ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND PHARMACOGNOSY

Quality Assessment of Antimicrobial Drugs: Amoxicillin Capsules, Amoxicillin + Clavulanate, Ciprofloxacin and Norfloxacin Tablets from Drug Retail Outlets of Selected Cities in Eastern Ethiopia.

By: Hailu Anjulo (B.Pharm)

A Thesis Submitted to the Department of Pharmaceutical Chemistry and Pharmacognosy in Partial Fulfillment for the Requirements of MSc Degree in Pharmaceutical Analysis and Quality Assurance.

March, 2023

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Abstract

Quality Assessment of Antimicrobial Drugs: Amoxicillin Capsules, Amoxicillin + Clavulanate, Ciprofloxacin and Norfloxacin Tablets from Drug Retail Outlets of Eastern Ethiopia.
By: Hailu Anjulo (B.Pharm), February 2023

Rising incidence of substandard and falsified antimicrobial drugs is an increasing concern in developing nations. The beta-lactams and fluoroquinolones are among the broad-spectrum antimicrobial agents that are most frequently prescribed, inexpensive, and readily available in Ethiopia, for the treatment of infectious diseases. The consumption of substandard and/or falsified antimicrobial drugs has the potential to result in treatment failure, emergence and development of Antimicrobial resistance (AMR), and ultimately patient mortality. The objective of this study is to assess the quality of four commonly used antimicrobials (ciprofloxacin, norfloxacin, and amoxicillin and its combination with clavulanic acid) marketed in Dire Dawa and Jijiga cities, and Togo-Wuchale town with high potential for illegal drug trade in Ethiopia due to porous border through Somaliland. A total of 54 different brands/products of amoxicillin, amoxicillin plus clavulanic acid, ciprofloxacin, and norfloxacin formulations were collected covertly from 43 facilities using a convenience sampling strategy from March 16 to March 29, 2022, from the selected locations. The samples were first screened using GPHF-minilab protocol and then analyzed with references to USP and BP methods. The quality evaluation showed that 14.28% (6/42) of all samples failed the GPHF-minilab screening test quantitatively, 27.27% (6/22) of amoxicillin samples failed weight variation test, and 22.73% (5/22) of amoxicillin and 40% (2/5) of amoxicillin/clavulanate products failed to meet the assay test. Furthermore, 12.96% of the samples failed the dissolution test. Overall, 22.22% of the products analyzed did not meet pharmacopoeial specifications. Additionally, 56.25% of amoxicillin samples, 60% of amoxicillin/clavulanate, 20% of ciprofloxacin, and 54.54% of norfloxacin samples were found to be pharmaceutically inequivalent with their respective comparator products in relation to dissolution profile studies. The study had shown that the quality of essential antimicrobial medicines in eastern Ethiopia is substandard. This affirms the need for regular post-market surveillance to inform on the situation of antibiotic quality in eastern Ethiopia. Based on the accumulated evidence regulatory actions and mechanisms had to be also in place to circumvent the challenge.

Keywords: Antimicrobials, quality assessment, pharmaceutical equivalence, Eastern Ethiopia

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Operational Definitions

Substandard medicines: Medicinal products that are substandard, sometimes known as "out of specification," are approved medical products that do not satisfy their quality standards. Substandard drug products are also commonly used interchangeably with the terms low- or poor-quality medicines.

Falsified medicines: Falsified drug products that falsely represent their identity, composition, or source. Any substitution, adulteration, reproduction, or manufacturing of an authorized medical product is considered purposeful deception.

Unregistered medicines: Medicinal products are referred to be "unregistered" or "unlicensed" if the NMRA has not examined and approved them for the markets in which they are distributed or used.

Abbreviations and Acronyms

API	Active Pharmaceutical Ingredient
AIC	Akaike Information Criteria
BA	Bioavailability
BP	British Pharmacopoeia
cGMP	Current Good Manufacturing Practice
EFDA	Ethiopian Food and Drug Authority
GSMS	Global Surveillance and Monitoring System
GPHF	Global Pharma Health Fund
HPLC	High Performance Liquid Chromatography
ICH	International Conference for Harmonization
IR	Infrared spectrophotometry
LMICs	Low- and-Middle-Income Countries
MA	Marketing Authorization
MSC	Model Selection Criteria
NMRA	National Medicines Regulatory Authorities
RDV	Rural Drug Vendor
PMS	Post-Marketing Surveillance
R&D	Research and Development
RSD	Relative Standard Deviation
SF	Substandard/falsified
SSFFC	Substandard/spurious/falsely labelled/falsified/counterfeit medical products
SST	System suitability test
TLC	Thin Layer Chromatography
UNESCO	United Nations Educational Scientific Cultural Organization
US FDA	United States Food and Drug Administration
USP/NF	The United States Pharmacopoeia and National Formulary
UV	Ultraviolet Spectrophotometry
WHO	World Health Organization

1. Introduction

1.1. Background

Medicines possess powerful effects; they can be beneficial, such as curing diseases, or they can be toxic. Thus, their quality should be assured consistently so that they can be used safely. The safety of medicines has multidimensional aspects, including the safety and quality of the drug substance referred as active pharmaceutical ingredient(s) (API(s)), the added substances called excipients, and the finished pharmaceutical products (FPPs), which makes their quality assurance system complex (Wirtz *et al*., 2017). Due to these complexities, consumers cannot reliably judge the quality of the medicines they use. As a result, governments have a mission to ensure the quality of pharmaceuticals circulating in a specific national market through the regulation of pharmaceutical manufacturing, registration, distribution, and use (CESCR, 2000).

Medicinal products should be fit for their intended use and not pose risks to patients. To achieve these objectives, different quality assurance systems have been implemented by governments *via* national medicines regulatory authorities (NMRAs). NMRAs provide manufacturing and marketing authorization upon evaluation of documentation and inspecting facilities to ensure that manufacturers are competent enough to produce safe, effective, and quality medicines. After manufacturing, the distribution, transportation, and storage practices are also controlled by inspection of facilities, as poor practices can affect their quality (WHO, 1997). After medicines are introduced to the market, their quality can be checked through laboratory testing, which is performed by post-marketing quality surveillance programs. However, regulating and testing for the quality of all medicines circulating in a country fully is extremely difficult, if not impossible (EFDA, 2020; Eissa, 2019).

Currently the majority of LMICs lack regulatory capability needed to adequately monitor and guarantee the quality of pharmaceuticals (Wirtz *et al*., 2017; Suleman et al., 2016). There are several studies reporting widespread distribution of substandard and/or falsified essential drug products all over the world (Nayyar *et al*., 2019; Ozawa *et al*., 2018; Renschler *et al*., 2015). Substandard and falsified medicines are manufactured, distributed, and sold all over the world every day, entering the global medicines supply chain systems (WHO, 2017b).

Recently incidents of pharmaceutical crime have been increasing at high rate (102%), posing a great danger to public health (PSI, 2019). Substandard and/or falsified essential antimicrobial drugs are commonly reported (Schäfermann *et al*., 2020; Kelesidis & Falagas, 2015), which can lead to increasing in the occurrence of antimicrobial resistance as well as associated socioeconomic costs and health risks (Callister, 2019; Nickerson *et al*., 2016; Ravinetto & Dujardin, 2016).

Therefore, quality surveys of essential antimicrobial medicines are important to identify the extent of the problem. This in turn helps to devise ways to reduce the threat of SF antimicrobial medicines. Besides it can help a lot in an effort to improve supply chain management, surveillance, and regulatory capacity in developing countries like Ethiopia. Therefore, this study is intended to assess the physico-chemical quality of four of the most commonly used antimicrobial medicines: amoxicillin, amoxicillin + clavulanate, ciprofloxacin, and norfloxacin oral solid dosage formulations circulating in health facilities and drug retail outlets within the market of selected locations in eastern Ethiopia.

1.2. The fluoroquinolone antibacterial agents

The fluoroquinolone antimicrobial drugs are a class of antibacterials with broad-range activity against aerobic gram-positive and gram-negative microbes. They are entirely synthetic antibacterial drugs with bactericidal activity that are generated from quinolone antimicrobials by adding a fluorine atom to their chemical structure (Figure 1.1) (Mitscher, 2005). Fluoroquinolones are the second largest chemotherapy medicines utilized in clinical practice around the globe for the treatment of wide range of bacterial disease (Hu *et al*., 2017).

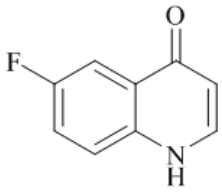


Figure 1.1: The basic chemical structure of fluoroquinolones.

Fluoroquinolone-class antimicrobials have gone through four generations of clinical development since the synthesis of nalidixic acid and discovery of its specific antibacterial action

in the early 1960s. Each generation has structural and activity profiles that distinguish it from other generations. Figure 1.2 depicts the structures of nalidixic acid and a few fluoroquinolones at various stages of development (Bisacchi, 2015).

The first-generation quinolone-class drugs were nalidixic acid and other related acids. First generation fluoroquinolones have activity against gram-negative bacteria; however, they have limited use because of the short serum half-lives associated with rapid renal elimination. Due to this, they can only be used to treat urinary tract infections caused by gram-negative bacteria, and they are no longer commonly utilized (Bisacchi, 2015). The modification of the bicyclic core structure in nalidixic acid to the quinolone core and the introduction of a fluorine atom at C-6 and piperazine at C-7 in second-generation derivatives, such as ciprofloxacin and norfloxacin, resulted in a greater spectrum of activity. They are active against a wider range of gram-negative bacteria, including *pseudomonas aeruginosa*, and gram-positive bacteria with longer serum half-lives than the first-generation fluoroquinolones (Bisacchi, 2015; Ball, 2000).

Third generation derivatives, such as levofloxacin, are developed by introducing additional C-7 rings to second generation derivatives or by chiral resolution of existing drugs. Third-generation derivatives have been proven to be effective against gram-positive bacteria as well as some penicillin-resistant infections. Current fourth generation fluoroquinolone drugs like moxifloxacin and gatifloxacin have the C-8 methoxy functionality that is included into their structures, which is attributed for enhanced activity against gram-positive pathogens as well as reduced phototoxicity. These drugs have been proven effective against anaerobic and ciprofloxacin-resistant bacteria (Bisacchi, 2015).

Quinolones and fluoroquinolones work by stabilizing the complex formed between DNA and topoisomerases, which inhibits bacterial DNA replication and transcription (Patrick G. 2017).

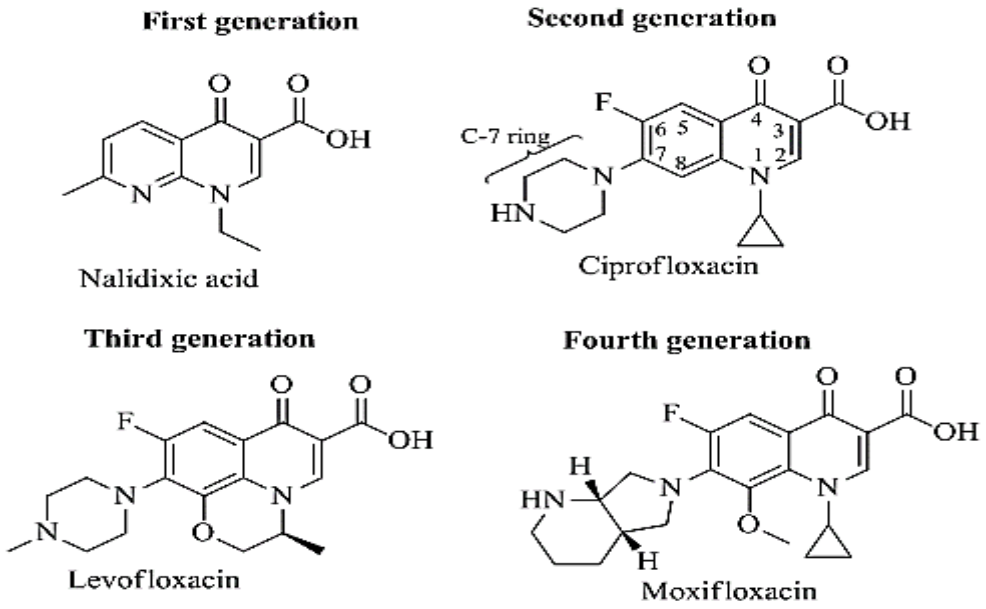


Figure 1.2: Fluoroquinolone structures by generation (Bisacchi, 2015)

1.2.1. Ciprofloxacin

Ciprofloxacin is a synthetic, second-generation broad-spectrum fluoroquinolone antimicrobial drug frequently used to treat a variety of bacterial infections affecting the urinary tract, respiratory tract, gastrointestinal tract, musculoskeletal system, skin, and soft tissues. Ciprofloxacin is the most effective fluoroquinolone drug against gram-negative bacteria. It has a cyclopropyl substituent at position 1 (Figure 1.2) which is attributed for its increased spectrum of activity and the nitrogen at position 8 in previous generations was replaced with carbon which minimized side effects while increasing effectiveness against *staphylococcus aureus*. Ciprofloxacin was first marketed in 1986 due to its additional pharmacokinetic profile and potent activity against different pathogens than its forerunners (Appelbaum & Hunter, 2000). Ciprofloxacin has a sound medical significance in treating infections caused by numerous *enterobacteriaceae* and other gram-negative *bacilli*. It represents the most potent of the fluoroquinolone agents for *pseudomonal* infections associated with cystic fibrosis (Solomon *et al*., 2001).

1.2.1.1. Chemistry of ciprofloxacin

Ciprofloxacin hydrochloride have a molecular formula $C_{17}H_{18}FN_3O_3.HCl.H_2O$ and a molecular weight of 385.82 amu. It exists as a light yellow, crystalline powder that have minimum

solubility in water, is slightly soluble in methanol, is very slightly soluble in dehydrated ethanol, and is practically insoluble in acetone, ethyl acetate, and dichloromethane. It is soluble in dilute (0.1N) hydrochloric acid. Ciprofloxacin hydrochloride is slightly hygroscopic and contains a variable quantity of water (LeBel, 1988). It is acidic, with a pKa of 6.09 at 25 °C and a melting point of 255–257 °C (Torniainen *et al*., 1997). The solubility of ciprofloxacin depends extremely on the pH value. At high pKa values (low pH), ciprofloxacin is more soluble since it is in the protonated form. It is almost insoluble in water and alcohol. At pH 4-5, it shows the highest solubility (>40 mg/mL). Ciprofloxacin is almost insoluble at neutral pH, but it becomes more soluble as the pH increases. The stability of the dry substance of ciprofloxacin is very high at room temperature (Hubicka *et al*., 2010).

Ciprofloxacin is susceptible to the photodegradation process, which may lead to a reduction and/or loss of antibacterial activity and thus it induces phototoxicity as a side effect. It photochemically decomposes in acidic aqueous solutions, forming two major degradation products: a quinoline carboxylic acid compound and an aromatic amino-compound. Ciprofloxacin should therefore be strictly protected from light during storage and administration (Torniainen *et al*., 1997).

1.2.2. Norfloxacin

Norfloxacin is a synthetic broad-spectrum fluoroquinolone with antibacterial activity against a wide range of aerobic gram-negative and gram-positive bacteria. In order to prevent bacterial DNA replication, the bactericidal drug norfloxacin binds to an enzyme called DNA gyrase. This prevents DNA replication from happening within the bacteria. The gram-negative enteric bacteria *Pseudomonas aeruginosa*, *Hemophilus influenzae*, and *Neisseria gonorrhoeae* are all susceptible to norfloxacin. In general, norfloxacin is less effective than ciprofloxacin, especially when used to treat *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Nix & DeVito, 1987).

1.2.2.1. Chemistry of norfloxacin

Norfloxacin is a quinoline monocarboxylic acid (Figure 1.3), with the molecular formula $C_{16}H_{18}FN_3O_3$ and a molecular weight of 319.33 amu. Norfloxacin is a crystalline powder that ranges in color from white to pale yellow and is hygroscopic and photosensitive. It is very barely

soluble in water, slightly soluble in alcohol and in acetone, freely soluble in acetic acid, sparingly soluble in chloroform, nearly insoluble in ether, and very barely soluble in ethyl acetate and in methyl alcohol (Martindale, 2014).

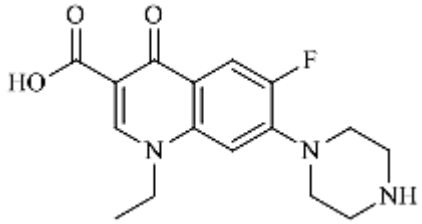


Figure 1.3: Chemical structure of norfloxacin (Martindale, 2014)

Norfloxacin exists in several solid forms: two anhydrous polymorphs and several hydrated forms. Different polymorphs exhibit different physicochemical properties such as solubility, dissolution rate, bioavailability, and chemical and physical stabilities. Many commercial samples of norfloxacin are provided in the metastable form at room temperature, and then undesirable transformations could occur (Chongcharoen *et al*., 2008). Norfloxacin is most stable at an acidic and basic pH, in darkness, and at a low temperature (Nangia *et al*., 1991). Therefore, it should be kept in airtight containers protected from light.

1.3. The penicillin antibacterial agents

Antibiotics with a four-membered cyclic amine (lactam) as a pharmacophore and a nitrogen atom linked to the *β*-carbon atom relative to the carbonyl group are known as β-lactams (*β*-lactams) (Hubschwerlen, 2007). The *β*-lactam antibiotics are classified into two groups: penicillin's and cephalosporins. The type of rings linked to the *β*-lactam moiety distinguishes the two groups. A highly strained 4-membered *β*-lactam ring is connected with a 5-membered thiazolidine ring in all penicillin molecules. The unstable *β*-lactam ring is primarily responsible for these compounds' antibiotic potency. The strained-lactam ring binds irreversibly to the enzyme transpeptidase, which is responsible for the bacterial cell wall's final cross-linking, inhibiting cell wall synthesis. The structural requirements for penicillins are depicted in Figure 1.4 (Foye *et al*., 2013).

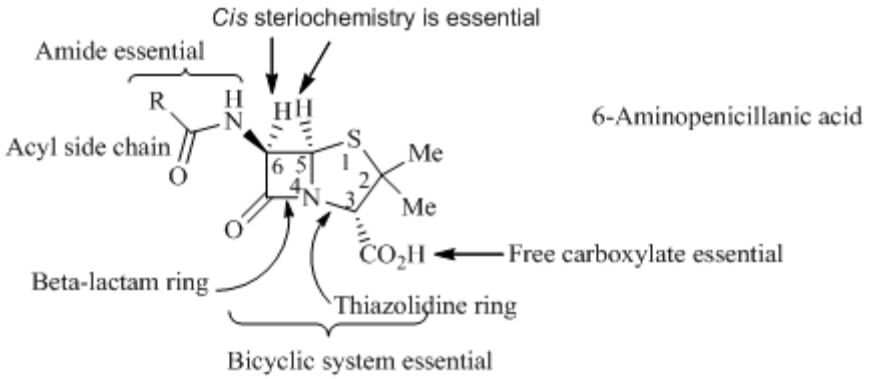


Figure 1.4: Structural features essential for activity of penicillin's (Foye *et al*., 2013).

The initial penicillin analogs, like penicillin G, were vulnerable to acid hydrolysis. Ampicillin analogues have demonstrated resistance to acid hydrolysis and can be given orally (Patrick, 2017). When the extensive use of penicillin-G led to a rise in penicillin-resistant s*taphylococcus aureus* infections in 1960s, the *β*-lactamase issue became important. The first semi-synthetic penicillin that was effective against the *staphylococcus aureus β*-lactamase enzyme was methicillin. Methicillin, on the other hand, needs to be administered intravenously since it is acid-sensitive (Patrick, 2017).

Penicillin's having broad-spectrum antibiotic activity against both gram-positive and gram-negative bacteria are categorized in to three classes. The aminopenicillins are one of the three classes of broad-spectrum antibiotics, all of which have an "α−hydrophilic group," which aids the passage of these penicillin's through the porins of the outer membrane of gram-negative bacteria. Ampicillin and amoxicillin, shown in Figure 1.5, are two antibiotics in this class that have a relatively similar structure with acid-resistance and which can be used orally (Patrick, 2017). Ampicillin is a benzylpenicillin analog in which the main amino group has been substituted for one of the hydrogen atoms in the side chain of methylene to generate an R-phenylglycine moiety. The antibacterial spectrum has been altered so that many common gram-negative organisms are sensitive to ampicillin, in addition to its strong acid stability. However, ampicillin showed limited oral efficacy for systemic infections owing to its amphoteric property, resulting in lower oral bioavailability. Amoxicillin was developed as a response to improve the limited oral bioavailability of ampicillin (Foye *et al*., 2013).

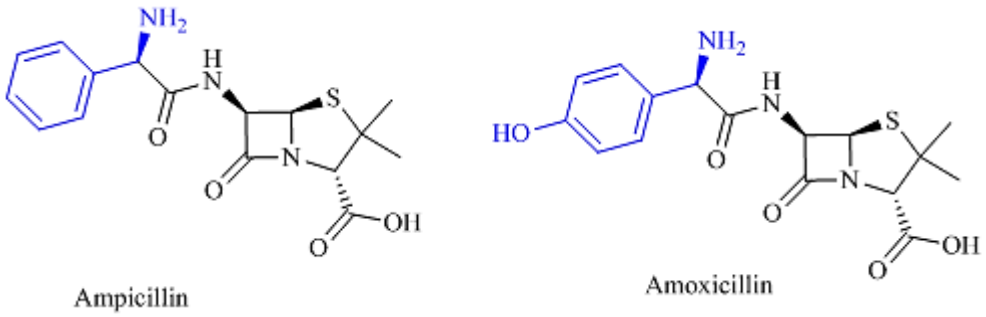


Figure 1.5: Penicillin derivatives containing electron withdrawing acyl side chains (Foye *et al*., 2013).

1.3.1. Amoxicillin

Amoxicillin is an acid-stable, semi-synthetic antibiotic in the aminopenicillin class of β-lactam antibiotics. It is a closely related analog of ampicillin that has a para-phenolic hydroxyl group added to the side-chain phenyl moiety. The added group changes the drug's isoelectric point to a more acidic value and is thought to be partly responsible for the higher blood levels found with amoxicillin compared to that of ampicillin, hence causes reduced disruption of the normal GIT flora (Foye *et al*., 2013).

1.3.1.1. Chemistry of amoxicillin

Amoxicillin exists in three different forms: anhydrous, sodium salt (white or slightly pink, amorphous, and extremely hygroscopic with a mild sulphurous odor) and trihydrate forms (Martindale, 2014). Its hydrate form having molecular formula of $C_{16}H_{19}N_3O_5S.3H_2O$ and relative molecular mass of 419.45 amu is used for the capsule pharmaceutical dosage forms. Amoxicillin in a trihydrate form is a white crystalline powder that is completely insoluble in fatty oils but only very barely soluble in alcohol and water. It is soluble in dilute acids and alkali hydroxide solutions. It must be kept in sealed, airtight containers (Ph. Int., 2021). Solubility of amoxicillin increases with increasing pH, and reported pKa values of amoxicillin are 2.67, 7.11, and 9.55 at 37 °C, with lowest solubility at a pH range of 4-6 (Rolinson & Geddes, 2007). Amoxicillin degrades with an increase in temperature, both in sealed and open containers. Amoxicillin degrades by first-order kinetics rate under controlled humidity conditions (Deshpande *et al*., 2004).

1.3.2. Amoxicillin + clavulanate combination drug

Amoxicillin is susceptible to hydrolysis by *β*-lactamases, which are produced by bacteria (Contreras-Martel *et al*., 2011). Clavulanic acid is a β-lactam compound isolated from the fermentation of *Streptomyces clavuligerus*, a gram-positive bacterium (Saudagar *et al*., 2008). Clavulanic acid binds to β-lactamases in an irreversible manner, preventing them from inactivating certain β-lactam antibiotics such as penicillin and cephalosporins. Thus, making them effective in treating gram-positive and gram-negative infections (Saudagar *et al*., 2008; Finlay *et al*., 2003). Clavulanic acid, shown in Figure 1.6, has a chemical formula of $C_8H_9NO_5$ (Saudagar *et al*., 2008). It functions as a pseudo-substrate, occupying the active site of *β*-lactamase for a long enough period of time to prevent the degradation of co-administered *β*-lactam antibiotics, by forming a stable acyl-enzyme complex. As a result, clavulanic acid increases the activity of *β*-lactam molecules, making the combination of amoxicillin and clavulanic acid more therapeutically useful and bacteriologically effective than amoxicillin alone (Finlay *et al*., 2003).

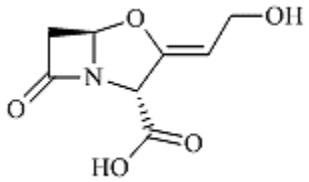


Figure 1.6: Chemical structure of clavulanic acid (Saudagar *et al*., 2008)

1.4. Pharmaceutical quality

In order for a pharmaceutical product to provide the intended therapeutic effect without exposing patients to an undesired adverse effect, it should be of the required quality. The quality standards of a drug are expressed in terms of identity (the product has the correct active ingredient), purity (the medicine is not contaminated with potentially harmful substances), potency (the product has the correct amount of active ingredient(s)), uniformity (the product has a consistent shape, weight, content, and size), bioavailability (the product has a consistent bioavailability (BA) to provide a predictable therapeutic result), and stability (the product is stable throughout its shelf life). These critical quality attributes of pharmaceutical products are developed and established based on data from clinical studies done on clinical batches with proven safety, efficacy, and

quality. The quality standards are consistently labeled as a label claim on the finished pharmaceutical product and published in relevant pharmacopoeias (Woodcock, 2004).

1.5. Quality testing of pharmaceutical products

The evaluation of the quality of pharmaceuticals is based on different quality control tests. 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).

1.6. Physicochemical quality parameters for oral solid (tablet and capsule) dosage forms

Pharmacopoeial methods and limits are used as compliance requirements to assess the quality of pharmaceutical products. Test material/sample of size as prescribed in the monograph that will be taken at any time during storage, distribution, and use within the accepted shelf-life is expected to meet all of the mandatory requirements for a product to comply with pharmacopoeial quality requirements (BP, 2021). 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 (Allen *et al*., 2010; Hansen *et al*., 2011).

1.6.1. Tests based on physical methods

1.6.1.1. Appearance or Description

Each of the dosage units of a product should be uniform in appearance. The color, size, shape, odor, labeling, and imprinted markings should be homogenous from dosage unit to dosage unit and from batch to batch to ensure the quality of the product (BP, 2021).

1.6.1.2. Uniformity of dosage Units:

The active pharmaceutical ingredients (APIs) and excipients in a product should be evenly distributed within a tight range around the label claim across each dosage unit in a batch so that the product can reliably provide the intended effect. Dosage unit consistency can be determined in two ways: by content uniformity and weight variation tests (USP 44-NF 39, 2021).

1.6.1.3. 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).

1.6.1.4. Hardness/resistance to crushing strength of tablets

Tablet dosage forms are subjected to a hardness test to determine their capacity to withstand mechanical shocks that may occur during manufacture, packing, and transportation. Also, a tablet's ability to disintegrate and dissolve is influenced to some extent by its hardness (USP 44-NF 39, 2021).

1.6.1.5. 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 (Karmakar & Kibria, 2012; Markl &

Zeitler, 2017). Figure 1.7 depicts the process of drug release from a tablet. (modified from Markl and Zeitler (2017)).

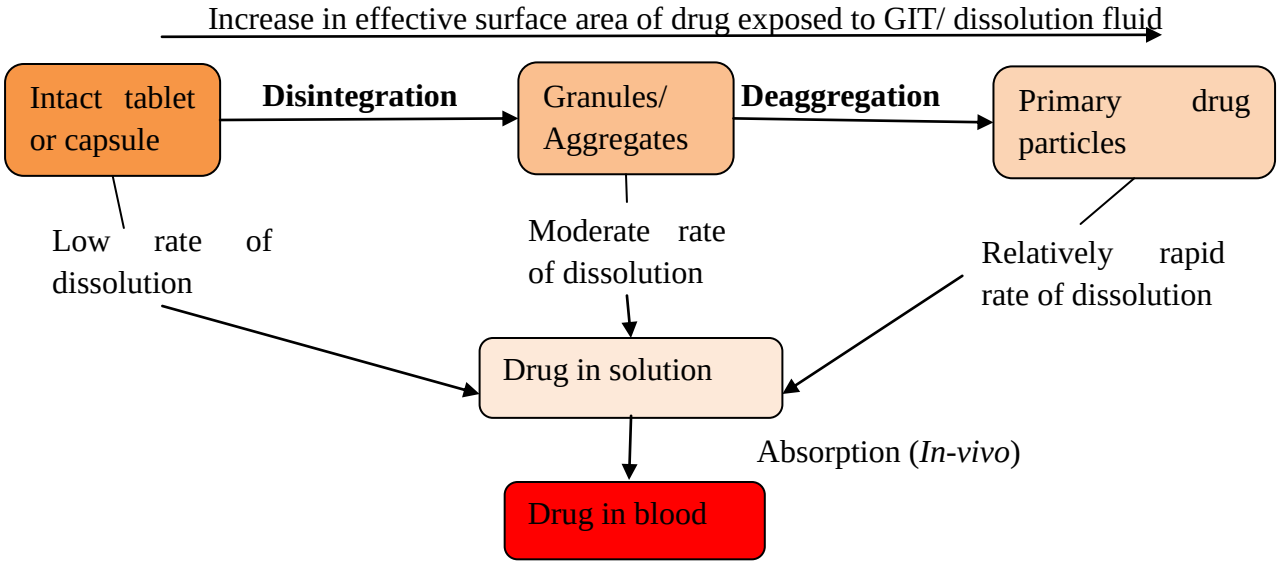


Figure 1.7: Schematic representation of the drug release process from a tablet.

1.6.2. Tests based on chemical methods

An oral solid-dosage formulation that has adequate physical quality alone does not achieve the intended therapeutic effect. As a result, determining the chemical makeup of a dosage form is required for assessing therapeutic efficacy. This is performed by employing chemical tests to evaluate the finished pharmaceutical product's composition (BP, 2021).

1.6.2.1. Dissolution test

To be absorbed through the GIT lumen and available in significant amounts in systemic circulation, a drug substance in a formulation must first be dissolved in a GIT fluid. A precondition for drug absorption is the rate and extent of drug substance dissolution in physiological fluid, which can be rate-limiting. As a result, the dosage form should release the drug substance at a consistent rate in order to achieve the desired therapeutic effect. *In-vitro* dissolution testing, which assesses the rate and amount of drug dissolving from a dosage form under standard test conditions, is used to keep track of this (Allen *et al*., 2010). As a quality control (QC) technique, the *in-vitro* dissolution test ensures consistent drug dissolution properties from batch to batch of a product (Zaborenko *et al*., 2019). An *in vitro* drug dissolution profile

comparison can be used to assess the *in vitro* equivalence or interchangeability of multisource generic products. There are several mathematical computational approaches available for comparing drug dissolution profiles, both model-dependent and model-independent.

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 equation 1.1:

$$Q_T = Q_0 + K_0 t \qquad \text{Equation 1.1}$$

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 equation 1.2:

$$\ln Q_t = \ln Q_0 + K_0 t \qquad \text{Equation 1.2}$$

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 equation 1.3:

$$Q_0^{1/3} + Q_t^{1/3} = K_d t \qquad \text{Equation 1.3}$$

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 equation 1.4:

$$Q = K_d \sqrt{t - t_0} \qquad \text{Equation 1.4}$$

Where K_d is the dissolution constant.

Makoid-Banakar Model: If the required hydrodynamic state and sink condition are maintained throughout the dissolution test, then the drug dissolution profile can be categorized into early-phase, middle-phase, and late-phase regions. This should reflect changes in release rates over the entire duration of the profile. The early region of an immediate-release solid dosage formulation with finite dose will follow a linear function with a positive slope, and the later region will follow an exponential function with a negative slope, reflecting a decrease in release rate. The Makoid-Banakar model combines zero-order and first-order drug dissolution kinetics. From the moment the dose unit is placed in the dissolution medium (t = 0) until all of the drug has been dissolved in the medium (t = ∞), the dissolution process follows a continuous function. It can be empirically determined using derivative functions based on these presumptions, and equation 1.5 can be used to determine the mechanics of this drug dissolution and release profile (Banakar, 2022):

$$F = B * t * [exp(-C * t)] \qquad \text{Equation 1.5}$$

Where F is the fraction of the drug dissolved at time t and B and C are the initial and later portion dissolution rate constants, respectively.

Weibull model: The dissolution rate can be investigated using the Weibull function, a mathematical model without a physicochemical basis. The following equation (1.6) represents the model:

$$\ln\left(\ln\frac{Q_\infty}{Q_\infty - Q}\right) = \beta \ln(t - t_0) - \beta \ln t_d \qquad \text{Equation 1.6}$$

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.

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 equation 1.7 (US-FDA, 1997):

$$f_1 = \{[\sum_{t=1}^{n} |R_t - T_t|]/[\sum_{t=1}^{n} R_t] * 100\}$$ Equation 1.7

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 equation 1.8 (US-FDA, 1997):

$$f_2 = 50\ x\ \log\left\{\left[\left[1 + (^1/_n)\sum_{t=1}^{n}(R_t - T_t)^2\right]\right]^{-0.5} x\ 100\right\}$$ Equation 1.8

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 $100 \leq f_2 \geq 50$.

1.6.2.2. Identification test

The purpose of identification testing is to verify that the product contains the API(s) that they claim to contain (BP, 2021; USP 44-NF 39, 2021). It confirms identity.

1.6.2.3. Assay

Assay is a method of determining the quantity of API(s) in a pharmaceutical product to guarantee that it has the correct amount of drug substance(s) within the allowed range per dosage unit in relation to the prescribed content (BP, 2021; USP 44-NF 39, 2021).

1.6.2.4. 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).

2. Literature Review

2.1. Poor quality medicines

Medicines of poor quality pose a substantial threat to public health. Poor-quality medical products are a local and worldwide health hazards. They are linked to manufacturing and supply chain integrity that must be properly addressed. Given the nature of the problem and procedures for combating low-quality medical products, collaboration among national, regional, and worldwide stakeholders is required (Newton, Amin, *et al*., 2011). In order to remove confusion among the stakeholders regarding the terms used to express poor quality medicines and to achieve a shared understanding among all levels and stakeholders and effectively prevent the market entry of substandard/spurious/falsely labelled/falsified/counterfeit medical products (SSFFC) medical items, these products are grouped within three adopted terms: substandard medical products, unregistered/unlicensed medical products, and falsified medical products (WHO, 2017b).

Medical items that are substandard, sometimes known as "out of specification," are approved medical products that do not satisfy their quality standards. Pharmaceutical products are referred to be "unregistered" or "unlicensed" if the NMRA has not examined and approved them for the markets in which they are distributed or used. Falsified drug products are those that falsely represent their identity (in terms of the name, labeling, packaging, or documents that support the authenticity of an authorized medical product), composition (in terms of any ingredient or component of the medical product), or source (identification, including name and address, of the market authorization (MA) holder, manufacturer, importer, exporter, or distributor). Any substitution, adulteration, reproduction, or manufacturing of an authorized medical product is considered purposeful deception (WHO, 2017a). The characteristics of substandard and falsified (SF) antimicrobials can be summarized as follows: formulations containing lower levels of API than claimed on the label are the most often reported quality concern with SF antibiotics (Almuzaini *et al*., 2013); medications with higher API levels than expected (Hadi *et al*., 2010); fake packaging (Kelesidis & Falagas, 2015); products that lack an API or replacing with cheap fillers (Almuzaini *et al*., 2013); *t*he label claim API has been replaced with another, such as erythromycin instead of artesunte (Newton *et al*., 2011); contamination with impurities,

chemicals or microbials (Frimpong *et al*., 2018); and products with variable mass or content (Almuzaini *et al*., 2013).

2.2. Global situation of poor-quality medicines: scope of the problem

A global effort has been made to reduce morbidity and mortality by improving access to vital medicines (GBD 2015 SDG Collaborators 2016). While access to healthcare and health outcomes has improved in recent decades, the problem of SF medicines is posing a severe threat to these efforts (Nayyar *et al*., 2012; Kaplan & Mathers, 2011). Despite the fact that many millions of people still lack access to essential medicines, the global pharmaceutical trade has grown dramatically in recent years. Unfortunately, this expansion has opened the door not only to high-quality, safe, and effective drugs but also to low-quality, potentially toxic medicines. The problem of SF pharmaceuticals is a global issue that affects countries of all sizes and economic levels, as well as drugs of any kind. The evidence available suggests that there are several issues with drug quality and product safety around the world that require immediate attention (WHO, 2017b).

The WHO estimates that 10% of medicines in low and middle-income countries (LMICs) are substandard and/or falsified, with antimicrobial pharmaceuticals making up 50% of these cases and developing countries accounting for 78% of these cases. Additionally, LMICs carry the majority of the burden due to inadequate pharmaceutical governance, technical proficiency, and supply-chain management (WHO, 2017b; Nayyar *et al*., 2012).

Overall, 13.6% of essential medicines in LMICs are of low-quality, with 18.7% in Africa and 13.7% in Asia. Antimalarials accounted for 19.1% of the total 13.6%, while 12.4% of antimicrobials were poor or fraudulent (Ozawa *et al*., 2018). According to a WHO study of drug product quality in African countries, 7.6% of major antimicrobial formulations have no active ingredient, while 17.8% of antibacterials and 13% of antiparasitic drugs are substandard (Reidenberg & Conner, 2001). According to the data from the Pharmaceutical Security Institute, the incidence of notifications of fake drugs has increased by more than tenfold between 2002 and 2012 (Kelesidis & Falagas, 2015).

Since 2011, the WHO has promoted and developed a standard form for reporting defective or falsified pharmaceuticals, and analyzes the reported data through the Rapid Alert System. By August 2016, the Rapid Alert System database had over 1000 reports. Anti-infectives were the most commonly reported classes of falsified medicines in the data system, which included practically all categories of pharmaceuticals as being substandard or falsified (Wirtz *et al*., 2017).

The problem of falsified drugs that are thought to cure illness is nearly as old as when commercial activities initiated, and it has been on the rise since then (WHO, 2017b). The term "pharmaceutical crime" covers the full spectrum of offenses involving drugs and medical equipment. The sale of substandard and falsified medications, nutritional supplements, and medical equipment is a basic definition of pharmaceutical crime, and it has risen tremendously in recent years, presenting an increased public health hazard (PSI, 2019). This is due to the fact that globalization has made the pharmaceutical supply chain more complex, allowing for various entry points for pharmaceuticals that were made unethically and illegally (WHO, 2017b). It's worth noting that the issue of fake medical products isn't just a problem in developing countries only; it's also growing rapidly in developed ones (Schäfermann *et al*., 2020).

2.2.1. Substandard and falsified antimicrobial drugs

Communicable diseases are currently one of the top ten causes of death worldwide, and thus antimicrobials remain essential life-saving drugs (WHO, 2019b). According to the WHO, beta-lactams, cephalosporins, quinolones, glycopeptides, and macrolides are among the most vital antimicrobials for human medicine (WHO, 2013). The global antimicrobial market had a worth $40.85 billion in 2020, and it is expected to expand by 4.6% between 2021 and 2027, resulting in a 55.96 billion increase by the end of the forecast period (Grand view research, 2022). Criminals are likely to target antimicrobials, which are essential medicines in high demand. As a result, SF antimicrobials are continuously detected globally. The illegal antimicrobial market was predicted to account 5% of the worldwide antimicrobial market (Delepierre *et al*., 2012).

Antimicrobial drugs are the most commonly falsified medical items in the world (WHO, 2017a), accounting for more than 25% of all SF medications (Delepierre *et al*., 2012). Since 2013, antibacterials and antimalarials have been the most often reported medications among the 10% of

SF medical products in LMICs (WHO, 2017b). Continent-wise, antibacterial agents that have been counterfeited are more common in Asia and Africa (Kelesidis & Falagas, 2015). According to a WHO warning, SF antimicrobials are not just a problem in developing nations only; they are also growing rapidly in developed nations (WHO, 2021). In 2012, it was reported that around 21,000 dosage units of SF antimicrobials were seized in 12 European nations in less than a week (Venhuis *et al*., 2016). According to the global surveillance and monitoring system (GSMS) of SF medical items, antimicrobials account for more than 40% of reported cases, and over 90% of reported antibacterials are essential antimicrobials. SF medicine reports came from all over the world: 42% from Africa, 21% from America, 21% from Europe, 8% from the Western Pacific, 6% from the Eastern Mediterranean, and 2% from Southeast Asia (WHO, 2017b).

The likelihood of finding SF antimicrobials grows as more efforts are made, and numerous case studies have evidently been published (Kelesidis & Falagas, 2015). The WHO issued medical product warning N° 9/2019, confirming the presence of counterfeit Augmentin (amoxicillin trihydrate and potassium clavulanate) in Uganda and Kenya, which contained no active ingredients (WHO, 2019a). These SF-antimicrobial cases are merely the tip of the iceberg. It's understandable that a significant proportion of incidents go unreported due to weak regulation and governance systems in developing countries.

According to the chemical classes of antibacterials, the most common low-quality products were β-lactams, quinolones, and macrolides: the top three categories of SF antimicrobials around the world, accounting for 50%, 12%, and 11%, respectively, of the 163 low-quality antimicrobials detected worldwide till 2009 (Delepierre *et al*., 2012). Amoxicillin is the most common SF antimicrobial, followed by ampicillin, tetracycline, and trimethoprim-sulfamethoxazole (Kelesidis & Falagas, 2015). According to dosage forms, oral solid dosage forms (tablets and capsules) are the most frequently reported to be SF, accounting for about 77% of poor-quality medicines. Amoxicillin in capsule formulations is the most frequently counterfeited active ingredient among antimicrobials in the world (Delepierre *et al*., 2012).

2.2.2. Quality of antimicrobial drugs in Ethiopia

Quality studies have been conducted in Ethiopia for decades, revealing failure rates that vary depending on the type of medicine, the manufacturer, and the place of origin. Antibacterials, antimalarials, and anti-TB drugs are of greatest concern, according to the studies. Ethiopian food and medicine authority (EFDA) have taken a number of steps, including issuing public alerts through the media, seizing the drugs, and apprehending the criminals (EFDA, 2021; KHN, 2003).

Several studies on the quality of antimicrobial products have been conducted in Ethiopia. 196 pharmaceutical samples of various therapeutic categories (represented mostly by amoxicillin, followed by ibuprofen and paracetamol) from both authorized and illegal drug sellers from 15 developing countries, particularly those with sub-Saharan tropical environmental conditions, including Ethiopia, were tested in the laboratories of the Department of Science and Technology (Faculty of Pharmacy, University of Turin, Italy). Significant percentages of samples (52%) have been reported as SF, with 2% of those being fraudulent items without the specified API. Antimicrobials made up 34.4% (74) of the total samples tested, and 29.7% (30) of those antimicrobials were reported to be counterfeit. Despite the small number of samples collected from Ethiopia, the study found that substandard and unregistered antimicrobials are present in the Ethiopian market. According to the study, 67% of the counterfeit product samples from Ethiopia were substandard formulations, while 37% of the products were illegally imported (parallel marketing), thus resulting in economic damage (Baratta *et al*., 2012). There is also a report of two main first-line anti-tuberculosis drugs, isoniazid and rifampicin, failing basic quality control tests (Bate *et al*., 2013).

The United States Pharmacopoeial Convention (USP) publishes the outcomes of data collected from medicine quality monitoring (MQM) activities in various countries throughout the world in a publicly accessible MQM database. From a total of 4473 antimicrobial samples examined between 2003 and 2013, 20.4% were determined to be poor quality or fake, according to data from five African countries, including Ethiopia. SF was found in 1.2% of amoxicillin samples tested throughout that time period (Hajjou *et al*., 2015). The database shows that samples of

several solid-dose antimalarial treatments (chloroquine and quinine) from Ethiopia failed quality tests between 2013 and 2017 (U.S PQM Programme, 2019).

According to Kahsay and G/Egziabher, (2010), an *in-vitro* comparative dissolution study revealed that some of the ciprofloxacin tablet formulations available in Tigray, Ethiopia, did not meet quality standards for dissolution and are non-interchangeable. In a research to evaluate the quality and physicochemical comparability of nine brands of norfloxacin tablets available in Jimma, Ethiopia, 22.2% of the samples did not meet the requirements of the compendial dissolution test (Hambisa *et. al*., 2019). An analysis and comparison of dissolution profiles for various products of amoxicillin capsules sold in the Ethiopian market also revealed that the majority of generic products of amoxicillin (62.5%) were reported to be non-interchangeable with the innovator brand (Kassaye & Genete, 2013).

3. Justification of the Study

3.1. Statement of the problem

Infectious diseases are the leading cause of public health concerns in Ethiopia. According to the Federal Ministry of Health of Ethiopia, 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 antibacterial medicinal products. However, there are reports showing suboptimal access to affordable, safe, and quality essential antimicrobial drug products in Ethiopia as a whole (Boche *et al*., 2020) and also in eastern Ethiopia as well (Sisay *et al*., 2021). When there is a gap between pharmaceutical supply and demand, SF medical items have the easiest time breaking into the market since they fill a void in supply (WHO, 2017b).

Most local medicine manufacturers in LMIC do not comply with WHO cGMP prequalification requirements, which can result in substandard medicines production (WHO, 2017a). Regulatory actions were launched against six local pharmaceutical manufacturing facilities in Ethiopia from 2017 to 2018 due to product quality problems (18 different products were recalled) (EFDA, 2018). Furthermore, maintaining the stability of pharmaceuticals that met quality standards when they left the factory might be problematic in tropical climates. Drug formulations which may be subjected to high temperatures combined with high humidity can have an adverse effect on their stability, as these conditions accelerate chemical breakdown (Kayumba *et al*., 2004; Risha *et al*., 2003, 2002).

Recently, it has been indicated that the regulatory capacity to effectively regulate and prevent SF medicines is insufficient in most LMICs, including Ethiopia (WHO, 2017b; Suleman *et al*., 2016). The length and porousness of Ethiopia's border with the Republic of Somalia make it difficult for the EFDA to adequately control and prohibit the circulation of SF medications in the Ethiopian market. With so many points of entry and the porous border, the illegal distribution network unfortunately merges seamlessly into the legal supply chain. Seizures of various illicit products, including illegal medicines, food products, and cosmetics, from cities in the Dire Dawa city administration, Harar, and Somali regions at various times indicate the possible availability of low-quality drugs in those localities (EFDA, 2021). SF drugs are therefore readily available in

the private marketplace and probably contribute to drug resistance. Thus, the ever-increasing evidence of drug quality problems justifies further investigation through large-scale studies of drug quality in all markets.

Therefore, the purpose of this study is to assess the quality of four commonly prescribed antibacterial drugs: amoxicillin capsules, amoxicillin plus clavulanic acid, ciprofloxacin, and norfloxacin tablet oral solid formulations circulating in selected locations of eastern Ethiopia, with the goal of detecting the existence of substandard and unsafe products thus contributing in public safety.

3.2. Significance of the study

The assessment of the physicochemical quality parameters of amoxicillin, amoxicillin + clavulanate, ciprofloxacin, and norfloxacin oral solid dosage formulations circulating in selected locations in Eastern Ethiopia will generate data indicating compliance or non-compliance with pharmacopoeial specifications.

The study's findings can provide a clue for therapeutic success or failure linked with the drugs studied since they can provide insight into the quality of these products circulating across the distribution chain and likely to be used by patients. Determining whether the quality of these drugs was affected by the origin, brand, and sample collection sites will help in the identification of manufacturers who are non-compliant with quality standards and identify locations that require regulatory focus.

4. Objectives

4.1. General objective

To evaluate the quality of selected antibacterial medicines collected from drug retail outlets with in selected locations of Eastern Ethiopia.

4.2. Specific objectives

The specific objectives of the study are:

- To perform visual inspection of samples and screen the samples with GPHF-minilab for identity and semi-quantitative determination.
- To carry out compendial tests in order to identify presence and extent of SF products
- To perform comparative dissolution analysis.

5. Methodology

5.1. Study area and study period

The research was carried out in two Eastern Ethiopia's major cities and one border town (Figure 5.1), which included Dire Dawa (Dire Dawa City Administration), Jijiga, and Togo-Wuchale (Somali Regional State). With a total size of 1288.02 km^2, Dire Dawa is situated 515 kilometers east of Addis Ababa, the capital of Ethiopia. The predominant climate features in the region are aridity, wind, and heat. According to the projections made based on the 2015 census, the total population of the administration is 383,529 – of whom 283,773 (74%) live in the urban part of the city. Jijiga is another significant city in eastern Ethiopia. According to data from the Central Statistics Agency (CSA) in 2015, Jijiga's population is thought to be 250,000. Togo-Wuchale is a border town in Somalia's regional state with a high potential for illicit drug trade. Samples were collected from March 16 to March 29, 2022.

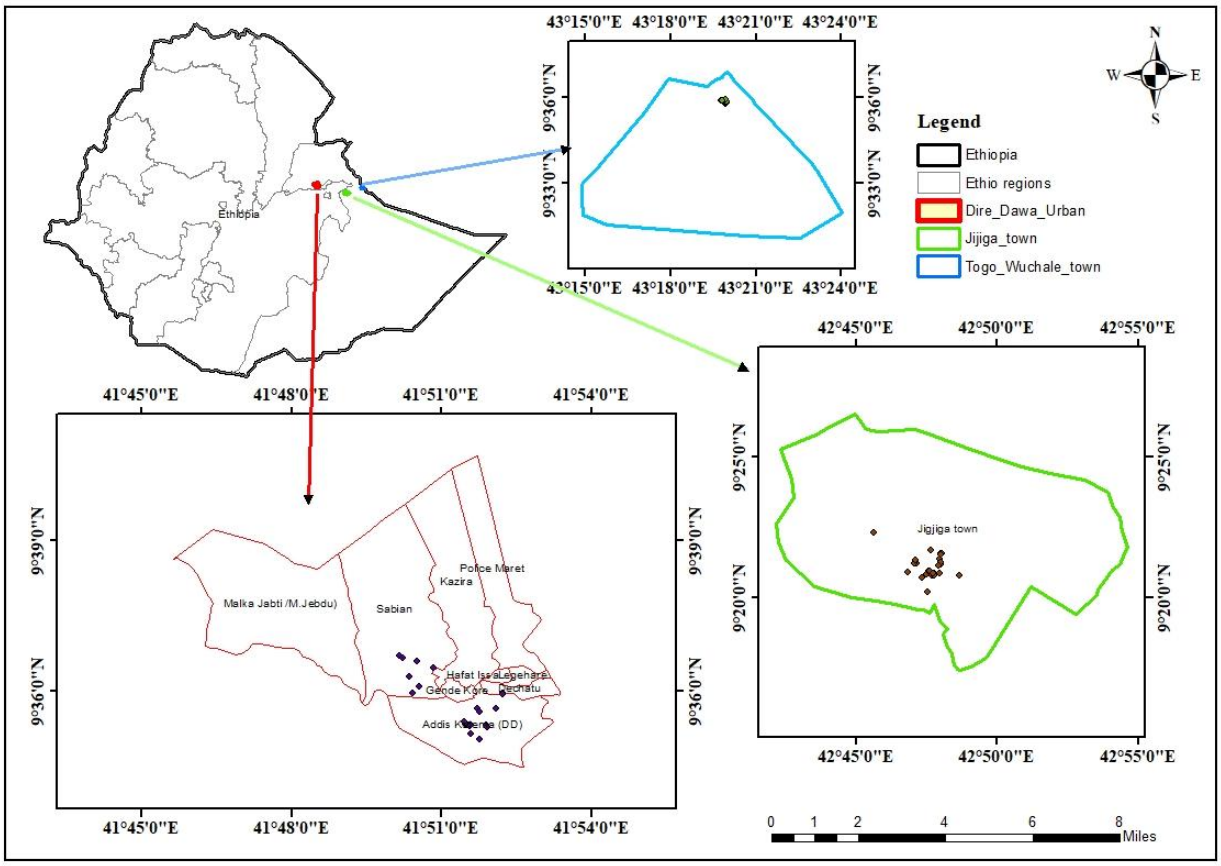


Figure 5.1: Map of the study area (OCHA, 2021).

5.2. Selection of antimicrobial drugs

The drugs were selected based on their relevance to public health, the likelihood of finding SF products, and the risk of antimicrobial resistance associated with the products. Based on reported country-wise consumption levels (Gutema *et al*., 2021) and reported highest risk of sub-standardization and falsification (Kelesidis & Falagas, 2015), the antimicrobial drugs amoxicillin and its combination with clavulanic acid, ciprofloxacin, norfloxacin, which were also listed in the national essential drugs, were selected for this study. Additionally, solid dosage formulations of the chosen antimicrobials for adults were examined in this study due to the highest reported frequency of SF antimicrobials linked to these dosage forms (Kelesidis & Falagas, 2015). Due to absence of baseline data on prevalence of SF antimicrobial drugs in the study area, considering available resources, predetermined 54 samples were collected from all of the locations included in the study. Based on reported country-wise consumption levels (Gutema *et al*., 2021) and reported highest risk of sub-standardization and falsification, (Kelesidis & Falagas, 2015) 40.74% of the samples were allocated for amoxicillin and 9.26% to its combination with clavulanic acid, 27.78% for ciprofloxacin and 22.22% for norfloxacin. Thus, a total of 54 oral solid preparations of amoxicillin 500 mg capsules (22), amoxicillin + clavulanic acid 625 mg (5), ciprofloxacin 500 mg (15), and norfloxacin 400 mg (12) tablets circulating in the markets of the selected study locations were investigated in this study.

5.3. Sampling

Samples were collected from drug retail outlets in the selected locations using the convenience sampling technique.

5.3.1. Types of sample collection sites

Sample collection was performed from drug retailers within the drug distribution chain in the selected locations, and the following sites were considered during sample collection: pharmacies, drug stores/shops, rural drug vendors (RDVs), public and private hospitals. These are the points in the drug supply chain where drugs are prepared for sale or dispensing and/or patient administration. This level of drug supply chain is not only vulnerable to SF medicines but also the degree of regulatory control lowers as a drug progresses through the supply chain where the number of stakeholders and transactions increases (WHO, 2017b).

5.3.2. Sampling design and mapping sample collection sites/areas

The locations for sample collection are mapped based on the potential for product smuggling and illegal border trade to take place in the sites, some of which have climatic conditions that affect the stability of medicines. The geographical locations that are considered to be major gateways for the unregulated entry of medicines into the country are prioritized. Within each region, the capital cities were sampled, as they are centers for drug distribution and could be representative for the entire region. In addition, the border town of Togo-Wuchale with neighboring country was considered. Convenience sampling strategies were employed for the selection of specific sampling facility from the types discussed in section 5.3.1. Private facilities near markets, where illegal drugs are very likely to be handled, and public health institutions that serve larger populations received more attention for sampling. Based on this, samples were collected from 39 private facilities and 4 public hospitals.

5.3.3. Sample

For this study's purpose, a sample was defined as the amount of a single product with the same batch number, brand, and manufacturer from each sampling facility that will allow conducting the planned pharmacopoeial tests.

5.3.4. Sample collection

The covert sampling (mystery shopping) technique was used in private and overt sampling technique was used in public facilities. From all of the products (branded or generic) available per facility, one randomly selected product was purchased covertly from the visited private facility. In covert sampling, a mystery shopper (principal investigator having training on the method) introduced himself as a practicing clinician owning a small clinic at small towns nearby the study areas and asked for all available brands of the target medicines and their price. Then, when the medicines are brought to display, maximum variation of manufacturers of the medicines were made to select medicines of different brands. This was not possible in public facilities where antimicrobials are not dispensed without prescription, thus, all brands of each product available in the facility were collected overtly, using a letter of cooperation issued from the department of pharmaceutical chemistry and pharmacognosy, School of pharmacy, Addis Ababa University. The identity and purpose of the buyer are not generally known by the outlet

being evaluated in a covert sampling technique, whereas they are explained in detail in an overt sampling strategy. Emphasis was given to different batch numbers, manufacturers, and countries of origin when moving from facility to facility to represent the quality of different products that are likely to be used by patients. In addition, unregistered products were given special attention (WHO, 2015). Each sample collected was provided with a code to ensure traceability, and for each sample, the sample collection form shown in Annex Ⅰ was completed.

5.4. Materials and methods

5.4.1. Chemicals and reagents

The following chemicals and reagents, supplied from EFDA QC directorate, were employed for the study: USP Amoxicillin trihydrate reference standards (Lot: R106H0 and LOK359, Spain and Lot: R05170, Germany), USP Ciprofloxacin RS (Lot: R12590, Germany), USP Clavulanic acid RS (Lot: R071W0, India) and USP Norfloxacin RS (Lot: R061R0, India), and USP Ciprofloxacin Ethylenediamine Analog RS (Lot: R013T0, India) were reference standards used for pharmacopoeial tests.

Amoxicillin 500 mg reference tablets (Lot No: 788230, GPHF, Germany), Clavulanic acid/Amoxicillin 125/500 mg reference tablets (Lot No: JY2838, GPHF, Germany), and Reference tablets containing an equivalent of 250 mg of Ciprofloxacin free base (Lot No: 80807V, GPHF, Germany), were authentic reference products used for screening tests.

HPLC grade acetonitrile (Fisher Scientific, U.K) and methanol (Fisher Scientific, U.K), analytical grade acetone (EMD Millipore corporation, Germany), toluene (EMD Millipore corporation, Germany), dichloromethane (Scharlab, Spain), phosphoric acid (Sigma-Aldrich, Switzerland), hydrochloric acid (37 %, ACS, ISO reagent grade, Scharlab, Spain), reagent grade glacial acetic acid (Sigma-Aldrich, Germany), potassium hydroxide pellets (Blulux Laboratories, India), sodium hydroxide pellets (Oxford Lab Fine Chem LLP, India), monobasic sodium phosphate (Sigma-Aldrich, Germany), potassium dihydrogen orthophosphate (Park scientific limited, U.K), ammonia (Scharlab, Spain), triethylamine (VWR chemicals, France), ethyl acetate (EMD Millipore corporation, Sweden), distilled water were reagents and solvents used in the analysis.

5.4.2. 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), ERWEKA disintegration test apparatus (Type: ZT 304, Germany), ERWEKA hardness, thickness and diameter tester (Type: TBH220, Germany) 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.

5.5. Methods

Quality of the samples was evaluated using a three-stage testing approach (WHO, 2015). The stages involved conducting visual inspection procedures, screening using GPHF Minilab, and full-scale compendial QC laboratory testing. In the first stage, all the collected samples were screened by visual inspection for compliance with the description of the dosage form, labeling, and packaging requirements. In this stage, samples were evaluated in connection with the compliance to the national requirements of the information accompanying products (on external and primary packaging, as well as in the package leaflet). In the second stage, samples were screened for identity and semiquantitative analysis for content using the GPHF-Minilab procedure (Jähnke & Dwornik, 2020). Finally, samples were assessed at full scale using compendial procedures for physicochemical quality parameters including hardness, weight variation, identification, assay, and dissolution. Except for the hardness, thickness, diameter, and disintegration tests, which were performed at the Ethiopian Pharmaceuticals Manufacturing Sh. Co. QC laboratory, all laboratory works were performed at the EFDA medicine QC laboratory at the head quarter, which is accredited for ISO-17025.

5.5.1. General requirements: appearance/description

Visual inspection: During visual inspection, at least 20 unpacked dosage units of each target medicine should meet the packaging and labeling requirements. The procedure was carried out according to the WHO checklist presented in Annex II.

5.5.2. Screening by GPHF- minilab®

All of the samples collected, except the norfloxacin samples for which the procedure was not available, were screened for quality with the Global Pharma Health Fund Minilab protocol for identity and a semiquantitative assay using a thin layer chromatographic method for initial quantification of the products (Jähnke & Dwornik, 2020). The screening test was conducted at the national quality control laboratory of the Ethiopian Food and Drug Authority. Merck TLC aluminium plates pre-coated with silica gel 60 F_{254} was a chromatoplate used for screening.

5.5.2.1. Verification of amoxicillin identity and content by thin-layer chromatography

Identity and semiquantitative determination of amoxicillin samples were performed according to the GPHF-Minilab® 2020 manual. Amoxicillin API was extracted from capsules with a known volume of diluted ammonia solution and determined by TLC with reference standard tablets containing 500 mg of Amoxicillin (Lot No: 788230) supplied with GPHF-Minilab®.

Preparation of the stock standard solution: One amoxicillin 500 mg reference tablet was wrapped in aluminum foil and crushed down to a fine powder using a pestle. The powder was then transferred to a 100 mL volumetric flask, and all leftover solid on the aluminum foil was gently washed down into the flask with 45 mL of water, followed by 5 mL of a 25% concentrated ammonia solution using the proper straight pipettes. The flask was then closed and sonicated until, after about three minutes, most of the solids were dissolved. After that, the solution was let to stand for five minutes to allow undissolved residues to settle below the supernatant liquid. For the next step, a clear or hazy supernatant liquid containing 10 mg/mL of amoxicillin stock standard solution was employed. This solution was freshly prepared for each test.

Preparation of the 100% working standard solution (upper working limit): One (1) mL of the stock standard solution was pipetted into a 10 mL vial and dissolved in 3 mL of methanol to obtain a final concentration of 2.5 mg/mL. This solution was used as the upper working limit to represent a product of good quality containing 100% of amoxicillin.

Preparation of 80% working standard solution (lower working limit): One (1) mL of the stock standard solution was pipetted into a 10 mL vial and dissolved in 4 mL of methanol to obtain a final concentration of 2 mg/mL. This solution was employed as the lower working limit to

represent a low-quality drug product that contained just 80% of the amoxicillin stated on the label.

Preparation of the stock sample solution: The powder obtained from one capsule from a product claiming to contain 500 mg of amoxicillin per unit was directly placed into a 100 mL volumetric flask, adding the cap and body shells last. The active ingredient was then extracted by adding 45 ml of water followed by 5 mL of ammonia solution (25%) into the flask using suitable graduated straight pipettes. To attain a complete dissolution of most of the solids, the flask was sonicated for about three minutes and allowed to stand for five minutes until undissolved residues settled below the supernatant liquid. The resultant clear or hazy supernatant liquid, containing 10 mg/mL of amoxicillin, was used as a stock sample solution.

Preparation of the 100% working sample solution: 1 mL of the stock sample solution was transferred into a 10 mL vial, and 3 mL of methanol was added to the solution. The vial was then closed and shaken well to obtain the expected concentration of 2.5 mg/mL amoxicillin in the working sample solution.

Preparation of the 80% working sample solution: 1 mL of the stock sample solution was transferred into a 10 mL vial, and 4 mL of methanol was added to the solution. The vial was then closed and shaken well to obtain the expected concentration of 2 mg/mL amoxicillin in the working sample solution.

Spotting: An origin line was marked parallel to and about 1.5 cm from the bottom edge of the chromatoplate, and 2 μL of each test and standard solution were applied side by side using the microcapillary pipettes. One chromatoplate was used for the screening of one sample, first spotting 100% working standard solution, followed by 100% sample solution, 80% working standard solution, and then 80% sample solution. After spotting, the uniformity of all spot diameters was checked with a UV light at 254 nm. The chromatoplate was held over the current of hot air just above the hot plate for about one minute to completely dry off all extraction solvent before development.

Development: 15 mL of ethyl acetate, 5 mL of 96% acetic acid solution, and 5 mL of water thoroughly mixed in the TLC developing chamber were used as the mobile phase. Filter papers were lined up against the chamber's walls and left to stand for 15 minutes to guarantee that the chamber was completely saturated with solvent vapor. The loaded chromatoplate was then placed carefully into the TLC developing chamber. The chamber was closed, and the chromatoplate developed until the solvent front had moved about three-quarters of the length of the plate. The TLC plate was taken from the chamber after development, and the solvent front was marked. After that, it was left to evaporate for about two minutes.

Detection: To identify and measure the amount of amoxicillin, the chromatoplate was subjected to UV light at a wavelength of 254 nm after being dried off completely.

Chromatogram reading: The travel distances of the principal spots of the drugs in the test and standard solutions were compared to identify the active ingredient in the sample solution. Samples having an identical travel distance to that of the principal spots of the standard are verified as having the active ingredient claimed on the label. Further, similarities in spot color, size, intensity, shape, and accompanying satellite spots are used as additional confirmation on the identity of the drug. After verification of drug identity, the spot sizes and intensities are further compared for a semi-quantitative reading in order to verify the label claim on drug content. Principal sample spots with spot sizes and intensities greater than a 100% standard spot and less than an 80% standard spot are determined to be noncompliant.

5.5.2.2. Screening of amoxicillin + clavulanic acid tablet products

5.5.2.2.1. *Verification of amoxicillin in amoxicillin/clavulanic acid tablets*

A similar procedure for screening amoxicillin in amoxicillin capsules (section 5.5.2.1) was used to verify the identity and content of amoxicillin in amoxicillin/clavulanate tablets.

5.5.2.2.2. *Verification of clavulanic acid identity and content by TLC*

Preparation of the stock standard solution: One reference tablet of Clavulanic acid/Amoxicillin 125/500 mg (Lot No.: JY2838, GPHF, Germany) was wrapped up in aluminum foil and crushed down into a fine powder using a pestle. The powder was then transferred into a 50 mL

volumetric flask, and the aluminum foil was washed down into the flask with 25 mL of distilled water. The flask was then closed and shaken for 3 minutes to dissolve most of the solids. The solution was then allowed to sit for 5 minutes to settle any undissolved residues below the supernatant liquid. This freshly prepared solution containing 5 mg/mL of clavulanic acid was used as a stock standard solution.

Preparation of the working standard solution 100% (Upper working limit): A 100% working standard solution of 1.25 mg/mL clavulanic acid was produced by diluting 1 mL of the stock standard solution with 3 mL of methanol in a volumetric flask of 10 mL.

Preparation of the working standard solution 80% (Lower working limit):1 mL of the stock standard solution was diluted with 4 mL of methanol in a 10 mL volumetric flask to produce an 80% working standard solution of 1 mg/mL clavulanic acid.

Preparation of the stock sample solution: One tablet of the sample wrapped in aluminum foil was crushed down to a fine powder using a pestle and transferred into a 50 mL volumetric flask. Then 25 mL of water was added to the flask and shaken for 3 minutes to extract the clavulanic acid. Then the solution was allowed to sit for 5 minutes to allow undissolved residues to settle below the supernatant. The resulting solution, containing 5 mg/mL of clavulanic acid, was used as the stock sample solution.

Preparation of the working sample solution 100% (Upper working limit): A 1 mL of the stock sample solution was diluted with 3 mL of methanol in a 10 mL volumetric flask to come up with a solution with 1.25 mg/mL clavulanic acid.

Preparation of the working sample solution 80% (Lower working limit): A 1 mL of the stock sample solution was diluted with 4 mL of methanol in a 10 mL volumetric flask to come up with a solution with 1 mg/mL clavulanic acid.

Spotting: From each of the test and standard solutions, 2 µL of the solutions were applied on the origin line, parallel to and about 1.5 cm from the bottom edge of the chromatoplate. Uniformity of all spots was checked under a UV light at 254 nm.

Development: The mobile phase used for development was composed of 15 mL ethyl acetate, 5 mL of 96% acetic acid solution, and 5 mL of water, which were thoroughly mixed in the developing chamber. The chamber's wall was then lined with filter paper and allowed to stand for 15 minutes to ensure saturation of the chamber with solvent vapor. Then, the loaded chromatoplate was developed for about 30 minutes, until the solvent front had moved up to three-quarters of the length of the plate. After removing the plate, the solvent front was immediately marked, and the plate was dried in order to remove the mobile phase before detection.

Detection: Using the battery-operated lamp included with the GPHF-minilab kit, the chromatoplate was examined under UV light at 254 nm and 366 nm after drying up all residual solvents.

5.5.2.3. Verification of ciprofloxacin identity and content by TLC

Ciprofloxacin and ciprofloxacin hydrochloride are extracted from tablets with a known volume of aqueous acetic acid solution and determined by TLC with reference to reference standard tablets containing an equivalent of 250 mg of ciprofloxacin free base supplied with minilab.

Preparation of the stalk standard solution: One reference tablet containing the equivalent of 250 mg of ciprofloxacin free base was wrapped up in aluminum foil and crushed down to a fine powder using a pestle. The powder was transferred into a volumetric flask with a 100 mL capacity, and all residual solids were washed down into the flask with 45 mL of water followed by 5 mL of acetic acid solution (96%). The flask was then closed and shaken for about 3 minutes, until most of the solids were dissolved. This solution was allowed to stand for 5 minutes until undissolved residues settled below the supernatant liquid. The resulting clear or hazy supernatant with 5 mg/mL ciprofloxacin was utilized as a stock standard solution.

Preparation of the working standard solution 100%: A 1 mL aliquot of the stock standard solution was diluted with 7 mL of methanol in a 10 mL volumetric flask to provide 0.625 mg/mL of ciprofloxacin upper limit (100%) working standard solution, which represents a medicinal product of good quality containing 100% of ciprofloxacin.

Preparation of the working standard solution 80%*:* A 1 mL aliquot of the stock standard solution was diluted with 9 mL of methanol in a 10 mL vial to provide a 0.5 mg/mL ciprofloxacin lower limit (80%) working standard solution, which represents just 80% of the amount of ciprofloxacin as stated on the product's label.

Preparation of the stock sample solution: One whole tablet of ciprofloxacin (500 mg) from each of the samples was wrapped up in aluminum foil and crushed down to a fine powder. All of the powder was transferred into a 25 mL volumetric flask, and ciprofloxacin was extracted by adding 90 mL of water followed by 10 mL of 96% acetic acid solution and shaking for about 3 minutes to dissolve most of the solids. This solution was left to stand for an additional 5 minutes until undissolved residues settled below the supernatant liquid. The resulting supernatant liquid, containing 5 mg/mL, was used as a stock sample solution. This solution was prepared freshly for each test.

Preparation of the 100% working sample solution: 1 mL of the stock sample solution was diluted with 7 mL of methanol in a 10 mL vial to obtain an expected 100% working sample solution containing 0.625 mg/mL of ciprofloxacin, which corresponds to the concentration of ciprofloxacin in the higher working standard solution.

Preparation of the 80% working sample solution: 1 mL of the stock sample solution was diluted with 9 mL of methanol in a 10 mL vial to obtain an expected 80% working sample solution containing 0.5 mg/mL of ciprofloxacin, which corresponds to the concentration of ciprofloxacin in the lower working standard solution.

Spotting: From each of the test and standard solutions, 2 µL of the solutions were applied on the origin line, parallel to and about 1.5 cm from the bottom edge of the chromatoplate. The uniformity of all spots was checked under UV light at 254 nm. Then all of the extraction solvents were dried off until no smell of acetic acid remained on the plate before chromatoplate development.

Development: 10 mL of methanol, 5 mL of acetone, 2.5 mL of toluene, and 5 mL of concentrated ammonia solution (25%) were thoroughly mixed in the jar being used as the TLC

developing chamber employed as the mobile phase. The wall of the chamber was lined with filter paper and allowed to stand for about 15 minutes, thus ensuring saturation of the chamber with solvent vapor. Then the spotted TLC plate was carefully placed into the jar and allowed to develop until the solvent front had moved at least three-quarters of the length of the plate. The solvent front was marked soon after withdrawing the plate from the chamber, and any excess solvent was allowed to evaporate before detection.

Detection: The chromatoplate was observed under UV light at 254 nm and 366 nm in a low- or no-light working environment for identification and quantification purposes.

5.5.3. Procedure for identification test

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 (BP, 2021; USP 44-NF 39, 2021).

5.5.4. Determination of uniformity of dosage units

Uniformity of dosage units was performed according to the procedure for weight variation in USP 44–NF 39 chapter <905> by accurately weighing 10 tablets individually (USP 44-NF 39, 2021). Then the expected percentage content of each dosage unit was calculated from the mean weight of 10 dosage units, the individual weight of each dosage unit, and the assay value. Then the acceptance value (AV) for each sample was calculated using equation 5.1:

$$AV = |M-\bar{X}| + KS \qquad \text{Equation 5.1}$$

Where, M is reference value, $\bar{X}$ average percentage content of 10 tablets, K Acceptability constant and, S – is sample standard deviation. According to USP, the calculated acceptance value for 10 dosage units of a sample should be less than or equal to L1% (15) to comply with the requirements for dosage form uniformity.

5.5.5. Procedure for testing hardness/breaking force, thickness and diameter

These procedures were performed for tablet formulations in accordance with the method in USP/NF. Ten tablets from each sample were tested for hardness by orienting each tablet

diametrically with respect to the direction of the application of force. The mean hardness was then determined by dividing the total hardness by the number of tablets (USP 44-NF 39, 2021).

5.5.6. Procedure for disintegration test

The test for disintegration was carried out according to the USP method for disintegration (USP 44-NF 39, 2021). 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 except for the soft mass of capsule shells with no pulpable firm core in the basket was recorded as the disintegration time.

5.5.7. Procedure for determination of content of API

5.5.7.1. Assay of amoxicillin capsules

An assay test for amoxicillin was conducted using the method mentioned in the USP monograph (USP, 2021a).

Preparation of the mobile phase: The mobile phase for the analysis of amoxicillin capsules consisted of acetonitrile and phosphate buffer. The buffer was prepared by dissolving 6.8 g of monobasic potassium phosphate salt in 1 L of distilled water and adjusted to pH 5.0 ± 0.1 using 45% w/v potassium hydroxide solution. The mobile phase was then prepared by mixing 1:24 v/v of acetonitrile and buffer. This solution was filtered *in vacuo* through a 0.45 µm nylon membrane filter and sonicated for about 20 minutes.

Preparation of the standard solution: Accurately weighed 24.0 mg of amoxicillin RS was dissolved in phosphate buffer in 20 mL volumetric flask and made up to mark to obtain 1.2 mg/mL solution.

Preparation of the sample solution: An equivalent of 200 mg of anhydrous amoxicillin from the combined contents of 20 capsules was transferred to 200 mL volumetric flask and dissolved and made up to mark with phosphate buffer. This solution was then transferred to 1.5 mL amber-

colored HPLC vials after being filtered through a 0.45 µm polytetrafluoroethylene (PTFE) syringe filter.

Chromatographic condition: reverse-phase HPLC equipped with a UV detector at 230 nm was used for the analysis. A Waters Spherisorb ODS2 C-18 column with 5 µm particle size, 250 mm length, and 4.6 mm internal diameter was used for analysis. The system was run at a flow rate of 1.5 mL/minute with an injection volume of 10 µL.

System Suitability solution: The standard solution was used to check the suitability of the system.

Analysis: The percentage content of amoxicillin in the unknown samples was calculated using equation 5.2:

$$\%Content = (r_u/r_s) * (C_S/C_U) * P * F * 100 \quad \text{Equation 5.2}$$

Where, r_u - peak area of sample solution; r_s - peak area of standard solution; C_S - concentration of amoxicillin RS in the standard solution (mg/mL); C_U - nominal concentration of amoxicillin in sample solution (mg/mL); P - potency of amoxicillin in amoxicillin RS (µg/mg); and F - conversion factor, 0.001 mg/µg. Acceptance criteria: 90.0%–120.0%

5.5.7.2. Assay test for amoxicillin + clavulanic acid

An assay test for amoxicillin plus clavulanic acid tablets was performed using the method mentioned in the USP monograph (USP, 2021b).

Preparation of the Mobile Phase: The mobile phase for the analysis of amoxicillin plus clavulanic acid tablets consisted of a phosphate buffer of pH 4.4 ± 0.1 and methanol in a composition of 19:1 v/v. The buffer was prepared by dissolving 7.8 g of monobasic sodium phosphate in 900 mL of distilled water. The buffer was then adjusted to pH 4.4 ± 0.1 using phosphoric acid, and the volume was made up to 1000 mL using distilled water. Then a 1:19 v/v composition was prepared by mixing methanol with buffer. The resultant solution was then filtered *in vacuo* through a 0.45 µm nylon membrane filter and sonicated for about 20 minutes.

Preparation of the standard solution: Accurately weighed 25.0 and 10.0 mg of amoxicillin RS and clavulanate lithium RS, respectively, mixed in a 50 mL volumetric flask were dissolved and made up to mark with distilled water to obtain 0.2 mg/mL of clavulanate lithium and 0.5 mg/mL of amoxicillin.

Preparation of sample solution: 10 tablets of a sample in a 2000 mL volumetric flask was dissolved in and made up to the mark with distilled water. 5 mL of this solution was diluted to 25 mL using distilled water.

Chromatographic condition: reverse-phase HPLC equipped with a UV detector at 220 nm was used for the analysis. A Waters Spherisorb ODS2 C-18 column with 5 µm particle size, 250 mm length, and 4.6 mm internal diameter was used for analysis. The system was run at a flow rate of 1 mL/minute with the injection volume of 20 µL.

System suitability test: Standard solution was used for system suitability test and the resolution between the amoxicillin and clavulanic acid peaks should be NLT 3.5, the tailing factor should be NMT 1.5 for each analyte peak, and the relative standard deviation should be NMT 2.0%.

The concentrations in the unknown samples were then calculated by using the area under the curve of each of the sample and standard preparations and the concentration of the standard solution using equation 5.3:

$$\%Content = (r_u/r_s) * (C_S/C_U) * P * F * 100 \qquad \text{Equation 5.3}$$

Where, r_u = peak response of sample solution; r_s - peak response of standard solution; C_S - concentration of amoxicillin RS in the Standard solution (mg/mL); C_U - nominal concentration of amoxicillin in sample solution (mg/mL); P - potency of amoxicillin in amoxicillin RS (µg/mg); and F - conversion factor, 0.001 mg/µg. Acceptance criteria: 90.0%–120.0%.
And the percentage content of clavulanic acid was calculated using equation 5.4:

$$\%Content = (r_U/r_S) * (C_S/C_U) * P * 100 \qquad \text{Equation 5.4}$$

Where, r_u is peak area of clavulanic acid of sample solution; r_s is peak area of clavulanic acid of standard solution; C_S is concentration of clavulanate lithium RS in the standard solution (mg/mL); C_U is nominal concentration of clavulanic acid in the sample solution (mg/mL); and P is potency of clavulanic acid in clavulanate lithium RS (mg/mg). Acceptance criteria: 90.0%–120.0%.

5.5.7.3. Assay test for ciprofloxacin

An assay test for ciprofloxacin was conducted using the method mentioned in the USP monograph (USP, 2021c).

Preparation of mobile phase: The mobile phase was made up of acetonitrile mixed in a 13:87 v/v ratio with a 0.025 M phosphoric acid solution that had previously been adjusted to a pH of 3.0 ± 0.1 with triethylamine. The resulting solution was filtered *in vacuo* through a 0.45 µm nylon filter and sonicated for 20 minutes.

Standard solution preparation: Accurately weighed 20.0 mg of the ciprofloxacin reference standard (RS) was dissolved in a 100 mL volumetric flask using the solvent prepared in the following manner: A solution of 0.025 M phosphoric acid, adjusted to a pH of 2.0 ± 0.1 using triethylamine, was mixed with acetonitrile in a ratio of 87:13. The volume was then brought up to the mark with the same solution to generate a ciprofloxacin solution containing 0.2 mg/mL.

System suitability solution: A 0.05 mg/mL ciprofloxacin ethylenediamine analog solution and the standard solution were used to check the suitability of the system. The requirements are the resolution between the ciprofloxacin ethylenediamine analog and ciprofloxacin should be NLT 6, tailing factor should be NMT 2.0, and relative standard deviation should be NMT 1.5%.

Sample preparation: 5 tablets of a sample were dissolved in 500 mL volumetric flask and made up to volume with the same diluent used to make the standard solution, and filtered through a 0.45 µm syringe filter to obtain a stock solution of 5 mg/mL. 2 mL of this solution was then diluted to 50 mL using the same diluent to obtain a 0.2 mg/mL ciprofloxacin solution.

Chromatographic condition: reverse-phase HPLC equipped with a UV detector at 278 nm was used for the analysis. Agilent's stainless-steel C-18 column with 5 µm particle size, 250 mm

length, and 4.6 mm internal diameter was used for analysis. The column temperature was set to 30 ^{o}C, and the system was run at a flow rate of 1.5 mL/minute with an injection volume of 10 µL.

The percentage content of ciprofloxacin in the unknown samples was then calculated by using the area under the curve of each of the sample and standard preparations and the concentration of the standard solution using equation 5.5:

$$\%Content = (r_U/r_S) * (C_S/C_U) * (M_{r1}/M_{r2}) * 100 \qquad \text{Equation 5.5}$$

Where, r_u = peak area of sample solution, r_s = peak area of standard solution, C_S = concentration of ciprofloxacin HCl RS in the standard solution (mg/mL), C_U = nominal concentration of ciprofloxacin in sample solution (mg/mL), M_{r1} = molecular weight of ciprofloxacin, 331.34, and M_{r2} = molecular weight of ciprofloxacin hydrochloride, 367.81. Acceptance criteria: 90.0% – 110.0%.

5.5.7.4. Assay test for norfloxacin

An assay test for norfloxacin was conducted using the method mentioned in the BP monograph (BP, 2021).

Preparation of the mobile phase: The mobile was prepared by mixing 150 volumes of acetonitrile with 850 volumes of 0.1% w/v orthophosphoric acid. This solution was filtered in *vacuo* through a 0.45 µm nylon membrane filter and sonicated for 20 minutes.

Chromatographic condition: an ACE C-18, 5 µm 150 x 3.9 mm column was preconditioned at a flow rate of 1.5 mL/minute for 8 hours using a 0.01M solution of anhydrous sodium dihydrogen orthophosphate previously adjusted to a pH of 4.0 with orthophosphoric acid. Before starting the chromatography, the column was equilibrated with the mobile phase for about 30 minutes.

Standard solution preparation: An accurately weighed 20.97 mg of norfloxacin RS, previously dried under vacuum at a pressure of 5 mm Hg at 100 ^{o}C, was dissolved in a 100 mL volumetric

flask using the mobile phase to obtain a standard solution of 0.2097 mg/mL norfloxacin. This solution, prepared in duplicate, was filtered through a 0.45 μm syringe PTFE membrane filter.

Preparation of the sample solution: An equivalent of 0.1 g of norfloxacin taken from homogenized powder of 20 tablets was dissolved in a 200 mL volumetric flask and made up to the mark with the mobile phase. Then 10 mL of this solution was diluted to 25 mL with the mobile phase to produce a 0.2 mg/mL working sample solution.

System suitability requirement: The symmetry factor of the principal peak should be less than 2.0 and the relative standard deviation of the area of the principal peak of six injections should not be more than 2.0%.

Analysis: the percentage content of the labeled amount of norfloxacin in the portion of tablet taken was calculated using equation 5.6:

$$\%Content = (r_u/r_s) * (C_S/C_U) * P * F * 100 \qquad \text{Equation 5.6}$$

Where, r_u – peak area of norfloxacin RS of sample solution, r_s -peak area of norfloxacin from the sample solution, C_S -concentration of norfloxacin RS in the standard solution (mg/mL), C_U - nominal concentration of norfloxacin in the sample solution (mg/mL), P- potency of norfloxacin in norfloxacin RS (μg/mg), and F- conversion factor, 0.001 mg/μg. Acceptance criteria: 95.0 - 105.0% of the labeled amount.

5.5.8. Dissolution test

For the dissolution test, only those samples that passed the assay test were subjected to the dissolution test, because products that did not contain acceptable quantity of the API will not release acceptable quantity of the API in dissolution testing. Therefore, a total of 48 samples of different products were tested for dissolution using current pharmacopoeial methods.

5.5.8.1. Dissolution profile test for amoxicillin capsules

A dissolution test was performed according to the method prescribed in the USP monograph using dissolution test equipment with a rotating paddle (USP apparatus 2) (USP, 2021a). The test was performed using 900 mL of distilled water at 37 ^{o}C, the paddle operating at 75 rpm. 5 mL of

the sample withdrawn at 5, 15, 30, 45, 60, and 75 minutes was replaced for each withdrawal. Then, the prefiltered samples through 0.45 μm syringe filters were diluted (3 mL to 10 mL) with distilled water and analyzed using a UV-visible spectrophotometer at 272 nm in a 1 cm^2 cell. The calibration curve equation was used to determine the concentration of amoxicillin in the unknown sample solutions.

Calibration standard preparation

Using a 50 mL volumetric flask, a stock standard solution was made by dissolving 28.05 mg of amoxicillin reference standard in 40 mL of distilled water and made up to the mark to obtain a solution containing 0.56 mg/mL of amoxicillin. From this solution, a series of six concentration levels (0.112, 0.140, 0.168, 0.196, 0.224, and 0.252 mg/mL) were prepared by diluting 2, 2.5, 3, 3.5, 4, and 4.5-mL aliquots of the stock standard solution to 10 mL with distilled water. The absorbances of these solutions were measured at 272 nm to generate a calibration curve.

5.5.8.2. Dissolution test for amoxicillin + clavulanic acid tablets

A dissolution test was performed according to the method prescribed in the USP monograph using dissolution test equipment with a rotating paddle (USP apparatus 2) at 75 rpm, 900 mL distilled water at 37 ^{o}C (USP, 2021b). A reference thermometer was used to monitor the temperature before and after the test. Then, 5 mL of sample withdrawn at 5, 10, 15, 20, 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.

System suitability solution: Accurately weighed 25.0 mg of RS amoxicillin and 10.0 mg RS clavulanate lithium were mixed in a 50 mL volumetric flask and dissolved using distilled water to make up the volume. This freshly prepared solution was then filtered through 0.45 μm PTFE syringe filters and placed in amber-colored HPLC vials for analysis.

Preparation of calibration standards: Stock standard solutions of amoxicillin and clavulanic acid were prepared by accurately weighing 40.0 and 50.0 mg reference standards, respectively. The standards for amoxicillin and clavulanic acid were dissolved in a 20 mL volumetric flask and a 50 mL volumetric flask using distilled water as a solvent to obtain 2 mg/mL and 1 mg/mL

solutions, respectively. Eight levels of calibration standard solutions of amoxicillin were prepared by taking 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4-mL aliquots of the stock solution and diluting to 10 mL with distilled water to obtain 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mg/mL of amoxicillin working standard solution. While seven levels of calibration standard solutions of clavulanic acid were prepared by taking 1.5, 3, 4.5, 6.0, 7.5, 9.0, and 10.5-mL aliquots of the stock solution and diluting to 50 mL with distilled water to obtain a final concentration of 0.03, 0.06, 0.09, 0.12, 0.15, 0.18, and 0.21 mg/mL solutions. All of the above solutions were filtered through 0.45 μm syringe filters and placed in amber-colored HPLC vials for analysis.

Mobile phase preparation: The mobile phase consisted of methanol and a phosphate buffer of pH 4.4 ± 0.1. The buffer was prepared by dissolving 7.8 g of monobasic sodium phosphate in 900 ml of distilled water and adjusted to a pH of 4.4 ± 0.1 using phosphoric acid. Then the mobile phase was prepared by mixing methanol and buffer in a ratio of 1 to 19 (1:19).

Analysis: Each of the calibration standards and samples were injected in duplicate for quantification. The mean areas under the curve recorded for each sample were used for quantification of the unknowns using the calibration equation generated previously.

5.5.8.3. Dissolution test for ciprofloxacin tablets

The dissolution test for ciprofloxacin 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 0.01N hydrochloric acid at 37 ± 0.5 °C (USP, 2021c). A reference thermometer was used to monitor the temperature before and after the test. Six tablets from one sample were placed in six of the dissolution vessels. Then, 5 mL of sample withdrawn at 5, 10, 15, 20, 30, and 45 minutes were replaced for each withdrawal. The samples prefiltered through 0.45 μm syringe filters diluted (2 mL to 200 mL) and were analyzed with a UV-visible spectrophotometer set at 276 nm using 1 cm^2 cells. The calibration curve equation was used to determine the concentration of ciprofloxacin in the unknown sample solutions.

Calibration standard solution: Stock standard solution was prepared by dissolving 20.00 mg of pure ciprofloxacin reference standard in 200 mL volumetric flask, using 180 mL of 0.01N HCl

solution and to make up to the mark to obtain 100 µg/mL. From this stock solution, a series of five concentration levels (2, 3, 4, 5, and 6 µg/mL) were prepared by diluting a 4, 6, 8, 10, and 12 -mL aliquot of the stock standard solution to 200 mL with 0.01N HCl in a 200 mL volumetric flask. The absorbances of these solutions were measured at 276 nm to generate a calibration curve.

5.5.8.4. Dissolution test for norfloxacin tablets

The dissolution test for norfloxacin was performed according to the method prescribed in the BP monograph using dissolution test equipment with a rotating paddle at 50 rpm, 750 mL, pH 4.0 acetate buffer at 37 ^{o}C (BP, 2021). A reference thermometer was used to monitor the temperature before and after the test. The 5 mL samples taken at 5, 10, 15, 20, 30, 35, and 45 minutes were replaced with an equal volume of fresh medium at 37 ± 0.5 ^{o}C. The samples filtered through 0.45 µm syringe filters were diluted (3 mL to 100 mL) using buffer solution and analyzed in 1 cm^2 glass cuvettes by a UV-visible spectrophotometer at 313 nm. The calibration curve equation was utilized to calculate the amount of norfloxacin in the unknown sample solutions.

Calibration Standard preparation

In a 100 mL volumetric flask, a stock standard solution was made by dissolving 16.00 mg of pure USP Norfloxacin reference standard in 90 mL of pH 4.0 acetate buffer. This was sonicated for about 5 minutes to ensure complete dissolution and made up to the mark with the buffer to give a concentration of 0.16 mg/mL. From this solution, a series of seven concentration levels covering 20% to 140% of target concentration (100%) were prepared by diluting 0.5, 1, 1.5, 2.0, 2.5, 3.0, and 3.5 mL aliquots of stock solution to 25 mL with the medium to obtain a concentration of 0.0032, 0.0064, 0.0096, 0.0128, 0.016, 0.196, and 0.0224 mg/mL, respectively. The absorbances of these solutions were taken at 313 nm to generate a calibration curve.

5.5.8.5. Comparison of dissolution profiles

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

5.5.8.5.1. Selection of comparator product

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. 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, Amoxapen 500 mg (ACD015—one of the samples), Augmentin 625 mg, CiproBay 500 mg, and Trizolin 400 mg (NTD052—one of the samples) were selected as a comparator product for respective multisource products based on the selection criteria.

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 pharmacopoeial QC media; hence, six dosage units of the reference and test products were tested in pharmacopoeial 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).

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, Makoid-Banakar, and Weibull models were among the model-dependent methods employed for profile comparison. The dissolution data were analyzed using the DDSolver® software (China Pharmaceutical University, China), 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).

5.6. Data Analysis

DDSolver® software (China Pharmaceutical University, China) and Microsoft Excel 2019 programs (Microsoft corporation, Seattle, USA) were used for the analysis of experimental data.

5.7. Ethical Consideration

Ethical approval for this study was obtained from the Ethics Committee of the School of Pharmacy, College Health Sciences, Addis Ababa University with reference number ERB/SOP/360/2021 dated September 29, 2021 (Annex Ⅷ).

6. Results and Discussion

6.1. Sample information, location, and frequency

Samples were collected from 43 facilities from Dire Dawa, Jijiga, and Togo-Wuchale. The distribution of facilities and collection sites is presented in Figure 6.1. From the total of the facilities, 41.90% were from the Dire Dawa city administration, 34.90% from Jijiga city, and 23.20% from Togo-Wuchale town. In general, 9.3% (a total of 4; one from Dire Dawa and 3 from Jijiga) were public facilities, and 90.7% (39) were private facilities. 2.56% (1 hospital-based pharmacy, from Dire Dawa), 48.72% (19 community pharmacies), 46.15% (15 drug shops), and 2.56% (1 rural drug vendor, from Togo-Wuchale) were private facilities. 57.89% of the community pharmacies were from Dire Dawa, 36.84% from Jijiga, and 5.26% from Togo-Wuchale, while drug shops consisted of 27.78% from Dire Dawa, 27.78% from Jijiga, and 44.44% from Togo-Wuchale town. Detailed information on samples collected is presented in Annex III.

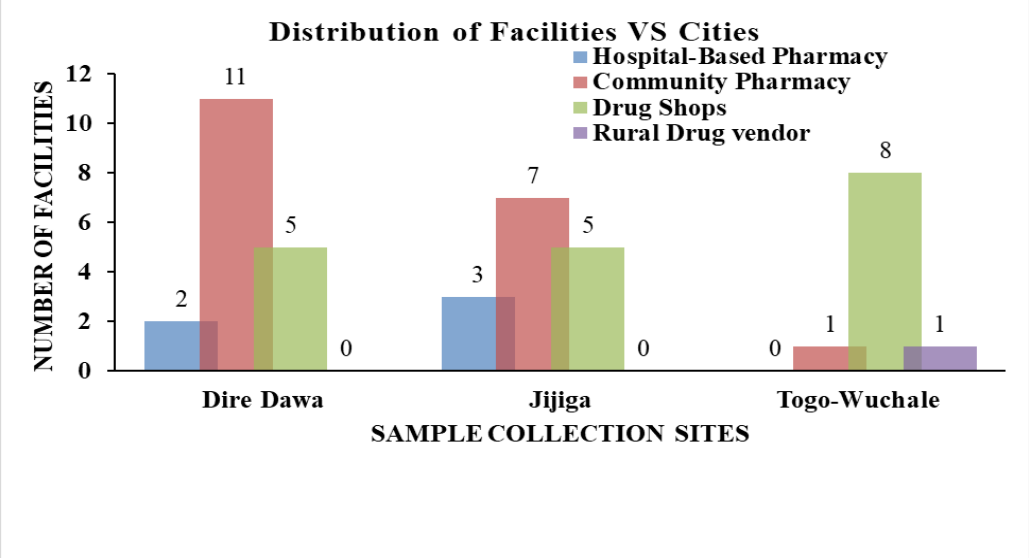


Figure 6.1: Distribution of sampling facilities in each of the sampling locations.

A total of 54 samples composed of four product types were collected from three selected sites. The distribution of number and types of products versus collection area is presented in Figure 6.2. From the total, 42.59 % of the samples (amoxicillin = 9; amoxicillin + clavulanic acid = 2; ciprofloxacin = 6; and norfloxacin = 6) were collected from Dire Dawa; 38.89 % of the samples (amoxicillin = 9; amoxicillin + clavulanic acid = 2; ciprofloxacin = 6; and norfloxacin = 4) were

collected from Jijiga; and 18.52 % of the samples (amoxicillin = 4; amoxicillin + clavulanic acid = 1; ciprofloxacin = 3; and norfloxacin = 2) were collected from Togo-Wuchale.

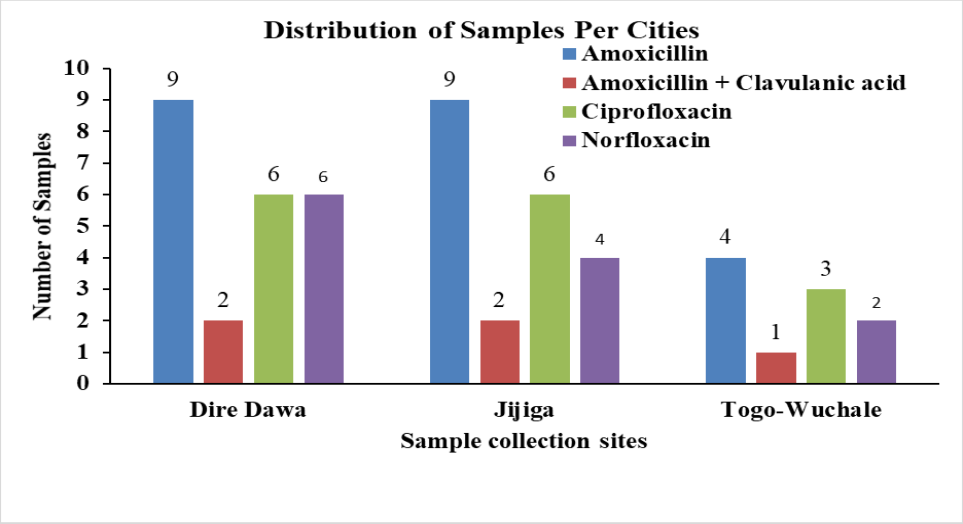


Figure 6.2: Distribution of samples in each sampling locations.

In terms of dosage form and strength, all the samples were solid oral dosage formulations consisting of amoxicillin 500 mg capsule and film coated tablets of amoxicillin/clavulanic acid 625 mg, ciprofloxacin 500 mg, and norfloxacin 400 mg. In terms of the stated country of origin on the label, 16.67% were locally manufactured products, while 33.33% were from China, 29.63% from India, 9.26% from Cyprus, 5.56% from Turkey, 1.85% from Kenya, 1.85% from Austria, and 1.85% did not have a label specifying their country of origin and storage condition. The distribution of samples and the country of origin claimed on the label are presented in Figure 6.3. Additionally, 14.81% (8/54) of the samples were not registered in the EFDA electronic medicine registration database to be marketed in the local market, all of which were collected from private facilities.

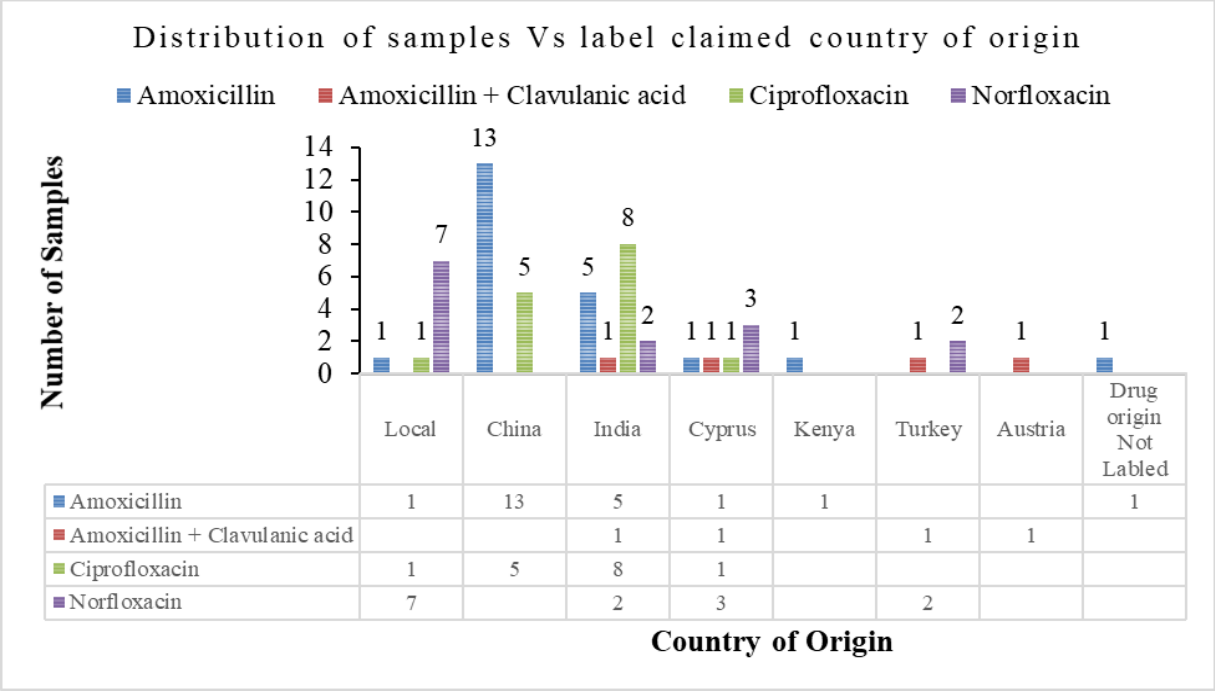


Figure 6.3: Distribution of countries of origin versus samples.

6.2. Visual inspection

Visual inspection revealed that all the samples met the labeling and physical appearance requirements, except one sample of amoxicillin 500 mg capsule (Sample ACW010) which was without full address of the manufacturer and no storage condition specified on the label. The visual inspection of the product and its packaging, often promoted as a first step in the detection of SF drugs, provides qualitative data on the quality and identity of the drug. Visual inspection of the samples showed no sign of falsification. However, the low level of detection of products that do not meet requirement for physical appearance, packaging and labeling may be due to the challenge associated with visual inspection process (Sherma, 2007). Similar outcomes have been reported in a study where visual assessment identified only a single falsified product out of 503 medicine samples (Schäfermann *et al*., 2020).

6.3. Screening with GPHF- minilab®

An identification test using the GPHF-minilab procedure had shown that all 42 samples, comprised of 22 amoxicillin, 5 amoxicillin/clavulanate, and 15 ciprofloxacin, contained the indicated API(s). Semi-quantitative determination of the amount of APIs in the products using

the GPHF-minilab TLC procedure revealed that six samples were observed to have either above or below the required quantity of the indicated APIs. Among the 22 samples of amoxicillin 500 mg capsules screened, 5 samples (ACJ007, ACJ008, ACW011, ACD013, and ACD017, were non-registered and imported products) had spot intensities lower than that of the 80% standard spots, indicating a lower quantity of amoxicillin API in these samples. 17 of the amoxicillin samples were found to comply with the current GPHF-minilab requirement for quantity. Except for sample ACTD026, all the samples of amoxicillin/clavulanic acid passed the screening test for quantity for both active ingredients. The spot for 100% sample solution for the sample ACTD026 was observed to be more intense than the spot for the 100% standard solution, and hence was suspect. All 15 samples of ciprofloxacin screened were observed to have comparable travel distance, intensities and color of the spots with those of the authentic reference product; hence, no suspect product of ciprofloxacin was found.

TLC chromatograms were routinely captured on camera during the analysis, and typical chromatograms are shown in Figure 6.4. In all of the chromatograms shown in the figure, the first and third spots are for 100% and 80% standard solutions, respectively, while the second and fourth spots are for 100% and 80% sample solutions, respectively.

Figure 6.4 (a), the chromatogram for amoxicillin (sample ACJ006), shows that the first spot observed at a retardation factor (R_f) value of 0.42 is for 100% amoxicillin standard, the second spot is for 100% amoxicillin sample spot, followed by 80% standard and sample solution, confirming the presence of amoxicillin in the samples. Also, the spots for 100% standard and sample solution and those for 80% standard and sample solution have comparable color, size, and intensity with each other, indicating that the sample is of an acceptable quality and quantity.

A representative chromatogram for screening amoxicillin/clavulanic acid tablet (sample ACTJ024) was presented in Figure 6.4 (b) for amoxicillin at 254 nm, (c) for clavulanic acid at 254 nm, and (d) for clavulanic acid at 366 nm. As it can be observed from Figure 6.4 (b) and (c), only the spots for amoxicillin were visible under UV light at 254 nm, and clavulanic acid was invisible at 254 nm. However, clavulanic acid becomes visible at 366 nm, where it appears as

bluish-white spots above the spots of amoxicillin. As it can be observed, all of the spots in the chromatograms shown have a similar R_f value and have comparable spot intensity.

As shown in Figure 6.4 (e) and (f), the chromatogram for ciprofloxacin (sample CTD037), a blue-violet principal spot under UV light at 254 nm indicates the presence of ciprofloxacin in the test solution. And when exposing the chromatogram to a UV light at 366 nm in the dark, all ciprofloxacin spots observed at 254 nm gave a bluish-white fluorescence. The ciprofloxacin spot in the chromatogram obtained with the test solution (100% and 80%) was comparable in terms of color, size, intensity, shape, and R_f value to that in the chromatogram obtained with the lower and higher standard solutions. Thus, the sample contain ciprofloxacin with an acceptable quantity. Figure 6.4 (g) shows a typical chromatogram for checking spot uniformity before development.

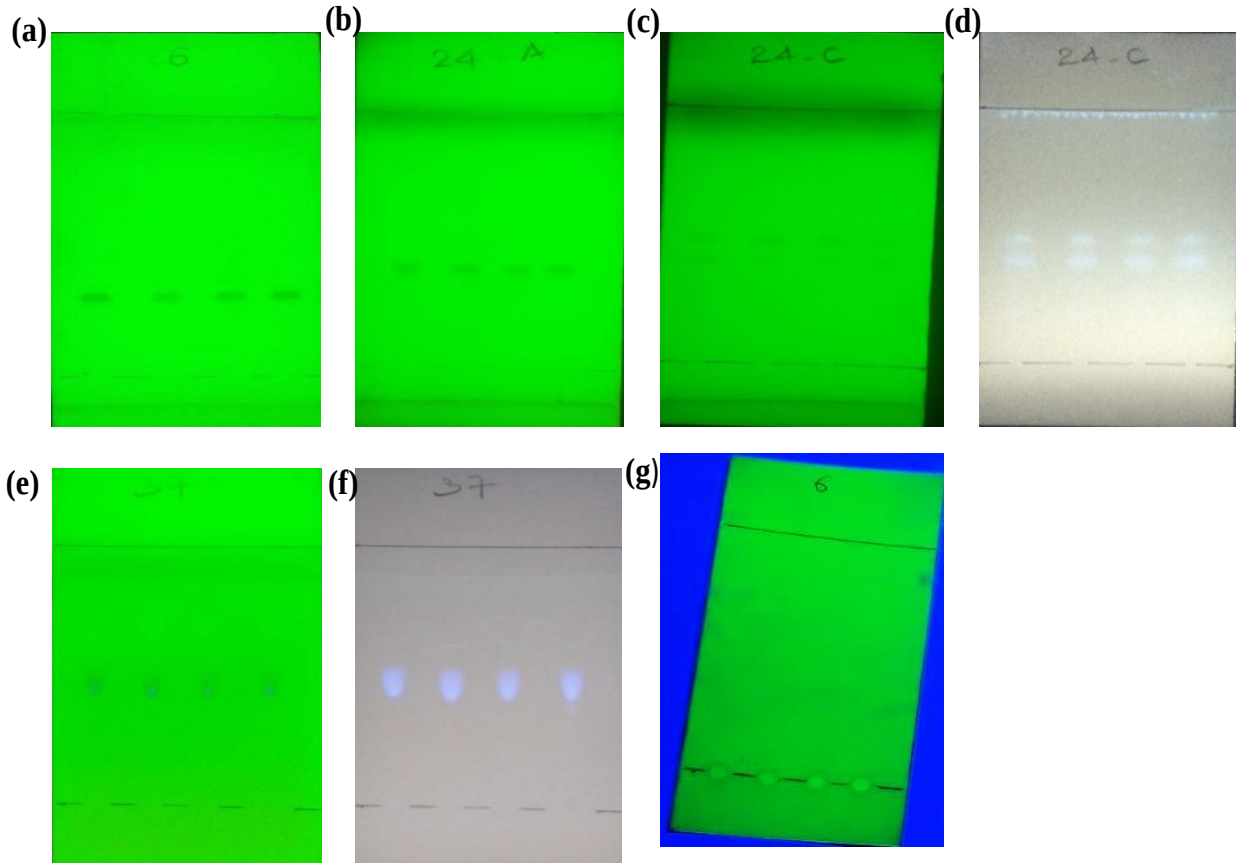


Figure 6.4: Representative TLC chromatograms of (a) amoxicillin at 254 nm from single dose product; (b) amoxicillin at 254 nm from combination product; (c) clavulanic acid at 254 nm; (d) clavulanic acid at 366 nm; (e) ciprofloxacin at 254 nm; (f) ciprofloxacin at 366 nm; and (g) checking uniformity of spotting.

6.4. Compendial Tests

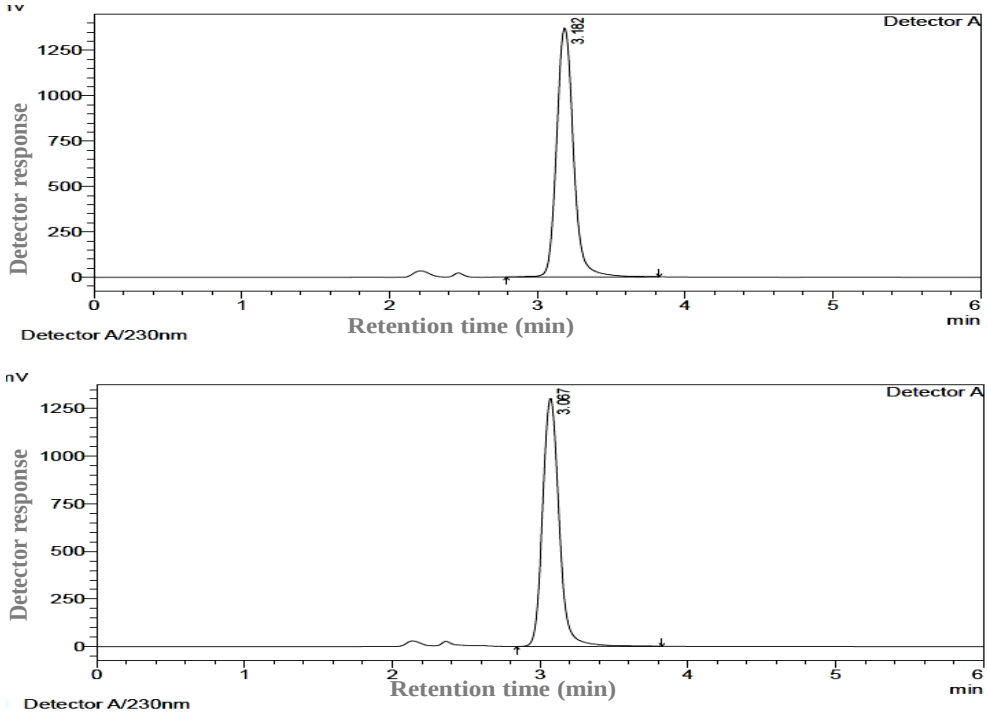
6.4.1. Identification

The result for identity test using high performance liquid chromatography showed that none of the samples failed to comply with the requirement for identity, so no falsified product was detected in this study.

6.4.1.1. Identification of amoxicillin capsules

The retention time of amoxicillin in the sample solution was compared with the retention time for amoxicillin 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 (3.067 minutes) as the major peak in the chromatogram obtained with the standard solution (3.182 minutes), as shown in Figures 6.5 (a) and (b). All the samples of amoxicillin in this study were identified to contain the required active pharmaceutical ingredient using the procedure described.

(a) Chromatogram of Amoxicillin standard



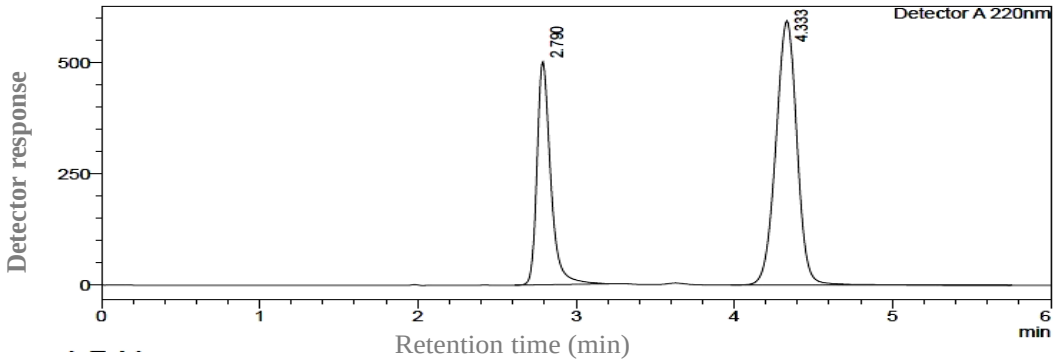
(b) Chromatogram of Amoxicillin sample (ACJ001)

Figure 6.5: HPLC chromatograms from analyzed samples of amoxicillin and amoxicillin standard

6.4.1.2. Identification of amoxicillin + clavulanate tablets

The test for identification in this study showed that all of the samples of amoxicillin/clavulanate tablets contained both active ingredients. The retention times for amoxicillin and clavulanic acid in sample solutions were equal for all samples, compared with the respective retention times of amoxicillin and clavulanic acid in standard solutions, resulting in positive identification for both active ingredients as shown in Figures 6.6 (a) and (b). The peaks at the retention times of 2.79 and 4.33 minutes in figure 6.6 (a) are for clavulanic acid and amoxicillin from standard solutions, respectively. While the peaks at the retention times of 2.788 and 4.324 minutes in figure 6.6 (b) are for clavulanic acid and amoxicillin from sample solutions, respectively.

(a) Chromatogram Amoxicillin/clavulanic acid standard



(b) Chromatogram Amoxicillin/clavulanic acid tablet sample (ACTJ023)

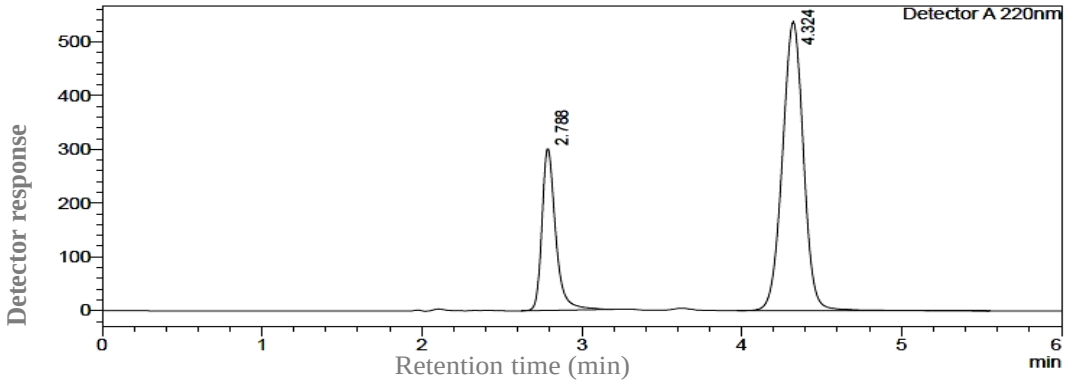


Figure 6.6: HPLC chromatogram of amoxicillin/clavulanic acid tablet sample and standard.

6.4.1.3. Identification of ciprofloxacin 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

ciprofloxacin. All of the ciprofloxacin samples had similar retention times as the reference standard solutions. Figures 6.7 (a) and (b) show the chromatogram of ciprofloxacin reference standard solution and the sample solution, with retention times of 6.513 and 6.467 for standard and sample, respectively.

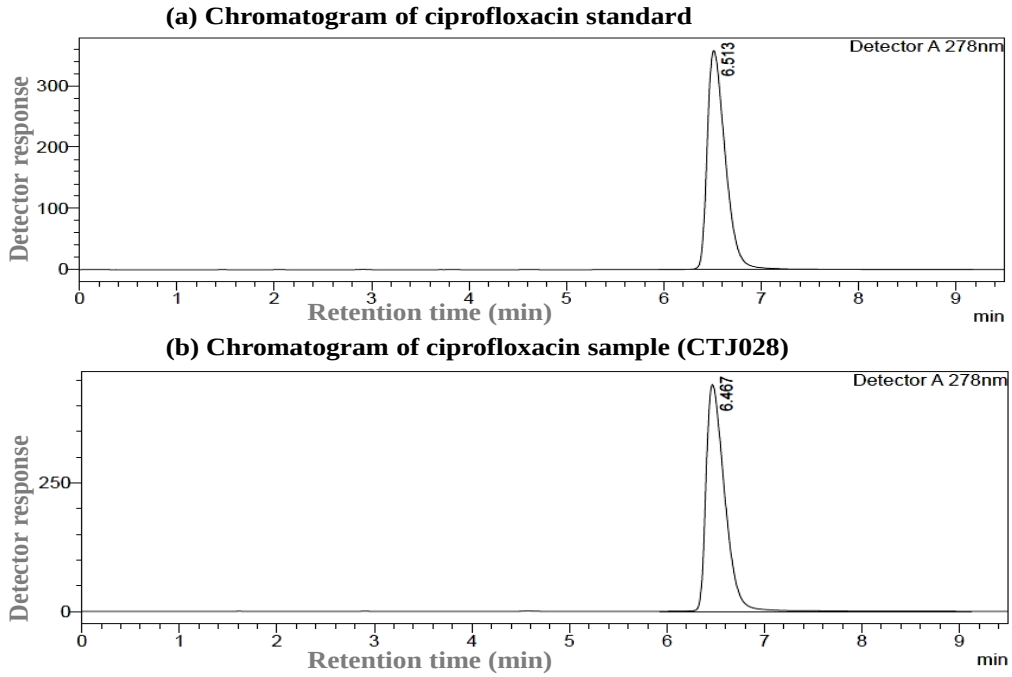
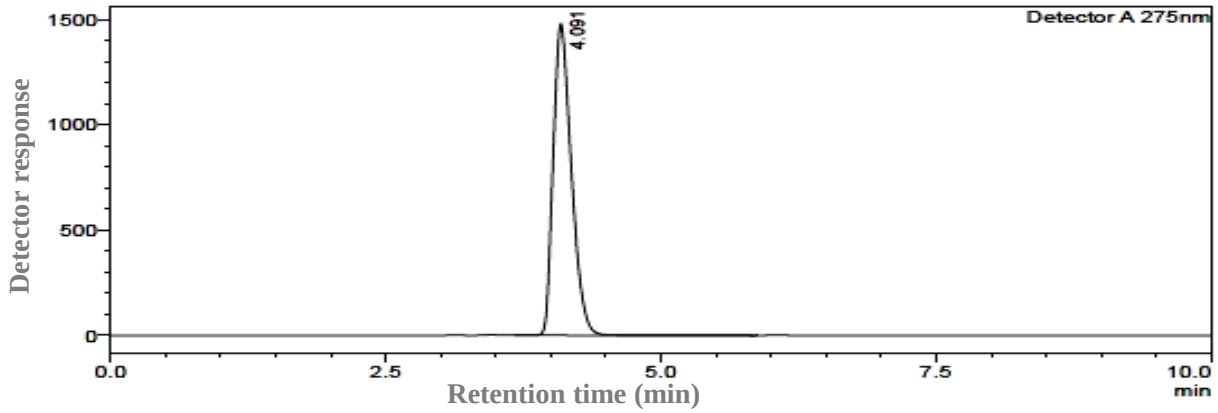


Figure 6.7: HPLC chromatograms from analyzed samples of ciprofloxacin tablets and ciprofloxacin reference standard.

6.4.1.4. Identification of norfloxacin tablets

The test for identification of norfloxacin was based on a comparison of the retention times of standard and sample solutions as described in the BP monograph for the assay of norfloxacin tablets (BP, 2021). The typical chromatograms obtained with standard and sample solutions are depicted in Figure 6.8 (a) and (b), having retention times of 4.091 and 4.116, respectively. Based on this procedure, all of the norfloxacin samples tested were identified as containing the required norfloxacin active ingredient.

(a) Chromatogram of Norfloxacin standard



(b) Chromatogram of Norfloxacin tablet sample (NTJ043)

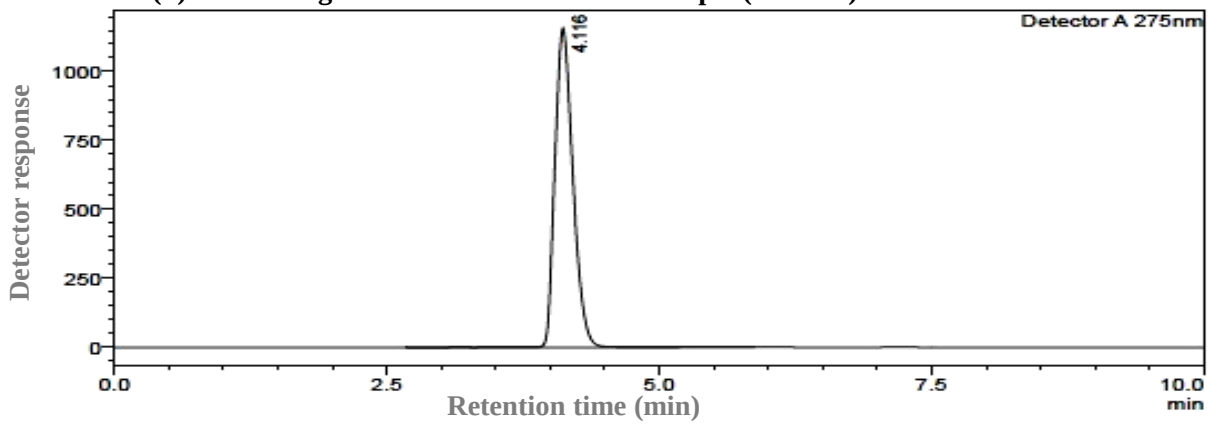


Figure 6.8: HPLC chromatograms from analyzed samples of norfloxacin tablets and norfloxacin reference standard.

6.4.2. Uniformity of dosage units

The USP weight variation procedure was applied to 10 dosage units of each sample by using the mean weight and assay value for each sample, then calculating the content of API in each dosage unit and the acceptance value. The USP specification states that dosage uniformity requirements are satisfied if the acceptance value of 10 dosage units is less than or equal to L1%, or 15% (USP 44-NF 39, 2021). A summary of the weight variation test results for amoxicillin single-dose formulation and amoxicillin/clavulanic acid fixed-dose combination products is shown in Table 6.1. As indicated in the table, 27.27% (6/22) of the amoxicillin samples did not meet the requirements for dosage form uniformity while all (5/5) samples of the amoxicillin and clavulanic acid combination complied with the specification.

Table 6.1: Summary of disintegration time and weight variation test results for amoxicillin single dose formulation and amoxicillin and clavulanic acid fixed dose combination products.

Sample Code	DT (min)	Weight Variation		
		Average wt.(mg) ±SD	Acceptance value (%)	Remark
ACJ001	9.3	607.9 ± 5.2	2.3	Passed
ACJ002	7.3	591.5 ± 5.9	3.5	Passed
ACJ003	9.9	591.0 ± 4.8	2.9	Passed
ACJ004	6.7	604.7 ± 16.5	9.0	Passed
ACJ005	3.5	593.5 ± 7.0	3.5	Passed
ACJ006	6.8	593.5 ± 7.0	14.0	Passed
ACJ007	8.5	568.2 ± 22.2	39.1	Failed
ACJ008	8.5	560.2 ± 23.0	44.4	Failed
ACJ009	8.7	593.1 ± 11.4	6.58	Passed
ACW010	11.1	585.0 ± 13.4	6.4	Passed
ACW011	7.5	555.0 ± 19.1	41.0	Failed
ACW012	11.2	603.5 ± 34.3	15.1	Failed
ACW013	7.3	572.9 ± 22.1	43.6	Failed
ACD014	11.2	607.0 ± 2.8	9.2	Passed
ACD015	8.5	582.9 ± 5.0	5.4	Passed
ACD016	6.2	593.1 ± 4.3	1.8	Passed
ACD017	8.1	541.4 ± 39.5	50.1	Failed
ACD018	8.4	598.3 ± 5.2	2.1	Passed
ACD019	8.2	600.3 ± 5.3	2.1	Passed
ACD020	7.2	581.1 ± 11.9	4.9	Passed
ACD021	7	597.4 ± 7.2	2.9	Passed
ACD022	7	586.7 ± 11.2	4.6	Passed
ACTJ023	8.6	1120.2 ± 9.1	1.9	Passed
ACTJ024	7.5	1018.9 ± 10.2	11.4	Passed
ACTW025	10.2	1036.8 ± 17.7	12.8	Passed
ACTD026	13.2	1094.2 ± 12.4	15.0	Passed
ACTD027	9.7	966.4 ± 12.4	10.7	Passed

NB: DT -Disintegration time; wt. – weight; SD -standard deviation

Weight variation test results for ciprofloxacin and norfloxacin tablet formulations is shown in table 6.3. All ciprofloxacin (15/15) and norfloxacin (12/12) samples met the pharmacopoeial acceptance requirements for dosage uniformity.

In the present study, among the 54 samples tested for weight variation 6 (11.11%) samples (all of which were imported amoxicillin samples) failed to meet the compendial requirement. The amount of API in the dosage units within a batch should be close enough to the strength of the drug so that each unit when administered can have the required therapeutic effect. Since a specific weight of an active substance is used for manufacturing a batch of the product, variations in the distribution of the substance can be explained in such a way that decrease in one unit is accounted by the gain in another dosage unit. This effect can be caused due to lose of control over manufacturing process. Too large variations can result in subtherapeutic amount in one unit and toxic amount in another unit. Therefore, weight variation is one of critical quality

parameters that should be controlled strictly during manufacturing to ensure uniform distribution of the drug substance among dosage units in a batch (USP 44-NF 39, 2021).

6.4.3. Disintegration test

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 amoxicillin capsule single dose and amoxicillin and clavulanic acid combination products are presented in Table 6.1 and those of ciprofloxacin and norfloxacin samples is presented in Table 6.2. As can be seen from the tables, all the samples comply with the requirement for disintegration time. The disintegration time for amoxicillin capsule formulations ranged from 3.5 to 11.2 minutes, while that of amoxicillin and clavulanic acid combination products ranged from 7.5 to 13.2 minutes. The lowest disintegration times observed were 1 and 0.7 minutes for ciprofloxacin and norfloxacin samples, respectively, while the longest disintegration times recorded were 17.5 and 19.73 minutes, respectively.

Table 6.2: Summary of disintegration time and weight variation test results for ciprofloxacin and norfloxacin tablet formulations.

Sample Code	DT (min)	Weight Variation		
		Average wt. (mg) ± SD	Acceptance value (%)	Remark
CTJ028	1.06	716.0 ± 1.9	4.50	Passed
CTJ029	12	686.2 ± 1.5	8.60	Passed
CTJ030	17.5	787.2 ±1.1	5.70	Passed
CTJ031	5.7	634.7 ± 0.7	2.60	Passed
CTJ032	3.15	772.7 ± 1.2	6.40	Passed
CTJ033	11.3	736.6 ± 1.5	6.60	Passed
CTJ034	1	798.2 ± 1.2	2.80	Passed
CTJ035	1.5	770.5 ± 0.5	8.10	Passed
CTD036	12.6	741.7 ± 1.4	8.20	Passed
CTD037	1.8	741.0 ± 0.004	4.78	passed
CTD038	2.7	686.2 ± 1.1	10.90	Passed
CTD039	3.03	643.9 ± 1.5	5.50	Passed
CTD040	7.83	637.8 ± 0.8	4.20	Passed
CTD041	4.43	776.5 ± 0.7	5.00	Passed
CTD042	6.23	797.9 ± 0.4	7.40	Passed
NTJ043	19.73	577.1 ± 1.4	3.69	Passed

Sample Code	DT (min)	Weight Variation		
		Average wt. (mg) ± SD	Acceptance value (%)	Remark
NTJ044	0.7	651.4 ± 1.1	2.85	Passed
NTJ045	6.83	684.3 ± 1.1	2.54	Passed
NTJ046	1.4	575.9 ± 1.0	1.99	Passed
NTW047	5	578.5 ± 0.4	0.89	Passed
NTW048	1.2	579.5 ± 0.7	2.67	Passed
NTD049	4.3	680.0 ± 0.8	2.11	Passed
NTD050	2	650.0 ± 1.1	4.12	Passed
NTD051	4.55	590.0 ± 1.5	5.88	Passed
NTD052	3.4	560.0 ± 0.8	1.97	Passed
NTD053	1.67	540.0 ± 0.5	2.12	Passed
NTD054	2.5	540.0 ± 0.4	1.03	Passed

NB: DT -Disintegration time; wt. – weight; SD -standard deviation

6.4.4. Hardness/breaking force, thickness and diameter

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).

Except for samples of amoxicillin capsules, all the remaining samples in tablet formulations were subjected to the test for breaking force/hardness using 10 tablets for each sample. The mean

hardness results of the test for breaking force for amoxicillin and clavulanic acid tablet combination products are given in Table 6.3 with their respective standard deviations. The minimum mean value of hardness was observed for sample ACTD027 (180 N), while the mean maximum value was 391.12 N for the comparator product.

Table 6.3: Hardness, thickness and diameter results of amoxicillin + clavulanic acid

Sample Code	Mean Hardness (N) (± SD)	Mean Thickness (mm) (±SD)	Mean Diameter (mm) (± SD)
ACTJ023	381.2 ± 15.4	7.08 ± 0.04	21.65 ± 0.05
ACTJ024	232.1 ± 17.6	6.94 ± 0.03	20.19 ± 0.04
ACTW025	328.1 ± 9.0	6.13 ± 0.02	20.46 ± 0.01
ACTD026	383.5 ± 20.3	6.29 ± 0.02	19.42 ± 0.02
ACTD027	180.7 ± 13.2	5.46 ± 0.03	21.13 ± 0.02
Augmentin	391.1 ± 9.7	6.87 ± 0.02	20.16 ± 0.01

The mean hardness results of the test for breaking force for ciprofloxacin tablet samples are given in Table 6.4 with their respective standard deviations. All the samples satisfy the minimum requirement for breaking force where the minimum mean value of hardness was observed in sample CTJ034 (115.35 N), while the maximum mean value was 227.26 N for the sample CTJ033.

Table 6.4: Hardness, thickness and diameter results of ciprofloxacin tablets

Sample Code	Mean H (N) (± SD)	Mean T (mm) (±SD)	Mean D (mm) (± SD)
CTJ028	193.84 ± 18.03	4.91 ± 0.03	17.42 ± 0.03
CTJ029	132.50 ± 8.55	6.01 ± 0.03	15.45 ± 0.04
CTJ030	135.73 ± 7.69	5.06 ± 0.07	18.12 ± 0.02
CTJ031	151.41 ± 9.87	4.91 ± 0.05	16.56 ± 0.03
CTJ032	177.87 ± 9.75	5.36 ± 0.02	18.14 ± 0.01
CTJ033	227.26 ± 19.73	5.40 ± 0.05	17.17 ± 0.02
CTJ034	115.35 ± 11.57	5.07 ± 0.04	18.12 ± 0.02
CTJ035	150.33 ± 6.93	5.36 ± 0.05	18.15 ± 0.02
CTD036	192.96 ± 25.49	5.44 ± 0.11	17.25 ± 0.02
CTD037	188.94 ± 11.27	5.38 ± 0.03	17.18 ± 0.01
CTD038	157.00 ± 13.64	5.91 ± 0.01	15.45 ± 0.03
CTD039	162.78 ± 26.33	5.20 ± 0.03	12.76 ± 0.02
CTD040	139.55 ± 10.48	4.95 ± 0.02	16.52 ± 0.10
CTD041	172.87 ± 4.06	5.35 ± 0.02	18.12 ± 0.02
CTD042	168.56 ± 6.23	6.07 ± 0.02	18.09 ± 0.03

NB: H -hardness in newtons (N); T -thickness; D -diameter

The mean hardness results of the test for breaking force for norfloxacin tablet samples are given in Table 6.5 with their respective standard deviations. All the samples satisfy the minimum requirement for breaking force where the minimum mean value of hardness was observed in sample NTJ044 (121.2 N), while the maximum value was 285.6 N for the sample NTJ051.

In summary, 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 285.6 N for the sample NTD051 of norfloxacin, 227.26 N for the sample CTJ033 of ciprofloxacin, and 383.47 N for the sample ACTD026 of amoxicillin/clavulanate. Despite the fact that the tablets are too hard, all of the analyzed products disintegrated within an acceptable range. A similar study conducted in Ethiopia reported that the majority of ciprofloxacin tablets had a mean hardness value above 480 N but acceptable disintegration times (Kahsay *et al*., 2007). A related result was also reported for amoxicillin/clavulanate tablets with hardness as high as 330 N but with acceptable disintegration time (Waqas *et al*., 2020).

Table 6.5: Hardness and diameter test results of norfloxacin tablets.

Sample Code	Mean Hardness (N) (± SD)	Mean Diameter (mm) (± SD)
NTJ043	123.1 ± 11.7	12.13 ± 0.02
NTJ044	121.2 ± 9.9	12.66 ± 0.02
NTJ045	192.7 ± 15.6	18.01 ± 0.10
NTJ046	155 ± 27.6	15.56 ± 0.05
NTW047	134.8 ± 12.0	17.13 ± 0.04
NTW048	132.3 ± 12.7	15.50 ± 0.03
NTD049	165.0 ± 21.3	17.99 ± 0.07
NTD050	213.1 ± 12.3	12.64 ± 0.08
NTD051	285.6 ± 4.22	15.57 ± 0.03
NTD052	170.2 ± 17.8	12.02 ± 0.09
NTD053	162.5 ± 9.9	14.22 ± 0.03
NTD054	161.6 ± 6.5	14.20 ± 0.02

All the ciprofloxacin, amoxicillin and clavulanic acid combination products were assessed for their thickness. The mean thickness results of 10 tablets of ciprofloxacin samples and amoxicillin with clavulanic acid combination products are presented in Tables 6.2 and 6.3, respectively. All of the tested samples were observed to have a mean thickness within 5% of the standard deviation, showing satisfactory manufacturing control over their thickness.

The test results for diameter are provided in Tables 6.3, 6.4, and 6.5 for all samples of amoxicillin with clavulanic acid combination products, ciprofloxacin and norfloxacin, respectively. Thickness and diameter should be inversely correlated for products of the same strength, or else tablet weight will be used to make up for any imbalance caused by inappropriate thickness and diameter. Also, the esophageal transit and administration methods are influenced by the tablet's diameter and shape. A minimum of 19.42 mm and a maximum of 21.65 mm diameter were observed for samples ACTD026 and ACTD023 for amoxicillin with clavulanic acid combination products. Whereas, the diameter of ciprofloxacin samples ranged from 12.76 mm for sample CTD039 and 18.15 mm for sample CTJ035. The diameter of norfloxacin tablet samples was within the range of 12.02 mm to 18.01mm for the samples NTD052 and NTJ045, respectively.

6.4.5. Results of assay

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.

6.4.5.1. Assay result of amoxicillin capsules

Amoxicillin was examined using the method outlined in the USP, and the monograph specifies that amoxicillin capsules should contain not less than 90.0% and not more than 120.0% of the labeled amount (USP, 2021a).

System suitability test:

The system suitability test results for assay of amoxicillin are provided in Table 6.6 with their respective specifications. According to the USP 2021, the tailing factor of the peak of amoxicillin standard solution should not be more than 2.5 and percentage relative standard deviation of six replicate injections of the standard solution should not be more than 2.0%. As shown in table 6.6, the tailing factor obtained was 1.2, the relative standard deviation was 0.86, and number of theoretical plates was 3381, which satisfies the system suitability requirement.

Table 6.6: System suitability test results for assay of amoxicillin capsules.

System Suitability Parameters of amoxicillin standard peak				
Standard Injections	Peak Area	Tailing Factor	TPN (N)	Retention Time
Reference Standard 1st inj.	10773345	1.173	3225.738	3.04
Reference Standard 2nd inj.	10797615	1.200	3352.52	3.05
Reference Standard 3rd inj.	10669331	1.193	3352.56	3.02
Reference Standard 4th inj.	10782043	1.188	3286.785	3.03
Reference Standard 5th inj.	10604214	1.181	3399.771	3.00
Reference standard 6^{th} inj.	10638066	1.179	3708.007	3.14
Mean Value	10711131	1.1864	3381.899	3.04
RSD	0.863394			
Limit	NMT 2%	NMT 2.5	NLT 2000	

The results of the analysis on 22 samples of amoxicillin capsules are presented in Table 6.7. It was noted that 77.3% (17 out of 22) of amoxicillin capsule samples met the specified USP limits for amoxicillin content. However, 22.73% (5 out of 22) failed to meet the USP limits for percentage content as presented in Table 6.7, and all were non-registered, similar-brand products imported through borders illegally. The samples that failed to meet the specification were ACJ007 (65.5%) and ACJ008 (60.0%), sampled from Jijiga; ACW011 and ACW013 (60.6%) were sampled from Togo-Wuchale; and ACD017 (58.7 %) was sampled from Dire Dawa, all of which were manufactured in China, as the label specifies and were collected from private facilities. Among the samples that complied with the compendial limit, the highest percentage content was obtained for sample ACJ006 (112.3%), while the least drug content was observed for sample ACJ004 (95.8%). 100% (5 out of 5) of the samples that failed to meet the limit were obtained from private facilities.

Table 6.7: Percentage content of API for amoxicillin capsule results.

Sample Code	**Brand**	**Country of Origin** [a]	**Collection site**	**Mean % Content (± %RSD)**	**Remark**
ACJ001	Amyn-500	India	Jijiga	98.3 ± 0.4	Passed
ACJ002	Amox-500	China	Jijiga *	97.3 ± 0.5	Passed
ACJ003	Amox-500	China	Jijiga	97.6 ± 0.5	Passed
ACJ004	Moxvid-500	India	Jijiga	95.8 ± 1.5	Passed
ACJ005	Amox-500	China	Jijiga *	97.7 ± 0.9	Passed
ACJ006	Amox-500	China	Jijiga*	112.3 ± 1.1	Passed
ACJ007	Sinomox	China	Jijiga	65.5 ± 1.6	Failed
ACJ008	Sinomox	China	Jijiga	60.0 ± 1.9	Failed
ACJ009	Miloxy-500	India	Jijiga	103.3 ± 0.4	Passed
ACW010	Amoxicillin BP	-	Togo-Wuchale	102.2 ± 0.5	Passed

Sample Code	Brand	Country of Origin [a]	Collection site	Mean % Content (± %RSD)	Remark
ACW011	Sinomox	China	Togo-Wuchale	62.7 ± 1.9	Failed
ACW012	Kemoxyl 500	Kenya	Togo-Wuchale	96.5 ± 0.4	Passed
ACW013	Sinomox	China	Togo-Wuchale	60.6 ± 2.9	Failed
ACD014	Amyn-500	India	Dire Dawa	109.5 ± 0.6	Passed
ACD015	Amoxapen	Cyprus	Dire Dawa	104.8 ± 1.1	Passed
ACD016	Amox-500	China	Dire Dawa	98.4 ± 0.2	Passed
ACD017	Sinomox	China	Dire Dawa	58.7 ± 2.6	Failed
ACD018	Amox-500	China	Dire Dawa *	101.4 ± 1.7	Passed
ACD019	Amox-500	China	Dire Dawa	99.8 ± 0.2	Passed
ACD020	Amoxycillin	Ethiopia	Dire Dawa	99.9 ± 1.8	Passed
ACD021	Miloxy-500	India	Dire Dawa	98.8 ± 0.2	Passed
ACD022	Amucap	China	Dire Dawa	100.7 ± 0.1	Passed

NB: %RSD-Percentage relative standard deviation, *-Public Facility, USP limit 90 - 120 % of label claim

6.4.5.2. Assay result of amoxicillin + clavulanic acid tablets

Quantitative determination of the content of tablet formulations claiming to contain amoxicillin 500 mg + clavulanic acid 125 mg tablet was conducted according to the method in the USP monograph for the specified medicine (USP, 2021b).

System suitability test result: The test results and system suitability requirements are shown Table 6.8. The resolution between amoxicillin and clavulanate was 10.4, and the tailing factor was 0.92 and 1.4, and the relative standard deviation was 0.2 and 0.05 for amoxicillin and clavulanate, respectively.

Table 6.8: System suitability test results for assay of amoxicillin/clavulanate

System Suitability Parameters							
Std Inj.	Peak Area		Tailing Factor		TPN (N)		Resolution B/n
	Amoxicillin	Clavulanate	Amoxicillin	Clavulanate	Amoxicillin	Clavulanate	Amoxicillin & Clavulanate
Ref.Std1st inj.	5269167	2800293	0.914	1.379	5770.696	4742.687	10.37
Ref std 2nd inj.	5246443	2797447	0.916	1.383	5802.123	4752.839	10.375
Ref std 3rd inj.	5271670	2798653	0.918	1.388	5808.914	4756.747	10.364
Ref std 4th inj.	5269151	2800106	0.919	1.394	5823.275	4777.728	10.37
Ref std 5th inj.	5267836	2800583	0.92	1.397	5843.658	4790.406	10.379
Mean	5264853.4	2799416	0.92	1.397	5843.658	4790.406	10.364
RSD	0.197249	0.047479					
Limit	NMT 2.0		NMT 1.5		NLT 2000		NLT 3.5

NB: TPN – number of theoretical plates; B/n -between

The monograph specifies that the tablets should contain an equivalent of NLT 90% and NMT 120% of amoxicillin and clavulanic acid. A total of 5 samples of amoxicillin and clavulanic acid combination products and one comparator product were analyzed. As shown in Table 6.9, 60.0% (3 out of 5) of the samples met the requirement for percentage content for both active ingredients, while 40.0% (2 out of 5) samples failed to meet the requirement for one of the active ingredients. Sample ACTJ024, despite meeting the requirement for clavulanic acid (110.5%), it failed to meet the requirement for amoxicillin (89.3%). The percentage level of clavulanic acid in sample ACTW025 was 44.7%, which does not meet the criterion, but sample ACTW025 conformed for amoxicillin (109.8%).

Table 6.9: Assay results of amoxicillin + clavulanic acid tablet samples.

Sample Code	Brand	Country of Origin[a]	Collection site	Mean % Content (± %RSD)		Remark
				Amoxicillin	Clavulanate	
ACTJ023	Clavomid	Cyprus	Jijiga *	98.8 ± 0.7	106.9 ± 0.4	Passed
ACTJ024	Koact 625	India	Jijiga	89.3 ± 0.4	110.5 ± 0.4	Failed
ACTW025	Amoklavin-bid	Turkey	Togo-Wuchale	109.8 ± 0.5	44.7 ± 0.4	Failed
ACTD026	Bactoclav-625	India	Dire Dawa	113.4 ± 2.0	113.1 ± 1.6	Passed
ACTD027	Curam	Austria	Dire Dawa	108.8 ± 0.4	116.4 ± 0.4	Passed
Comparator	Augmentin	UK	Addis Ababa	96.6 ± 0.4	107.9 ± 0.4	Passed

NB: USP limits 90 – 120% of label claim for both API's; a- label claimed; *- Public facility.

6.4.5.3. Assay result of ciprofloxacin tablets

All 15 samples of ciprofloxacin tablets were analyzed for percentage content of ciprofloxacin according to the method outlined in the USP. Ciprofloxacin tablets should contain between 90 and 110% of the specified quantity. All the samples of ciprofloxacin tablets analyzed were within the USP limit, and the data is presented in Table 6.10. The minimum percentage content was observed for sample CTJ035 (91.6%) and the maximum was observed for sample CTD038 (109.9%).

Table 6.10: Results of percentage content of ciprofloxacin in ciprofloxacin samples.

Sample Code	Brand	Country of Origin[a]	Collection site	Mean % Content (± %RSD)	Remark
CTJ028	Akcipro 500	India	Jijiga	101.0 ± 0.1	Passed
CTJ029	Galcipro 500	India	Jijiga	106.5 ± 0.2	Passed
CTJ030	Cipoflox-500	China	Jijiga	104.4 ± 0.3	Passed
CTJ031	Ciproquin-500	India	Jijiga*	97.5 ± 0.8	Passed

Sample Code	Brand	Country of Origin[a]	Collection site	Mean % Content (± %RSD)	Remark
CTJ032	Ciprofloxacin	China	Jijiga*	105.0 ± 1.3	Passed
CTJ033	Ciprobid 500	India	Jijiga	104.5 ± 0.3	Passed
CTJ034	Cipoflox-500	China	TW	100.4 ± 3.3	Passed
CTJ035	Ciprofloxacin	China	TW	91.6 ± 3.6	Passed
CTD036	Ciprobid 500	India	TW	106.2 ± 0.7	Passed
CTD037	Ciprobid 500	India	Dire Dawa	104.8 ± 1.2	Passed
CTD038	Galcipro 500	India	Dire Dawa	109.9 ± 1.5	Passed
CTD039	Cipro-Epharm	Ethiopia	Dire Dawa	103.3 ± 0.9	Passed
CTD040	Ciproquin-500	India	Dire Dawa*	103.9 ± 0.9	Passed
CTD041	Ciprofloxacin	China	Dire Dawa	104.7 ± 1.8	Passed
CTD042	Zindolin	Cyprus	Dire Dawa	107.9 ± 0.7	Passed
Comparator	CiproBay	Germany	Addis Ababa	100.8 ± 0.8	Passed

NB: a- label claimed; *- public facility; USP limit – 90 – 110 % of label claim, TW- Togo-Wuchale.

System suitability test result:

The system is suitable for assay of ciprofloxacin tablets states the resolution between the peaks of ciprofloxacin ethylenediamine analog and ciprofloxacin is not less than 6, the tailing factor of ciprofloxacin is not more than 2.0 and relative standard deviation of six injections of standard solution is not more than 1.5. As presented in Table 6.11, the tailing factor was 1.58 and %RSD of six injections was 0.113%.

Table 6.11: System suitability test results for assay of ciprofloxacin

	System suitability parameters of ciprofloxacin			
	Peak Area	Retention time	Tailing factor	TPN
Reference Std 1st inj.	4626346	6.5	1.58	5868
Reference Std 2nd inj.	4638949	6.5	1.58	5852
Reference Std 3rd inj.	4626382	6.5	1.58	5866
Reference Std 4th inj.	4634952	6.5	1.58	5862
Reference Std 5th inj.	4634596	6.5	1.58	5867
Reference Std 6th inj.	4628977	6.5	1.58	5876
Mean	4631700.3	6.5	1.58	5865.17
%RSD	0.113			
Limit	NMT 1.5	NA	NMT 2.0	NLT 2000

The resolution between ciprofloxacin ethylenediamine analog and ciprofloxacin was 8.4, as shown in Figure 6.9.

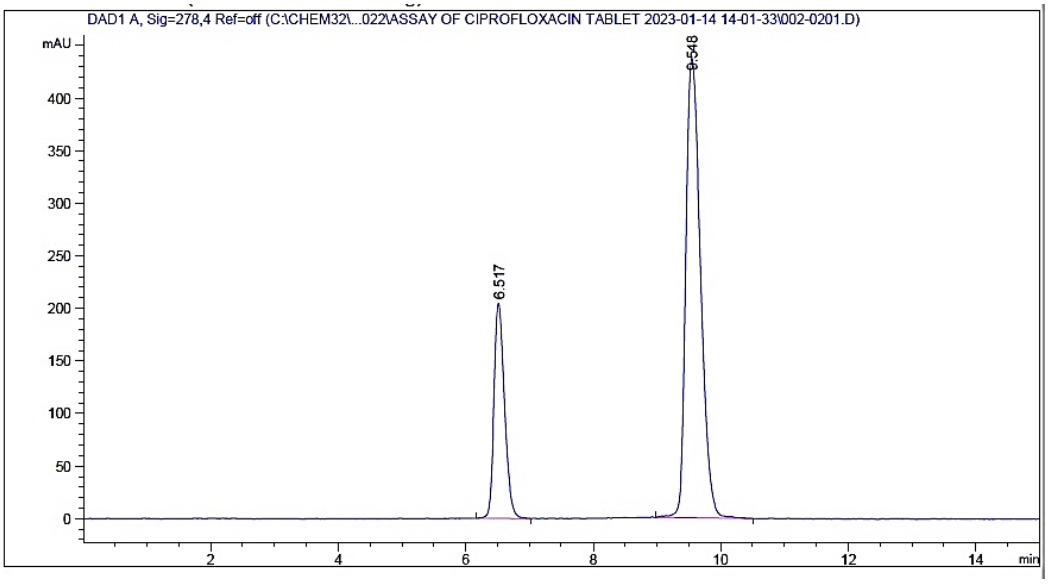


Figure 6.9: Resolution between ciprofloxacin and ciprofloxacin ethylenediamine analog

6.4.5.4. Assay result of norfloxacin tablets

Samples (12) of norfloxacin tablets were analyzed according to the method outlined in the BP (BP, 2021). The BP states that norfloxacin tablets should contain between 95.0 and 105.0 percent of the label claimed amount. All the samples of norfloxacin tablets analyzed were within the BP limit, and the data is presented in Table 6.12. The minimum percentage content was observed for sample NTD051 (96.2%) and the maximum was observed for samples NTJ043 and NTJ044 (101.8 %).

Table 6.12: Assay results of norfloxacin tablet samples.

Sample Code	Brand	Stated Country of Origin	Collection site	Mean % Content (± %RSD)	Remark
NTJ043	Norflo-SSP 400	Ethiopia	Jijiga*	101.8 ± 0.9	Passed
NTJ044	Norfloxacin	India	Jijiga*	101.8 ± 0.6	Passed
NTJ045	Norflox 400	Ethiopia	Jijiga	100.4 ± 0.3	Passed
NTJ046	Norfen 400	Ethiopia	Jijiga	99.8 ± 0.5	Passed
NTW047	Norflo-SSP 400	Ethiopia	Togo-Wuchale	100.9 ± 0.8	Passed
NTW048	Norfen 400	Ethiopia	Togo-Wuchale	97.5 ± 0.4	Passed
NTD049	Norflox 400	Ethiopia	Dire Dawa	98.4 ± 1.1	Passed
NTD050	Norfloxacin	India	Dire Dawa*	96.9 ± 1.2	Passed
NTD051	Norfen 400	Ethiopia	Dire Dawa	96.2 ± 0.5	Passed
NTD052	Trizolin	Cyprus	Dire Dawa	100.8 ± 0.5	Passed
NTD053	Gyrablock	Cyprus	Dire Dawa	97.6 ± 0.4	Passed
NTD054	Gyrablock	Cyprus	Dire Dawa	99.1 ± 1.0	Passed

NB: *-Public facility; BP limit- 95 – 105 % of label claim.

System suitability test result: The requirement for system suitability is met if the symmetry factor of the principal peak of the standard solution of norfloxacin is less than 2.0 and the relative standard deviation of six injections of the standard solution is not more than 2.0%. As shown in Table 6.13, the symmetry factor obtained was 1.4 and relative standard deviation of the six injections was 0.045, satisfying the system suitability requirement.

Table 6.13: System suitability test results for assay of norfloxacin

	System Suitability Parameters			
Standard Injections	Peak Area	Symmetry factor	TPN	Retention Time
Reference Standard 1st inj.	16971544	1.4	2682	4.1
Reference Standard 2nd inj.	16976942	1.4	2664	4.1
Reference Standard 3rd inj.	16970565	1.4	2675	4.1
Reference Standard 4th inj.	16984112	1.4	2673	4.1
Reference Standard 5th inj.	16963522	1.4	2674	4.1
Reference Standard 6th inj.	16977573	1.4	2670	4.1
Mean Value	16973337	1.4	2673.6	4.1
RSD	0.045286			
Limit	NMT 2.0%	NMT 2.0	NLT 1500	NA

Overall, 7 of the samples (12.96%) (5 amoxicillin capsules and 2 amoxicillin/clavulanate tablets) out of the 54 samples tested for content of active ingredient in the formulation failed to meet compendial specification. Many factors can influence the content of a drug product, including the conditions of transportation during distribution, storage conditions, poor manufacturing conditions, compatibility of excipients with API's, nature of the active ingredient and stability of the formulation which can enhance chemical degradation of the drug product (Kelesidis *et al*., 2007). The content of 5 amoxicillin capsule samples failed (all contained below 65% of label claim) and one of amoxicillin/clavulanate tablets containing as low as 44.72% of label claimed clavulanic acid which are far away from lower pharmacopoeial limit. This may indicate that the products might be fraudulently sub-standardized or might be due to instability of clavulanic acid under tropical climatic conditions. However, it should be noted that the percentage content of remaining amoxicillin/clavulanate (89.27%) was much closer to the USP pharmacopoeia's lower limit, which can be attributed to ineffective manufacturing and/or distribution procedures (Kelesidis *et al*., 2007).

Similar results to the current study were reported where all the samples of ciprofloxacin (Hintsa & Murad, 2018), norfloxacin (Hambisa *et al*., 2019) samples met the compendial requirements for content of active ingredient. Another study showed that some of amoxicillin capsule (Corazza *et al*., 2015) and amoxicillin/clavulanic acid tablet (Khan *et al*., 2013) products were reported to fail pharmacopoeial requirement for content of the active substance. However, results of the current study are against the findings of a study in Kenya (Koech *et al*., 2020), where all of the samples of amoxicillin capsule and amoxicillin/clavulanic acid tablets analyzed fulfilled the compendial requirement for content of API. In general, antimicrobials that are of poor quality or that are falsified can have negative effects on patients as well as the general health of the public. These implications can include increased antimicrobial resistance, unsuccessful treatments, and adverse effects (Kelesidis & Falagas, 2015).

6.4.6. In-vitro dissolution profile

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, 49 samples of four product types were examined for dissolution profiles according to the methods described in the USP and BP.

6.4.6.1. Dissolution profile of amoxicillin capsules

A total of 17 different amoxicillin samples that passed the assay were tested for dissolution using 900 mL of distilled water at 37^{o}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 (Figure 6.10) for amoxicillin demonstrated linearity in the concentration range of 112 µg/mL to 252 µg/mL with the linear regression equation y = 0.0032x - 0.0121 and a correlation coefficient (r^2) of 0.9906. The curve demonstrated a good linear relationship between the concentration of the tested samples and the absorbance values over the concentration range of 112 – 252 µg/mL (R^2 = 0.9906).

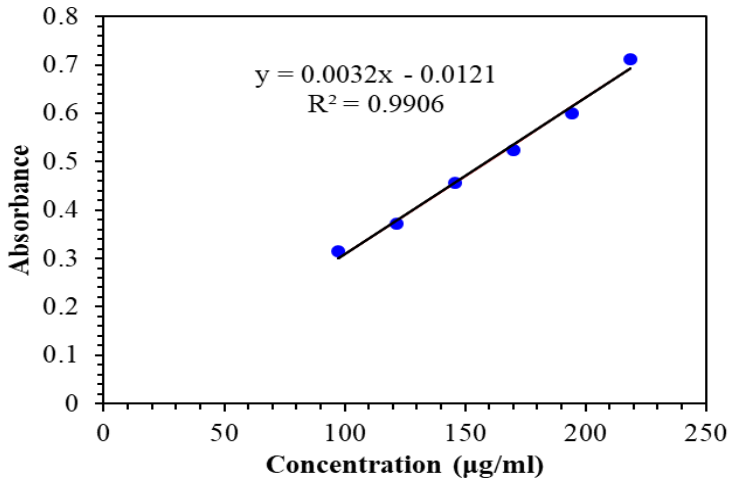


Figure 6.10: Calibration curve of amoxicillin reference standard in the concentration range of 112–252 µg/mL.

The USP/NF specifies that the amount of amoxicillin released after 60 minutes should not be less than 80% of the stated amount. Among the samples tested, 94.12% (16 out of 17) of samples complied with the requirement after 60 minutes for single point dissolution test, while sample ACJ005 (imported product collected from public facility in Jijiga) released only 65.5% of the stated amount after 60 minutes. It failed to meet the specification. Summary of the dissolution data for the samples tested is presented in Table 6.14 and Figure 6.11.

Table 6.14: Cumulative percentage drug release and similarity of amoxicillin capsules.

Sample Code	Mean % CDR (±%RSD) (n=6)						Similarity	
	Time in minutes							
	5	15	30	45	60	75	f_1	f_2
ACJ001	45.6 (17.8)	65.6 (3.9)	78.8 (1.8)	87.4 (5.2)	92.7 (2.4)	96.9 (2.4)	13.09	46.87
ACJ002	50.6 (7.3)	62.5 (2.6)	103.4 (1.1)	106.5 (1.0)	107.3 (1.2)	107.0 (0.8)	11.93	42.47
ACJ003	55.9 (4.8)	66.0 (2.9)	101.6 (1.6)	105.7 (1.3)	109.0 (1.2)	109.0 (1.1)	13.17	41.41
ACJ004	51.3 (4.0)	72.7 (4.3)	85.8 (2.0)	92.8 (2.7)	98.7 (2.4)	100.8 (1.8)	14.02	46.16
ACJ005	27.3 (3.9)	64.6 (1.9)	67.1 (1.2)	65.7 (1.9)	65.5 (0.4)	65.5 (0.5)	28.58	26.63
ACJ006	54.2 (10.7)	92.6 (4.0)	95.5 (2.8)	97.4 (1.6)	96.9 (2.0)	96.1 (1.2)	21.05	35.70
ACJ009	46.2 (11.4)	80.9 (6.5)	96.1 (4.2)	103.8 (3.4)	107.6 (2.4)	111.7 (3.0)	12.03	43.84
ACW010	32.4 (20.2)	73.3 (4.1)	87.8 (2.3)	97.4 (1.7)	103.5 (2.0)	108.1 (2.2)	7.52	55.14
ACW012	42.0 (7.5)	72.2 (6.4)	91.2 (5.0)	98.5 (3.5)	107.2 (1.2)	110.1 (1.1)	8.58	51.85
ACD014	53.2 (5.6)	64.3 (1.7)	102.5 (0.9)	106.1 (0.9)	108.2 (0.9)	108.0 (0.7)	12.55	42.04
ACD015	33.0 (6.2)	60.8 (2.2)	74.5 (2.3)	101.2 (0.9)	106.8 (1.7)	111.3 (0.7)	c	c
ACD016	70.2 (9.5)	106.9 (2.4)	111.9 (0.6)	116.1 (1.2)	118.3 (1.1)	118.5 (0.7)	31.64	26.31

ACD018	44.7 (17.7)	94.8 (3.3)	98.2 (1.5)	98.8 (2.1)	102.5 (1.4)	101.5 (2.1)	17.58	37.07
ACD019	44.7 (10.5)	88.6 (8.2)	105.3 (1.8)	112.8 (0.8)	115.7 (1.4)	116.8 (0.9)	19.74	36.39
ACD020	63.2 (7.3)	89.8 (4.9)	104.3 (4.3)	110.0 (1.4)	115.2 (1.3)	117.6 (1.6)	23.08	33.13
ACD021	52.6 (6.9)	77.8 (5.8)	96.1 (4.7)	106.9 (4.3)	112.6 (2.4)	117.9 (3.0)	15.66	41.98
ACD022	50.5 (3.3)	75.0 (2.7)	93.3 (3.3)	104.5 (4.4)	114.9 (4.2)	115.1 (3.4)	13.46	44.94

NB: CDR- cumulative drug release; c- comparator product

Samples ACJ006, ACD016, ACD018, ACD019, and ACD020 released more than 85% of the drug during the first 15 minutes, demonstrating that the products are rapidly releasing. All samples except sample ACJ005 released more than 90% of the drug within 60 minutes; however, sample ACJ005 remained at less than 70% throughout the whole test. While samples ACJ001 and ACD015 released slowly for the first 30 minutes, they then increased in percentage release to 96.9% and 111.3%, respectively, at the end point. Percentage drug dissolved for sample ACJ005 decreased after 30 minutes from 67% to 65%, which could be due to difference in particle size, since amoxicillin do not exhibit polymorphism and only have single crystal structure. Similarly decreasing profile for amoxicillin after 30 minutes was reported by Thambavita *et al*., (2017).

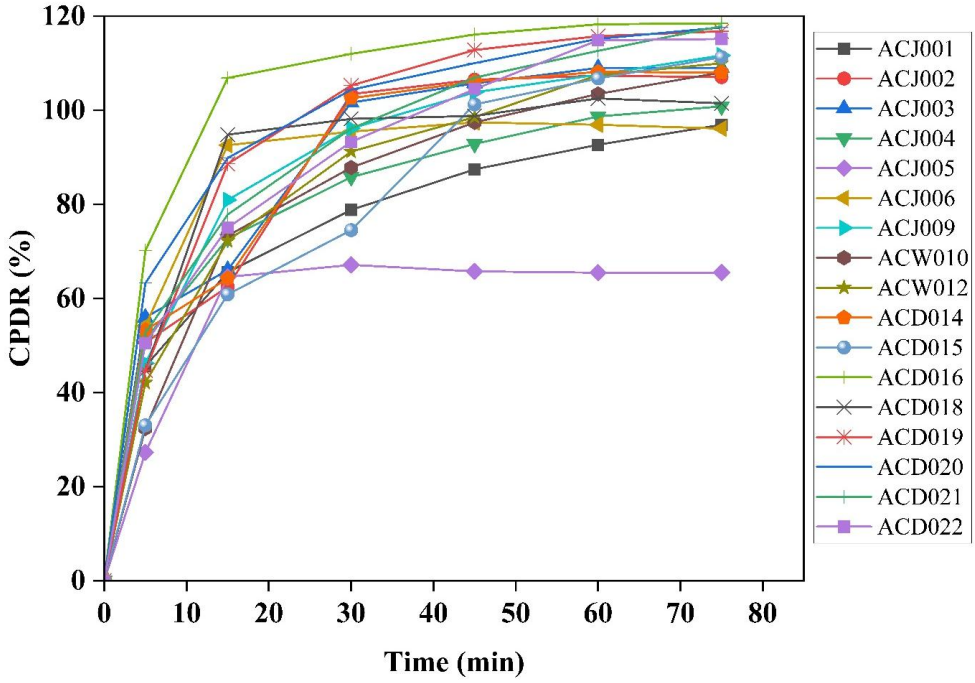


Figure 6.11: Dissolution profiles of amoxicillin samples using distilled water as a medium.

The sample ACD015 was used as a comparator product for amoxicillin capsule samples to calculate the similarity (f_2) and difference (f_1) factors. The values for f_2 and f_1 were calculated using all observed points and the results are presented in Table 6.14. To be considered similar to the comparator product, f_2-values should be between 50 and 100, whereas f_1-values should be 0 to 15. Out of the 17 samples analyzed, only two samples (ACW010 and ACW012) were found to have f_2 values above 50 and f_1 values below 15, hence are equivalent with the comparator product. 5 of the samples (ACJ006, ACD016, ACD018, ACD019, and ACD020) released more than 85% of the drug within the first 15 minutes and do not require profile comparison to show their equivalence to the comparator product. The remaining 7 samples (ACJ001, ACJ002, ACJ003, ACJ004, ACJ009, ACD014 and ACD022) may be considered equivalent to the comparator, since the products met the requirement for difference factor.

In summary, considering the two samples that have f_2 value >50 and the 5 rapidly releasing samples, only 43.75% of the samples can be considered as pharmaceutically equivalent with the comparator product. Thus, 56.25% of the samples are pharmaceutically inequivalent with the comparator product and cannot be used interchangeably. Comparable findings to this study were reported in a comparative dissolution study of different brands of amoxicillin in Ethiopia, which reported that 62.5% of the products were inequivalent to the comparator product (Kassaye & Genete, 2013). Another study in Tigray, Ethiopia, reported that 80% of different brands of amoxicillin were not equivalent to the comparator product (Hailu *et al*., 2013). The differences in the findings could be due to the sample size assessed, where the study by Kassaye & Genete found 5 dissimilar products from 8 brands assessed, while the study by Hailu *et al* assessed only 5 brands and found one similar product to the comparator product. Despite the differences, the findings of this study show the need for regulatory bodies to continuously monitor and oversee the marketing of pharmaceuticals in order to ensure bioequivalence, particularly for drugs like amoxicillin, for which there is evidence of non-bioequivalence from various firms, leading to efficacy concerns.

Different kinetic models were also fitted into the dissolution data of the comparator and samples in the current study to further explain the overall release of the drug from the dosage forms. The dissolution data were analyzed using the DDSolver® software, and the release mechanisms were

established through model fitting using various built-in models (Zhang *et al.*, 2010). 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 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 lowest AIC, highest R^2, and highest MSC were applied (Usta & Incecayir, 2022).

Zero-order kinetics, first-order kinetics, Higuchi, Hixson-Crowell, Makoid-Banakar, and the Weibull are among the models used to fit the dissolution data of amoxicillin samples. The model parameters used for the selection of best kinetic model for the dissolution data of amoxicillin are presented in Table 6.15.

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 samples of amoxicillin. However, the Makoid-Banakar model also properly describes the release kinetics of all samples except for sample ACJ005. Samples ACJ006, ACJ009, ACW010, ACW012 and ACD018 can also be explained by first-order kinetics, while the dissolution data of sample ACW010 can also be explained by Hixson-Crowell model. The dissolution data of the comparator product (sample ACD015) was also properly described by Higuchi model. Different mathematical models can be used to describe the dissolution data of different products. However, since the Weibull model best fits the dissolution data of all samples and the comparator product, it can be used to characterize the dissolution profile of all the study samples. The predicted and observed dissolution profiles of reference and test products of amoxicillin for Weibull model are given in Annex Ⅳ.

Table 6.15: Model selection parameters of amoxicillin capsule samples

Sample code	Stat	ACJ001	ACJ002	ACJ003	ACJ004	ACJ005	ACJ006	ACJ009	ACW010	ACW012	ACD014	ACD015	ACD016	ACD018	ACD019	ACD020	ACD021	ACD022
Zero order	R^2	-1.599	-1.010	-1.512	-2.519	-2.541	-5.859	-1.319	-0.230	-0.619	-1.242	0.3441	-6.948	-2.695	-1.144	-3.649	-1.214	-0.925
	AIC	52.924	54.895	55.213	54.434	52.294	57.231	55.078	52.910	53.565	55.046	50.252	59.139	56.807	56.135	57.153	54.931	54.407
	MSC	-1.288	-1.032	-1.254	-1.591	-1.597	-2.259	-1.174	-0.540	-0.815	-1.141	0.0884	-2.406	-1.640	-1.096	-1.870	-1.128	-0.988
First order	R^2	0.7606	0.8229	0.7756	0.8353	-0.622	0.9669	0.9262	0.9727	0.9391	0.8023	0.9110	0.3233	0.9636	0.8005	0.6759	0.7973	0.8176
	AIC	38.613	40.320	40.720	36.061	47.612	25.235	34.391	30.065	33.877	40.474	38.266	44.358	29.087	41.884	41.171	40.583	40.265
	MSC	1.0964	1.3978	1.1611	1.4705	-0.817	3.0737	2.2734	3.2667	2.4660	1.2878	2.0861	0.0571	2.9797	1.2787	0.7935	1.2628	1.3685
Higuchi	R^2	0.5404	0.5872	0.4850	0.2664	-0.123	-1.151	0.5682	0.8222	0.7729	0.5427	0.9573	-1.375	-0.065	0.5677	-0.103	0.6418	0.7147
	AIC	42.526	45.398	45.704	45.025	45.403	50.273	44.993	41.302	41.779	45.506	33.864	51.891	49.346	46.525	48.525	43.999	42.950
	MSC	0.4442	0.5514	0.3303	-0.024	-0.449	-1.099	0.5064	1.3938	1.1491	0.4492	2.8197	-1.198	-0.397	0.5053	-0.432	0.6934	0.9209
Hixson crowell	R^2	0.5534	0.7698	0.6515	0.5117	-1.162	-0.205	0.8013	0.9618	0.9050	0.7182	0.9124	-1.201	0.4866	0.7165	0.0810	0.7334	0.7698
	AIC	42.354	41.894	43.361	42.583	49.333	46.796	40.337	32.079	36.548	42.602	38.176	51.436	44.964	43.994	47.425	42.227	41.663
	MSC	0.4728	1.1353	0.7209	0.3834	-1.104	-0.519	1.2824	2.9310	2.0208	0.9331	2.1011	-1.122	0.3334	0.9271	-0.248	0.9887	1.1354
Makoid-Banakar	R^2	0.9996	0.9274	0.9337	0.9989	0.8799	0.9249	0.9889	0.9780	0.9957	0.9303	0.9827	0.9535	0.8979	0.9837	0.9966	0.9997	0.9967
	AIC	4.5333	38.969	37.406	10.061	35.991	34.140	27.037	32.775	21.957	38.223	32.453	32.292	39.274	30.852	17.876	4.7784	20.229
	MSC	6.7764	1.6228	1.7134	5.8039	1.1193	1.5895	3.4991	2.8149	4.4527	1.6629	3.0550	2.0680	1.2819	3.1175	4.6760	7.2303	4.7078
Weibull	R^2	**0.9999**	**0.9619**	**0.9655**	**0.9995**	**0.9985**	**0.9990**	**0.9998**	**0.9999**	**0.9986**	**0.9628**	**0.9825**	**0.9991**	**0.9986**	**0.9998**	**0.9996**	**0.9999**	**0.9965**
	AIC	-1.818	37.097	35.488	7.7048	11.816	10.338	3.9978	3.6810	17.330	36.445	34.519	10.333	15.696	6.149	6.7842	3.2752	22.520
	MSC	7.8351	1.9349	2.0331	6.1966	5.1484	5.5565	7.3390	7.6640	5.2239	1.9593	2.7106	5.7280	5.2114	7.2346	6.5247	7.4808	4.3260
	α	4.2360	22999	25408.	3.3200	13.746	0.9770	2.5937	6.3740	4.3603	36975.	31.766	1.0487	0.9455	3.096	2.4871	5.6128	10.540
	β	0.4086	3.1066	2.5784	0.4309	1.5145	0.4704	0.3956	0.2442	0.5039	2.7021	0.9851	0.1263	0.0817	0.573	0.4216	0.5721	0.7567
	Ti	0.5961	-39.02	-38.06	0.6397	1.2747	4.3754	3.5049	4.7375	1.9986	-35.42	-5.507	4.9558	4.9999	3.102	1.5598	-1.081	-4.336
	Fmax	129.81573	108.36894	109.85009	118.44922	65.966662	96.814648	127.11979	302.38687	127.82455	109.12734	124.15373	148.03429	131.32035	119.9633	128.58394	133.64064	125.91382

6.4.6.2. Dissolution profile of amoxicillin + clavulanic acid tablets

The dissolution test for amoxicillin and clavulanic acid combination tablets was carried out according to the LC method in the USP monograph by employing 900 mL of distilled water at 37^{o}C with a paddle operating at 75 rpm (USP 44-NF 39, 2021).

The calibration curve for amoxicillin reference standard (Figure 6.12 (a)) showed linearity in the concentration range of 0.1 to 0.8 mg/mL with the linear regression equation y = 783.76x + 7509.5 and a correlation coefficient (r^2) of 0.9992. The clavulanic acid reference standard calibration curve (Figure 6.12 (b)) also demonstrated linearity in the concentration range of 0.03 mg/mL to 0.21 mg/mL with the linear regression equation y = 932.57x + 9670.5 and a correlation coefficient (r^2) of 0.9881.

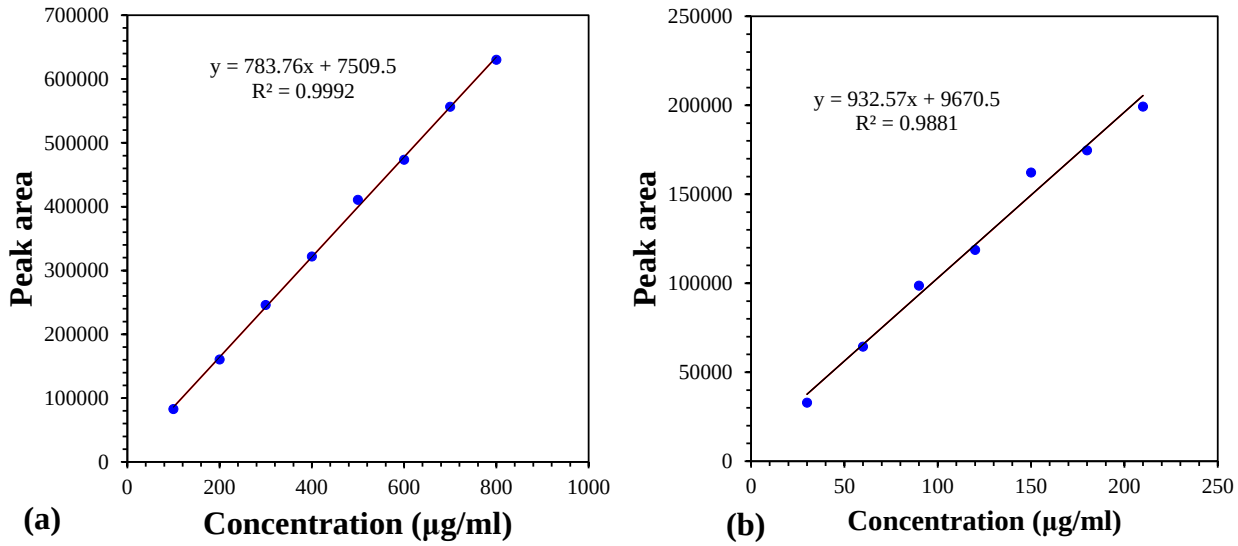


Figure 6.12: Calibration curve: (a) amoxicillin reference standard at the concentration range of 0.1 - 0.8 mg/mL (b) clavulanic acid reference standard at the concentration range of 0.03 - 0.21 mg/mL.

According to the USP, amoxicillin + clavulanic acid tablets should release NLT 85% (Q) of the labeled amount of amoxicillin and NLT 80% (Q) of the labeled amount clavulanic acid after 30 minutes for single point dissolution test. A summary of the dissolution data for 5 samples and 1 innovator product is presented in Table 6.16, and Figures 6.13 (a) and (b). The samples ACTJ024 and ACTW025 (both of which are imported products collected from private facilities)

failed to comply with official requirement after 30 minutes for clavulanic acid, releasing only 69.5% and 75.6%, respectively, of label claim. Therefore, only 60.0% of the samples met the dissolution specification in 30 minutes.

Table 6.16: Cumulative percentage drug release and similarity of amoxicillin + clavulanic acid tablets.

Sample code	Mean cumulative %age release of Amoxicillin (±%RSD) (n=6)							Similarity	
	Time in minute								
	5	10	15	20	30	45	60	f_1	f_2
CP	38.9 (11.5)	78.6 (3.4)	94.8 (1.2)	96.2 (0.8)	94.4 (3.3)	95.6 (1.0)	93.3 (4.6)	-	-
A	33.8 (6.2)	75.5 (3.7)	92.9 (1.6)	98.9 (2.1)	105.6 (1.7)	108.7 (1.5)	109.8 (1.5)	9.0	51.3
B	51.6 (24.8)	88.4 (5.7)	90.9 (1.2)	90.8 (0.8)	91.6 (2.1)	89.5 (1.9)	88.8 (1.2)	7.6	56.9
C	36.2 (14.6)	68.7 (6.9)	81.3 (1.9)	85.2 (4.1)	91.8 (1.2)	94.3 (1.9)	100.4 (5.2)	8.1	54.2
D	25.2 (10.6)	58.9 (7.0)	80.4 (1.5)	89.9 (2.1)	95.9 (1.2)	98.3 (0.9)	97.9 (1.0)	10.6	47.7
E	33.2 (19.8)	69.1 (10.5)	93.2 (4.0)	107.8 (2.3)	111.3 (3.5)	109.5 (4.5)	110.1 (4.3)	12.89	45.76
	Mean cumulative %age release of Clavulanic acid (±%RSD) (n=6)								
CP	42.9 (13.9)	89.5 (3.6)	109.1 (1.5)	110.2 (1.1)	107.7 (3.0)	110.0 (1.4)	107.7 (4.9)	-	-
A	42.4 (4.1)	84.5 (3.1)	100.9 (1.2)	99.6 (1.4)	98.7 (1.5)	97.8 (1.4)	97.1 (1.9)	8.3	52.6
B	29.7 (34.1)	65.1 (10.7)	68.9 (0.8)	69.1 (0.8)	69.5 (2.5)	67.9 (1.4)	67.7 (1.1)	35.3	22.4
C	36.4 (18.9)	63.5 (10.8)	73.0 (4.7)	73.6 (5.5)	75.6 (3.4)	75.3 (2.2)	78.9 (7.3)	29.6	25.9
D	35.5 (8.2)	71.0 (5.3)	90.6 (1.4)	97.6 (1.4)	97.9 (0.8)	97.2 (0.9)	96.6 (1.0)	13.40	43.38
E	27.1 (21.8)	61.4 (11.4)	87.9 (6.9)	104.5 (2.6)	108.9 (3.9)	107.2 (4.2)	107.2 (3.3)	11.10	41.48

NB: CP-comparator product; A- ACTJ023; B- ACTJ024; C- ACTW025; D- ACTD026; E- ACTD027

Similar to the comparator product, the samples ACTJ023, ACTJ024, and ACTD027 released more than 85% of the amoxicillin during the first 15 minutes. Within the first 20 minutes, all of the samples and the comparator product released more than 85% of amoxicillin, satisfying the compendial specification. After 45 minutes, the release of amoxicillin was decreased gradually for samples ACTJ024, ACD026 and the comparator product, while others samples continued to release.

Samples ACTJ023, ACTD026, ACTD027, and the comparator product released more than 85% of clavulanic acid during the first 15 minutes. All of the samples and the comparator released more than 95% of clavulanic acid during the first 30 minutes, except the samples ACTJ024 and ACTW025. The percentage amount of clavulanic acid dissolved remained below 80% until to the final sampling point for samples ACTJ024 and ACTW025.

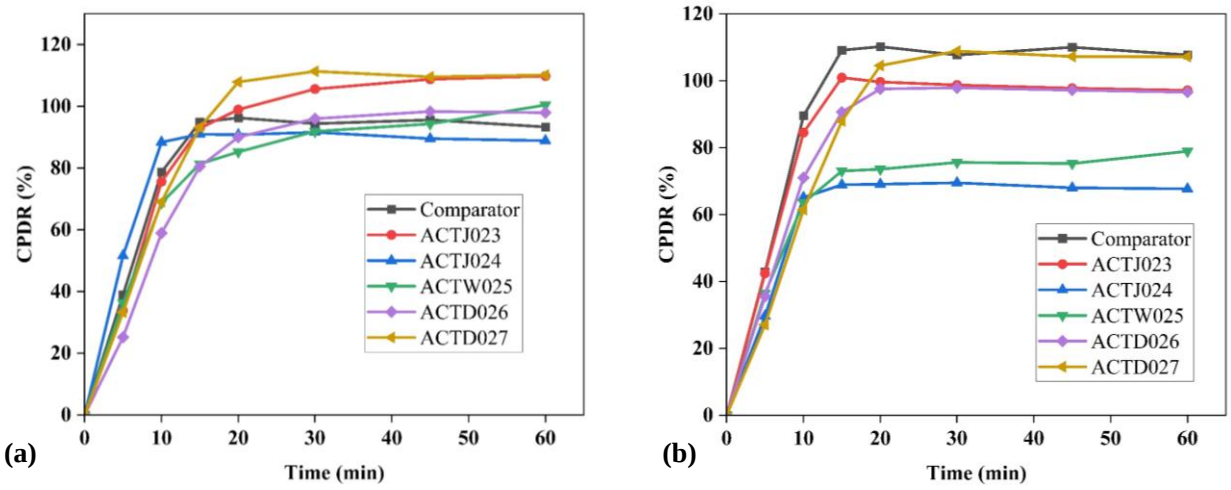


Figure 6.13: Dissolution profile of (a) amoxicillin and (b) clavulanic acid; in amoxicillin + clavulanic acid tablets using distilled water as a medium.

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 Table 6.16. Only one sample (ACTJ023) was found to be similar to comparator product for both API's, while samples ACTJ024 and ACTW025 met the requirement for f_1 (below 15) and f_2 (above 50) for only amoxicillin. The other samples ACTD026 and ACTD027 met the requirement for only f_1 and may be similar to the comparator.

In summary, considering the sample ACTD027 that released more than 85% of both active ingredients within 15 minutes and the sample ACTJ023, which showed similarity for both f_1 and f_2 factors, 60% (3/5) of the samples are not equivalent to the comparator product. A study in Nigeria that assessed 5 amoxicillin/clavulanate products reported 40% of the products to be inequivalent to the comparator product (Al-Tabakha *et al*., 2017).

The dissolution data of amoxicillin/clavulanic acid 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 amoxicillin/clavulanic acid samples and the comparator product. The model parameters used for the selection of best kinetic model for the dissolution data are presented in Table 6.17. The

model fitting revealed that the first-order kinetic model can properly fit the dissolution data of amoxicillin for samples ACTW025 and ACTD026, and the dissolution data of clavulanic acid for sample ACTD026. However, 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 amoxicillin/clavulanate for Weibull model are given in Annex V.

Table 6.17: Model selection parameters of amoxicillin/clavulanate tablets

		Amoxicillin						Clavulanate					
Sample code		Comparator	ACTJ023	ACTJ024	ACTW025	ACTD026	ACTD027	Comparator	ACTJ023	ACTJ024	ACTW025	ACTD026	ACTD027
Zero-Order	R^2	-3.778	-1.469	-10.124	-1.983	-0.775	-1.212	-3.433	-4.395	-4.865	-4.869	-2.435	-0.715
	AIC	68.14	67.11	68.91	65.29	64.80	67.49	69.95	69.06	64.43	64.58	67.29	66.49
	MSC	-1.85	-1.19	-2.69	-1.38	-0.86	-1.08	-1.78	-1.97	-2.05	-2.06	-1.52	-0.82
First-Order	R^2	0.896	0.880	0.684	0.970	0.952	0.829	0.743	0.885	-0.739	0.052	0.937	0.858
	AIC	41.32	45.91	43.98	32.89	39.51	49.55	50.01	42.11	55.92	51.82	39.24	49.02
	MSC	1.98	1.84	0.87	3.25	2.75	1.48	1.07	1.88	-0.84	-0.23	2.49	1.67
Higuchi	R^2	-0.351	0.476	-2.489	0.429	0.623	0.495	-0.247	-0.581	-0.802	-0.493	0.129	0.598
	AIC	59.30	56.25	60.79	53.71	53.95	57.15	61.07	60.47	56.17	55.01	57.68	56.33
	MSC	-0.59	0.36	-1.54	0.28	0.69	0.39	-0.51	-0.74	-0.88	-0.69	-0.15	0.63
Hixson-Crowell	R^2	0.502	0.828	-0.760	0.869	0.951	0.814	0.304	0.332	-1.340	-0.377	0.764	0.887
	AIC	52.31	48.43	56.01	43.37	39.64	50.15	56.98	54.42	57.99	54.44	48.53	47.39
	MSC	0.41	1.48	-0.85	1.75	2.74	1.39	0.08	0.12	-1.14	-0.61	1.16	1.90
Weibull	**R^2**	**0.996**	**0.991**	**0.996**	**0.980**	**0.999**	**0.997**	**0.997**	**0.995**	**0.998**	**0.992**	**0.999**	**0.999**
	AIC	22.41	31.19	17.16	34.15	14.99	24.65	23.07	26.04	13.92	24.14	12.99	20.03
	MSC	4.68	3.94	4.69	3.07	6.26	5.04	4.92	4.17	5.16	3.72	6.24	5.81

6.4.6.3. Dissolution profile of ciprofloxacin tablets

In this study, the dissolution profiles of the fifteen samples of ciprofloxacin and one innovator product were tested following the method outlined in the USP monograph using 900 mL of 0.01N HCl as a medium with the paddle operating at 50 rpm. The samples withdrawn at 5, 10, 15, 20, 30 and 45 minutes were analyzed at wavelength of 276 nm spectrophotometrically. The readings taken for each sample were quantified for ciprofloxacin using the calibration curve equation. The ciprofloxacin reference standard calibration curve (Figure 6.14) also demonstrated linearity in the concentration range of 2 μg/mL to 6 μg/mL with the linear regression equation $y = 0.1041x + 0.0197$ and a correlation coefficient (r^2) of 0.9995.

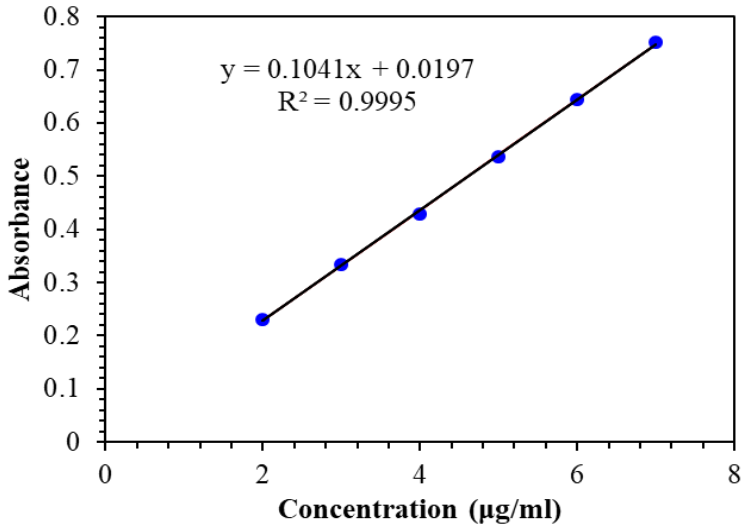


Figure 6.14: Calibration curve of ciprofloxacin reference standard in the concentration range of 2 to 6 µg/mL.

A summary of the data for tested samples is presented in Table 6.18 and Figure 6.15. According to the USP, at least 80% of the indicated amount of ciprofloxacin should be released within 30 minutes. Among the samples tested, only 86.67% (13 out of 15) released ciprofloxacin above the required amount (80%), while 13.33% failed to meet the requirement. It can be seen from the table that samples CTJ030 and CTJ031 released only 71.4% and 60.4% of the labeled amount, respectively, which does not comply with the requirement.

As shown in the figure, various ciprofloxacin samples followed varying drug release patterns at different time points. In the first 5 minutes, 53.33% (8/15) of the samples had released more than 80% of the drug, while the CTJ031 and CTD040 samples had the slowest rate of dissolution in the first 10 minutes. Further, 66.67% (10/15) of the samples and the comparator product released more than 80% of the drug within 10 minutes. Furthermore, in 0.01N HCl medium, majority of the samples (80%) and the comparator product released more than 90% of the drug within 15 minutes, indicating that the products are rapidly releasing. A total of 86.67% of the samples and the comparator product released more than 85% of the drug in the first 20 minutes, whereas 13.33% of the samples released less than 65% of the drug within 20 minutes. Overall, samples CTJ030, CTJ031, and CTD040 had shown the slowest release rate, which is in line with the disintegration test result for CTJ030 (17.5 min, the highest DT time for ciprofloxacin).

Table 6.18: Cumulative percentage drug release and similarity for ciprofloxacin tablets.

Sample Code	Mean %age drug release (± %RSD)						Similarity	
	Time (min)							
	5	10	15	20	30	45	f_1	f_2
CiproBay	71.1 (1.0)	93.6 (4.2)	97.2 (5.6)	101.6 (1.2)	102.6 (1.7)	100.7 (1.5)	-	-
CTJ028	34.2 (13.9)	73.2 (7.6)	97.2 (3.7)	110.5 (3.1)	112.9 (2.5)	117.1 (1.4)	16.40	35.71
CTJ029	99.5 (1.6)	105.9 (1.0)	104.7 (0.2)	103.1 (1.0)	104.9 (0.5)	104.8 (0.8)	9.89*	43.99
CTJ030	19.8 (13.4)	57.2 (11.9)	77.7(5.4)	89.4 (4.0)	71.4 (3.5)	74.5 (0.6)	31.20	24.73
CTJ031	5.8 (29.9)	27.1 (79.3)	32.9 (58.8)	42.6 (45.9)	60.4 (26.4)	74.7 (15.2)	57.01	12.63
CTJ032	97.5 (5.6)	100.3 (0.8)	99.6 (3.4)	99.7 (1.4)	103.9 (5.3)	106.7 (1.1)	7.85*	46.98
CTJ033	88.3 (8.5)	99.6 (2.1)	102.3 (0.6)	101.1 (1.9)	100.5 (0.6)	101.7 (2.5)	5.62*	55.27*
CTW034	35.6 (23.3)	73.2 (7.9)	93.3 (3.7)	96.4 (2.0)	100.0 (2.3)	100.5 (1.9)	11.98*	38.48
CTW035	103.4 (3.1)	110.7 (1.4)	111.9 (1.6)	111.1 (1.0)	110.5 (0.8)	110.2 (1.2)	16.05	38.05
CTW036	87.5 (1.1)	97.7 (7.1)	103.1 (2.1)	104.5 (3.2)	102.2 (1.8)	104.3 (1.2)	5.87*	55.88*
CTD037	86.8(4.4)	97.8 (1.8)	99.7 (1.8)	100.5 (1.1)	99.5 (1.6)	100.0 (1.3)	4.83*	58.02*
CTD038	99.0 (1.4)	102.5 (1.8)	102.1 (1.6)	104.3 (0.7)	104.4 (0.7)	105.1 (0.9)	8.94*	45.36
CTD039	69.8 (27.9)	97.2 (0.2)	101.9 (3.1)	104.1 (1.4)	104.1 (1.6)	103.5 (2.4)	2.89*	75.19*
CTD040	15.6 (49.0)	32.9 (45.6)	47.0 (30.7)	62.2 (21.9)	83.0 (9.4)	98.3 (1.2)	40.18	18.21
CTD041	90.8 (3.8)	99.8 (4.0)	100.9 (0.6)	104.9 (5.3)	105.1 (0.7)	104.2 (0.8)	6.86*	52.54*
CTD042	54.0 (10.6)	85.0 (6.1)	94.9 (1.2)	97.9 (1.3)	101.7 (2.0)	103.2 (2.4)	6.19*	54.50*

The type and/or quantity of the excipients employed, together with formulation and processing factors, may all have a role in the variability in drug release patterns. Further, the rapid release profile of ciprofloxacin could be due to its "U"-shaped pH-solubility profile, with maximum solubility at pH levels below 5 and above 10, and minimum solubility close to the isoelectric point, which is close to neutral (Olivera *et al*., 2011).

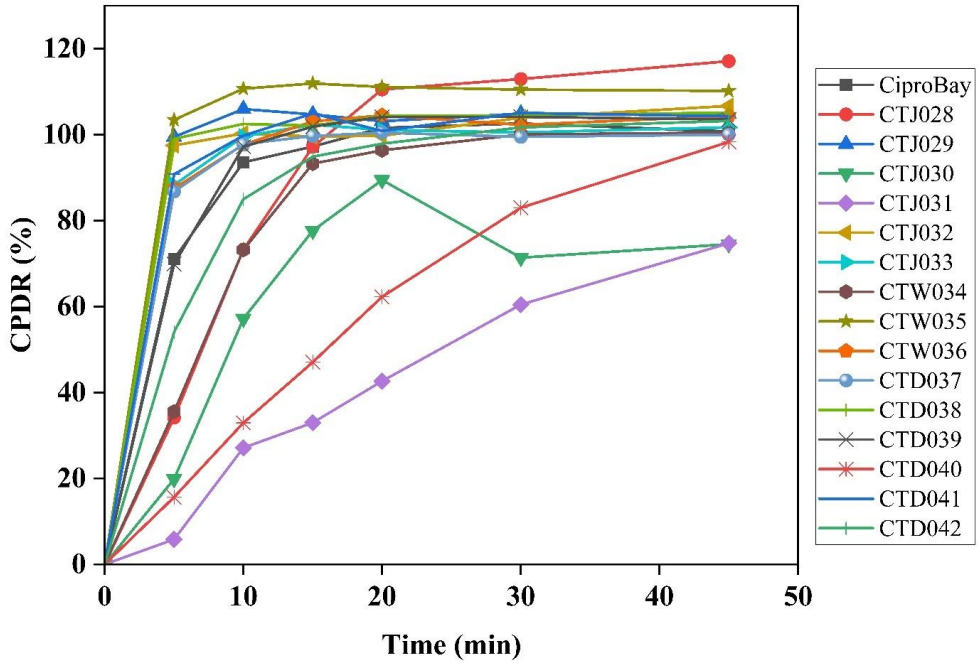


Figure 6.15: Dissolution profiles of ciprofloxacin tablet samples in 0.01 N HCl as a medium.

Based on the analysis of the difference (f_1) and similarity (f_2) factors, only six out of fifteen products showed similarity with the reference product in 0.01 N HCl medium. Furthermore, analysis of difference factor (f_1) demonstrated *in-vitro* bioequivalence of 10 products (out of 15) with the reference product as shown in Table 6.18. Considering the 6 rapidly releasing products and the 6 products that showed similarity in both factors, 80% (12/15) of the products are equivalent to the comparator product and can therefore be used interchangeably and 20% of the products are pharmaceutically inequivalent to the comparator product and may have differences in therapeutic efficacy. A similar study was conducted in Ethiopia in 2018 using the same experimental conditions as in the present study. All of the generic ciprofloxacin tablets used in the investigation were found to be bioequivalent to the innovator brand when the dissolution profiles of four brands were compared to those of the innovator brand (Hintsa & Murad, 2018). Even though similar media was employed in the current study, the obtained results were different.

As it is observed in this study, most of the samples of ciprofloxacin HCl tablets are shown to be pharmaceutically equivalent with the reference product. However, the biopharmaceutical

classification system classifies ciprofloxacin hydrochloride as class IV. Therefore, it was recommended that the interchangeability of generic multisource ciprofloxacin hydrochloride immediate release tablets for biowaiver should be based on *in-vivo* studies (Olivera *et al*., 2011).

The overall release of the drug from the dosage forms were also further explained by fitting the data into various kinetic models. The mean cumulative dissolution data at each sampling point of each sample and reference product were used for model fitting. To choose the best model, the goodness of fit criteria of lowest AIC, highest R^2, and highest MSC were applied (Usta & Incecayir, 2022). A summary of modeling parameters and descriptive statistics for the data on dissolution of ciprofloxacin is provided in Table 6.19.

Table 6.19: Modeling parameters and statistics for the data on dissolution of ciprofloxacin.

Sample code	**Zero -order**			**Higuchi**			**Makoid-Banakar**			**Weibull**		
Stat	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC	R^2	AIC	MSC
CiproBay	-16.54	58.58	-3.19	-3.66	50.63	-1.87	0.963	25.59	2.30	**0.991**	17.13	3.71
CTJ028	-0.372	55.15	-0.65	0.748	44.98	1.05	0.976	34.79	2.74	**0.998**	18.99	5.38
CTJ029	-686.052	60.90	-6.87	-235.4	54.50	-5.79	0.538	21.07	-0.23	**0.845**	14.49	0.87
CTJ030	-0.911	53.90	-0.98	0.352	47.41	0.10	0.874	41.54	1.08	**0.935**	37.57	1.74
CTJ031	0.9196	34.93	2.19	0.8599	38.26	1.63	0.985	28.80	3.21	**0.985**	28.78	3.21
CTJ032	-285.18	60.21	-5.99	-92.73	53.51	-4.87	0.924	14.79	1.58	**0.814**	20.16	0.68
CTJ033	-111.48	59.99	-5.06	-34.99	53.15	-3.91	0.865	23.63	1.00	**0.984**	10.66	3.16
CTW034	-1.19647	55.19	-1.12	0.547	45.72	0.46	0.958	35.46	2.17	**0.998**	15.71	5.46
CTW035	-425.618	61.53	-6.39	-143.9	55.05	-5.31	0.860	17.40	0.97	**0.962**	9.43	2.29
CTW036	-70.889	59.86	-4.61	-20.95	52.74	-3.42	0.939	21.40	1.80	**0.969**	17.24	2.49
CTD037	-107.986	59.76	-5.02	-33.63	52.89	-3.88	0.918	20.59	1.50	**0.996**	2.47	4.52
CTD038	-698.358	60.65	-6.89	-234.3	54.11	-5.79	0.926	9.67	1.61	**0.932**	9.20	1.69
CTD039	-13.497	58.89	-3.01	-2.868	50.96	-1.69	0.929	30.96	1.65	**0.998**	6.90	5.66
CTD040	0.8797	40.14	1.78	0.901	38.98	1.98	0.999	10.68	6.70	**0.999**	12.93	6.32
CTD041	-106.353	60.07	-5.01	-32.43	53.06	-3.84	0.972	14.36	2.61	**0.968**	15.32	2.45
CTD042	-4.395	56.79	-2.02	-0.125	47.39	-0.45	0.959	31.50	2.19	**0.995**	18.04	4.44

The model fitting showed that the dissolution data of drug products were able to be properly described by only two of the different kinetic equations according to the model goodness of fit. Therefore, the Weibull and Makoid-Banakar models were most predominantly found to fit the release data of the drug products. Based on lowest AIC and highest MSC values, the Weibull kinetic model was found to be the best fit for the dissolution data of the reference and the

samples, except samples CTJ029 and CTJ032. Therefore, based on model dependent methods of comparison, all of the samples were found to follow similar mechanism of drug release with the reference product, except the two samples. The predicted and observed dissolution profiles of reference and test products of ciprofloxacin for Weibull model are given in Annex VI.

6.4.6.4. Dissolution profile of norfloxacin tablets

In the present study, the dissolution profiles of the 12 samples of norfloxacin were tested according to the method outlined in the BP monograph using 750 mL of pH 4.0 acetate buffer as a medium with the paddle operating at 50 rpm (BP, 2021). The samples withdrawn at 5, 10, 15, 20, 30, 35 and 45 minutes were analyzed spectrophotometrically at wavelength of 313 nm. The readings taken for each sample were quantified for norfloxacin using calibration curve equation. The norfloxacin reference standard calibration curve (Figure 6.16) also demonstrated linearity in the concentration range of 3.2 µg/mL to 22.4 µg/mL with the linear regression equation y = 0.0333x + 0.014 and a correlation coefficient (r^2) of 0.9996.

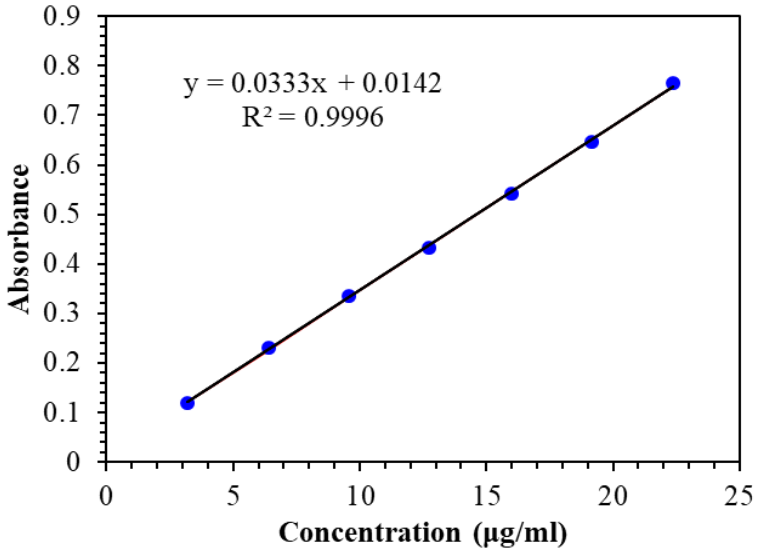


Figure 6.16: Calibration curve for norfloxacin reference standard in the concentration range of 3.2 to 22.4 µg/mL.

A summary of the data for tested samples is presented in Table 6.20 and Figure 6.17. According to the BP, at least 80% of the specified amount of norfloxacin should be released within 30 minutes. Among the samples tested, only 6.67% (1 out of 12) of the samples (sample NTJ043

releasing only 19.9 %) failed to comply with the BP limit within 30 minutes, while 93.33% (11 out of 12) of the samples complied with the limit.

Table 6.20: Cumulative percentage drug release and similarity of norfloxacin tablets.

Sample code	Time in minute							Similarity	
	5	10	15	20	30	35	45		
	Mean Cumulative Percentage Drug Release (±%RSD) (n = 6)							f_2	f_1
NTJ043	1.9 (16.8)	7.6 (18.7)	13. 0 (9.2)	13.0 (9.4)	19.9 (6.7)	25.9 (3.2)	28.0 (4.5)	4.72	83.57
NTJ044	58.6 (13.4)	76.8 (8.0)	80.2 (6.0)	85.9 (4.5)	88.8 (2.8)	94.6 (5.9)	97.4 (3.8)	41.76	13.36
NTJ045	95.7 (3.2)	99.9 (2.5)	102.9 (1.2)	104.8 (0.7)	106.6 (1.2)	109.6 (1.4)	107.6 (1.9)	40.40	9.13
NTJ046	56.9 (6.5)	69.8 (9.2)	78.6 (8.7)	87.4 (6.4)	94.8 (4.8)	97.0 (4.2)	106.2 (5.8)	40.76	13.19
NTW047	58.0 (11.8)	64.9 (10.6)	69.2 (7.0)	73.0 (6.8)	81.5 (4.0)	83.5 (4.0)	89.8 (3.7)	31.01	22.57
NTW048	72.5 (7.2)	96.1 (2.2)	101.2 (0.9)	103.7 (0.4)	105.3 (0.7)	106.7 (0.5)	107.2 (1.3)	**58.12**	**4.38**
NTD049	102.4 (3.2)	106.4 (0.6)	107.7 (0.7)	109.0 (1.1)	110.3 (1.1)	111.8 (0.9)	112.6 (1.0)	35.79	14.12
NTD050	76.2 (6.4)	84.9 (2.4)	88.9 (1.8)	92.9 (0.8)	97.6 (1.5)	103.4 (1.9)	105.4 (1.5)	47.38	9.97
NTD051	28.4 (4.6)	64.2 (1.3)	80.8 (4.4)	91.5 (4.3)	95.6 (5.1)	98.8 (5.9)	101.2 (6.4)	36.50	15.93
NTD052	56.0 (19.8)	94.9 (2.2)	102.2 (3.1)	104.1 (2.1)	104.8 (0.8)	103.4 (1.4)	100.9 (2.9)	_	_
NTD053	79.0 (1.1)	83.0 (2.4)	84.6 (4.2)	90.2 (3.1)	97.0 (3.1)	97.8 (1.8)	98.9 (4.7)	43.48	12.27
NTD054	80.0 (3.5)	85.6 (2.6)	91.8 (2.5)	94.4 (2.7)	98.5 (2.2)	99.6 (3.2)	101.0 (3.1)	46.99	9.52

As shown in Figure 6.17, norfloxacin samples followed varying drug release patterns at different time points. Six of the samples (NTJ045, NTW048, NTD049, NTD050, NTD052, NTD053, and NTD054) released more than 85% of norfloxacin during the first 15 minutes. The sample NTJ043 showed slowest drug release during the entire sampling points, while NTW047 showed the second slowest drug release profile, both of which are manufactured locally by the same manufacturer with different batch numbers.

Despite having moderate hardness (123.1 N) compared to the other norfloxacin samples, sample NTJ043 was observed to have the longest disintegration time (19.73 minutes, as shown in Table 6.3). This could be one of the reasons for the delayed drug dissolution rate observed with this product (Gupta *et al*., 2009). However, sample NTW047, having a hardness of 134.8 N, has been observed to have a disintegration time of 5 minutes. In spite of the fact that this sample showed the second-slowest drug dissolution rate, this product has met the pharmacopoeial 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 products of the same brand (Gupta *et al.*, 2009). This may indicate that there might be loss of control over critical process parameters and critical quality parameters, hence low &/or non-compliance to cGMP requirements (ICH Q13, 2022).

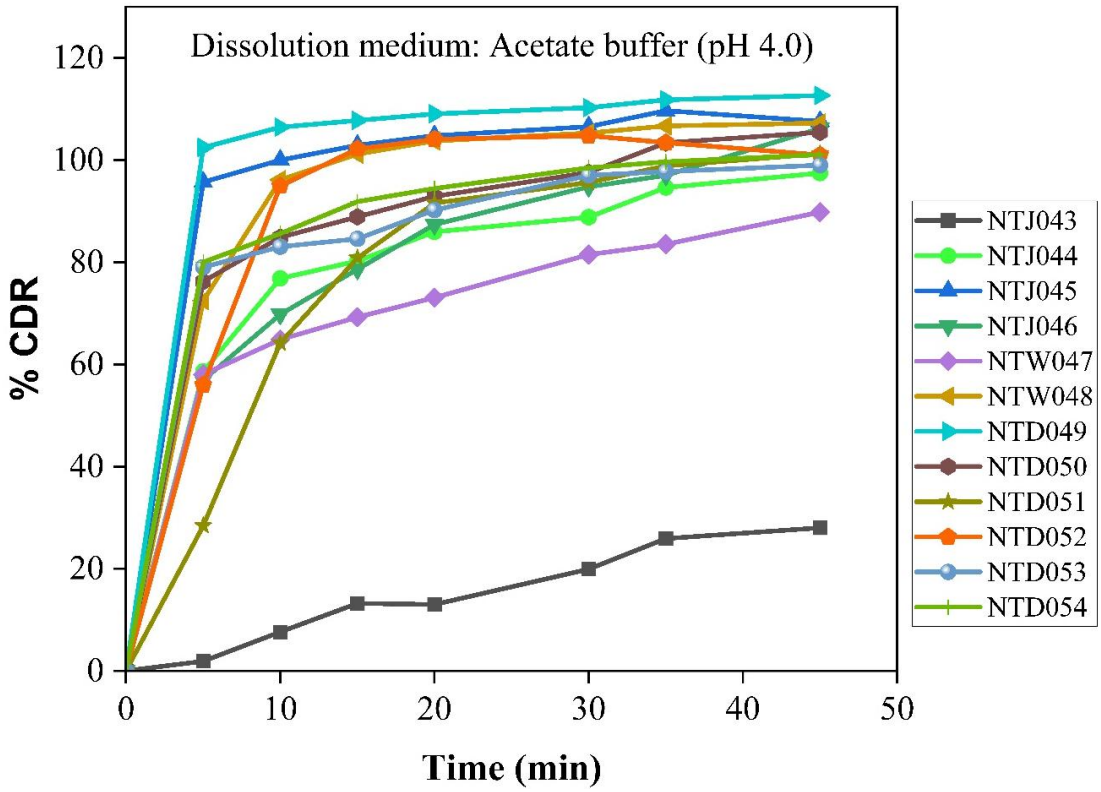


Figure 6.17: Dissolution profiles of norfloxacin samples using acetate buffer of pH 4.0 dissolution medium.

The dissolution profiles of norfloxacin 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 NTD052 as a comparator product, selected based on the WHO recommendation due to the absence of reference product in the local market, and including all observed points, with the findings displayed in Table 6.20. Only the sample NTW048 showed the values of f_2 and f_1 within the acceptable range. With regard to the value of difference factor, 8 of the samples were found to have acceptable similarity with the comparator product and may be equivalent to the comparator. Whereas 3 of the samples failed to meet the requirement for both parameters.

In summary, considering the 4 rapidly releasing products and the sample NTW048 that showed acceptable similarity in both parameters, 45.45% (5/11) of the samples can be considered equivalent to the comparator product, and 54.54% (6/11) are not equivalent. An *in vitro* comparative quality study of norfloxacin in Jimma, Ethiopia, is in good agreement with this study, which reported 85% of a total of 8 brands assessed were inequivalent to the comparator product (Hambisa *et al*., 2019). These findings in general indicate that there is a need for regulatory bodies to continuously monitor and oversee the marketing of pharmaceuticals in order to ensure bioequivalence, particularly for drugs like norfloxacin, for which there is evidence of non-bioequivalence from various firms, leading to efficacy concerns.

The dissolution data was also further characterized by fitting into different drug release kinetic models. Zero-order kinetics, Higuchi, Makoid-Banakar and the Weibull models were employed to fit the dissolution data of norfloxacin tablet samples. The model selection parameters for all samples with their respective values are presented in Table 6.21. Among the models, the Makoid-Banakar model and the Weibull model can properly explain the release kinetics of all samples of norfloxacin. In addition to these models, the sample NTJ043 can also be described by zero-order kinetics. However, based on the value of r^2, lowest AIC and highest MSC values, the Weibull model best fits the dissolution data of all samples. Therefore, it can be deduced that all of the samples follow similar drug release mechanism. The predicted and observed dissolution profiles of reference and test products of norfloxacin for Weibull model are given in Annex VII.

Table 6.21: Modeling parameters for the data on dissolution of norfloxacin.

Sample code	**Zero-order**			**Higuchi**			**Makoid-Banakar**			**Weibull**		
Stat	R2	AIC	MSC	R2	AIC	MSC	R2	AIC	MSC	R2	AIC	MSC
NTJ043	0.956	24.141	2.849	0.819	34.099	1.426	0.971	25.189	2.699	**0.975**	26.163	2.560
NTJ044	-6.30	64.460	-2.27	-0.534	53.537	-0.71	0.970	29.979	2.651	**0.988**	25.391	3.307
NTJ045	-120.2	70.078	-5.08	-38.64	62.258	-3.96	0.962	17.559	2.419	**0.966**	18.754	2.249
NTJ046	-2.4937	63.040	-1.536	0.498	49.457	0.40	0.994	21.443	4.405	**0.995**	23.019	4.180
NTW047	-6.5090	62.534	-2.302	-0.547	51.475	-0.72	0.998	7.7577	5.523	**0.998**	10.625	5.114
NTW048	-13.331	68.301	-2.948	-2.841	59.085	-1.63	0.949	32.742	2.132	**0.999**	1.1118	6.650
NTD049	-259.6	70.963	-5.85	-87.8	63.427	-4.77	0.989	4.1835	3.691	**0.991**	4.3638	3.665
NTD050	-15.2	66.807	-3.07	-3.10	57.192	-1.69	0.99	19.055	3.751	**0.989**	21.368	3.420
NTD051	-0.18	61.3885	-0.452	0.7998	48.9622	1.32	0.972	39.2012	2.7171	**0.998**	21.3735	5.2639
NTD052	-5.49	67.781	-2.16	-0.77	58.683	-0.86	0.919	41.076	1.658	**0.995**	22.874	4.259

Sample code	Zero-order			Higuchi			Makoid-Banakar			Weibull		
Stat	R2	AIC	MSC	R2	AIC	MSC	R2	AIC	MSC	R2	AIC	MSC
NTD053	-26.7	66.985	-3.60	-6.80	58.109	-2.34	0.9586	25.4391	2.3271	**0.973**	24.4409	2.4697
NTD054	-31.2	67.639	-3.76	-8.21	58.86	-2.51	0.993	12.59	4.10	**0.996**	9.098	4.602

6.5. Prevalence of substandard and non-registered products

In this study, out of a total of 54 samples collected from public and private facilities within Dire Dawa, Jijiga, and Togo-Wuchale, the percentage of drugs that failed to meet pharmacopoeial requirements for the percentage content of the API, the dissolution of the API, and the uniformity of the dosage units was 12.96%, 12.24%, and 11.11%, respectively. Similar findings were reported in the WHO QAMSA research, which examined antimalarial drugs in six African nations and found failure rates in assay, dissolution, and uniformity of dosage units of 10.9%, 15.0%, and 6.4%, respectively (WHO, 2011). Overall, 22.22% (12/54) of the drugs had failed to comply with one or more of the aforementioned quality specifications. This failure rate is comparable with the 18.7% estimate for the prevalence of SF medications given by Ozawa *et al*., (2018) from a meta-analysis of more than 40 medicine quality studies in Africa. Out of the total substandard products, 58% (7/12) was amoxicillin, 17% was amoxicillin/clavulanate, 17% was ciprofloxacin, and 8% was norfloxacin, which is consistent with the finding of systematic review on SF antimicrobial agents that reported highest risk of SF products for beta-lactam antibiotics followed by quinolone antimicrobial agents (Kelesidis & Falagas, 2015).

A total of 11.11% (8/54) samples were not registered by the EFDA to be marketed in the country, which may hold the risk of being substandard and falsified, as evidenced by the results of the compendial quality analysis. All the non-registered products failed the pharmacopoeial tests, except one sample, for either weight variation, assay, or dissolution. All the samples screened with the TLC minilab and analyzed with HPLC were positively identified for their respective APIs. However, 14.28% (6/42) of the samples failed to comply with the current GPHF minilab requirement for quantity of the API(s). The 5 samples of amoxicillin that were suspect in the GPHF-minilab TLC quantitation also failed the HPLC content analysis. Two samples of amoxicillin/clavulanic acid tablets that passed the minilab screening failed in pharmacopoeial HPLC analysis for content of one of the active ingredients, one for amoxicillin (89.3%) and the other for clavulanic acid (44.7%). And one of the amoxicillin/clavulanate

samples (ACTD026) that was suspect in the TLC analysis was found to contain both of the active substances within the USP limit. The product that contained 89.3% of amoxicillin was rightly not detected by TLC screening, since the procedure only detects products containing less than 80% of the label claim. Overall, this result agrees with the results of a study conducted on amoxicillin, amoxicillin/clavulanate, and ciprofloxacin which reported 15 samples to fail the screening test, and similar results were also reported on the low sensitivity of the GPHF minilab in detecting some drugs (Schäfermann *et al*., 2020).

From a total of 11 samples collected from governmental facilities, 20.37% (3/11) were substandard, failing to comply with the specification for dissolution, and 20.93% (9/43) of the samples collected from private facilities were found to be substandard. In addition, proportion of samples failed per facility type was 8%, 23%, 31%, and 38% for rural drug vendor, hospital pharmacy, community pharmacy, and drug shops, respectively. These are the points in the drug supply chain where drugs are prepared for sale, dispensing, or patient administration. This finding agrees with the idea that the drug supply chain is most vulnerable towards the point of use. SF medical products may enter it more easily, as evidenced by comparable findings in Latin America, which reported detection rates of 46.0%, 21.8%, and 10% SF medicines in drug shops, pharmacies, and hospitals, respectively (Rojas-Cortés, 2020). This level of drug supply chain is not only vulnerable to SF medicines but also the degree of regulatory control lowers as a drug progresses through the supply chain where the number of stakeholders and transactions increases (WHO, 2017b). The prevalence of SF products as analyzed by different factors like country of origin, collection sites so on is also shown in Figure 6.18.

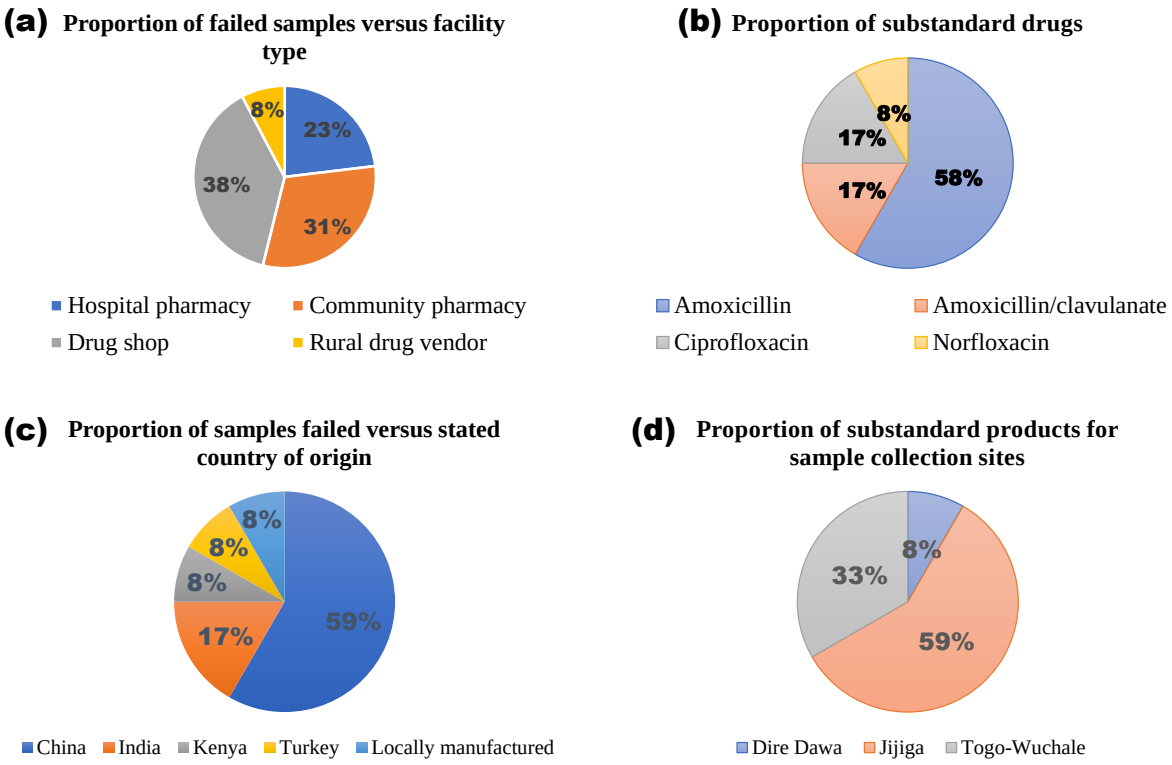


Figure 6.18: Prevalence of substandard products

According to the stated country of origin, 59%, 17%, 8%, 8%, and 8% of the substandard products evaluated in this study were stated to be from China, India, Kenya, Turkey, and locally manufactured, respectively. This finding is similar with the systematic review report of Kelesidis & Falagas, (2015) in that the majority of SF antimicrobials originate from Asia and Africa. With respect to the location of sample collection, the observed failure rate was 4.35%, 33.33%, and 40%, respectively, in Dire Dawa, Jijiga, and Togo-Wuchale.

7. Conclusion and Recommendation

7.1. Conclusion

In this study, an effort was made to evaluate the physico-chemical quality of 54 samples comprised of 22 samples of amoxicillin, 5 samples of amoxicillin/clavulanate, 15 samples of ciprofloxacin, and 12 samples of norfloxacin oral solid products collected from Dire-Dawa, Jijiga, and Togo-Wuchale cities in Eastern Ethiopia. The pharmacopoeial quality parameters such as identity, uniformity of weight, hardness, thickness, diameter, disintegration, assay, and dissolution tests were carried out according to USP and BP methods. The quality assessment revealed that all the products met the standards for visual inspection, identity, thickness, hardness, diameter, and disintegration tests. However, 14.28% (6/42) of the samples failed the GPHF-minilab screening test for semi-quantitative investigation, 27.27% (6/22) of amoxicillin samples failed to meet the requirement for weight variation, and 22.73% (5/22) of amoxicillin and 40% (2/5) of amoxicillin/clavulanate products failed to meet the requirement for percentage content of the active substance. Furthermore, 12.24% (6/49) of the samples failed to comply with the pharmacopoeial specification for dissolution, of which 1 was amoxicillin, 2 were amoxicillin/clavulanate, 2 were ciprofloxacin, and 1 was norfloxacin. Overall, 22.22% (12/54) of the products analyzed did not meet pharmacopoeial specifications, indicating that these products are of substandard quality. To sum up, substandard and unregistered products were found in this study. These drugs being antibacterials which are prescribed for life saving conditions it affects public health and therapeutic outcome. It may be a contributing factor for antimicrobial resistance. Hence the regulatory authority and other stake holders should devise mechanisms to enforce medicine regulation in porous border areas of the country.

Comparative dissolution testing using pharmacopoeial quality control media showed that most of the products analyzed were observed to be inequivalent to the comparator product and hence could not be used interchangeably. 56.25% (9/16) of amoxicillin, 60% (3/5) of amoxicillin/clavulanate, 20% (3/15) of ciprofloxacin, and 54.54% (6/11) of norfloxacin samples were found to be pharmaceutically inequivalent with respective comparator products. Therefore, health professionals should avoid assuming that formulations supplied across national borders are therapeutically similar, even if they are labeled with the same pharmaceutical active ingredient and strength.

7.2. Recommendation

One of the biggest threats to public health is the distribution of fraudulent and/or substandard medicines, which could lead to avoidable morbidity, mortality, and drug resistance. Therefore, EFDA, with its regional branch offices, and regional health regulatory bodies need to conduct post-marketing quality surveillance regularly to determine the quality status of the drug on the market. To prevent the occurrence of essential antimicrobial medicines of poor quality, the authority and other stakeholders should take the right action. Additionally, Ethiopia's federal and regional regulatory bodies have to increase their dedication to tightening licensing requirements for local manufacturers, importers, pharmacies, and drug stores and their cGMP compliance. Local manufacturer licensing should be enhanced to meet international standards, and more-strict requirements, including routine quality control testing, should be implemented to provide quality assurance. Local manufacturers are recommended to properly validate manufacturing processes; routine in-process quality control and efforts to achieve cGMP compliance with international standards are essential to ensuring the quality of the medicines. Further, extensive post-marketing quality surveillance studies of essential antimicrobial medicines, including amoxicillin, ciprofloxacin, and norfloxacin, in the studied locations and surrounding rural areas are recommended. The comparison of the results of three-level QC test schemes showed that screening with the GPHF-minilab protocol can be effectively implemented to assess the quality of essential medicines at a lower cost of analysis. The results of the study also showed porous border areas are susceptible to illegal drug smuggling of substandard quality. Therefore, strengthening border control and cross-border cooperation with neighboring countries is recommended. Drugs of substandard quality are not only found in private facilities but also in public facilities; therefore, similar standards of regulatory focus to ensure the quality of medicines should be given to public facilities. It is also important to conduct more research on how storage conditions and environmental factors affect the quality of these drugs. Furthermore, it is recommended to conduct additional studies on the tested products using the three pH levels (pH 1.2, pH 4.5, and pH 6.8) specified by the WHO in order to draw a more precise conclusion regarding the interchangeability of the generic products with the innovator product. The evaluation of generic products' *in-vivo* bioequivalence is also essential.

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Annexes

Annex I: Sample Collection Form

1. *Name of survey site (region, city):* _____

Code given to the sample: _____

Name of retail out let: _____

2. *Name, type and address of collection site/point (specify if the site is private or public, as well as the type, e.g. hospital, clinic, public dispensary, wholesaler, pharmacy, other retail outlet, NGO facility or informal market; in the case of an informal market, please describe):* _____
3. *Commercial name of product:* _____
4. *INN of active ingredients:* _____
5. *List of excipients:* _____
6. *Dosage form (e.g. tablet, capsule):* _____
7. *Strength per unit dose (e.g. mg/tablet):* _____
8. *Type and packaging material (primary container):* _____
9. *Quantity collected per sample, with specification of the package size:*_____
10. *Batch number:* _____
11. *Manufacturing date:* _____
12. *Expiry date:* _____
13. *Name of manufacturer:* _____
14. *Country of origin of manufacturer:* _____
15. *Regulatory status of the product in the country (on the basis of NMRA records), i.e. registered, unregistered or other and, if registered, marketing authorization holder and number:* _____

Date of sample collection: _____

Name of sample collector: _____ Signature: _____

Note: Samples collected must be in their original containers, intact and unopened. Package leaflet must be included.

Annex II: A checklist for visual inspection of medicines in order to identify suspicious products for further examination.

Packaging: Any drug should be packaged in a container, which can be anything from a glass bottle to a blister pack, to a tube of glass, plastic or metal. A folding carton bearing the label very often protects the container. Check the type of packaging & compare it to known containers for the same drug from the same manufacturer. The packaging & the labelling of pharmaceutical products is a very complex and expensive business. Thus, the process & the quality of packaging material are very difficult to counterfeit. This is why a thorough visual inspection could be an important screening step for drug quality control. However, producers of counterfeit drugs are quick to copy special labelling & holograms.

Label: The information written on the label is very important. The information can be printed on a label adhered to the container, or printed directly onto the container itself, but all information must be legible and indelible.

The manufacturer's full address: *All manufacturers are required by international law to print their complete address on the label. Many companies making substandard or counterfeit drugs do not have a traceable address on the label.*

The batch (or lot) number: *Drugs under the same batch/lot number are expected to be equivalent.* In a continuous process, a batch corresponds to a defined portion of the production, based on time or quantity. Drugs from the same batch number should have the same history of manufacturing, processing, packing, and coding. All drug quality control testing should be based on batch/lot numbers.

The date of manufacture and the expiry date: *An expired drug should not be sold under any circumstances.*

Leaflet or package insert: *All drug packages should contain a leaflet explaining dosage, the drug content, the adverse affects, the drug actions, & how the drug should be taken. The only exceptions are where the packaging includes all the information that would otherwise be in the leaflet.*

Physical characteristics of tablets/capsules:

All types of medicines can be and have been counterfeited from cough syrups to injections. As mentioned, it is important to check the packaging of these drugs. Additionally, medicines in the form of tablets or capsules can be checked for signs of moisture, dirty marks, abrasion erosion, cracks, or any other adulteration.

	Yes	No	Other observations
Packaging			
1.1 Container and closure			
Do the container & closure protect the drug fromthe outside environment e.g. properly sealed?			
Do they assure that the drug will meet the properspecifications throughout its shelf life?			
Are the container and the closure appropriate forthe drug inside?			
Is the container safely sealed?			
1.2 Label			
If there is a carton protecting the container, does the label on the carton match the label on the container?			
Is all information on the label legible and indelible?			
1.2.1 Trade Name			
Is the trade name spelled correctly?			
Is the drug (trade name) registered in the country by the DRA (drug regulatory authority)?			
Is the drug legally sold in the country?			
Does the symbol ® follow the trade name?			
1.2.2 The API Name (Scientific name)			
Is the active ingredient name spelled correctly?			
Do the trade name and the active ingredient namecorrespond to the registered drug?			
1.2.3 The Manufacturer's Name & Logo			
Are the manufacturer's name and logo legible and correct?			
Does the logo or hologram (if applicable)look authentic?			
Does it change colour when viewed fromdifferent angles?			
1.2.4 Manufacturer's Full Name			
Is the manufacturer's full address legible and correct?			
Has the company or its agent registered the drug inthe country?			
1.2.5 The drug strength (mg/unit)			
Is the strength - the amount of active ingredientper unit - clearly stated on the label?			
1.2.6 The dosage form (e.g., tablet/capsule)			
Is the dosage clearly indicated?			
Is the indicated drug under this dosage form is registered and authorized for sale in the country?			
1.2.7 The number of units per container			
Does the number of tablets listed on the label matchthe number of tablets stated on the container?			
1.2.8 The batch or lot number			
Does the numbering system on the package correspond to that of the producing company?			
1.2.9 The date of manufacture and the expiry date			
Are the manufacture and expiry dates clearlyindicated on the label?			
1.3 Leaflet or package insert			
Is the package insert printed on the same colored or same quality paper as the original?			
Is the ink on the package insert or packagingsmudge-proof?			
Physical characteristics of tablets/capsules			
2.1 Uniformity of shape			
Are the tablets/capsules uniform in shape?			
2.2 Uniformity of size			
Are the tablets/capsules uniform in size?			

2.3 Uniformity of color			
Are the tablets/capsules uniform in color?			
2.4 Uniformity of texture			
Do the tablets have a uniform coating?			
Is the base of the tablets fully covered?			
Are the tablets uniformly polished, free of powder,and non-sticking?			
2.5 Markings (scoring, letters, etc)			
Are markings uniform and identical?			
2.6 Breaks, Cracks and Splits			
Are the tablets/capsules free of breaks, cracks, splitsor pinholes?			
2.7 Embedded surface spots or contamination			
Are the tablets/capsules free of embedded surface spots and foreign particle contamination?			
2.8 Presence of empty capsules in the case of a sample of capsules			
Is the sample examined free of empty capsules?			
2.9 Smell			
Does the medicine smell the same as the original?			

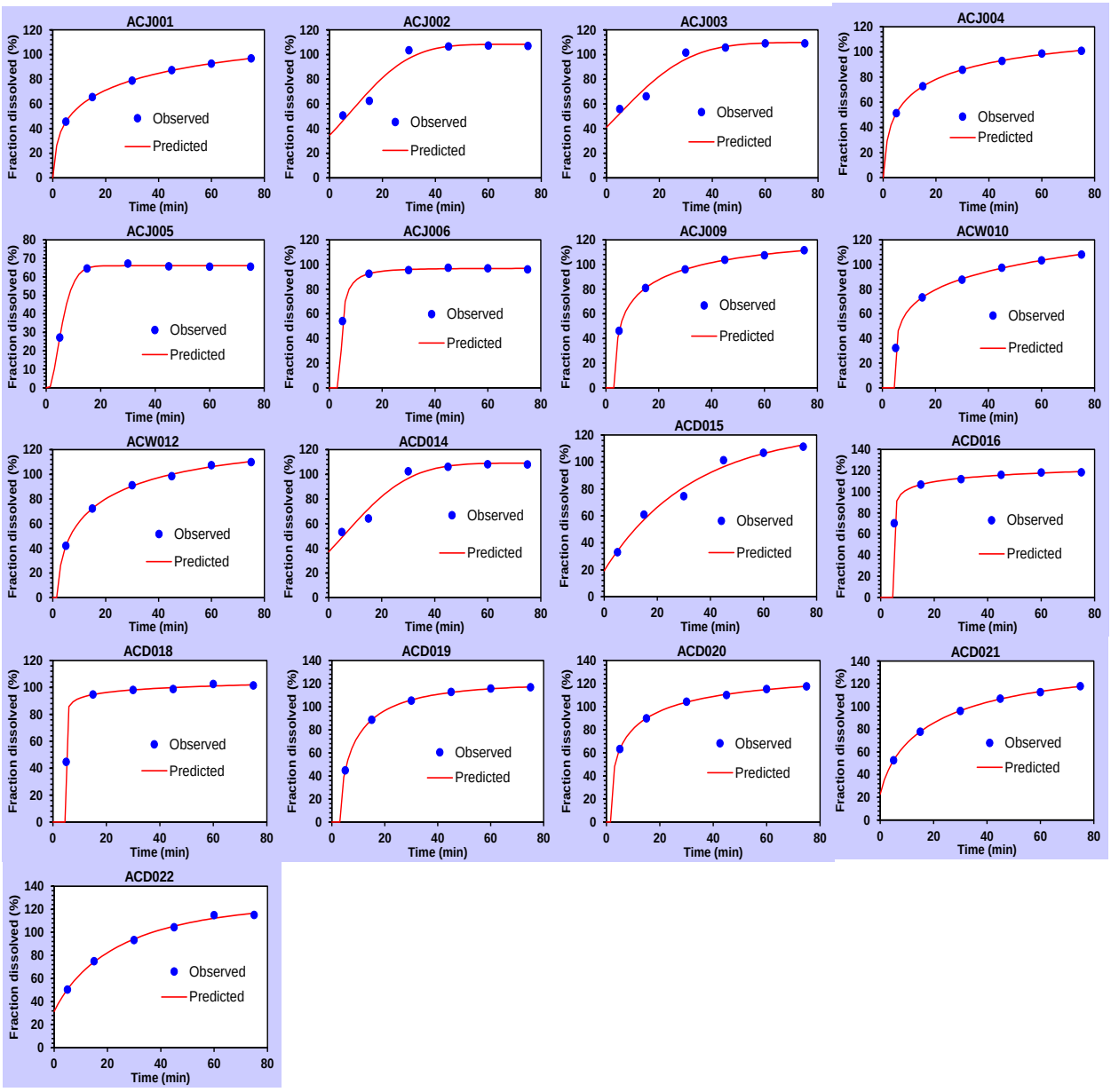
Annex III: Information on samples collected

Product Name	Brand	Batch Number	Manufacturing Date	Expiry Date	Sampling Area	Stated Manufacturer	Country of origin	Sample code
Amoxicillin	Amyn	S36521044	Nov-21	Oct-24	Jijiga	Kopran Limited	India	ACJ001
	Amox	706210538	May-21	May-24	Jijiga	CSPC zhongnuo	China	ACJ002
	Amox	706210550	May-21	May-24	Jijiga	CSPC zhongnuo	China	ACJ003
	Moxvid		Jul-21	Jun-24	Jijiga	Scott Edil	India	ACJ004
	Amox	706210538	May-21	May-24	Jijiga	CSPC zhongnuo	China	ACJ005
	Amox	706210538	May-21	May-24	Jijiga	CSPC zhongnuo	China	ACJ006
	Sinomox	211048	Oct-21	Oct-26	Jijiga	Sinochem	China	ACJ007
	Sinomox	210748	Jul-21	Jul-26	Jijiga	Sinochem	China	ACJ008
	Miloxy	MP21407	Jun-21	May-24	Jijiga	Milan laboratories	India	ACJ009
	Amoxicillin	211120	Nov-21	Nov-24	Togo-Wuchale	Guangdong	-	ACW010
	Sinomox	21074	Jul-21	Jul-26	Togo-Wuchale	Sinochem	China	ACW011
	Kemoxyl	79463	Jul-21	Jun-24	Togo-Wuchale	Laboratory & Allied	Kenya	ACW012
	Sinomox	211048	Oct-21	Oct-26	Togo-Wuchale	Sinochem	China	ACW013
	Amyn	S36521026	Sep-21	Aug-24	Dire Dawa	Kopran Limited	India	ACD014
	Amoxapen	87844	Apr-20	Apr-25	Dire Dawa	Remedica	Cyprus	ACD015[C]
	Amox	706210404	Apr-21	Apr-24	Dire Dawa	CSPC zhongnuo	China	ACD016
	Sinomox	210748	Jul-21	Jul-26	Dire Dawa	Sinochem	China	ACD017
	Amox	706219549	Aug-21	May-24	Dire Dawa	CSPC zhongnuo	China	ACD018
	Amox	706210570	May-21	May-24	Dire Dawa	CSPC zhongnuo	China	ACD019
	Amoxycillin	2020041	-	Feb-26	Dire Dawa	EPHARM	Ethiopia	ACD020
	Miloxy	MP21490	Jul-21	Jun-24	Dire Dawa	Milan laboratories	India	ACD021
	Amucap	2110022	Oct-21	Oct-24	Dire Dawa	North China	China	ACD022
Amoxicillin Clavulanic acid	Clavomid	92285	Feb-21	Feb-23	Jijiga	Remedica	Cyprus	ACTJ023
	Koact	EL5021013A	Mar-21	Feb-23	Jijiga	Aurobindo Pharma	India	ACTJ024
	Amoklavin	A093497	-	Feb-25	Togo-Wuchale	Deva Holding	Turkey	ACTW02
	Bactoclav	BBBBV0004	Jun-21	May-23	Dire Dawa	Microlabs Ltd	India	ACTD026
	Curam	KC6159	Oct-19	Oct-22	Dire Dawa	Sandoz GmbH	Austria	ACTD027
	Augmentin	B25C	Sep-21	Sep-24	Addis Ababa	GSK	UK	C
Ciprofloxacin	Akcipro	ATB070	Mar-21	Feb-24	Jijiga	Akriti pvt. Ltd.	India	CTJ028
	Galcipro	GCDT20038	Dec-20	Nov-23	Jijiga	Galpha Laboratories	India	CTJ029
	Cipoflox	203121691	Sep-20	Aug-23	Jijiga	Ningbo Inopha	China	CTJ030
	Ciproquin	k114210902	Apr-21	Mar-24	Jijiga	Kopran Limited	India	CTJ031

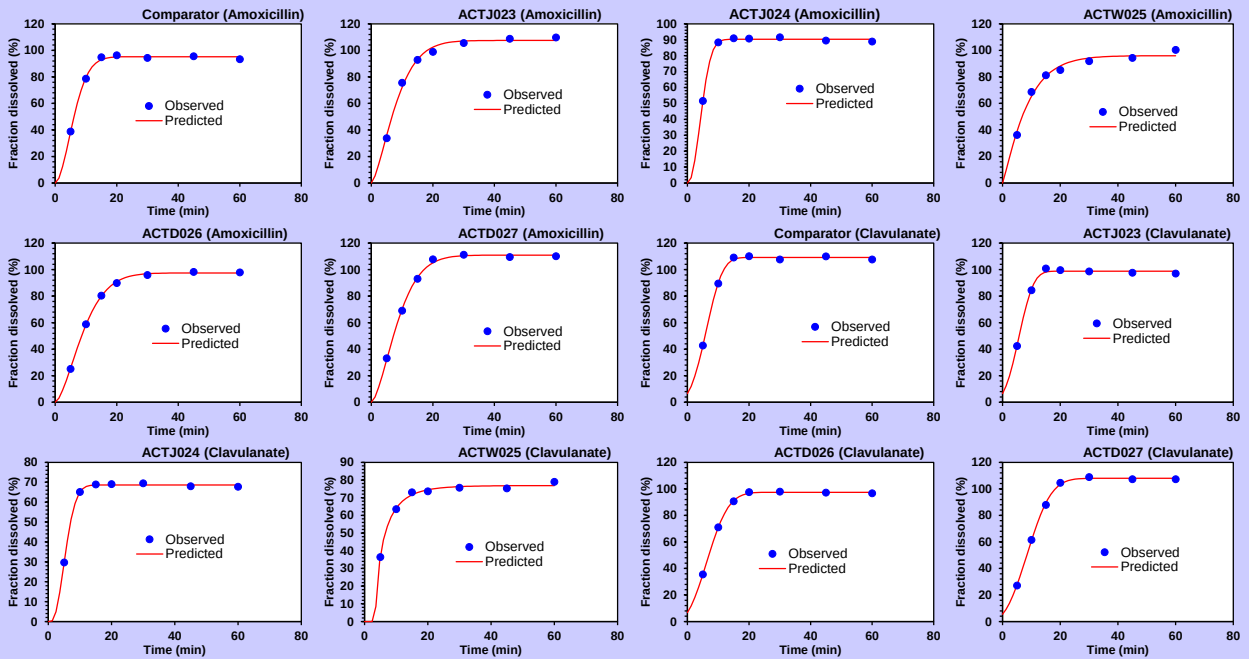
Product Name	Brand	Batch Number	Manufacturing Date	Expiry Date	Sampling Area	Stated Manufacturer	Country of origin	Sample code
	Ciprofloxaci	B200204	Feb-20	Jan-23	Jijiga	Zhejiang Jingxin Ltd.	China	CTJ032
	Ciprobid	T9327	Nov-19	Oct-22	Jijiga	Cadila	India	CTJ033
	Cipoflox	213121053	Mar-21	Feb-24	Togo-Wuchale	Ningbo Inopha	China	CTW034
	Ciprofloxaci	B200201	Feb-20	Jan-23	Togo-Wuchale	Zhejiang Jingxin Ltd.	China	CTW035
	Ciprobid	T9333	Nov-19	Oct-22	Togo-Wuchale	Cadila	India	CTW036
	Ciprobid	T9327	Nov-19	Oct-22	Dire Dawa	Cadila	India	CTD037
	Galcipro	GCDT20031	Dec-20	Nov-23	Dire Dawa	Galpha Laboratories	India	CTD038
	Cipro-	9040313	Apr-19	Apr-23	Dire Dawa	EPHARM	Ethiopia	CTD039
	Ciproquin	k11421042	Feb-21	Jan-24	Dire Dawa	Kopran Limited	India	CTD040
	Ciprofloxaci	B200204	Feb-20	Jan-23	Dire Dawa	Zhejiang Jingxin Ltd.	China	CTD041
	Zindolin	90713	Oct-20	Oct-25	Dire Dawa	Remedica	Cyprus	CTD042
	CiproBay®	BXJBB41	21/06/19	21/06/24	Addis Ababa	Bayer	Germany	C
Norfloxacin 500 mg	Norflo-SSP	D1520110020	11/18/2020	Oct-22	Jijiga	Sansheng	Ethiopia	NTJ043
	Norfloxacin	NT10108	Mar-21	Feb-24	Jijiga	Medicamen Biotech	India	NTJ044
	Norflox	KD102	Apr-21	May-24	Jijiga	East African	Ethiopia	NTJ045
	Norfen	D20008BX64	Jul-20	Jun-23	Jijiga	Cadila	Ethiopia	NTJ046
	Norflo-SSP	D1521030010	3/1/2021	Feb-23	Togo-Wuchale	Sansheng	Ethiopia	NTW047
	Norfen	D20007BX64	Jul-20	Jun-23	Togo-Wuchale	Cadila	Ethiopia	NTW048
	Norflox	KC 111	Mar-21	Feb-23	Dire Dawa	East African	Ethiopia	NTD049
	Norfloxacin	NT10089	Mar-21	Feb-24	Dire Dawa	Medicamen Biotech	India	NTD050
	Norfen	D19010BX64	Jul-19	Jun-23	Dire Dawa	Cadila	Ethiopia	NTD051
	Trizolin	91037	Nov-20	Nov-23	Dire Dawa	Remedica	Cyprus	NTD052[C]
	Gyrablock	A4A002	Jan-20	Jan-23	Dire Dawa	Medochemie Ltd	Cyprus	NTD053
	Gyrablock	A4A002	Jan-20	Jan-23	Dire Dawa	Medochemie Ltd	Cyprus	NTD054

NB: C-Comparator product; Amoxicillin 500 mg was hard gelatin capsule; Amoxicillin/clavulanate 625 mg, Ciprofloxacin 500 mg, and Norfloxacin 400 mg were film coated tablets

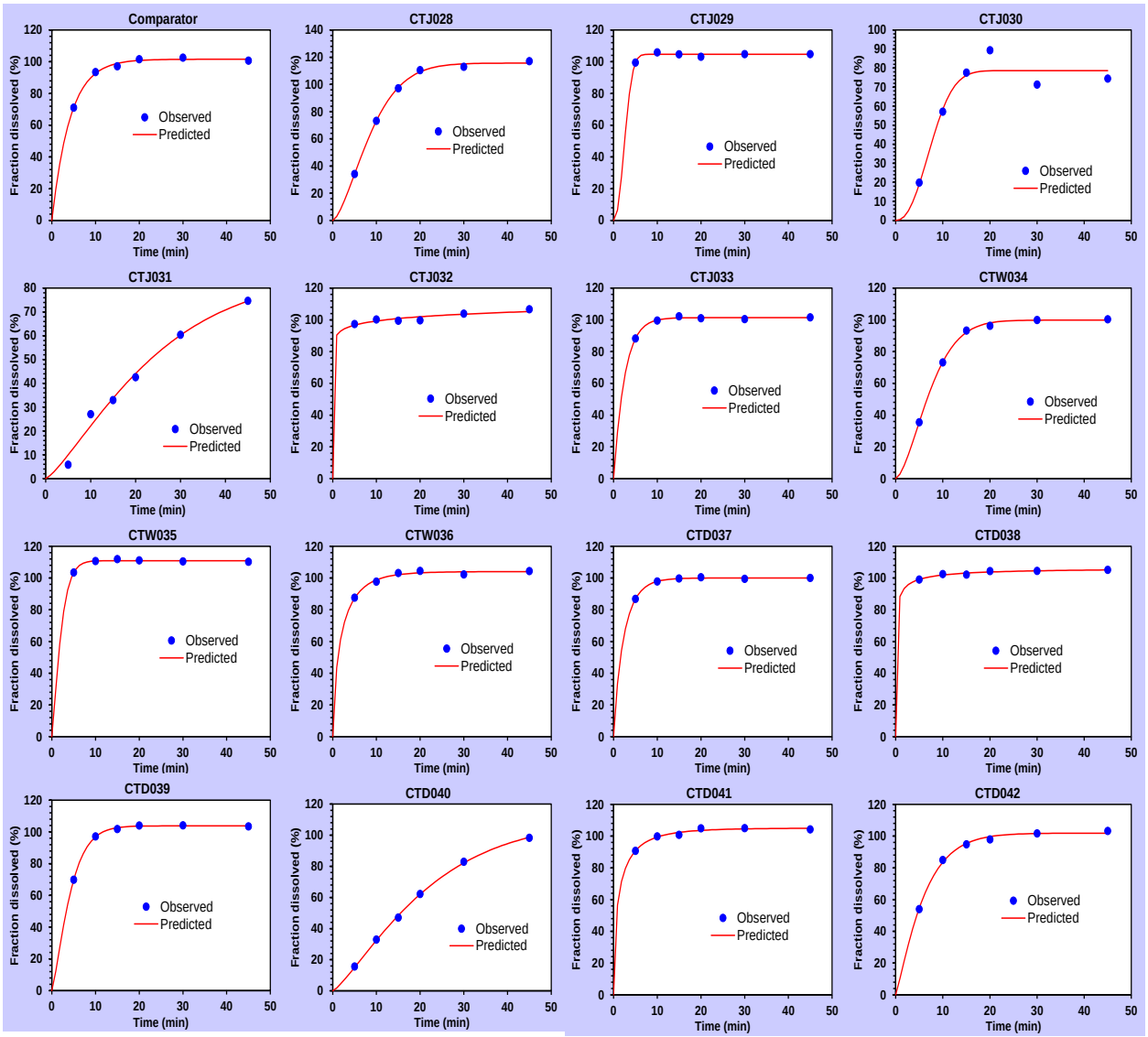
Annex IV: Dissolution profiles of reference and test products amoxicillin predicted for Weibull model (n = 6).



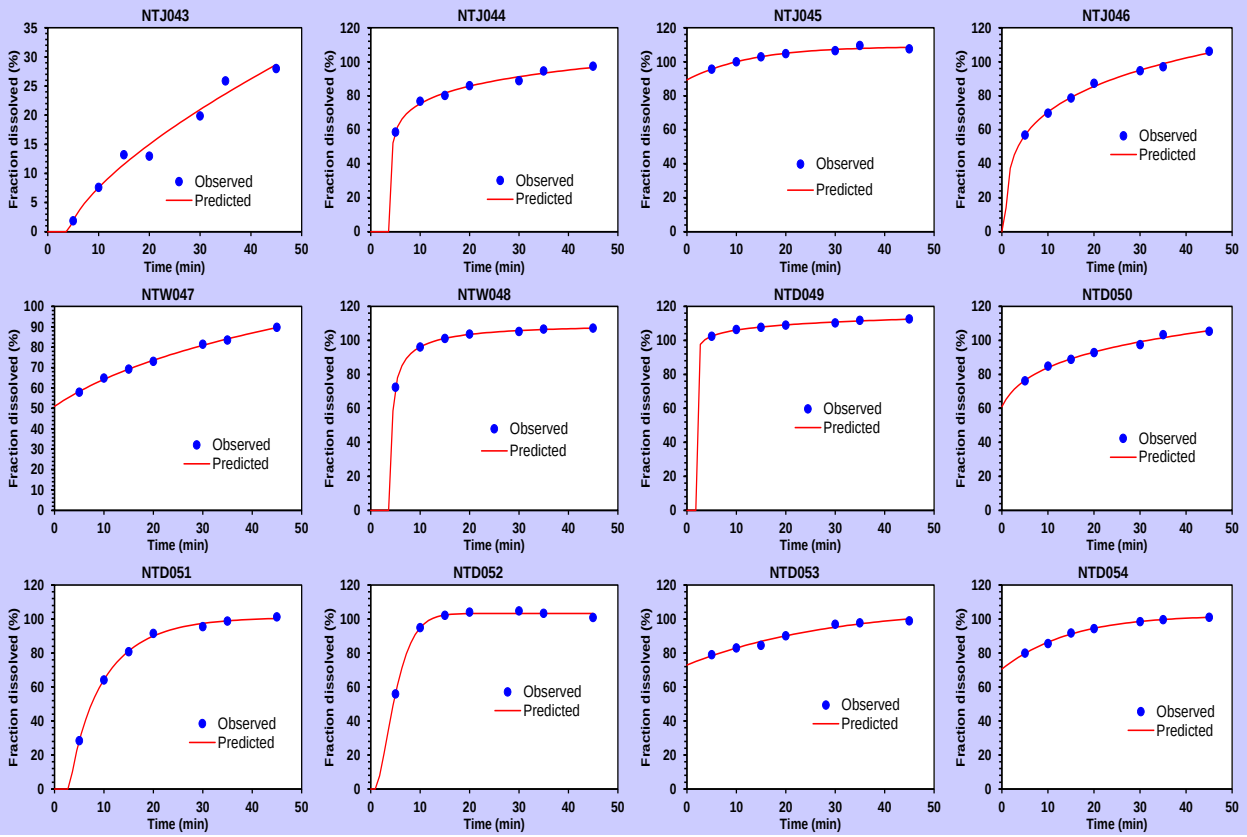
Annex V: Dissolution profiles of reference and test products of amoxicillin/clavulanate predicted for Weibull model (n = 6).



Annex VI: Dissolution profiles of reference and test products of ciprofloxacin predicted for Weibull model (n=6).



Annex VII: Dissolution profiles of reference and test products of norfloxacin predicted for Weibull model (n=6).



Annex VIII: Ethical clearance

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Addis Ababa University

School of Pharmacy
Ethical Review Committee

ቀን Date: September 29, 2021

ቁጥር Ref. No.: ERB/SOP/360/13/2021

To: **Hailu Anjulo**
School of Pharmacy

Re: Ethical Clearance

It is to be recalled that you submitted a research proposal entitled "**Quality Assessment of Antimicrobial Drugs: Amoxicillin Capsules, Amoxicillin+Clavulanate, Ciprofloxacin and Norfloxacin Tablets from Drug Retail Outlets of Eastern Ethiopia.**" The committee thoroughly reviewed the proposal based on its operational guidelines and found that it fulfills all the ethical requirements stipulated in the guidelines. This is, therefore, to inform you that the proposal is ethically approved for implementation.

With best regards,

Shemsu Umer (PhD)
Chairperson, ERC
School of Pharmacy
College of Health Sciences
Addis Ababa University

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