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Post-marketing Quality Surveillance of Selected Antibacterial Agents Marketed in Setit Humera and west Gondar Zones, North West Ethiopia.

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**Post-marketing Quality Surveillance of Selected Antibacterial Agents
Marketed in Setit Humera and west Gondar Zones, North West Ethiopia.**

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This is to certify that the thesis prepared by Tewodros Denekeew Haile, entitled “Post-marketing quality surveillance (PMS) of selected antibacterial agents marketed in Setit Humera and west Gondar Zones, North West Ethiopia” is original and complies with the regulation of Addis Ababa University.

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Abstract

Background: The presence of low-quality medicines is becoming increasingly problematic in many parts of the world, particularly in developing countries. Antibiotics are among the most common anti-infective medicines that are falsified.

Objective: The purpose of this study was to assess the quality of selected antibacterials that are marketed in Setit Humera and west Gondar Zones, North West Ethiopia.

Methods: Seventy-one samples of the antibacterials were collected from six sites in Setit Humera and west Gondar Zones, North West Ethiopia. A mystery shopper collection system was used for sample collection. Visual inspection and confirmation of the registration status were carried out using the WHO/FIP/USP checklist and the EFDA's MRIS database, respectively. Then Pharmacopeial methods (USP, BP) were employed to assess the physicochemical parameters.

Results: From 71 samples, 25.35% (18/71) were not registered and 15.49% (11/71) were registered but the license period was expired i.e not in line with the EFDA's specification. Some (74.65% (53/71) observed samples) did not meet the visual inspection criteria outlined in the joint WHO/FIP/USP checklists. The physicochemical evaluation showed that all the samples studied met the quality specification with respect to the identification and hardness test. From 71 samples tested with regard to assay, dissolution, uniformity of dosage units, disintegration and friability tests, 27.49% (23/71), 16.9%, and 14.08% (10/71), 2.82% (2/71) and 8.57% (3/35) of samples were found to be substandard respectively. Overall, 56.33 % (40/71) of the samples tested were of poor-quality.

Conclusion: The results indicated that some (56.33 %) antibacterial medicines circulating in the market did not meet the required quality. Therefore, the regulatory body (EFDA) should focus on enforcing the necessary measures to ensure that antibacterial drugs on the market meet the required mandatory standards.

Keywords: Antibacterial, poor-quality medicine, Setit Humera, West Gondar, Post-marketing surveillance.

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List of Abbreviations and Acronyms

API	Active Pharmaceutical Ingredient
BP	British Pharmacopoeia
EFDA	Ethiopian Food and Drug Authority
FIP	International Pharmaceutical Federation
GMP	Good manufacturing practice
HPLC	High-Performance Liquid Chromatography
IR	Infrared Spectroscopy
MRIS	Medicine Registration Information System
NMRA	National Medicine Regulatory Authority
QC	Quality control
TLC	Thin Layer Chromatography
USP	United States Pharmacopoeia
UV–Vis	Ultraviolet-visible spectroscopy

1. Introduction

1.1. Background

The term antibiotic was derived from the Greek word antibiosis, which means against life. Antibiotics are generally classified as antibacterial, antifungal, or antivirals (Etebu and Ariekpar, 2016; Verduin-Muttiganzi, 1998). They are strong and effective treatments in the fight against infectious diseases brought on by bacteria (Jayakar *et al.*, 2011). Antibiotics can also be defined as medicines used for treating infectious diseases such as bacterial infections, fungal infections, and some parasitic infections, but unwise use may contribute to resistance development and poor management of infectious diseases (Endale *et al.*, 2013). Antibiotics are life-saving medicine thus an ideal antibiotic therapy is a prerequisite to treating the systemic spread of infection and preventing its complications (Khan and Khar, 2015).

Antibiotics are among the most counterfeited anti-infectious medicines in developing countries (Koech *et al.*, 2020). Low-quality antibiotics also increase the risk of microorganism resistance, treatment failure, loss of trust in healthcare systems, and the associated socioeconomic consequences (Bizimana *et al.*, 2022).

The term post-marketing surveillance (PMS) study denotes a scientifically rigorous investigation of a product that has been given national registration approval with the goal of generating accurate data about drug safety (Yadav, 2008). Continuous post-market quality evaluation contributes to the production of clear information on the current quality status of various brands of a given drug, introducing a biopharmaceutical and therapeutically equivalent list of products to prescribers and users (Abraham *et al.*, 2021).

Universal definition of quality includes conformity to identity, purity, and other requirements as well as the suitability of medicines and products for their intended uses, which is established by their efficacy and safety, adhering to label claims, or as advertised

or promoted (Chavan *et al.*, 2018). The International Conference on Harmonization defines pharmaceutical drug quality as a substance's or product's suitability for use, including attributes like identity, strength, and purity, without any unintended side effects (Haleem *et al.*, 2015). Medicines may be as old as mankind, and ideas about how to ensure their quality have gradually evolved over time (Rägo and Santoso, 2008).

According to the International Standard of Organization (ISO), quality control refers to the operational techniques and activities used to meet quality standards. All requirements are satisfied since the producer conducts in-process quality control on the entire product. The requirement that the product is safe is the most crucial one (Chavan *et al.*, 2018). Quality control (QC) is also the part of GMP that is concerned with sampling, specifications, testing, documentation, and release procedures which ensure that the necessary and relevant tests are carried out and that materials are not released for use sale, or supply until their quality has been judged to be satisfactory according to specification (Uddin *et al.*, 2016). Poor-quality medicines are a growing global problem since they have a reduced amount of active drugs, and the majority of them are manufactured in India, China, and Sub-Saharan Africa (Kelesidis and Falagas, 2015).

1.2. Statement of the problem

The WHO estimates that approximately 10% of all global pharmaceutical supply is fake and of poor-quality reaching up to 50% of the supply in developing countries and as low as 1% in the developed world (Alghannam *et al.*, 2014). The marketing of poor-quality drugs is high especially in Africa and Asia because of weak drug regulatory systems. Thus the quality of marketed drug products cannot be guaranteed (Wertheimer *et al.*, 2003). Many countries in sub-Saharan Africa are highly affected by infectious diseases. Even if these countries account for only 12% of the world population, they account for 50% of deaths due to infectious diseases. The high rate of infectious diseases morbidity is a factor inciting traffickers of counterfeit drugs to sell their products in these countries. Ethiopia is one of the destination countries for counterfeit antibiotics (Delepierre *et al.*, 2012). According to the U.S. Food and Drug Administration (FDA), up to 25% of all antibiotics

in sub-Saharan African countries have a low quality (Delepierre *et al.*, 2012). In Ethiopia, there is inadequate PMS and regulatory enforcement and lack of comprehensive study about the problem (Belachew *et al.*, 2021). Amoxicillin, amoxicillin-clavulanate potassium, and ciprofloxacin are the most widely prescribed antibiotics in Ethiopia for the control of common bacterial species (Endale *et al.*, 2013; Yimenu *et al.*, 2019).

Despite the considerable use in Ethiopia, there are not enough studies conducted on overall quality and comparative in vitro quality evaluation on different brands of these drugs that are marketed in Setit Humera and west Gondar Zones, North West Ethiopia. Therefore, the study assessed the quality of selected antibacterial agents marketed in Setit Humera and west Gondar Zones. It assesses the presence of poor-quality drugs which is a very important assessment to confirm the presence of poor-quality products, especially at the border of entry Setit Humera and west Gondar Zones the place where the smuggling of such products is suspected to be very high. The study sites are geographically located as borders for Sudan and Eritrea.

1.3. Research questions

The purpose of the assessment was to answer the following questions: -

- ✓ Are exist non-registered drugs in the study area?
- ✓ Are low-quality medicines available in the Setit Humera and West Gondar Zones of North West Ethiopia?
- ✓ What percentage of the drugs tested failed quality test parameters?

1.4. Hypothesis

Null hypothesis: The selected antibacterial agent samples collected from the study area will pass the required quality control test.

Alternative hypothesis: The samples of selected antibacterial agents collected from the study area do not pass the quality control test parameters.

1.5. Significance of the study

Low-quality medicines are common in developing countries, but they cause problems around the world (Alghannam *et al.*, 2014). To aid in the improvement of the healthcare system, there is a need for reliable, unbiased research on the prevalence of low-quality medicines in developing countries. The global production, distribution, and consumption of low-quality antibiotics pose a significant public health risk to the global drug supply chain system. It poses a particularly serious threat to developing countries (Mackey and Nayyar, 2017). Because there have been no previously published studies that have reported the quality of antibacterials in the study area, this study will aid in determining the scope of the problem in the study site as well as the country. This research will have far-reaching implications for various organizations and the community at large. The findings of this study will serve as reference material for other researchers in the field, as baseline data for future research on the subject, and as evidence to inform policymakers. As a result, the Federal Ministry of Health, the Ethiopian Food and Drug Authority (EFDA), and the Customs and Revenue Authority will all benefit directly from this research. Furthermore, it might deliver consciousness to the community, health professionals, and local governments thus it will help them to design ways to proactively detect poor-quality medicines and take appropriate measures towards securing the market and the supply chain.

2. Literature review

2.1. Antibiotic quality assessment

Antibiotics are the most counterfeited medicines, accounting for 28% of all counterfeit medications worldwide. Antibiotic counterfeiting is estimated to account for 5% of the global antibiotic market. Antibiotic counterfeiting appears to be widespread throughout the world. However, these are uncommon in developed countries, whereas the strong demand for antibiotics in emerging countries creates a highly attractive market for counterfeiters. As a result, South-East Asia is the source of 78% of all counterfeit

antibiotics, and the majority of their destinations are in developing countries (44% in South-East Asia, 30% in Sub-Saharan Africa, 9% in Europe, and 16% in other countries) (Delepierre *et al.*, 2012). In developing nations including Ethiopia antibiotics are the most commonly counterfeited pharmaceutical products, particularly those used to treat patients with fatal diseases (Harper, 2006).

According to the World Trade Organization, India manufactured 54% of counterfeit drugs confiscated and recorded by customs worldwide in 2006, confirming its leadership in the manufacture of counterfeit drugs (Patrignani *et al.*, 2009). China comes in second, accounting for 21% of global production of low-quality drugs. China's production appears to be more sophisticated than India's. Low-quality drugs account for 10% to 30% of all drugs sold in developing countries (United Nations Office on Drugs and Crime, 2010). According to a WHO study of drug product quality in African developing countries, 7.6% of major antibiotic formulations contained no active ingredient (Kelesidis and Falagas, 2015).

Falsified antibiotics cause hundreds of deaths every year worldwide, especially in South-East Asia and in Sub-Saharan Africa (Delepierre *et al.*, 2012). Falsified antibiotics directly promotes the emergence of acquired bacterial resistance against antibiotics (Kelesidis *et al.*, 2007). Thus, falsified drugs are to blame for patients' mistrust of real medications, the pharmaceutical industry, and public health authorities in addition to the financial costs of stealing market share from real medicines [World Health Organization, 2000].

Even though the pharmaceutical industry invests heavily in the development of tools or technologies for the prevention and detection of SF drugs, as well as procedures to assist healthcare professionals and patients in distinguishing between real and falsified drugs, the international collaboration required to ensure the application and development of such mechanisms is frequently lacking. Developing countries are also concerned about a lack of awareness about the dangers that purchasing falsified drugs may expose them to (Mondiale de la Santé, 2012).

Reasons for low-quality drugs include widespread counterfeiting of medicines in less developed countries, excessive decomposition of active ingredients as a result of high temperature and humidity, and poor-quality assurance during medicinal product manufacturing. Thus, low-quality drugs may contribute to global microbial resistance, increased burden for health workers, therapeutic failure and pose a serious threat to human health (Abraham *et al.*, 2021 ; Taylor *et al.*, 2001). Antibiotic resistance is currently one of the most pressing issues in global public health. As a result, a better understanding of the existing evidence on antibiotic knowledge and attitudes in the general population worldwide is recommended (Gualano *et al.*, 2015) and it is important to regularly evaluate the pharmaceutical quality of medicines. Low-quality drugs can be detected by using various methods such as inspection, colorimetric methods, chromatography, and spectrometry (Kelesidis and Falagas, 2015).

3. Objectives

3.1. General Objective

- To assess the quality of selected antibacterial agents that are marketed in Setit Humera and west Gondar Zones, North West Ethiopia.

3.2. Specific Objectives

- ✓ To assess the registration status of different brands of the selected medicines.
- ✓ To evaluate the physicochemical quality status of the selected medicines.
- ✓ To determining the prevalence of poor-quality medicines in the study area.

4. Materials and methods

4.1. Description of the study area and study period

The study was conducted in Setit Humera and west Gondar Zones, North West Ethiopia from August to September 2021. The sample sites include Humera, Mai Kadra, Metema, Gendawuha, Kokit, and Midre Genet towns. Both zones are located in the Amhara regional state of Ethiopia. Setit Humera is a zone bordering Sudan and Eritrea. Because of their geographical location, both Setit Humera and West Gondar Zones have the potential for medicine smuggling and illegal border trade. During data collection, there were 47 health facilities (7 hospitals: 3 from West Gondar zones, 4 from Setit humera zones, and 40 health centers: 18 from West Gondar zones, 22 from Setit humera zones) and 57 private drug retail outlets (1 pharmacy, 34 drug stores from West Gondar zones, and 22 drug stores from Setit humera zones) in the study area where samples were collected.

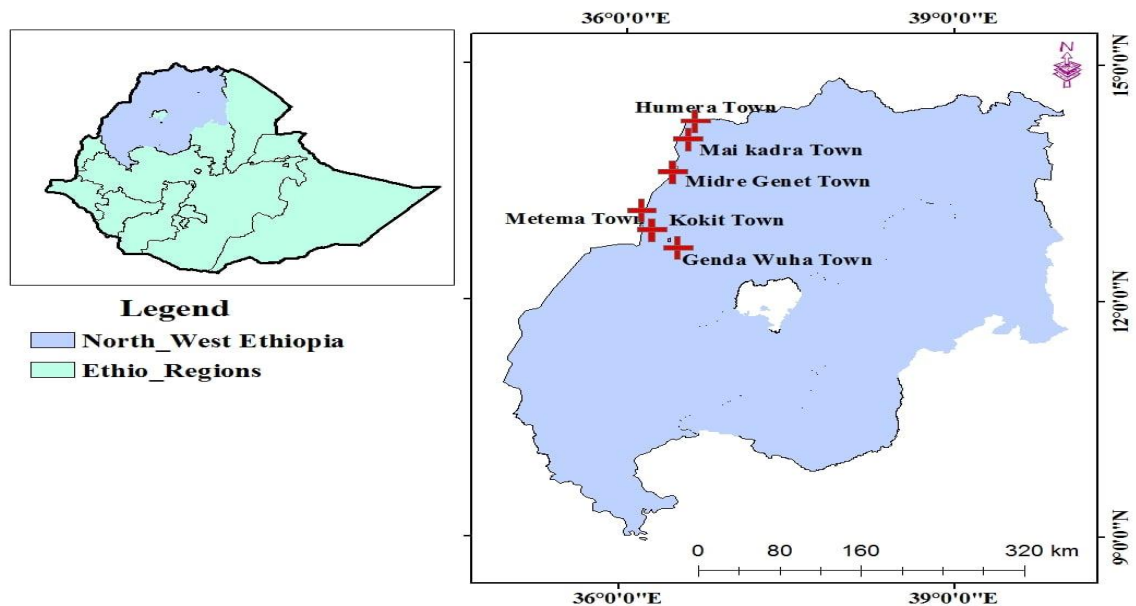


Figure 1: Map of the study area (Boundaries <https://data.humdata.org/m/dataset/cod-ab-eth>)

4.2. Materials/Reagents/Chemicals

The following instruments were used in the experiments: HPLC (Shimadzu, Japan) Instrunment ID: PHY-HPLC 012, column (25 cm × 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography (5 µm) (Nucleosil 120-C18 and LiChrospher 100 RP 18, Germany), Ultrasonic cleaner (S. No 0083311134PO04, Dahan scientific Co., Ltd, Korea), pH meter (S. No 31092, Biby scientific Ltd. Co., UK), shaker (S. No 100077495220240V, Germany), UV-VIS detector (S. No. L20145302582AE, Shimadzu Corporation, Japan), UV- VIS spectrophotometer (S. No. 50038, UK), Disintegration apparatus (model DIST, S.no 241002), Erweka dissolution tester apparatus (S. No. 135658-2217, Germany), Caleva hardness tester (model THT2, S.No. 111436), Erweka friability tester (S. No. 101821, Germany). Pharmaceutical standards USP amoxicillin RS (Lot No. R106H0, Spain with potency of 86.9%), USP clavulanate lithium RS (Lot No. R071W0, India with potency of 96.9%), USP ciprofloxacin RS (Lot No. R05170, Germany with potency of 98%), USP doxycycline RS (Lot No. R065H0, Portugal with potency of 98%), HPLC grade methanol (B. No. MH209, made in India) and glacial acetic acid (B. No. STBG7804, made in India) were used.

4.3. Sample size and sampling technique

Depending on the type, nature, and purpose of the study, a convenience sampling technique was used to identify sample collection sites based on WHO technical report series No. 996, 2016. The Zones of Setit Humera and West Gondar were chosen because they are extremely vulnerable to drug smuggling and penetration into the country. The samples were collected from 15 private drug stores, 3 health center pharmacies, and 1 hospital pharmacy. All samples were purchased from governmental and private drug retail outlets at six sites in both zones. From all sites, a total of 71 samples of amoxicillin capsules, amoxicillin and clavulanate potassium tablets, ciprofloxacin tablets, and doxycycline capsules were collected based on the convenience sampling technique. Out of 71 samples, 26 samples were amoxicillin capsules, 12 samples were amoxicillin and clavulanate potassium tablets, 23 were ciprofloxacin tablets, and 10 samples were doxycycline ca-+

psules.

The prescription papers were obtained from the University of Gondar, and the samples were collected using a mystery shopping sampling technique. The collected samples for the study were coded (amoxicillin = A, amoxicillin and clavulanate potassium = AU, ciprofloxacin = C, and doxycycline = D), labeled, and stored under the storage conditions specified on the label.

4.4. Inclusion criteria

- All capsule dosage forms of amoxicillin and doxycycline.
- All tablet dosage forms of amoxicillin and clavulanate potassium and ciprofloxacin.

4.5. Exclusion criteria

- The same product with a similar batch number at the same sample collection site was excluded from this study.

4.6. Background of medicines assessed from different sites

Table 1 shows that 71 samples of four antibiotics (amoxicillin, amoxicillin and clavulanate potassium, ciprofloxacin, and doxycycline) were collected from Setit Humera and west Gondar Zones including Humera, Mai kadra, Midre-genet, Gendwiha, Kokit, and Metema towns. The samples were collected from private drug retail outlets 76.06% (54/71) and governmental health facilities 23.9% (17/71). Two of these medicines were in tablet form, while the other two were in capsule form. Table 1 also includes the dosage forms, number of brands, and number of medicine samples collected in the study area.

Table 1: Type of dosage form, number of brands, and number of samples collected, in Setit Humera and west Gondar Zones

S/NO	Medicine type	Medicine form	dosage	Strength of samples	Number of brands	The number of samples collected	Sample collected sources		
							Community pharmacy	Hospital pharmacy	Health center
1	Amoxicillin and clavulanate potassium	Tablet		625mg	04	09	06	01	02
				375mg	02	03	01	01	01
2	Amoxicillin	Capsule		500mg	03	19	14	02	03
				250mg	02	07	05	-	02
3	Ciprofloxacin	Tablet		500mg	08	21	17	02	02
				250mg	01	02	01	-	01
4	Doxycycline	Capsule		100mg	04	10	10	-	-
Total					24	71	54	06	11

4.7. Quality assessment test methods

4.7.1. Visual inspection

This test includes a physical description of a drug to evaluate its size, shape, color, breaks, cracks, splits, surface spot or contamination, packing information (medicinal strength, dosage statement, batch/lot number, manufacture, expiry date, storage information), and leaflet (Allen, 2013b). For this study by using WHO/FIP/USP checklist the physical properties of the dosage form, packaging, and labeling information were investigated (Anisfeld, 2006, Bizimana *et al.*, 2022). All samples were visually inspected for the presence of a trading name, active ingredient name, manufacturer's name, and full address. Each collected sample's registration status was checked using the registration database, which is the EFDA's medicine registration information system.

4.7.2. Identification test

To confirm the identity of the pharmaceutical active ingredient (API) in the pharmaceutical products, this test was carried out. This test should be able to recognize substances with structurally similar compounds (Allen, 2013a).

4.7.2.1. Identification of amoxicillin

Identification of amoxicillin was performed according to BP methods (BP, 2019).

Infrared absorption: A portion of the capsule's contents containing the same amount of amoxicillin as 0.5 g in 5 mL of water for 5 minutes was filtered, the residue was swilled with absolute ethanol and then ether, and it was dried for an hour at a pressure no greater than 0.7 kilopascal.

Acceptance criteria: The infrared absorption spectrum of the amoxicillin sample matched that of the amoxicillin reference standard (BP, 2019).

4.7.2.2. Identification of amoxicillin and clavulanate potassium

Identification of amoxicillin and potassium clavulanate were performed according to USP 2020 and BP 2019 methods.

Identification by retention times: The chromatogram of the assay preparation's major peaks for amoxicillin and potassium clavulanate corresponds to the chromatogram of the standard preparation.

Identification by thin layer chromatography (TLC)

Preparation of sample solution: Shake a quantity of the powdered tablets containing the equivalent of 0.4 g of clavulanate potassium in 100 mL of a mixture of 4 volumes of methanol and 6 volumes of 0.1 M mixed phosphate buffer pH 7.0 and filter. 0.4 g to 0.1 g and 100 mL to 25 mL.

Preparation of 0.1 M mixed phosphate buffer pH 7.0: Dissolve 6.805 g of potassium dihydrogen phosphate in water and dilute to 500 mL with the same solvent. Adjust the pH using a 35 g/L solution of disodium hydrogen phosphate dodecahydrate.

Preparation of standard solution: 0.8% w/v of amoxicillin and 0.4% w/v of clavulanate potassium mixture: 40 mg of the amoxicillin USP reference standard and 20 mg of USP clavulanate potassium reference standard powder were weighed and transferred into 25 mL volumetric flask of in mixture of 4 volume of methanol and 6 volumes of 0.1 M mixed phosphate buffer pH 7.0 to produce a known concentration of 1.6 mg/mL of amoxicillin and 0.8 mg/mL of clavulanate potassium.

Preparation of mobile phase: In a mixed phosphate buffer pH 4.0, 1 volume of butan-1-ol, 2 volumes of a 0.1% w/v solution of disodium edetate, 6 volumes of glacial acetic acid, and 10 volumes of butyl acetate were mixed.

Preparation of phosphate buffer pH 4.0, mixed: Weighed 5.04 g of disodium hydrogen orthophosphate and 3.01 g of potassium dihydrogen orthophosphate were dissolved in sufficient water to produce 1000 mL and adjusted the pH with glacial acetic acid.

Preparation of 0.1% w/v solution of disodium edetate: weighed 500 mg of disodium edetate and dissolved in 500 mL of mixed phosphate buffer pH 4.0.

Confirmation: The principal spots in the chromatogram obtained with the sample solution correspond in position and color to those in the chromatogram obtained with a standard solution.

4.7.2.3. Identification of ciprofloxacin

Identification of ciprofloxacin samples were performed according to USP 2020 and BP 2019 methods.

Identification by retention times: The chromatogram of the assay preparation's obtained ciprofloxacin principal peak retention times match those of the standard preparation's chromatogram.

4.7.2.4. Identification of doxycycline

Identification of doxycycline samples were performed according to BP 2019 methods.

Identification by chemical reaction: Weighed 5 mg of the contents of the capsules powder and added 10 mL of sulphuric acid.

Confirmation: A yellow color was produced.

4.7.3. Assay

This test, also known as a content test, determines the strength or content of the API in the pharmaceutical product (Chavan *et al.*, 2018). Products should be manufactured in such a way that the API is present in the specified amount, ensuring the active ingredient content, which is indicative of the product's efficacy and stability (Vipul and Devesh, 2012). The assay was performed by HPLC for all investigated medicines per the respective monographs of the USP 2020 and BP 2019. The contents of the API of all amoxicillin and doxycycline were determined using HPLC according to BP specifications, and amoxicillin and clavulanate potassium, and ciprofloxacin samples were performed using HPLC according to both BP 2019 and USP 2020 specifications based on their label claim.

4.7.3.1. Assay for amoxicillin

Construction of calibration curve for assay of amoxicillin: A stock solution (200%) was prepared by dissolving 70 mg of amoxicillin USP RS in 50 mL of mobile phase A. From this stock solution, five different concentration levels 0.84 mg/mL (120%), 0.77 mg/mL (110%), 0.7 mg/mL (100%), 0.63 mg/mL (90%), and 0.56 mg/mL (80%), were prepared by diluting (6, 5.5, 5, 4.5, and 4) mL to 10 mL respectively in mobile phase A. Then concentrations of amoxicillin against the peak area were plotted to obtain the calibration curves.

Mobile phase preparation: A filtered mixture of 400 mL of mobile phase B, and 1840 mL of mobile phase A was mixed in a ratio of 8:92 respectively.

Mobile phase A: A filtered mixture of 20 mL of acetonitrile and 1980 mL of a 25% v/v solution of 0.2 M potassium dihydrogen orthophosphate were mixed and adjusted to pH 5.0 with 2 M sodium hydroxide (Acetonitrile and Buffer) (1:99).

Mobile phase B: A filtered mixture of 400 mL of acetonitrile and 1600 volume of a 25% v/v solution of 0.2 M potassium dihydrogen orthophosphate were mixed and adjusted to pH 5.0 with 2 M sodium hydroxide.

Sample preparation (1): Approximately 30 mg of amoxicillin equivalent to 20 mixed powder amoxicillin capsules were transferred to a 50 mL volumetric flask, and 40 mL of mobile phase A was added. Mixed with the aid of ultrasound for 1 minute, and added sufficient mobile phase A to produce 50 mL, and mixed. After that, the solution was filtered through 0.45 µm filter paper, and the clear filtrate was used as the assay preparation. Equal volumes of the standard preparation and the assay preparation were separately injected into the chromatograph, the chromatograms were recorded, and the responses were measured for the major peaks.

Preparation of 0.0004% w/v of cefadroxil USP RS and 0.003% w/v of amoxicillin trihydrate USP RS (2): Weighed 4 mg of cefadroxil USP RS and dissolved in 100 mL of mobile phase A. Transfer 10 mL stock solution to 100 mL volumetric flask then added 3 mg of amoxicillin trihydrate USP RS powder, mixed and dissolved in mobile phase A to produce 0.004 mg/mL cefadroxil BPCRS and 0.03 mg/mL amoxicillin trihydrate USP RS.

Preparation 0.070% w/v of amoxicillin trihydrate USP RS (3): Weighed 17.5 mg amoxicillin trihydrate USP RS and dissolved in 100 mL of mobile phase A to produce 0.7 mg/mL of amoxicillin trihydrate USP RS.

Chromatographic systems/conditions: The liquid chromatography was equipped with a 254 nm UV detector and used a stainless steel column (25 cm × 4.6 mm) packed with octadecylsilyl silica gel for chromatography (5 µm) was used. The inject volume and flow rate was 50 µL and 1 mL per minute respectively.

Determination of content: Calculated the content of $C_{16}H_{19}N_3O_5S$ in the capsules from the chromatograms obtained and from the declared content of $C_{16}H_{19}N_3O_5S$ in amoxicillin trihydrate USP RS.

4.7.3.2. Assay for amoxicillin and clavulanate potassium

Assay by USP methods

Preparation of different concentrations for calibration curve /linearity: 50 mg of the amoxicillin USP reference standard and 20 mg of USP clavulanate reference standard powders were weighed and transferred into 50 mL volumetric flask and made up to mark with distilled water and (3 mL, 4 mL, 5 mL, 6 mL and 7 mL) of stock solution was transferred into a 10 mL volumetric flask to prepare 60% (0.3 mg/mL), 80% (0.4 mg/mL), 100% (0.5 mg/mL), 120% (0.6 mg/mL) and 140% (0.7 mg/mL) respectively.

Preparation of the buffer solutions: Monobasic sodium phosphate (15.6090 gm) was dissolved in 1800 mL of water and adjusted with phosphoric acid to a pH of 4.4 ± 0.1 and was diluted with water to produce 2000 mL (USP, 2020).

Preparation of mobile phase: Prepared from a filtered mixture of 100 mL of methanol and 1950 mL of buffer (1:19).

Preparation of standard solution: 0.5 mg/mL of amoxicillin and 0.2 mg/mL of clavulanate potassium mixture: 25 mg of the amoxicillin USP reference standard and 10 mg of USP clavulanate reference standard powder were weighed and transferred into 50 mL volumetric flask and makeup to mark with distilled water to produce a concentration of 0.5 mg/mL of amoxicillin and 0.2 mg/mL of clavulanate.

Preparation of sample stock solution: Five tablets containing 500/125 mg and 250/125 (amoxicillin and clavulanate potassium) each were mixed and dissolved in a 2000 mL and

1000 mL volumetric flask respectively with water using a shaker to give a concentration of 1.25 mg/mL amoxicillin and 0.625 mg/mL clavulanate.

Preparation of sample solution: 10 mL of sample stock solution in combination (amoxicillin and clavulanate potassium) was transferred into a 25 mL volumetric flask, mixed, and made to volume with distilled water, given a concentration of 0.5 mg/mL amoxicillin and 0.625 mg/mL of clavulanate. The solution was sonicated by using an ultrasonic bath to remove air bubbles and kept in a vial bottle and injected into the HPLC machine after filtering in a syringe filter.

Chromatographic system: The liquid chromatography was equipped with a 220 nm UV detector and used a stainless-steel column: 4 mm × 30 cm, 3 to 10 µm packing L1 was used. The inject volume and flow rate was 20 µL and 1.8 mL per minute respectively.

The formula used to calculate the active content and percentage content of the tablets using HPLC chromatogram is shown: Samples: Standard solution and sample solution.

$$\text{Results} = \left(\frac{r_u}{r_s} \right) \times \left(\frac{C_s}{C_u} \right) \times 100 \dots\dots\dots \text{Equation 1.}$$

Where:

C_s = concentration of a standard solution (mg/mL)

C_u = nominal concentration of sample solution (mg/mL)

r_u = peak area of samples solution

r_s = peak area of standards solution

Assay by BP methods

Preparation of different concentrations for calibration curve /linearity: Acurately 50 mg of the amoxicillin USP reference standard and 20 mg of USP clavulanate reference standard powder were weighed and transferred into 50 mL volumetric flask and made up

to mark with distilled water and from this were taken 3 mL, 4 mL, 5 mL, 6 mL and 7 mL of stock solution in combination (amoxicillin and clavulanate potassium) was transferred into a 10 mL volumetric flask to prepare 60% (0.3 mg/mL), 80% (0.4 mg/mL), 100% (0.5 mg/mL), 120% (0.6 mg/mL) and 140% (0.7 mg/mL) respectively. Then concentrations of amoxicillin and clavulanate potassium against peak height were plotted to obtain the calibration curves.

Preparation of the buffer Solutions: Sodium dihydrogen orthophosphate monohydrate (17.76 gm) was dissolved in 1800 mL of water and adjusted with adjusted to pH 4.4 with orthophosphoric acid and diluted with water to produce 2000 mL (BP, 2019).

Mobile phase: A filtered mixture of 100 mL of methanol and 1900 mL of buffer was mixed in the ratio (5:95).

Preparation of standard solution: An accurately weighed 50.15 mg of USP amoxicillin RS and 20.04 mg of potassium clavulanate were dissolved in 100 mL of water. The solution was sonicated to dissolve completely and 5.0 mL of the solution was taken into a 10 mL volumetric flask and diluted to 10 mL with water and mixed to obtain a solution having a known concentration of about 0.5 mg/mL amoxicillin trihydrate USP RS and 0.2 mg/mL of lithium clavulanate USP RS.

Sample preparation: About 20 tablets were weighed and finely powdered. An accurately weighed portion of the powder, equivalent to about 125 mg of amoxicillin and potassium clavulanate powder was transferred to a 200 mL volumetric flask. Sonicated until the powder has dissolved and added sufficient water to produce 250 mL, mixed and filtered.

Chromatographic system: The liquid chromatography was equipped with a 220 nm UV detector and used a stainless-steel column: (25 cm × 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography was used. The inject volume and flow rate was 20 µL and 2 mL per minute respectively. The Formula used to calculate the active content

and percentage content of the tablets using HPLC chromatogram is shown:
Samples: Standard solution and sample solution.

$$\text{Results} = \left(\frac{r_u}{r_s} \right) \times \left(\frac{C_s}{C_u} \right) \times 100 \dots\dots\dots \text{Equation 2}$$

Where:

Cs = concentration of a standard solution (mg/mL)

Cu = nominal concentration of sample solution (mg/mL)

ru = peak area of sample solution

rs = peak area of standard solution.

4.7.3.3. Assay for ciprofloxacin

Assay by USP methods

Preparation of different concentrations for calibration curve /linearity: To prepare 80% (0.16 mg/mL), 90% (0.18 mg/mL), 100% (0.2 mg/mL), 110% (0.22 mg/mL), and 120% (0.24 mg/mL) of the ciprofloxacin hydrochloride USP reference standard, 40.3 mg of the ciprofloxacin hydrochloride USP reference standard was weighed, transferred into a 100 mL volumetric flask, and made up to the mark with to create the calibration curves, ciprofloxacin hydrochloride concentrations were then plotted against peak height.

Mobile phase: A filtered mixture of 650 mL acetonitrile and 4350 mL solution A was mixed in the ratio (13:87)

Solution A: Measured 8.5 mL of 0.025 M phosphoric acid and mixed with 5000 mL of water and adjusted with triethylamine to a pH of 2.0 ± 0.1 .

Solution B: A filtered mixture of 650 mL acetonitrile and 4350 mL solution A was mixed in the ratio (13:87).

Solution C: Measured 8.5 mL of 0.025 M phosphoric acid and mixed with 5000 mL of water and adjusted with triethylamine to a pH of 3.0 ± 0.1 .

Standard solution: Accurately 40.3 mg of the ciprofloxacin hydrochloride USP reference standard powder were weighed and transferred into 100 mL volumetric flask and made up to mark with solution B, and from this were taken 5 mL ciprofloxacin hydrochloride solution into a 10 mL volumetric flask to obtained known concentration, (0.5 mg/mL) ciprofloxacin hydrochloride.

System suitability solution: Accurately 10 mg of the USP ciprofloxacin ethylenediamine analog RS were weighed and transferred into a 200 mL volumetric flask and made up to mark within the standard solution to obtain known concentration, (0.2 mg/mL).

Sample solution: Five tablets were transferred into a 500 mL volumetric flask. Approximately 400 mL of solution B was added and sonicated for about 20 minutes.

Dilute with solution B to volume, a magnetic stirrer was added, mixed, and a portion of the solution was passed through a membrane filter of 0.45 μm pore size. After filtrate transfer 2 mL (V_1) from a stock solution (5 mg/mL) into a 50 mL volumetric flask in a solvent. 2.0 mL of the filtrate (5 mg/mL) was taken into a 50 mL volumetric flask and diluted to 50 mL with solution B to obtain a known concentration, of 0.20 mg/mL of ciprofloxacin hydrochloride.

Chromatographic system: The liquid chromatography was equipped with a 278 nm UV detector and column: 4.6 mm $\times$ 25 cm, packing L1 was used. The inject volume and flow rate was 10 μL and 1.5 mL per minute respectively and the column temperature was $30 \pm 1^\circ$. The percentage of $\text{C}_{17}\text{H}_{18}\text{FN}_3\text{O}_3$ in the formula's tablet portion was calculated:

$$\text{Result} = \left(\frac{r_u}{r_s} \right) \times \left(\frac{C_s}{C_u} \right) \times \left(\frac{Mr_1}{Mr_2} \right) \times 100 \dots\dots\dots \text{Equation 3}$$

Where:

C_s = anhydrous concentration of USP ciprofloxacin hydrochloride RS in standard solution (mg/mL).

C_u = ciprofloxacin concentration (mg/mL) in the sample solution

r_u = peak area of sample solution

r_s = peak area of standards solution.

M_{r1} = ciprofloxacin's molecular weight, 331.34

M_{r2} = 367.81 molecular weight of anhydrous ciprofloxacin hydrochloride

Assay by BP methods

Preparation of different concentrations for calibration curve /linearity: Accurately 50 mg of the ciprofloxacin hydrochloride USP reference standard were weighed and transferred into 50 mL volumetric flask and made up to mark with mobile phase and from this were taken (3, 4, 5, 6 and 7) mL of stock solution was transferred into a 10 mL volumetric flask to prepare 60% (0.3 mg/mL), 80% (0.4 mg/mL), 100% (0.5 mg/mL), 120% (0.6 mg/mL) and 140% (0.7 mg/mL) respectively. Then concentrations of ciprofloxacin hydrochloride against peak height were plotted to obtain the calibration curves.

Preparation of the buffer Solutions: 14.4 mL of solution of orthophosphoric acid was mixed with 5000 mL of water and adjusted with adjusted to pH 3.0 with triethylamine.

Mobile phase: A filtered mixture of 650 mL acetonitrile and 4350 mL of a 0.245% w/v solution of orthophosphoric acid (buffer) was mixed in the ratio (13:87).

Standard solution: Accurately 58.02 mg of the ciprofloxacin hydrochloride USP reference standard powder were weighed and transferred into 50 mL volumetric flask and made up to mark with the mobile phase and from this were taken 5 mL ciprofloxacin

hydrochloride solution into a 10 mL volumetric flask to obtained known concentration, (0.58 mg/mL) ciprofloxacin hydrochloride.

Sample preparation: A quantity of the powdered tablets containing the equivalent to about 0.5 gm of ciprofloxacin was transferred to a 250 mL volumetric flask, and 187.5 mL of the mobile phase was added and shaken for a minute. Mixed with the aid of ultrasound for 20 minutes, and added sufficient mobile phase to produce 250 mL and mixed. After that, the solution was filtered through a 0.45 μm filter paper, and from the clear filtrate, 2.5 mL for 500 mg and 5 mL for 250 mg tablet were taken into a 10 mL volumetric flask and diluted the filtrate with sufficient mobile phase to produce a solution containing the equivalent of 0.05% w/v of ciprofloxacin hydrochloride.

Chromatographic system: The liquid chromatography was equipped with a 278 nm UV detector and column: a stainless steel column (25 cm $\times$ 4.6 mm) packed with base-deactivated octadecylsilyl silica gel for chromatography (5 μm) (Nucleosil 120-C18 and LiChrospher 100 RP 18 are suitable) was used. Isocratic elution and the following mobile phase described were used: a flow rate of 1.5 mL per minute was used, a detection wavelength of 278 nm was used, 5 μL of each solution was injected.

4.7.3.4. Assay for doxycycline

Preparation of different concentrations for calibration curve /linearity: Accurately 16 mg of the doxycycline USP reference standard were weighed and transferred into a 100 mL volumetric flask and made up to mark with 0.01 M hydrochloric acid and from this were taken (3, 4, 5, 6 and 7) mL of stock solution in was transferred into a 10 mL volumetric flask to prepare 60% (0.048 mg/mL), 80% (0.064 mg/mL), 100% (0.08 mg/mL), 120% (0.096 mg/mL), and 140% (0.112 mg/mL) respectively. The calibration curves were then obtained by plotting doxycycline concentrations against peak height.

Buffer: Tetrabutylammonium hydrogen sulfate (135.8 gm) was dissolved in 1800 mL of water and adjusted to pH 7.0 with concentrated ammonia and diluted with water to produce 2000 mL.

Solution A: Sodium edetate (223.2 g) was dissolved in 1800 mL of water and adjusted to pH 7.0 with concentrated ammonia and diluted with water to produce 2000 mL.

Mobile phase: A filtered mixture of 520 mL of acetonitrile, 680 mL of water, 1400 mL of a 6.79% w/v solution of tetrabutylammonium hydrogen sulfate, and 1400 mL of solution A was mixed in the ratio (13:17:35:35) respectively.

Standard solution: Accurately 16.02 mg of the doxycycline USP reference standard powder were weighed and transferred into a 100 mL volumetric flask and made up to mark with the 0.01 M hydrochloric acid, and from this were taken 5 mL doxycycline solution into a 10 mL volumetric flask to obtain known concentration, 0.08 mg/mL (0.008% w/v) doxycycline USP reference standard.

Sample preparation: The contents of 20 capsules were removed and weighed accurately. An accurately weighed quantity, equivalent to about 140 mg of anhydrous doxycycline of the combined contents was mixed, transferred to a 200 mL volumetric flask, 200 mL of 0.01 M hydrochloric acid was added to the volume, mixed, and sonicated for 15 minutes to ensure complete dissolution. A portion of this solution passed through a suitable filter having a 0.45 μm and diluted 2 mL of the filtrate into 20 mL with 0.01 M hydrochloric acid.

Chromatographic system: The liquid chromatography was equipped with a 280 nm UV detector and column: Use a stainless-steel column (25 cm 4.6 mm) packed with (5 L) polar-embedded octadecyl silyl amorphous organosilica polymer (Xterra RP18). Isocratic elution and the mobile phase described below were used: A flow rate of 1 mL per minute was used,

an amber column temperature of 35°C was used, and 50 L of each solution was injected, allowing the chromatography to continue for twice the doxycycline retention time.

Table 2: System suitability test parameters and acceptance criteria for assay

Sample name	System suitability test parameters				Acceptance criteria
	Resolution	Tailing factor	RSD	Theoretical plate	
Amoxicillin	#2.0%	*2	*3.0%	# 2000	92.5-110% (BP-2019)
Amox/clav K	#3.5	*1.5	*2.0%	# 2000	90-120% (USP-2020)
Ciprofloxacin	#3.5	*1.5	*2.0%	#2000	90-105%(BP-2019)
	-	*1.5	*3.0%	# 2000	95-105% (BP-2019)
	#6	*2	*1.5%	#2500	90-110% (USP-2020)
Doxycycline	-	*1.5	*3.0%	# 2000	95-105% (BP-2019)

= Not less than, * = Not more than, K= potassium and RSD = Relative standard deviation

4.7.4. Dissolution profile

Dissolution of the API was quantified by UV spectroscopy for all investigated medicines except amoxicillin and clavulanate potassium, which were quantified by HPLC.

4.7.4.1. Dissolution profiles of amoxicillin

Calibration curve for amoxicillin dissolution test method: A stock solution was prepared by dissolving 55 mg of amoxicillin USP RS in 50 mL of water. The seven concentration levels were 0.66 mg/mL (120%), 0.55 mg/mL (100%), 0.44 mg/mL (80%), 0.33 mg/mL (60%), 0.22 mg/mL (40%), 0.11 mg/mL (20%), 0.055 mg/mL (10%) were then prepared from the stock by diluting (6, 5, 4, 3, 2, 1 and 0.5) mL of the stock solution to 10 mL with a water solution. Their absorbances were determined spectrophotometrically. Besides, concentrations of amoxicillin against absorbance were plotted to obtain the calibration curves.

The dissolutions of amoxicillin capsules were carried out using dissolution apparatus 1 (basket apparatus), for capsules containing 250 mg and apparatus 2 (paddle) for capsules containing 500 mg following the United States Pharmacopoeia protocol (USP, 2020). The dissolution medium (900 mL of water) was transferred to vessels of the dissolution apparatus. The temperature and the spindle rotation speed were set to $37 \pm 0.5^{\circ}\text{C}$ and 75 rpm, for capsules containing 500 mg and 100 rpm, and for capsules containing 250 mg for 60 minutes. Capsules from each brand were randomly assigned to the six dissolution vessels. Ten (10.0) mL samples were withdrawn at predetermined time points (5, 10, 15, 20, 30, 45, 60, 75 and 90 minutes). After each withdrawal, an equal volume of water medium that had been maintained at the same temperature was replaced to maintain the total volume of the medium constant. The samples (10 mL) were immediately filtered using a syringe filter and measured the absorbance of each sample at 272 nm using a UV/Visible spectrophotometer. water was used as the blank solution. The content in mg of $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$ dissolved was calculated by the formula:

$$\% \text{ Release} =$$

$$\frac{\text{Absorbance of sample} \times \text{concentration of reference standard} \times \text{sample dilution factor} \times 100}{\text{The absorbance of reference standard} \times \text{label claim of sample}}$$

.....Equation 4

Standard solution preparation: Weighed 55 mg of amoxicillin USP RS and was transferred to a 50 mL volumetric flask. Then 50 mL of water was added and dissolved. 5 mL of stock solution was transferred to 10 mL to give a final concentration of 0.55 mg/mL (USP, 2020). Then the absorbance of the solution was determined at 272 nm using a UV/Visible spectrophotometer.

4.7.4.2. Dissolution profiles of amoxicillin and clavulanate potassium

Dissolution by USP methods: The dissolutions of amoxicillin and clavulanate potassium tablets were evaluated using dissolution apparatus 2 (paddle apparatus) per the United

States Pharmacopoeia protocol (USP, 2020). The dissolution medium (900 mL of water) was transferred to vessels of the dissolution apparatus. The temperature and the spindle rotation speed were set to $37 \pm 0.5^{\circ}\text{C}$ and 75 rpm for 30 minutes. Each brand's tablets were assigned at random to the six dissolution vessels. At predetermined time intervals, ten (10.0) mL samples were drawn (5, 10, 15, 30, 45, and 60 minutes). To keep the total volume of the medium constant, an equal volume of water medium that had been kept at the same temperature was replaced after each withdrawal. The samples (10 mL) were immediately filtered using a syringe filter and determined the dissolved amount of $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$ and $\text{C}_8\text{H}_9\text{NO}_5$, by HPLC. water was used as the blank solution. The content in mg of $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$ and $\text{C}_8\text{H}_9\text{NO}_5$ dissolved was calculated by the formula:

Determine the amount of: $\text{C}_{16}\text{H}_{19}\text{N}_3\text{O}_5\text{S}$ in each tablet:

$$\text{Result} = \frac{r_u}{r_s} \times \frac{C_s}{C_u} \times P \times F \times 100 \dots\dots\dots \text{Equation 5}$$

r_u = peak amoxicillin response from the sample solution

r_s = maximum amoxicillin response from standard solution

C_s = amoxicillin RS concentration in standard solution (mg/mL)

C_u = nominal amoxicillin concentration in sample solution (mg/mL).

P = potency of USP amoxicillin RS ($\mu\text{g}/\text{mg}$) and F = conversion factor, 0.001 mg/ μg

Determine the amount of $\text{C}_8\text{H}_9\text{NO}_5$ in each tablet:

$$\text{Result} = \left(\frac{r_u}{r_s} \right) \times \left(\frac{C_s}{C_u} \right) \times P \times 100 \dots\dots\dots \text{Equation 6}$$

r_u = clavulanic acid peak response from sample solution

r_s = clavulanic acid peak response from standard solution

C_s = USP clavulanate lithium RS concentration in standard solution (mg/mL)

C_u = nominal clavulanic acid concentration in sample solution (mg/mL)

P = clavulanic acid potency in USP clavulanate lithium RS (mg/mg)

Buffer: Monobasic sodium phosphate (15.6090 gm) was dissolved in 1800 mL distilled water and sonicated for a minute and adjusted to a pH of 4.4 ± 0.1 with phosphoric acid, and diluted the solution with water to 2000 mL.

Mobile phase: Prepared from the filtered mixture of 1900 mL of buffer and 100 mL of methanol in the ratio of buffer and methanol (19:1).

Preparation of standard solution: An accurately weighed 25.08 mg of USP amoxicillin RS and 10.04 mg of potassium clavulanate were dissolved in 50 mL of water to obtain a solution having a known concentration of about 0.5 mg/mL amoxicillin and 0.2 mg/mL of potassium clavulanate.

Sample solution: The withdrawal samples (10 mL) were immediately filtered using a syringe filter and determined the dissolved amount of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$, by HPLC. Water was used as the blank solution. The final concentration was 0.5 mg/mL of amoxicillin and 0.2 mg/mL of clavulanate potassium.

Chromatographic system: The liquid chromatography equipped with a 230 nm UV detector and column of phenomex/Luna, C18-161 with a size of 4 mm x 30 cm of 771035-3 serial number was used. The flow rate was 1.3 mL/min..

Tolerances: Not less than 85% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ and NLT 80% (Q) of the labeled amount of $C_8H_9NO_5$ were dissolved in 30 minutes (USP, 2020).

Dissolution by BP methods: The dissolutions of amoxicillin and clavulanate potassium tablets were evaluated using dissolution apparatus 2 (paddle apparatus), for following the British Pharmacopoeia protocol (BP, 2019). The dissolution medium (900 mL of water) was transferred to vessels of the dissolution apparatus. The temperature and the spindle rotation speed were set to $37 \pm 0.5^\circ\text{C}$ and 75 rpm for 45 minutes. The six dissolution vessels received tablets from every brand at random. Ten (10.0) mL samples were withdrawn at

predetermined time points (5, 10, 15, 30, 45, and 60 minutes). After each withdrawal, an equal volume of water medium that had been maintained at the same temperature was replaced to maintain the total volume of the medium constant. The samples (10 mL) were immediately filtered using a syringe filter through 0.45 µm membrane and determined the dissolved amount of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$, by HPLC. water was used as the blank solution. The content in mg of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$ dissolved was calculated by the formula similar to the above USP method:

Buffer: Monobasic sodium phosphate (15.6090 gm) was dissolved in 1800 mL of distilled water, sonicated for a minute, and the pH was then adjusted with phosphoric acid to 4.4 ± 0.1 before the solution was diluted to 2000 mL with water.

Mobile phase: Prepared from the filtered mixture of 1900 mL of 0.78% w/v solution of sodium dihydrogen orthophosphate monohydrate and 100 mL of methanol in a ratio of (95:5).

Preparation of 0.025% w/v of amoxicillin trihydrate and 0.01% w/v of lithium clavulanate standard solution: An accurately weighed 25.06 mg of USP amoxicillin RS and 10.05 mg of clavulanate potassium were dissolved in 100 mL of water to obtain a solution having a known concentration of about 0.25 mg/mL amoxicillin and 0.1 mg/mL clavulanate potassium.

Sample solution: The withdrawal samples (10 mL) were immediately filtered using a syringe filter and diluted 5 mL of the filtrate to 25 mL in water to produce a known concentration of 0.25 mg/mL amoxicillin and 0.1 mg/mL of clavulanate potassium and determined the dissolved amount of $C_{16}H_{19}N_3O_5S$ and $C_8H_9NO_5$, by liquid chromatography (HPLC). water was used as the blank solution.

Chromatographic system: The liquid chromatography equipped with a 220 nm UV detector and column of phenomex/Luna, C18-161 with a size of 4 mm x 30 cm was used. The inject volume and flow rate was 20 µL and 1.8 mL per minute respectively.

Tolerances: Not less than 85% (Q) of the labeled amount of $C_{16}H_{19}N_3O_5S$ and NLT 80% (Q) of the labeled amount of $C_8H_9NO_5$ were dissolved in 45 minutes (BP, 2019).

System suitability requirements: The assay was not valid unless the resolution factor between the peaks due to amoxicillin and lithium clavulanate was at least 3.5 and the symmetry factor of the peak due to lithium clavulanate was at most 1.5.

4.7.4.3. Dissolution profiles of ciprofloxacin

Calibration curve for ciprofloxacin dissolution test method: A stock solution was prepared by dissolving 55 mg of ciprofloxacin USP RS in 50 mL of 0.01 M hydrochloric acid. The seven concentration levels 0.66 mg/mL (120%), 0.55 mg/mL (100%), 0.44 mg/mL (80%), 0.33 mg/mL (60%), 0.22 mg/mL (40%), 0.11 mg/mL (20%), 0.055 mg/mL (10%) were then prepared from the stock by diluting (6, 5, 4, 3, 2, 1 and 0.5) mL of the stock solution to 10 mL with 0.01 M hydrochloric acid solution. Their absorbances were determined spectrophotometrically. Besides, concentrations of ciprofloxacin against absorbance were plotted to obtain the calibration curves.

The dissolutions of ciprofloxacin tablets were evaluated using dissolution apparatus 2 (paddle) for following the United States Pharmacopoeia protocol (USP, 2020). The dissolution medium (900 mL of 0.01 M hydrochloric acid) was transferred to vessels of the dissolution apparatus. The temperature and the spindle rotation speed were set to $37 \pm 0.5^\circ\text{C}$ and 50 rpm, for 30 minutes. The six dissolution vessels received tablets from each brand at random. Ten 10.0 mL samples were withdrawn at predetermined time points (5, 10, 15, 20, 30, 45, and 60 minutes). After each withdrawal, an equal volume of water medium that had been maintained at the same temperature was replaced to maintain the

total volume of the medium constant. The samples 10 mL were immediately filtered using a syringe filter and 2 mL of the filtered sample solution was quantitatively taken into a 100 mL volumetric flask. It was diluted to volume with 0.1 M hydrochloric acid to produce 0.011 mg/mL and measured the absorbance of each sample solution was determined at 276 nm using a UV/Visible spectrophotometer. 0.01 M hydrochloric acid was used as the blank solution. The amount of ciprofloxacin hydrochloride in mg ($C_{17}H_{18}FN_3O_3 \cdot HCl$) dissolved was calculated by the formula (Equation 4).

Standard solution preparation: Accurately weighed 55.0 mg of ciprofloxacin USP RS was transferred to a 50 mL volumetric flask. 40 mL of 0.01 M hydrochloric acid was added and dissolved. Then it was diluted with 0.01 M HCl to volume and mixed. From stock solution (5 mL) was transferred to a 10 mL volumetric flask and diluted with 0.01 M hydrochloric acid and then 2 mL of dilute solution was transferred to a 100 mL volumetric flask to produce a final concentration of 0.011 mg/mL (USP, 2020). Then the absorbance of the solution was determined at 276 nm using a UV/Visible spectrophotometer.

Tolerances: An amount of ciprofloxacin hydrochloride ($C_{17}H_{18}FN_3O_3 \cdot HCl$) equivalent to not less than 80% (Q) of the labeled amount of ciprofloxacin ($C_{17}H_{18}FN_3O_3$) was dissolved.

4.7.4.4. Dissolution profiles of doxycycline

Calibration curve for doxycycline dissolution test method: A stock solution was prepared by dissolving 22.5 mg of doxycycline USP RS in 500 mL of 0.1 M hydrochloric acid. Eight concentration levels, 0.02886 mg/mL (130%), 0.02664 mg/mL (120%), 0.0222 mg/mL (100%), 0.01776 mg/mL (80%), 0.01332 mg/mL (60%), 0.00888 mg/mL (40%), 0.00444 mg/mL (20%), 0.00222 mg/mL (10%) were prepared. Then from the stock by diluting (13, 12, 10, 8, 6, 4, 2, and 1) mL of the stock solution to 20 mL with 0.1 M hydrochloric acid solution. Their absorbances were determined spectrophotometrically. Besides, concentrations of doxycycline against absorbance were plotted to obtain the calibration curves.

The dissolution of the doxycycline capsules was evaluated using dissolution apparatus 1 (basket) to follow the British Pharmacopoeia protocol (BP, 2019). The dissolution medium (900 mL of 0.1 M hydrochloric acid) was transferred to vessels of the dissolution apparatus. The temperature and the basket rotation speed were set to $37 \pm 0.5^{\circ}\text{C}$ and 100 revolutions per minute (rpm), for 30 minutes. Capsules from each brand were randomly assigned to the six dissolution vessels. Ten 10.0 mL samples were withdrawn at predetermined time points (5, 10, 15, 30, 45, and 60 minutes). After each withdrawal, an equal volume of water medium that had been maintained at the same temperature was replaced to maintain the total volume of the medium constant. The samples (10 mL) were immediately filtered using a syringe filter and 5 mL of the filtered sample solution was quantitatively taken into a 25 mL volumetric flask. It was diluted to volume with 0.10 M hydrochloric acid to produce 0.0222 mg/mL and measured the absorbance of each sample solution was determined at 345 nm using a UV/Visible spectrophotometer. Hydrochloric acid (0.1 M) was used as the blank solution. The amount of doxycycline hyclate capsule in mg $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ dissolved was calculated by the formula (Equation 4).

Standard solution preparation: Accurately weighed (22.5 mg) of doxycycline USP RS was transferred to a 500 mL volumetric flask. 400 mL of 0.1 M hydrochloric acid was added and dissolved. Then it was diluted with 0.1 M hydrochloric acid to volume and mixed. From stock solution (5 mL) was transferred to a 10 mL volumetric flask and diluted with 0.01 M hydrochloric acid to produce a final concentration of 0.0222 mg/mL (BP, 2019). Then, the absorbance of the solution was determined at 345 nm using a UV/Visible spectrophotometer.

Acceptance limit: Not less than 85% (*Q*) of the labeled amount of $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_8$ was dissolved in at 30 minutes.

4.7.5. Uniformity of dosage units

The term uniformity of dosage unit is defined as the degree of uniformity in the amount of the drug substance among dosage units. The uniformity of dosage units of amoxicillin, amoxicillin and clavulanate potassium, ciprofloxacin, and doxycycline was demonstrated by weight variation as their active ingredient content is greater than 25 mg (USP, 2020). A dosage uniformity test were performed according to monographs of the USP 2020 method specifications. The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to L1%, which is 15%. If the acceptance value is greater than L1%, test the next 20 units and calculate the acceptance value. The requirements are met if the final acceptance value of the 30 dosage units is less than or equal to L1% and all individual dosage units fall within the ranges calculated using L2 factor. First, 10 capsules/tablets were selected randomly. Each capsules/tablet were weighed individually. Then the average weight of 10 capsules/tablets was determined. Then the content (assay) of the individual capsule (% Xi), was obtained by the formula:

$$\% \text{ Xi} = \frac{\text{weight of capsule}}{\text{The average weight of 10 capsules}} \times A \dots\dots\dots \text{Equation 7}$$

Where

% Xi is the content percentage of each tablet/capsule

A is a total percent assay that is obtained from an assay conducted on representative drug samples of each brand as indicated on the monograph for tablet/capsule. Finally, the average (X) for ten capsules/tablets was determined by dividing the sum of individual capsules' content percentage % Xi by 10, which is the number of capsules/tablets used for the weight uniformity test in the study.

4.7.6. Disintegration time

The test is used to evaluate the quality of oral dosage forms like tablets and capsules. It is used to calculate how long it takes for a solid oral dosage form, such as a tablet, to

disintegrate completely. The disintegration time is a quality indicator. This is due to the fact that a long disintegration time, for example, indicates that the tablet is too tightly packed or that the capsule shell gelatin is not of pharmacopeial quality, among other things. Furthermore, if the disintegration time is not uniform across a set of samples, it indicates batch inconsistency and lack of uniformity. A disintegration apparatus performs the test. The pharmacopeia includes a disintegration test for each dosage form (Kostewicz *et al.*, 2014). Six capsules/tablets of each brand of amoxicillin capsules, amoxicillin and clavulanate potassium tablets, ciprofloxacin tablets, and doxycycline capsules were randomly selected and placed in one of six separate tubes in this study. The basket rack assembly was programmed to rise and fall. The medium (900 mL of distilled water) temperature was maintained at $37 \pm 1^\circ\text{C}$. The disintegration time was taken to be the time when no particles remained in the basket. The time in minutes required for each capsule/tablet to disintegrate was recorded. The average disintegration time for each product was calculated.

4.7.7. Friability test

The tendency of a tablet to chip, crumble, or break during compression is referred to as friability. The friability test, which is closely related to tablet hardness, is designed to assess the tablet's ability to withstand abrasion during packaging, handling, and shipping. It is typically measured using the friability apparatus (Shirsand *et al.*, 2009). Ten amoxicillin and clavulanate potassium tablets and ciprofloxacin tablets were chosen at random and weighed before being friability tested on an analytical balance. The tablets were then placed in the tester's drum, which was set to rotate at 25 rpm for 4 minutes, for a total of 100 revolutions. After the procedure was completed, the tablets were dedusted and reweighed. Friability was calculated as the percent loss in weight. The weight loss should not exceed 1%, according to USP for friability. The percentage of weight loss was calculated (USP, 2020).

$$\% \text{ Friability} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \dots\dots\dots \text{Equation 8}$$

4.7.8. Hardness test

This test measures a tablet's ability to resistance mechanical shocks during manufacturing, packaging, and shipping (Karmakar and Kibria, 2012). Ten tablets from each brand of samples were randomly taken and each tablet was then placed in the hardness tester. The tester was programmed to crush the tablet, and the force required to do so was measured and recorded in newtons (Abebe *et al.*, 2020).

4.8. Data quality control

Completeness and consistency of the data were checked. System suitability tests, single injections, blank solutions, standard quality controls, method linearity (calibration curve), repeatability, and strict adherence to the procedures outlined in the pharmacopeia method specification monographs were performed to ensure the quality of the experimental results. Each experiment was carried out in triplicate.

4.9. Data management and analysis

A worksheet created with Microsoft Excel 2016 contained the coded data. Then, Excel and Origin Pro 8.5 were used for the analysis. To summarize the data, descriptive statistics were used. In order to assess the quality of amoxicillin capsules, amoxicillin and clavulanate potassium tablets, ciprofloxacin tablets, and doxycycline capsules, the mean, standard deviation, and relative standard deviation (RSD) were used.

4.10. Ethical consideration

Prior to collecting samples, the ethical clearance approval letter (Ref. No. ERB/SOP/324/13/2021) were obtained from Addis Ababa University's College of Health Sciences, School of Pharmacy.

4.11. Definition of terms

Poor quality medicines: Medicines that do not meet official specifications for strength, quality, purity, packaging, or labeling (whether legally registered, generics, or counterfeits).

Quality: The extent to which a product, system, or process's inherent properties meet requirements.

Not registered: The national regulatory authority does not grant market authorization to not registered product. Even if it is of high quality, not registered product is illegal.

5. Results

5.1. Visual Inspection

As indicated in Annex 7 the results of the visual inspection were obtained after inspecting each sample physically by using a visual inspection checklist. The packaging, labeling and package leaflets of all samples indicates that for all samples the label on the carton protecting the container was in line with the label on the containers, and all capsules or tablets from each product were uniform in shape, size and free of breaks and cracks. However, 7.04% (5/71) samples were intact, and in the case of capsules: they were not easy to uncap, 1.41% (1/71) sample had problem on the packaging, 8.45% (6/71) samples were not legible, 7.04% (5/71) samples had no the manufacturer's name. Overall, 74.65% (53/71) observed samples were not complied with criteria given in the joint WHO/FIP/USP checklists.

5.2. Registration status of medicines

From the total 24 brands included in this study, only 11 (45.83%) brands were registered but 5 (20.83%) not registered, and 8 (33.33%) brands registered before but their license-were expired during the study period. In terms of brands of each medicine surveyed, it was confirmed that 2 (40%) of amoxicillin, 3 (50%) of amoxicillin and clavulanate potassium, 4 (44.44%) ciprofloxacin and 2 (50%) doxycycline were registered. From the total 71 samples included in this study, 42 (59.15%) samples were registered, 18 (25.35%) were not registered and 11 (15.49%) were registered in the earlier years but the licenses were expired during the study period and need re-licensure of registration by the regulatory authority i.e. EFDA. When this was expressed in terms of samples, 18 (69.2%) of amoxicillin, 8 (66.66%) of amoxicillin and clavulanate potassium, 12 (52.17%) ciprofloxacin and 4 (40%) doxycycline were registered.

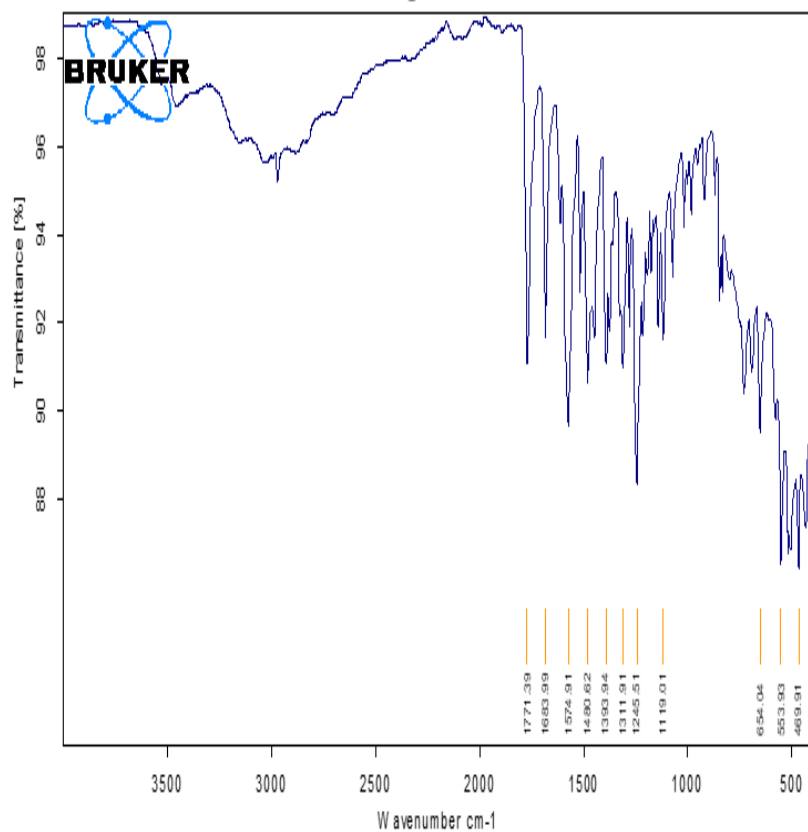
Of the total collected samples, 53.52% (38/71) were manufactured outside Ethiopia, 7.89% (3/38) of the imported samples were not registered and 46.47% (33/71) of the collected samples were manufactured in Ethiopia and from this 45.45% (15/33) were not registered. A license for 21.21% (7/33) of the samples manufactured in Ethiopia were registered but the license period was expired and 10.52% (4/38) were manufactured outside Ethiopia and were registered but the license expired. When examined individually, 30.77% (8/26) amoxicillin samples manufactured in Ethiopia were not registered, 33.33% (4/12) amoxicillin and clavulanate potassium samples manufactured in India were registered but the license expired, 30.43% (7/23) ciprofloxacin samples manufactured in Ethiopia were not registered, among the total ciprofloxacin samples 8.7% (2/23) manufactured in China were not registered, 4.35% (1/23) manufactured in Ghana were not registered and 40% (4/10) doxycycline samples manufactured in India were not registered.

5.3. Identification test

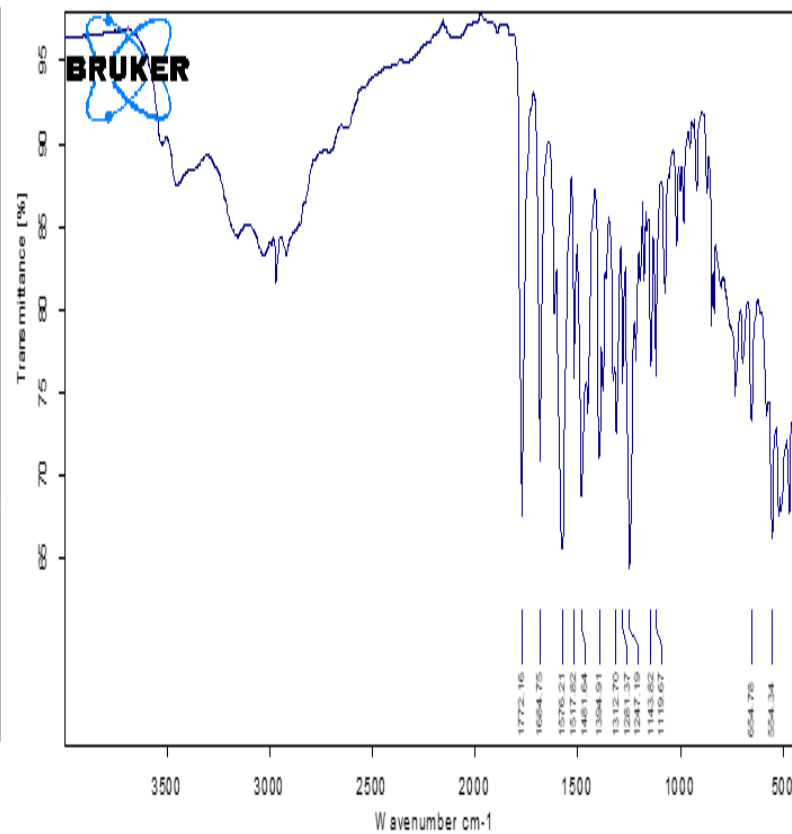
5.3.1. Identification of amoxicillin

IR absorption spectra of the reference standard and the samples were illustrated in Figure 2. As indicated in the Figures, the IR absorption spectra of the standard and the samples were similar pattern of spectrum. As a result, all samples of amoxicillin passed the identification test.

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C:\Tewodros research\Amoxicillin USP RS.0	Amoxicillin USP RS	Powder	14/04/2022
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C:\Tewodros research\AA-02-500.0	AA-02-500	Amoxicillin Capsule powder	14/04/2022
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Figure 2: IR Spectrum of amoxicillin USP RS and amoxiocllin sample AA_02_500

5.3.2. Identification of amoxicillin and clavulanate potassium

The identification test of amoxicillin and clavulanate potassium was performed by comparing the retention of the samples with the reference standards from the HPLC chromatograms and the retardation factor of the sample with the reference standards from the thin layer chromatography respectively. As a result, all of the amoxicillin and clavulanate potassium samples passed the identification test (Figures 3 and 4).

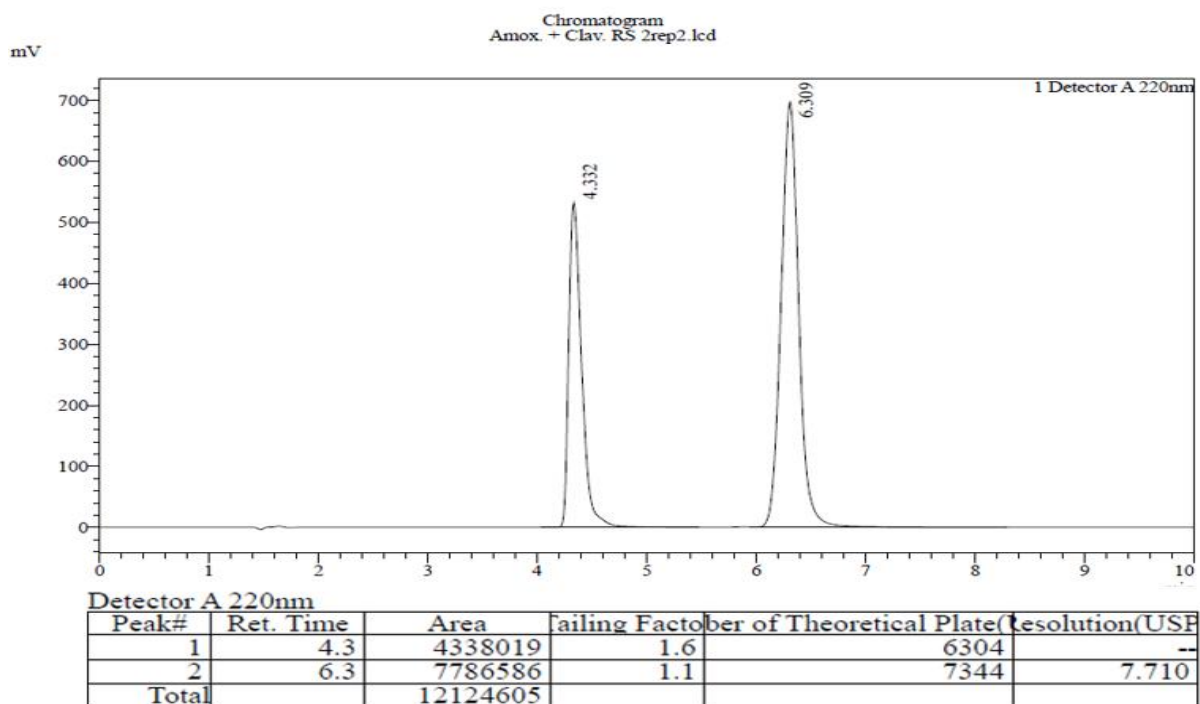


Figure 3: HPLC chromatogram of amoxicillin and clavulanate potassium USP RS

From the above Figure, the first peak at a retention time of 4.3 minute is the peak response for clavulanate potassium. The second peak at a retention time of 6.3 minute is a peak for amoxicillin.

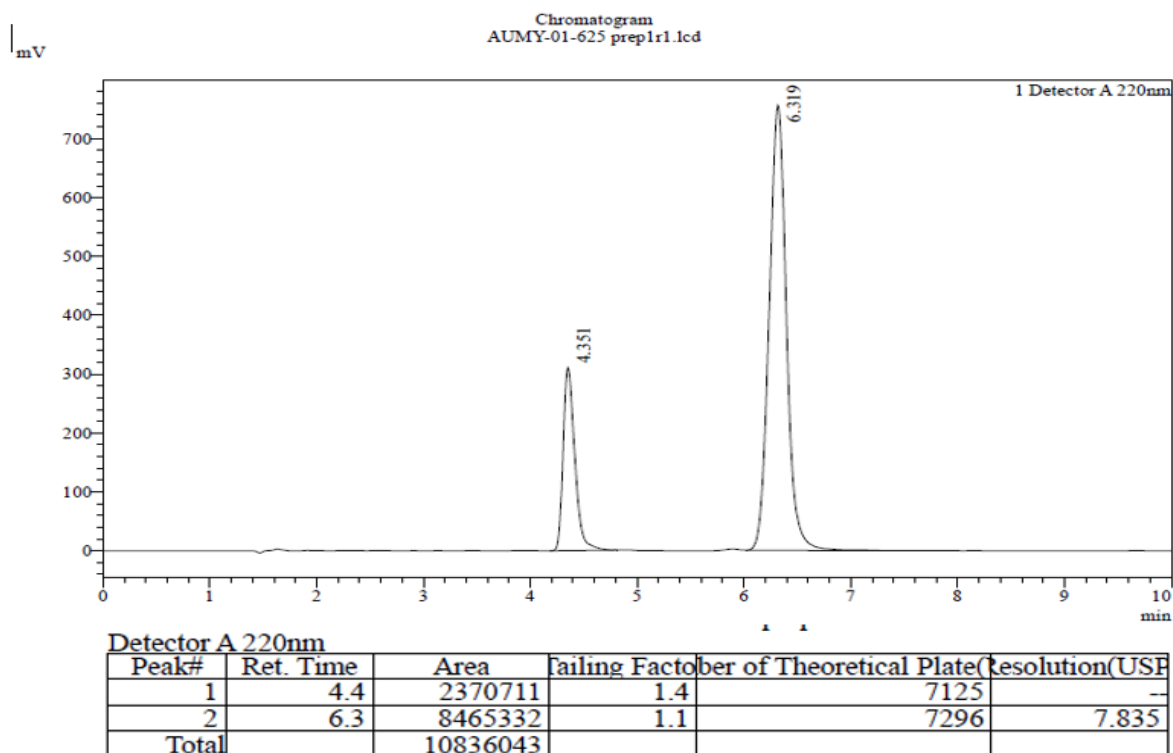


Figure 4: HPLC chromatogram of amoxicillin and clavulanate potassium sample

From the above Figure, the first peak at a retention time of 4.3 minute is the peak response for clavulanate potassium. The second peak at the retention time of 6.3 minute is a peak for amoxicillin.

The identity of the sample needs to be verified before further experimental work. Identification tests performed as per the BP test method indicated the principal spot in the chromatogram obtained with the test solution was compared to the corresponding spot in the chromatogram obtained with the reference solution by the retardation factor of both spots. This indicates that the entire samples of amoxicillin and clavulanate potassium examined for the identity of the active ingredient passed the identification test as per the BP specifications. The retardation factor is defined as the ratio of the distance from the point of application to the centre of the spot and the distance travelled by the solvent front

from the point of application. The TLC results for the identification test of amoxicillin and clavulanate potassium samples are shown in Table 3

Table 3: TLC identification test result of amoxicillin and clavulanate potassium

S/no	Code	RF + SD (n = 2)		% Deviation of Rf from RS		Status
		Amoxicillin	Clavulanate	Amoxicillin	Clavulanate	
1	RS	0.146 0.0014	± 0.2 ± 0.0	0	0	-
2	AUG_01_625	0.153 0.0014	± 0.190 ± 0.001	0.8	-0.9	Pass
3	AUAG_01_625	0.146 0.0021	± 0.2 ± 0.007	0.1	0.5	Pass
4	AUG_02_625	0.146 0.0021	± 0.196 ± 0.001	0.15	-0.3	Pass
5	AUHG_01_625	0.146 0.0007	± 0.193 ± 0.001	0.05	-0.65	Pass
6	AUA_01_625	0.150 0.0007	± 0.2 ± 0.004	0.3	0.25	Pass
7	AUMYG_01_625	0.146 0.0021	± 0.196 ± 0.002	0.15	-0.25	Pass

5.3.3. Identification of ciprofloxacin

The sample ciprofloxacin identification test was conducted in accordance with USP-2020 and BP-2019 based on the indicated of sample level claim. The identities of the tested products were confirmed by comparing their retention times to the ciprofloxacin reference standard. All ciprofloxacin samples tested showed chromatogram retention times that corresponded to the respective reference standards.

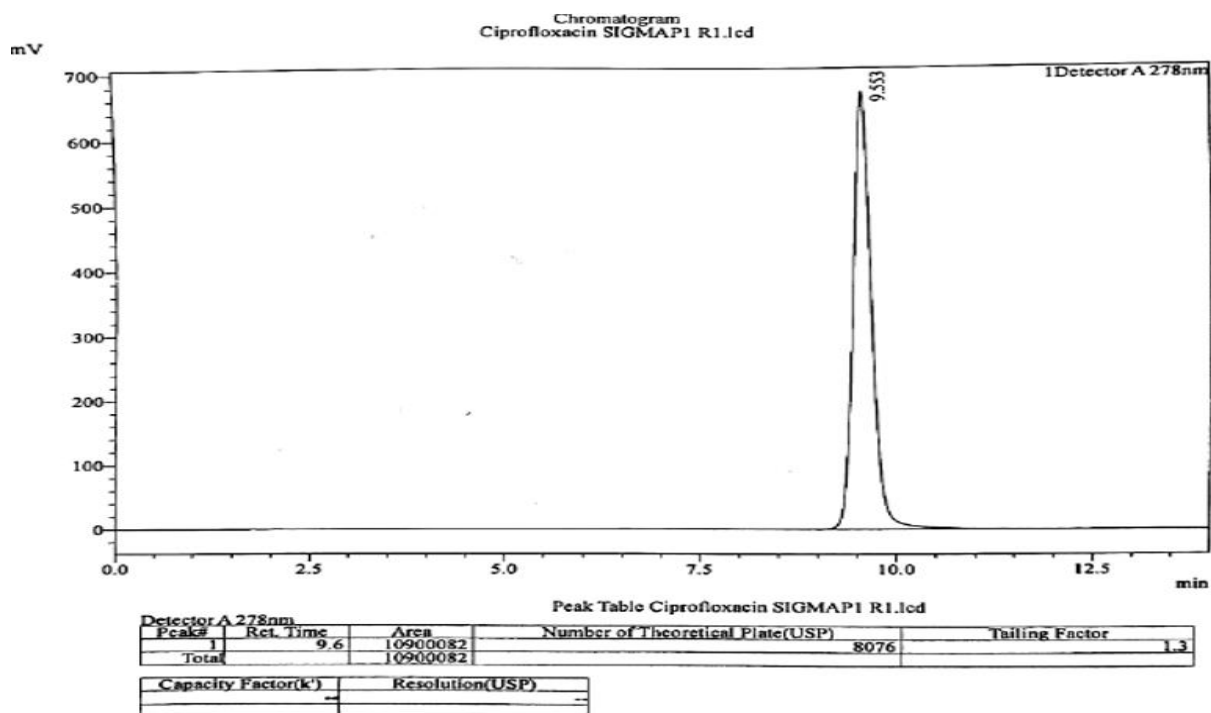


Figure 5: HPLC chromatogram of ciprofloxacin USP RS (USP method)

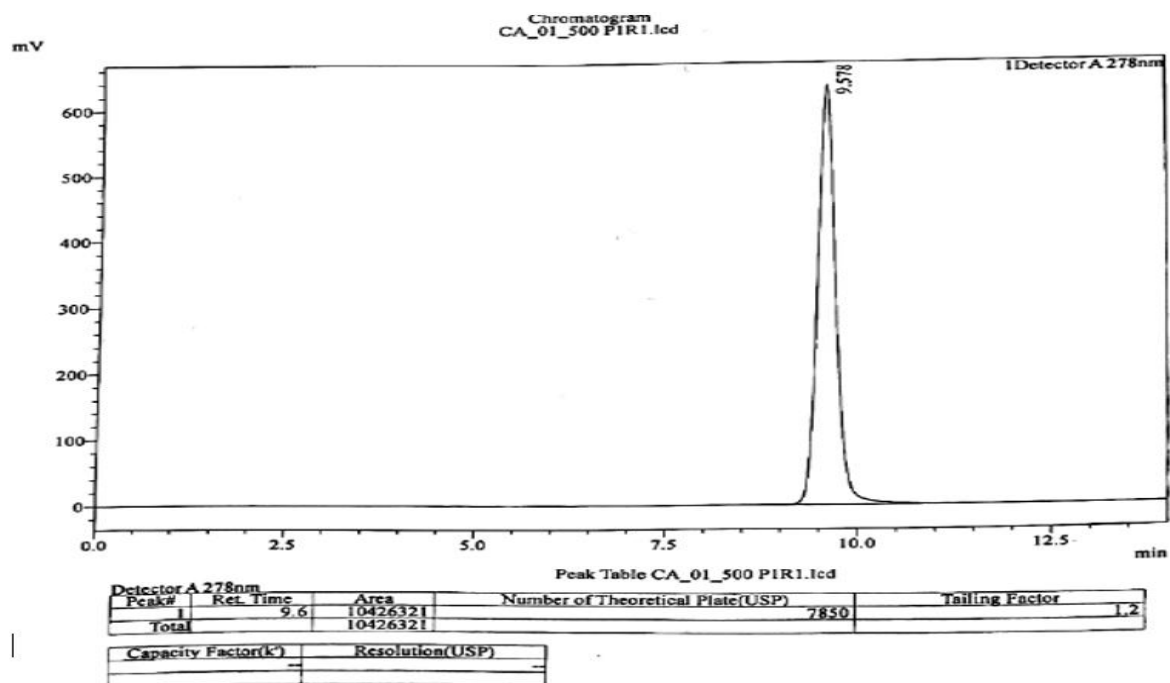


Figure 6: HPLC chromatogram of ciprofloxacin sample (USP method)

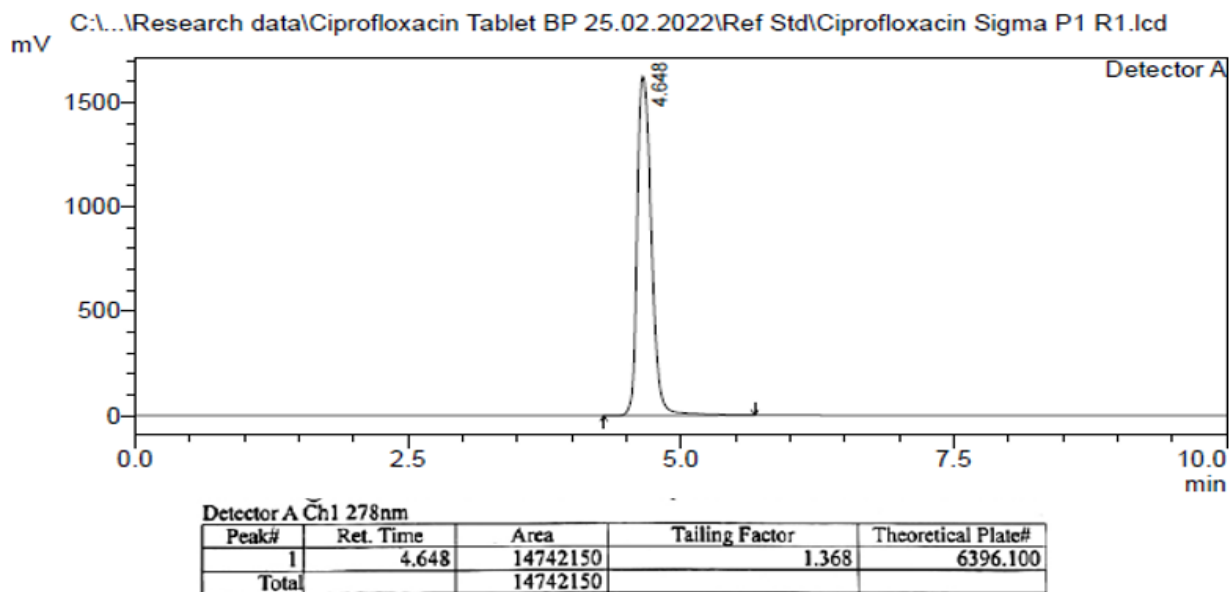


Figure 7: HPLC chromatogram of ciprofloxacin USP RS (BP method)

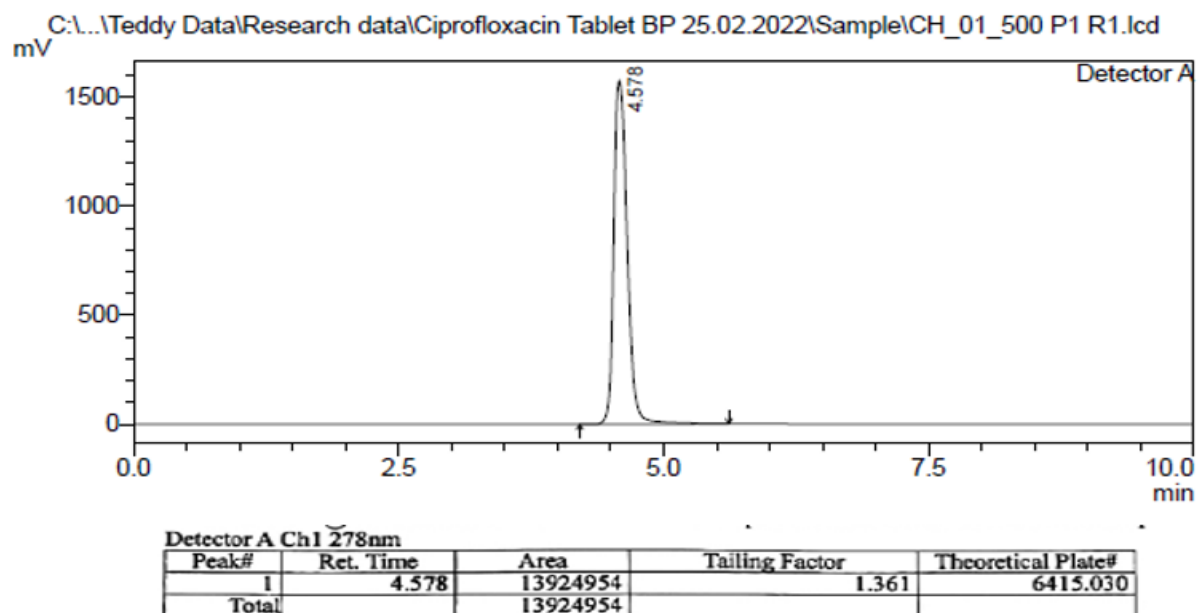


Figure 8: HPLC chromatogram of ciprofloxacin sample (BP method)

Before proceeding with further experiments, the sample's identity must be confirmed. The identification test performed according to USP and BP test methods revealed that the

retention times of the peaks in the chromatograms of all ciprofloxacin samples were similar to their respective RS. This means that all ciprofloxacin samples tested for active ingredient identity passed the identification test as specified by the USP and BP methods.

5.3.4. Identification of doxycycline

The doxycycline identification test was carried out by BP-2019, which means that 5mg of capsule powder was weighed and mixed with 10 mL of sulphuric acid. The tested products were confirmed by the chemical reaction result, which means that the required yellow color was produced by the chemical reaction. All the samples of doxycycline passed the test.

5.4. Assay

5.4.1. Limits for system suitability parameters

As shown in Table 4, theoretical plate values for amoxicillin capsules were 2779, 7957.88 for amoxicillin tablet, and 7114.75 for clavulanate potassium tablet by USP, and 8339 for amoxicillin tablet and 7276 for clavulanate potassium tablet by BP for amoxicillin and clavulanate potassium tablets, respectively. Doxycycline's theoretical plate value was 2323. By USP and BP methods, the resolution factor for amoxicillin capsules was 3.6, 9.9 for amoxicillin and clavulanate potassium tablets, and 9.5 and 7.6 for ciprofloxacin tablets. The tailing factor for amoxicillin capsules was one, 0.96 and 1.44 for amoxicillin and clavulanate potassium tablets, respectively, 1.3 and 1.5 for ciprofloxacin tablets by USP and BP methods, and one for doxycycline capsules. The relative standard deviation was 0.039% for amoxicillin capsules, 0.12% for amoxicillin and 0.1% for clavulanate potassium by USP and BP methods, 0.15 for amoxicillin and 0.1% for clavulanic acid, 0.2% and 1.5% for ciprofloxacin, respectively, and 0.24% for doxycycline capsules.

Table 4: Limits for system suitability tests

S.no	System suitability parameters	Standard limit	From Assay value of SS (n = 6)
1	Column efficiency	>2000 Theoretical plate (for A)	2779
		≥ 2000 Theoretical plate (for Au)	7957.88 (for Amox) and 7114.75 (clavulanic acid) from USP-2020
			8339 (for Amox) and 7276 (clavulanic acid) for BP-2019
		≥ 2500 Theoretical plate (for C)	8805 From USP-2020 and 8794.1 From BP-2019
		>2000 Theoretical plate (for D)	2323
2	Resolution factor	≥ 2 (for A)	3.6
		≥ 3.5 (for Au)	9.9
		>6 (For C)	9.5 From USP-2020 and 7.6 From BP-2019
3	Tailing factor	<2 (for A)	1
		<1.5 (for Au)	0.96 for amox and 1.44 (for clavulanic acid)
		<2 (for C)	1.3 For USP-2020 and 1.5 For BP-2019
		<1.5 (for D)	1
4	The relative standard deviation	<3.0% (for A)	0.039%
		<2.0% (for Au)	0.12% for amox and 0.1% (for clavulanic acid) from USP-2020
			0.15% for amox and 0.1% (for clavulanic acid) from BP-2019
		$\leq 1.5\%$ (for C)	0.2% USP-2020 and 1.5 BP-2019
		$\leq 3\%$ (for D)	0.24%

A = amoxicillin, Au = amoxicillin-clavulanic acid, C = ciprofloxacin, D = doxycycline

The column's effective performance was suggested by the large number of theoretical plates. Under the same operating conditions, the low relative standard deviation of the peak areas of five replicate injections denotes good precision. The tailing factor's system suitability test result of 1 showed that the peaks were symmetrical.

5.4.2. Construction of linearity/calibration line

Various concentrations of ciprofloxacin, amoxicillin, and doxycycline were prepared by dissolving reference standards in diluents, and their corresponding peak areas at 254 nm, 220 nm, 278 nm, and 280 nm were prepared using chromatography. In the regression equations, y is the peak response and x is the concentration in mg per ml (Figure 9-16).

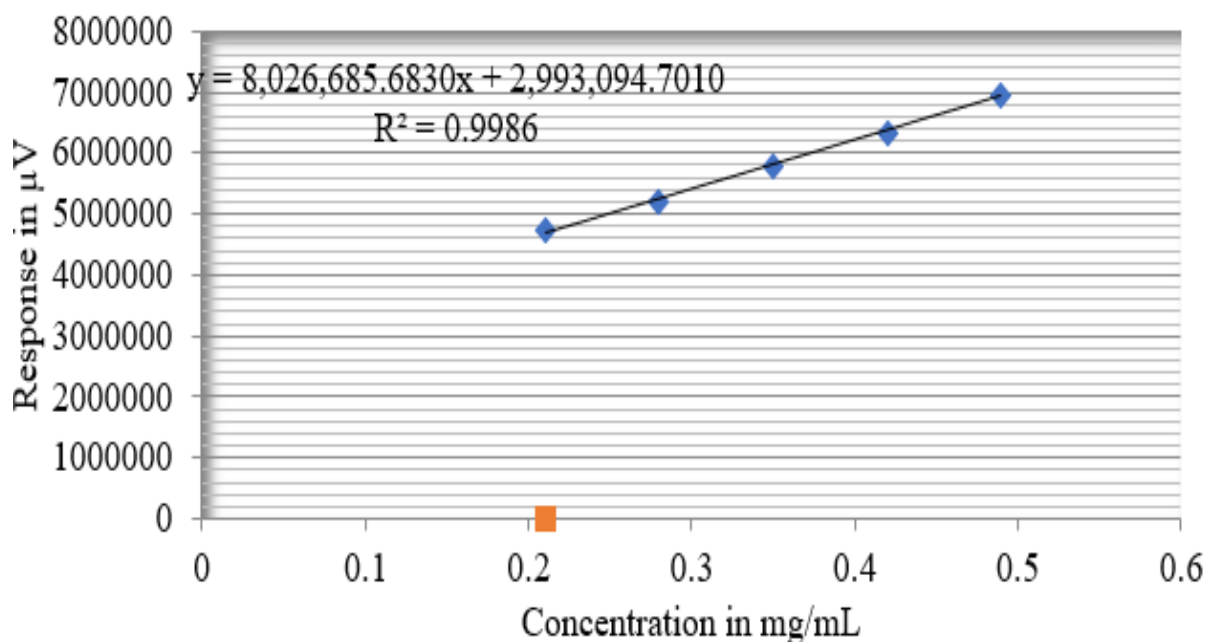


Figure 9: Calibration curve of amoxicillin RS in the concentration ranges of 0.84 mg/mL to 0.56 mg/mL for assay

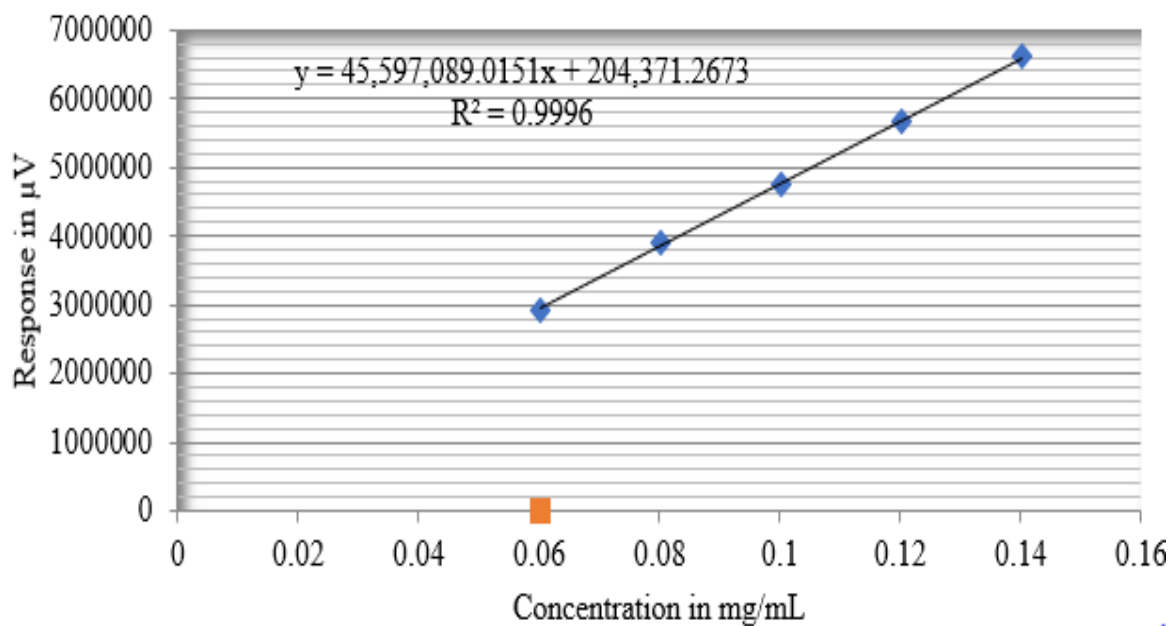


Figure 10: Calibration curve of amoxicillin and clavulanate potassium RS in the concentration ranges of 0.48 mg/mL to 0.72 mg/mL (amoxicillin) (USP-2020) for assay

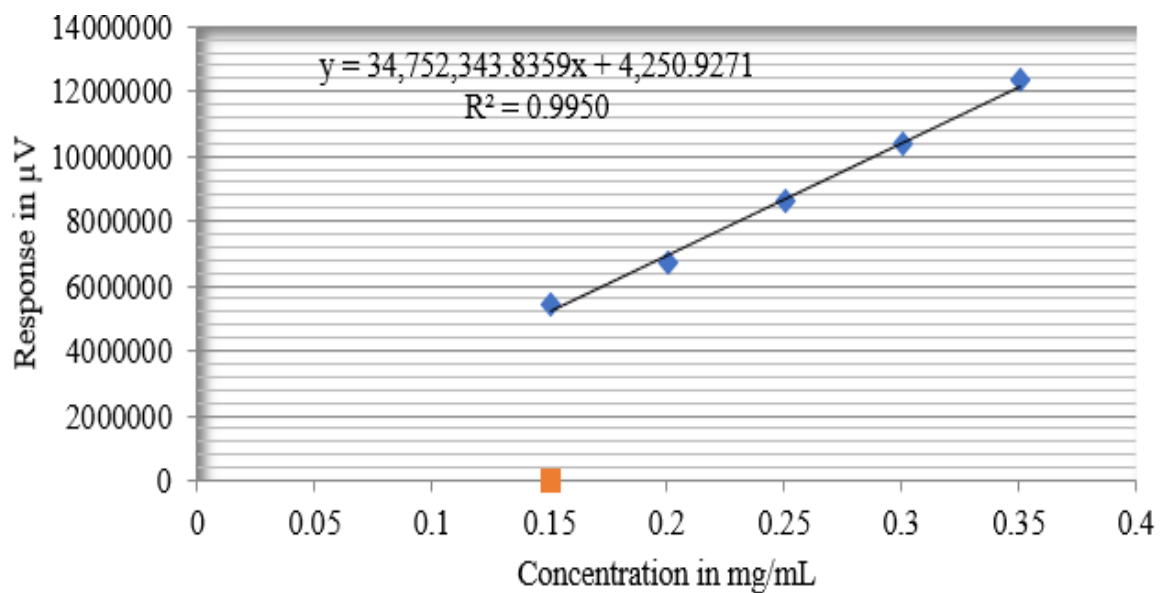


Figure 11: Calibration curve of amoxicillin and clavulanate potassium RS in the concentration ranges of 0.48 mg/mL to 0.72 mg/mL (amoxicillin) (BP-2019) for assay.

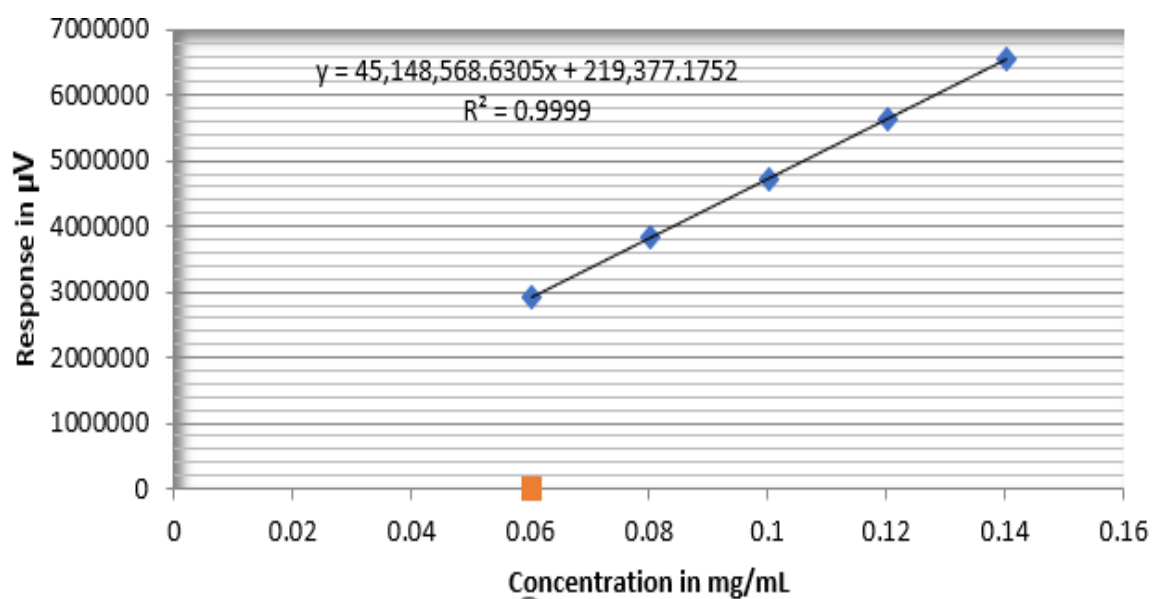


Figure 12: Calibration curve of amoxicillin and clavulanate potassium USP RS in the concentration ranges of 0.3 -0.7 mg/mL (clavulanate potassium) (USP-2020) for assay.

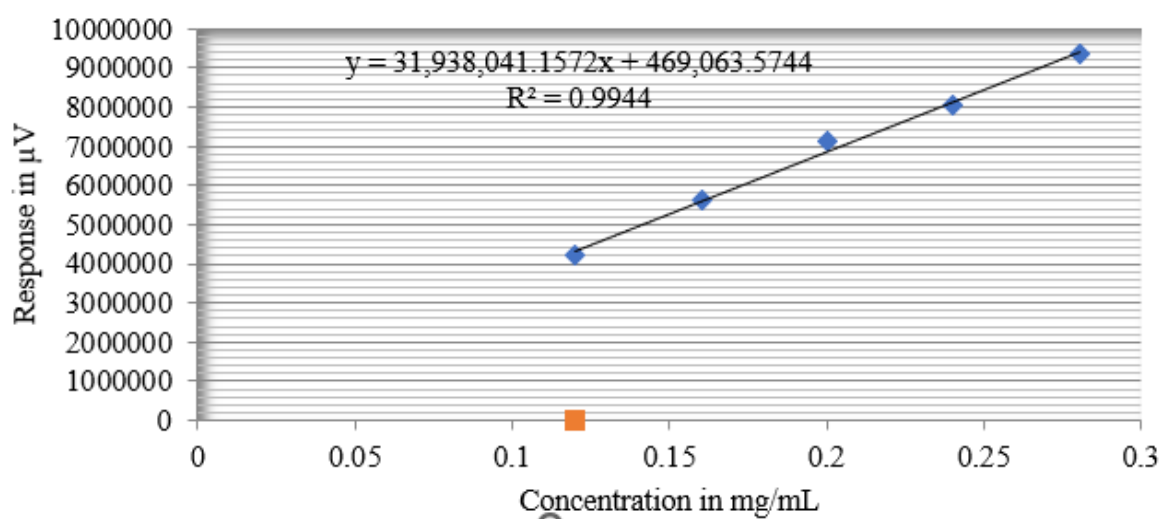


Figure 13: Calibration curve of amoxicillin and clavulanate potassium USP RS in the concentration ranges of 0.3 -0.7 mg/mL (clavulanate potassium) (BP-2019) for assay.

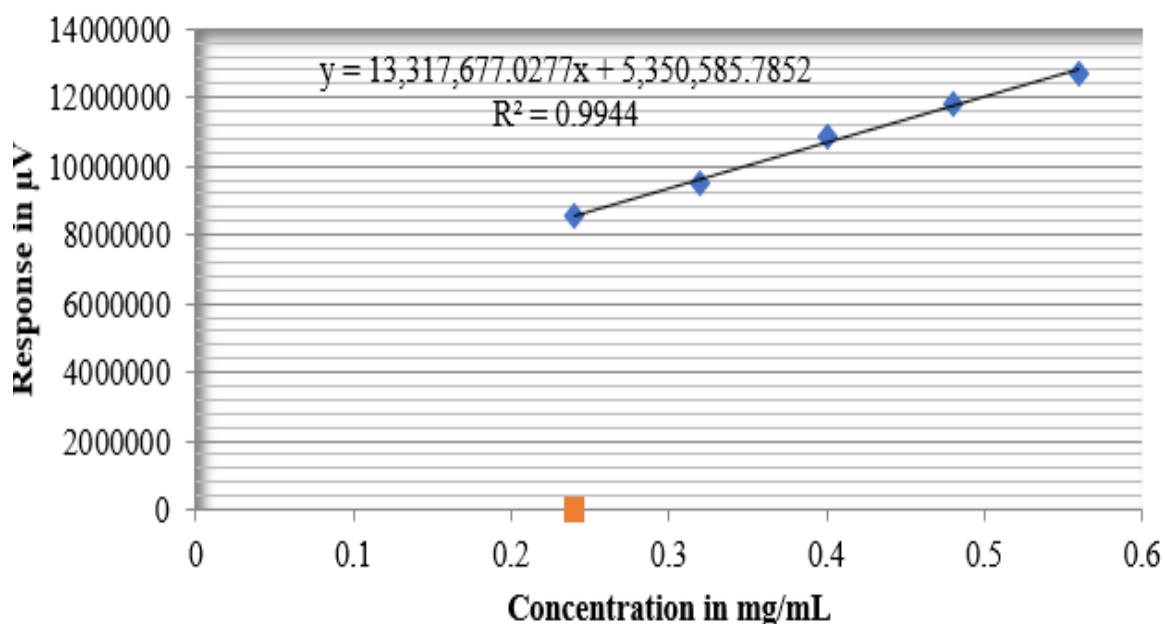


Figure 14: Calibration curve of ciprofloxacin sigma RS in the concentration ranges of 0.16 mg/mL (80%) to 0.24 mg/mL (120%) (USP-2020) for assay

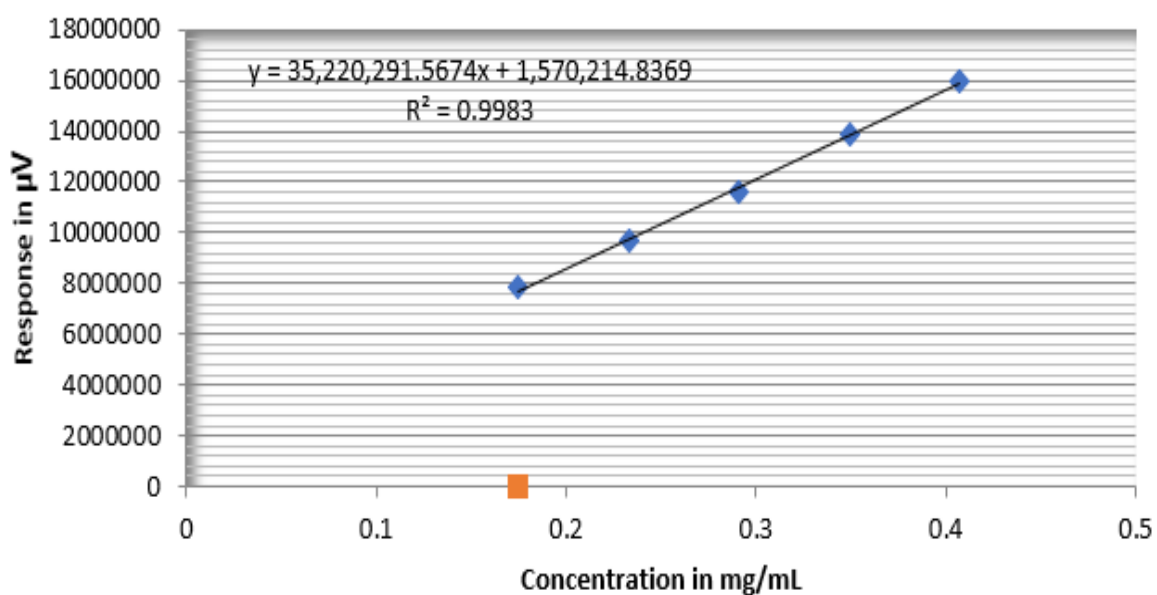


Figure 15: Calibration curve of ciprofloxacin sigma RS in the concentration ranges of 0.3 mg/mL (60%) to 0.7 mg/mL (120%) (BP-2019) for assay

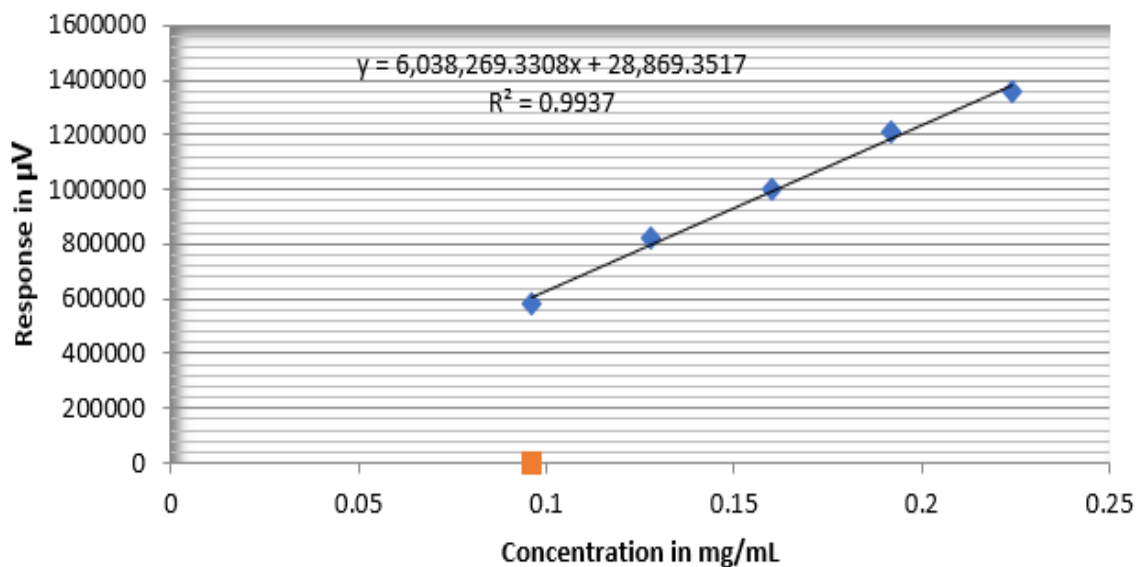


Figure 16: Calibration curve of doxycycline RS in the concentration ranges of 0.048 mg/mL (60%) to 0.1152 mg/mL (140%) for assay

5.4.3. Assay results

5.4.3.1. Assay result of amoxicillin

The assay values for amoxicillin drug products ranged from 97.22 to 105% (Table 5). This result showed that all samples comply with the BP specification limit (92.5 to 110%).

Table 5: Assay value, collection site, brands, and samples of amoxicillin samples

S/NO	Brand name	Batch No	Manufactured country	Collection site	Code	Assay value (%) - Content Specifications [92.5%-110.0%]	Conclusion
1	AMOXID cap 500	28228	Ethiopia	Humera	AHG_01_500	102.4	Passed
2	Amox – 500	706210313	China	Kokit	AK-01_500	101.4	Passed
3	AMOXID cap 500	31423	Ethiopia	Abderafi	AAG_01_500	100.7	Passed
4	AMOXID cap 500	32490	Ethiopia	Metema	AM_02_500	102.93	Passed
5	AMOXID cap 500	32476	Ethiopia	Mai kadra	AMY_01_500	103.1	Passed
6	Amox-500	706201101	China	Gendwuha	AG_01_500	99.85	Passed
7	Amox-500	706210316	China	Abderafi	AA_02_500	101.55	Passed
8	Amox-500	706210316	China	Mai kadra	AMY_02_500	101.6	Passed
9	Amox-500	706210427	China	Metema	AM_01_500	98.34	Passed
10	Amoxicillin 500mg	1030391	Ethiopia	Kokit	AK_02_500	99.7	Passed
11	Amoxicillin 500mg	1030121	Ethiopia	Metema	AM_03_500	97.22	Passed
12	AMOXID cap 500	31074	Ethiopia	Humera	AH_02_500	101.5	Passed
13	AMOXID cap 500	31286	Ethiopia	Gendwuha	AG_03_500	102.2	Passed

S/NO	Brand name	Batch No	Manufactured country	Collection site	Code	Assay value (%) - Content Specifications [92.5%-110.0%]	Conclusion
14	Amoxicillin 500mg	1030431	Ethiopia	Humera	AHG_02_500	103.2	Passed
15	Amoxicillin 500mg	1030431	Ethiopia	Mai kadra	AMY_03_500	103.8	Passed
16	Amoxicillin 500mg	1030431	Ethiopia	Humera	AH_01_500	102.5	Passed
17	Amoxicillin 500mg	1030391	Ethiopia	Mai kadra	AMYG_01_500	102.1	Passed
18	AMOXID cap 500	26619	Ethiopia	Abderafi	AA_01_500	102.75	Passed
19	Amoxicillin 500mg	1030391	Ethiopia	Metema	AMG_01_500	99.9	Passed
20	Amoxicillin 500mg	1010361	Ethiopia	Gendwuha	AG_02_500	104.2	Passed
21	AMYN – 250	S36420032	India	Mai kadra	AMY_01_250	102.4	Passed
22	AMOXID – 250	26506	Ethiopia	Mai kadra	AMYG_01_250	105	Passed
23	AMYN – 250	S36420039	India	Gendwuha	AG_01_250	103.9	Passed
24	AMOXID – 250	22253	Ethiopia	Abderafi	AAG_01_250	103.8	Passed
25	AMOXID – 250	29916	Ethiopia	Humera	AH_03_250	100.13	Passed
26	AMOXID – 250	29916	Ethiopia	Abderafi	AA_01_250	100	Passed

5.4.3.2. Assay result of amoxicillin and clavulanate potassium

The assay results for the different brands and samples of amoxicillin and clavulanate potassium included in the study are shown in Table 6. The products were assayed according to the method outlined in BP and USP. The BP states that amoxicillin and clavulanate potassium must contain not less than 90% and not more than 105% of the level claim of amoxicillin. The USP states that amoxicillin and clavulanate potassium must contain not less than 90% and not more than 120% of the label claim of amoxicillin and clavulanate potassium. This study revealed that 50% (6/12) of amoxicillin and clavulanate potassium samples did not meet both of the pharmacopoeial acceptance specifications for the assay, and thus are formally classified as substandard medicines.

Table 6: Assay value, collection site, brands, and samples of amoxicillin and clavulanic acid

S/NO	Brand name	Batch No	Manufactured country	Collection site	Code	Assay value (%)		Conclusion	
						Amoxi cillin	Clavulanate lithium	Amoxici llin	Clavulan ate lithium
1	INDCLAV 625	T1150007ET	India	Mai kadra	AUMY_01_625	94.98	98.48	Passed	Passed
2	INDCLAV 375	T1170001ET	India	Humera	AUHG_01_375	84.74	40.74	Failed	Failed
3	INDCLAV 625	T1150002ET	India	Humera	AUH_02_625	94.92	89.54	Passed	Passed
4	INDCLAV 625	T1150009ET	India	Humera	AUH_01_625	96.46	85.82	Passed	Failed
5	INDCLAV 375	T1130019ET	India	Abderafi	AUAG_01_375	88.97	71.35	Failed	Failed
6	ACINET 375	19150567	India	Mai kadra	AUMY_01_375	93.82	84.50	Passed	Failed
7	KOACT 625	EL5019041-C	India	Gendwuha	AUG_01_625	99.83	91.28	Passed	Passed
8	KOACT 625	EL5020013-A	India	Abderafi	AUAG_01_625	99.81	108.78	Passed	Failed
9	CLAVAMYN-625	K55320019	India	Gendwuha	AUG_02_625	97.23	105.61	Passed	Passed
10	KOACT 625	EL5020013-A	India	Humera	AUHG_01_625	100.30	104.36	Passed	Passed
11	CLAVAMYN-625	K55320006	India	Abderafi	AUA_01_625	101.49	102.23	Passed	Passed
12	Clavomid 625	92283	Cyprus	Mai Kadra	AUMYG_01_625	99.33	109.38	Passed	Failed

5.4.3.3. Assay result of ciprofloxacin

The assay results for the different brands and samples of ciprofloxacin included in the study are shown in Table 7. The BP states that the ciprofloxacin must contain not less than 95% and not more than 105% of the label claim of ciprofloxacin. The USP states that the ciprofloxacin must contain not less than 90% and not more than 110% of the label claim of ciprofloxacin (Table 8). This study revealed that 47.8% (11/23) of ciprofloxacin samples did not meet both of the pharmacopoeial acceptance specifications for the assay, and thus are formally classified as substandard medicines. Ciprofloxacin assay test results by product brand and batch and place of the collection are presented in Table 7.

Table 7: Assay value, collection site, brands, and samples of ciprofloxacin tablet

S/NO	Brand Name	Batch No	Manufactured country	Collection Site	Code	Assay value (%)	Conclusion
1	AKCIPO 500	ATB057	India	Abderafi	CA_01_500	87.1	Failed
2	Ciproqun 500	K11420006	India	Abderafi	CA_03_500	88.13	Failed
3	Ciprofloxacin usp 500	B190807	China	Abderafi	CAG_01_500	107.7	Passed
4	AKCIPRO 500	ATB024	India	Gendwuha	CG_04_500	84.8	Failed
5	Ciproquin 500	K11420038	India	Gendwuha	CG_03_500	169.8	Failed
6	Ciproquin 500	K11419108	India	Gendwuha	CG_02_500	92.1	Failed
7	CIPRDAC 500	D21001BY38	Ethiopia	Metema	CM_01-500	90.1	Failed
8	Ciproquin 500	K11420008	India	Metema	CM_02_500	91.3	Failed
9	Ciproquin 500	K11419116	India	Kokit	CK_01_500	91.4	Failed
10	AKCIPRO 500	ATB022	India	Kokit	CK_02_500	88.73	Failed
11	G_CIPROX 500	HB190201	Ghana	Humera	CHG_02_500	92.7	Passed
12	Ciproflacin tab USP 500	B190809	China	Humera	CH_02_500	94.6	Passed
13	CIPOFLOX-500	203121099	China	Humera	CH_03_500	95.8	Passed
14	CIPOFLOX-500	193121262	China	Metema	CM_03_500	96.2	Passed
15	CIPRO-SSP 500	00321010010	Ethiopia	Gendwuha	CG_01_500	95.1	Passed
16	CIPRO-SSP 500	00321040060	Ethiopia	Abderafi	CA_02_500	95.4	Passed

S/N o	Brand Name	Batch No	Manufactured country	Collection Site	Code	Assay value (%)	Conclusion
17	CIPOFLOX-500	193121260	China	Mai kadra	CMY_01_500	99.2	Passed
18	Cipro-SSP 500	00320110020	Ethiopia	Humera	CH_01_500	108.1	Passed
19	Ciproleb-500	T3579	India	Humera	CH_04_500	105.54	Passed
20	Cipro-SSP 500	00320110070	Ethiopia	Humera	CHG_01_500	107.35	Passed
21	Cipro-SSP 500	00320110050	Ethiopia	Abderafi	CAG_02_500	105.2	Passed
22	Cipro-SSP 250	00320120030	Ethiopia	Gendwuha	CG_01_250	80.21	Failed
23	Cipro-SSP 250	00320120020	Ethiopia	Abderafi	CAG_01_250	80.3	Failed

5.4.3.4. Assay result of doxycycline

The assay results for the different brands and samples of doxycycline included in the study are shown in Table 8. The BP states that the doxycycline must contain not less than 95% and not more than 105% of the label claim of doxycycline. This study revealed that 60% (6/10) of doxycycline samples did not meet the pharmacopoeial acceptance specification for the assay, and thus are formally classified as substandard medicines.

Table 8: Assay value, collection site, samples of doxycycline

S.NO	Brand name	Batch no	Manufactured country	Collection site	Code	Assay value (%)	Conclusion content specifications [95.0-105.0%]
1	Kodoxy	S55621002	India	Abderafi	DA_02	91.8	Failed
2	Doxycap	26255	Ethiopia	Abderafi	DA_01	95	Passed
3	Doxycap 100mg	32221	Ethiopia	Mai kadra	DMY_02	170.1	Failed
4	Doxyzim 100	Fbw4a106	India	Humera	DH_02	99.7	Passed
5	Doxycap 100mg	32219	Ethiopia	Metema	DM_02	94.3	Failed
6	Eapadoxine 100	Ia206	Ethiopia	Mai kadra	DMY_03	53.6	Failed
7	Doxycap 100mg	32233	Ethiopia	Metema	DM_01	75.4	Failed
8	Doxycap	26267	Ethiopia	Gendwuha	DG_01	95	Passed
9	Kodoxy	S55620003	India	Humera	DH_01	94.6	Failed
10	Kodoxy	S55621002	India	Mai kadra	DMY_01	95.2	Passed

5.5. Dissolution profile

5.5.1. Dissolution profiles of amoxicillin

Calibration curve for amoxicillin dissolution test method: As revealed on the calibration curve (Figure 17), Where Y is the absorbance and X is the concentration in mg/mL.

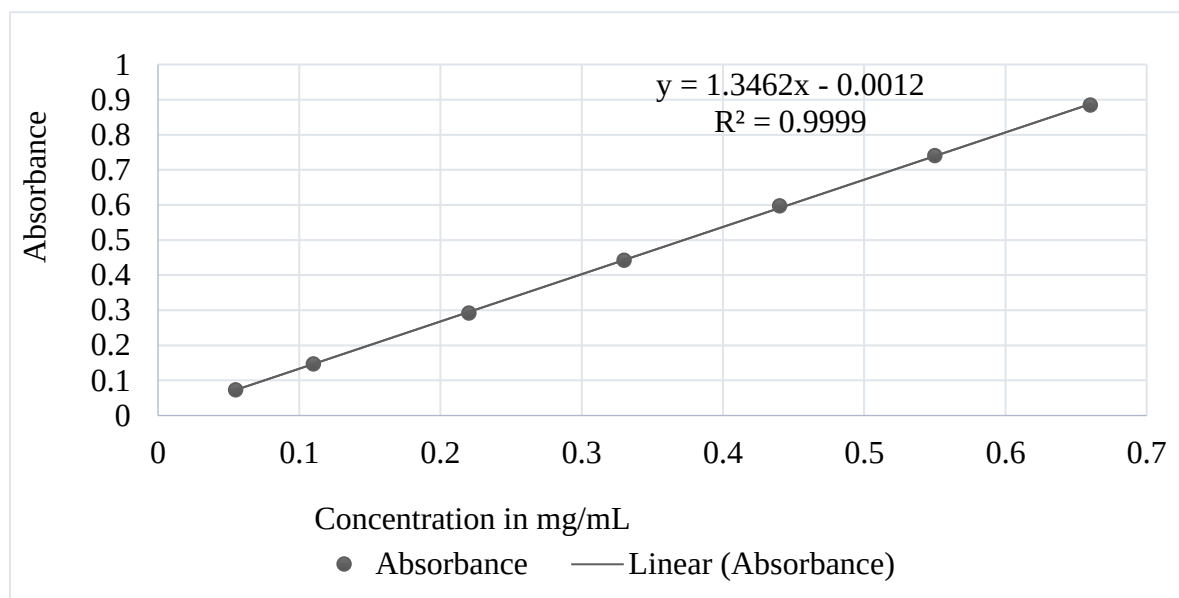


Figure 17: Calibration curve for amoxicillin dissolution test

The dissolution profiles of all samples of amoxicillin had passed to comply with the USP 2020 specification limits. As per the USP 2020 specification, amoxicillin had released more than 80% of the labelled amount within 60 minutes. Time-dependent dissolution profiles of all samples of amoxicillin were illustrated in Figures 18a and b.

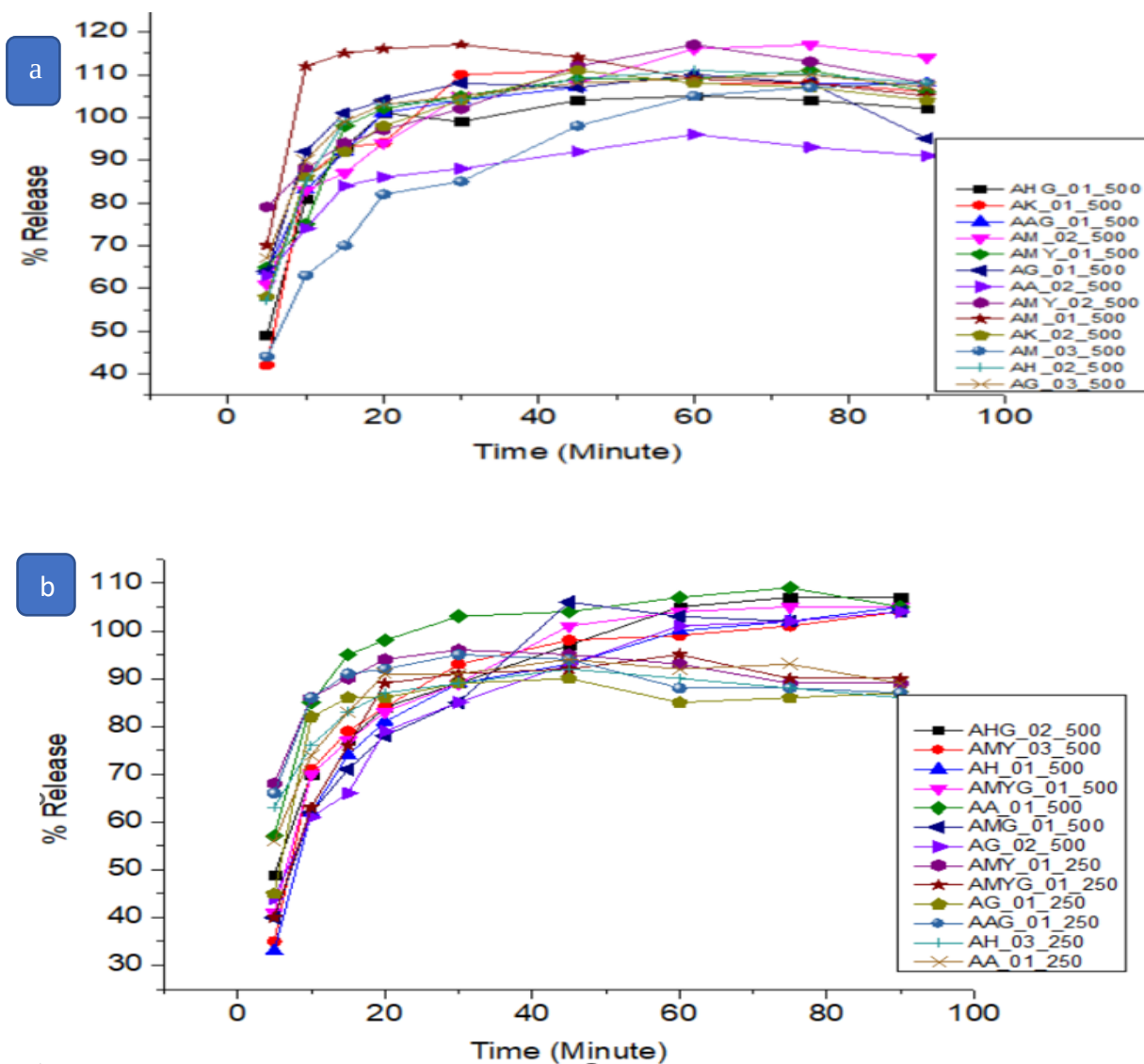


Figure 18a and b: Dissolution profiles of amoxicillin and clavulanate potassium

5.5.2. Dissolution profiles of amoxicillin and clavulanate potassium

Calibration curve for amoxicillin and clavulanate potassium USP dissolution test method:

As revealed on the calibration curve (Figures 19 and 20), a linear regression equation, where Y is the absorbance and X is the concentration in mg/mL. The response function (calibration curve)

showed a strong linear relationship between the concentration of the tested samples and the absorbance values over the concentration range of 0.3 (60%) - 0.7 mg/mL (140%).

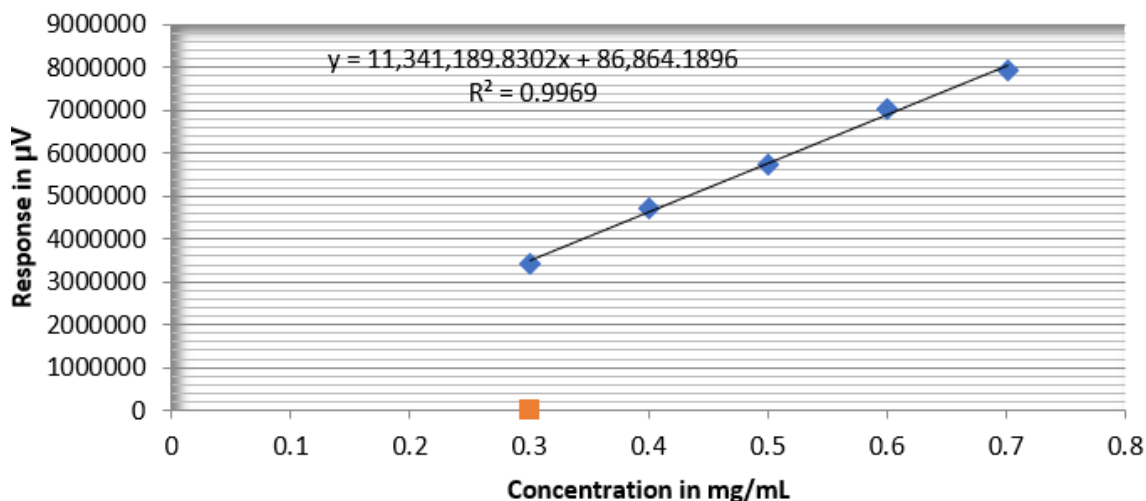


Figure 19: Calibration curve for amoxicillin and clavulanate potassium USP dissolution test method (amoxicillin)

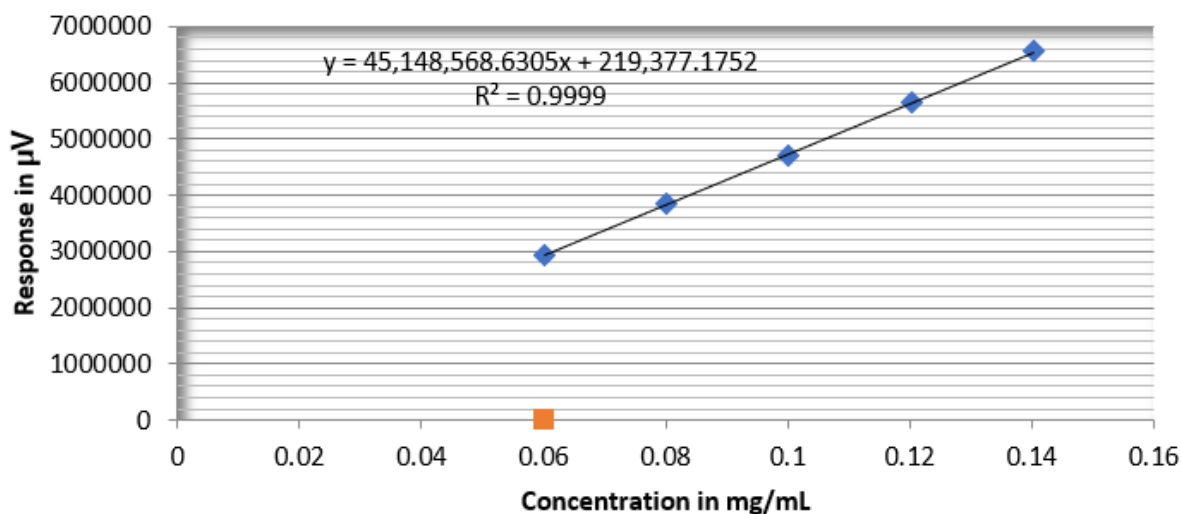


Figure 20: Calibration curve for amoxicillin and clavulanate potassium USP dissolution test method (clavulanate potassium)

The dissolution profiles of all brands of amoxicillin and clavulanate potassium did not comply with the USP 2020 and BP-2019 specification limits. The dissolution profile of amoxicillin and

clavulanic acid tablets was illustrated in Figures 21 and 22. Due to resource-limitations dissolution profile was performed only for brands.

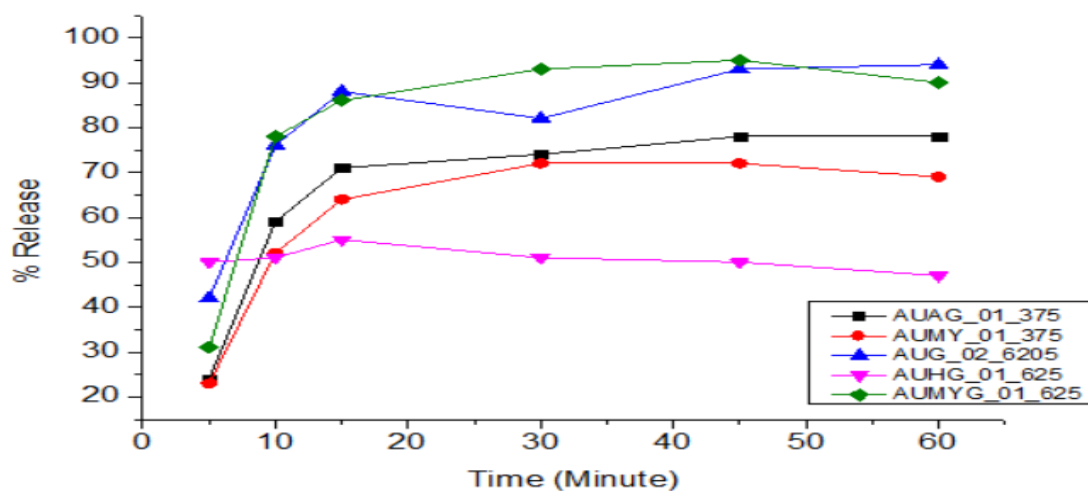


Figure 21: Time-dependent-dissolution profiles of amoxicillin and clavulanic acid (amoxicillin)

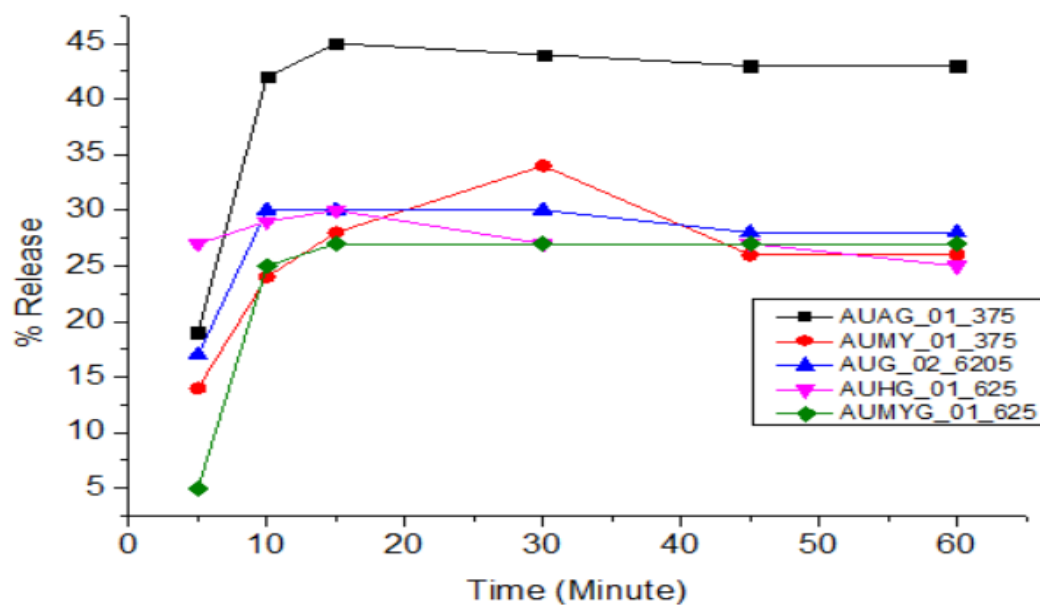


Figure 22: Time-dependent-dissolution profiles of amoxicillin and clavulanic acid (clavulanic)

Calibration curve for amoxicillin and clavulanate potassium BP dissolution test method: The concentration range of 0.3 mg/mL (60%) - 0.7 mg/mL (140%) (Figure 23 and 24).

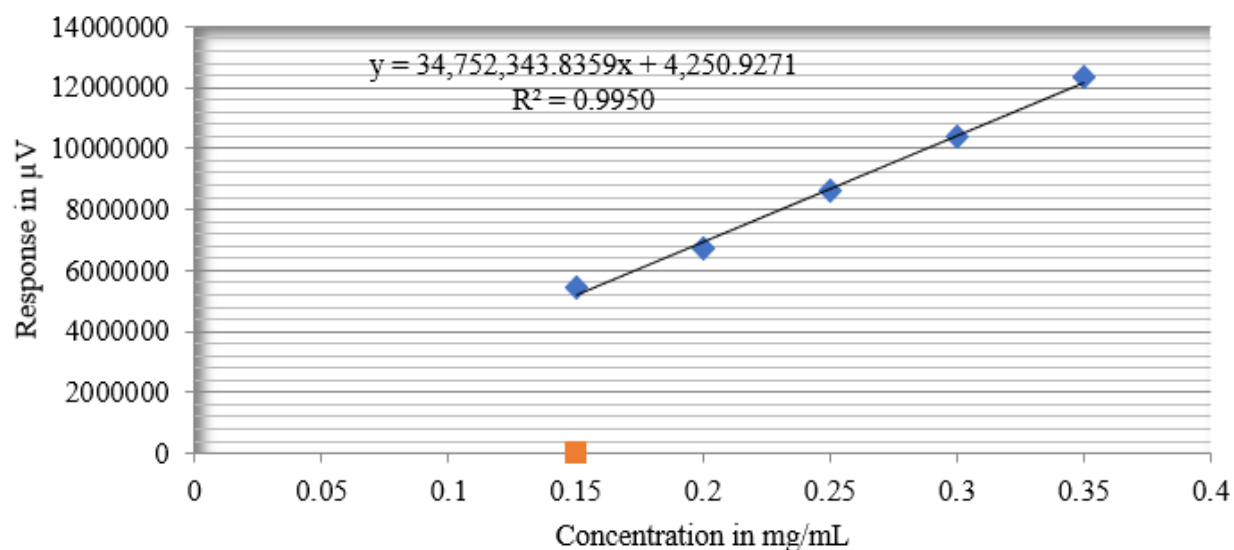


Figure 23: Calibration curve for amoxicillin and clavulanate potassium BP dissolution test method (amoxicillin)

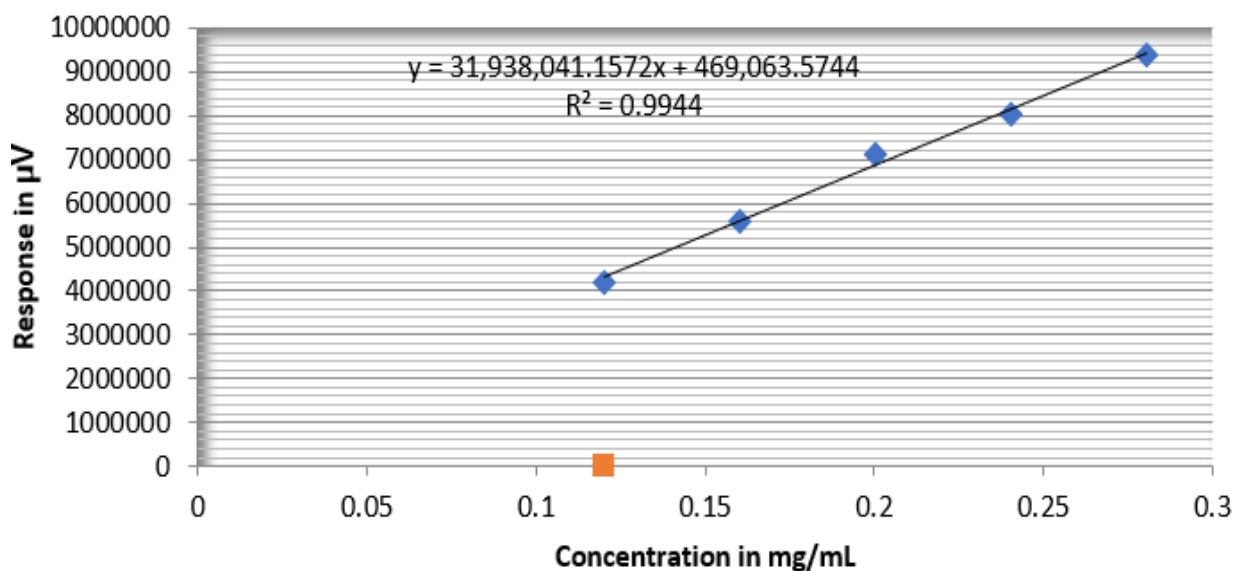


Figure 24: Calibration curve for amoxicillin and clavulanate potassium BP dissolution test method (clavulanate potassium)

5.5.3. Dissolution profiles of ciprofloxacin

Calibration curve for ciprofloxacin dissolution test method: The concentration of the tested samples and the absorbance values over the concentration range of 0.055 mg/mL (10%) - 0.66 mg/mL (120%) (Figure 25).

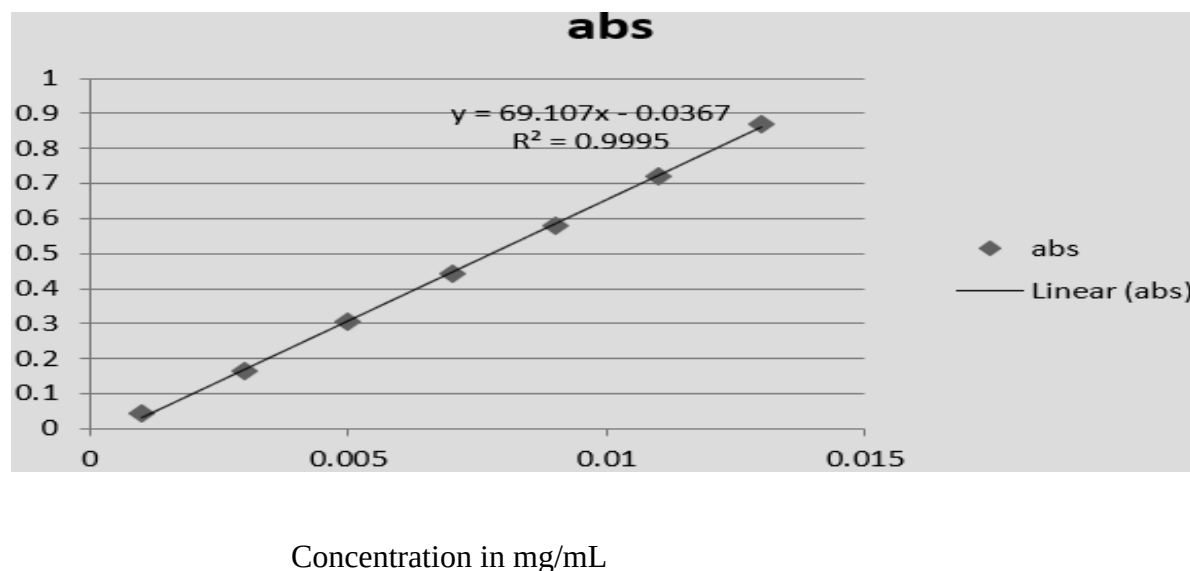


Figure 25: Calibration curve for ciprofloxacin dissolution test method

The average values of dissolution of the different samples of ciprofloxacin tested are shown in Figures 26 a and b. The obtained dissolution content at 30 minutes was found to range from $95.30\% \pm 0.65\%$ to $109.76\% \pm 1.10\%$. All samples of ciprofloxacin showed more than 80% drug release after 30 minutes. At 30 minutes, all the samples released more than the pharmacopeia requirement of 80% active pharmaceutical ingredient (USP, 2020).

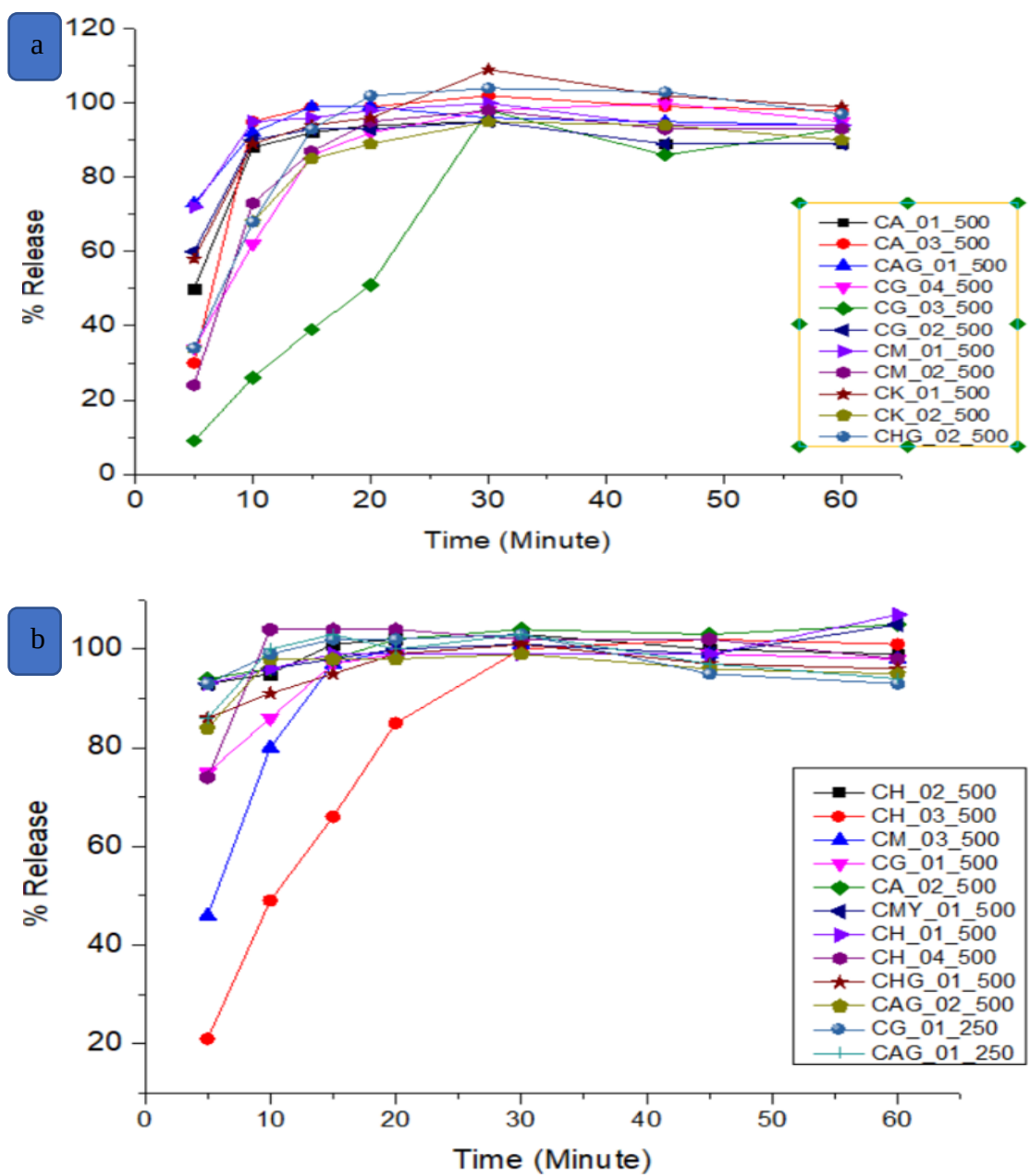


Figure 26a and b: Time-dependent-dissolution profiles of different samples of ciprofloxacin

Calibration curve for ciprofloxacin dissolution test method: The concentration of the tested samples and the absorbance values over the concentration range of 0.055 mg/mL (10%) - 0.66 mg/mL (120%) (Figure 27).

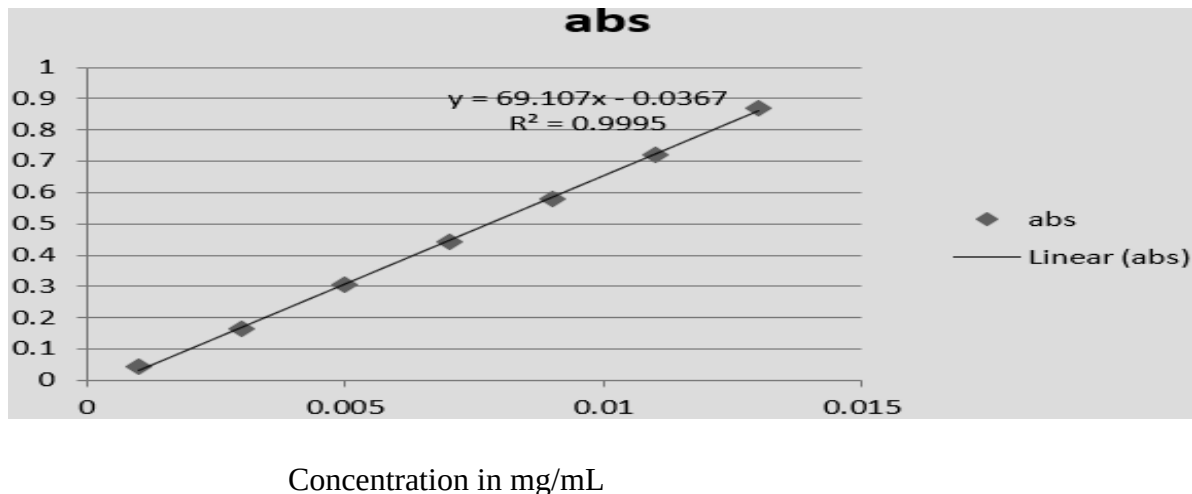


Figure 27: Calibration curve for ciprofloxacin dissolution test method

5.5.4. Dissolution profiles of doxycycline

Calibration curve for doxycycline dissolution test method: The concentration of the tested samples and the absorbance values over the concentration range of 0.00222 mg/mL (10%) - 0.03108 mg/mL (Figure 28).

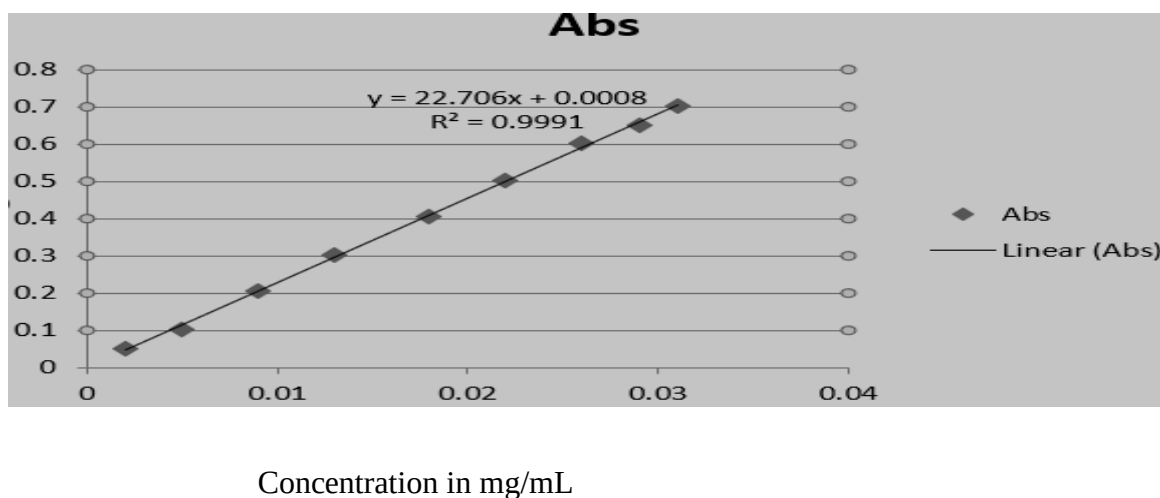


Figure 28: Calibration curve for doxycycline dissolution test method

The average values of dissolution of the different samples of doxycycline tested are shown in Figure 29. The obtained dissolution content at 30 minutes was found to be range from $94.81\% \pm 0.90\%$ to $101.68\% \pm 0.91\%$. All samples of doxycycline showed more than 80% drug release after 30 minutes. At 30 minutes, all the samples released more than the pharmacopeia requirement of 80% active pharmaceutical ingredient.

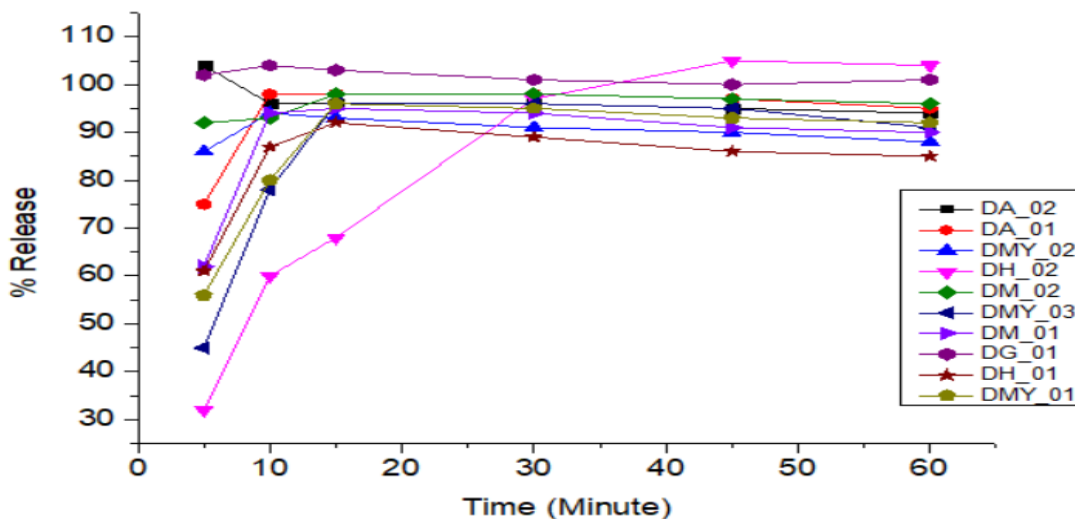


Figure 29: Time-dependent-dissolution profiles of doxycycline

5.6. Weight Variation test

5.6.1. Uniformity of dosage units of amoxicillin

A weight variation test was performed using a method specified in USP 2020 to ensure the uniformity of amoxicillin dosage units. The requirements for dosage uniformity are met if the acceptance value of the first 10 dosage units is less than or equal to L1%, which is 15 according to the USP (2020) specification (USP, 2020). All amoxicillin samples met pharmacopoeial dosage uniformity acceptance criteria (Table 9).

Table 9: Uniformity of weight of the different samples of amoxicillin

S/NO	Sample code	Mean weight (mg)+/- SD	Acceptance Value (%)	Comply to USP Specification (AV for the first 10 individual content is NMT L1 = 15.0%)
1	AHG_01_500	595.85 ± 0.022	6.2	Passed
2	AK-01_500	577.04 ± 0.0095	2.3	Passed
3	AAG_01_500	595.51 ± 0.0175	4.2	Passed
4	AM_02_500	587.79 ± 0.02	6.21	Passed
5	AMY_01_500	593.84± 0.021	6.6	Passed
6	AG_01_500	589.11± 0.013	3.2	Passed
7	AA_02_500	597.02±0.022	5.4	Passed
8	AMY_02_500	595.33±0.0087	2.2	Passed
9	AM_01_500	599.94±0.0058	1.6	Passed
10	AK_02_500	591.98 ± 0.0097	2.340	Passed
11	AM_03_500	583.80 ±0.0057	2.7	Passed
12	AH_02_500	584.1 ±0.0188	4.6	Passed
13	AG_03_500	588.24±0.014	4.1	Passed
14	AHG_02_500	580.17±0.008	3.83	Passed
15	AMY_03_500	581.65±0.0157	6.04	Passed
16	AH_01_500	580.54 ±0.0102	3.5	Passed
17	AMYG_01_500	582.53±0.0235	6.21	Passed
18	AA_01_500	586.03±0.017	5.3	Passed
19	AMG_01_500	577.09±0.005	1.2	Passed
20	AG_02_500	586.9±0.031	10.1	Passed
21	AMY_01_250	302.71±0.02	5.3	Passed
22	AMYG_01_250	305.43±0.021	8.4	Passed
23	AG_01_250	298.15 ±0.025	8.4	Passed
24	AAG_01_250	294.40±0.04	10.99	Passed
25	AH_03_250	293.88±0.03	6.9	Passed
26	AA_01_250	288.46 ± 0.0242	5.805	Passed

5.6.2. Uniformity of dosage units of amoxicillin and clavulanate potassium

As Table 10 shows that 25% (3/12) of the amoxicillin and clavulanate potassium samples did not meet these requirements.

Table 10: Uniformity of weight of the different amoxicillin and clavulanate potassium

S/NO	Sample code	Mean weight (mg) $\pm$ SD	Acceptance Value (%)	Comply to USP Specification (AV for the first 10 individual content is NMT L1 = 15.0%)
1	AUMY_01_625	1037.41 $\pm$ 0.009	2.4	Passed
2	AUHG_01_375	791.43 $\pm$ 0.006	58.7	Failed
3	AUH_02_625	1043.21 $\pm$ 0.005	5.6	Passed
4	AUH_01_625	1062.68 $\pm$ 0.005	14.9	Passed
5	AUAG_01_375	784.78 $\pm$ 0.007	28.8	Failed
6	AUMY_01_375	776.58 $\pm$ 0.009	17.0	Failed
7	AUG_01_625	1018.24 $\pm$ 0.006	1.5	Passed
8	AUAG_01_625	1017.43 $\pm$ 0.008	1.9	Passed
9	AUG_02_625	1036.42 $\pm$ 0.017	5.1	Passed
10	AUHG_01_625	1016.92 $\pm$ 0.011	3.5	Passed
11	AUA_01_625	1021.76 $\pm$ 0.009	2.2	Passed
12	AUMYG_01_625	1133.37 $\pm$ 0.011	2.7	Passed

5.6.3. Uniformity of dosage units of ciprofloxacin

A dosage uniformity test for ciprofloxacin dosage units was conducted using a method specified in USP (2020). Table 11 shows that 17.39% (4/23) of the ciprofloxacin samples did not meet these requirements.

Table 11: Uniformity of weight of the different ciprofloxacin

S/NO	Sample code	Mean weight (mg)+/-SD	Acceptance Value (%)	Comply to USP Specification (AV for the first 10 individual content is NMT L1 = 15.0%)
1	CA_01_500	703.08 ± 0.009	13.7	Passed
2	CA_03_500	645.7 ± 0.007	12	Passed
3	CAG_01_500	784.4050 ± 0.021	11.3	Passed
4	CG_04_500	705.3630 ± 0.011	16.2	Failed
5	CG_03_500	633.6910 ± 0.009	15.9	Failed
6	CG_02_500	640.1860 ± 0.008	8.3	Passed
7	CM_01-500	664.9430 ± 0.018	12.7	Passed
8	CM_02_500	638.7760 ± 0.006	9.9	Passed
9	CK_01_500	644.8990 ± 0.007	8.7	Passed
10	CK_02_500	705.2390 ± 0.034	17.9	Failed
11	CHG_02_500	618.5200 ± 0.006	7.2	Passed
12	CH_02_500	796.4470 ± 0.013	7.1	Passed
13	CH_03_500	780.7860 ± 0.015	5.8	Passed
14	CM_03_500	795.5490 ± 0.009	4.6	Passed
15	CG_01_500	806.9210 ± 0.016	7.4	Passed
16	CA_02_500	807.1380 ± 0.047	14.5	Passed
17	CMY_01_500	791.5970 ± 0.013	3.1	Passed
18	CH_01_500	808.7850 ± 0.024	12.3	Passed
19	CH_04_500	696.0050 ± 0.025	10.1	Passed
20	CHG_01_500	817.3100 ± 0.011	8.5	Passed
21	CAG_02_500	818.1850 ± 0.025	10.3	Passed
22	CG_01_250	400.1 ± 0.004	19.4	Failed
23	CAG_01_250	401.1 ± 0.005	19.3	Failed

5.6.4. Uniformity of dosage units of doxycycline

A dosage uniformity test was performed on doxycycline dosage units using a method specified in USP (2020). Table 12 shows that 30% (3/10) of doxycycline samples did not meet these specifications.

Table 12: Uniformity of weight of different doxycycline

S/NO	Sample code	Mean weight (mg) $\pm$ SD	Acceptance Value (%)	Comply to USP Specification (AV for the first 10 individual content is NMT L1 = 15.0%)
1	DA_02	242.04 $\pm$ 0.02	8.01	Passed
2	DA_01	223.00 $\pm$ 0.014	10.11	Passed
3	DMY_02	227.44 $\pm$ 0.04	78.04	Failed
4	DH_02	249.47 $\pm$ 0.031	7.4	Passed
5	DM_02	224.34 $\pm$ 0.02	8.21	Passed
6	DMY_03	129.64 $\pm$ 0.012	47.9	Failed
7	DM_01	229.34 $\pm$ 0.0214	28.3	Failed
8	DG_01	226.83 $\pm$ 0.025	9.6	Passed
9	DH_01	243.01 $\pm$ 0.0335	11.9	Passed
10	DMY_01	240.12 $\pm$ 0.02	7.8	Passed

5.7. Disintegration time

Amoxicillin capsules disintegrated in less than 15 minutes in all samples. Furthermore, all samples of amoxicillin and clavulanate potassium, ciprofloxacin, and doxycycline disintegrated in less than 15 minutes and met the specification limit. However, two samples of amoxicillin and clavulanate potassium failed to meet the USP disintegration time limit.

5.8. Friability test

Three samples of ciprofloxacin 500mg tablets were broken in this study during the friability test. According to the USP (2020), if there are cracked, cleaved, or broken tablets in the tablet sample after tumbling, the sample fails the test. Except for three samples of ciprofloxacin 500 mg tablets (CHG_02_500, CMY_01_500, and CM_03_500), all samples of ciprofloxacin were complied to meet USP specifications for friability testing. In addition, all samples of amoxicillin and clavulanate potassium had to meet USP standards. Tables 14 and 15 show the friability results for amoxicillin and clavulanate potassium and ciprofloxacin samples.

5.9. Hardness test

The hardness test results for amoxicillin and clavulanate potassium, and ciprofloxacin are shown in Tables 14 and 15. All brands and samples of amoxicillin and clavulanate potassium, and ciprofloxacin tested positive for hardness values greater than 40 N, indicating that all products met the hardness test requirement (Olayemi *et al.*, 2012).

Table 13: Disintegration test values of amoxicillin

S/NO	Sample code	Disintegration time (minutes) $\pm$ SD
1	AHG_01_500	5.15 $\pm$ 0.21
2	AK-01_500	7.3 $\pm$ 0.23
3	AAG_01_500	5.32 $\pm$ 0.2
4	AM_02_500	6.6 $\pm$ 0.31
5	AMY_01_500	7.57 $\pm$ 0.33
6	AG_01_500	5.38 $\pm$ 0.09
7	AA_02_500	6.235 $\pm$ 0.36
8	AMY_02_500	5.8 $\pm$ 0.408
9	AM_01_500	5.73 $\pm$ 0.3
10	AK_02_500	6.2 $\pm$ 0.23
11	AM_03_500	6.92 $\pm$ 0.34
12	AH_02_500	6.51 $\pm$ 0.5
13	AG_03_500	6.4 $\pm$ 0.43
14	AHG_02_500	5.72 $\pm$ 0.43
15	AMY_03_500	6.6 $\pm$ 0.3
16	AH_01_500	6.31 $\pm$ 0.2
17	AMYG_01_500	6.07 $\pm$ 0.09
18	AA_01_500	6.4 $\pm$ 0.43
19	AMG_01_500	6.7 $\pm$ 0.47
20	AG_02_500	7.23 $\pm$ 0.205
21	AMY_01_250	6.12 $\pm$ 0.2
22	AMYG_01_250	5.75 $\pm$ 0.3
23	AG_01_250	5.8 $\pm$ 0.24
24	AAG_01_250	7.2 $\pm$ 0.2
25	AH_03_250	6.75 $\pm$ 0.25
26	AA_01_250	6.3 $\pm$ 0.2

Table 14: Hardness, disintegration, and friability of amoxicillin and clavulanate potassium

S/NO	Sample code	Average hardness (N) ($\pm$ SD)	Average diameter ($\pm$ SD)	Disintegration time (minutes) $\pm$ SD	% Friability
1	AUMY_01_625	204.5 $\pm$ 28.4	19.9 $\pm$ 0.07	22.7 $\pm$ 0.5	0.008
2	AUHG_01_375	227.9 $\pm$ 11.8	19.03 $\pm$ 0.02	11.7 $\pm$ 0.9	0.006
3	AUH_02_625	244.8 $\pm$ 15.2	19.9 $\pm$ 0.05	9.5 $\pm$ 0.5	0.0095
4	AUH_01_625	261.8 $\pm$ 14.8	19.9 $\pm$ 0.02	9.5 $\pm$ 0.5	0.004
5	AUAG_01_375	200.7 $\pm$ 12.6	19 $\pm$ 0.04	24.3 $\pm$ 0.5	0.0063
6	AUMY_01_375	244.2 $\pm$ 24.4	20.2 $\pm$ 0.05	25.7 $\pm$ 2.5	0.005
7	AUG_01_625	233.9 $\pm$ 8.2	20.2 $\pm$ 0.05	21.5 $\pm$ 0.9	0.002
8	AUAG_01_625	258.8 $\pm$ 10.6	20.2 $\pm$ 0.02	22.5 $\pm$ 0.5	0.008
9	AUG_02_625	339.7 $\pm$ 21.6	19.7 $\pm$ 0.03	48.3 $\pm$ 0.5	0.009
10	AUHG_01_625	206.4 $\pm$ 13.2	20.02 $\pm$ 0.06	6.3 $\pm$ 0.5	0.003
11	AUA_01_625	253.9 $\pm$ 28.7	19.8 $\pm$ 0.04	28.5 $\pm$ 1.1	0.005
12	AUMYG_01_625	356.6 $\pm$ 18.8	21.7 $\pm$ 0.03	41.3 $\pm$ 2.5	0.003

Table 15: Hardness, disintegration, and friability of ciprofloxacin

S. No.	Sample code	Average hardness(N) ($\pm$ SD)	Average diameter (N) ($\pm$ SD)	Disintegration time (minutes) $\pm$ SD	% Friability
1	CA_01_500	272.2 $\pm$ 20.9	16.954 $\pm$ 1.2	3.5 $\pm$ 0.5	0.3
2	CA_03_500	225 $\pm$ 12.5	16.548 $\pm$ 0.06	5.3 $\pm$ 0.5	0.08
3	CAG_01_500	167 $\pm$ 11.9	18.102 $\pm$ 0.025	2.3 $\pm$ 0.5	0.03
4	CG_04_500	288.4 $\pm$ 12.4	17.404 $\pm$ 0.03	3.3 $\pm$ 0.5	0.05
5	CG_03_500	189.1 $\pm$ 11.2	16.447 $\pm$ 0.03	7.66 $\pm$ 0.5	0.08
6	CG_02_500	196.4 $\pm$ 3.7	16.519 $\pm$ 0.02	3.33 $\pm$ 0.5	0.05
7	CM_01_500	216.8 $\pm$ 21.8	16.446 $\pm$ 0.03	3.7 $\pm$ 0.75	0.008
8	CM_02_500	207.9 $\pm$ 7.8	16.529 $\pm$ 0.03	5.8 $\pm$ 0.4	0.05
9	CK_01_500	203.7 $\pm$ 8.4	16.526 $\pm$ 0.03	4 $\pm$ 0.82	0.02
10	CK_02_500	290.3 $\pm$ 6.9	17.344 $\pm$ 0.03	3.83 $\pm$ 0.85	0.02
11	CHG_02_500	194.2 $\pm$ 7.4	17.819 $\pm$ 0.04	9.33 $\pm$ 0.74	Broken
12	CH_02_500	152.5 $\pm$ 22.6	17.816 $\pm$ 0.94	1.7 $\pm$ 0.24	0.05
13	CH_03_500	108.8 $\pm$ 15.9	18.13 $\pm$ 0.02	13.6 $\pm$ 0.54	0.02
14	CM_03_500	93.4 $\pm$ 9.02	18.106 $\pm$ 0.03	3.2 $\pm$ 0.24	Broken
15	CG_01_500	116.7 $\pm$ 14.0	18.122 $\pm$ 0.01	0.72 $\pm$ 0.3	0.015
16	CA_02_500	132.8 $\pm$ 7.2	18.074 $\pm$ 0.02	1.4 $\pm$ 0.08	0.015
17	CMY_01_500	86 $\pm$ 9.6	18.117 $\pm$ 0.02	2.5 $\pm$ 0.0	Broken
18	CH_01_500	120.4 $\pm$ 3.9	18.059 $\pm$ 0.02	0.625 $\pm$ 0.3	0.24
19	CH_04_500	196.7 $\pm$ 12.53	16.9 $\pm$ 0.01	1.325 $\pm$ 0.23	0.05
20	CHG_01_500	196.7 $\pm$ 12.5	16.924 $\pm$ 0.01	1.33 $\pm$ 0.01	0.05
21	CAG_02_500	154.3 $\pm$ 11.3	18.146 $\pm$ 0.02	0.465 $\pm$ 0.015	0.04
22	CG_01_250	145.7 $\pm$ 16.9	18.067 $\pm$ 0.03	0.43 $\pm$ 0.03	0.08
23	CAG_01_250	98.2 $\pm$ 5.8	11.012 $\pm$ 0.01	0.5 $\pm$ 0.03	0.06

Table 16: Disintegration test values of the doxycycline

S. No	Sample code	Disintegration time (minutes) $\pm$ SD
1	DA_02	8.3 ± 0.24
2	DA_01	6.75 ± 0.25
3	DMY_02	8.13 ± 1.44
4	DH_02	5.65 ± 0.4
5	DM_02	5.9 ± 0.3
6	DMY_03	11.42 ± 0.34
7	DM_01	6.5 ± 0.41
8	DG_01	6.3 ± 0.4
9	DH_01	7.3 ± 0.3
10	DMY_01	7.2 ± 0.2

As shown in Table 17, all of the amoxicillin and clavulanate potassium samples were poor-quality. However, all amoxicillin samples (26/71) passed the quality control parameters. Overall, 56.33 % (40/71) of the samples tested were of poor-quality. Because all brands and samples of amoxicillin and clavulanate potassium failed the dissolution test (100%), and (47.8 %, 21.74 %, 13.04 %) of ciprofloxacin samples failed the assay, uniformity of dosage units and friability test respectively and 60% failed the assay test and 30% failed uniformity of dosage units for doxycycline.

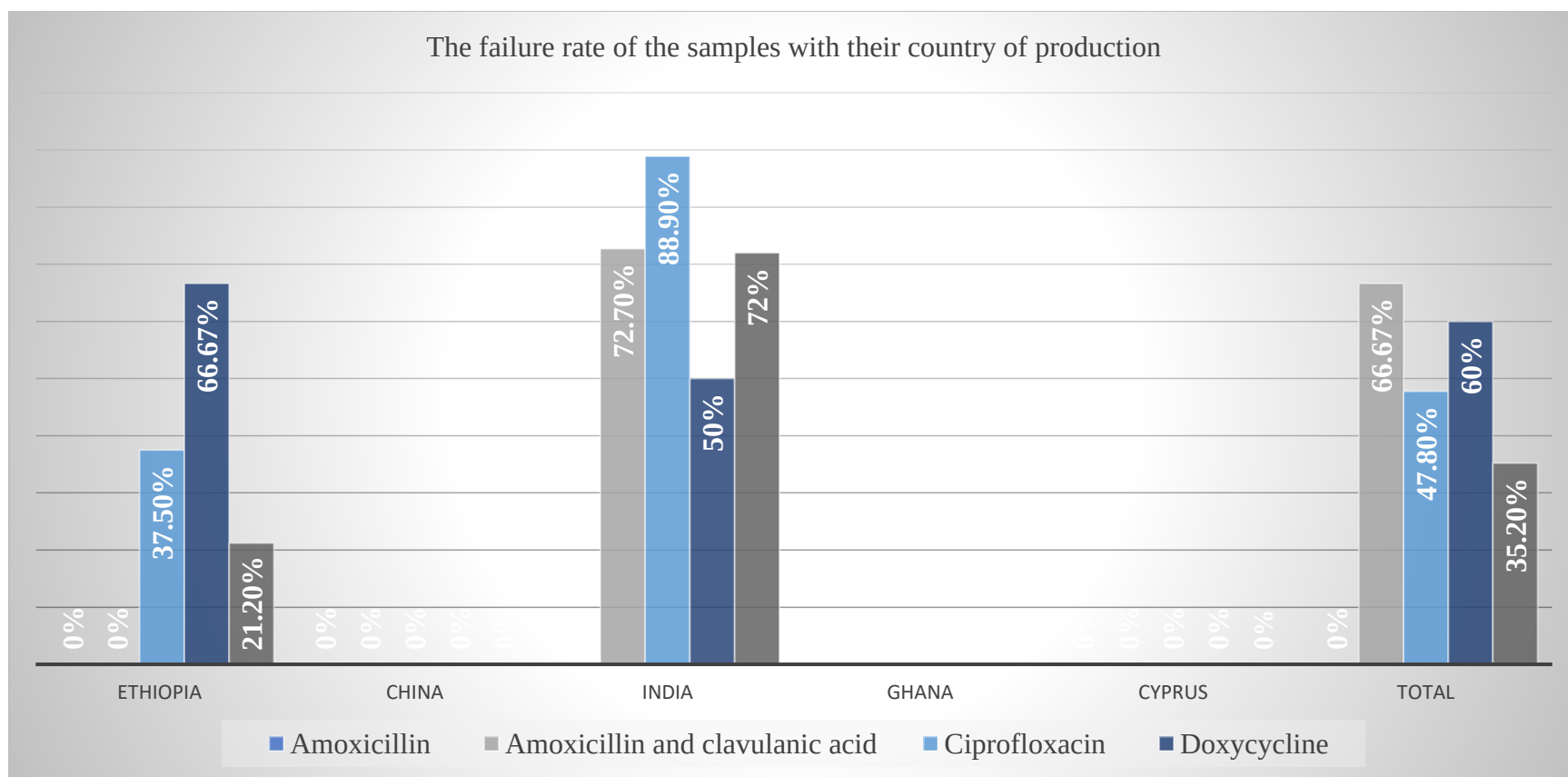


Figure 30: The failure rate of overall quality parameters of the samples tested in relation of country of manufacture (PMS-study in Setit humera and west Gondar zones, north west Ethiopia, 2021)

Table 17: Overall Summary of the quality test results of products (samples)

Product (sample)	Samples failing parameters test							
	Identification	Dissolution	Assay	Uniformity of dosage units	Disintegration	Friability	Hardness	Overall
Amoxicillin	*	*	*	*	*	-	-	*
Amox/clav k	*	100 % failed	50 % (6/12)	25 % (3/12)	16.66 % (2/12)	*	*	100 %
Ciprofloxacin	*	*	47.8 % (11/23)	21.74 % (5/23)	*	13.04 % (3/23)	*	82.61 %
Doxycycline	*	*	60 % (6/10)	30 % (3/10)	*	-	-	90 %
Overall	*	16.9 % (12/71)	27.49 % (23/71)	14.08 % (10/71)	2.82 % (2/71)	8.57 % (3/35)	*	56.33 % (40/71)

* = 0 % (not failed) and k = potassium

6. Discussion

Since antibiotics are life-saving medicines, appropriate antibiotic treatment is required to treat the systemic spread of infection and avoid its consequences (Khan and Khar, 2015). Antibiotics are among the most commonly falsified anti-infective medications in developing countries (Koech *et al.*, 2020). This study evaluated the pharmaceutical quality of commonly available samples of amoxicillin, amoxicillin and clavulanate potassium, ciprofloxacin, and doxycycline in Ethiopia's Setit Humera and west Gondar zones. This study provides important information about the quality of some antibacterial drugs available in Ethiopia.

The primary and secondary packaging, labeling, and package leaflets of all samples demonstrate that the labels on the cartons protecting the containers matched the labels on the containers, and that all of the capsules or tablets from each product were the same size and shape, without breaks or cracks. However, visual inspection results from some of the analyzed samples revealed that they were fake medicines. The packaging was a problem for 1.41% (1/71) of the sample, 8.45% (6/71) samples were not legible, 50.70% (36/71) samples did not have the symbol ® follow the trade name, 7.04% (5/71) samples did not have the manufacturer's name, and only 7.04% (5/71) samples of this falsified medicine were intact. Similar results have been reported in the labeling of different brands of amoxicillin, amoxicillin and clavulanate potassium, ciprofloxacin, and doxycycline in Rwanda (Bizimana *et al.*, 2022). In contrast to the finding of this study, problems were not observed with labeling and physical characteristic of doxycycline capsules in Jimma Town, Ethiopia, as reported by Abraham *et al.*, (2021).

All brands and samples of amoxicillin, amoxicillin and clavulanate potassium, ciprofloxacin, and doxycycline passed the identity tests in this study. Similar studies were conducted in Nairobi, Kenya (Koech *et al.*, 2020), Katsina State, Nigeria (Lawal *et al.*, 2019), Abu Dhabi, United Arab Emirates (Al-Tabakha *et al.*, 2017), Tigray, Ethiopia (Hailu *et al.*, 2013), Jounieh, Ghana (Nettey *et al.*, 2014), Lebanon (Hobeika *et al.*, 2020), Jimma Town, South-West Ethiopia (Abraham *et al.*, 2021) and Pakistan (Humayoon *et al.*, 2014).

The results of this study showed that all samples of amoxicillin samples comply with the BP specification for assay tests. Comparable findings were reported in Rwanda (Bizimana *et al.*, 2022), Lebanon (Hobeika *et al.*, 2020), and Ethiopia (Hailu *et al.*, 2013). In contrast to these findings a study conducted in Nigeria (Lawal *et al.*, 2019) 30.2% of samples were outside the pharmacopeial limit, in Boston, USA (Kelesidis and Falagas, 2015), and in Nairobi, Kenya (Koech *et al.*, 2020) failed 30% of the assay test. In this study, the assay results showed that 50% of amoxicillin and clavulanate potassium samples did not meet both the BP and USP specifications. From the total failed of 50% (6/12) samples 33.33% (2/6) samples of both amoxicillin and clavulanate potassium failed and 66.67% (4/6) failed only clavulanic acid. Although amoxicillin passed the assay test, it was considered substandard medicine because of the combination of drugs. This result was similar to a study carried out in Nigeria's Katsina State (Lawal *et al.*, 2019) 38.8% outside the pharmacopeial limit, and Ghana (Nettey *et al.*, 2014) the results showed 100% failure (14 out of 14) for amoxicillin and 14% failure (2 out of 14) for clavulanate potassium. But, in contrast to these findings a study was conducted in Dubai, United Arab Emirates (Nessa *et al.*, 2020) for amoxicillin and clavulanate potassium tablets. This study revealed that 52.17% of samples of ciprofloxacin tablets met the standard criteria of the specification in terms of active ingredient assay. However, 47.8% of the total samples failed to meet USP specifications. Similar results were reported in Katsina State, Nigeria (Lawal *et al.*, 2019), and Boston, USA (Kelesidis and Falagas, 2015) 30.0% and 18% of ciprofloxacin had not met the specifications respectively. In contrast to these findings, a study was conducted in Jounieh, Lebanon (Hobeika *et al.*, 2020). The results of this study showed that 40% of doxycycline samples comply with the BP specification. However, 60% of the samples failed the assay test. Of the total failed samples five of them were lower than 95% (53.6 – 75.4%) and one sample of them was higher than 105% of the label claim, and thus are formally classified as poor-quality medicines. Similar findings were reported in a study conducted Pakistan (Humayoon *et al.*, 2014). Contrasting results were reported Jimma Town, South-West Ethiopia (Abraham *et al.*, 2021) 100% (7/7) samples were passed the assay test.

In this study, the dissolution profile of all samples of amoxicillin, ciprofloxacin, and

doxycycline products comply with the acceptance specifications. However, all samples of amoxicillin and clavulanate potassium drugs failed to meet the acceptance criteria. All samples could not release amoxicillin and clavulanate potassium from their respective tablets up to the specified percentage within the specified minutes. The dissolved amount of amoxicillin and clavulanate potassium was in the range of 34.77-72.92% within 30 minutes (USP 2020) and 27.45-28.14% for clavulanate potassium and 50.98-95.93% for amoxicillin brands (BP 2019). From this 100% failed samples (5 out of 5) for clavulanate potassium and 40% failed (2 out of 5) for amoxicillin brands. Even though 60% of amoxicillin brands had met the specification but due to the failure of clavulanate potassium the whole combination of drugs was considered poor-quality medicines. Similar studies were conducted in Pakistan (Waqas *et al.*, 2020), Yemen (Alyahawi and Abdulmajed, 2018), Tigray, Ethiopia (Hailu *et al.*, 2013), Jimma, Ethiopia (Abraham *et al.*, 2021), and Karachi, Pakistan (Naveed and Waheed, 2014). However, Contrasting results were reported in Ghana (Nettey *et al.*, 2014) where tablet dissolution tests revealed that there was a 93% (13 out of 14) pass rate for both amoxicillin and clavulanate potassium acid and in Boston, USA (Kelesidis and Falagas, 2015), 8% of ciprofloxacin failed samples for the dissolution test, and 58% of amoxicillin failed samples in Hawassa, Ethiopia (Endashaw *et al.*, 2022) and Dubai, United Arab Emirates (Nessa *et al.*, 2020). Dissolution (drug release from the dosage form) is critical for all conventionally constructed, solid oral dosage forms in general, and modified-release dosage forms in particular, and can be the rate-limiting step in oral drug absorption (Dressman and Krämer, 2005). Most oral solid dosage forms' *in-vivo* bioavailability is estimated primarily by the results of dissolution tests, which is a crucial quality factor in evaluating the drug formulation (Waqas *et al.*, 2020).

In this study, all amoxicillin samples met the standards for dosage uniformity. However, 25% (3/12) of amoxicillin and clavulanate potassium samples, 17.39% (4/23) of ciprofloxacin samples, and 30% (3/10) of doxycycline samples failed to meet the accepted standards. Similar studies were reported in Yemen (Alyahawi and Abdulmajed, 2018), Lebanon (Hobeika *et al.*, 2020), Karachi, Pakistan (Naveed and Waheed, 2014), Tigray, Ethiopia (Hailu *et al.*, 2013), and Nigeria (Igboasoiyi *et al.*, 2018) for amoxicillin. In contrast to these findings, the absence of variation in dosage uniformity was reported in

Jimma, Ethiopia (Abraham *et al.*, 2021) for ciprofloxacin, Karachi, Pakistan (Naveed and Waheed, 2014) for doxycycline, Dubai, United Arab Emirates (Nessa *et al.*, 2020) for amoxicillin/clavulanic acid. Dosage uniformity is measured to ensure a consistent dose of the API between individual dosage units in a batch (Abraham *et al.*, 2021). To ensure dosage uniformity, each unit in a given batch should contain the active drug within a narrow range around the label claim (Zaid *et al.*, 2013). The uniformity of dosage units can be assessed by measuring either the content uniformity or the weight of the tested units. The weight variation test applies to hard capsules, uncoated tablets, and film-coated tablets containing 25 mg or more of a drug substance constituting 25% or more, by weight, of the dosage unit or, in the case of hard capsules, the capsule contents, with the exception that uniformity of other drug substances present in lesser proportions is demonstrated by meeting the content uniformity requirements.

In this study, all amoxicillin, ciprofloxacin, and doxycycline samples disintegrated in less than 15 minutes. However, two of the film coated amoxicillin and clavulanate potassium samples (AUG _02 _625 and AUMYG_ 01_ 625) did not meet the specification. The maximum mean disintegration time for amoxicillin, ciprofloxacin, and doxycycline were 6.31 ± 0.29 , 3.59 ± 0.37 , and 73.45 ± 0.43 minutes respectively. Even though the disintegration time is up to 15 minutes for uncoated tablets and 30 minutes for coated tablets and capsules, all drugs disintegrate below this time (USP, 2020). The rapid disintegration time exhibited by these samples might be due to the type and amount of disintegrant used in the formulation. This result is in agreement with the study conducted in Yemen (Alyahawi and Abdulmajed, 2018), Tigray, Ethiopia (Hailu *et al.*, 2013), Jimma, Ethiopia (Abraham *et al.*, 2021), Karachi, Pakistan (Naveed and Waheed, 2014), Dubai, United Arab Emirates (Nessa *et al.*, 2020), Ghana (Nettey *et al.*, 2014) and Nigeria (Igboasoiiyi *et al.*, 2018). Contrasting results were reported in Boston, USA (Kelesidis and Falagas, 2015) where 8% of ciprofloxacin samples failed for disintegration test.

In this study, all samples of amoxicillin and clavulanate potassium tablets and twenty samples of ciprofloxacin tablets met the specification for the friability test. However, three samples of ciprofloxacin samples (CHG_02_500, CM_03_500, and CMY_01_500) were

broken during the friability test and failed to comply. Failure to meet the friability test specification may be due to low binder concentration, resulting in loose inter particulate bonding, or the use of low compression pressure in the tablet machine. Friability ranges from 0.002% to 0.0095% for amoxicillin and clavulanate potassium tablets and 0.008% to 0.3% for ciprofloxacin tablets. The friability was less than 1%, indicating that it is within the compendia limits, indicating that the tablets have good mechanical strength. This also demonstrated that all brands and samples could withstand abrasion without losing tablet integrity. Similar results were reported in a study conducted in Yemen (Alyahawi and Abdulmajed, 2018) and Nigeria (Igboasoiki *et al.*, 2018) for ciprofloxacin tablets, and Dubai, United Arab Emirates (Nessa *et al.*, 2020) for amoxicillin and clavulanate potassium tablets. Tablet hardness is not an absolute indicator of tablet strength because some formulations cap or laminate on attrition, losing their crown part. When subjected to stresses from collision and tablets sliding towards one another, as well as other solid substances, tablets may be able to withstand abrasion. As a result, another indicator of tablet strength is its friability. The abrasion loss is a measure of tablet friability (Hailu *et al.*, 2013). According to the pharmacopeia, the friability value of tablets should be less than 1% (USP 2020).

The results of this study showed that the hardness value for all amoxicillin and clavulanate potassium, and ciprofloxacin samples were in the range of 200.7 - 356.6 and 86-290.3 respectively. Which is greater than the minimum value of 40 Newton (Ngwuluka *et al.*, 2009). According to these findings, all samples of amoxicillin and clavulanate potassium, and ciprofloxacin fulfilled the limits of the hardness test. Similar results were reported in a study conducted in Yemen (Alyahawi and Abdulmajed, 2018) and Nigeria (Igboasoiki *et al.*, 2018) for ciprofloxacin tablets, and in Dubai, United Arab Emirates (Nessa *et al.*, 2020) for amoxicillin and clavulanate potassium tablets. The hardness test is a critical quality assurance parameter (Nessa *et al.*, 2020). The ability of the tablets to resist chipping, abrasion, or breakage under conditions of storage, transportation, and handling prior to storage is determined in large part by their crushing strength (Alyahawi and Abdulmajed, 2018).

In this study, 56.33 % (40/71) of the tested samples were of low quality. Because all brands and samples of amoxicillin and clavulanate potassium failed the dissolution test (100%), (47.8 %, 21.74 %, 13.04 %) failed assay, uniformity of dosage units and friability test of ciprofloxacin respectively and 60% failed the assay test and 30% failed uniformity of dosage units for doxycycline. Poor-quality drugs are those that did not meet the correct scientific specification limits for quality attributes (Khan and Khar, 2015).

6.1. Limitation of the study

The study's findings could have been influenced by the following limitations. To begin, only capsules and tablets were tested. As a result, the findings are inapplicable to other dosage forms or medications. Second, because of the small number of samples collected and the data collected by convenience sampling technique which is poor representativeness and the study findings is difficult to represent the entire country

7. Conclusion and Recommendation

7.1. Conclusion

The findings indicate that some 39.44% of the samples from the four antibiotics were found to be of poor quality. Of the total samples, 25.35% (18/71) were not registered, while 15.49% (11/71) were registered but the license was expired, and 74.65% (53/71) observed samples did not meet the criteria specified in the joint WHO/FIP/USP visual inspection checklists. This study indicates, overall, 56.33 % (40/71) of the tested samples, were of poor quality (do not meet the pharmacopeial quality specification limits). Therefore, the regulatory body (EFDA) should focus on enforcing the necessary measures to ensure that antibacterial drugs on the market meet the required mandatory standards.

7.2. Recommendations

- The medicine registration system should be strengthened to ensure that all drugs, both domestically produced and imported, are evaluated before they are placed on the market.
- Drug regulatory bodies (EFDA) and regional branches should conduct post-market quality assessment studies on a regular basis to determine the quality status of the drug on the market.
- All concerned MRAs on packaging, labelling and package leaflets of different brands of drug should do regular and emergency inspection periodically in order to identify suspicious products for further examination (EFDA and Regional regulatory body).
- Pharmacists and distribution channels must understand their responsibilities under the Drug Supply Chain Security Act, in order to protect patients from receiving low-quality drug products.
- The national drug regulatory system should be strengthened at all levels, as well as coordinated with other government agencies in implementing pharmaceutical-related regulations to protect illegal traders, with a focus on borderlines.

8. References

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9. Annexes

Annex 1: Visual inspection tool for packaging and labeling of pharmaceutical formulations; modified from WHO/FIP/USP checklist

Sample code..... Product nameDate of inspection:.....

	Yes	No	Other Observations
1. Packaging			
Any medication should be packaged in a container, which can range from a glass bottle to a blister pack to a glass, plastic, or metal tube. A folding carton with the label frequently protects the container. Examine the packaging and compare it to known containers from the same manufacturer for the same drug. Pharmaceutical packaging and labeling is a highly complex and costly business. As a result, the process and the quality of the packaging material are extremely difficult to imitate. As a result, a thorough visual inspection may be a necessary screening step for drug quality control. However, counterfeit drug manufacturers are quick to replicate special labeling and holograms.			
1.1 Container and Closure			
Do the container and closure protect the product from the outside environment; e.g. is the container properly sealed?			
Do they guarantee that the product will meet the required specifications throughout its shelf life?			
Are the container and closure appropriate for the product inside?			
Is the container securely sealed?			
1.1 Container and Closure			

Does the packaging—for instance, is the container properly sealed—protect the product from the outside environment?			
1.2 Label The information on the label is crucial. The information can be printed on a label that is attached to the container or directly on the container itself, but it must be legible and indelible.			
Does the label on the carton that contains the blister pack, if one is present, match the label on the blister pack?			
Is all of the label's text readable and permanent?			
1.2.1 The trade/Brand name			
Is the company name spelled correctly?			
Is the drug (trade name) registered with the country's DRA (drug regulatory authority)?			
Is the drug legally available in the country?			
Does the ® symbol appear after the trade name?			
Is the trade name indelibly impressed or imprinted onto the strip for blister or foil strip-packed products?			
1.2.2 The active ingredient name (scientific/Generic name)			
Is the spelling of the name of the active ingredient accurate?			
Do the registered drug's trade name and the name of the active ingredient match?			
1.2.3 The manufacturer's name and logo			
Is the name and logo of the manufacturer legible and correct?			
Does the logo or hologram (if applicable) appear genuine?			

Does the logo or hologram (if applicable) change color when viewed from different angles?			
1.2.4 The manufacturer's full address			
International law requires all manufacturers to print their complete address on the label. Many companies that manufacture substandard or counterfeit goods do not include a traceable address on the label.			
Is the full address of the manufacturer legible and correct?			
Is this company or its agent registered in the country?			
1.2.5 The drug strength (mg/unit)			
Is the strength, or the quantity of the active ingredient in each unit, specified on the label?			
Is the medicine strength permanently impressed or imprinted onto the foil strip for blister- or blister-packaged products?			
1.2.6 The dosage form			
Is the dosage specified on the container label?			
Does the dosage form stated on the label correspond to the medication's actual dosage form?			
Is the indicated drug in this dosage form registered and authorized for sale in the country?			
1.2.7 The number of units per container			
Is the number of tablets on the label consistent with the number of tablets on the container?			
1.2.8 Dosage statement (if appropriate)			

Is the dose specified on the label?			
Is the labeled dosage appropriate for the medicine in this form and strength?			
Is the product registered and legal to sell in the country at this dosage?			
1.2.9 The batch number (or lot number)			
Medicines with the same batch/lot number should be the same. A batch is a defined portion of the production in a continuous process, based on time or quantity. The same batch number should have the same manufacturing, processing, packing, and coding history. Batch/lot numbers should be used for all product quality control testing.			
Does the numbering system on the package correspond to the one used by the manufacturer?			
1.2.10 The manufacture date and the expiry date			
Under no circumstances should an expired product be sold.			
1.2.11 Storage information:			
Are the storage conditions specified on the label?			
Has the product been properly stored?			
1.3 Leaflet or package insert: Every product package includes a leaflet outlining dosage instructions, the medication's ingredients, potential side effects, how the medication works, and other pertinent information. The only exceptions are situations where the packaging already contains all the details that would otherwise be in the leaflet.			

If available for comparison, is the package insert printed on the same color or caliber paper as the original, or does it appear to be a copy?			
Is the package insert's or packaging's ink smudge-proof?			
Do the details on the product container and the package insert correspond?			
2. Physical characteristics of tablets/capsules From cough syrups to injections, all types of medications can and have been counterfeited. As previously stated, it is critical to inspect the packaging of these medications. Additionally, tablets or capsules can be examined for moisture, dirty marks, abrasion erosion, cracks, or other signs of adulteration.			
2.1 Shape uniformity			
Are the pills/tablets all the same shape?			
2.2 Size uniformity			
Are the pills/tablets all the same size?			
2.3 Color consistency			
1.2.11 Information about storage:			
Are the storage conditions mentioned on the label?			
Has the item been stored properly?			
1.3 Leaflet or package insert:			

Each product pack includes a leaflet that describes the dosage, the medication's ingredients, any possible side effects, how the medication works, and how it should be taken. The only exception is when the packaging contains all the information that would otherwise be in the leaflet.			
Is the package insert printed on the same color or caliber paper as the original, if possible, or does it just look...off?			
Is the ink on the packaging smudge-resistant? Is the package insert inked?			
2. Physical characteristics of tablets/capsules From cough syrup to injections, all types of medications can and have been counterfeited. As previously stated, it is critical to examine the packaging of these medications. Additionally, tablets or capsules can be examined for signs of moisture, dirty marks, abrasion erosion, cracks, or other adulteration.			
2.1 Uniformity of shape			
Is the shape of the tablets/capsules consistent?			
2.2 Uniformity of size			
Is the size of the tablets/capsules uniform?			
2.3 Uniformity of color			
Is the color of the tablets/capsules uniform?			
2.4 Texture Uniformity:			
Tablets can be film-coated, sugar-coated, or enteric-coated.			

Is the coating on the tablets consistent?			
Is the tablet's base completely covered?			
2.5 Markings (scoring, letters, etc):			
Are markings uniform and identical?			
Does the logo (if present) match that of the manufacturing company?			
2.6 Breaks, Cracks, and Splits:			
Are there any breaks, cracks, splits, or pinholes in the tablets or capsules?			
2.7 Embedded surface spots or contamination:			
Are there any embedded surface spots or foreign particle contamination in the tablets/capsules?			
2.8 Presence of empty capsules in the case of a sample of capsules:			
Is the examined sample free of empty capsules?			
2.9 Smell			
Is the medicine's odor identical to the original (if available)?			
Is the odor unusual?			
Is the color of the tablets/capsules uniform?			

Annex 2: Sample collection form

Area/region/country: _____ Sample code: _____

(Area/region/country code/medicine abbreviation/sequence number/sampling date dd/mm/yy)

Where the sample was collected and its name: _____

Address (including phone number, email address, and GPS coordinates, if applicable): _____

Organization and names of people who collected the sample:

1. _____

2. _____

Sample product name: _____

Name and strength of active pharmaceutical ingredient(s): _____

Dosage form (tablet, capsule): _____

Size, type, and material of the container's packaging: _____

Lot number: _____

Manufacture date: _____ Expiry date: _____

Regulatory status in the country, and, if applicable, registration number:

Name and address of the manufacturer: _____

Quantity collected (number of tablets/ampoules/vials and packages): _____

Initial first page:

Product name: _____ Sample code:

Date the batch was received at the location: _____

Storage and climatic conditions at the sampling site:

Conditions controlled? Yes _____ No _____

Temperature and humidity in the place where the sample was stored at the time of sample collection: _____

Comments on suitability of premises where products are stored, abnormalities, remarks or observations that may be considered relevant, if any _____

Date:

Signature of a person(s) taking samples Signature of representative of the facility where a sample was taken

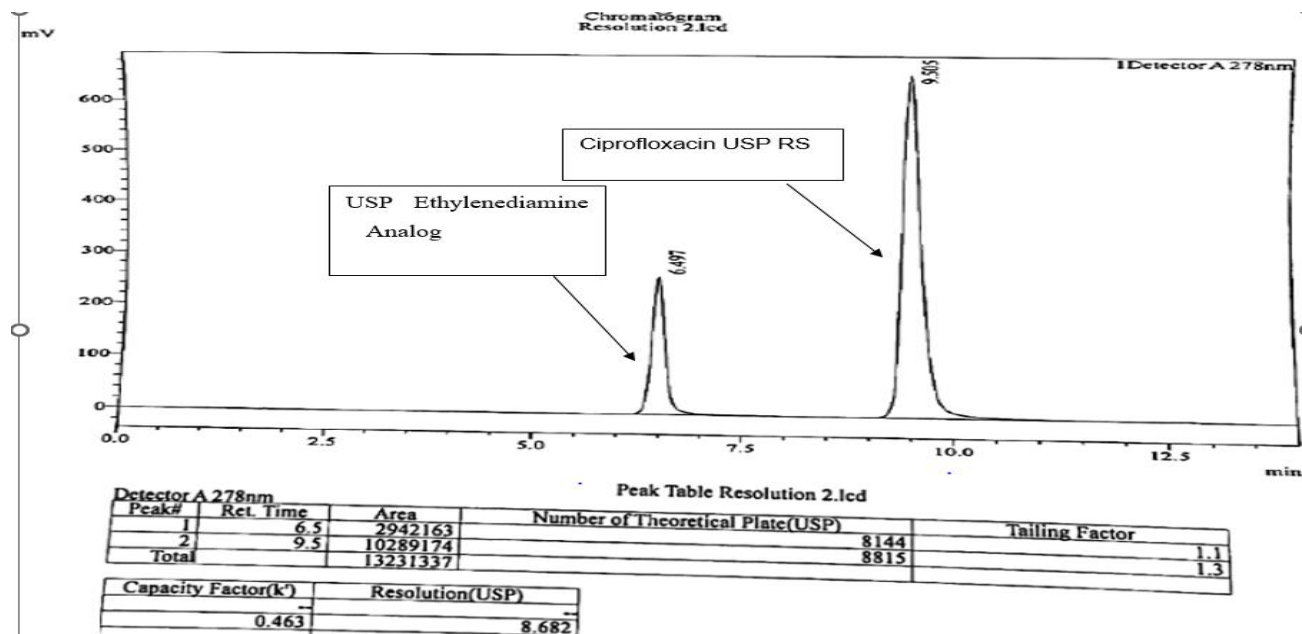
1. _____

2. _____

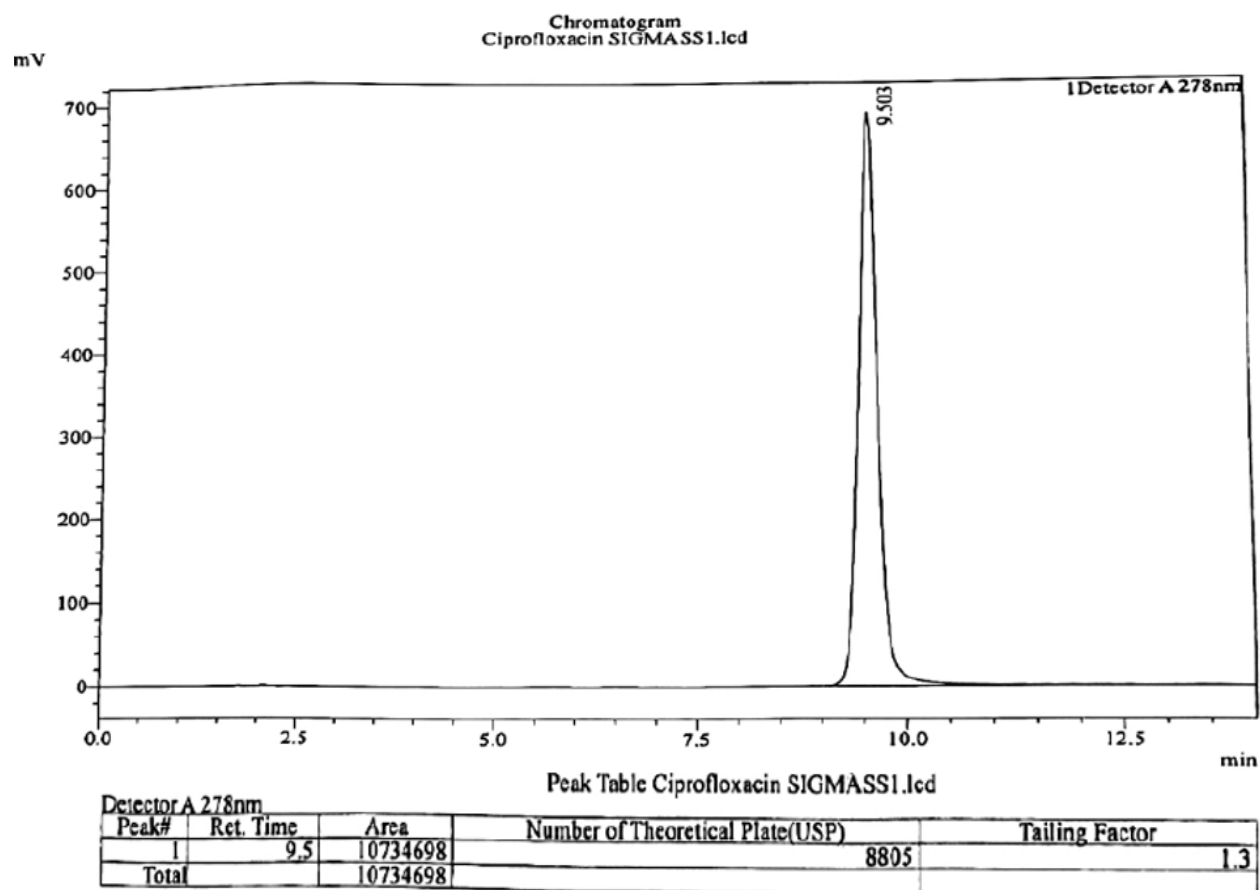
Note: Samples collected must remain in their original primary and secondary packaging, intact and unopened.

The sample collection form should always be kept with the collected sample.

Annex 3: HPLC Chromatogram of Ciprofloxacin Resolution



Annex 4: HPLC Chromatogram of Ciprofloxacin SIGMA system suitability

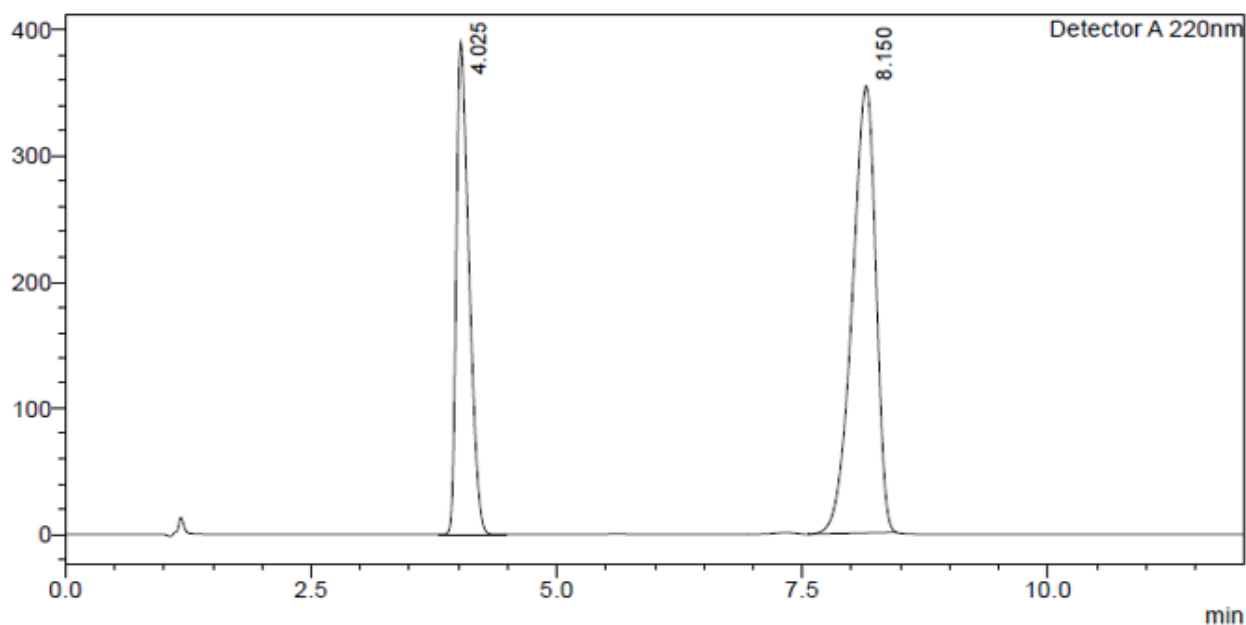


Annex 5: HPLC Chromatogram of amoxicillin and clavulanate potassium Reference Standards

Sample Name : Amoxicillin USP RS &Clavulanate K USP RS
 Sample ID : Amoxicillin USP RS &Clav P1R1
 Data Filename : Amoxicillin USP RS &Clav P1R1.lcd
 Method Filename : Amoxicillin and Clavulanate potassium tablet Dissolution.lcm
 Batch Filename : Amoxicillin and Clavulanate potassium tablet.lcb
 Vial # : 1-2
 Injection Volume : 20 uL
 Date Acquired : 3/22/2022 5:44:22 PM
 Date Processed : 4/8/2022 11:59:39 AM
 Sample Type : Standard
 Level : 1
 Acquired by : System Administrator
 Processed by : System Administrator

<Chromatogram>

mV



Detector A 220nm

Ret. Time	Area	Number of Theoretical Plate(USP)	Tailing Factor	Resolution(USP)
4.025	3507236	4025	1.520	--
8.150	5910283	5231	0.826	11.709
	9417519			

Annex 6: Ethical clearance



ቀን
Date August 10, 2021

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Ref. No. ERB/SOP/324/13/2021

To: Tewodros Denekeu
School of Pharmacy

Re: Ethical Clearance

It is to be recalled that you submitted a research proposal entitled “**Post Marketing Quality Surveillance of Selected Antibacterial Agents (Amoxicillin capsule, Doxycycline capsule and Ciprofloxacin tablet) Marketed in Gondar, Ethiopia**”. The committee thoroughly reviewed the proposal based on its operational guidelines and found that it fulfills all 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



Annex 7: shows defects observed on the samples collected by visual inspection

S.no	Parameter	Defect observed	Sample code with stated defect
1	Is the drug registered with the country's drug regulatory authority?	Not registered in the country	AG_02_500, AH_01_500, AMY_03_500, AM_03_500, AHG_02_500, AK_02_500, AMYG_01_500, AMG_01_500, DMY_02, DM_01, DM_02, DA01, DG_01, DMY_03, CAG_01_250, CAG_02_500, CHG_01_500, CH_01_500, CG_01_500, CA_02_500, CG_01_250, CAG_01_500, CH_02_500, CHG_02_500, CM_01_500, AUMY_021, AUHG_01, AUAG_01,AUG_01
2	Problem with the packaging	Package with damage	DH_01
3	Are the manufacture and expiry dates clearly stated on the label?	The manufacture expiry dates are not clearly indicated on the label.	DMY_01, DH_01, DA-02
4	Are the name and logo of the manufacturer legible and correct?	Not legible and correct manufacturer's name and logo	DMY_01, DH_01, DA_02, CAG_01_250, CAG_02_500, CHG_01_500, CH_01_500, CG_01_500, CA_02_500, CG_01_250
5	Is the label's information legible and permanent?	Not readable	CAG_01_250, CAG_02_500, CHG_01_500, CH_01_500, CG_01_500, CA_02_500, CG_01_250
6	Does the symbol ® appear after the trade name?	Doesn't the symbol ® appear after the trade name?	AMY_01_250, AG_01_250, AM_01_500, AK_01_500, AA_02_500, AG_01_500, AMY_02_500, DMY_02, DM_01, DM_02, DA-01, DG-0, DA_01, DM_02, CK_01_500, CM_02_500, CG_03_500, CG_02_500, CA_03_500, CMY_01_500, CH_03_500, CM_03_500, CA_01_500, CG_01_500, CK_02_500, CM_01_500, CHG_02_500, AUMY_01_375, AUHG_01_625, AUAG_01_625, AUG_01_625, AUHG_01_625, AUG_02_625, AUA_01_625, AUMYG_01_625, AUMY_01_625.

Annex 8: Overall Information of collected sample

S. No	Sample code	Sample site	Brand name	Dosage form	Manufacturer name & address	Batch no	MFD	EXD
1	AHG_01_500	Humera	AMOXID cap 500	Capsule	APF PLC, Adigrat, Ethiopia	28228	01 2019	01 2024
2	AK-01_500	Kokit	Amox – 500	Capsule	CSPC Shijiazhuang co.,Ltd, China	706210313	03 2021	03 2024
3	AAG_01_500	Abderafi	AMOXID cap 500	Capsule	APF PLC, Adigrat, Ethiopia	31423	-	04 2024
4	AM_02_500	Metema	AMOXID cap 500	Capsule	APF PLC, Adigrat, Ethiopia.	32490	11 2020	11 2025
5	AMY_01_500	Mai kadra	AMOXID cap 500	Capsule	APF PLC,Adigrat, Ethiopia	32476	10 2020	10 2025
6	AG_01_500	Gendwuha	Amox-500	Capsule	CSPC Shijiazhuang co., Ltd, China	706201101	11 2020	11 2025
7	AA_02_500	Abderafi	Amox-500	Capsule	CSPC Shijiazhuang co., Ltd, China	706210316	03 2021	03 2024
8	AMY_02_500	Mai kadra	Amox-500	Capsule	CSPC Shijiazhuang co., Ltd, China	706210316	03 2021	03 2024
9	AM_01_500	Metema	Amox-500	Capsule	CSPC Shijiazhuang co., Ltd, China	706210427	04 2021	04 2024
10	AK_02_500	Kokit	Amoxicillin 500mg	Capsule	Epharmethiopia	1030391	03 2021	03 2025
11	AM_03_500	Metema	Amoxicillin 500mg	Capsule	Epharmethiopia	1030121	03 2020	03 2025
12	AH_02_500	Humera	AMOXID cap 500	Capsule	APF PLC, Adigrat, Ethiopia	31074	02 2020	03 2025
13	AG_03_500	Gendwuha	AMOXID cap 500	Capsule	APF PLC, Adigrat, Ethiopia.	31286	03 2020	02 2025
14	AHG_02_500	Humera	Amoxicillin 500mg	Capsule	Epharmethiopia	1030431	03 2021	03 2025
15	AMY_03_500	Mai kadra	Amoxicillin 500mg	Capsule	Epharmethiopia	1030431	03 2021	03 2025
16	AH_01_500	Humera	Amoxicillin 500mg	Capsule	Epharmethiopia	1030431	03 2021	03 2025
17	AMYG_01_500	Mai kadra	Amoxicillin 500mg	Capsule	Epharmethiopia	1030391	03 2021	03 2025

18	AA_01_500	Abderafi	AMOXID cap 500	Capsule	APF PLC, Adigrat, Ethiopia	26619	07 2018	03 2025
19	AMG_01_500	Metema	Amoxicillin 500mg	Capsule	Epharmethiopia	1030391	03 2021	07 2023
20	AG_02_500	Gendwuha	Amoxicillin 500mg	Capsule	Epharmethiopia	1010361	-	03 2025
21	AMY_01_250	Mai kadra	AMYN – 250	Capsule	KOPRAN LIMITED, Khalapur, India	S36420032	08 2020	01 2025
22	AMYG_01_250	Mai kadra	AMOXID – 250	Capsule	APF PLC, Addigrat, Ethiopia.	26506	01 2018	07 2023
23	AG_01_250	Gendwuha	AMYN – 250	Capsule	KOPRAN LIMITED, Khalapur, India	S36420039	10 2020	07 2023
24	AAG_01_250	Abderafi	AMOXID – 250	Capsule	APF PLC, Adigrat, Ethiopia	22253	-	11 2023
25	AH_03_250	Humera	AMOXID – 250	Capsule	APF PLC, Adigrat, Ethiopia	29916	-	03 2022
26	AA_01_250	Abderafi	AMOXID – 250	Capsule	APF PLC, Adigrat, Ethiopia	29916	10 2019	10 2024
27	AUMY_01_625	Mai kadra	INDCLAV 625	Tablet	Indchemie Health Specialities Pvt. Ltd., India	T1150007ET	02 2020	10 2024
28	AUHG_01_375	Humera	INDCLAV 375	Tablet	Indchemie Health Specialities Pvt. Ltd., India	T1170001ET	02 2020	01 2022
29	AUH_02_625	Humera	INDCLAV 625	Tablet	Indchemie Health Specialities Pvt. Ltd., India	T1150002ET	02 2020	01 2022
30	AUH_01_625	Humera	INDCLAV 625	Tablet	Indchemie Health Specialities Pvt. Ltd., India	T1150009ET	12 2020	01 2022
31	AUAG_01_375	Abderafi	INDCLAV 375	Tablet	Indchemie Health Specialities Pvt. Ltd., India	T1130019ET	08 2020	11 2022
32	AUMY_01_375	Mai kadra	ACINET 375	Tablet	Alkem laboratories ltd	19150567	10 2019	07 2022
33	AUG_01_625	Gendwuha	KOACT 625	Tablet	Aurobindo pharma ltd, India	EL5019041-C	12 2019	09 2021
34	AUAG_01_625	Abderafi	KOACT 625	Tablet	Aurobindo pharma ltd, India	EL5020013-A	04 2020	11 2021
35	AUG_02_625	Gendwuha	CLAVAMYN-625	Tablet	Kopran limited	K55320019	06 2020	03 2022
36	AUHG_01_625	Humera	KOACT 625	Tablet	Aurobindo pharma ltd, India	EL5020013-A	04 2020	05 2022
37	AUA_01_625	Abderafi	CLAVAMYN-625	Tablet	KOPRAN LIMITED, Khalapur, India	K55320006	04 2020	03 2022
38	AUMYG_01_625	Mai Kadra	Clavomid 625	Tablet	Remedica ltd	92283	01 2021	03 2022
39	CA_01_500	Abderafi	AKCIPO 500	Tablet	Akriti pharmaceuticals pvt., Ltd, India	ATB057	03 2021	01 2023

40	CA_03_500	Abderafi	Ciproqun 500	Tablet	KOPRAN LIMITED, Khalapur, India	K11420006	01 2020	02 2024
41	CAG_01_500	Abderafi	Ciprofloxacin usp 500	Tablet	Zhejiang jingxin pharmaceutical co., ltd, China	B190807	08 2019	12 2022
42	CG_04_500	Gendwuha	AKCIPRO 500	Tablet	Akriti pharmaceuticals pvt., Ltd, India	ATB024	02 2021	07 2022
43	CG_03_500	Gendwuha	Ciproquin 500	Tablet	KOPRAN LIMITED, Khalapur, India	K11420038	09 2020	01 2024
44	CG_02_500	Gendwuha	Ciproquin 500	Tablet	KOPRAN LIMITED, Khalapur, India	K11419108	12 2019	08 2023
45	CM_01-500	Metema	CIPRDAC 500	Tablet	Cadila Pharmaceuticals Plc, Ethiopia	D21001BY38	01 2021	11 2022
46	CM_02_500	Metema	Ciproquin 500	Tablet	KOPRAN LIMITED, Khalapur, India	K11420008	02 2020	12 2023
47	CK_01_500	Kokit	Ciproquin 500	Tablet	Akriti Pharmaceuticals Pvt., Ltd, India	K11419116	12 2019	01 2023
48	CK_02_500	Kokit	AKCIPRO 500	Tablet	Akriti pharmaceuticals pvt. Ltd	ATB022	02 2021	11 2022
49	CHG_02_500	Humera	G_CIPROX 500	Tablet	Guilian Pharmaceutical co., Ltd, Ghana	HB190201	02 2019	01 2024
50	CH_02_500	Humera	Ciproflacin USP 500	Tablet	Zhejiang jingxin pharmaceutical co., Ltd, China	B190809	08 2019	02 2022
51	CH_03_500	Humera	CIPOFLOX-500	Tablet	Reyoung pharmaceuticeutical co., Ltd, China	203121099	04 2020	07 2022
52	CM_03_500	Metema	CIPOFLOX-500	Tablet	Reyoung pharmaceuticeutical co., Ltd, China	193121262	12 2019	03 2023
53	CG_01_500	Gendwuha	CIPRO-SSP 500	Tablet	Sansheng Pharmaceutical Plc, Ethiopia	00321010010	01 2021	11 2022
54	CA_02_500	Abderafi	CIPRO-SSP 500	Tablet	Sansheng Pharmaceutical Plc, Ethiopia	00321040060	28 2021	12 2022
55	CMY_01_500	Mai kadra	CIPOFLOX-500	Tablet	Reyoung pharmaceutical co., Ltd, China	193121260	12 2019	03 2023
56	CH_01_500	Humera	Cipro-SSP 500	Tablet	Sansheng Pharmaceutical Plc, Ethiopia	00320110020	03 2020	11 2022
57	CH_04_500	Humera	Ciproleb-500	Tablet	Leben laboratories pvt., Ltd, India	T3579	12 2019	10 2022
58	CHG_01_500	Humera	Cipro-SSP 500	Tablet	Sansheng Pharmaceutical Plc, Ethiopia	00320110070	09 2020	11 2022
59	CAG_02_500	Abderafi	Cipro-SSP 500	Tablet	Sansheng Pharmaceutical Plc, Ethiopia	00320110050	06 2020	10 2022
60	CG_01_250	Gendwuha	Cipro-SSP 250	Tablet	Sansheng Pharmaceutical Plc, Ethiopia	00320120030	07 2020	10 2022

61	CAG_01_250	Abderafi	Cipro-SSP 250	Tablet	Sansheng Pharmaceutical Plc, Ethiopia	00320120020	06 2020	11 2022
62	DA_02	Abderafi	Kodoxy	Capsule	KOPRAN LIMITED, Khalapur, India	S55621002	01 2021	11 2022
63	DA_01	Abderafi	Doxycap	Capsule	APF PLC, Addigrat, Ethiopia	26255	06 2018	06 2022
64	DMY_02	Mai kadra	Doxycap 100mg	Capsule	APF PLC, Adigrat, Ethiopia	32221	09 2020	09 2024
65	DH_02	Humera	Doxyzim 100	Capsule	Zim Laboratories Limited, India	Fbw4a106	01 2021	12 2022
66	DM_02	Metema	Doxycap 100mg	Capsule	APF PLC, Adigrat, Ethiopia	32219	09 2020	12 2024
67	DMY_03	Mai kadra	Eapadoxine 100	Capsule	East African Phltical Factory, AA, Ethiopia	Ia206	01 2019	12 2021
68	DM_01	Metema	Doxycap 100mg	Capsule	APF PLC, Adigrat, Ethiopia	32233	09 2020	09 2024
69	DG_01	Gendwuha	Doxycap	Capsule	APF PLC, Adigrat, Ethiopia	26267	06 2018	05 2022
70	DH_01	Humera	Kodoxy	Capsule	KOPRAN LIMITED, Khalapur, India	S55620003	-	05 2023
71	DMY_01	Mai kadra	Kodoxy	Capsule	KOPRAN LIMITED, Khalapur, India	S55621002	01 2021	12 2023