KYAMBOGO  UNIVERSITY

ASSESSMENT OF THE QUALITY OF LEVONORGESTREL EMERGENCY CONTRACEPIVE TABLETS (MORNING - AFTER PILL) SOLD IN UGANDA

BY

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DECLARATION

I, Babirye Lydia Brenda**,** declare that this dissertation is my original work and has not been submitted to any other university for a master of chemistry award.

Signed ……. Date ……

APPROVAL

This dissertation has been submitted to the Directorate of Research and Graduate Training for examination with the approval of the following university supervisors.

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DEDICATION

With great love, I dedicate this work to my parents Mr. & Mrs. Tenywa David and for their unconditional love, support and encouragement throughout my studies.

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*"**God bless you all**"*

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LIST OF ABBREVIATIONS

API	Active Pharmaceutical Ingredient
ECs	Emergency Contraceptives
CI	Confidence Interval
GMP	Good Manufacturing Practices
HPTLC	High Performance Thin Layer Chromatograph
HPLC	High Performance Liquid Chromatograph
IUD	Intrauterine device
IUPAC	International Union of Pure and Applied Chemistry
NDA	National Drug Authority
WHO	World Health Organization
Uv-vis	Ultra-Violet visible
TLC	Thin Layer Chromatograph
RS	Reference Standard
RT	Retention Time
mL	Milliliter
µL	Microliter

mm	Millimeter
USA	United States of America

DEFINITION OF TERMS

Active Pharmaceutical Ingredient (API): This is a substance intended to be used in the manufacture of a drug product.

Batch (or lot): A defined amount of starting material, packaging material, or product manufactured in a single process and expected to be homogeneous.

Batch number (or lot number): A distinctive combination of numbers /letters which specifically identifies a batch on the labels, the batch records, the certificates of analysis, etc.

Brand: A name given by the company that makes the drug for purposes of marketing and selling of that drug.

Label claim: Amount of active pharmaceutical ingredient the drug contains.

Monograph: Compiled information of APIs or finished products with its quality control tests such as assay, identification, dissolution etc.

Pharmaceutical outlet. A licensed or unlicensed sale counter for provision of medicines.

Specification: A list of test parameters with their respective appropriate acceptance criteria in numerical limits, ranges or statements.

Validation: A collection of objective evidence that a given item fulfils the specified criteria.

ABSTRACT

Use of poor quality emergency contraceptives may lead to unwanted pregnancies. Many unwanted pregnancies lead to illegal abortions having a devastating impact on the health of the girl child and women at large. This study was aimed at assessing the quality of levonorgestrel tablets sold on the Ugandan market.

The quality of levonorgestrel tablets was established through collection of samples from the Eastern, Western, Central and Northern regions of Uganda. The High Performance Thin Layer Chromatography (HPTLC) and the High Performance Liquid Chromatography (HPLC) analytical techniques were used to identify and determine the content of levonorgestrel, respectively. The quality parameters assessed included visual inspection, identification, content uniformity, assay and dissolution.

Results showed that thirteen percent of samples found on the market were counterfeits and all belonged to brand G. The samples that had been confirmed counterfeits further failed the uniformity of content, assay and dissolution tests. Eighty-seven percent of the samples found on the market passed the assay and uniformity of content test and the statistical analysis conducted at 95% CI revealed significant differences (ANOVA, $p < 0.05$) within the brands in the mean uniformity of content and assay results.

Further findings showed that 30% of the samples on market exhibited inadequate release of levonorgestrel by dissolution testing whereas 13% showed no release of levonorgestrel. Similarly, the statistical analysis conducted using the one-way ANOVA at 95% CI revealed that there was a significant difference ($p < 0.05$) in the drug release of the different brands of levonorgestrel tablets examined in the study.

From the study it was observed that only 57% provided the stated level of contraceptive action, 30% provided questionable results whereas 13% offered no prevention against unwanted pregnancy. The statistically significant differences observed further indicated the availability of levonorgestrel tablets on the market that are not therapeutically equivalent.

It was concluded that there is need to emphasize regular assessment of the quality of emergency contraceptives on the market in order to lower the risk of patients being exposed to products of poor quality, safety, and efficacy.

CHAPTER ONE

INTRODUCTION

1.1 Background

Globalization of the pharmaceutical industry has the potential to rapidly spread substandard and counterfeited medicines before adequate detection and intervention are possible. The export of pharmaceutical products which used to be direct from a manufacturing country to an importing country, is now taking place from stocks held in one or more intermediate countries or through trading houses via duty-free ports/zones (World Health Organization (WHO), 1999a). Drugs that move through intermediate countries may be repackaged or relabeled, and

with the ineffective drug regulation and poor quality control procedures in many countries ,this has facilitated the common appearance of poor quality medicines on the market (WHO, 1999a).

Poor quality medicines are a critical global health issue with much of the burden falling on low- and middle-income countries. Most of the burden falls on these countries because of poor pharmaceutical governance, lack of institutional capacity in regulation and the cost of legitimate drugs being beyond the reach for much of the population (WHO, 2017a). World Health Organization has made an estimated 1 in 10 medical products circulating in low and middle-income countries being either substandard or counterfeited (WHO, 2017a). Such medicines are a danger to patients and may result in treatment failure, development of drug resistance, increased costs for patients and the health system and at worst even cause serious illness or even death (Ozawa et al., 2018; WHO, 2017a).

Reproductive health has also been affected by substandard health products such as counterfeited emergency contraceptives and poor quality condoms. Towards the end of June 2013, a batch of falsified postinor 2 was discovered at Lagos International Airport, Nigeria, containing no levonorgestrel (active pharmaceutical ingredient) (WHO, 2013). In August 2015, the National Drug Authority of Uganda notified WHO of the seizure of falsified Postinor-2 discovered in Kampala, Uganda (WHO, 2015). A study on emergency contraceptives in Peru found that twenty eight percent of the samples analyzed were either of substandard quality or falsified (Monge et al., 2014). This meant that the emergency contraceptives circulating in different parts of the world may not be providing the recommended level of contraceptive action in preventing unwanted pregnancy.

Emergency contraceptives (ECs) are a birth control method that women can use immediately after unprotected sex (WHO, 2018a). The use of ECs amongst women to prevent pregnancies has steadily increased over years. In the US, about 5.8 million women aged 15–44 years used ECs between 2006 and 2010, compared with 4.2% of women in 2002 and less than 1% in 1995 (Haeger et al., 2018). In Uganda, it has been reported that 62% of active youth have used ECs (Babirye, 2013). Women have found the use of ECs as a very convenient method of family planning because it is taken as a single dose and can only be used if someone has had unprotected sex, unlike other methods of preventing unwanted pregnancies that require consistent use of the medicine (Merten & Rokicki, 2018). Furthermore, an estimated 52% of pregnancies are unintended, and about a quarter of these end up in abortion. It has been further reported that 8% of maternal deaths are due to unsafe abortions which result in serious injuries and illness (Guttmacher Institute, 2017). The use of ECs is a strategy to help women avoid unwanted pregnancies hence reduce unsafe abortions.

The high demand of ECs, coupled with a growing number of registered levonorgestrel tablets (National Drug Authority (NDA), 2019a) may increase the risk of substandard, degraded or falsified products on the Ugandan market. The unwitting use of poor quality levonorgestrel tablets may result in unwanted pregnancy, and diverse medical and social consequences such as, quitting school, rejection from family members and physical harm as a result of illegal abortions (Monge et al., 2014). To protect the safety and health of consumers from the emerging threat of poor quality levonorgestrel tablets, there was need to assess the quality of levonorgestrel tablets sold on the Ugandan market.

1.2 Problem statement

The use of poor quality levonorgestrel tablets may lead to unwanted pregnancies which may in turn lead to illegal abortions having a devastating impact on the health of the girl child and women at large. Despite the popular and increasing use of levonorgestrel tablets as an EC in Uganda, little information about their quality is available. Therefore, there was need to assess the quality of the levonorgestrel tablets sold on the Ugandan market in Uganda.

1.3 Objectives

1.3.1 General Objective

The overall objective of the study was to assess the quality of levonorgestrel tablets sold on the Ugandan market.

1.3.2 Specific Objectives

i. To physically inspect the tablets and packaging information for any suspected counterfeits.
ii. To identify and quantify (assay) levonorgestrel in levonorgestrel tablets.
iii. To determine uniformity of content of levonorgestrel in levonorgestrel tablets.
iv. To determine the dissolution of levonorgestrel tablets.

1.4 Scope of the study

The study was aimed at assessing the quality of levonorgestrel tablets on the Ugandan market. Samples were randomly collected from only registered pharmacy outlets, from the four main regions of Uganda (Central, Eastern, Western and Northern). The samples were collected for a period of two months, from September to November 2019.The tests performed included visual inspection, identification, uniformity of content, assay and dissolution. The analytical techniques employed were HPTLC and HPLC. The study was done for a period of two years, from August 2019 to August 2021.

1.5 Significance for the study

Despite the increase in poor quality medicines on the market worldwide, there is not enough data to do a correct estimation of the extent of the problem and the impact on public health (Newton et al., 2006). Similarly, there is not much information about the quality of levonorgestrel tablets sold on the Ugandan market despite the several allegations that have been made about its therapeutic failure. A study on the quality of levonorgestrel tablets on market will generate information that will be used to assure users of the quality of levonorgestrel tablets and also generate information that will be used as evidence during policy making and development of regulations and standards by relevant government agencies. In addition, the study will provide information that will form the basis for investing in dissemination of public information and regular surveys on the quality of medicines on the Ugandan market.

1.6 Justification of the study

The use of poor quality levonorgestrel tablets may result in unwanted pregnancies. Unwanted pregnancies are the leading cause of illegal abortions which have various medical and social consequences. This study will provide information on the quality of levonorgestrel tablets on

the market which will further inform the relevant government agencies on the steps to be taken to ensure that Ugandans access safe, efficacious and quality levonorgestrel tablets.

1.7 Hypothesis

All brands of levonorgestrel tablets on the market are therapeutically equivalent in regards to their quality parameters (assay, dissolution and uniformity of content) irrespective of the manufacturer. To test this hypothesis, the mean results obtained were statistically evaluated using one-way ANOVA and the student t-test.

The null hypothesis (Ho) was that, there was no significant difference in the mean drug content amongst the brands of levonorgestrel tablets found on the market, whereas the alternate hypothesis (Ha) was that, there was a significant difference in the mean drug content amongst the brands of levonorgestrel tablets found on the market. The statistical evaluations were done with a 95% CI consideration and significant differences were considered when the value of p was less than 0.05 ($p < 0.05$).

CHAPTER TWO

LITERATURE REVIEW

2.1 Emergency Contraceptives

Emergency contraceptives are medicines used to prevent pregnancy immediately after sexual intercourse. There are four main types of ECs, namely, levonorgestrel, ulipristal acetate, combined oral contraceptive pills and copper-bearing intrauterine devices (IUDs) (Reproductive Health Supply Coalition (RHSC), 2018; WHO, 2018a). Levonorgestrel is the most widely used and is regarded as the 'gold standard' emergency contraception regimen (Millán & Castañeda, 2014). Levonorgestrel is readily available at all pharmacies without showing a prescription whereas other types of ECs need a prescription to take it, hence not a preferred regimen of contraceptives (Ajayi et al., 2016).

2.1.1 Ulipristal acetate

Ulipristal acetate as an emergency contraceptive should be used within 120 hours after sexual intercourse. Ulipristal acetate is a progesterone that is taken as a single dose of 30mg (Jadav & Parmar, 2012). Ulipristal acetate was approved for emergency contraception by the European Medicines Agency in 2009 and by the United States Food and Drug Administration (FDA) in 2010 (European Medicines Agency (EMEA), 2009; FDA, 2010). However, it is not widely used in most countries because it is not readily available over the counter without showing a prescription (Shigesato et al., 2018).

2.1.2 Combined oral contraceptives

The Combined oral contraceptive is taken in two doses that are 12 hours apart and should be taken within 72 hours of unprotected sexual intercourse (RHSC, 2018). It is a combination of an estrogen (usually ethinylestradiol) and a progestin and these are taken as a split dose, with one dose of 100 μg of ethinyl estradiol plus 0.50 mg of levonorgestrel followed by a second identical dose 12 hours later (WHO, 2018a).

2.1.3 Copper-bearing intrauterine device (IUD)

The IUD can be used as an emergency contraceptive if used within 5 days after unprotected sex, however it can also be used as a permanent family planning method to prevent pregnancy (WHO, 2018a). The copper-bearing intrauterine device is a small T- shaped flexible plastic frame with copper wires that is inserted into a woman's uterus through her vagina and cervix (WHO, 2018b). The IUD is the second most commonly used method of preventing pregnancy, and in 1986, it was estimated that about 83 million women were using the IUD, with 71% being Chinese (Rosenfield, 1989).

2.1.4 Levonorgestrel

Levonorgestrel is a synthetic progestin used as an emergency contraceptive and taken within 3 days of unprotected sex. It is taken orally as a single dose of 1.5mg or alternatively as 0.75mg doses each separated by 12 hours (RHSC, 2018; WHO, 2018a). Progestins are synthetic steroidal hormonal forms of the body's naturally occurring hormone progesterone. Progestins are present in all kinds of hormonal birth control, either alone (like in implants, hormonal IUDs or injections) or with an estrogen (like in most pills, patches, vaginal rings and some injections) (Ray, 2019). Levonorgestrel is either used as a single agent in emergency contraception or as a hormonal contraceptive released from the IUD (Shohel et

al., 2014). Levonorgestrel has been listed as an essential health care medicine by the World Health Organization (WHO, 2019b).

Levonorgestrel is a levorotatory stereoisomer and enantiopure form of norgestrel; it is a racemic mixture containing levonorgestrel and dextronorgestrel ("Levonorgestrel," 2023). Norgestrel was the first progestogen to be manufactured and its discovery by Hughes and colleagues at Wyeth in 1963 was via structural modification of norethisterone (17α-ethynyl-19-nortestosterone). Norgestrel was later licensed to Schering AG who separated the racemic mixture into its optical isomers and identified levonorgestrel as the active component of the mixture (Aigner et al., 1996).

The IUPAC name of Levonorgestrel is 13-ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one. Levonorgestrel has a molecular formula of $C_{21}H_{28}O_2$ and molecular weight 312.5g/mol. Levonorgestrel is practically insoluble in water, sparingly soluble in methylene chloride and slightly soluble in ethanol (Medicines and Healthcare products Regulatory Agency (MHRA), 2019a; United States Pharmacopeial Convention (USPC), 2019a). Figure 2.1 gives the structural formula for Levonorgestrel (MHRA, 2019a; USPC, 2019a).

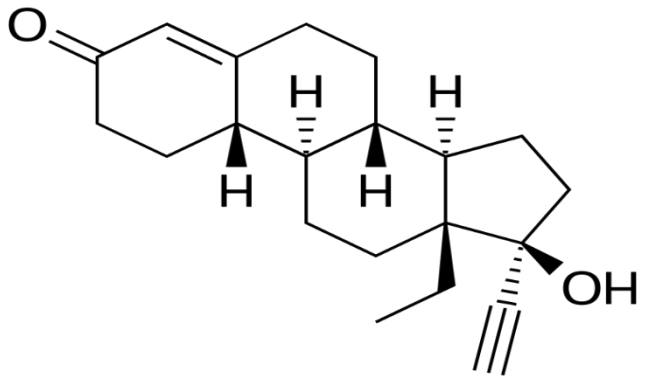


Figure 2.1: Structure of Levonorgestrel

According to the International Consortium for Emergency Contraception (ICEC), there are about 218 different brands of ECs containing levonorgestrel, mifepristone and ulipristal

acetate manufactured in over 25 countries (ICEC, n.d.). Uganda has over 26 registered brands of ECs containing levonorgestrel and a combination of levonorgestrel and ethinylestradiol that are manufactured in over 8 different countries. Levonorgestrel-only emergency contraceptives take up a percentage of 62% (about 16 brands) of the registered ECs, hence making it the most available type on the market (NDA, 2019a). Appendix 1 gives a list of ECs containing levonorgestrel registered to be sold on the Ugandan market.

2.2 Mechanism (Mode of Action) of emergency contraceptives

Levonorgestrel and Ulipristal acetate interfere with the ovulation process if taken before the pre-ovulatory luteinizing hormone (LH) surge has started (Brache et al., 2010; Croxatto et al., 2001). The LH rise triggers the start of ovulation. Levonorgestrel will hinder the rise of LH making it difficult for the egg to mature or be released. Ulipristal acetate on the other hand has been shown to prevent ovulation both before and after the surge of LH has started, delaying follicular rupture for at least five days (Brache et al., 2010; Gemzell-Danielsson et al., 2013).

Levonorgestrel and Ulipristal acetate have not been demonstrated to be effective as ECs when taken after ovulation. Emergency contraception taken after implantation cannot reverse an established pregnancy or harm a developing embryo hence do not induce an abortion (De Santis et al., 2010; Gemzell-Danielsson et al., 2013).

The copper-bearing intrauterine device causes a chemical change in sperm and egg before they meet hence preventing fertilization. The copper ions released from the copper IUD in the genital tract enhance are toxic for spermatozoa and this affects the function and viability of gametes and prevents fertilization (Ortiz et al., 1996).

Studies have showed that women who have used ECs with ulipristal acetate have had a pregnancy rate of 1.3% and ECs with levonorgestrel a pregnancy rate of 1.2% to 2.2% (Von

Hertzen et al., 2002). It has further been reported that ECs should be taken as early as possible after sex and within 72 hours, however ECs with ulipristal acetate are licensed for use up to 120 hours (RHSC, 2018; WHO, 2018a). A copper-bearing intrauterine device used within 120 hours of unprotected intercourse is 99% effective in preventing pregnancy and this is the most effective form of emergency contraception method available (Kulier et al., 2005).

ECs are safe and the side effects are minor which may include irregular bleeding, vomiting, headache, abdominal pain, breast tenderness, dizziness or fatigue. Medicines used for emergency contraception do not harm future fertility and do not cause delay in the return to fertility (RHSC, 2018; WHO, 2018a).

2.3 History and sources of emergency contraceptives in Uganda

A woman's choice to terminate a pregnancy is often related to a number of reasons such as, rape, social stigma, economic struggles or being young and wishing to postpone a child birth. Unwanted pregnancies/births could lead to quitting school, facing rejection from one's family and community, and in some societies even being forced to marry or experiencing serious physical harm (Atuyambe et al., 2005).

Unsafe abortions are illegal in Uganda, and it has been estimated that nearly one third of the maternal deaths among the country's young people are linked to unsafe abortions (Larsson et al., 2015). Reports from studies found out that twenty-three percent of women between the ages fifteen and twenty-four that had been pregnant had at least one abortion, and 35 % of maternal deaths in Uganda were linked to unsafe abortions (Skuster, 2004). Furthermore, as reported in the Ugandan Ministry of Health Annual Health Sector Performance Report of 2017-2018, about 5.3% of all maternal deaths result from abortion complications (Ministry of Health, 2018).

With women lacking access to safe and legal abortions, many of them turn to unsafe abortion practices, such as self-induced abortions (Larsson et al., 2015). The high number of maternal deaths still remains a public health concern with unsafe abortion contributing significantly to this health problem. The wide spread awareness on the use of family planning methods and emergency contraceptive is a strategy to lower the incidence of unintended pregnancies. Use of the available family planning methods and emergency contraceptives over time has led to a reduction in pregnancy related deaths in Uganda (the maternal mortality ratio dropped from 684 per 100,000 live births in 1995 to 343 per 100,000 in 2015) (Guttmacher Institute, 2017).

The Ministry of Health of Uganda approved the use of ECs in 1998 and the method was used three years later as a socially marketed product. However, there was resistance and religious leaders requested the government to stop any efforts of promoting ECs and to deem ECs as illegal under the country's abortion laws. The Solicitor General declared ECs illegal under the laws restricting abortion in 2002 but this restriction ended five years later, as pharmacies in Kampala began to sell ECs again (Advancing Partners and Communities, 2016; RHSC, 2015; Skuster, 2004). In 2012, the Ugandan government approved levonorgestrel as an essential medicine (Ministry of Health, 2016).

Emergency contraceptives are available for free in Uganda through the public health sectors and for sale through the private sector. According to USAID, stock status reports indicated that about 218,799 units of levonorgestrel tablets were available for use through the public sectors (Advancing Partners and Communities, 2016).

Studies have showed that over 50% of active youth in Uganda have used Emergency Contraceptives (Babirye, 2013). This means that the consumption of these medicines is steadily increasing over time which in turn increases the demand. Counterfeiters are more

likely to only invest in high demand products with the aim of making big profits. With the increase in the demand of levonorgestrel tablets, they have become targets for counterfeiters in Uganda. Several alerts have been made by the Uganda National Drug Authority of the suspected counterfeited levonorgestrel tablets on the market (Atukunda, 2019; Kasujja, 2017; WHO, 2015).

However, despite all the efforts that have been made to alert the public of suspected counterfeits of levonorgestrel tablets, there is no published data showing the extent of the problem and its impact on public health in Uganda. Hence, to protect the safety and health of consumers from the emerging threat of poor quality levonorgestrel tablets, there was an urgent need to assess the quality of levonorgestrel tablets sold on the Ugandan market so as to assure users of their safety and therapeutic action.

2.4. Regulation of medicines

A medicine, also called a pharmaceutical drug is defined by the Federal Food Drug and Cosmetic Act (FD&C Act) and FDA as a chemical substance used in the treatment, cure, mitigation, prevention or diagnosis of a disease (FDA, 2021). Because medicines are not the ordinary consumer goods, consumers do not have enough knowledge to make informed choices about when to use a particular medicine how to use them, and do not also have adequate information to weigh potential benefits against the risk of side effects. It is therefore important that governments effectively regulate the manufacture, distribution and use of medical products to protect and promote public health.

Regulation is key to the health and safety of the public in ensuring that products are of the required quality, safety and efficacy. Medicine regulatory frameworks are laid down by legislation to be enforced by government agencies known as National Medicines Regulatory

Authorities (NMRAs). A functioning regulatory system is a prerequisite for ensuring that products are appropriately manufactured, stored, distributed (WHO, 2003).

In Uganda, the National Drug Authority (NDA) has the responsibility to ensure that the manufacture, distribution and use of medicinal products are regulated effectively to protect and promote public health. NDA requires that before any medicinal product is imported in Uganda, it is subjected to marketing authorization prior its availability on the market. The procedure entails an assessment of a dossier, in which the manufacturer or authorized supplier provides evidence of the safety, efficacy, and quality of the product. In addition, the details of the patient information leaflet such as indications, side effects, dosage are assessed (NDA, 2018).

A product that is given marketing authorization is considered registered in Uganda. Once a product is registered, it means that it has met the national and internationally accepted quality, safety and efficacy standards and hence can be imported into the country for human consumption (NDA, 2019a). However, it is important to note that this activity is done once and the products are not subjected to testing, rather the Authority relies on the accuracy of the documentation submitted.

2.5. Poor quality medicines: A public health problem

Poor quality medicines are two major categories, that is, substandard medicines and counterfeit/falsified medicines. Substandard medicines are genuine medicines which do not meet the acceptance criteria set for them by a recognized standard whereas counterfeit medicines are medicines that are deliberately and fraudulently mislabeled with respect to identity and /or source. Counterfeit drugs ‘may not’ contain the active ingredient, or may contain ‘wrong ingredients’ or may even contain toxic compounds. Substandard medicines often contain less than the stated active pharmaceutical ingredient and this is usually due to

poor manufacturing practices, lack of expertise or insufficient infrastructure (Attaran et al., 2012; Caudron et al., 2008; WHO, 1999a, 2017b).

A 2017 WHO study estimated approximately 10.5% failure rate of substandard and falsified medicines in developing countries (WHO, 2017a). Poor quality medicines deter the treatment of chronic and infectious diseases, and any efforts to improve public health by developing new medicines or by changing treatment policies will have no purpose if the medicines taken contain less or incorrect ingredients (Caudron et al., 2008; Newton et al., 2006).

The use of poor-quality medicines leads to therapeutic failure and drug resistance (Keoluangkhot et al., 2008; WHO, 2003). Not only is this an increased cost to health care systems, these products can cause serious illness or even death. In 2008, in Singapore, a total of 150 non-diabetic patients with hypoglycemia were admitted to hospital where four of them died and seven suffered severe brain damage. It was discovered that these people had taken counterfeit copies of drugs claimed to treat erectile dysfunction but which contained a large dose of glyburide, used for treating diabetes (Kao et al., 2009). It was further reported in the Africa's Medical Media Digest that more than 80 children in Nigeria were killed in 2009 by a teething syrup tainted with a chemical normally used in engine coolant and blamed for causing kidney failure (Africa's Medical Media Digest, 2018).

Despite the high prevalence of poor quality medicines, there is little published data enabling estimation of the extent of the problem and the impact on public health. Reports have indicated that only 5–15% of the 191 member states of WHO report cases of counterfeit drugs (Newton et al., 2006). The International Medical Products Anti-Counterfeiting Taskforce (IMPACT) has also further reported that the "off-quoted estimate of 10% of the global supply being counterfeit may be inaccurate, and suggested that many developing

countries of Africa, parts of Asia, and parts of Latin America have areas where >30% of the medicines on sale can be counterfeit " (IMPACT, 2006; Newton et al., 2010).

There are a number of measures medicine regulatory authorities can adopt to reduce the availability of poor quality medicines. These may include but not limited to; implementation of a robust marketing authorization process, inspection and surveillance of medicines manufacturers to ensure that they adhere to Good Manufacturing Practices, routine inspection of importers, wholesalers and dispensers of medicines to ensure that the medicines offered for sale are registered and that medicines distributors adhere to Good Distribution Practices, and the last but most important, monitoring the quality of medicines on the market (Post market surveillance) (Rägo & Santoso, 2008; WHO, 2016).

2.6 Post market surveillance

Post Market Surveillance (PMS) is the practice of monitoring the quality, safety and efficacy of a pharmaceutical drug or medical device after it has been released on the market. Regular sampling and surveying of both the regulated and unregulated supply chains is a way of identifying poor quality medicines on the market. Different methodologies are used to sample the market and these range from random sampling through to target sampling of particular products and outlets (WHO, 2016).

Samples that are collected are subjected to quality tests which should provide a detailed information on the quality of the target medicines. WHO recommends that quality tests should be referenced to a pharmacopoeia monograph and these include but not limited to: sample description, identification, assay, degraded compounds, content uniformity, pH, extractable, sterility (WHO, 2016).

Data collected in post market surveys gives a snapshot of the medicine quality on the market, and hence vital in implementing effective interventions to improve the quality of medicines. The quality of a medicine is dependent on its compliance with the laid down pharmacopoeia specifications. “A pharmacopoeia is an official (legally binding) publication containing recommended quality specifications for the analysis and determination of drug substances, specific dosage forms, excipients and finished drug products”. “Quality specifications are composed of appropriate tests for confirming the identity and purity of medicinal products, ascertaining the amount of active pharmaceutical ingredients and the performance characteristics of medicinal products” (Rägo & Santoso, 2008).

Post market surveillance studies published in various journals point towards a significant proportion of medicines circulating in the healthcare market of the low income countries being of poor quality (Frimpong et al., 2018; Habyalimana et al., 2015; Othman, 2018; Seifu et al., 2019). A recent study done by the University of North Carolina further indicated that most post market studies have tended to focus on only antibiotics and antimalarial. The study analyzed 96 previous studies of falsified and substandard medicines and found that in low-income and middle-income countries, 19 percent of antimalarial and 12 percent of antibiotics are substandard or falsified (New study finds fake, 2018). There is little information that has been published on the quality of ECs circulating the market of low income countries yet several claims of counterfeited contraceptives have been reported on the market (Atukunda, 2019; Kasujja, 2017; WHO, 2015). It is therefore imperative that post market quality studies are conducted to establish the quality of emergency contraceptives circulating the Ugandan market.

2.7 General methods of analysis

2.7.1 Physical Inspection (Visual Inspection)

Visual inspection is the recommended first step in identifying suspicious medicines. Visual inspection is done on the physical characteristics of packaging, outer containers' labeling, closures, sealing, as well as the appearance of a medicine itself by its color, smell and consistency (WHO, 1999b). Different checklists for visual inspection of medicines have been designed by internationally recognized bodies such as United States Pharmacopoeia to help health professionals carry out visual inspections of medicines for signs of counterfeiting. During visual inspection, critical alterations done to a medicinal product such as spoiled tablets, oral suspensions that harden, fluids leaking or containing particles/molds can easily be detected and these are sufficient signs to decide the non-conformity of a medicine without carrying out further laboratory tests tablets (Habyalimana et al., 2015). WHO further recommends that during visual inspection, a comparison with an authentic sample when available can be done and the focus should be on the differences in packaging, labeling information and appearance of the medicine (WHO, 1999b).

2.7.2 Identification test

The purpose of identification tests is to establish the identity of an API in a product. There are three ways in which the true identity of a drug can be determined, namely: determination of physical constants, chromatographic tests and lastly the chemical tests. The physical constants may include but not limited to: infra-red absorption, the melting point, solubility boiling point, specific optical rotation, light absorption, viscosity, UV-Vis absorbance spectra, etc. The chromatographic tests include column chromatography, Gas Chromatography (GC), paper chromatography, thin-layer chromatography including high-HPTLC and pressurized liquid chromatography commonly called HPLC. Chemical tests are

categorized separately under tests for inorganic substances and organic substances (U.S. Department of Justice, Office of Justice programs, 2005).

The ICH Q6A guidelines specifies that "identification tests should discriminate between compounds of closely related structure which are likely to be present and that identification by a single technique is not regarded as specific enough". It is therefore necessary to perform a combination of tests to obtain the required level of specificity (EMEA, 2000). The most widely used identification tests for dosage forms in the known internationally recognized pharmacopoeias are the chromatographic tests specifically the TLC including HPTLC and the HPLC (MHRA, 2019b; USPC, 2019b; WHO, 2019a). The HPTLC was the analytical technique used to identify for levonorgestrel in levonorgestrel tablets.

High performance thin layer chromatography is an improved form of thin layer chromatography. Thin-layer chromatography is a separation technique for separating dissolved chemical substances. The stationary phase is an appropriate material spread in a uniform thin layer on a support (plate) of glass, metal or plastic. Prior to development, solutions of analytes are deposited on the plate and the solutes migrate in a solvent or a suitable mixture of solvents through the thin layer (MHRA, 2019c). The separation relies on the relative affinity of compounds and is based on adsorption, partition and ion-exchange or on combinations of these mechanisms. Evaluation of results is done by comparing the principal spot in the chromatogram obtained with the test solution with that obtained with the reference solution. The comparison is based on the Retardation Factor (RF), color and the size of both the sample and reference standard spots. The RF is the ratio of the distance from the point of application to the center of the spot and the distance travelled by the solvent front from the point of application (MHRA, 2019c; USPC, 2019c).

The HPTLC is comprised of five major components, namely: the automatic TLC sampler, developing chamber, derivatizer, TLC visualizer and the TLC scanner. The cycle starts with a specified volume of sample being automatically injected on to the plate using the Automatic TLC sampler (ATS). The plate is then placed in the Automatic Developing chamber (ADC) for development and separation of samples into their components. The separated components are then visualized under an ultraviolet (UV) light source suitable for observations under short (254 nm) and long (365 nm) wavelength UV light. The TLC visualizer further enables an image-based evaluation of chromatograms for quantitative evaluation. Molecules without chromophores or fluorophores are visualized or made detectable through derivatization using a derivatizer. The observed TLC/HPTLC chromatograms from the TLC visualizer can be densitometrically evaluated using the TLC Scanner (Camag, n.d.).

2.7.3 Assay

An assay is an analytical procedure for qualitatively assessing or quantitatively measuring the presence, amount, or functional activity of a target entity. Assay is a major critical quality attribute of a pharmaceutical dosage form which helps to check the quality, safety and efficacy of the drug substances and drug products (Lakka & Kuppan, 2020). The active pharmaceutical ingredient in medicines must be within the specified amounts in order to furnish pharmacological activity or have a direct effect in restoring, correcting or modifying physiological functions in human beings. Analytical techniques used in quantification of a drug substance include but not limited to, titration, high performance liquid chromatography (HPLC), ultra violet spectrophotometry and gas chromatography (GC). The most commonly used technique for quantitative determination of drug substances is the HPLC (MHRA, 2019b; USPC, 2019b; WHO, 2019a).

2.7.3.1 High Performance Liquid Chromatography (HPLC)

The HPLC is an advanced form of column chromatography used to separate, identify and quantify each component in a solvent mixture. The separation is based on the difference in the distribution of species between 2 non-miscible phases, in which the mobile phase is a liquid which percolates through a stationary phase contained in a column. Separations are achieved by adsorption, mass distribution, ion exchange, size exclusion or stereo chemical interaction (MHRA, 2019c; USPC, 2019c). The Retention Time (RT) measured under particular conditions is an identifying characteristic of a given analyte, and evaluation of results is done by comparing the retention time of the principal spot in the chromatogram obtained with the test solution with that obtained with the reference standard solution. RT is a measure of the time taken for a solute to pass through a chromatography column. It is calculated as the time from injection of a sample to detection of substances in that sample (USPC, 2019c).

Chromatography can be operated in two separation modes, namely, the normal phase and the reversed phase chromatography. In reversed phase chromatography the mobile phase is polar and stationary phase is non-polar where as in normal-phase chromatography, the mobile phase is nonpolar and stationary phase is polar. The choice of stationary phase and mode of separation is dependent on the structure of the molecule which will determine whether the molecule is polar or non-polar (Lakka & Kuppan, 2020; Waters, n.d.).

The reversed phase separation mode and the octadecly (C18) stationary phase were employed in the identification and quantification of levonorgestrel in the samples. The choice of these separation modes was on the basis that levonorgestrel with empirical formula $C_{21}H_{28}O_2$ is a polar molecule. Reversed phase chromatography uses a hydrophobic stationary phase (C18) which is non polar in nature. Since the stationary phase is hydrophobic, molecules with

hydrophobic properties in the polar mobile phase (Acetonitrile: Water) will have a strong affinity for the stationary phase and hence adsorb to the column packing while the hydrophilic molecules (levonorgestrel being polar in nature) will pass through the column and be eluted first. In summary, a mobile phase and particle stationary phase with appropriately opposite polarities has to be selected to ensure that as the sample analytes move through the column, the principle *like attracts like* is respected to determine which analytes slow down and which proceed at a faster speed (Waters, n.d.).

The detection of compounds present in the eluent coming from the HPLC column is done by the detector. Detectors determine the identity and concentration of eluting compounds in the mobile phase. The choice of detector is critical to guarantee that all the components are detected. One of the widely used detectors in HPLC is the UV detector including diode array detector which is capable of monitoring several wavelengths concurrently (Choudhary, 2016).

The UV detector was the selected choice of detector to be used in the identification and quantification of levonorgestrel in the samples. Levonorgestrel is an aromatic compound with chromophores which will absorb light in the UV–vis region, and this comes as an advantage in quantifying and analyzing the molecules and its associated impurities. The HPLC is comprised of four main components and a data acquisition system, namely: pumping system, injector, chromatographic column and a detector (MHRA, 2019c; USPC, 2019c).

2.7.4 Uniformity of content

Uniformity of content assesses the degree of uniformity in the amount of the drug substance among dosage units. Dosage units are defined as dosage forms containing a single dose or a part of a dose of drug substance in each unit (USPC, 2019d). Ten tablets are selected randomly and a suitable analytical method is used to assay the individual content of the active

ingredient in each capsule or tablet. According to the International Pharmacopoeia, this test should be performed where the declared quantity of the active ingredient in the various dosage forms (tablets, capsules, oral powders, single dose oral suspensions) is 5mg or less (WHO, 2019c). Levonorgestrel tablets contain 0.75mg or 1.5mg of the active ingredient, and as per the International Pharmacopoeia requirement above, it was prudent that this test be performed.

2.7.5 Dissolution

Dissolution testing is performed to determine the bioavailability and therapeutic effectiveness of drug. Since dissolution predicts the in-vitro performance of a drug, it provides crucial information that is routinely used for quality control and quality assurance purposes in the pharmaceutical industry. The test involves quantifying the active ingredient(s) released from a solid oral dosage form under specified conditions using a known volume of dissolution medium within a predetermined length of time (MHRA, 2019d). It is critical for procedures to be standardized to properly evaluate the dissolution of drug products. On that note, if a pharmacopoeial method is available for a product, then it is recommended that this method be adopted and used without any changes (Anand et al., 2011).

Dissolution is run in three stages and a sample can only be declared non-compliant if it fails at all the three stages. In the first stage (S1), six tablets are run for the specified time period, sampled and analyzed for the dissolved amount of active ingredient (Q). The quantity, *Q*, is the specified amount of dissolved active ingredient expressed as a percentage of the labelled content. The requirement for S1 is that the dissolved amount of each tablet should not be less that Q + 5%. If any of the tablets is found below this limit, then the analysis proceeds to stage 2 (S2) (MHRA, 2019d).

An additional six tablets is tested in S2 and the requirement is that the average of 12 tablets should not be less than Q and no tablet should be less than Q-15%. If any unit is stll found below the S2 criteria, then the analysis proceeds to stage 3 (S3). In S3 stage, twelve additional units are analyzed for dissolved active content and evaluated against the criteria for S3 stage. At this stage, the average of all the 24 tablets should not be less than Q, only two tablets should be less than Q-15% and no tablet should be less than Q-25% (MHRA, 2019d).

The choice of apparatus to be used is solely dependent on the type of product. Paddles and baskets are the most commonly used apparatus for oral dosage forms (Boda, n.d.). Apparatus 2 (paddle) was the choice of apparatus used in the evaluation of the dissolution of levonorgestrel tablets and a standardized method from the International pharmacopeia was adopted as the method of analysis.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The study was done in the four main regions of Uganda, that is, Central (Kampala and Wakiso), Western (Mbarara and Kasese), Eastern (Jinja, Iganga, Busia and Mbale) and Northern (Arua and Gulu). Figure 3.1 below displays the Uganda map with study areas indicated**.**

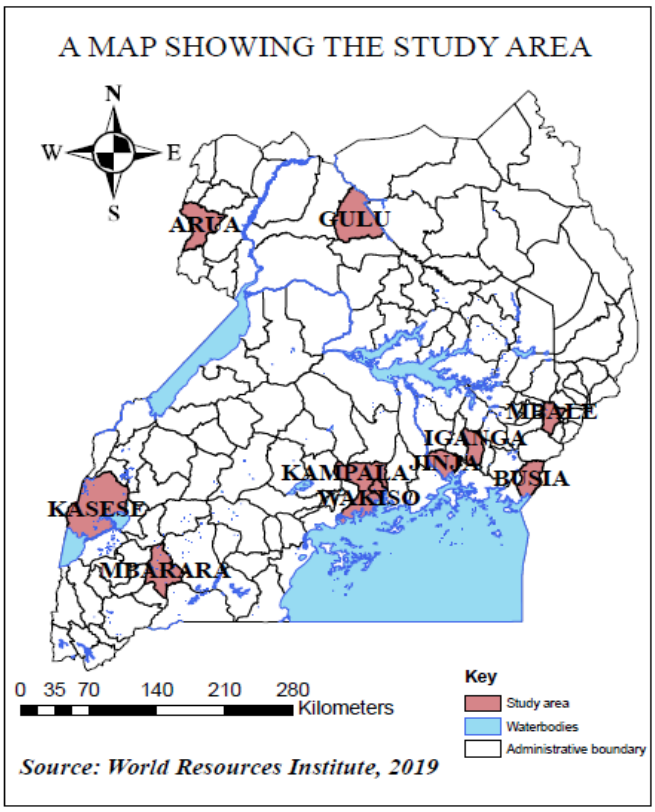


Figure 3.1: A location map showing the study area.

3.2 Sample collection

Samples were collected from licensed pharmacies located within the four regions of Uganda. The simple random technique was employed when collecting samples from the randomly selected pharmacies. This technique ensured that samples were chosen randomly, and hence giving reliable estimates of the quality of medicines on the market (WHO, 2016).

The total number of samples collected was based on availability, and everything available on the market was collected at the time of sampling. At least four samples were collected from each region from the different pharmacies. A total of 23 samples (Table 3.1) each comprising of 100 tablets were purchased between September and November 2019. In order to eliminate bias, all levonorgestrel tablets stocked in each sampled pharmacy were purchased. The regions from which the samples were picked was not considered for the study, since the

samples were picked from licensed pharmacies that met the Good Distribution Practices for medicinal products. This meant that the region had no impact on the results obtained.

Table 3.1: Distribution of samples collected per region.

Region	**Samples collected per region**	**Tablets per region**
Central	8	800
Northern	4	400
Eastern	6	600
Western	5	500
Total	**23**	**2300**

From the 23 samples collected, 9 of them were of 0.75 mg levonorgestrel tablets and the remaining 14 were of 1.5 mg levonorgestrel tablets. Seven brands of levonorgestrel tablets (Table 3.2), were available on the market at the time of sampling and all these were registered by NDA.

Table 3.2: Distribution of samples per brand

Brand Name	**Quantity of samples collected per brand**
Back-up	3
Unosure 72	7
Easy pill	1
Lydia	2
1-Pill	1
P2	5
Postinor	4

A sample was defined as that number of tablets bearing the same name, content of active pharmaceutical ingredients, dosage form, batch number and manufacturer, and collected at a specific pharmacy (WHO, 2016). Collection of samples was done through the covert method where covert shoppers were engaged to pose as customers.

Sample collection forms were filled to capture the details of the sample as indicated in Appendix II. Prior to analysis, the samples were coded as X-1, where X represented a one-

digit incremental letter of the alphabet for a particular brand and 1 being the first sample of that brand. Samples codes were randomly assigned as indicated in table 3.3. All samples coded were within the expiry limits throughout the study.

Table 3.3: Sample codes assigned per brand

Sample code	Brand Name	API content (mg)	Expiry date
A-1	Back-up	1.5	Mar-20
A-2	Back-up	1.5	Aug-20
A-3	Back-up	1.5	Aug-20
B-1	Unosure 72	1.5	Feb-21
B-2	Unosure 72	1.5	Feb-21
B-3	Unosure 72	1.5	Feb-21
B-4	Unosure 72	1.5	Feb-21
B-5	Unosure 72	1.5	Feb-21
B-6	Unosure 72	1.5	Feb-21
B-7	Unosure 72	1.5	Aug-22
C	Easy pill	1.5	Apr-22
D-1	Lydia postpil	1.5	Feb-21
D-2	Lydia	1.5	Feb-21
E	1-Pill	1.5	Jan-21
F-1	P2	0.75	Mar-20
F-2	P2	0.75	Mar-20
F-3	P2	0.75	Mar-20
F-4	P2	0.75	Mar-20
F-5	P2	0.75	Mar-20
G-1	Postinor	0.75	Aug-23
G-2	Postinor	0.75	Jan-22
G-3	Postinor	0.75	Mar-22
G-4	Postinor	0.75	Aug-23

3.3 Validity of results

The methods of analysis were verified to ensure that they could be used for their intended purpose, under the actual conditions of use. The parameters performed included; specificity, precision, linearity and system suitability as indicated. Appendix III &IV gives a summary of the validation parameters performed for the Assay and Dissolution test. The equipment used were qualified, maintained and within their calibration due dates. The data generated was reviewed and calculations were done using validated excel calculation sheet. The standard

preparations were done in duplicate and five replicate injections of each were run on the HPLC. To ensure that the equipment was suitable prior to running the analysis, system suitability parameters were assessed for each test performed. Table 3.3 shows the system suitability parameters assessed for one of the Assay tests run on HPLC.

Table 3.4: System suitability results for the assay chromatographic method

System suitability	Value	Limit	Compliance
% RSD for peak area response of levonorgestrel for 5 replicate injections	0.2	NMT 2%	Compliant
Symmetry factor	0.9	NMT 1.6	Compliant
column efficiency	7795	≥ 5000	Compliant

3.4 Methods of Analysis

The methods of analysis (Identification, Uniformity of content, dissolution and Assay) were adopted from the monograph of levonorgestrel tablets of the International pharmacopoeia (2019). The International Pharmacopoeial methods were chosen since they cut across all countries and serve all national and regional regulatory authorities in the United Nations system.

3.4.1 Physical Inspection (Visual Inspection)

Samples were visually inspected for physical characteristics of shape, color, breaks, cracks and splits, packaging and the labeling information using the International Pharmaceutical Federation checklist for visual inspection of medicines. A copy of the checklist used to visually inspect the samples is given in Appendix V. Furthermore, a comparison with an authentic sample received by NDA at the time of registration was done and the focus was on the differences in the packaging and labeling information.

3.4.2 Identification and assay for levonorgestrel

3.4.2.1 Identification for levonorgestrel

For identification of levonorgestrel, Thin Layer Chromatography was performed on 10 cm × 20 cm silica gel 60 F254 HPTLC plates (Merck, Darmstadt, Germany) using a mobile phase of cyclohexane (VWR, Leuven, Belgium): acetone (Sigma-Aldrich, Stenheim, German) in the ratio of 7:3 %v/v. Ten microliter of levonorgestrel standard 99.8 % of purity (USP, Rockville, USA) and sample solutions were applied to the plates by means of a TLC Sampler 4 (Camag, Muttenz, Switzerland). Plates were then developed to a distance of 15cm, in linear ascending mode, in a Camag automatic development chamber. The separated spots were examined with a Camag TLC visualizer controlled by vision cuts software (version 1.4.6) at a wavelength of 254 nm. Densitometric scanning was further performed with a Camag TLC Scanner 4 to obtain UV spectra of the prominent spots in the sample and reference standard solution.

Samples were prepared by dissolving a quantity of powdered tablets equivalent to 1.5 mg of Levonorgestrel in 5 mL of acetonitrile (VWR, Leuven, Belgium), followed by filtration. Standard solutions of 0.3 mg per ml of Levonorgestrel RS were prepared in acetonitrile (VWR, Leuven, Belgium). The specification was that the principal spot obtained with the sample solution should correspond in position (retardation factor (RF)), appearance and intensity to that obtained with standard solution. The RF and RF sample error for samples that contained levonorgestrel was calculated using the formula below;

$$\mathrm{RF} = \frac{\mathrm{Ds}}{\mathrm{Dm}}$$

$$\text{RF sample error} = \frac{\text{RF of sample x } 100\%}{\text{RF of reference standard}}$$

Migration distance of the substance

Where;

Ds =

Dm = Migration distance of the mobile phase

As outlined in the Global Pharma Health Fund (GPHF) min-Lab manual second edition, a percentage RF sample error of less than or equal to ± 5% indicated a pass, a percentage RF sample error of more or equal to ± 10% indicated a fail and a percentage RF sample error between 5% and 10% indicated a doubtful result.

The HPLC technique was further used to confirm for the presence or absence of levonorgestrel in the samples. The details of analysis were as described in the assay method

3.4.2.2 Assay (Quantification of levonorgestrel)

The HPLC technique was used for quantification (assay) of levonorgestrel in the samples. Assay was conducted using an Agilent 1260 Infinity series high performance liquid chromatograph (Agilent Technologies, Deutschland, Germany) supported by Open-Lab software reversion C.01.07 SR1 (113). The liquid chromatograph was equipped with a G13298 Agilent 1260 autosampler for introduction of 25µL of sample and standard solutions into the flowing mobile phase pumped at a flow rate of 1.3 mL /minute with a G1311C Agilent 1260 quant pump. Chromatography was performed on 150 x 4.6 mm, particle size 5 µm Luna C18 stationary phase (Phenomenex, Madrid, Spain) using a mobile phase of acetonitrile (VWR, Leuven, Belgium): HPLC water in the ratio of 50:50 %v/v. The temperature of the stationary phase was controlled using a G1316A Agilent 1260 TCC column compartment. Detection of the separated analytes in the sample and standard solutions was done at a wavelength of 220nm using a G1314B Agilent 1260 Infinity variable wavelength detector with a wavelength range of 190-600 nm.

Test samples were prepared by transferring one weighed powdered tablet to a 50 mL volumetric flask and mobile phase added up to the mark. The suspension was sonicated for 45 minutes with a fast-clean ultrasonic cleaner (Life-care Pvt., Mumbai, India), shaken for 15 minutes with an orbit 1000 orbital shaker (Labnet, Woodbridge, United States) and centrifuged at 5000 RPM with a CenceL600 orbital shaker (Hunan Xiangyi Ltd., Hunan, China) for 10 minutes. The supernatant was further diluted to a concentration of 6µg/ml with mobile phase and mixed. This procedure was repeated for 9 more tablets. Standard solutions of 6µg/ml of Levonorgestrel reference standard (USP, Rockville, USA) were prepared in mobile phase. The content of Levonorgestrel in each tablet was calculated using the formula as stated in the uniformity of content test and the assay value was obtained by averaging the 10 individual results obtained using the formula below:

$$\% \text{ Assay} = \frac{(\% \text{ content } 1 + \% \text{ content } 2 \ldots\ldots\ldots + \% \text{ content } 10)}{10}$$

The acceptance criteria for the assay test required that the sample should contain not less than 90.0% and not more than 110.0% of the amount of levonorgestrel claimed to be present on the package label of the medicine.

3.4.3 Uniformity of content of levonorgestrel tablets

The test for uniformity of content was conducted using an Agilent 1260 Infinity series high performance liquid chromatograph with the same equipment details as indicated in the assay test above.

Test samples were prepared by transferring one weighed powdered tablet to a 50 mL volumetric flask and mobile phase added up to the mark. The suspension was sonicated for 45 minutes with a fast-clean ultrasonic cleaner (Life-care Pvt., Mumbai, India), shaken for 15 minutes with an orbit 1000 orbital shaker (Labnet, Woodbridge, United States) and

centrifuged at 5000 RPM with a CenceL600 orbital shaker (Hunan Xiangyi Ltd., Hunan, China) for 10 minutes. The supernatant was further diluted to a concentration of 6µg/ml with mobile phase and mixed. This procedure was repeated for 9 more tablets. Standard solutions of 6µg/ml of Levonorgestrel reference standard (USP, Rockville, USA) were prepared in mobile phase.

The acceptance criteria for the uniformity of content test required that each single tablet should contain within ±15%of the average amount of the active ingredient. The content of Levonorgestrel in each tablet was calculated using the declared content of the chemical reference standard using the formula below:

$$\%\ \text{Content} = \frac{\text{Rsp} \times \text{Wst} \times 2 \times 50 \times 10 \times \text{Avwt.} \times \text{Pst} \times 100}{\text{Rst} \times 100 \times 50 \times 2 \times \text{Wsp} \times \text{LC}}$$

Where;

Wst = Weight of standard in standard solution (mg)

Pst = Purity of standard, mg/mg

Rsp = Peak area for sample solution

Avwt.= Average weight, g

Rst = Average peak area for standard solution

Wsp = Weight of sample taken (g)

LC = Labeled amount of levonorgestrel (mg/tablet)

3.4.4 Dissolution of levonorgestrel tablets

Drug release from tablets during a specific period of time was determined by dissolution testing. The dissolution test was performed using USP dissolution type II apparatus (Erweka, Langen, Germany; Model: DT 1614) filled with 500 mL of 0.1% solution of sodium dodecyl sulfate in 0.1M HCl dissolution medium, maintained at 37 ± 0.5^{o}C and operated at 75 rpm

(WHO, 2019a). A sample aliquot of 20 mL was withdrawn from each of the six bowls after 30 minutes and filtered through millex 0.45µm PTFE syringe filters (Merck KGaA, Darmstadt, Germany). The obtained sample was diluted to a concentration of 1.5µg/mL. Standard solutions of 1.5µg/ml of levonorgestrel reference standard (USP, Rockville, USA) were prepared. The amount of levonorgestrel dissolved was quantified using the Agilent 1260 Infinity series high performance liquid chromatograph (Agilent Technologies, Deutschland, Germany) under the same HPLC conditions utilized for the assay test.

The dissolution testing was performed in a series of stages. If the initial sample analysis, known as S1 or stage 1 testing failed to meet the acceptable value, then additional testing known as stage 2 (S2) and stage 3 (S3) testing was required. S3 testing was performed only if S2 testing still failed the acceptable parameter. For samples that deviated from the acceptable Q values at S3, an Out of Specification (OOS) investigation was initiated.

The acceptance criteria for the dissolution test required that the amount of levonorgestrel in solution for each tablet should not be less than 80% for stage 1; stage 2 and stage 3 required that the average result for the additional tablets tested respectively should not be less than 75%. The percentage of dissolution of levonorgestrel in each tablet was calculated using the formula below:

$$\%\ \text{Dissolution} = \frac{\text{Rsp} \times \text{Wst} \times 1 \times 100 \times 10 \times 500 \times \text{Pst} \times 100}{\text{Rst} \times 100 \times 100 \times 5 \times \text{LC}}$$

Where;

Wst = Weight of standard in standard solution (mg)

Pst = Purity of standard, mg/mg

Rsp = Peak area for sample solution

Rst = Average peak area for standard solution

LC = Labeled amount of levonorgestrel (mg/tablet)

3.5 Data Analysis

The analytical data obtained was analyzed using Microsoft excel 2016 and descriptive statistics (mean and standard deviation) were used to summarize the data. One-way analysis of variance (ANOVA) and the student-t test using Microsoft excel 2016 was employed to determine whether there were any significant differences in the mean drug content amongst the different brands of levonorgestrel tablets.

The Student-t test was used to determine if there were any variances in the mean drug content between two groups of samples, whereas one-way ANOVA was used where the groups of samples were more than two. The use of one-way ANOVA instead of two-way ANOVA was based on the fact that only a single independent variable was being compared in this study.

The choice of these statistical tools used was based on the fact that each sample was drawn independently of the other samples, data was normally distributed and there was equality of variance in standard deviation for the data sets to be compared.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Physical Inspection (Visual Inspection)

Brands A, B, C, D, E and F passed all the attributes of visual inspection as indicated in Table 4.1 and 4.2. Assessment of the physical characteristics of the tablets for samples of Brand G showed a 50% failure for the breaks, cracks and splits attribute as indicated in table 4.1 below. The tablets of samples G-2 and G-3 were observed to have breaks and cracks contrary to the requirements outlined in the checklist for physical inspection of samples (see appendix IV). Therefore, sample G2 and G3 did not meet the requirements for the physical characteristics of the tablets.

Table 4.1: Physical characteristics of the tablets

Sample code	Uniformity of shape	Uniformity of color	Breaks, cracks and splits	Surface spots
A-1	Yes	Yes	No	No
A-2	Yes	Yes	No	No
A-3	Yes	Yes	No	No
B-1	Yes	Yes	No	No
B-2	Yes	Yes	No	No
B-3	Yes	Yes	No	No
B-4	Yes	Yes	No	No
B-5	Yes	Yes	No	No
B-6	Yes	Yes	No	No
B-7	Yes	Yes	No	No
C	Yes	Yes	No	No
D-1	Yes	Yes	No	No
D-2	Yes	Yes	No	No
E	Yes	Yes	No	No
F-1	Yes	Yes	No	No
F-2	Yes	Yes	No	No
F-3	Yes	Yes	No	No
F-4	Yes	Yes	No	No
F-5	Yes	Yes	No	No
G-1	Yes	Yes	No	No
G-2	Yes	Yes	**Yes[a]**	No
G-3	Yes	Yes	**Yes[a]**	No
G-4	Yes	Yes	No	No

[a]Failed the visual inspection test for physical characteristics of tablets.

Brands A, B, C, D, E and F passed all the attributes of visual inspection as indicated in Table 4.1 and 4.2. Assessment of the physical characteristics of the tablets for samples of Brand G showed a 50% failure for the breaks, cracks and splits attribute as indicated in table 4.1 below. The tablets of samples G-2 and G-3 were observed to have breaks and cracks contrary to the requirements outlined in the checklist for physical inspection of samples (see appendix IV). Therefore, sample G2 and G3 did not meet the requirements for the physical characteristics of the tablets.

Furthermore, as indicated in Table 4.2, brand G showed a 75% failure rate when assessed for the packaging and labeling information requirements for the attributes of the presence of batch/lot number, manufacturing and expiring date. These samples were noted not to have in prints of expiry date, manufacturing date and batch number on their primary packaging contrary to the authentic sample as shown in appendix VII. However,100% pass rate was observed for brand G when assessed for the container closure, dosage statement, medicine strength, storage information and leaflet (package insert) attributes of the packaging and labeling information as shown in table 4.2.

Table 4.2: Physical characteristics of the Packaging and Labeling Information.

Sample code	Container and closure appropriate	Dosage statement	Medicine strength	Batch/ Lot number	Mfg. and expiry date	Storage information	Leaflet insert
A-1	Yes	Yes	Yes	Yes	Yes	Yes	Yes
A-2	Yes	Yes	Yes	Yes	Yes	Yes	Yes
A-3	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B-1	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B-2	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B-3	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B-4	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B-5	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B-6	Yes	Yes	Yes	Yes	Yes	Yes	Yes
B-7	Yes	Yes	Yes	Yes	Yes	Yes	Yes
C	Yes	Yes	Yes	Yes	Yes	Yes	Yes
D-1	Yes	Yes	Yes	Yes	Yes	Yes	Yes
D-2	Yes	Yes	Yes	Yes	Yes	Yes	Yes
E	Yes	Yes	Yes	Yes	Yes	Yes	Yes
F-1	Yes	Yes	Yes	Yes	Yes	Yes	Yes
F-2	Yes	Yes	Yes	Yes	Yes	Yes	Yes
F-3	Yes	Yes	Yes	Yes	Yes	Yes	Yes
F-4	Yes	Yes	Yes	Yes	Yes	Yes	Yes
F-5	Yes	Yes	Yes	Yes	Yes	Yes	Yes
G-1	Yes	Yes	Yes	Yes	Yes	Yes	Yes
G-2	Yes	Yes	Yes	**No**[b]	**No**[b]	Yes	Yes
G-3	Yes	Yes	Yes	**No**[b]	**No**[b]	Yes	Yes
G-4	Yes	Yes	Yes	**No**[b]	**No**[b]	Yes	Yes

[b]Failed the visual inspection test for the Packaging and Labeling information

It is a requirement that all medicinal products shall be identified by labeling, as required by the national legislation, bearing information such as, the product name, list and amount of API present, batch number assigned by the manufacturer, the indications, contra-indications, warnings, storage information, manufacturing date, expiration date, manufacturer name and address and leaflet insert (WHO, 2002). In this study, problems were observed with the labelling of 3 samples of brand G, where these were noted not to bear the manufacturing date, expiration date and batch number.

Additionally, tablets should be checked for uniformity of shape, uniformity of color, signs of moisture, absence of breaks, cracks, splits, or any other adulteration to ensure that they are in compliance with the national legislations on medical products (WHO, 1999b). Samples of brand G (2 samples) were observed to have issues with the physical characteristics of the tablets, where they were noted to have breaks contrary to the requirements of the physical appearance of tablets.

The absence of the manufacturing date, expiration date and batch number on the primary package of samples G2, G3 and G4; and presence of breaks for tablets of samples G2 and G3 indicated the presence of suspected counterfeits of samples of brand G. Similar to the findings in this study, a case study on antimalarial medicines in Rwanda revealed the presence of suspected counterfeits and/or substandard antimalarial when the product's physical appearance presented evident deviations to the quality standards. This study, revealed the presence of discolored film coated quinine tablets, different batch numbers of artesunate powder for injection on the vials and outer packaging boxes and last but not least fake packaging of artemether-lumefantrine blister tablets (Habyalimana et al., 2015). Another study in Cambodia on anthelmintic revealed that two of the suspect samples contained two types of tablets in each container, one had a differently colored label on the container than that of an authentic sample and some were noted to have loose packs (Khan et al., 2010).

The studies above confirm the usefulness of visual inspection at first line in decision making on drug quality. Visual inspection is a simple and inexpensive technology which can be adopted by both patients and health professionals to rapidly detect suspect poor quality medicines on market (Mohamed et al., 2020). In a pharmaceutical world where there are numerous quality standards, complex distribution networks and weaknesses of the pharmaceutical systems (Caudron et al., 2008), visual inspection can provide guidance on

how to timely recall suspicious medicines, officially cancel marketing authorizations and protect public health. Through visual inspection, the World Health Organization had issued medical product alerts citing the discrepancies observed in the physical appearance of the suspected medicines (WHO, 2013, 2015).

4.2 Identification and assay for levonorgestrel

4.2.1 Identification for levonorgestrel

The High Performance Thin Layer chromatograph was used to confirm for the presence of levonorgestrel in the samples. Levonorgestrel in all the samples collected was confirmed by comparing the retardation factor, (RF), appearance, ultra-violet spectrum and intensity of the sample spots to that of the levonorgestrel standard. While brands A, B, C, D, E and F passed all the attributes of the identity test, brand G showed a 75% failure rate for this test. Three out of four of the samples found on the market did not show any presence of levonorgestrel, and all these belonged to brand G. These three samples did not show any spot in the chromatoplate (Figure 4.1 and 4.2). Evaluation of the different travel distances (retardation factor) of the 20 samples that showed spots in the chromatoplate indicated the presence of levonorgestrel. Samples A-2 and A-3 had the highest percentage retardation factor sample error of 3.4% whereas sample B-5, D-1, E, F-2 and G-1 had the lowest percentage retardation factor sample error of 0%. Table 4.3 and 4.4 shows the percentage retardation factors for the levonorgestrel samples run on the same day but on different plates; one plate could take a maximum of 15 samples hence two separate plates were used in this analysis to cater for all the samples.

Table 4.3: Percentage Retardation factors for the levonorgestrel samples run on plate 1.

Sample code	**Retardation factor (RF)**	**% RF sample error** (0.298± 5%)
Standard	0.298	**N/A**
A1	0.306	2.7
A3	0.308	3.4
A2	0.308	3.4
B1	0.300	0.7
B2	0.306	2.7
B3	0.306	2.7
B4	0.304	2.0
C	0.306	2.7
F1	0.304	2.0
G2	**No spot observed**	
G3	**No spot observed**	

Table 4.4: Percentage Retardation factors for the levonorgestrel samples run on plate 2.

Sample code	**Retardation factor (RF)**	**% RF sample error** (0.273± 5%)
Standard	0.273	**N/A**
B5	0.273	0.0
B6	0.277	1.5
B7	0.277	1.5
D1	0.273	0.0
D2	0.275	0.7
E	0.273	0.0
F2	0.273	0.0
F3	0.275	0.7
F4	0.279	2.2
F5	0.277	1.5
G1	0.273	0.0
G4	**No spot observed**	

N/A; Not applicable for the standard

Samples that contained levonorgestrel showed spots that appeared as dark bands against the fluorescent background at 254 nm, while those that did not contain levonorgestrel did not show

any dark spot against the fluorescent background as illustrated in Figure 4.1 and Figure 4.2 below:

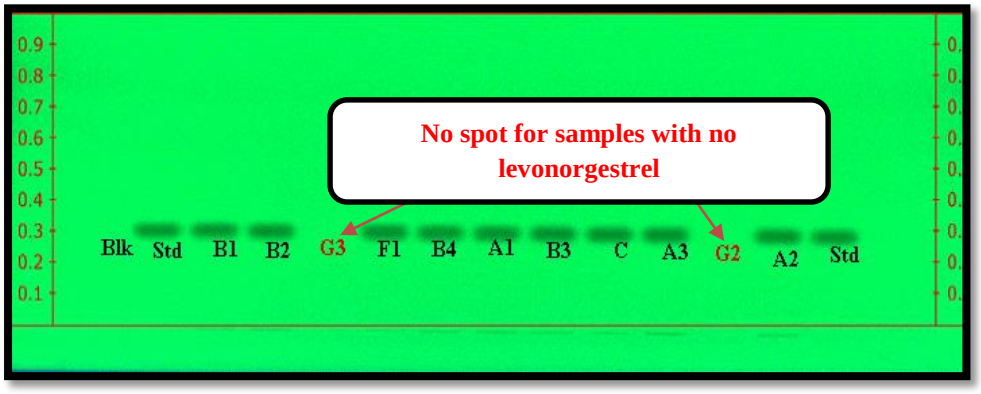


Figure 4.1: HPTLC chromatoplate showing reference standards and sample chromatographic spots on illumination at 254 nm.

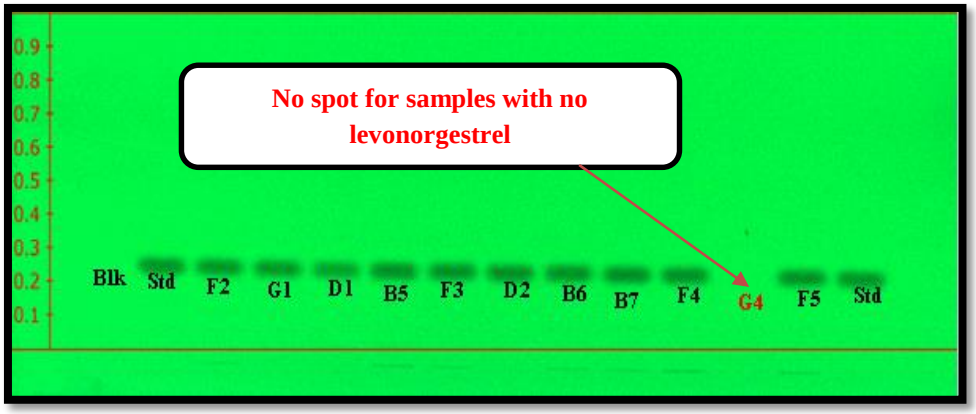


Figure 4.2: HPTLC chromatoplate showing reference standards and sample chromatographic spots on illumination at 254 nm.

Densitometric scanning of the obtained chromatographic spots was carried out in the range of 190 nm to 400 nm and this resulted into ultra-violet (UV) spectra that were inspected for their shape and absorption maxima at about 240 nm for levonorgestrel (Rizk et al., 2017) .The UV spectra for the samples that contained levonorgestrel were comparable in shape and UV maxima to that of the levonorgestrel reference standard; samples that did not contain levonorgestrel showed no absorption maxima. The spectra obtained further confirmed that the chromatographic spots observed on the chromatoplate for the samples tested as shown in figure 4.1 and 4.2 above were indeed for levonorgestrel API. Figure 4.3 and 4.4 below show a

comparison of a sample with and without levonorgestrel with that of the levonorgestrel reference standard.

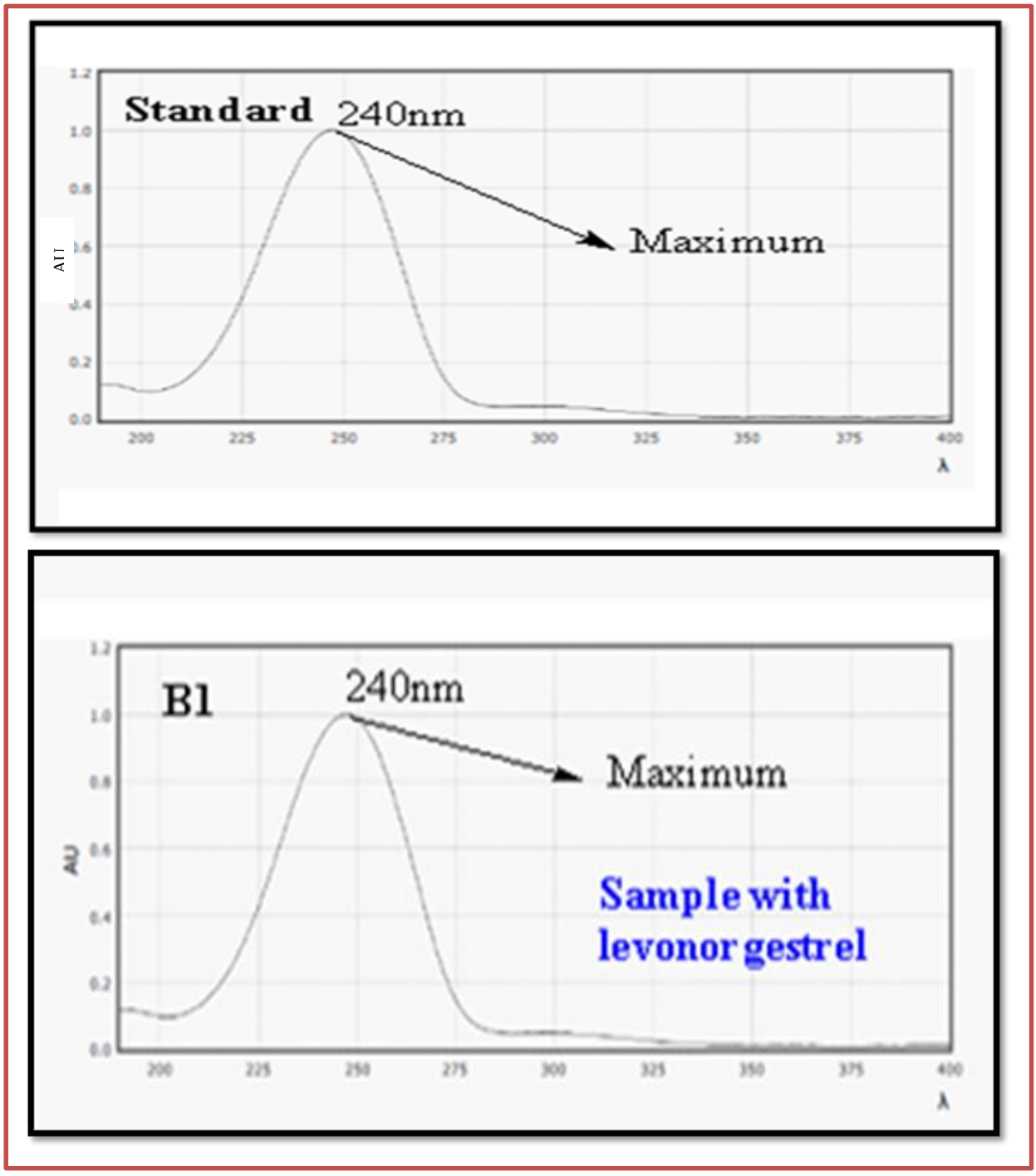


Figure 4.3: Comparison of the HTPLC ***UV spectra*** for sample B1 with that of the levonorgestrel reference standard.

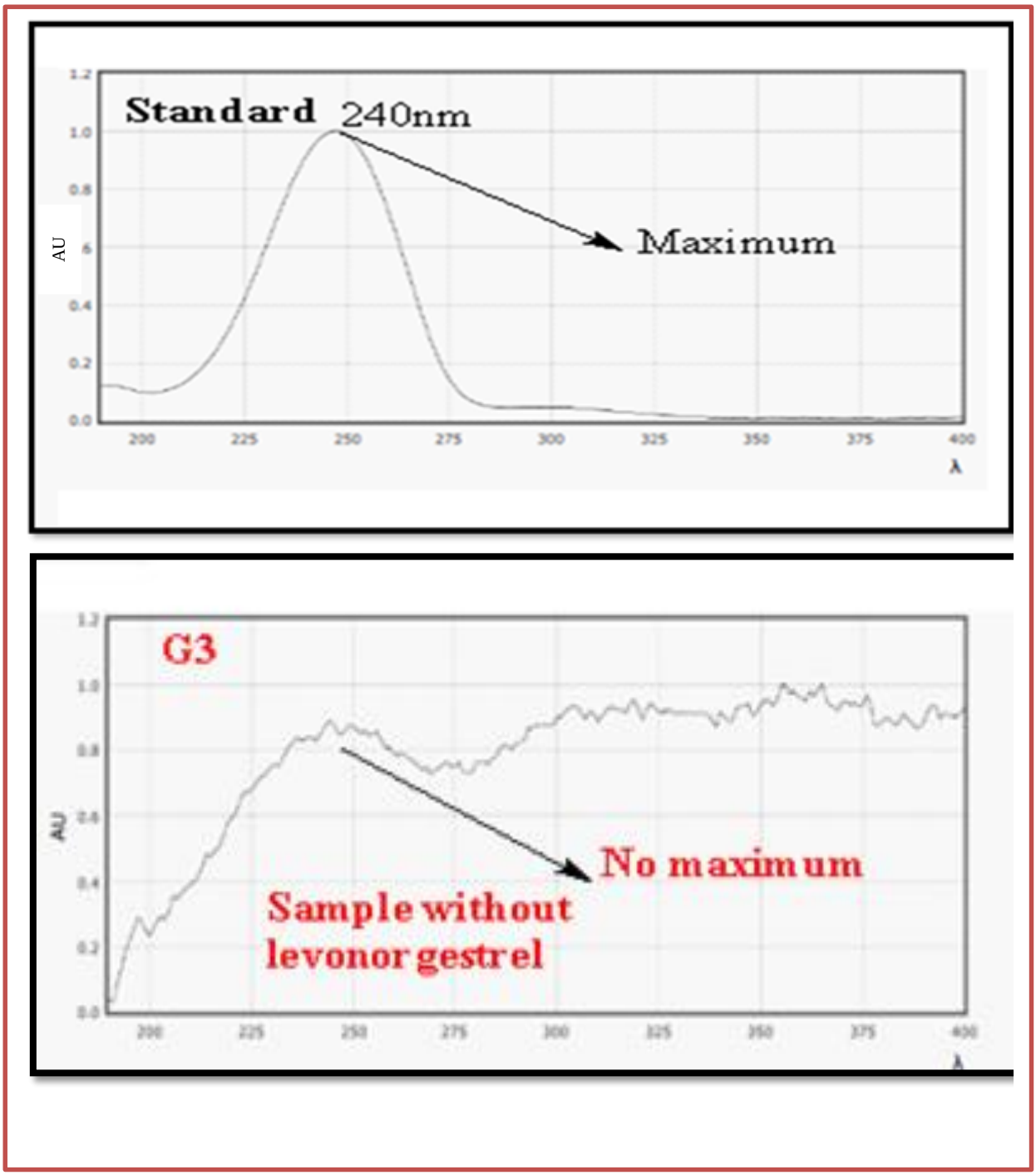


Figure 4.4: Comparison of the HPTLC ***UV spectra*** for sample G3 with that of the levonorgestrel reference standard.

To further confirm that indeed the three samples tested with the HPTLC analytical technique did not contain levonorgestrel, they were run on the HPLC using the assay method of analysis. The test required that to confirm the active ingredient was present; the retention time of the levonorgestrel peak in the chromatogram obtained with the sample solution

should be similar to that in the chromatogram obtained with the levonorgestrel reference standard solution.

The chromatograms of samples that contained levonorgestrel showed principal peaks (levonorgestrel) that were similar in retention time to that of the levonorgestrel reference standard (Figure 4.5), whereas the chromatograms of samples that did not contain levonorgestrel gave just a straight line without any principal peak (Figure 4.6). The chromatograms of these three samples were similar to those of the blank (diluent used to extract the levonorgestrel from the sample matrix).

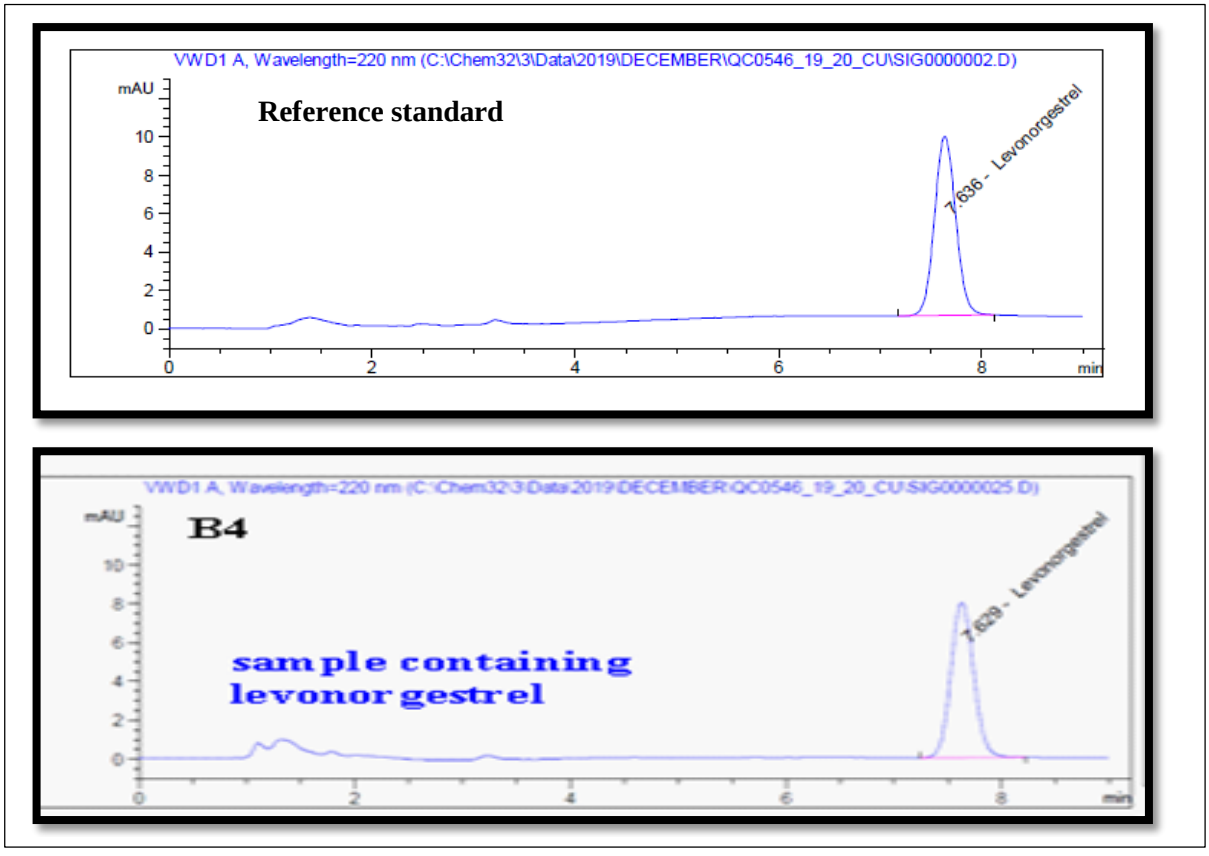


Figure 4.5: Comparison of the HPLC ***chromatogram*** for sample B4 with that of the levonorgestrel reference standard.

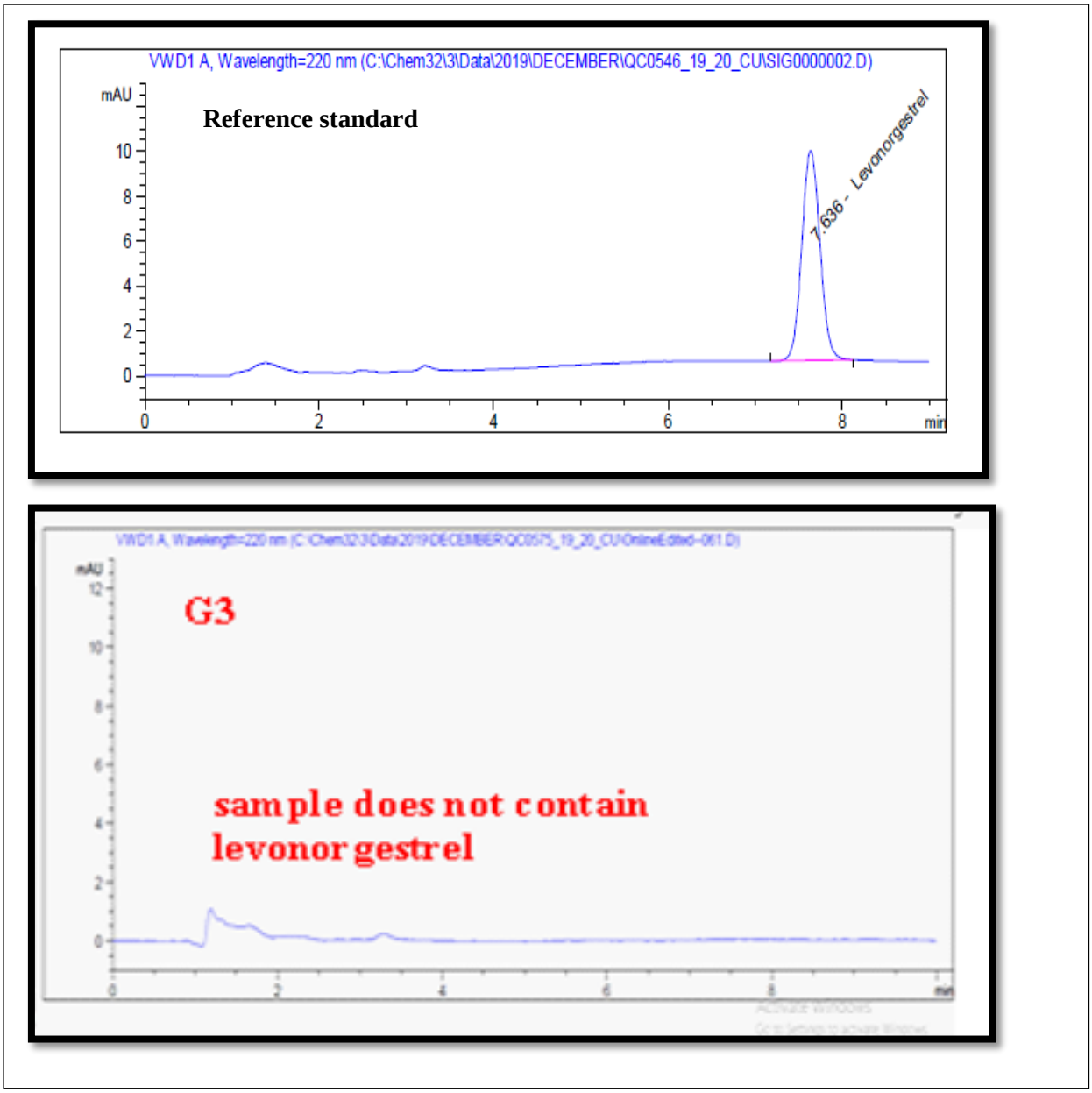


Figure 4.6: Comparison of the HPLC ***chromatogram*** for sample G3 with that of the levonorgestrel reference standard.

In summary, the samples initially suspected to be counterfeits in the visual inspection test were indeed confirmed to show no presence of levonorgestrel when subjected to the test of identification for levonorgestrel using the HPTLC and HPLC technique. Hence, the postinor brand of levonorgestrel tablets was confirmed to have counterfeited samples.

Therapeutic effect of any medicine can only be achieved if the right active pharmaceutical ingredient is present. Active pharmaceutical ingredients are intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment or prevention of disease. Samples G-2, G-3 and G-4 did not show any presence of the active ingredient (levonorgestrel) and hence would not be expected to have any therapeutic effect. Medicines that are deliberately and fraudulently produced and/or mislabeled with respect to identity and/or source to make it appear to be a genuine product are termed as counterfeits drugs (Attaran et al., 2012; Caudron et al., 2008). Counterfeit drugs are a global health concern because of the risks it poses to the public. Not only will consumers pay for medicines that have little or no medical value, these medicines will also lead to unresolved health problems, and death (Ozawa et al., 2018; WHO, 2017a).

A number of studies have reported the dangers inherent in counterfeit drugs. The New England Journal of Medicine reported an outbreak of hypoglycaemia in patients using counterfeit sexual enhancement drugs. Of the 150 non-diabetic patients admitted to hospitals in Singapore, seven of them went into permanent coma and four died. The sexual enhancement herbal drug they had taken was contaminated with glyburide, a powerful drug used for the treatment of diabetes (Kao et al., 2009). In another example, at least 51children in Bangladesh died as a result of taking a paracetamol syrup that was contaminated with diethylene glycol (Hanif et al., 1995).

Samples G2, G3 and G4 not showing the presence of the active ingredient would pose the highest risk to the consumer, since it would not prevent pregnancy as expected and certainly might cause other health related problems. A similar study done in Peru aimed at investigating the quality of ECs on the market confirmed the presence of a batch that contained a wrong active ingredient with no detectable levonorgestrel (Monge et al., 2014).

Another study in Cambodia confirmed the presence of 4.2% counterfeit anthelmintic drugs on market (Khan et al., 2010).

In conclusion, previous reports and studies indicate how counterfeits are a global health problem particularly affecting poorer countries. They are a major cause of unnecessary mortality, drug resistance and loss of public confidence in medicines and health structures. National Drug Regulatory bodies need to step up in the fight against counterfeits circulating the market.

4.2.2 Assay for levonorgestrel

The content of levonorgestrel in the samples was determined using the HPLC and the drug content was determined in respect to the label claim of the tablets. The study revealed that brands A, B, C, D, E and F passed the assay test with 100% pass rate (Table 4.5). Three out of the four samples (75%) for brand G failed the assay test. Samples G2, G3 and G4 gave the most worrisome result with no evidence of levonorgestrel in any of the assays conducted. The lack of the active pharmaceutical ingredient was first noted in the identification test as indicated in Figure 4.1 and Figure 4.2.

For the samples that passed this test (87%), sample F-2 had the highest percentage content of active ingredient of 102.1% and sample B-3 had the lowest percentage content of active ingredient of 92.7%. As outlined in the International Pharmacopoeia specifications, levonorgestrel content should not be less than 90.0% and not more than 110.0% of the amount stated on the label.

Table 4.5: Assay for the 7 brands of levonorgestrel tablets in the study.

Sample code	Label dose (strength) of API (mg/tablet)	Label claimed (%)	Mean Assay (mg/tablet)	Assay (%mean ± SD)	Assay test (90.0 - 110.0%)
A-1	1.5	100	1.46	97.3 ± 0.59	Pass
A-2	1.5	100	1.511	100.7 ± 2.5	Pass
A-3	1.5	100	1.478	98.5 ± 1.6	Pass
B-1	1.5	100	1.430	95.3 ± 0.90	Pass
B-2	1.5	100	1.400	93.3 ± 0.85	Pass
B-3	1.5	100	1.390	92.7 ± 0.98	Pass
B-4	1.5	100	1.393	92.9 ± 1.00	Pass
B-5	1.5	100	1.415	94.3 ± 1.21	Pass
B-6	1.5	100	1.412	94.1 ± 0.96	Pass
B-7	1.5	100	1.487	99.1 ± 0.96	Pass
C	1.5	100	1.487	99.1 ± 1.18	Pass
D-1	1.5	100	1.514	100.9 ± 0.71	Pass
D-2	1.5	100	1.517	101.1 ± 0.87	Pass
E	1.5	100	1.512	100.8 ± 0.99	Pass
F-1	0.75	100	0.748	99.7 ± 1.24	Pass
F-2	0.75	100	0.766	102.1 ± 0.91	Pass
F-3	0.75	100	0.74	98.7 ± 0.52	Pass
F-4	0.75	100	0.731	97.4 ± 0.75	Pass
F-5	0.75	100	0.737	98.2 ± 2.62	Pass
G-1	0.75	100	0.759	101.2 ± 0.82	Pass
G-2	0.75	100	NC	NC	**Fail**
G-3	0.75	100	NC	NC	**Fail**
G-4	0.75	100	NC	NC	**Fail**

NC, Non-compliant sample with no evidence of levonorgestrel.

Statistical analysis conducted using the one-way ANOVA for the mean differences of the drug content (assay) revealed that with a 95% CI, there was a significant difference ($p < 0.05$) in the drug content among the different brands of levonorgestrel tablets examined in the study.

Medicines are formulated with the intent to provide 100% of the quality of each active ingredient stated on the label. No matter how careful the processing is done, variations are possible and do occur, and that is why the International Pharmacopoeias come up with ranges within which the assay of a product should lie. This study revealed that three samples were neither outside nor within the range recommended by the International Pharmacopoeia 2019; the samples did not show any presence of the active ingredient (levonorgestrel).

Levonorgestrel tablets are indicated for prevention of pregnancy after unprotected sex, and when taken as instructed, they can reduce the risk of pregnancy by approximately 98% (Von Hertzen et al., 2002). However, this effect can only be achieved if levonorgestrel as an active ingredient is available and in the right amounts. Hence, samples G-2, G-3 and G-4 would present the highest risk to the consumer due to the lack of expected contraceptive action.

The assay results of this finding are similar to previous studies that have been done. A study conducted on levonorgestrel tablets in Peru found that 1/25 samples analyzed showed no presence of levonorgestrel in the assay conducted (Monge et al., 2014). Another study on albendazole tablets in Ethiopia found that 3/10 samples had less than 90% of the active pharmaceutical ingredient (Seifu et al., 2019). A study in Lubumbashi, Democratic Republic of Congo found that 26% of the albendazole tablets tested failed the assay test (Mwamba et al., 2016). Results of these studies indicate a general failure of the assay test, and hence the need for drug regulators to frequently monitor and test the quality of medicines on the market.

Contrary to this study, previous research scholars have indicated that there were no significant differences among the mean assay results of the different brands of cotrimoxazole tablets (Hailu et al., 2011) and norfloxacin tablets (Hambisa et al., 2019) that were found on market. The significant differences observed among the brands of levonorgestrel tablets

indicate that the levonorgestrel tablets available on the Ugandan market might not provide the same therapeutic effect to the consumer and hence the choice of brand of medicine taken matters. This points to the fact that manufacturers of this medicine are not regularly monitored by drug regulators to ensure that they produce therapeutically equivalent levonorgestrel tablets so as to save the patient the burden of looking for which brand performs best.

4.3 Uniformity of content of levonorgestrel tablets

As indicated in Table 4.6, brands A, B, C, D, E and F passed the uniformity of content test with 100% pass rate. Brand G registered a 75% failure rate of this test. The three samples that failed this test did not show any presence of levonorgestrel as was noted in the assay test. For the 20 samples that passed this test, samples A-2 and F-5 recorded the highest percentage deviation of 4.5% whereas samples B-2, B-3, B-4, B-5 and D-1 had the lowest percentage deviation of 0.0%. The International pharmacopoeia required that for the content uniformity test to pass, each of the single tablets should contain within ±15% of the average amount of the active ingredient.

Table 4.6: Percentage uniformity of content for the 7 brands of levonorgestrel

Sample code	**% content of levonorgestrel per tablet (Min - Max)**	**Content of levonorgestrel (%mean ± SD) (n=10)**	**% deviation of individual content from average per tablet (Min-Max)**	**Uniformity of content test (±15% from the average content)**
A-1	96.1 – 98.2	97.3 ± 0.59	0.3 – 0.9	Pass
A-2	97.4 – 105.2	100.7 ± 2.5	0.5 - 4.5	Pass
A-3	95.7 – 100.8	98.5 ± 1.6	0.1 – 2.8	Pass
B-1	94.3 – 96.7	95.3 ± 0.90	0.3 – 1.5	Pass
B-2	91.9 – 94.4	93.3 ± 0.85	0.0 – 1.5	Pass
B-3	91.6 – 94.8	92.7 ± 0.98	0.0 – 2.3	Pass
B-4	91.3 – 94.8	92.9 ± 1.00	0.0 – 2.0	Pass
B-5	92.2 – 96.1	94.3 ± 1.21	0.0 – 2.2	Pass
B-6	93.4 – 96.1	94.1 ± 0.96	0.1 – 2.1	Pass

B-7	97.6 – 100.3	99.1 ± 0.96	0.2 – 1.5	Pass
C	97.6 – 100.9	99.1 ± 1.18	0.1 – 1.9	Pass
D-1	99.6 – 102.3	100.9 ± 0.71	0.0 – 1.4	Pass
D-2	100.0 – 103.0	101.1 ± 0.87	0.1 – 1.9	Pass
E	98.7 – 102.2	100.8 ± 0.99	0.1 – 2.0	Pass
F-1	98.0 – 101.8	99.7 ± 1.24	0.1 – 2.1	Pass
F-2	100.8 – 103.3	102.1 ± 0.91	0.4 – 1.2	Pass
F-3	98.0 – 99.4	98.7 ± 0.52	0.1 – 0.7	Pass
F-4	96.5 – 98.6	97.4 ± 0.75	0.1 – 1.2	Pass
F-5	93.8 – 101.8	98.2 ± 2.62	0.1 – 4.5	Pass
G-1	100.2 – 103.0	101.2 ± 0.82	0.1 – 1.8	Pass
G-2	**NC**	**NC**	**NC**	**Fail**
G-3	**NC**	**NC**	**NC**	**Fail**
G-4	**NC**	**NC**	**NC**	**Fail**

NC, non-compliant sample with no evidence of levonorgestrel.

Statistical analysis conducted at 95% confidence interval revealed that brand D did not show any significant difference (Student-t, $p > 0.05$) among the mean percentage drug content of samples within that brand, whereas brands A, B and F revealed significant differences (ANOVA, $p < 0.05$) among the mean percentage drug content of samples within the same brands. Brands C, E were not statistically evaluated since only one sample within the same brand was collected at the time of sampling. For brand G, the other three samples of the same brand did not show any presence of Levonorgestrel, hence no meaningful comparison could be done.

Uniformity of content helps ensure that the strength of a medicine remains within specified acceptance limits. “When considering single- dose preparations, it is fundamental that the patient receives in his individual dose an amount of drug close to that claimed on the label. For that reason, pharmacopeia standards and specifications have been established to provide limits for permissible variations in the amount of active ingredient of individual single-dose units” (USPC, 2019c).

The findings of the study showed that all samples that contained levonorgestrel passed the uniformity of content test. Similar to this study, previous research scholars have also reported 100% compliance for the uniformity of dosage unit test (Hambisa et al., 2019; Othman, 2018; Uddin et al., 2017). In summary, manufacturers of medicines have tried to ensure that they produce tablets whose dosage contents are within the permissible variations, so that a patient taking a single dose prescription gets the right amount of the drug.

The studies further showed that despite the samples having dosage contents that were within the permissible variations, significant differences were observed among the mean percentage drug content of samples within the same brand (A, B and F). The significant differences observed among samples from the same manufacturer could indicate that, the respective manufacturing processes are not reproducible as expected.

Manufacturers of medicinal products are required to validate their manufacturing processes to ensure that the process is capable of consistently delivering quality products. Hence, "before any batch from a manufacturing process is commercially distributed for use by consumers, a manufacturer should have gained a high degree of assurance in the performance of the manufacturing process such that it will consistently produce drug products meeting those attributes relating to identity, strength, quality, purity, and potency" (FDA, 2011). With the process validation done right, we would not expect to have samples from the same manufacturer (represented by brand) having significant differences in the dosage content like it was observed in brands A, B and F. Various excipients such as diluents, binders, disintegrants, lubricants and others used in solid dosage forms may alter quality control parameters of tablets within the same batch. Hence, the variations observed could be related to the pharmaceutical manufacturer's formulation conditions such as mixing, blending, lubrication, granulation, and the amount of excipient added (Jean-Louis, 2010). Previous

studies have also further indicated that manufacturing process parameters indeed do have an impact on the uniformity of content of dosage formulations (Muselík et al., 2014).

The significant differences observed further indicate that a consumer consistently procuring medicines of the same manufacturer (brand) may not receive the same therapeutic effect as expected since the variances observed indicate that the manufacturer is not consistently producing batches of the same quality (Muselík et al., 2014). Frequent inspection of the manufacturing processes by drug regulators is key to ensure that there is no batch-to-batch variability from the same manufacturer.

4.4 Dissolution of levonorgestrel tablets

The release of the drug substance from a solid dosage form has a major impact on its rate and extent of absorption. In-vitro dissolution of an immediate release product is one of the most important tools in assuring the batch to batch quality of a drug product (Le, 2022). Hence, establishing appropriate dissolution specifications will assure that the manufacture of the dosage form is consistent and successful throughout the products life cycle. Due to the crucial role that dissolution plays in the bioavailability of the drug, in vitro dissolution can serve as an indicator of the efficiency of the in vivo performance of the drug product (Xie et al., 2014).

The study showed that all samples of brands A, D, E and F passed the dissolution test with 100% pass rate, whereas some samples of brands B (85%, 6/7), C (100%, 1/1) and G (75%, 3/4) failed the dissolution test, releasing less that the accepted amount of the levonorgestrel content within the time allowed (Table 4.7).

Compliance for the dissolution test with the International pharmacopoeia specifications was observed for 13 of 23 samples (57%, Table 4.7). Eleven samples passed at S1 stage with

results ranging from 80 to 100% and two samples passed at stage S2 with results of 75% for both samples. For the ten non-compliant samples, 7 failed at all dissolution stages S1, S2 and S3 with results ranging from 45% to 76% whereas three samples gave results with no evidence of levonorgestrel. As outlined in the International Pharmacopoeia, the specifications for each stage were: Stage 1= individual values of 6 tablets must be greater than or equal to 80%, Stage 2= mean value of 12 tablets must be greater than or equal to 75%, and no tablets can be less than 60%, Stage 3 = mean value of 24 tablets must be greater than or equal to 75%, no more than 2 tablets can be less than 60%, and no tablet can be less than 50%.

Table 4.7: Percentage dissolution of the levonorgestrel tablets in the study

Sample code	**API level**	**%Dissolution (Min-Max)**	**Dissolution (%mean ± SD)**	**Dissolution (Not less than 80% at S1)**
A1	1.5mg	92 -96	S1;93 ±1.34	Pass
A3	1.5mg	90 -98	S1;95 ±2.53	Pass
A2	1.5mg	94 -100	S1;97 ±2.01	Pass
B1	1.5mg	56 -67	S3;62 [c] ±2.93	**Fail**
B2	1.5mg	50 – 76	S3;59 [c] ±6.49	**Fail**
B3	1.5mg	56 – 69	S3;61 [c] ±3.89	**Fail**
B4	1.5mg	54 – 68	S3;63 [c] ±3.77	**Fail**
B5	1.5mg	58 – 66	S3;62 [c] ±2.19	**Fail**
B6	1.5mg	57 – 70	S3;63 [c] ±2.69	**Fail**
B7	1.5mg	87 - 92	S1;89 ±1.65	Pass
C	1.5mg	45 – 68	S3;60 [c] ±7.04	**Fail**
D1	1.5mg	66 - 82	S2;75 ±5.04	Pass
D2	1.5mg	67 - 85	S2;75 ±5.00	Pass
E	1.5mg	87 - 90	S1;88 ±1.07	Pass
F1	0.75mg	94 - 99	S1;97 ±1.90	Pass
F2	0.75mg	94 - 99	S1;96 ±2.30	Pass
F3	0.75mg	89 - 94	S1;91 ±1.62	Pass
F4	0.75mg	86 - 89	S1;88 ±1.13	Pass
F5	0.75mg	81 - 89	S1;83 ±3.12	Pass
G1	0.75mg	80 - 85	S1;82 ±2.31	Pass
G2	0.75mg	**NC**	**NC**	**Fail**
G3	0.75mg	**NC**	**NC**	**Fail**
G4	0.75mg	**NC**	**NC**	**Fail**

NC, non-compliant sample with no evidence of levonorgestrel.

[c] Failed the dissolution test as per International Pharmacopoeia specifications.

Statistical analysis conducted using the one-way ANOVA at 95% CI for the pharmacopoeially specified time (30 minutes) revealed that there was a significant difference ($p<0.05$) in the drug release of the different brands of levonorgestrel tablets examined in the study

Levonorgestrel tablets for emergency contraception are indicated to be used within 72 hours after unprotected sex and before ovulation. However, some evidence suggests that increased effectiveness is achieved when taken as soon as possible (Novikova et al., 2007; Von Hertzen et al., 2002).The low levels of levonorgestrel release for samples B1, B2, B3, B4, B5, B6 and C (see Table 11) suggest that these particular product batches may not yield the full level of contraceptive effectiveness expected, especially considering the suggested importance of taking the medication within a time frame of 72 hours after intercourse. The dissolution test has been performed in previous studies indicating similar results. A study done in Peru indicated that 7 out of 25 samples showed inadequate release of levonorgestrel (Monge et al., 2014). Another study conducted in Yemen on albendazole tablets reported a 29% failure of the dissolution test (Othman, 2018).

The statistically significant difference (ANOVA, $p < 0.05$) observed in the drug release of the different brands of levonorgestrel tablets indicates the presence of levonorgestrel products that are not equivalent statistically with respect to their in vitro release. These results are consistent with other studies that have been done on various marketed brands of norfloxacin Hambisa et al., 2019), cotrimoxazole (Hailu et al., 2011) and amoxicillin formulations (Koech, 2020).

The findings in this study indicate a general failure of the dissolution test for medicines circulating Africa. Dissolution is a very useful physiochemical test for assessment of drug product quality and performance. The effectiveness of an oral dosage form relies on its ability to dissolve in fluids of the gastrointestinal tract prior to it being absorbed into circulation. Therefore, it is important that the rate of dissolution of a tablet or capsule is within the recommended limits. The results of this study further highlight the need for drug regulatory agencies to effectively monitor the quality of drugs by focusing on the post-marketing evaluation of medicines circulating the market.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

A study to evaluate the quality of levonorgestrel tablets on the Ugandan market indicated that only 57% of the samples collected complied with the tests performed as per the International Pharmacopoeia 2019. Out of the 23 samples analysed,10 samples were non-compliant with the product specifications; mainly due to undetectable levonorgestrel and release of less than the accepted amount of the levonorgestrel when subjected to the identity and dissolution test respectively.

The visual inspection test revealed three suspected counterfeits for the postinor brand of levonorgestrel tablets. This was further confirmed by the identity test for levonorgestrel when these samples did not show any detectable levonorgestrel, hence confirmed with no doubt to be counterfeits of brand G of levonorgestrel tablets.

The statistical evaluations performed on the samples for the assay, content uniformity and dissolution test indicated the presence of brands of levonorgestrel tablets that are not therapeutically equivalent to each other, hence these medicines cannot be used

interchangeably. This leaves the burden on the consumer to look out for the levonorgestrel brands that perform best and further rely on only that particular brand.

The study concluded that from the samples analyzed, only 57% will provide the stated level of contraceptive action, 30% may or may not prevent pregnancy and 13% will offer no prevention against unwanted pregnancy. The results obtained in this study emphasize the need for constant surveillance of the quality of levonorgestrel tablets on the market with the intent to reduce on the prevalence of substandard and counterfeited levonorgestrel tablets on the market.

5.2 Recommendation

There's need to increase on the frequency of enforcements carried out by the National Medicines Regulatory body to be able to detect and immediately impound suspected counterfeits found on the market.

There is need for immediate re-call of the substandard medicines found on the market.

There is need to adequately monitor good manufacturing and distribution practices of pharmaceutical manufacturers to ensure batch to batch quality.

There's need to increase on the awareness of the use of the visual inspection and possibly translate it into our local languages so that the consumer can possibly appreciate it and join in the fight against counterfeits.

There is need to extend the study to non-registered pharmacies, black markets and all entry points for drugs to allow an accurate estimation of the extent of the problem of counterfeited and substandard levonorgestrel tablets.

There is need for regular post market surveillance to monitor the quality of drugs in the Ugandan market.

REFERENCES

Aigner, Z., Dombi, G., & Kata, M. (1996). Increasing the solubility characteristics of D-norgestrel with cyclodextrins. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry*, *25*, 145–148. https://doi.org/10.1007/BF01041556

Ajayi, A. I., Nwokocha, E. E., Akpan, W., & Adeniyi, O. V. (2016). Use of non-emergency contraceptive pills and concoctions as emergency contraception among Nigerian University students: results of a qualitative study. *BMC Public Health*, *16*(1), 6-7. https://doi:10.1186/s12889-016-3707-4

Advancing Partners and Communities. (2016). Providing Emergency Contraception through Community Health Workers in Uganda: A Formative Assessment. https://www.advancingpartners.org/sites/default/files/sites/default/files/resources/apc_uganda_cb-ec_brief_12.2.16_tagged.pdf

Africa's Medical Media Digest. (2018, November 21). Africa bears the brunt of the counterfeit medicine curse. https://www.medicalbrief.co.za/archives/africa-bears-brunt-counterfeit-medicine-curse/

Anand, O., Yu, L. X., Conner, D. P., & Davit, B. M. (2011). Dissolution testing for generic drugs: An FDA perspective. *AAPS Journal*, *13*(3), 328–335. https://doi.org/10.1208/s12248-011-9272-y

Attaran, A., Barry, D., Basheer, S., Bate, R., Benton, D., Chauvin, J., Garrett, L., Kickbusch, I., Kohler, J. C., Midha, K., Newton, P. N., Nishtar, S., Orhii, P., & McKee, M. (2012). How to achieve international action on falsified and substandard medicines. *BMJ*, *345*, 1–6. https://doi.org/10.1136/bmj.e7381

Atukunda, Norbert. (2019, May 2). Fake family planning pills found on market. *The Daily monitor*. https://www.monitor.co.ug/uganda/news/national/fake-family-planning-pills-found-on-market-1823432

Atuyambe, L., Mirembe, F., Johansson, A., Kirumira, E. K., & Faxelid, E. (2005). Experiences of pregnant adolescents--voices from Wakiso district, Uganda. *Afr Health Sci.*, *5*(4), 304-9. doi: 10.5555/afhs.2005.5.4.304

Babirye Kayongo, Susan. (2013). Uptake of modern contraception among youths (15-24) at community level in Busia district, Uganda. [Unpublished master's thesis]. Makerere University.

Boda, Ken. (n.d.). Transferring Dissolution Methods Session 1 – Building a Robust Dissolution Method. https://www.agilent.com/Library/eseminars/Public/Building%20a%20Robust%20Dissolution%20Method.pdf

Brache, V., Cochon, L., Jesam, C., Maldonado, R., Salvatierra, A. M., Levy, D. P., Gainer, E., & Croxatto, H. B. (2010). Immediate pre-ovulatory administration of 30 mg ulipristal acetate significantly delays follicular rupture. *Human Reproduction*, *25*(9), 2256–2263. https://doi:10.1093/humrep/deq157

Borges, E. M., & Euerby, M. R. (2013). An appraisal of the chemical and thermal stability of silica based reversed-phase liquid chromatographic stationary phases employed within the pharmaceutical environment. *Journal of Pharmaceutical and Biomedical Analysis*, *77*, 100–115. https://doi:10.1016/j.jpba.2013.01.013

Camag. (n.d.) HPTLC instruments for qualitative and quantitative analyses. https://www.camag.com/products/hptlc

Caudron, J. M., Ford, N., Henkens, M., Macé, C., Kiddle-Monroe, R., & Pinel, J. (2008). Substandard medicines in resource-poor settings: A problem that can no longer be ignored. *Tropical Medicine and International Health*, *13*(8), 1062–1072. https://doi.org/10.1111/j.1365-3156.2008.02106.x

Choudhary, A. (2016, January). Different types of HPLC detectors. pharmaguideline. https://www.pharmaguideline.com/2016/01/different-types-of-hplc-detectors.html

Cockburn, R., Newton, P. N., Agyarko, E. K., Akunyili, D., & White, N. J. (2005). The global threat of counterfeit drugs: Why industry and governments must communicate the dangers. *PLoS Medicine*, *2*(4), 0302–0308. https://doi.org/10.1371/journal.pmed.0020100

Croxatto, H. B., Devoto, L., Durand, M., Ezcurra, E., Larrea, F., Nagle, C., … von Hertzen, H. (2001). Mechanism of action of hormonal preparations used for emergency contraception: a review of the literature. *Contraception*, *63*(3), 111–121. https://doi:10.1016/s0010-7824(01)00184-6

De Santis, M., Cavaliere, A. F., Straface, G., Carducci, B., & Caruso, A. (2005). Failure of the emergency contraceptive levonorgestrel and the risk of adverse effects in pregnancy

and on fetal development: an observational cohort study. *Fertility and Sterility*, *84*(2), 296–299. https://doi:10.1016/j.fertnstert.2005.01.136

European Medicines Agency. (2000, May). ICH Topic Q 6 A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-6-test-procedures-acceptance-criteria-new-drug-substances-new-drug-products-chemical_en.pdf

European Medicines Agency. (2009). Ellaone: Ulipristal acetate. https://www.ema.europa.eu/en/documents/overview/ellaone-epar-summary-public_en.pdf

Frimpong, G., Ofori-Kwakye, K., Kuntworbe, N., Buabeng, K. O., Osei, Y. A., El Boakye-Gyasi, M., & Adi-Dako, O. (2018). Quality Assessment of Some Essential Children's Medicines Sold in Licensed Outlets in Ashanti Region, Ghana. *Journal of Tropical Medicine*, 2018, 1-14. https://doi.org/10.1155/2018/1494957

Gemzell-Danielsson, K., Berger, C., & Lalitkumar, P.G.L. (2013). Emergency contraception — mechanisms of action. *Contraception*, *87*(3), 300–308. https://doi:10.1016/j.contraception.2012.08.021

Guttmacher Institute. (2017). Abortion and Postabortion Care in Uganda. https://www.guttmacher.org/sites/default/files/factsheet/abortion-and-postabortion-care-uganda.pdf

Habyalimana, V., Mbinze, J. K., Tshilombo, N. K., Dispas, A., Loconon, A. Y., Sacré, P.-Y., Widart, J., De Tullio, P., Counerotte, S., Ntokamunda, J.-L. K., Ziemons, E., Hubert, P., & Djang'eing'a, R. M. (2015). Analytical Tools and Strategic Approach to Detect Poor Quality Medicines, Identify Unknown Components, and Timely Alerts for Appropriate Measures: Case Study of Antimalarial Medicines. *American Journal of Analytical Chemistry*, *06*(13), 977–994. https://doi.org/10.4236/ajac.2015.613093

Haeger, K. O., Lamme, J., & Cleland, K. (2018). State of emergency contraception in the U.S. *Contraception and Reproductive Medicine*, *3*(1), 1–12. https://doi.org/10.1186/s40834-018-0067-8

Hailu, G. S., Gutema, G. B., Asfaw, A. A., Dagim, A. H., & Hadera, M. G. (2011). Comparative assessment of the physicochemical and in-vitro bioavailability equivalence of cotrimoxazole tablets marketed in Tigray, Ethiopia. *International Journal of Pharmaceutical Sciences and Research*, *2*, 3210-3218. https://doi.org/10.13040/IJPSR.0975-8232.2(12).3210-18

Hambisa, S., Belew, S., & Suleman, S. (2019). In vitro comparative quality assessment of different brands of norfloxacin tablets available in Jimma, SouthWest Ethiopia. *Drug Design, Development and Therapy*, *13*, 1241–1249. https://doi.org/10.2147/DDDT.S189524

Hanif, M., Mobarak, M. R., Ronan, A., Rahman, D., Donovan, J. J. Jr., & Bennish, M. L. (1995). Fatal renal failure caused by diethylene glycol in paracetamol elixir: the Bangladesh epidemic. *BMJ*. *311*(6997), 88-91. https://doi: 10.1136/bmj.311.6997.88

IMPACT. (2006). Counterfeit Medicines: an update on estimates. https://www.yumpu.com/en/document/view/37643951/counterfeit-medicines-an-update-on-estimates-world-health-

ICEC. (n.d.). EC pill types and countries of availability, by brand. Retrieved June 11, 2019, from https://www.cecinfo.org/country-by-country-information/status-availability-database/ec-pill-types-and-countries-of-availability-by-brand/

Jadav, S. P., & Parmar, D. M. (2012). Ulipristal acetate, a progesterone receptor modulator for emergency contraception. *Journal of Pharmacology and Pharmacotherapeutics*, *3*(2), 109–111. https://doi.org/10.4103/0976-500X.95504

Jean-Louis, Robert. (2010). Q8(R2): Pharmaceutical Development. https://admin.ich.org/sites/ default/files/inline-files/Q8_Pharma_development_JL.Robert.pdf

Kao, S. L., Chan, C. L., Tan, B., Lim, C. C. T., Dalan, R., Gardner, D., Pratt, E., Lee, M., & Lee, K. O. (2009). An Unusual Outbreak of Hypoglycemia — A Correction. *New England Journal of Medicine*, *360*(23), 2482–2483. https://doi.org/10.1056/nejmc096117

Kasujja, Carol. (2017, June 14). Deadly Postinor 2 on the market. *The New vision.* https://www.newvision.co.ug/news/1455558/deadly-postinor-market

Keoluangkhot, V., Green, M.D., Nyadong, L., Fernandez, F.M., Mayxay, M., & Newton, P.N. (2008). Impaired clinical response in a patient with uncomplicated falciparum malaria who received poor-quality and under dosed intramuscular artemether. *The American Journal of Tropical Medicine and Hygiene*, *78*(4), 552–555. https://doi.org/10.4269/ajtmh.2008.78.552

Khan, M. H., Okumura, J., Sovannarith, T., Nivanna, N., Akazawa, M., & Kimura, K. (2010). Prevalence of counterfeit anthelminthic medicines: A cross-sectional survey in Cambodia. *Tropical Medicine and International Health*, *15*(5), 639–644. https://doi.org/10.1111/j.1365-3156.2010.02494.x

Koech, L. C., Irungu, B. N., Ng'ang'a, M. M., Ondicho, J. M., & Keter, L. K. (2020). Quality and Brands of Amoxicillin Formulations in Nairobi, Kenya. *BioMed Research International*, *2020*(4), 1–14. https://doi:10.1155/2020/7091278

Kulier, R., Helmerhorst, F., O'Brien, P., Usher-Patel, M., & d'Arcangues, C. (2005). Copper containing intra-uterine devices for contraception. Cochrane Database of Systematic Reviews, 3. https://doi.org/10.1002/14651858.cd005347

Lakka, N., & Kuppan, C. (2020). Principles of Chromatography Method Development. In O. M. Boldura, C. Baltă & N. S. Awwad. *Biochemical Analysis Tools - Methods for Bio-Molecules Studies*. https://doi.org/10.5772/intechopen.89501

Larsson, S., Eliasson, M., Klingberg Allvin, M., Faxelid, E., Atuyambe, L., & Fritzell, S. (2015). The discourses on induced abortion in Ugandan daily newspapers: a discourse analysis. *Reproductive Health*, *12*(1), 2-3. https://doi:10.1186/s12978-015-0049-0

Le, J. (2022). Drug Absorption. In Merck & Co, Inc. (Ed.), *MSD Manual*. https://www.msdmanuals.com/home/drugs/administration-and-kinetics-of-drugs/drug-distribution

Levonorgestrel. (2023, June 1). In Wikipedia. https://en.wikipedia.org/wiki/Levonorgestrel

Medicines and Healthcare products Regulatory Agency. (2019a). Levonorgestrel. In *British pharmacopoeia* (pp. 108-10).

Medicines and Healthcare products Regulatory Agency. (2019b). General monographs. In *British pharmacopoeia* (pp. 39-1385).

Medicines and Healthcare products Regulatory Agency. (2019c). Appendix III: Chromatographic Separation Techniques. In *British pharmacopoeia* (pp. 223-39).

Medicines and Healthcare products Regulatory Agency. (2019d). Appendix XII B: Dissolution. In *British pharmacopoeia* (pp. 395-402).

Merten, S., & Rokicki, S. (2018). The context of emergency contraception use among young unmarried women in Accra, Ghana: a qualitative study. *Reproductive Health*, *15*(212), 1-2. https://doi.org/10.1186/s12978-018-0656-7

Millán, M. M., & Castañeda, S. (2015). Sex Hormones and Related Compounds, Including Hormonal Contraceptives. *Side Effects of Drugs Annual, 36*, 615-619. https://doi.org/10.1016/B978-0-444-63407-8.00040-X

Ministry of Health. (2016). Essential Medicines and Health Supplies List for Uganda. http://www.medicinestransparency.org/fileadmin/uploads/News/Essential Medicines & Health Supplies List 2012.pdf

Ministry of Health. (2018). Annual Health Sector Performance Report: Financial Year2017/18. http://library.health.go.ug/sites/default/files/resources/AHSPR%202017_18%20FY.pdf

Mohamed, A. G. K., Ravinetto, R., & Alfadl, A. A. (2020). The importance of visual inspection in national quality assurance systems for medicines. *Journal of Pharmaceutical Policy and Practice, 13*(52), 1–3. https://doi.org/10.1186/s40545-020-00264-w

Monge, M. E., Dwivedi, P., Zhou, M., Payne, M., Harris, C., House, B., Juggins, Y., Cizmarik, P., Newton, P. N., Fernández, F. M., & Jenkins, D. (2014). A tiered analytical approach for investigating poor quality emergency contraceptives. *PLoS ONE, 9*(4), 4-8 https://doi.org/10.1371/journal. pone.0095353

Muselík, J., Franc, A., Doležel, P., Goněc, R., Krondlová, A., & Lukášová, I. (2014). Influence of process parameters on content uniformity of a low dose active pharmaceutical ingredient in a tablet formulation according to GMP. *Acta Pharmaceutica, 64*(3), 355–367.

Mwamba, P., Duez, P., & Kalonji, N. J. B. (2016). Preliminary survey of counterfeiting of albendazole and metronidazole marketed in Lubumbashi. International Journal of Pharmacy and Pharmaceutical Sciences, 8(7), 282–287.

National Drug Authority. (2018). Guidelines on Submission of Documentation for Marketing Authorization of a Pharmaceutical Product for Human Use (Doc. No. PAR/GDL/004, Rev. 3). Retrieved June 29, 2023, from https://www.nda.or.ug/wp-content/uploads/2023/02/PAR-GDL-004-Guidelines-on-Submission-of-Documentation-for-Registration-of-a-Pharmaceutical-Product-for-Human-Use_Rev-3.pdf

National Drug Authority. (2019a, July). Drug Register. Retrieved August 3, 2019, from https://www.nda.or.ug/drug-register/#1539148991734-31a96a97-315e

National Drug Authority. (2021). Drug Recalls 2018-2021. Retrieved October 4, 2021, from https://www.nda.or.ug/drug-recalls-2018-2020/

New study finds fake, low-quality medicines prevalent in the developing world. (2018, August 10). Science Daily. Retrieved February 15, 2021, from www.sciencedaily.com/releases/ 2018/08/180810120019.htm

Newton, P. N., Green, M. D., Fernández, F. M., Day, N. P., & White, N. J. (2006). Counterfeit anti-infective drugs. *Lancet Infectious Diseases*, *6*(9), 602–613. https://doi.org/10.1016/S1473-3099(06)70581-3

Newton, P. N., Green, M. D., & Fernández, F. M. (2010). Impact of poor-quality medicines in the "developing" world. *Trends in Pharmacological Sciences*, *31*(3), 99–101. https://doi.org/10.1016/j.tips.2009.11.005

Novikova, N., Weisberg, E., Stanczyk, F. Z., Croxatto, H. B., & Fraser, I. S. (2007). Effectiveness of levonorgestrel emergency contraception given before or after ovulation - a pilot study. *Contraception*, *75*(2), 112–118. https://doi.org/10.1016/j.contraception.2006.08.015

Ortiz, M. E., Croxatto, H. B., & Bardin, C. W. (1996). Mechanisms of action of intrauterine devices. *Obstet Gynecol Surv.*, *51*(12 Suppl), 42-51. https://doi:10.1097/00006254-199612000-00014.

Othman, G. Q. (2018). Quality Assessment of Seven Brands of Albendazole Tablets Marketed in Yemen. *Yemeni Journal for Medical Sciences*, *11*(1), 46–52. https://doi.org/10.20428/yjms.v11i1.1223

Ozawa, S., Evans, D. R., Bessias, S., Haynie, D. G., Yemeke, T. T., Laing, S. K., & Herrington, J. E. (2018). Prevalence and Estimated Economic Burden of Substandard and Falsified Medicines in Low- and Middle-Income Countries. *JAMA Network Open*, *1*(4), 1-2. https://doi:10.1001/jamanetworkopen.2018.1662

Rägo, L., & Santoso, B. (2008). Drug regulation: History, present and future. In C.J. van Boxtel, B. Santoso & I.R. Edwards (Eds.), *Drug Benefits and Risks* (pp. 65-77). Wiley.

Ray Laurie. (2019, June 27). Progestins 101. https://helloclue.com/articles/sex/progestins-101#:~:text=Progestins%20are%20present%20in%20all,some%20injections)%20(4).

RHSC. (2015). Counting what counts: Tracking access to emergency Contraception in Uganda. https://www.rhsupplies.org/uploads/tx_rhscpublications/ICEC_Uganda-factsheet_2015.pdf

RHSC. (2018). Emergency Contraceptive Pills: Medical and Service Delivery Guidance (4th Edition). https://www.rhsupplies.org/uploads/tx_rhscpublications/ICEC-guides_FINAL.pdf

Rizk, M. S., Sultan, M., Mohamed, D., & Tony, R. M. (2017). Simultaneous determination of levonorgestrel and ethinyl estradiol in combined dosage form utilizing spectrophotometric methods and high performance thin layer chromatographic method on nanosilica gel plates. *European Journal of Chemistry*, *8*(1), 52–59. https://doi.org/10.5155/eurjchem.8.1.52-59.1523

Rosenfield, A. (1989). Modern contraception: A 1989 update. *Annual Review of Public Health*, *10*, 385–401. https://doi.org/10.1146/annurev.pu.10.050189.002125

Seifu, A., Kebede, E., Bacha, B., Melaku, A., & Setegn, T. (2019). Quality of albendazole tablets legally circulating in the pharmaceutical market of Addis Ababa, Ethiopia: Physicochemical evaluation. *BMC Pharmacology and Toxicology*, *20*(1), 1–7. https://doi.org/10.1186/s40360-019-0299-5

Shigesato, M., Elia, J., Tschann, M., Bullock, H., Hurwitz, E., Wu, Y. Y., & Salcedo, J. (2018). Pharmacy access to Ulipristal acetate in major cities throughout the United States. *Contraception*, *97*(3), 264–269. https://doi.org/10.1016/j.contraception.2017.10.009

Shohel, M., Rahman, M. M., Zaman, A., Uddin, M. M. N., Al-Amin, M. M., & Reza, H. M. (2014). A systematic review of effectiveness and safety of different regimens of levonorgestrel oral tablets for emergency contraception. *BMC Women's Health*, *14*(1), 2-3. doi:10.1186/1472-6874-14-54

Skuster, P. (2004). Advocacy in Whispers: The Impact of the USAID Global Gag Rule Upon Free Speech and Free Association in the Context of Abortion Law Reform in Three East African Countries. *Michigan Journal of Gender & Law*, *11*(1), 97–126. https://repository.law.umich.edu/mjgl/vol11/iss1/4

Uddin, M. S., Mamun, A. A., Hossain, M. S., Asaduzzaman, M. D., Sarwar, M. S., Rashid, M., & Herrera-Calderon, O. (2017). In vitro quality evaluation of leading brands of ciprofloxacin tablets available in Bangladesh. *BMC Research Notes*, *10*(1), 1–9. https://doi.org/10.1186/s13104-017-2507-y

United States Food and Drug Administration. (2010). Ella: Ulipristal acetate 30 mg. https://www.fda.gov/media/83203/download

United States Food and Drug Administration. (2011). Process Validation: General Principles and Practices. https://www.fda.gov/files/drugs/published/Process-Validation--General-Principles-and-Practices.pdf

United States Food and Drug Administration. (2021, March 5). Human Drugs. https://www.fda.gov/industry/regulated-products/human-drugs#drug

U.S. Department of Justice, Office of Justice programs. (2005, January). Methods of Analysis/Drug Identification. https://www.ojp.gov/ncjrs/virtual-library/abstracts/methods-analysisdrug-identification

United States Pharmacopeial Convention. (2019a). Levonorgestrel. In *United States Pharmacopeia and National Formulary* (USP 42–NF 37) (pp. 2558-2560).

United States Pharmacopeial Convention. (2019b). Official monograph. In *United States Pharmacopeia and National Formulary* (USP 42–NF 37) (pp. 19-2480).

United States Pharmacopeial Convention. (2019c). Chromatography <621>. In *United States Pharmacopeia and National Formulary* (USP 42–NF 37) (pp. 6781-92).

United States Pharmacopeial Convention. (2019d). Uniformity of Dosage Units <905>. In United States Pharmacopeia and National Formulary (USP 42–NF 37) (pp. 7183-86).

Von Hertzen, H., Piaggio, G., Ding, J., Chen, J., Song, S., Bártfai, G., Ng, E., Gemzell-Danielsson, K., Oyunbileg, A., Wu, S., Cheng, W., Lüdicke, F., Pretnar-Darovec, A., Kirkman, R., Mittal, S., Khomassuridze, A., Apter, D., & Peregoudov, A. (2002). Low dose mifepristone and two regimens of levonorgestrel for emergency contraception: A WHO multicentre randomised trial. *Lancet*, *360*(9348), 1803–1810. https://doi.org/10.1016/S0140-6736(02)11767-3

Water. (n.d.). HPLC Separation Modes. Retrieved January 24, 2021, from https://www.waters.com/waters/en_US/HPLC-Separation-Modes/nav.htm?cid=10049076&locale=en_US

World Health Organization. (1999a). Counterfeit and substandard drugs in Myanmar and Viet Nam: Report of a study carried out in cooperation with the Governments of Myanmar and Viet Nam / Eshetu Wondemagegnehu. https://apps.who.int/iris/bitstream/handle/10665/66032/WHO_EDM_QSM_99.3.pdf?sequence=1&isAllowed=y

World Health Organization. (1999b). Counterfeit Drugs: Guidelines for the Development of Measures to Combat Counterfeit Drugs. https://apps.who.int/iris/bitstream/handle/10665/65892/WHO_EDM_QSM_99.1.pdf?sequence=1&isAllowed=y

World Health Organization. (2002). Annex 9: Guidelines on packaging for pharmaceutical https://cdn.who.int/media/docs/default-source/medicines/norms-and-standards/guidelines/regulatory-standards/trs902-annex9.pdf?sfvrsn=82b4c57d_2

World Health Organization. (2003). Effective medicines regulation: ensuring safety, efficacy and quality. https://apps.who.int/iris/bitstream/handle/10665/68391/WHO_EDM_2003.2.pdf?sequence=1&isAllowed=y

World Health Organization. (2013). Falsified batches of Postinor 2 recently discovered in https://cdn.who.int/media/docs/default-source/pvg/drug-alerts/da128---alert_128_information_postinor_sp_26july.pdf?sfvrsn=21f4e22b_4

World Health Organization. (2015). Falsified Emergency Contraceptive circulating in East Africa. http://www.fdd.gov.la/download/documents/Alert%205%202015%20Nov%2020 15_Falsified%20Postinor2_FINAL_EN%20(1).pdf

World Health Organization. (2016, October 2016). Annex 7: Guidelines on the conduct of surveys of the quality of medicines. https://cdn.who.int/media/docs/default-source/medicine s/norms-and-standards/guidelines/distribution/trs966-annex7-who-guidelines-on-the-conduct-of-surveys-of-the-quality-of-medicines.pdf?sfvrsn=4dbb5519_2&download=true

World Health Organization. (2017a). WHO Global Surveillance and Monitoring System for Substandard and Falsified Products. https://apps.who.int/iris/bitstream/handle/10665/326708/9789241513425-eng.pdf?sequence=1&isAllowed=y

World Health Organization. (2017b, January 10). Member State mechanism on substandard/spurious/falsely-labelled/falsified/counterfeit medical products. https://apps.who.int/iris/bitstream/handle/10665/273272/B140_23-en.pdf?sequence=1&isAllowed=y

World Health Organization. (2018a, November 9). Emergency contraceptive Pills. https://www.who.int/news-room/fact-sheets/detail/emergency-contraception

World Health Organization. (2018b, May 31). Family Planning: A Global Handbook for Providers. https://www.who.int/publications/i/item/9780999203705

World Health Organization. (2019a). Monographs: Specific monographs. In *the International Pharmacopoeia (9thEdition).* https://digicollections.net/phint/2022/index.html#d/b.1

World Health Organization WHO. (2019b). WHO model list of essential medicines. https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf

World Health Organization. (2019c). 5.1: Uniformity of content for single-dose preparations. In *the International Pharmacopoeia (9thEdition).* https://digicollections.net/phint/2022/index.html#d/b.7.5.2

Xie, F., Ji, S., & Cheng, Z. (2015). In vitro dissolution similarity factor (f2) and in vivo bioequivalence criteria, how and when do they match? Using a BCS class II drug as a simulation example. *European Journal of Pharmaceutical Sciences*, *66*, 163–172. https://doi.org/10.1016/j.ejps.2014.10.002

APPENDICES

Appendix I: List of ECs containing levonorgestrel registered to be sold on the Ugandan market, Source: National Drug Authority Register 2021.

Name of drug	Generic name	Strength of drug	Manufacturer	Country of manufacture
Depregdina	Levonorgestrel	1.5mg	Acme formulation PVT LTD	India
Jadellesine	Levonorgestrel	1.5mg	Bayer OY	Finland
Microgynonfe	Levonorgestrel /Ethinylestradiol	0.15mg/0.03mg	Bayer Pharma	German
Mirena	Levonorgestrel	52mg	Bayer OY	Finland
Microlut	Levonorgestrel	0.03mg	Bayer Pharma	German
Combination 3	Levonorgestrel /Ethinylestradiol /ferrous fumarate	0.15mg/0.03mg/75mg	Del pharma SAS	German
Unosure 72	Levonorgestrel	1.5mg	Akums drugs & Pharmaceuticals Ltd.	India
Back up	Levonorgestrel	1.5mg	Acme Formulation	India

			PVT Ltd.	
Lagest	Levonorgestrel	1.5mg	Corona remedies Pvt.Ltd.	India
Pronta 1	Levonorgestrel	1.5mg	Mylan Laboratories Ltd.	India
Easy pill	Levonorgestrel	1.5mg	Corona Remedies Pvt. Ltd.	India
I-Free 72	Levonorgestrel	1.5mg	Synokem Pharmaceuticals Limited	India
Lydia postpil	Levonorgestrel	1.5mg	Naari Pharma private Ltd.	India
Sure-72	Levonorgestrel	1.5mg	Synokem Pharmaceuticals Limited	India
Avibela	Levonorgestrel	52mg/system	Odyssea Pharma	Belgium
Well-plan	Levonorgestrel /Ethinylestradiol	0.15mg/0.03mg	Renata Ltd	Bangladesh
Postinor	Levonorgestrel	0.75mg	Gideon Richter PLC	Hugary
1-pill	Levonorgestrel	1.5mg	CIPLA Ltd.	India
P2	Levonorgestrel	0.75mg	Famy care Ltd	India

Appendix II: Sample collection form

SAMPLE COLLECTION FORM

QC NUMBER ……

A. Product information

1. Trade name (if any) ……

2. Generic name ……

3. Dosage-form …… 4. Storage condition ……
(As per manufacturer's instructions)

5. Strength/Size …… 6. Pharmacopoeia status ..……

7. Unit pack size…… 8. Batch/lot no.
……

10. Date of manufacture ……. 11. Date of expiry ……

12. Name & address of manufacturer ……

13. Name & amount of API on label ……

B. Sample information

1. Sample size ……

2. Sample source ……..

3. Sampled by ……

6. Signature ……

7. Date
……

Appendix III: Analytical method validation report for the Assay method

1.Title of protocol:	Analytical method verification protocol for testing levonorgestrel tablets
2.Protocol Number:	NDA/DLS/PRT/140
3.Performance Parameters:	Specificity, Precision, Linearity and System suitability

4. System suitability results

System suitability	Value	Limit	compliance
% RSD for peak area response of levonorgestrel for 5 replicates injections	0.2	NMT 2%	compliant
Symmetry factor	0.9	NMT 1.6	compliant
column efficiency	7795	≥ 5000	compliant

Conclusion: From the above observations it is concluded that the chromatographic system is suitable for the intended analysis.

5. Specificity results

System suitability	Value	Observation
Blank	-----	There was no interference observed at the retention time of levonorgestrel.
Sample Retention time	7.376	
Standard Retention time	7.375	

Conclusion: From the above observations it is concluded that the method is specific for determination of levonorgestrel in levonorgestrel tablets.

5. Precision results (Repeatability)

HPLC used	Agilent 1260 Infinity with a VWD detector	
		Limit
Range (%)	95.3 – 99.3	90.0% to 110.0%
Mean (%)	96.0	90.0% to 110.0%
Individual %RSD	1.6	NMT 2.0%

Conclusion: The analytical method was found to be precise for the determination of levonorgestrel in levonorgestrel tablets.

6. Precision results (Ruggedness)

	Analyst 1	Analyst 2	Limit
HPLC used	Agilent 1260 Infinity with a VWD detector	Agilent 1100 Series with a VWD detector	NA
Range (%)	95.3 – 99.3	92.8 – 94.5	90.0% to 110.0%
Mean (%)	96.0	94.0	90.0% to 110.0%
Inter-Analyst /System %RSD	1.5		NMT 2.0%

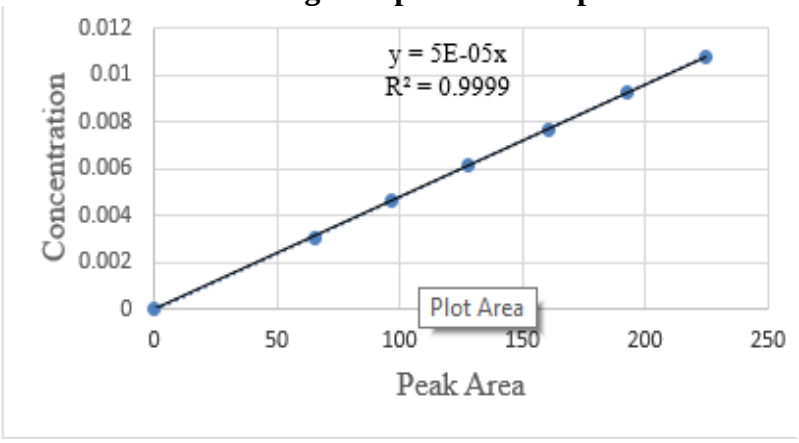
Conclusion: The analytical method was found to be precise for the determination of levonorgestrel in levonorgestrel tablets

7. Linearity

Linearity concentrations and observations

Peak area of levonorgestrel	**levonorgestrel Concentration**	**R^2**	**Limit**
0	0	1.000	NLT 0.995
65.37927	0.00308		
96.90821	0.00462		
128.00896	0.00616		
160.8812	0.00770		
192.33003	0.00924		
224.64497	0.01078		

Plot of concentration against peak area response



Conclusion: The Analytical method was found to be linear in the range of 0-150%

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Appendix IV: Analytical method validation report for the Dissolution method

1.Title of protocol: Analytical method verification protocol for testing levonorgestrel tablets

2.Protocol Number: NDA/DLS/PRT/140

3.Performance Parameters: Specificity, Precision, Linearity and System suitability

4. System suitability results

System suitability	Value	Limit	compliance
% RSD for peak area response of levonorgestrel for 5 replicates injections	1.6	NMT 2%	compliant
Symmetry factor	0.9	NMT 1.6	compliant
column efficiency	11034	≥ 5000	compliant

Conclusion: From the above observations it is concluded that the chromatographic system is suitable for the intended analysis.

5. Specificity results

System suitability	Value	Observation
Blank	-----	There was no interference observed at the retention time of levonorgestrel.
Sample Retention time	7.400	
Standard Retention time	7.397	

Conclusion: From the above observations it is concluded that the method is specific for determination of levonorgestrel in levonorgestrel tablets.

5. Precision results (Repeatability)

HPLC used	Agilent 1260 Infinity with a VWD detector	
		Limit
Range (%)	87 – 89	NLT 80%
Mean (%)	88	NLT 80%
Individual %RSD	0.9	NMT 2.0%

Conclusion: The analytical method was found to be precise for the determination of levonorgestrel in levonorgestrel tablets.

6. Precision results (Ruggedness)

	Analyst 1	Analyst 2	Limit
HPLC used	Agilent 1260 Infinity with a VWD detector	Agilent 1100 Series with a VWD detector	NA
Range (%)	87 – 91	83 – 91	NLT 80%
Mean (%)	89	88	NLT 80%.0%
Inter-Analyst /System %RSD	0.8		NMT 10.0%

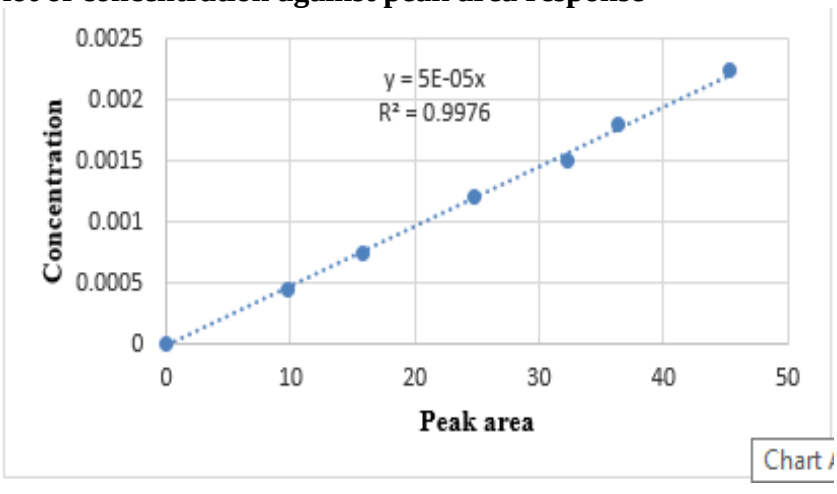
Conclusion: The analytical method was found to be precise for the determination of levonorgestrel in levonorgestrel tablets.

7. Linearity

Linearity concentrations and observations

Peak area of levonorgestrel	**levonorgestrel concentration**	**R^2**	**Limit**
0	0	0.998	NLT 0.995
9.80000	0.00045		
15.85479	0.00075		
24.84583	0.00120		
32.23471	0.00150		
36.40258	0.00180		
45.30000	0.00225		

Plot of concentration against peak area response



Conclusion: The Analytical method was found to be linear in the range of 0-150%

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Appendix V: Checklist used to visually inspect the samples

CHECKLIST FOR VISUAL INSPECTION OF MEDICINES				
Container and closure	**Assessment**	**Yes**	**No**	**NA**
	Does the container and closure protect the product from the outside environment; e.g. is the container properly sealed?			
	Are the container and the closure appropriate for the product inside?			
	Is the container safely sealed?			
The medicine strength (mg/unit)	Is the dosage clearly indicated?			
	Is the strength - the amount of active ingredient per unit - clearly stated			
	For blister or foil strip packed products, is the medicine strength indelibly impressed or imprinted onto the strip?			
Dosage statement	Is the dosage stated on the label appropriate for the medicine in this form and strength?			
	Is the product registered and authorized for sale in the country with this dosage?			
The batch (or lot) number	Does the numbering system on the package correspond to that of the producing company?			
	Does the numbering system on the package correspond to that of the producing company?			
	For blister or foil strip packed medicines, is the batch number indelibly impressed or imprinted onto the strip?			
The date of manufacture and the expiry date	Are the manufacturing and expiry dates clearly indicated?			
	For blister or foil strip packed products, is the expiry date indelibly impressed or imprinted onto the strip?			
Storage information	Are the storage conditions indicated?			
	Has the product been properly stored?			
Leaflet or package insert	Is the package insert printed on the same colored or same quality paper as the original (If available to compare) or does it look familiar?			
	Is the ink on the package insert or packaging smudge-proof?			
	Does the information on the package insert match the information on the product container?			
Physical characteristics of tablets	Are the tablets uniform in shape?			
	Are the tablets uniform in size?			
	Are the tablets uniform in color			
	Are the tablets free of breaks, cracks, splits or pinholes?			
	Are the tablets free of embedded surface spots and foreign particle contamination?			

Appendix VII: Pictorial

Figure 1: Samples documented in the study

Figure 2: Samples documented in the study

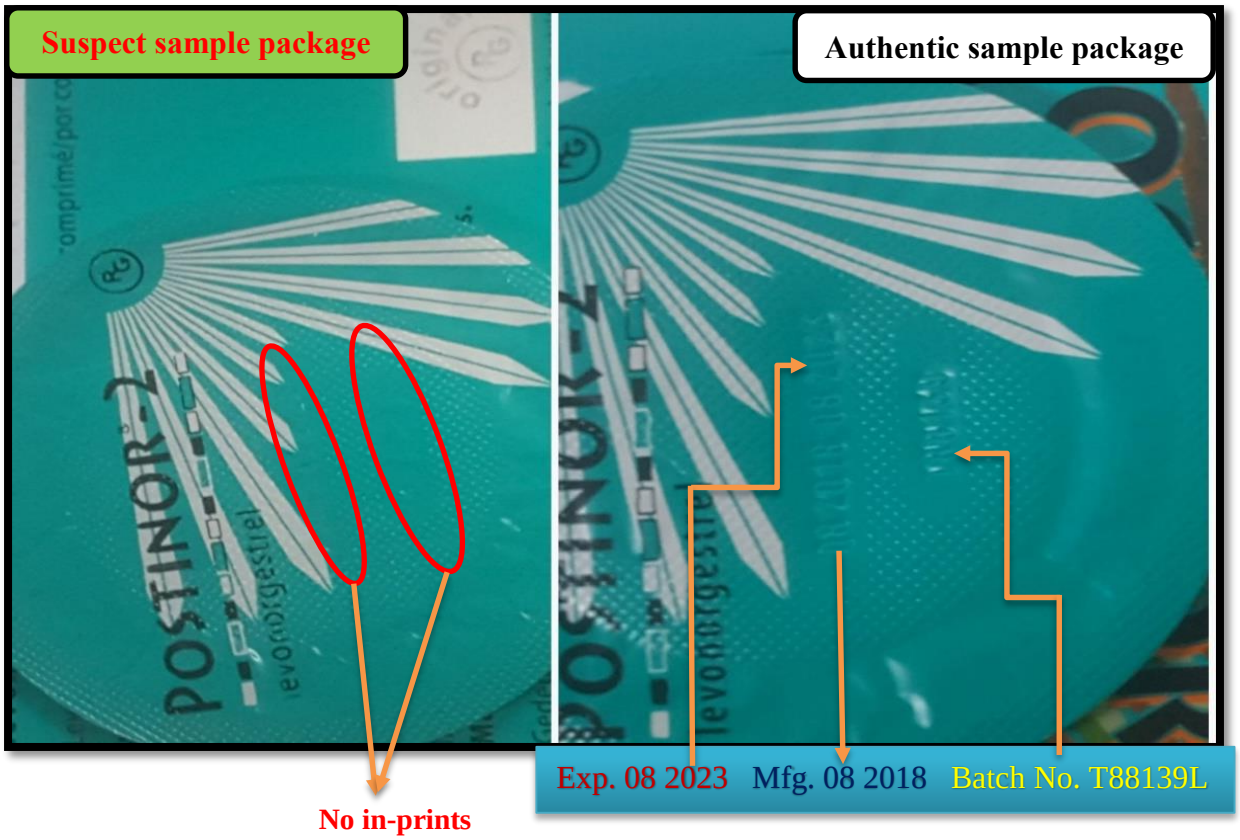

Figure 3: Back side of the primary package of an authentic and a suspect sample

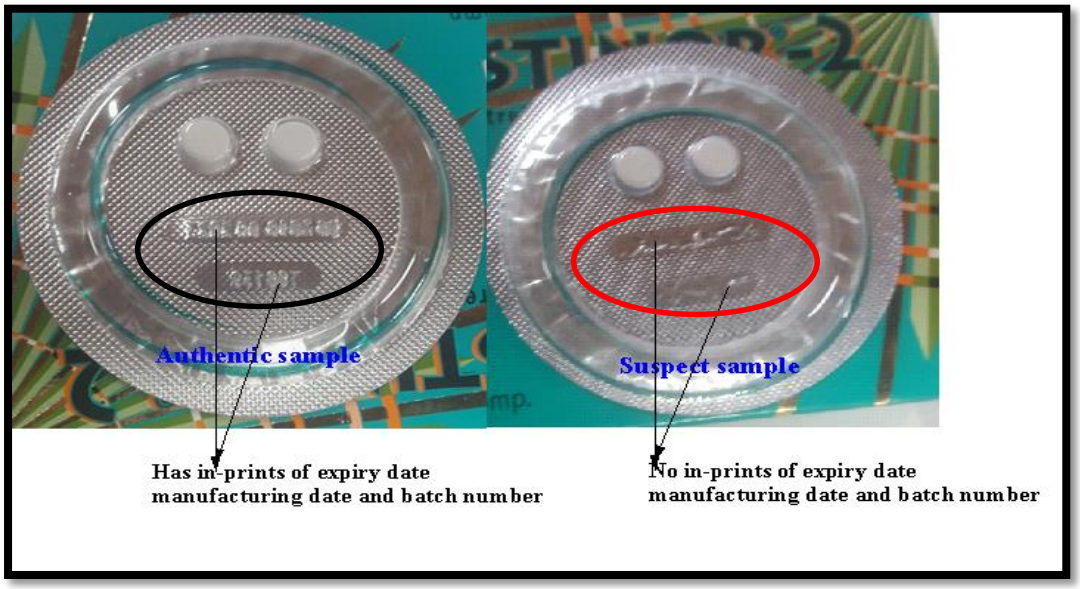

Figure 4: Pictorial of the Front side of the primary package of an authentic and a suspect sample

Appendix VIII: Statistical analysis conducted using one-way ANOVA at 95% confidence interval for mean drug content of brand B

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
B1	10	952.568	95.256791	0.814791
B2	10	932.817	93.281718	0.724817
B3	10	926.756	92.675633	0.954412
B4	10	929.256	92.925614	0.995177
B5	10	943.068	94.306844	1.470498
B6	10	941.174	94.117374	0.917302
B7	10	991.1	99.11	0.909889

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	292.954	6	48.82573	50.35891	3.45323E-22	2.246408
Within Groups	61.082	63	0.969555			
Total	354.036	69				

Appendix IX: Statistical analysis conducted using the student-t test at 95% confidence interval for mean drug content of brand D

t-Test: Two-Sample Assuming Equal Variances

	D1	*D2*
Mean	100.92	101.13
Variance	0.515111	0.744555556
Observations	10	10
Pooled Variance	0.629833	
Hypothesized Mean Difference	0	
df	18	
t Stat	**-0.591686**	
P(T<=t) one-tail	**0.28071**	
t Critical one-tail	1.734064	
P(T<=t) two-tail	**0.561419**	
t Critical two-tail	**2.100922**	