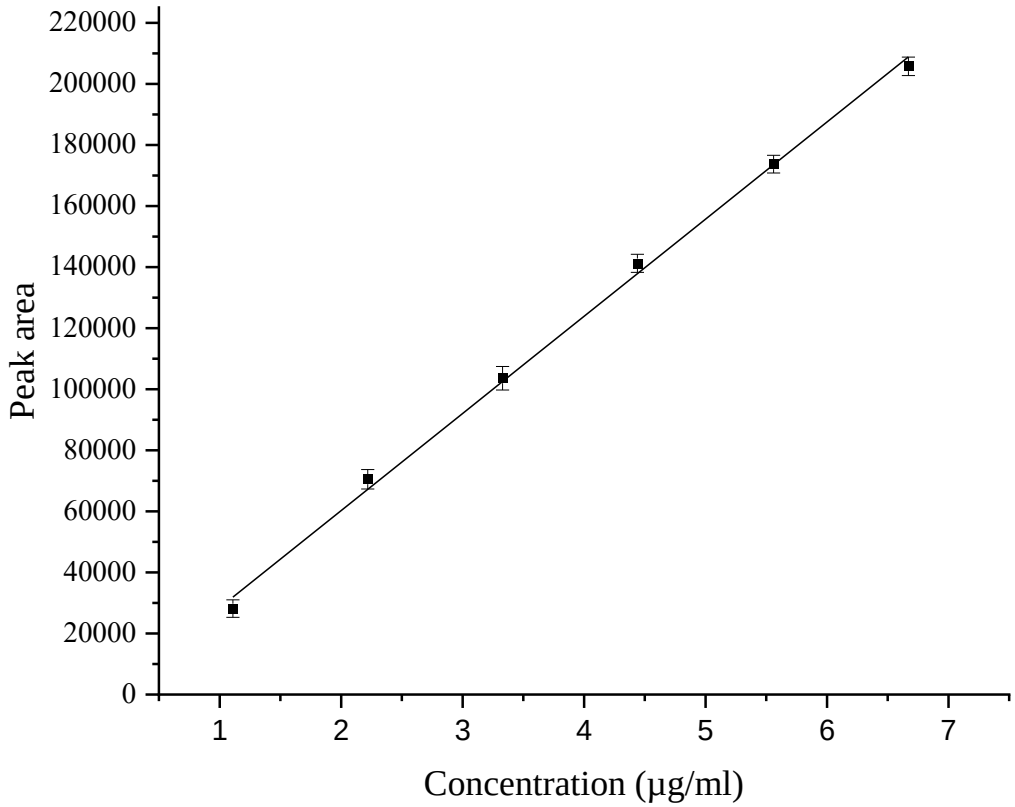

Figure 18: Standard calibration curve of glibenclamide in phosphate buffer (pH=7.5 at λmax 254 nm over the concentration range of 1.11-6.67 µg/ml.

As per the USP specification, the release of glibenclamide from the tablet formulation should not less than 75% of label claim within 45min. 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 glibenclamide to lower glucose levels in the short term. In this study, out of three generic and one innovator products of glibenclamide tested all of them passed the specifications of the single-point dissolution test as indicated below in Table 20 and Fig. 17. The results indicate that all the glibenclamide samples, including the innovator drug product (Daonil), release over 30% of the drug within the first 10 min. At the end of 45 min, GLB 4 released maximum drug content (85.62. However, GLB2 exhibited lower release of 76.88%.

The comparative analysis of dissolution profiles of various glibenclamide brands was done by one-way ANOVA (95%CI) with the application of Dunnett's test (Table 20). At various time points, the subsequent post hoc Dunnett test outcome revealed that there is a significant difference between the tested samples and the reference product ($p<0.05$). This difference could potentially stem from variations in the manufacturing process and the parameters of dissolution testing.

The dissolution profiles of glibenclamide samples were also assessed for similarity with the comparator product using similarity (f_2) and difference (f_1) factors. The values of f_2 and f_1 were calculated using the sample GLB4 as a comparator product due to it is an innovator product. As revealed in Table 21, all the tested sample showed the values of f_2 and f_1 within the acceptable range. With regard to the value of difference in DE all of the samples were found to have acceptable difference with the comparator product and may be equivalent to the comparator.

In the meantime, MDT, t50% and t90% time ware considered to analyze dissolution profile of glibenclamide products. The value of MDT, t50% and t90% of glibenclamide are given (Table 21), and it showed that all the sample had comparable value with the drug comparator product. The dissolution data was also further characterized by fitting into different drug release kinetic models. The model selection parameters for all samples with their respective values are presented in Table 22. Among the models, the Weibull model can properly explain the release kinetics of all samples of glibenclamide. The predicted and observed dissolution profiles of reference and test products of glibenclamide for Weibull model are given in Annex V.

Table 20: Cumulative percentage of dissolution profiles of glibenclamide tablet in phosphate buffer pH=7.5 at maximum wavelength 254 nm and 37± 0.5 ºC and ANOVA -post-hoc Dennett's test result

Sample code	Sampling time (min)					Mean Difference (I-J)	95% CI		Pvalue
	10	15	30	45	60		Lower bound	Upper bound	
	Mean Cumulative Percentage Drug Release (±%RSD) (n = 5)								
GLB1	37.37± 0.35	48.56±0.54	62.38±0.59	81.13±0.84	91.92±1.11	-4.493	-6.844	-2.142	0.001
GLB2	39.22±0.90	46.49±1.21	56.04±1.38	76.88±0.86	84.95±1.65	-8.743	-11.09	-6.392	0.000
GLB3	40.64±0.64	49.99±0.88	65.51±0.82	82.56±0.89	86.71±1.15	-3.063	-5.414	-.7121	0.014
GLB4	41.67±0.89	54.7±1.21	66.44±0.97	85.62±1.02	95.51±1.05				

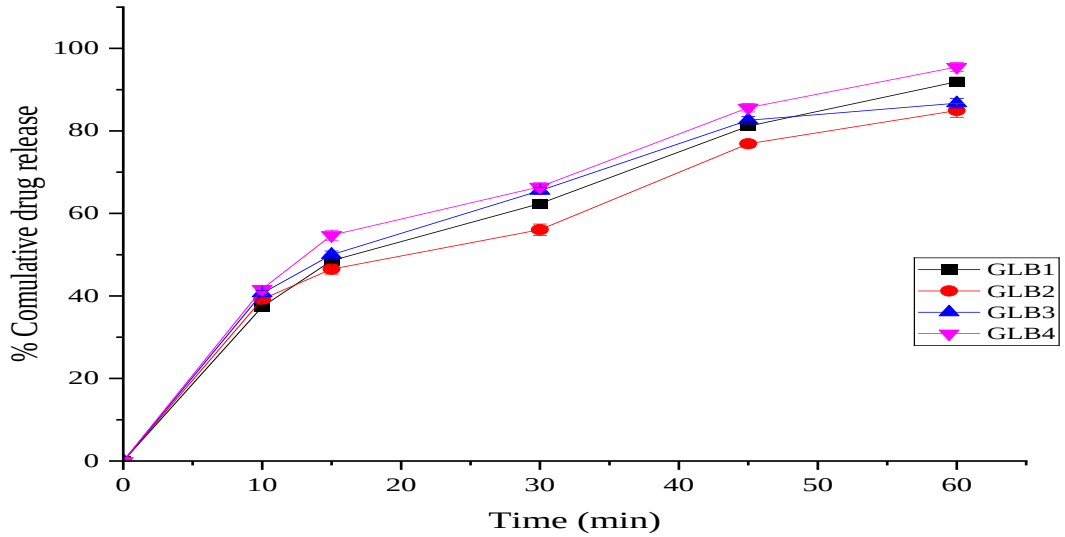

Figure 19: Time dependent dissolution profiles of different brands of glibenclamide tablet in phosphate buffer (pH=7.50) λmax 254 nm.

Table 21: Model-independent approaches (f1, f2 and DE) and Dissolution parameter (t50% MDT and t90%) of glibenclamide tablets

Sample code	f1	f2	Dissolution Efficiency (DE)	Difference in DE	t50%	MDT	t90%	Remark
GLB1	6.56	68.25	82.83	-2.34	16.65	25.44	61.60	Similar
GLB2	11.73	55.09	78.89	1.6	17.79	29.29	68.8	Similar
GLB3	5.38	67.75	74.72	5.77	14.48	24.94	67.20	Similar
GLB4			77.58		13.88	21.45	58.8	

Table 22: Modelling parameters for the data on dissolution of glibenclamide

Sample code	**Kinetic model**														
	Zero order			First order			Hickson Crowell			Higuchi			Weibull model		
	Parameters			Parameters			Parameters			Parameters			Parameters		
	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC
GLB1	0.787	44.358	0.468	0.983	29.143	3.004	0.961	34.045	2.187	0.989	30.465	2.784	0.997	18.553	4.769
GLB2	0.731	44.718	0.171	0.952	34.329	1.902	0.965	37.807	1.322	0.981	32.680	2.177	0.987	26.389	3.225
GLB3	0.553	47.723	-0.458	0.934	36.231	1.454	0.870	40.311	0.776	0.948	34.789	1.679	0.984	31.478	2.248
GLB4	0.723	46.366	0.132	0.975	30.917	2.707	0.953	35.632	1.921	0.986	32.277	2.481	0.991	25.517	3.606

6 Conclusion

In this study, a comparative *in vitro* equivalence study of 21 samples of BCS class II drug products comprising of 5 samples of atorvastatin calcium, 6 samples of azithromycin, 6 samples of clarithromycin and 4 samples glibenclamide tablets were made. The pharmacopeial quality parameters such as identity, mass uniformity, hardness, thickness, diameter, disintegration, assay, and dissolution tests were carried out according to USP and BP methods. The findings from the quality evaluations revealed that all products complied with the stipulated standards for various parameters such as identity, weight uniformity, hardness, thickness, diameter, disintegration, and assay tests. With the exception of a single generic atorvastatin product (ATO4) and two generic clarithromycin products (CLM2 and CLM3), the drug products investigated in this study met the dissolution test criteria outlined in USP 44 /NF39. Hence the regulatory authority and other stake holders should devise mechanisms to enforce medicine regulation in porous border areas of the country. The comparison of dissolution profiles using fit factors indicated that, aside from sample ATO4 from atorvastatin and CLM2 and CLM3 from clarithromycin tablets, all analyzed products exhibited *In-vitro* availability equivalence to their respective reference products, suggesting interchangeability. A model-dependent approach revealed that the drug release data of all tested drug products follows the Weibull, kinetic models. The overall outcomes of the *in vitro* dissolution assessment suggested that the interchangeable use of the evaluated drug products with their reference counterparts is feasible in clinical settings.

Suggestions for further work

The *in vivo* bioavailability assessment for the examined drug products should be studied to obtain a complete picture on their bioequivalence and clinical efficacy.

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Annexes

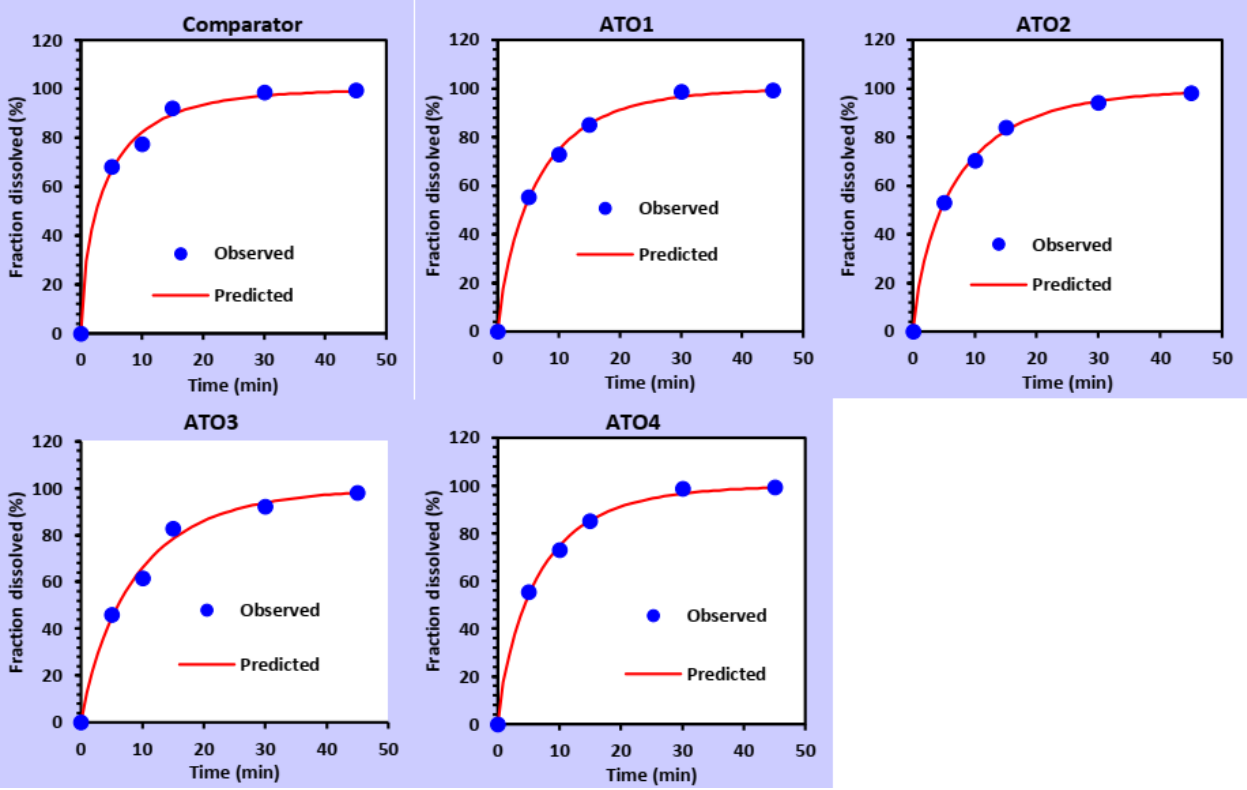
Annex I: General information on samples used in this study

Drug products	Brand name (code)	Manufacturer	Country of origin	Batch number	Mfg. date	Exp. date
Atorvastatin 40 mg tablet	ATORVA40 (ATO1)	East Africa Pharmaceuticals PLC	Ethiopia	BF109	06/22	05/24
	LORVAST (ATO2)	Tabuk Pharmaceuticals Mfg. Co	Saudi Arabia	1NM139	10/22	10/24
	ATSTAT40 (ATO 3)	Ind-Swift Ltd.	India	AHX10121	08 /21	07/23
	VASTPIN40 (ATO4)	Pinnacle life Science Private Ltd.	India	T2161	03/22	02/24
	ATEROZ(ATO5)*	Bilim Pharmaceuticals	Turkey	20199017A	12/21	12/24
Azithromycin 500 mg tablet	Zycin (AZM 1)	Cadilla Pharmaceuticals PLC	Ethiopia	D21001T355	06/21	05/24
	Azik 500(AZM2)	GLOCARE Pharma	Ethiopia	TB032	05/23	04/26
	Azeescot (AZM3)	SCOTTEDIL PHARMACIA LTD	India	2216346T004	01/22	05/25
	Azito (AZM4)	Zim Laboratories Ltd.	India	FAG4B304	01/23	01/26
	Azitro (AZM 5)	Deva Holding	Turkey	A102614	03/ 23	10/25
	Zithrotel (AZM 6)*	Anfarm Hellas	Greece	21C080	10/21	10/24
Clarithromycin 500 mg tablet	Clari-SSP500(CLM1)	Sansheng Pharmaceuticals PLC	Ethiopia	00421070010	Jun 2021	Jun 2023

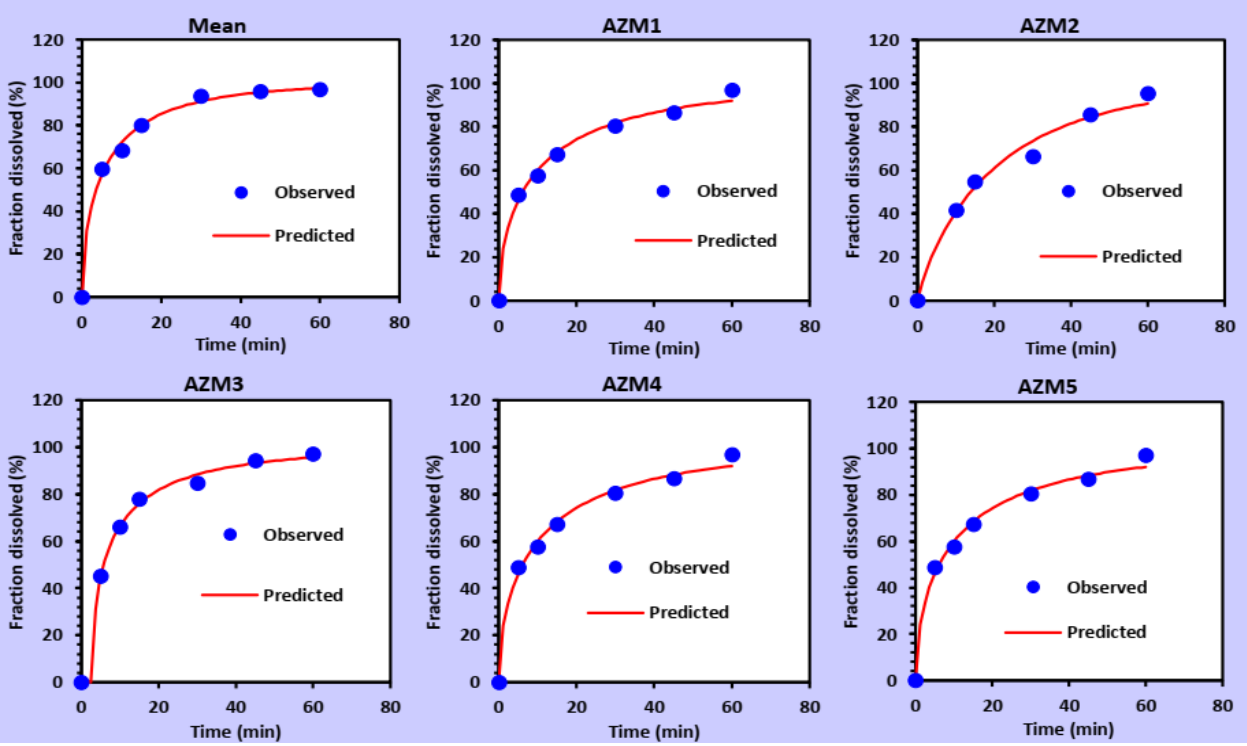
	Clarimycin (CLM2)	YSP M Industries Sdn Bhd	Malaysia	DJ005	10/22	10/24
	Clarzim (CLM3)	Zim Laboratories Ltd.	India	FOF2N102N	12/21	07/24
	Clarimin (CLM4)	United Biotech (p) Ltd.	India	552820002	01/21	01/24
	KLERIMED (CLM5)	MEDOCHEMIE Ltd	Cyprus	A5K031	10/21	10/24
	Claricide (CLM 6)*	Bilim Pharmaceuticals	Turkey	20113015A	12/20	12/23
Glibenclamide 5 mg tablet	Glamide (GLB1)	Cadilla Pharmaceuticals PLC	Ethiopia	0200048N75	03/20	02/24
	Gliben-SSP(GLB2)	Sansheng Pharmaceuticals PLC	Ethiopia	85022106610	05/22	09/24
	Glitsol (GLB3	REMEDICA Ltd.	Cyprus	A3L122	09/21	01/26
	Daonil (GLB4) *	Sanofi- Aventis	France	2MW1C	01/22	05/24

***Comparator drug products.**

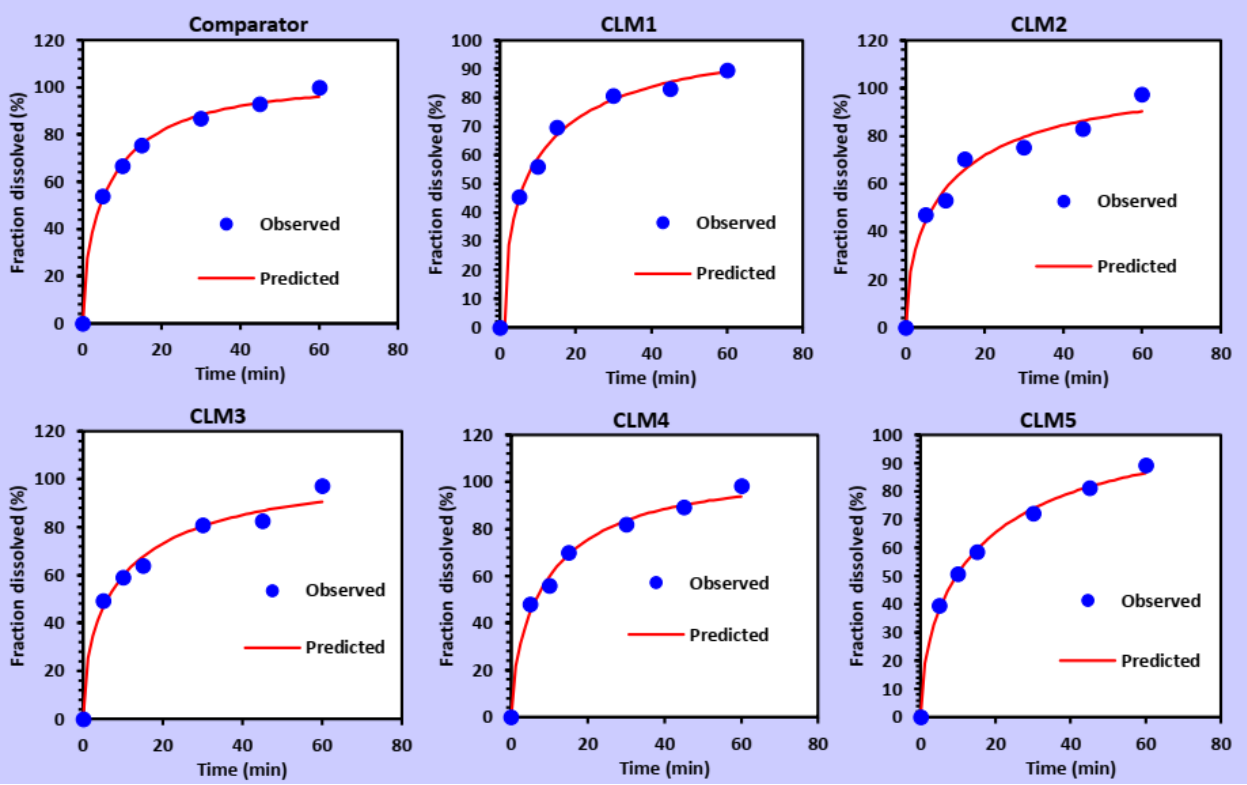
Annex II: Dissolution profiles of reference and test products of atorvastatin tablets observed vs predicted values as per Weibull kinetic model.



Annex III: Dissolution profiles of reference and test products of azithromycin tablets – observed vs predicted values according to Weibull model



Annex IV: Dissolution profiles of reference and test products of clarithromycin tablets – observed vs predicted values according to Weibull kinetic model.



Annex V: Dissolution profiles of reference and test products of glibenclamide tablets – observed vs predicted values according to Weibull kinetic model.