College of Health Sciences

School of Pharmacy

Department of Pharmaceutical Chemistry and Pharmacognosy

Investigation of the Quality of Some Selected Chemotherapeutic Drugs (Cisplatin, Oxaliplatin, Doxorubicin and Methotrexate) using Paper Analytical Device and Pharmacopeial Methods from Samples Collected in Addis Ababa, Central-Ethiopia.

Adugnaw Demesie (B.Pharm)

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Addis Ababa, Ethiopia

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By:

Adugnaw Demesie

A thesis submitted to the Department of Pharmaceutical Chemistry and Pharmacognosy, School of Pharmacy, College of Health Sciences, Addis Ababa University in partial fulfillment of the requirements of the Master of Science (M.Sc.) in Pharmaceutical Analysis and Quality Assurance.

Advisor: Mr. Ayenew Ashenef (Assistant Professor)

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Adugnaw Demesie, entitled, "**Investigation of the Quality of Some Selected Chemotherapeutic Drugs (Cisplatin, Oxaliplatin, Doxorubicin and Methotrexate) using Paper Analytical Device and Pharmacopeial Methods from Samples Collected in Addis Ababa, Central-Ethiopia**", submitted in partial fulfillment of the requirements for M.Sc. Degree in Pharmaceutical Analysis and Quality Assurance complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

Advisor:

Ayenew Ashenef (Assistant Professor) Signature _____Date_____

Examiner:

Internal Examiner Professor Ariaya Hymete Signature _____ _____Date_____

External Examiner Zelalem Mamo Signature _____Date_____

_____

Head of Department

Abstract

Introduction: Cancer is a group of disorders in which aberrant cells proliferate and spread uncontrollably. Earlier diagnosis brings more hope for cancer treatment. However, increases in demand for chemotherapeutic drugs had the potential to opportunities for unethical manufacturers and suppliers to infiltrate and avail poor quality products.

Objective: The aim of the study was to investigate the quality of some selected chemotherapeutic drugs (cisplatin, oxaliplatin, doxorubicin and methotrexate) using paper analytical device and pharmacopeial methods from samples collected in Addis Ababa, Central-Ethiopia.

Materials and Methods: A total of 98 vials of cisplatin, oxaliplatin, doxorubicin and methotrexate were collected in Tikur Anbessa Specialized Hospital (TASH) and Girum hospital, both located in Addis Ababa from April, 2021 to December, 2021. Visual inspection was performed with criteria given in the joint WHO/FIP/USP checklist. Then, representatives' samples were taken for ChemoPAD screening with purposive sampling design. Confirmatory assays were done on suspected analytes with HPLC.

Results: The results of visual inspection on the four parenteral preparation (cisplatin, oxaliplatin, doxorubicin and methotrexate) did not show any signs of defect. Among the four different brands of cisplatin samples, two brands failed the Chemo-PAD screening test. Only one brand for oxaliplatin, when visually compared to a typical bar-code, failed the screening test. For doxorubicin and methotrexate, there was no any failures by Chemo-PAD screening. HPLC confirmatory assay values of cisplatin samples were 103% (accepted) and 137% (over and not acceptable). The assay values for doxorubicin products were substandard (83.44%). Methotrexate has slightly higher API content (118.9%). The assay value for oxaliplatin product was of good quality (109. 9%).

Conclusion: The study investigated the quality of some selected parenteral chemotherapeutic drugs (cisplatin, oxaliplatin, doxorubicin and methotrexate) in Addis Ababa. Visual inspection revealed no defects based on WHO/FIP/USP checklist. The results of ChemoPAD screening and

HPLC confirmatory assays revealed the presence of some poor quality chemotherapeutic drugs at the outlets.
Key-words: Cancer, Chemotherapeutic drugs, Quality, Substandard, Counterfeited, ChemoPAD, HPLC.

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List of Abbreviations

ACS	American Cancer Society
APIs	Active Pharmaceutical Ingredients
DNA	Deoxyribonucleic acid
EFDA	Ethiopian Food and Drug Authority
FMoH	Federal Ministry of Health
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
LMICs	Low- and Middle-Income Countries
NCDs	Non-Communicable Diseases
NMQCL	National Medicines Quality Control Laboratory
NLT	Not less than
NMT	Not More Than
NS	Not specified
Pt	Platinum
RNA	Ribonucleic acid
RS	Reference Standard
RSD	Relative Standard Deviation
Rt	Retention time
SST	System Suitability Test
TASH	Tikur Anbessa Specialized Hospital
UND	University of Notre Dame
US	United States
USP	United States Pharmacopoeia
WHO	World Health Organization

1. Introduction

Cancer is a group of disorders in which aberrant cells proliferate and spread uncontrollably (Aggarwal *et al*., 2018). These diseases are thought to develop from a cell in which the normal mechanisms for control of growth and proliferation are altered (Katzung, 2012, Dipiro *et al*., 2014). Moreover, cancer is a disease in which some of the body's cells grow uncontrollably and spread to other parts of the body. Widespread metastases are the most frequent cause of cancer-related mortality. Cancer is also called a genetic disease, because it is caused by mutations in genes that control how our cells divide and grow. In 2018, cancer accounted for 9.6 million deaths globally, or one in every six deaths. It is the second leading cause of mortality worldwide (WHO, 2018).

Cancer is not restricted to humans; animals can get cancer that makes treatment difficult. It affects all human beings. However, being the most complex and diverse disease; the type and prevalence of occurrence vary depending on geographical location, socio-economic status, sex, age, life style, genetic variation, race and other risk factors (Katzung, 2012, Woldu *et al*., 2017). Chemical carcinogens (specifically those in tobacco smoke) as well as aflatoxins, azo dyes, benzene, asbestos and radon have been clearly implicated in cancer induction in humans and animals (Katzung, 2012).

Cancer is non-communicable diseases (NCDs) and an important contributor to the international burden of disease (Aggarwal *et al*., 2018). Although many Low- and Middle-Income Countries (LMICs) still see a high death rate from communicable diseases, the incidence and mortality from NCDs, particularly cancer, are rising at an alarming rate. This has resulted in a two-fold burden of the diseases there and imposing strain on existing health care system (FMoH, 2015b).

The number of people affected by cancer is rising worldwide, causing a tremendous financial, emotional, and physical burden on patients, families, communities, and health systems. Many health systems in LMICs are not ready to handle the burden. Thus, many cancer patients around the world lack timely access to high-quality diagnosis and treatment(American Cancer Society, 2015)

1.1. Epidemiology of cancer

Cancer is a major cause of morbidity and mortality in all regions of the world *(*Tigeneh *et al., 2015*). In 2018, there were 9.6 million fatalities and 18.1 million new cases of cancer worldwide, according to estimates. Even though Europe only has 9.0% of the world's population, it accounts for 20.3% of cancer fatalities and 23.4% of cancer diagnoses worldwide. The United States accounts for 14.4% of global mortality and 21.0% of global incidence, with 13.3% of the world's population. In contrast to other world regions, the proportions of cancer deaths in Asia and in Africa (57.3% and 7.3%, respectively) are higher than the proportions of incident cases (48.4% and 5.8%, respectively). This is because, in addition to having less access to timely diagnosis and therapy in many nations, these locations have higher frequencies of specific cancer types linked to worse prognoses and higher death rates. (WHO, 2018).

According to Aggarwal *et al.,* worldwide, around 12% deaths are due to cancer, it causes more death than AIDS, tuberculosis and malaria combined(Aggarwal *et al*., 2018). By 2030, the number of new cases and deaths from cancer worldwide is predicted to reach 21.7 million and 13 million, respectively. This number will probably increase in the future due to the result of life style change that increases cancer risk such as smoking, poor diet and physical inactivity in developing countries(American Cancer Society, 2015).

Even if the recent global cancer case prevalence rates remain unchanged, the estimated incidence of 12.7 million new cancer cases in 2008 will rise to 21.4 million by 2030, with nearly 67% of all cancer diagnoses occurring in LMICs (Tigeneh *et al*., 2015, Aggarwal *et al*., 2018) and 13.2 million cancer deaths, the majority of which occur in these countries. Furthermore, according to Shah *et al.,* by 2040, this may increase to 29.5 million new cancer diagnoses and 16.5 million cancer-related deaths annually (Shah *et al*., 2019).

In Ethiopia, cancer accounts for about 5.8% of total national mortality. The annual incidence of cancer is estimated around 60,960 cases and over 44,000 people die from the disease. For people under the age of 75 years, the risk of being diagnosed with cancer is 11·3% and the risk of dying from the disease is 9·4%. Cervical cancer (13.4%), colorectal cancer (5.7%), and breast cancer (30.2%) are the most common cancers among adult population in Ethiopia. It is estimated that

women account for two thirds of cancer-related deaths each year (FMoH, 2015a). Specifically, the prevalence of cancer was still higher in the central part of Ethiopia (Woldu *et al*., 2017).

1.2. Management of Cancer

Owing to its nature, cancer is difficult to treat. However, it is possible to significantly reduce and prevent it. Effective cancer case treatments rely on early and accurate diagnosis and access to effective chemotherapeutic products(Smith *et al*., 2018, Shah *et al*., 2019). According to Globocan 2012 estimates, about 40% of the cases are preventable (FMoH, 2015a). Cancer is treated in a variety of ways. The types of treatment received will depend on the type of cancer. Surgery, chemotherapy, hormone therapy, and radiation therapy are the most effective classical cancer treatment procedures (Abbas and Rehman, 2018).

Cancer patients' treatment outcomes are influenced by the tumor's biology, patient characteristics, cancer type and stage. Accordingly, the ultimate goal of cancer treatment is to relief the pain and search for cure for the patient. To this end, the field is facing several challenges. Common obstacles in the field of cancer treatment include the continually rising cost of chemotherapy, treatment methods that are unavailable or too expensive, drug resistance in cancer cells, and the complexity and high cost of radiation therapy centers and equipment. These are more pronounced and complex problems for patients and clinicians in LMICs (Zugazagoitia *et al*., 2016).

1.2.1. Chemotherapeutics Drugs

Chemotherapy is the use and administration of pharmaceuticals that have been created or obtained from synthetic or natural sources in order to inhibit growth and treat cancer. The medications are most often known as chemotherapy medications. Anticancer medications are made to suppress malignant cells through a variety of molecular pathways, causing them to gradually grow slower and eventually stop progressing (Mullaney, 2012). Almost two out of every three people with the disease are cured when modern therapeutic methods (such as surgery, radiation therapy, chemotherapy, and biological therapy) are used (Dipiro *et a*l., 2014).There are five major classes of anticancer drugs; these include alkylating agents, antimetabolites, antibiotics, mitotic inhibitors, and hormones. In addition, there are a number of

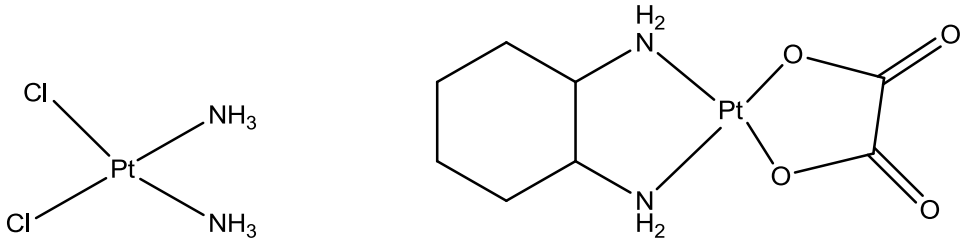
drugs that do not fall within those classes categorized as anticancer drugs (Espinosa *et al*., 2003).

Table 1: Classifications of anticancer drug (Espinosa *et al*., 2003).

S. N_o	Types of anticancer drugs	Mechanism of Action (MoA)	Drug example
1	Alkylating agents	Interferes with the growth of cancer cells and slows their growth and spread in the body	Cyclophosphamide
2	Antimetabolites/ Nucleoside Analogs	Affect DNA synthesis by acting as a substitute to the actual metabolites that would be used in the normal metabolism	5-fluorouracil, methotrexate
3	Mitotic inhibitors	Inhibition of mitosis at metaphase	Vincristine, Vinblastine
4	Hormone therapy	Inhibition of protein kinase, which prevents DNA synthesis	Tamoxifen
5	Antibiotics	Antimitotic and cytotoxic activity through a number of proposed mechanisms of action	Doxorubicin, Bleomycin
6	Platinum-based agents	Interferes with DNA replication	Cisplatin, Oxaliplatin Carboplatin
7	Miscellaneous Antineoplastic Agents	Inhibits DNA synthesis through the inhibition of ribonucleoside diphosphate reductase.	Hydroxyurea

1.2.2. Platinum-based Chemotherapeutic Drugs

Platinum (Pt)-based anticancer drugs (cisplatin, carboplatin and oxaliplatin) are the commonly used anticancer medication in clinical cancer treatment. However, their serious adverse effects and medication resistance significantly reduced their efficacy. Pt-containing medicines' anticancer effects can be largely attributed to the kinetics of their ligand displacement processes. Pt-containing drugs primary target is believed to be nitrogen donor atoms in the nucleobases of Deoxyribonucleic acid (DNA). The bonds formed between the metal ion and these atoms must be sufficiently extended to interfere with the process of cell division. They trigger the intracellular mechanisms that recognize irreversible damage to a cell. Bonds between the nucleobase nitrogen atoms and Pt clearly fulfill this requirement. Metal ions that create labile bonds with nitrogen atoms in the nucleobase are unable to function similarly and do not produce active molecules. On the other hand, the metal-based drug that is injected must undergo in a relatively short time to initiate a sequence of reactions that allows the leaving groups on the initial compound to be replaced by the DNA base nitrogen atoms. Compounds that are totally inert are also inactive (Espinosa *et al*., 2003, Dipiro *et al*., 2014, Dai and Wang, 2020).



(A) (B)

Figure 1: Structural formula of some Pt based chemotherapeutic drugs: A. Cisplatin- Pt based alkylating agent, B. Oxaliplatin- cisplatin analog

1.2.3. Antimetabolites/ Nucleoside Analogs

Nucleoside analogues are a large class of agents (anticancer) that include drugs like methotrexate. Antimetabolites interfere with DNA and Ribonucleic acid (RNA) synthesis by acting as false metabolites, which are incorporated into the DNA strand or block essential

enzymes, so that DNA synthesis is prevented(Espinosa *et al.*, 2003, Katzung, 2012, Dipiro *et al*., 2014)

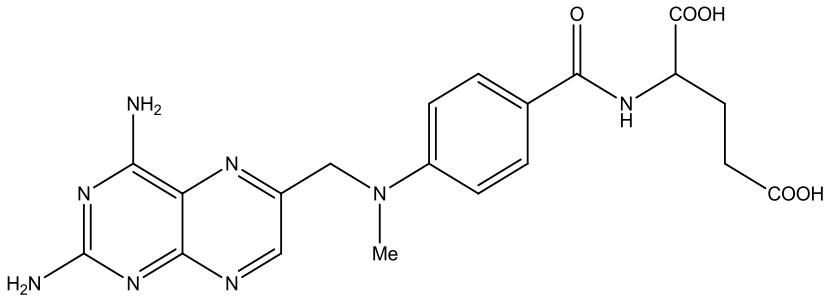


Figure 2: Structural formula of methotrexate - dihydrofolate reductase

1.2.4. Antibiotic Chemotherapeutic Drugs

Doxorubicin is an anthracycline which counteracts cancer cells growth by blocking an enzyme called topo-isomerase 2. Anthracyclines are in the class of chemotherapeutic medication group that are particularly successful in treating human malignancies (Katzung, 2012, Dipiro *et al*., 2014, Bozza *et al*., 2021). In-fact, these medication are differ from the antibiotics used to treat infections. They function by altering the DNA of cancer cells to prevent them from proliferating. Anthracyclines are anti-tumor antibiotics that interfere with enzymes involved in copying DNA during the cell cycle. They bind with DNA so it cannot make copies of itself, and a cell cannot reproduce. They are among the most effective chemotherapy agents and cover a significant number of cancers types (Bozza *et al*., 2021).

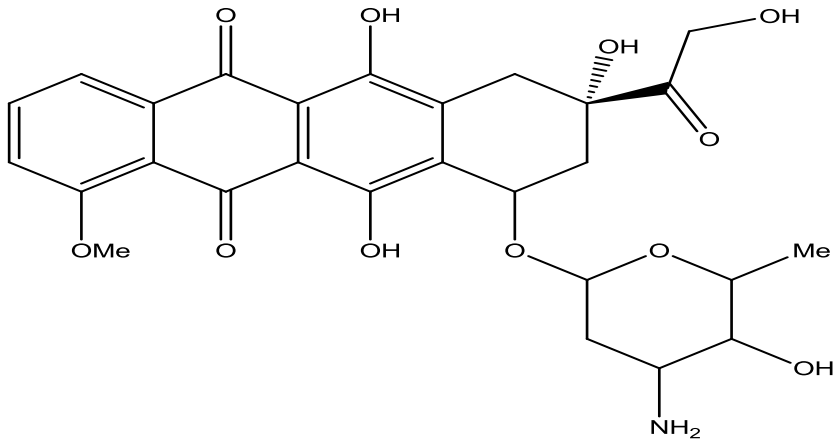


Figure 3: Structural formula of doxorubicin (anthracycline)

1.3. Therapeutic Effectiveness and Quality of Pharmaceuticals

Substandard and falsified medical products are medical products that are not up to their requirements or quality standards, or both and dishonestly misrepresent their identity, composition or source. Patients who live in areas with restricted access to safe and high-quality medical products are more likely to receive substandard and fake products. The situations favorable include presence of poor governance, weak technical capacity to perform proper regulation, and instability. Medical products that are counterfeit are produced throughout the world in a wide range of nations. An estimated 10% medical products in LMICs is substandard and falsified. (Ozawa *et al*., 2018, WHO, 2018).

Substandard and falsified medical products raise risks of morbidity and mortality by prolonging illnesses, and increase the risk of treatment failure, can cause poisoning, and increase in frequency of adverse drug interactions. The distribution of substandard and falsified medications with minimal therapeutic efficacy additionally exposes entire populations to the risk of developing drug resistance. Additionally, it will erode public confidence in the medical system and its accredited healthcare providers (WHO, 2017).

According to Cockburn *et al*., there is no accurate statistics that represents the prevalence of substandard and falsified medicines, partly due to lack of sufficient resources and poor reporting practices (Cockburn *et al*., 2005).

Products having the wrong ingredients, no active ingredient, the improper dosages of active ingredients, or fake packaging can all be considered falsified medications. Substandard medicines are products whose composition and ingredients do not adhere to the appropriate scientific specifications. They are consequently ineffective and often harmful to the patient. Negligence or human error may result in substandard products. Although they are produced legally, they may have quality issues or be the result of falsified. During transportation, storage, and environmental effects, drugs may degrade and become substandard *(Nanjwade et al., 2010, WHO, 2017, Khan et al.,* 2013)

One major problem for worldwide public health is the use of substandard and falsified pharmaceuticals. It reduce the confidence of the patient in the health systems, in the health

professionals, in pharmaceutical manufacturers and in distributors, leading to emergence of drug resistance, disability, injury and death. Anti-infective and anti-parasite medication classes rank as the two most frequently substandard and falsified categories of pharmaceuticals in developing countries (Amin and Kokwaro, 2007, Khan *et al*., 2013). In a 1991–1993 study on the quality of medications that were distributed in three African nations, it was discovered that 18% of the evaluated samples were below standard (Gbotosho *et al*., 2009). This alarming situation underscores the need for medicines regulatory agencies to quickly introduce powerful post-marketing surveillance mechanisms for critical chemotherapeutic drugs in strategic sites like Addis Ababa.

1.4. Quality of Anticancer Drugs in the World

Up to two billion people around the world lack access to essential medicines that creates a vacuum which is too often filled by substandard and falsified products (WHO, 2017, Leisinger *et al*., 2012). This problem is growing as global drug supply chains become more complex (Ravinetto *et a*l., 2016). There are many pharmaceutical plants and distribution channels of pharmaceutical products which improves the health care system. Unfortunately, circulation of substandard and falsified products increases significantly as a result of ineffective regulation of manufacturing and trading of pharmaceutical products (Wega, 2016, Giralt *et al*., 2017).

In recent years, the number of substandard and falsified drugs has increased dramatically. Anticancer drugs are attractive targets for counterfeiting due to their high selling prices and low availability (WHO, 2018). Many LMIC regulators, including the Ethiopian Food and Drug Authority (EFDA), lack safety equipments and standard operating procedures to handle chemotherapy agents safely in the drug analysis laboratory. They have limited capacity to perform post market surveillance on these products.

A particularly serious case involved falsified versions of bevacizumab (Avastin), a cancer-fighting medication. In February 2012, Roche, the company that makes Avastin, alerted doctors about a fake bevacizumab that only included starch and salt instead of the medication's active ingredient. FDA laboratory tests have confirmed that at least one batch of a counterfeit version of Roche's Altuzan distributed in the United States (US) no active ingredient present (Blackstone

et al., 2014, Pitts, 2020). The study done at Tikur Anbessa Specialized Hospital (TASH), deploying Chemotherapeutic Paper Analytical Device (ChemoPAD) had showed that multiple lots of a particular cisplatin product were suspect. Confirmatory testing by High Performance Liquid Chromatography (HPLC) confirmed that this product contained 54% of the stated amount of active ingredients (Eberle *et al*., 2020).

1.5. Analytical Methods of Analysis

Poor health outcomes (lack of cure) can erode trust to the pharmaceutical plant of genuine pharmaceutical products. It is often challenging to differentiate from substandard or falsified ones without the use of verification technologies (Kovacs *et al*., 2014, Ozawa *et al*., 2018). Hence, there is a need for efficient, fast, simple and transferable analytical methods that can be used for detection and analysis of substandard and/or falsified chemotherapeutic product (Smith *et al*., 2018).

Analytical methods provide various levels of qualitative and quantitative information about pharmaceutical products quality (Gaudin *et al.*, 2009). Qualitative methods reveal details about a drug's identity, including its labeling, color, and active ingredient. Quantitative methods offer details on the quantity or concentration of a pharmaceutical products and how the body will absorb it. Qualitative tests can be used to detect absence or presence of active ingredient of drugs, such as those with the incorrect or no active ingredients (Martino *et al*., 2010). Quantitative values such as above level limit of impurities or unacceptably low or high dosage of active ingredients are more common among substandard drugs (Staub *et al*., 2010). Hence, visual inspection followed by analytical methods had become paramount in judging the quality of chemothepeutic products.

1.5.1. Visual Inspection

The usual first step in any drug quality analysis is visual inspection of a product and its packaging by someone who is familiar about the qualities of the standard drug or who can compare the sample to the original product (Martino *et al*., 2010). Qualitative information regarding the identities of pharmaceuticals is provided by these visual inspections. Differences from the standard products in size, color, shape, and packaging indicate a possible falsified or

substandard drug. These differences range from minor to severe differences as compared to the standards ones (Venhuis and De Kaste, 2012).

The Minilab toolkit from the Global Pharma Health Fund encourages visual inspection as the initial step in identifying falsified and substandard drugs , although even professionals find it difficult to do this (Green *et al*., 2015). Falsified medication packaging may contain numerous other differences from authentic packaging, such as misplaced or absent expiration dates, missing manufacture information or instructions, no batch number, or none at all. Sometimes improperly written instructions and spelling errors expose fake medicines; low quality inks may discolor in water. Similarly, the drugs may have different color, size, or shape, have the wrong markings on them, have a different texture, or be otherwise different from the standard products or drugs(Kaur *et al*., 2010).

1.5.2. ChemoPAD Methods

Modern pharmaceutical testing methods, including mass spectrometry or HPLC, offer strong instruments for determining active pharmaceutical ingredients (APIs). However, it is costly and requires the use of qualified experts, dependable laboratory equipment, and funds for the purchase, use, maintenance, and repair of instruments. To address the problem of substandard medicines around the world, finding low-quality medications in areas with limited resources is a critical public health concern. Hence there is a need of cheap, user-friendly tools to increase screening capacity within the pharmaceutical supply chain (Smith *et al.*, 2018, Eberle *et al*., 2020).

Ethiopia has over 25 Minilabs at multiple EFDA branches, at Addis Ababa University (AAU) and at customs sites, but it is not an option for field screening of chemotherapeutic products. This is because chemotherapeutic drugs are cytotoxic and no chemotherapeutic agents are included in the Minilab's protocol drug lists (Smith *et al*., 2018). Currently, patients' number receiving chemotherapeutic products has considerably increased. Given the toxicity of cytotoxic products to clients, the development and application of cheap, accessible and reliable analytical methods to screen and analyze these products become necessary. Among all the analytical methods, Paper Analytical Device (PAD) method hold an important position for the determination of the most commonly used chemotherapeutic products in our country's, Ethiopia, setting (Smith *et al*.,

2018, Eberle *et al*., 2020). These paper tests pioneered the development of new analytical tools for an on-site pharmaceutical product analysis (Ozer *et a*l., 2020).

Paper test strips such as litmus paper have a long history. These tests don't need any special equipment or a lab environment, and they are cheaper and simple to use. A qualitative chemical profile of pharmaceutical formulations can be obtained by PAD. The paper device is patterned using wax printing with many channels to allow multiplexing, while mill fluidic flow assembles reagents in *situ*. PADs are a novel technology for rapid field screening of medicines in the developing world. Simple equipment can be used to make test cards on a small scale, making them suitable for local production in some areas where low-quality drugs are a problem(Weaver *et al*., 2013).

Development of color tests used to detect chemotherapy agents: The principle of operation of PAD is that multiple chemical color tests are carrying out in parallel on the analyte. Hydrophobic barriers are printed onto the paper to create twelve lanes, and each chemical color reaction's specific reagents are kept in dry form on their own lanes of the PAD. By pushing the application strip into the lanes, a few microliters of the analyte are deposited about two thirds of the way up each of the lanes. All tests are activated when the PAD's bottom edge is immersed in water. By use of capillary action, water ascends the 12 lanes and carries the reagents to the analyte. The reactions between the reagents and the analyte create a color bar code, which can be matched to bar codes from known good samples. Several lanes are included on the chemo-PAD to detect possible adulterants, fillers, or substitute APIs that might be included in a falsified chemotherapy product. These lanes contain a timer lane as well as lanes for testing nucleophiles, tertiary and primary amines, -lactams, starch, and carbonates. Additional lanes were created to detect the APIs in the four targeted chemotherapy drugs (Eberle *et a*l., 2020).

Detection of cisplatin and oxaliplatin: Strong reductant stannous chloride has been used as a color test to detect Pt compounds. Cisplatin and oxaliplatin react with it to form yellow products. The reagents for the reaction are stored by allowing a solution of stannous chloride to dry in the designated lane of the ChemoPAD. This lane test was found to be semi quantitative. Therefore it is useful both to detect severely diluted drugs that have less than 50% of the stated API content and to distinguish between the common dosages forms of cisplatin and oxaliplatin, which are

provided at different concentrations (1 mg/mL and 5 mg/mL, respectively) (Eberle *et al*., 2020, PAD, 2020),.

Detection of doxorubicin: Doxorubicin is an organic polyphenol that forms colored complexes with different divalent and trivalent transition metals. Several lanes containing copper (II), nickel (II), and iron (III) were already available on the ChemoPAD for the detection of potential API substitutes. When doxorubicin was added to the copper (II) lane, it consistently produced a dark purple color signal at dosage form-typical values of 2 mg/mL. Two new test lanes were added, lane G containing Vanadyl (IV) in the form of vandyl sulfate and lane K containing Zink (II). In the presence of base (provided *via* a pot of 1 M NaOH at the bottom of the lanes), these metals react with doxorubicin on the test card to form purple colors(Eberle *et al*., 2020).

Detection of methotrexate: Methotrexate contains several chelating nitrogens and produce a green complex with copper. Copper was present on the PAD in a lane which uses copper (II) and potassium carbonate to detect beta lactam groups. In contrast to the blue hue of the copper ions and the dark green or black color found with beta lactam antibiotics, methotrexate creates a green color in this lane (Eberle *et al*., 2020).

A. 

B. 

C. 

Fig 4. The chemoPAD "barcode" for cisplatin (A), oxaliplatin (A), doxorubicin (B), and methotrexate (C) are shown with the white boxes indicating areas of interest for identification of each drug

1.5.3. High Performance Liquid Chromatography

HPLC, also known as High Pressure Liquid Chromatography, is a common analytical method for separating, identifying, and quantifying each component of a mixture. It is a sophisticated method of column liquid chromatography (Martin and Guiochon, 2005, Horváth, 2013). Chromatography separates mixtures into their individual components based on a variety of chemical and physical properties. It can be used to separate drug constituents for further testing and, when used with appropriate detectors, gives both qualitative and quantitative information about active ingredients and impurities(Kaale *et al*., 2011).

HPLC the most often utilized analytical technique for drug evaluations (Martino et al., 2010). Normally, the solvent moves through a column with the aid of gravity. However, in the HPLC process, the solvent is forced through a column at pressures as high as 400 atmospheres, allowing the sample to be separated into various constituents using differences in relative affinities. (Horváth, 2013, Ravinetto *et al*., 2016).

In many pharmaceutical applications, including formulation evaluation, purity testing, process modification monitoring, scale-up, and post-marketing surveillance, high performance liquid chromatography (HPLC) is an essential tool. There are a number of literatures confirming their

study with this (ChemoPAD) technique as golden reference standard (Woodcock, 2004, Weaver *et al*., 2013, Smith *et al*., 2018, Eberle et al., 2020).

1.6. Statement of the Problem

The majority of African nations are plagued by numerous issues requiring immediate, full attention. The spread and use of substandard and falsified medications is one of them. The healthcare systems and treatment outcomes across the African continent have been severely impacted by this (Aminu *et a*l., 2017).

Poor-quality medicines can reach the market through inadequate manufacturing of approved pharmaceuticals, due to inadequate quality-control processes during manufacture, in addition to by deliberately fraudulent practices. Marketing of substandard and falsified drugs is high in developing countries, especially in Asia and Sub-Saharan Africa including in Ethiopia because of weak drug regulatory systems. Therefore, it is impossible to ensure the quality of drug products offered in nations with weak drug regulatory controls (Höllein *et al*., 2016). The global issue of substandard and falsified drugs has damaged the credibility of the healthcare system and has the potential to cause serious harm to consumers, including disease, incapacity, and even death (Chinwendu, 2008).

Substandard and falsified pharmaceutical products are a global public health concern that lead to harm, impairment, and death. Inadequate amounts of active chemicals, hazardous substances added, or no active ingredient at all are common features of low-quality pharmaceutical products. With these, the patient doesn't get the full benefits of the treatment and will either recover slowly or not at all. This results in illness and death as well as a heavy financial burden (WHO, 2017).

Although instrumental analysis methods remain the best choice for assessing overall drug quality defects, they are time consuming. This might be a factor for patients' exposure to substandard and falsified product(s) (Wega, 2016). Clinical workers wait some time until quality checking was performed. Thus, on site fast quality screening tools can fill the gap. Currently, none of the instrumental analysis methods are available for screening chemotherapeutic products at the point of use in oncology ward at TASH (Smith *et al*., 2018).Hence, the quality of these drugs might be

under question as full quality control mechanisms are not in place. A patient who receives a substandard and/or falsified chemotherapeutic products may risk a poor treatment outcome which might be the confounding factor for death (Blackstone *et al*., 2014, Smith *et al*., 2018).

1.7. Significance of the Study

A safe medicines supply is fundamental for public health but most challenging in the world today is the manufacture and distributions of substandard and/or falsified drug due to lack of effective regulatory system to control drug quality. Counterfeiters are targeting cancer drugs because of the high profits to be made than other substandard and falsified drugs. Simple analytical method is needed that can perform with available resources. This study will be important to assess anticancer drugs by using simple analytical method that can be done at any health sector without need of any complicated laboratory and instrument and also save cost and time. The method can be used by regulatory agencies to implement low cost, higher frequency testing of pharmaceuticals along the supply chain.

2. Objective

2.1. General Objective

- ✓ Investigation of the quality of some selected chemotherapeutic drugs (cisplatin, oxaliplatin, doxorubicin and methotrexate) from Addis Ababa, Central-Ethiopia using paper analytical device and pharmacopeia methods.

2.2. Specific Objectives

- ✓ To investigate of the quality cisplatin, oxaliplatin, doxorubicin and methotrexate with visual inspection.
- ✓ To screen the selected chemotherapy drugs with paper analytical device at the oncology ward of TASH and Girum hospital in Addis Ababa, Ethiopia.
- ✓ To assay and evaluate the suspect chemotherapeutic drug(s) by HPLC using pharmacopoeial assay methods.

3. Materials and Methods

3.1. Materials

3.1.1. Drugs, Chemicals and Solvents

The following drugs, chemicals and solvents were used in the experiments: Platol (cisplatin 1mg/ml, Venus remedies limited, India), cisplat (cisplatin 1mg/ml, Khandelwal labratoties pvt ltd, India), Namanaplatin (cisplatin 1mg/ml, Namapharmadruds, India) Platinox (cisplatin 1mg/ml, Khandel laboratories pvt.ltd, India), Zolon (oxaliplatin, Zuvius life sciences pvt ltd, India), Oxaliplit (oxaliplatin, Zee laboratories ltd, India), Zildox (oxaliplatin VHB Medi sciences limited, India), Oxali-100 (oxaliplatin, Esperer Bioresearch pvt. Ltd, India) Oxol (oxaliplatin Venus Remedies limited, India), Curatinox (Oxaliplatin, Fortius Pharmaceutical,Turkey), Adrosal (doxorubicin, VHB Medi science ltd, India), Doxotero (doxorubicin, Hetro healthcare ltd, India), Adridox (doxorubicin, Bruck pharma private limited, India), Namantrex (methotrexate, Naman-pharma drugs, India),

Ethyl acetate (Carlo Erba Reagents S.A.S, 412612000, France), dimethylformamide (Carlo Erba Reagents SPA 444926, France), anhydrous monobasic sodium phosphate (Research Lab Fine Chem Industries, 10027-21-6, Mumbai, India), monobasic potassium phosphate (sigma-Aldrich, 90662, Germany), sodium hydroxide (Research Lab Fine Chem Industries, 1310-73-2, Mumbai, India), Methanol (Sisco Research Laboratories Pvt Ltd, 59029, Mumbai, India), acetonitrile (Carlo Erba Reagents S.A.S, 412382000, France), trifluoroacetic acid (sigma-Aldrich, 302031, Germany), lactose monohydrate solution (Loba Chem Pvt Ltd, 320371912, Mumbai, India), 1-pentanesulfonic acid sodium (Research Lab Fine Chem Industries, 7664939, Mumbai India). triethylamine (sigma-Aldrich, 246713), distilled and/or degassed water and dimethyl sulfoxide (67-68-5) were used to perform the experiment.

All were of analytical and HPLC grade and stored at ambient temperature. USP Cisplatin (CAS: 15663-27-1), USP Oxaliplatin (CAS: 1481204), USP Doxorubicin (CAS: 1225703) and Methotrexate (CAS: 59-05-2) Reference Standards (RS) were kindly supplied from UND, Indiana, USA and the United States Pharmacopoeia (USP), USA.

3.1.2. Instruments

The following instruments were used for the experiments: Analytical balance (Mettler Toledo, Switzerland), refrigerator (GN-M352YVD), pH meter (Mettler Toledo, Switzerland), vacuum degasser (2F3, A2306-04), and HPLC (Schimadzu Corporation, Japan), waters symmetry column, C18, 15 cm x 3.9 mm (Ireland), UV-VIS detector (Schimadzu Corporation, Japan).

3.1.3. Study Period, Area and Sample Collection

Sample collection was performed from April, 2021 to December, 2021 and chemotherapeutic drugs were obtained from TASH and Girum hospital, Addis Ababa, Central Ethiopia. From TASH 54 vials and from Girum hospital 44 vials, altogether 94 vials were collected. Purposive sampling was used in the collection of the samples from selected sites. Microsoft Excel® 2019 was utilized for statistical analysis. Table 2 shows collection site, origin and manufacturer of the samples included in the study.

Table 2: Label description of the four chemotherapeutic product samples for screening and assay

Drug Name	Brand Name	Batch Number	Manufacturing date	Expiry date	Manufacturer	N_o of Vial	Sites Collected
Cisplatin	Platol	X0BE012A	02-2020	01-2022	Venus remedies limited, India	5	Girum hospital
	Cisplat	IcsB01127	11- 2020	10. 2023	Khandelwal labratoties pvt ltd, India	4	Girum hospital
	Namanaplatin	308001A	09-2019	08-2022	Namapharmadruds, India	6	Girum hospital
	Platinox	ICSB90716	07-. 2019	06. 2022	Khandel laboratories pvt.ltd, India	7	TASH
Oxaliplatin	Zolon	0X1H20B21-A	08-2020	07-2022	Zuvius life sciences pvt ltd, India	4	TASH
	Oxaliplit	321-192	03-2021	02-2023	Zee laboratories ltd, India	10	TASH
	Zildox	NN9349A	08-2019	04-2022	VHB Medi sciences limited, India	4	TASH
	EsOxali-100	Oxlc21B5-A	03-2021	02-2023	Esperer Bioresearch pvt. Ltd, India	7	Girum hospital
	Oxol	X9G012D	02-2021	01-2022	Venus Remedies limited, India	6	Girum hospital
	Curatinox	00203710	10 -2020	09 – 2022	Fortius Pharmaceutical,Turkey	9	TASH
Doxorubicin	Adrosal	V284015	09. 2020	08 -.2022	VHB Medi science ltd, India	10	TASH
	Adrosal	V284016	11 -2020	09 -2022	VHB Medi science ltd, India	10	TASH
	Doxotero	HHBL2014DR	10-2020	09-2022	Hetro healthcare ltd, India	6	Girum hospital
	Adridox	2172008E	12-2020	11-2022	Bruck pharma private limited, India	5	Girum hospital
Methotrexate	NAMANTREX	3292002B	09-2020	08-2023	Naman-pharma drugs, India	5	Girum hospital

3.1.4. Eligibility Criteria

All prescribed parenterals of cisplatin, oxaliplatin, doxorubicin and methotrexate were included in the study. Those vials with small amount of left overs which are not enough for screening with ChemoPAD, by considering sample for HPLC, were excluded in this study.

3.1.5. Ethical Consideration

This study did not involve any human subjects; the main ethical issues were communication with the appropriate decision makers and safe handling of the toxic chemotherapeutic products. Ethical approval for this study was obtained from the School of Pharmacy, Addis Ababa University Ethical Review Board- ERB/SOP/273/13/2021 (Annex 1). The oncology staffs at TASH and Girum hospital were notified about this study in a letter written from the Department of Pharmaceutical chemistry and Pharmacongosy, School of Pharmacy, CHS, AAU. .

3.2. Methods

3.2.1. Physical Characteristics, Packaging and Labeling Information

Visual inspection of the dosage form's physical properties, packaging, and labeling details was carried out. The joint WHO/FIP/USP check list form was used (Annex 2).

Visual inspection was performed on each sample to verify the trade name, active ingredient name, manufacturer, and complete address. Labeled medicine strength, dosage form, number of units per container, dosage statement, batch/lot number, manufacturing and expiry dates were assessed. Similarly storage information, presence of leaflets/package insert, consistency of color and visual contaminations were investigated.

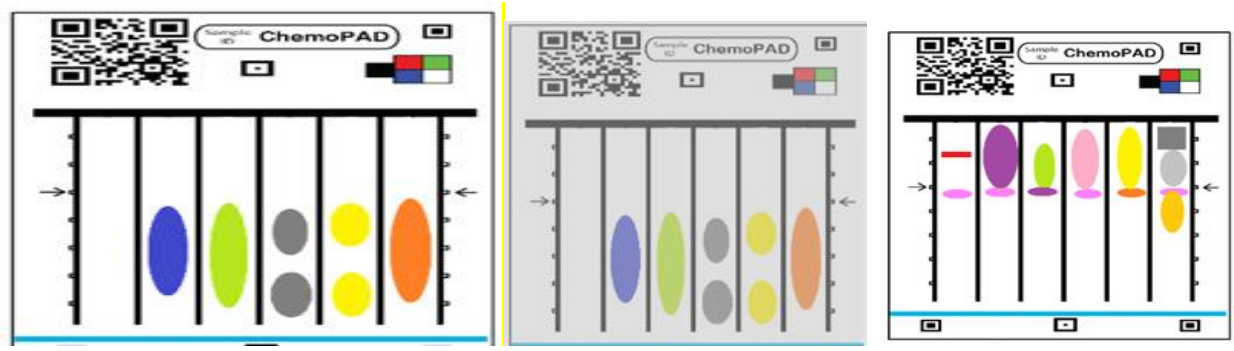
3.2.2. ChemoPAD Tests

After visual inspection, the different samples collected were tested by ChemoPAD for qualitative and semi-quantitative analysis (PAD, 2020).

The chemo-PAD test card was designed to see whether cisplatin, doxorubicin, methotrexate, and oxaliplatin were present or absent. The chemoPAD is covered with a plastic sheet that creates a physical barrier between the user and the drugs. The design incorporates a sample application

strip made from absorbant paper, which the user can load by removing 65 µL from the vial with a syringe and applying to the strip. The side of the card containing the sample application strip is then folded over and pushed into the chemical testing portion of the card, so a few microliters of the sample are moved to each of the twelve lanes. The bottom edge of the chemoPAD was put in into 1 cm depth of water-dish.

The chemoPAD was holed upright against a support for about 3 minutes. The water was seen moving up the lanes. When a red dot appeared at the top of lane A, the PAD was removed and laid flat on a clean piece of paper. The ChemoPAD was laid for 3 minute then photographed the PAD. Finally the sample image was compared to the standard image.



Test card containing preload dry reagents | Apply sample and dip card in water for 3 minutes | Colors develop in 3 minutes

Fig 5. Chemo-PAD screening procedure

3.2.3. Assay - High Performance Liquid Chromatography

The suspected drugs of cisplatin, oxaliplatin, doxorubicin and methotrexate, identified from ChemoPAD screening were assayed according to the method procedures outlined in each individual drug monographs of USP (2020) for confirmatory purpose.

3.2.3.1. Cisplatin

The API contents of cisplatin were determined using HPLC according to USP specification (USP-43, 2020b).

Mobile phase preparation: A mixture of methanol and water (10:90 ratio) was prepared and degassed.

Standard preparation: An precisely weighed quantity of 12.5mg cisplatin RS was dissolved in 25 mL 0.9% normal saline to obtain a solution containing 0.5mg/mL.

Sample preparation: 5ml of cisplatin was added in to 10 mL volumetric flask and diluted with normal saline to the volume. A small amount of the solution was passed through chemically resistant syringe filter before to injection.

Chromatographic system: The liquid chromatograph was adjusted to a 230-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate was 1 mL per minute.. Room temperature was maintained for the column.

Assay: 40 µL of the standard preparation and the analyte were injected into the chromatograph sequentially. The chromatograms were recorded, and the peak areas for component cisplatin sample component cisplatin RS were recorded. The content of cisplatin was calculated from the peak areas of the chromatograms of the test and reference standard solutions by the formula below (Equation 1).

$$(ru/rS) * (CS/CU) * 100 \quad \text{.....Equation 1}$$

Where:

r_U: peak response of cisplatin from the sample solution.

r_S: peak response of cisplatin from the standard solution.

C_S: concentration of Cisplatin USP RS in the standard solution (µg/mL).

C_U: nominal concentration of cisplatin in the sample solution (µg/mL).

Five replicate of 40µl cisplatin USP RS solutions were injected to the HPLC system and the chromatograms were recorded to evaluate the system suitability parameters like tailing factor (NMT 2), theoretical plate number (NLT 1500 for cisplatin), and % Relative RSD (NMT 2.0).

3.2.3.2. Assay of Oxaliplatin

Mobile phase preparation: First, 1.36 g monobasic potassium phosphate, anhydrous and 0.9 g 1-pentanesulfonic acid sodium salt was dissolved in 1 L of water. After sonication, 250 µL triethylamine was added. The pH was adjusted to 4.3 by added 5 drops of phosphoric acid,

mixed, and filtered by filtration unit (buffered). Finally, mixture of methanol and buffer (15:85) was prepared and degassed. The clear filtrate was degassed before used (USP-43, 2020d).

Standard preparation: The standard was prepared by dissolving 10mg of USP Oxaliplatin RS in 25mL lactose monohydrate (40mg per mL) of solvent to obtain 0.4 mg per mL solution.

Sample preparation: 2mL of oxaliplatin sample was added in to 25 ml volumetric flask. Lactose monohydrate (40mg per ml) was added to the volume and 0.4 mg/mL of oxaliplatin injections were prepared.

Chromatographic system: The liquid chromatograph was adjusted to a 210-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate was 1 mL/minute. Room temperature was maintained for the column.

Assay: 40 µL of the standard preparation and the analyte were injected into the chromatograph sequentially. The flow rate was adjusted to 1 mL/minute. Finally, the responses for the major peaks were measured and the chromatograms were recorded. The percentage of component oxaliplatin sample plus component oxaliplatin RS were calculated as a percentage of the label claim of oxaliplatin (Equation 1)

Where:

r_U: peak response of oxaliplatin from the sample solution.

r_S: peak response of oxaliplatin from the standard solution.

C_S: concentration of oxaliplatin USP RS in the standard solution (mg/mL).

C_U: nominal concentration of oxaliplatin in the sample solution (mg/mL)

3.2.3.3. Doxorubicin

Mobile phase preparation: Solution A (0.1% trifluoroacetic acid) and solution B (acetonitrile, methanol and trifluoroacetic acid in the ratio of 800:200:1), diluent a mixture of solutions A and B in 50:50 ratio, flow rate of 0.5 mL/minute and injection volume 2 µL.

Standard preparation: Standard solutions were prepared by dissolving 10 mg of doxorubicin USP RS in a100ml of mobile phase to obtain a solution having a concentration of 0.1 mg/mL.

Sample preparation: 2.5ml of sample was added into a 25 mL volumetric flask and dissolve it by mobile phase to the volume. The portion of preparations were sonicated and filtered with syringe filter before injection.

Chromatographic system: The liquid chromatograph was adjusted to a 254-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing L1. The flow rate was about 1.5 mL/minute. Room temperature was maintained for the column.

Assay: 20 µL of the standard preparation and the sample preparation were injected into the chromatograph sequentially. The chromatograms were recorded, and the peak areas for component doxorubicin plus component doxorubicin RS were measured. The content of doxorubicin was calculated from the peak areas of the chromatograms of the samples and reference standard solutions (Equation 1).

Where:

r_U: peak response of doxorubicin from the sample solution.

r_S: peak response of doxorubicin from the standard solution.

C_S: concentration of USP doxorubicin RS in the standard solution (mg/mL).

C_U: nominal concentration of doxorubicin in the sample solution (mg/mL).

P: Potency of doxorubicin in doxorubicin USP RS (µg/mg).

F: Conversion factor, 0.001 mg/ µg.

3.2.3.4. Methotrexate

Mobile phase preparation: The mobile phase was prepared from acetonitrile and buffer. Buffer was prepared by mixing 0.2 M dibasic sodium phosphate and 0.1 M citric acid in a 630:370 ratio, while mobile phase was prepared by mixing acetonitrile and buffer solution in a 10:90 ratio (USP-43, 2020a).

Standard preparation: The standard was prepared by dissolving 12.5mg of USP methotrexate RS in 50 ml of solvent (mobile phase) to obtain 0.25 mg/ml solution.

Sample preparation: 0.5ml of methotrexate sample was added in to 50 mL volumetric flask and diluted with mobile phase to the volume. A portion of the solution was passed through chemically resistant filter paper prior to injection.

Chromatography systems: The liquid chromatograph was adjusted to a 302-nm detector and a 4.6-mm × 25-cm column that contains 5-µm packing. It operated at a flow rate of approximately 1.2 mL/minute and maintained a column temperature at room temperature.

Assay: 20 µl of the standard preparation and the sample preparation were injected into the chromatograph sequentially. The chromatograms were recorded, and the peak areas for component methotrexate plus component methotrexate RS were measured (Equation 1).

Where:

r_U: peak response of methotrexate from the sample solution.

r_S: peak response of methotrexate from the standard solution.

C_S: concentration of methotrexate USP RS in the standard solution (µg/mL).

C_U: nominal concentration of methotrexate in the sample solution (µg/mL).

4. Results and Discussion

4.1. Results

4.1.1. Physical Characteristics, Packaging and Labeling Information

The results of visual inspection of packaging and labelling information on the tested brands of cisplatin, oxaliplatin, doxorubicin and methotrexate did not show any signs of defect based on WHO/ FIP/USP checklist of visual inspection tool. The pharmaceutical products safety system relies on pharmaceutical companies providing complete, descriptive and accurate label information including warnings and contraindications to physicians, health-care givers and clients.

4.1.2. Chemo-PAD Tests

Chemo-PAD results of cisplatin: Among the four different brands of cisplatin samples, two brands (Platol & Cisplat) failed the Chemo-PAD screening test which were both collected from Girum hospital leftovers. As shown at Figure 6(A) and 6(B) at lane C and D does not show strong yellow color, the absence of a strong yellow color in the sample indicates a deficient platinum content. In other brands (Namanaplatin & Platinox), strong yellow color was present at lanes C and D indicating a sufficient platinum content of the formulation (Figures 6C & 6D).

A. 

B. 

C. 

D 

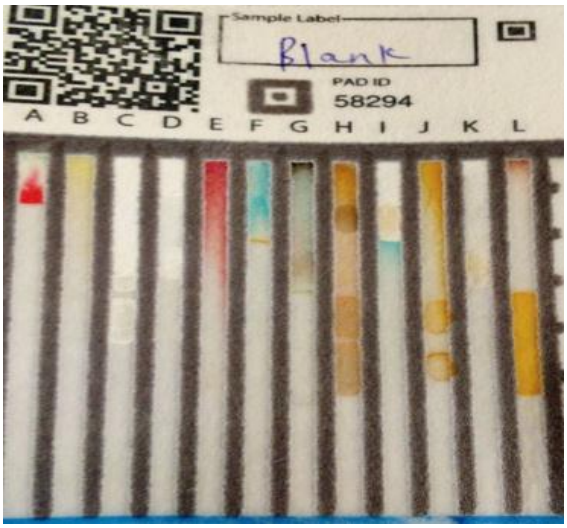
E. 

Figure 6: Results of cisplatin sample ChemoPAD screened upshots; A. Platol, B. Cisplat, C.Namanaplatin, D. Platinox, and E. Blank

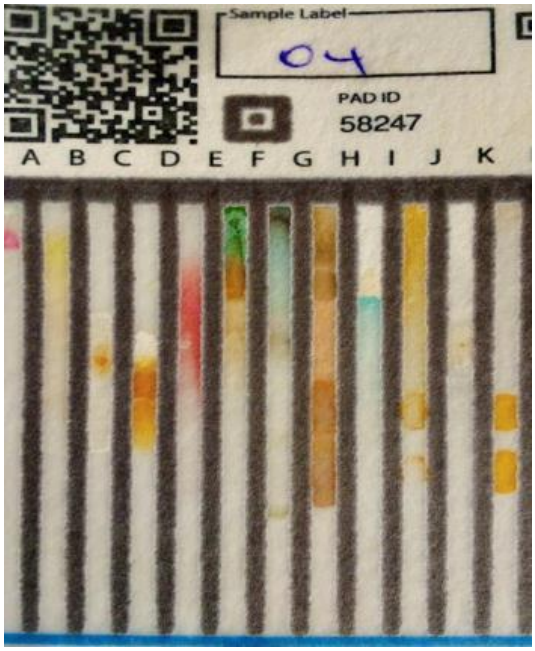
ChemoPAD result of Oxaliplatin: Six brands were included in the chemoPAD screening test for oxaliplatin. Only one brand (Zolon), when visually compared to a typical bar-code, failed the ChemoPAD screening test (Figure 7A). As shown from the figure, at lane C and D does not show strong yellow color; the absence of a strong yellow color in the sample indicates a deficient platinum content. In other samples (Oxaliplit, Zildox, EsOxali-100, Oxol, Curatinox from

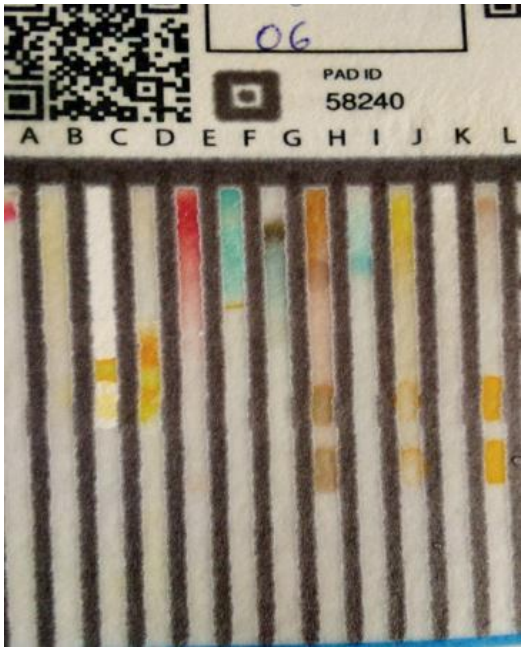
Figure7 B-E, respectively) strong yellow color is present in lanes C and D, this indicates a sufficient platinum content (Figure 7).

A. 

B. 

C. 

D. 

E. 

F. 

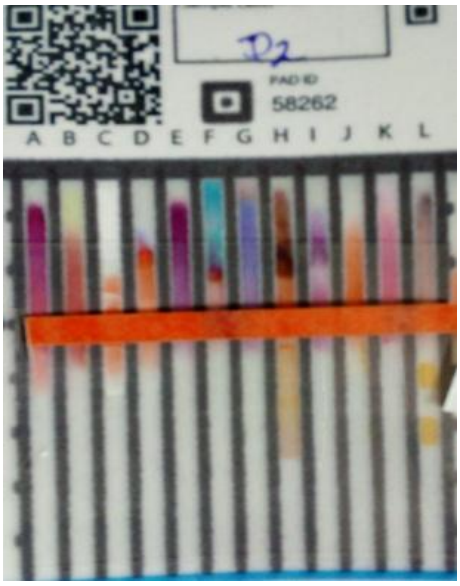
Figure 7: Results of oxaliplatin sample ChemoPAD screened upshots; A. Zolon, B. Oxaliplit, C. Zildox, D. EsOxali-100, E. Oxol, F. Curatinox.

Stannous chloride is a strong reductant that has been used as a color test to detect Pt compounds (Smith *et al*., 2018, Eberle *et al.*, 2020). Cisplatin and oxaliplatin interact with it to form yellow products at λ_{max} 475 nm. The color bar code is therefore due to the reaction of cisplatin and oxaliplatin with tin chloride. Tin chloride (approximately 2 µL of 75 mg/mL solution) was pre-dosed from the ChemoPAD at lanes C & D and cisplatin and oxaliplatin contain the element Pt causing pale yellow color bar-code.

For cisplatin and oxaliplatin, the strength or weakness of colors on the ChemoPAD can identify products that may be seriously substandard or have undergone significant degradation at available doses (Eberle *et al*., 2020). Even though, half of cisplatin brands (1 mg/mL) and 83.3% oxaliplatin (2 mg/mL) passed the screening, it is not sure that it contains the label claim doses as the test is qualitative and semi-quantitative.

Doxorubicin Chemo-PAD test: Three brands of doxorubicin were tested for chemo-PAD screening (Adrosal, Dexos, and Adridox). Two samples of Adrosal with different lot numbers (V284015 & V284016) (Table 2). All samples (100%) screening had passed the test (Figure 8).

A. 

B. 

C. 

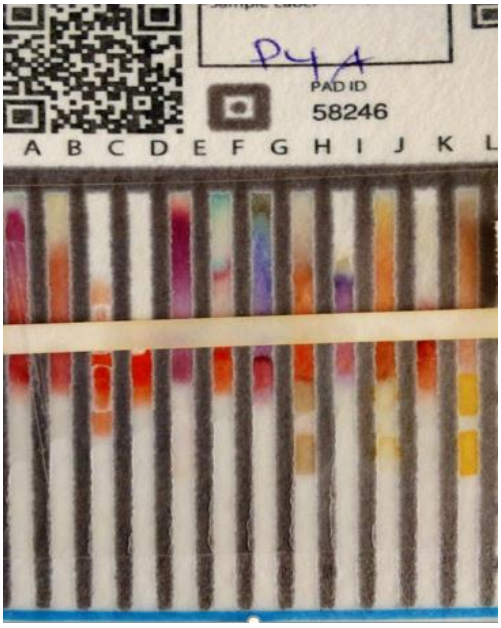
D. 

Figure 8: Results of doxorubicin sample ChemoPAD screened upshots; A. Adrosal (V284015), B. Adrosal (V284016}, C. Dexos D. Adridox).

The ChemoPAD already had several lanes pre-dosed with dry reagents of reactive heavy metallic compound such as 0.2M nickel chlorides at lane A, tin chloride at lane D, Cobalt (II) thiocyanate at lane E, Vanadyl sulfate at lane G and Iron (III) chloride at lane L. In addition, it contains ninhydrin and potassium carbonate at lane B, copper sulfate at lane F and sodium hydroxide at lane H. Doxorubicin is an anthracycline characterized by having a tetracyclic quinone containing

ring nucleus which is attached to a unique daunosamine sugar (Figure 2) (Katzung, 2012, Bozza *et al*., 2021). According to Smith *et al.*, it is an organic polyphenol, many phenolic groups, that forms colored complexes with various divalent and trivalent transition metals (Smith *et al*., 2018). Polyphenols by their nature are used for dyes, so doxorubicin can easily form color with heavy metals. These chemicals react with doxorubicin at available dose (2 mg/mL) to form their respective color on the card (Figure 8).

Methotrexate Chemo-PAD test: Methotrexate is antifolate antimetabolite chemotherapeutic drug (Katzung, 2012, Dipiro *et al*., 2014) with orange color. It is composed of a pteridine ring, p-aminobenzoic acid plus glutamic acid. Methotrexate reacts with various heavy metallic compounds pre-dosed from ChemoPAD to form respective colors (Figure 9). It has multiple chelating nitrogens and creates a green copper complex, which distinguishes it from other lane tests (Smith *et al*., 2018). One brand (Namatroxate) was used in the methotrexate screening test; the presence of a green mark on line F confirms the presence of methotrexate (Figure 9).

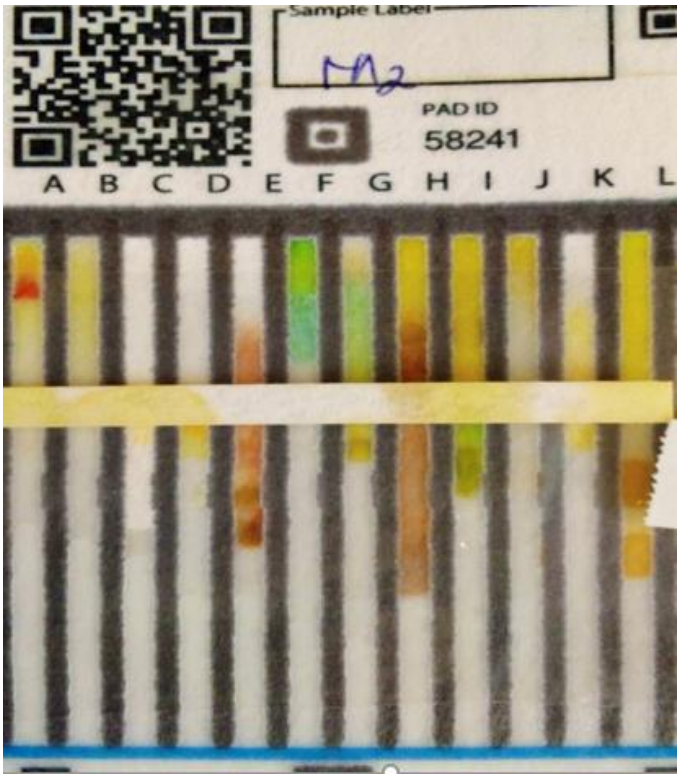


Figure 9: Chemo-PAD result of methotrexate (Namatroxate).

4.1.3. Assay - High Performance Liquid Chromatography

4.1.3.1. Cisplatin

System Suitability Test (SST): According to USP 2020, the system is suitable if the Relative Standard Deviation (RSD) is not more than 2%. SST result for RSD of the peak heights of five replicate injections was 0.2703%. This small RSD value shows a good precision under the same operating conditions. The number of theoretical plates (NTP) was 1602.23. This number of theoretical plates indicates the efficient performance of the column. The average retention time (Rt) was 2.59 minutes.

Table 3: System suitability test for Cisplatin USP RS

	System Suitability Parameters		
Standard Injections	Peak Area	NTP	R_t
Reference Standard 1st Injection	2752262	1780.685	2.589
Reference Standard 2nd Injection	2765560	1675.515	2.588
Reference Standard 3rd Injection	2762221	1565.229	2.579
Reference Standard 4th Injection	2769956	1543.053	2.583
Reference Standard 5th Injection	2770676	1446.648	2.584
Mean Value	2764135	1602.226	2.5846
SD	7472.129	128.7377	0.004037
RSD (%)	0.270324	8.03493	0.156207
Limit	NMT 2	NA	NA

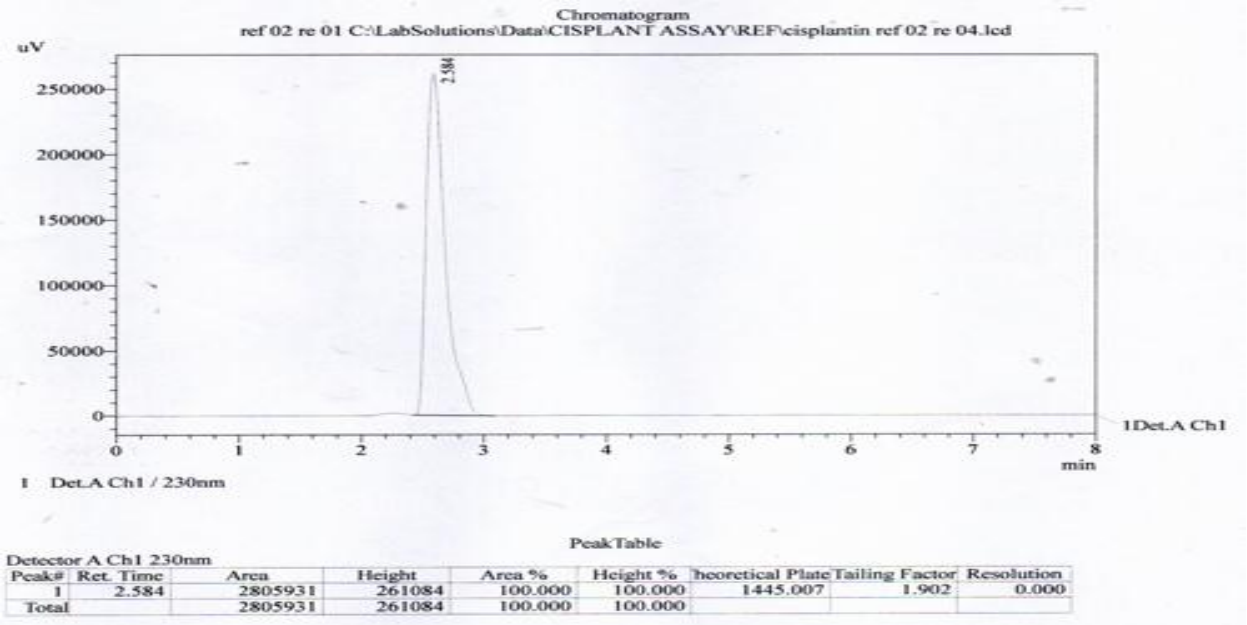
SST is used to confirm that an analytical procure was appropriate for its intended purpose the day the analysis was done. It is an crucial parameter to ensure the quality of the procedure for correct measurements (ICH, 2005). SST is run each time an analysis is carried out and each SST is specific to an individual procedure with pre-defined acceptance criteria for certain parameters such as RSD, resolution, Rt, tailing factor, theoretical plate number (USP-43, 2020b). The method determined to be suitable for a specific formulation directly impacts the confidence in all future assay procedures for that product. The formulation must stay the same for the

method. Changes to any of the components of the formulation or sub-formulation(s) can impact the acceptability of the assay method.

Assay Results: The assay values for cisplatin products (Platol and Cisplat) were 103% and 137% respectively. The specification outlined in USP (USP-43, 2020b) (2020) indicated that the product assay (i.e. drug content) should lie in a range of 90% to 110% of label claims. From the results of assay of cisplatin, Platol (103.44) passed the USP specification i.e. they contain the required amount of cisplatin while Cisplat (137.4) fail to pass the specification on percentage content as it has higher percentage than the USP specification limit.

Table 4: Cisplatin (Cisplat and Platol) injection peak areas and content of the API

	Reference standard		Sample Name					
	standard	standard	Cisplat			Platol		
Parameters	1	2	sample1	sample 2	sample 3	sample 1	sample 2	sample 3
Peak area1	2768342	2805931	3812323	3828397	3810449	2807120	2820089	3016920
Peak area2	2754404	2780676	3822939	3831260	3817117	2798376	2807961	3019555
Peak area3	2763173	2818956	3820024	3828514	3819309	2796519	2818945	3002628
	2761973	2801854	3818429	3829390	3815625	2800672	2815665	3013034.333
Mean area		2781913.667			3821148			2914349.667
SD	7046.06	19462.89	5484.859	1620.235	4614.591	5661.083	6696.335	9107.943584
%RSD	0.25511	0.694643	0.143642	0.042311	0.120939	0.202133	0.237824	0.302284759
%Content			137.2591	137.6531	137.1583	100.6743	101.2132	108.3079741
Mean %Content					137.3568147			103.3984999
Judgment					Failed			Passed

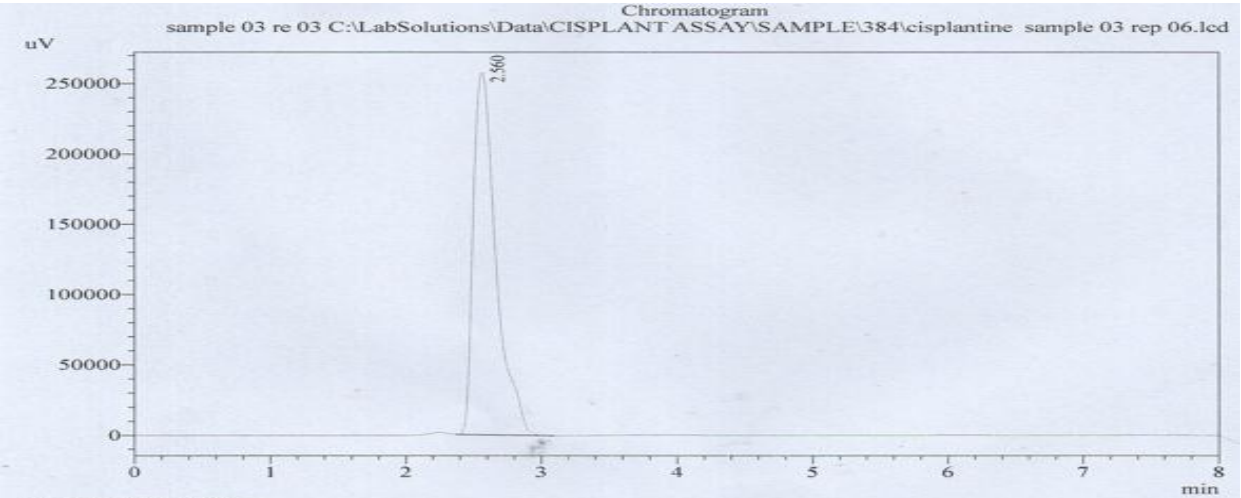


PeakTable

Detector A Ch1 230nm

Peak#	Ret. Time	Area	Height	Area %	Height %	heoretical Plate	Tailing Factor	Resolution
1	2.584	2805931	261084	100.000	100.000	1445.007	1.902	0.000
Total		2805931	261084	100.000	100.000			

Figure 10: HPLC Chromatogram of Cisplatin USP RS

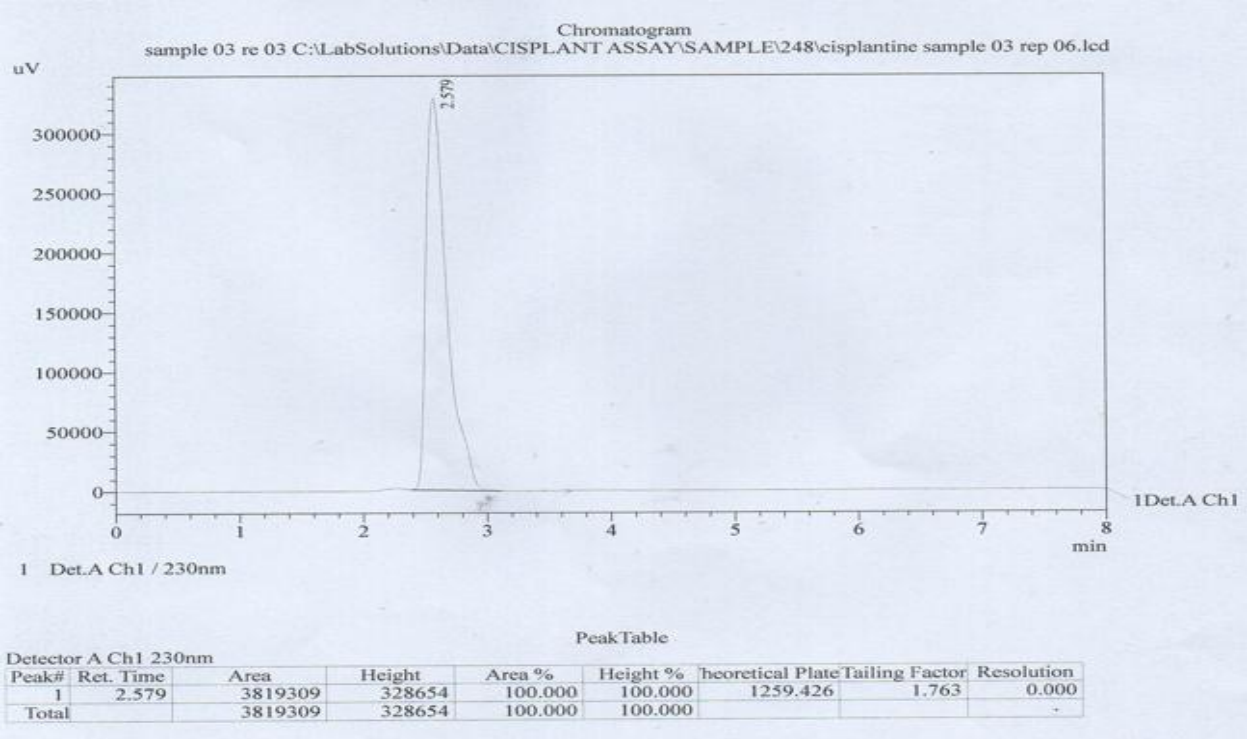


PeakTable

Detector A Ch1 230nm

Peak#	Ret. Time	Area	Height	Area %	Height %	heoretical Plate	Tailing Factor	Resolution
1	2.560	3002628	257542	100.000	100.000	1243.965	1.800	0.000
Total		3002628	257542	100.000	100.000			

Figure 11: HPLC Chromatogram of Cisplatin (Cisplat)



PeakTable

Detector A Ch1 230nm

Peak#	Ret. Time	Area	Height	Area %	Height %	heoretical Plate	Tailing Factor	Resolution
1	2.579	3819309	328654	100.000	100.000	1259.426	1.763	0.000
Total		3819309	328654	100.000	100.000			

Figure 12: HPLC Chromatogram of Cisplatin (Platol)

4.1.3.2. Doxorubicin

SST: As indicated in Table 5, SST result for RSD of the peak heights of five replicate injections was 0.912%, which indicates good precision under the same operating conditions and that of tailing factor was 1.12, which indicated the symmetric nature of the peaks. NTPs were 26436.6. This high number of theoretical plates suggested the efficient performance of the column (USP-43, 2020c) (Table 5).

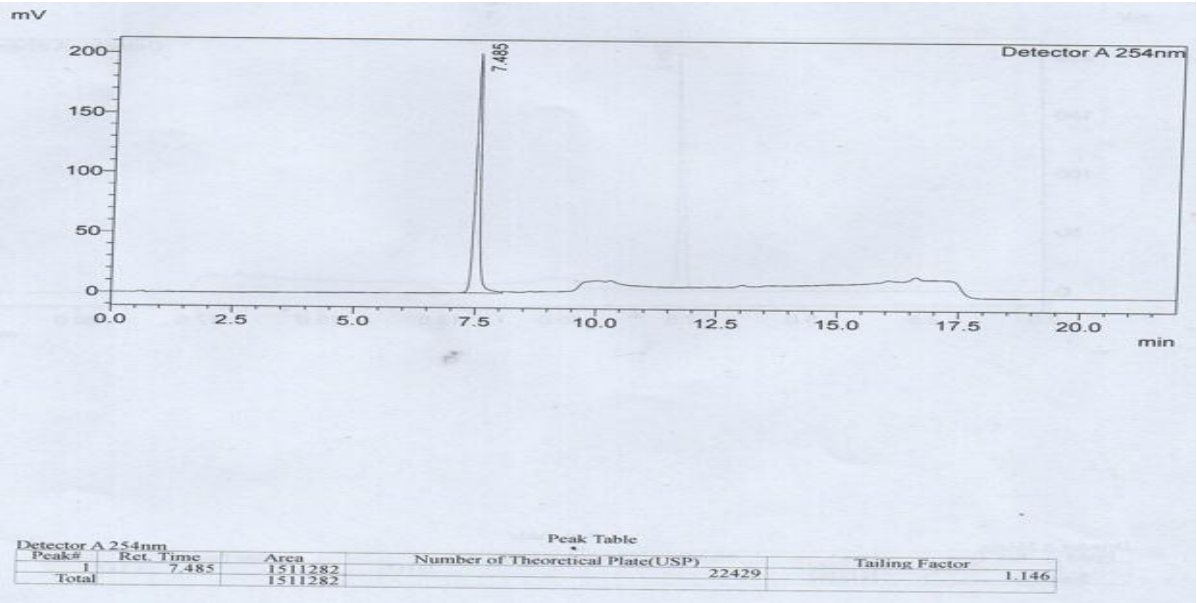
Assay Results: The assay values for doxorubicin drug (Adrosal) products is 83.44% (Table 6). This result showed that doxorubicin assay not comply with the USP specification limit (90–110%). The HPLC chromatogram of reference standards and doxorubicin samples were indicated on Figures 13 and 14, respectively.

Table 5: System suitability test for Doxorubicin USP RS

	System Suitability Parameters			
Standard Injections	Peak Area	NTP	Tailing Factor	Rt
Reference Standard 1st injection	1429184	27904	1.108	7.197
Reference Standard 2nd injection	1423925	27232	1.117	7.255
Reference Standard 3rd injection	1407952	26524	1.118	7.302
Reference Standard 4th injection	1401489	25655	1.122	7.351
Reference Standard 5th injection	1401793	24868	1.123	7.394
Mean Value	1412869	26436.6	1.1176	7.2998
SD	12891.6	1210.885	0.005941	0.07758
RSD	0.912442	4.580335	0.53162	1.062773
Limit	NMT 2%	NA	NMT 2	NA

Table 6: Doxorubicin (Adrosal) injection peak areas and content of the API

	Sample Name			
		Doxorubicin		
Parameters	Standard	Sample 1	Sample 2	Sample 3
Peak Area 1	1484947	1221420	1227651	1293656
Peak Area 2	1510696	1245321	1211404	1312072
Peak Area 3	1511282	1242004	1206251	1321944
		1236248	1215102	1309224
Mean Area	1502308			1253524.778
SD	15038.21	12948.37	11168.99	14357.44
%RSD	1.001007	1.047392	0.919182	1.096637
%Content		82.28992	80.88233	87.14749
Mean %Content				83.4399
Judgment				Failed

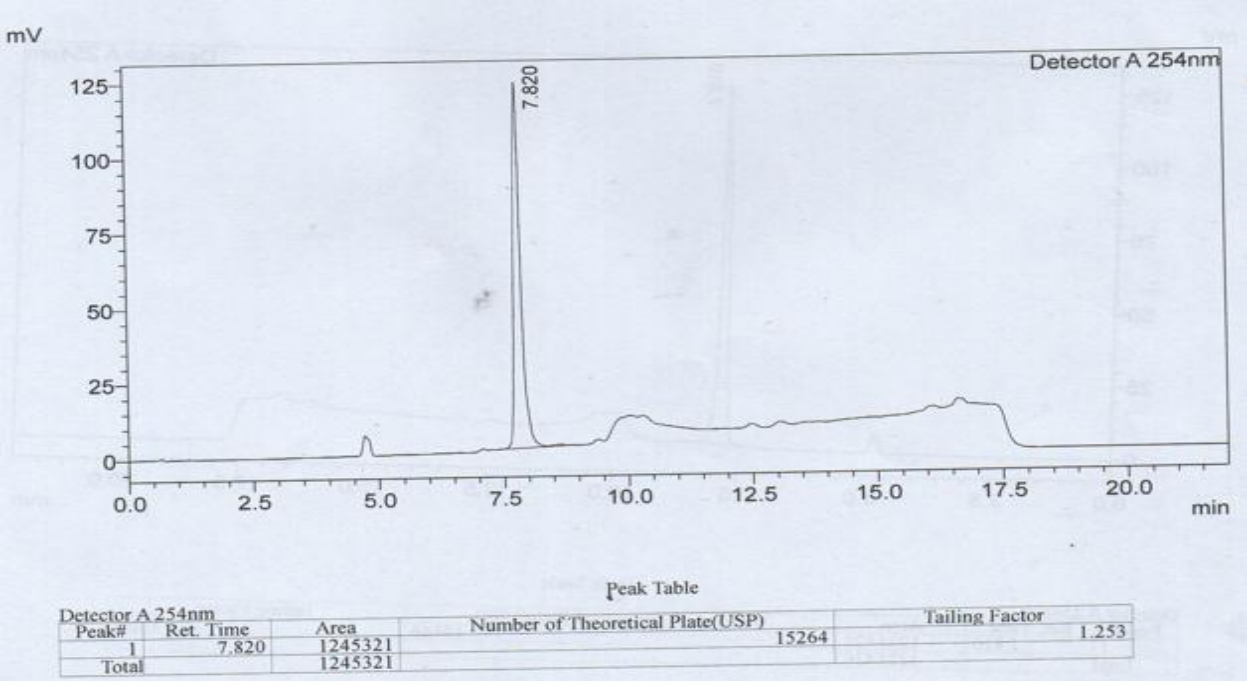


Peak Table

Detector A 254nm

Peak#	Ret. Time	Area	Number of Theoretical Plate(USP)	Tailing Factor
1	7.485	1511282	22429	1.146
Total		1511282		

Figure 13: HPLC Chromatogram of doxorubicin USP RS:



Peak Table

Detector A 254nm

Peak#	Ret. Time	Area	Number of Theoretical Plate(USP)	Tailing Factor
1	7.820	1245321	15264	1.253
Total		1245321		

Figure 14: HPLC Chromatogram of doxorubicin samples (Adrosal)

4.1.3.3. Methotrexate

SST: According to USP (USP-43, 2020a), the system is suitable if the column efficiency determined from methotrexate peaks is not less than 1500 theoretical plates, the tailing factor for the methotrexate peak is not more than 2, and the relative standard deviation for replicate injections for the methotrexate peak is not more than 2.5%. As indicated in Table 7, SST result for tailing factor was 1.20, which indicated the symmetric nature of the peaks. The numbers of theoretical plates were 24868. This high number of theoretical plates show as the efficient performance of the column. RSD of the peak areas of five replicate injections was 1.85%. These parameters are below the limit, indicating that the approach is suitable (Table 7).

Table 7: System suitability test for Methotrexate USP RS

	System Suitability Parameters			
Standard Injections	Peak Area	Tailing Factor	NTP	Rt
Reference Standard 1st injection	1791105	1.205	25081.39	8.28
Reference Standard 2nd injection	1734843	1.205	24946.01	8.228
Reference Standard 3rd injection	1824688	1.21	24901.11	8.175
Reference Standard 4th injection	1786014	1.21	24705.99	8.08
Reference Standard 5th injection	1803249	1.213	24709.21	8.114
Mean Value	1787980	1.2086	24868.74	8.1754
SD	33233.23	0.003507	161.3809	0.081516
RSD	1.858703	0.290182	0.648931	0.997084
Limit	NMT 2.5%	NMT 2	NLT 1500	

Assay Results: The assay value for methotrexate drug products was 118.9% (Table 8). This result showed that it does not comply with the USP specification limit (90–110%). The specification outlined in USP indicated that the product assay (i.e. drug content) should lie in a range of 90% to110% of label claims (USP-43, 2020a). The HPLC chromatograms of selected methotrexate standard and methotrexate samples are displayed in Figures 15 and 16, respectively.

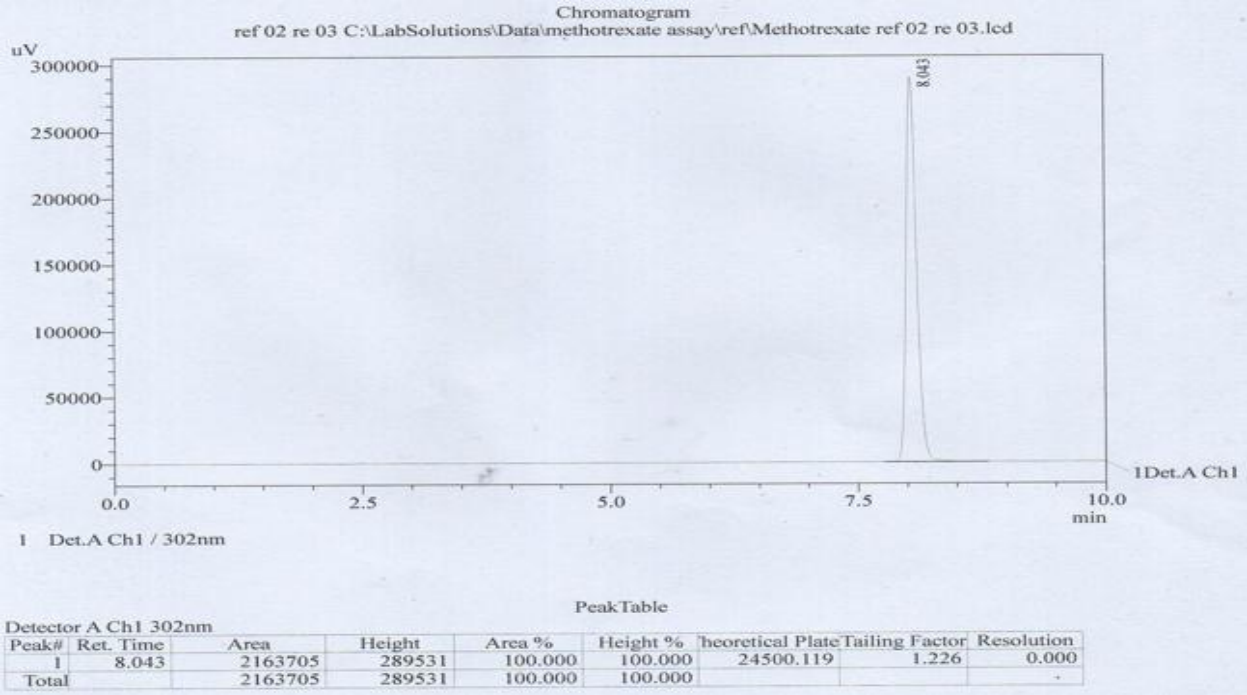
Table 8: Methotrexate (Namathrexate) injection peak areas and content of the API

	Reference Standard		Sample Name		
			Methotrexate		
Parameters	Standard 1	Standard 2	Sample1	Sample 2	Sample 3
Peak Area1	2183705	2204656	2513155	2599955	2617705
Peak Area2	1953161	2286278	2444852	2589136	2649815
Peak Area3	2115517	2163705	2515985	2516295	2586365
	2084128	2218213	2491331	2568462	2617962
Mean Area		2151170.333			2559251.444
SD	118434	62400.96	40276.57	45500.66	31725.78
%RSD	5.682663	2.813118	1.616669	1.771514	1.21185
%Content			115.8128	119.3984	121.6994
Mean Content%					118.9701905
Judgment					Failed

4.1.3.4. Oxaliplatin

SST: Oxaliplatin SST results for RSD of the peak heights of five replicate injections was 0.8692% and that of the tailing factor was 0.5041 (Table 9). These parameters are far below the limit, indicating that the approach is suitable. NTP was 2165. This higher figure of the plate implies the efficient performance of the machine which was far greater than 1500 specified in the method (Table 9).

Assay Results: The assay value for oxaliplatin product (brand Zolon) was 109. 9%. The specification outlined in USP (USP-43, 2020d) indicated that the product's assay (i.e. drug content) should lie in a range of 90% to110% of label claims. From the results of assay of oxaliplatin, (109.9) passed the USP specification (Table 10) i.e. they contain the required amount of oxaliplatin. The HPLC chromatograms of selected Oxaliplatin standard and Oxaliplatin samples are displayed in Figures 17 and 18, respectively.

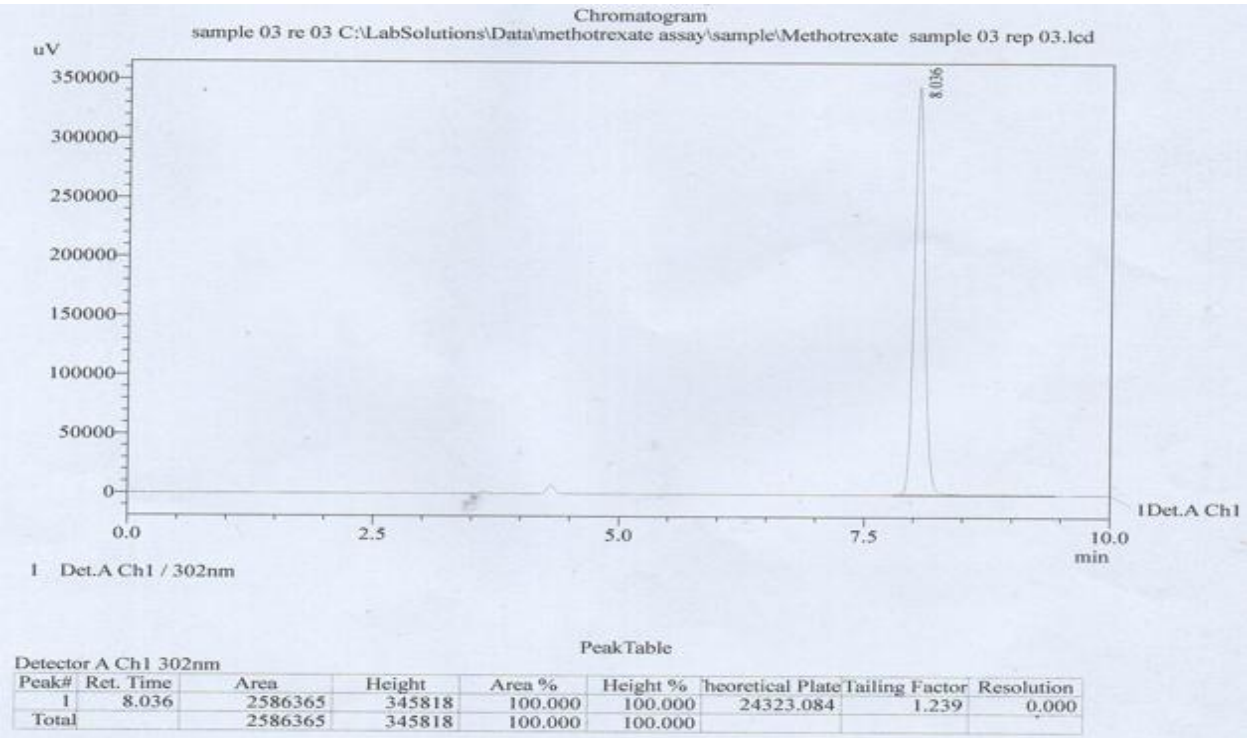


PeakTable

Detector A Ch1 302nm

Peak#	Ret. Time	Area	Height	Area %	Height %	heoretical Plate	Tailing Factor	Resolution
1	8.043	2163705	289531	100.000	100.000	24500.119	1.226	0.000
Total		2163705	289531	100.000	100.000			

Figure 15: HPLC Chromatogram of Methotrexate USP RS



PeakTable

Detector A Ch1 302nm

Peak#	Ret. Time	Area	Height	Area %	Height %	heoretical Plate	Tailing Factor	Resolution
1	8.036	2586365	345818	100.000	100.000	24323.084	1.239	0.000
Total		2586365	345818	100.000	100.000			

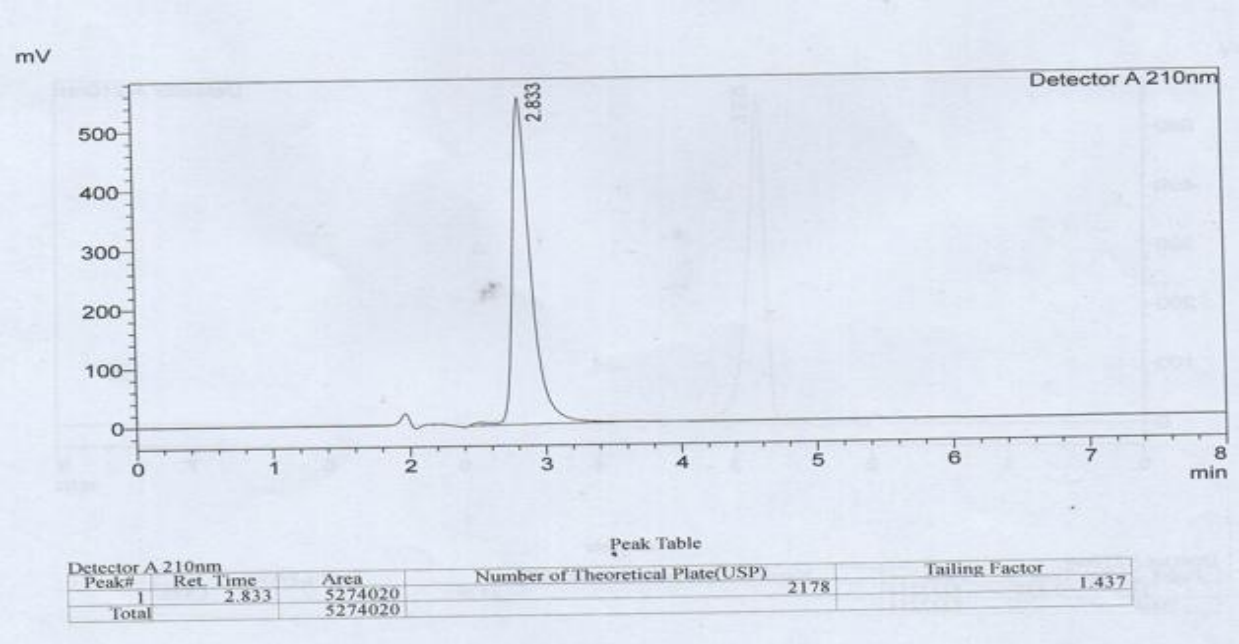
Figure 16. HPLC Chromatogram of methotrexate sample

Table 9: System suitability test for Oxaliplatin USP RS

Standard Injections	System Suitability Parameters			
	Peak area	NTP	Tailing Factor	RT
Reference Standard 1st injection	6271541	2158	1.432	2.834
Reference Standard 2nd injection	6246858	2166	1.423	2.833
Reference Standard 3rd injection	6356772	2159	1.439	2.832
Reference Standard 4th injection	6358465	2169	1.44	2.832
Reference Standard 5th injection	6359314	2176	1.439	2.833
Mean Value	6318590	2165.6	1.4346	2.8328
SD	54921.34	7.436397	0.007232	0.000837
RSD	0.869202	0.343387	0.504104	0.029535
Limit	NMT 2%	NLT 1500	NMT 2	

Table 10: Oxaliplatin (Zolon) injection peak areas and content of the API

Parameters	Standard	Sample Name Oxaliplatin	
		Sample 1	Sample 2
Peak Area1	5358731	5846528	5797294
Peak Area2	5332254	6144175	5734816
Peak Area3	5274020	5822792	5773287
		5937832	5768466
Mean Area	5321668	5853148.667	
%RSD	0.814336	3.016122	0.546364
%Content		111.5784	108.3958
Mean %Content		109.9870999	
Judgment		Passed	

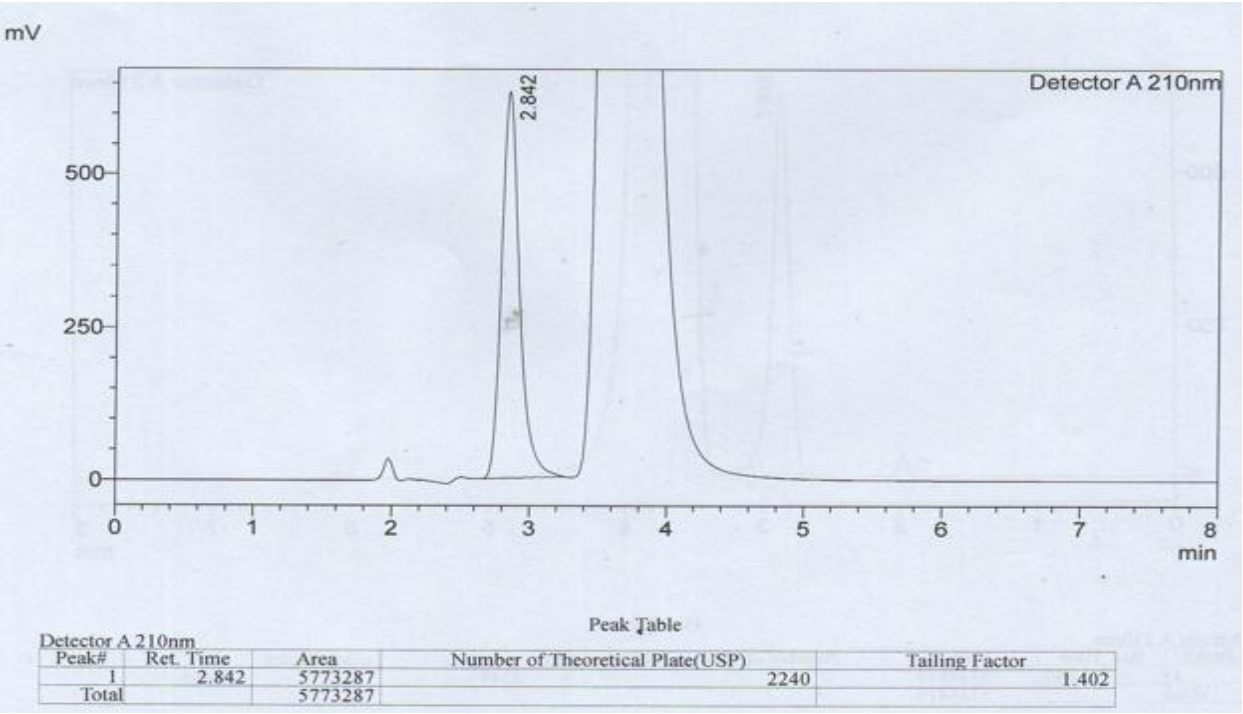


Peak Table

Detector A 210nm

Peak#	Ret. Time	Area	Number of Theoretical Plate(USP)	Tailing Factor
1	2.833	5274020	2178	1.437
Total		5274020		

Figure 17. HPLC Chromatogram of Oxaliplatin USP RS



Peak Table

Detector A 210nm

Peak#	Ret. Time	Area	Number of Theoretical Plate(USP)	Tailing Factor
1	2.842	5773287	2240	1.402
Total		5773287		

Figure 18: HPLC Chromatogram of Oxaliplatin samples

4.2. Discussion

Chemotherapeutic medicines are essential in the fight against cancer (Espinosa *et al.*, 2017). Evaluating the quality of chemotherapeutic medicines circulating in the market is important to reduce the risk of having poor-quality medicines in the supply chain (Khan *et al.*, 2010). In this study, the pharmaceutical quality of commonly available brands of cisplatin, oxaliplatin, doxorubicin and methotrexate in Addis Ababa, Ethiopia were assessed.

The visual inspection results on the analyzed samples did not show any signs of counterfeit medicines. Similar results have been reported for compliance in packaging and labelling in different brands of albendazole tablets marketed in Jimma, Ethiopia (Belew *et al.*, 2018). In contrast to the finding of this study, problems were observed with labelling and physical characteristic of chloroquine in south west Ethiopia, as reported by Abuye *et al.* (2020) and a study conducted in Malawi, 9 in 10 amoxicillin analytes were losing their own packages and/or package inserts (Chikowe *et al*., 2018).

The PAD was developed as a cost-effective tool for field screening of a wide variety of pharmaceutical products. Among the four different brands of cisplatin samples, two brands (Platol &Cisplat) failed the Chemo-PAD screening test which were both collected from Girum hospital, Addis Ababa, as leftovers. Only one brand for oxaliplatin (Zolon), when visually compared to a typical bar-code, failed the screening test, collected at TASH, Addis Ababa. For doxorubicin and methotrexate, no sample had failed the Chemo-PAD screening. Similar study done at TASH show that substandard Cisteen were found while screening with ChemoPAD (Eberle *et al.*, 2020). In contrast to the finding of this study, all brands of amoxicillin analyzed by PAD passed an identity test in a study done at Blantyre urban townships, Malawi, (Chikowe *et al*., 2018). The other screening done with PAD at Eldoret, in Kenya identified many lots of amoxicillin and doxycycline adulterated with talcum powder, counterfeited paracetamol and substandard losartan(USAID, 2016). There are limited published data on screening of chemotherapeutic products with PAD (ChemoPAD).

The intent of HPLC assay in this study is to confirm the ChemoPAD screened results as it is a golden standard method. It provides quantitative findings in order to monitor and analyze the

infiltration and spread of low-quality chemotherapeutic product. Basically, drug assay is a critical quality control parameter required to confirm the labeled dose on the required container. Any deviation from the acceptable range is poor quality pharmaceutical product (USP-43, 2020b, USP-43, 2020d, USP-43, 2020c, USP-43, 2020a).

The assay values for cisplatin products (Cisplat and Platol) were 103% and 137%, respectively. From the results of assay, Cisplat (103.44) passed the USP specification i.e. they contain the required amount of cisplatin while Platol (137.4) fail to pass the specification it has higher content than the USP specification limit. The assay values for doxorubicin drug (Adrosal) products was substandard (83.44%). Doxorubicin by its nature have phenolic groups that are capable generating reactive species like radical OH and super oxide radical O-O- anion (Foye, 2013). Moreover, it is known for instability in solution form (Eberle *et a*l., 2020). The lower HPLC assay results found in the study might be due to degradation during long period of storage in solution form. Methotrexate had slightly higher API content (118.9%). The assay value for oxaliplatin product (brand Zolon) was good quality (109. 9%) as indicated by the product's assay (i.e. drug content) fall in a range of 90% to110% of label claims.

A similar survey conducted on anti helimenitic drugs 45.3% of the analytes of albendazole, mebendazole and tinidazole were substandard (Suleman *et al*, 2005,). Furthermore, substandard Tegral (generic name- carbamazepine) (Suleman, 2005) and Asmol (generic name-acetaminophen) (Teklu *et al.,* 2014) were found circulating in the central part of Ethiopia, Addis Ababa.

The probable factors for the presence of such poor quality drugs in developing countries like Ethiopia might be due several reason. Factors contributing to this problem include poor storage conditions, insufficient quality assurance, poor compliance with good manufacturing and storage practice standards, lack of scientific expertise in the quality control sector, limited technical capacity and insufficiently well-developed regulatory system to evaluate and take action to solve the problems related to drug quality (Kelesidis *et al.,* 2007).

The source of the chemotherapeutic products used in this screening were from India. According to Vorrath and Voss, India is the major source of poor-quality pharmaceutical products (Vorrath

and Voss, 2019). The cause for screening failures might be poor manufacturing practice and absence of quality control measures. The study previously done at TASH on chemotherapeutic products founds substandard Cisteen (generic name - cisplatin) and doxorubicin (Eberle *et al*., 2020 and smith *et al*., 2018)

Inadequate API in the dosage form leads to under-dosed medication whereas excessive amounts of API lead to over-dosage and increased adverse drug responses. In both case, there might be treatment failure whatever accurate enough the diagnosis is perdormed. There are mounting evidence for poor-quality pharmaceutical products accelerating the burden of morbidly and mortality. For instance, one study estimated that more than 122,000 malaria deaths in children younger than five years, that is, 3.8% of all deaths in that age group across the 39 countries studied, were associated with consumption of poor-quality antimalarials (Renschler *et al.,* 2015). Besides, over 2,000 children in Kenya and 9,000 children in Bangladesh (who could have recovered if they were good quality) died by pneumonia, because they were given substandard amoxicillin (USAID, 2016).

Chemotherapy drugs are often expensive due to the high costs associated with research, development, manufacturing, and regulatory approvals. This makes them a particularly attractive target for substandard and falsified medicines. The high price of these drugs creates a lucrative market for counterfeiters looking to exploit the demand for affordable alternatives, particularly in regions with limited access to quality healthcare. Patients and healthcare providers in low- and middle-income countries may unknowingly purchase substandard and falsified chemotherapy drugs, leading to severe health consequences, treatment failures, and increased resistance to effective medications. Addressing this issue requires robust regulatory frameworks, improved supply chain monitoring, and global collaboration to ensure the integrity of chemotherapy treatments.

Availability issues at public hospitals often lead to significant challenges for patients, particularly when it comes to accessing essential treatments like chemotherapy medications. In many cases, due to shortages or logistical problems, patients turn to community pharmacies that may supply these medicines in informal ways. Such practices, while filling immediate gaps, can accelerate the circulation of poor-quality or counterfeit chemotherapy drugs. This undermines

treatment efficacy, compromises patient safety, and exacerbates public health concerns, especially for vulnerable populations who cannot afford alternative options. Strengthening supply chains and regulatory oversight is crucial to addressing these issues and ensuring the availability of high-quality medicines in public healthcare settings.

Hence, the presence of substandard chemotherapeutic products might be the confounding factor for high rate of morbidity and mortality due to cancer in Addis Ababa. The findings revealed that there is a need for continuous assessment of the chemotherapeutic product imported and circulating in the market of Ethiopia to ensure the safety, efficacy and quality.

5. Conclusion and Recommendations

The study investigated the quality of some selected parenteral chemotherapeutic drugs (cisplatin, oxaliplatin, doxorubicin and methotrexate) at Addis Ababa. Visual inspection revealed no defects based on WHO/FIP/USP checklist. The results of ChemoPAD screening and HPLC confirmatory assays revealed the presence of some poor quality chemotherapeutic products/samples at the chemo centers included in this study. The outcome of this study augments a concern regarding quality of some chemo-drugs assessed in this study

There is need to conduct continuous country-wide post-market check up to determine the quality status of chemotherapeutic products.

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Annexes

Annex 1: Ethical clearance letter

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Addis Ababa University

School of Pharmacy
Ethical Review Committee

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Date April 16, 2021

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Ref. No ERB/SOP/273/13/2021

To: **Adugnaw Demesie**
School of Pharmacy

Re: Ethical Clearance

It is to be recalled that you submitted a research proposal entitled "**Investigation of the Quality of Some Selected Chemotherapeutic Drugs Using Paper Analytical Device and Pharmacopoeia Methods from Addis Ababa, 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 (Ph
Chairperson, ERC
School of Pharmacy
College of Health Sciences
Addis Ababa University

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Telex: 21205

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Fax: 00251(11)1558566

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Cable: AAUNIV

Annex 2: label analysis tool for visual inspection of chemotherapeutic drugs; modified from WHO/FIP/USP checklist. The visual inspection results were for all vials.

Label information (all from package till ampules)	Collected parenteral Chemotherapeutic Drugs			
	Cisplatin	Doxorubicin	Methotrexate	Oxaliplatin
Container				
Does the container and closure protect the product from the outside environment; is the container properly sealed	X	X	X	X
Do they assure that the product will meet the proper specifications throughout its shelf life?	X	X	X	X
Are the container and the closure appropriate for the product inside?	X	X	X	X
Is the container safely sealed?	X	X	X	X
Label on package				
If there is a carton protecting the container, does the label on the carton match the label on the container?	X	X	X	X
Is all information on the label legible and indelible?	X	X	X	X
Brand name				
Is the trade name spelled correctly?	X	X	X	X
Is the medicinal product (trade name) registered in the country by the Drug Regulatory Authority)? Is the product legally sold in the country?	X	X	X	X
Does the symbol ® follow the trade name?	X	X	X	X

Active ingredient name				
Is the active ingredient name spelt correctly?	X	X	X	X
Do the trade name and the active ingredient names correspond to the registered product?	X	X	X	X
Manufacturer's name and Address				
Are the manufacturer's name and logo legible and correct?	X	X	X	X
Does the logo or hologram (if applicable) look authentic?	X	X	X	X
Does the logo or hologram (if applicable) change colour when viewed from different angles?	X	X	X	X
Is the manufacturer's full address legible and correct?	X	X	X	X
Has this company or its agent registered the product in the country?	X	X	X	X
Medicine strength (mg/unit) and dosage form				
Is the strength - the amount of active ingredient per unit - clearly stated on the label?	X	X	X	X
Is the dosage form clearly indicated on the container label?	X	X	X	X
Does the dosage form stated on the label match the actual dosage form of the medication?	X	X	X	X
Is the indicated medicine under this dosage form registered and authorised for sale in the country?	X	X	X	X

The batch number, manufacturing and expire dates	X	X	X	X
Does the numbering system on the package correspond to that of the producing company?	X	X	X	X
Are the manufacture and expiry dates clearly indicated on the label?	X	X	X	X
Storage information				
Are the storage conditions indicated on the label?	X	X	X	X
Has the product been properly stored?				
Leaflet				
s the package insert printed on the same coloured or same quality paper as the original (If available to compare) or does it look familiar?	X	X	X	X
Is the ink on the package insert or packaging smudge-proof?	X	X	X	X
Does the information on the package insert match the information on the product container?	X	X	X	X

The sign **'x'** indicates the product meets to the specifications of the checklist.