A COMPARATIVE QUALITY CONTROL STUDY OF IBUPROFEN TABLETS and AMOXICILIN CAPSULES AVAILABLE ON THE MARKET IN HARARE (ZIMBABWE): RP-HPLC AND SPECTROSCOPIC (UV-Vis and FTIR) CHARACTERISATION.

BY

NANCY NYARADZO GWAZIWA (B1335618)

SUPERVISOR: DR M. MUPA

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APPROVAL FORM

The undersigned certify that they have supervised, read and recommend to the Bindura University of Science Education for acceptance a research project entitled:

A comparative quality control study of ibuprofen tablets and amoxicillin capsules available on the market in Harare (Zimbabwe): RP-HPLC and spectroscopic (UV-Vis and FTIR) characterization.

Submitted by Nancy Nyaradzo Gwaziwa

In partial fulfilment of the requirements for the MASTERS OF SCIENCE ANALYTICAL CHEMISTRY (MSc ACH)

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(Signature of Student)  Date

..........................................................  …/…………/…………/  
(Signature of Supervisor)  Date

..........................................................  …/…………/…………/  
(Signature of the Chairperson)  Date
DECLARATION

I Nancy Nyaradzo Gwaziwa declare that this research project is my own work and has not been copied from any source without the acknowledgement of the source.

Signed……………………………………………………………………………………….
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<table>
<thead>
<tr>
<th>TABLE OF CONTENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHAPTER ONE: INTRODUCTION</strong></td>
</tr>
<tr>
<td>1.0 Background</td>
</tr>
<tr>
<td>1.1 Statement of the problem</td>
</tr>
<tr>
<td>1.2 Research Question</td>
</tr>
<tr>
<td>1.3 Aim of the study</td>
</tr>
<tr>
<td>1.4 Specific objectives of the study</td>
</tr>
<tr>
<td>1.5 Rationale</td>
</tr>
<tr>
<td>1.6 Significance of the study</td>
</tr>
<tr>
<td>1.7 Limitations</td>
</tr>
<tr>
<td>1.8 Delimitations</td>
</tr>
<tr>
<td><strong>CHAPTER TWO: LITERATURE REVIEW</strong></td>
</tr>
<tr>
<td>2.0 Introduction</td>
</tr>
<tr>
<td>2.1 Effects of counterfeit and substandard medicines</td>
</tr>
<tr>
<td>2.2 Reported cases of counterfeited medicines</td>
</tr>
<tr>
<td>2.3 Quality assessment studies on medicinal products</td>
</tr>
<tr>
<td>2.4 Detection of counterfeits in pharmaceutical products</td>
</tr>
<tr>
<td>2.4.1 Visual inspection</td>
</tr>
<tr>
<td>2.4.2 Spectroscopic techniques</td>
</tr>
<tr>
<td>2.4.3 Chromatographic techniques</td>
</tr>
<tr>
<td>2.4.4 Hyphenated techniques</td>
</tr>
<tr>
<td><strong>CHAPTER THREE: MATERIALS AND METHODS</strong></td>
</tr>
<tr>
<td>3.0 Introduction</td>
</tr>
<tr>
<td>3.1 Sample collection</td>
</tr>
<tr>
<td>3.2 Reagents</td>
</tr>
<tr>
<td>3.2.1 Preparation of Potassium Phosphate buffer pH 7.2</td>
</tr>
<tr>
<td>3.2.2 Preparation of Potassium Phosphate buffer pH 5.0</td>
</tr>
<tr>
<td>3.3 Equipment</td>
</tr>
<tr>
<td>3.4 Sample characterisation methods</td>
</tr>
<tr>
<td>3.4.1 Appearance</td>
</tr>
<tr>
<td>3.4.2 Uniformity of weight</td>
</tr>
</tbody>
</table>
CHAPTER FOUR: RESULTS

4.0 Introduction .................................................................................................................. 36
4.1 Appearance ..................................................................................................................... 36
4.2 Uniformity of mass ......................................................................................................... 37
4.3 Friability ........................................................................................................................ 38
4.4 Disintegration time ........................................................................................................ 39
4.5 Identification .................................................................................................................. 40
4.6 Quantitative HPLC test results ..................................................................................... 47
4.7 Dissolution test results .................................................................................................. 48
4.8 Statistical analysis of results ........................................................................................ 51
  4.8.1 ANOVA for ibuprofen quantitative chemical test results .................................... 51
    4.8.1.1 ANOVA for quantitative chemical test for all ibuprofen samples .................... 51
    4.8.1.3 ANOVA for quantitative chemical test for ibuprofen batch consistency ........... 52
    4.8.1.4 ANOVA for consecutive ibuprofen samples batch consistency ....................... 52
  4.8.2 ANOVA for amoxicillin quantitative chemical test results ..................................... 53
    4.8.2.1 ANOVA for for all the amoxicillin samples analysed ....................................... 53
  4.8.3 ANOVA for ibuprofen dissolution test results ......................................................... 54
    4.8.3.1 ANOVA for quantitative chemical test for all ibuprofen samples .................... 55
    4.8.3.2 ANOVA for ibuprofen dissolution test for the different brands ......................... 55
    4.8.3.3 ANOVA for ibuprofen dissolution test batch consistency ................................. 56
  4.8.4 ANOVA for amoxicillin dissolution test results ...................................................... 56
    4.8.4.1 ANOVA for dissolution test for all the amoxicillin samples analysed ............... 56
    4.8.4.2 ANOVA for dissolution test for amoxicillin batch consistency ........................ 57

CHAPTER FIVE: DISCUSSION ............................................................................................ 58

5.0 Introduction .................................................................................................................... 58
5.1 Appearance .................................................................................................................... 58
5.2 Uniformity of mass ........................................................................................................ 59
| 5.3  | Friability ............................................................................................................. 61 |
| 5.4  | Disintegration ......................................................................................................... 62 |
| 5.5  | Identification ......................................................................................................... 63 |
| 5.6  | Quantitative HPLC assay .......................................................................................... 66 |
| 5.7  | Dissolution ............................................................................................................... 69 |
| 5.8  | Conclusion .................................................................................................................. 73 |
| 5.9  | Recommendations ....................................................................................................... 74 |
LIST OF TABLES

Table 2.1 Examples of reported cases of counterfeit medicines.................................15
Table 3.1 List of ibuprofen samples analysed.....................................................................26
Table 3.2 List of amoxicillin samples analysed.................................................................26
Table 3.3 List of reagents used..........................................................................................27
Table 4.1 Results for % Friability of Ibuprofen Tablets...................................................37
Table 4.2 Ibuprofen assay test results...............................................................................46
Table 4.3 Amoxicillin assay test results.............................................................................46
Table 4.4 Dissolution test results......................................................................................47
Table 4.5 ANOVA for content API for all ibuprofen samples...........................................50
Table 4.6 ANOVA for content API for the different ibuprofen brands sampled..............51
Table 4.7 ANOVA for content API for different batches by same manufacturer..............51
Table 4.8 ANOVA for content API for products manufactured in the same month.........52
Table 4.9 ANOVA for content API for all amoxicillin samples.......................................53
Table 4.10 ANOVA for content API for different batches by same manufacturer.............53
Table 4.11 ANOVA for dissolution test for all ibuprofen samples....................................54
Table 4.12 ANOVA for dissolution test for different ibuprofen brands............................54
Table 4.13 ANOVA for dissolution test for the same manufacturer..................................55
Table 4.14 ANOVA for dissolution test for all amoxicillin samples..................................56
Table 4.15 ANOVA for dissolution for different batches by same manufacturer..............56
LIST OF FIGURES

Figure 2.1 Structure of ibuprofen.................................................................8
Figure 2.2 Structure of amoxicillin...............................................................9
Figure 2.3 Distribution of substandard and falsified medicines globally.........9
Figure 4.1 Ibuprofen Samples..............................................................35
Figure 4.2 Amoxicillin Samples ..........................................................36
Figure 4.3 Uniformity of mass results for Amoxicillin Capsules samples......36
Figure 4.4 Uniformity of mass results for Ibuprofen Tablets samples.........37
Figure 4.5 Disintegration time results.........................................................38
Figure 4.6 Amoxicillin HPLC profiles.....................................................39
Figure 4.7 Amoxicillin HPLC comparability results.................................40
Figure 4.8 Ibuprofen HPLC profiles .......................................................40
Figure 4.9 Ibuprofen UV profiles.............................................................41
Figure 4.10 Ibuprofen HPLC comparability...............................................42
Figure 4.11 Ibuprofen UV comparability results.........................................42
Figure 4.12 Amoxicillin UV profiles..........................................................43
Figure 4.13 Amoxicillin UV comparability results......................................44
Figure 4.14 Amoxicillin FTIR profiles.......................................................44
Figure 4.15  Ibuprofen FTIR profiles.................................................................45

Figure 4.16  Brand comparison of Ibuprofen dissolution profiles......................48

Figure 4.17  Brand consistency of ibuprofen dissolution profiles......................49

Figure 4.18  Brand comparison of Amoxicillin dissolution profiles....................49
LIST OF ACRONYMS

API          Active Pharmaceutical Ingredient
OTC         Over – the - counter
MCAZ       Medicines Control Authority of Zimbabwe
WHO         World Health Organisation
ART        Antiretroviral Therapy
HIV         Human Immuno-deficiency Virus
HPLC      High Performance Liquid Chromatography
UV-Vis    Ultra violet-visible
Ph.Int    International Pharmacopoeia
USP      United States Pharmacopoeia
BP          British Pharmacopoeia
PMS        Post Market Surveillance
ADR        Adverse drug reaction
ANOVA      Analysis of variance
SSFFC    Substandard, spurious, falsely labelled, falsified and counterfeit
Abstract

This research provided quantitative data of the quality of randomly selected ibuprofen tablets and amoxicillin capsules that are currently being sold on both the formal and informal market in Harare (Zimbabwe). Fourteen samples of ibuprofen tablets and eight of amoxicillin capsules were randomly selected from retail pharmacies and informal traders over a period of six months. Quality control tests which included physical appearance, uniformity of mass, disintegration test, friability, in-vitro dissolution test, assay by HPLC, spectroscopic (UV-Vis and FTIR) and HPLC profiling of the samples were also carried out.

Three out of the fourteen samples of ibuprofen tablets failed the uniformity of weight test. These three samples that failed were from the informal market. One out of these three that failed the uniformity of weight test also failed the quantitative chemical assay test and the dissolution test. Four other ibuprofen samples were from the same manufacturer but had differences in the packaging used therefore raising suspicion of the possibility of counterfeiting. Two more ibuprofen samples were from the same manufacturer but the appearance of these samples differed in shape size and colour and therefore failed the appearance test. The other samples of ibuprofen tablets passed all the tests done.

All the amoxicillin capsules samples passed all the tests done however ANOVA tests at 95 % confidence level showed that there’s a significant difference between the content of active pharmaceutical ingredient (API) and percentage drug release therefore samples did not contain equivalent amounts of API. All the samples tested contained the (API) ibuprofen and amoxicillin trihydrate as stated on the sample labels. There is need for a large scale study of different medicines found in Zimbabwe to properly quantify the extent of the problem.
Key words: ibuprofen, amoxicillin, quality control, API, friability, pharmacopeia, HPLC, FTIR
CHAPTER ONE: INTRODUCTION

1.0 Background

Pharmaceuticals are synthetic or natural chemicals that contain active ingredients that have been designed to have pharmacological effects (WHO, 2006). They can be found in prescription medicines, over-the-counter therapeutic drugs and veterinary drugs. With many new medicinal products released into the market regularly, it is difficult to keep track of the safety of every product (Avbunudiogba et al., 2013). Failure to keep track of medicinal products may lead to influx of poorly manufactured pharmaceutical products that may be classified as either substandard or counterfeit.

According to Deconinck et al. (2013), a counterfeit drug is one that is “deliberately and fraudulently mislabelled with respect to identity and/or source,” and substandard drugs are “genuine drug products which do not meet quality specifications set for them.” Counterfeits may include preparations with exact composition, or with substituted components, missing or lacking active component, or unknown products in false packing (Ali, 2000). Counterfeit products may either imitate original drugs or represent newly issued products (generics).

Ibuprofen is an over-the-counter anti-inflammatory drug that is widely used for the relief of pain caused by headache, toothache, backache, menstrual pains, minor injuries and arthritis (Giri et al., 2013; Okunlola et al., 2009; Hapse et al., 2011). The ability of ibuprofen to treat a wide range of mild to serious pain makes it a popular medicine and therefore prone to counterfeiting.

Amoxicillin trihydrate is an antibiotic and unlike ibuprofen which is an over-the-counter medicine, amoxicillin is a prescription medicine. It is used to treat bacterial infections, ulcers,
sinus infections, urinary tract infections and sexually transmitted infections in men and women. Generally, prolonged exposure to antibiotics may lead to an increased risk of treatment failure, relapse and the development of drug-resistant strains (Huda et al., 2009). According to a report by the WHO (2016), there are a number of the antibiotics that are falsified globally, but amoxicillin had the highest percentage (24 %) making it also more prone to counterfeiting. It is then followed by sulfamethoxazole (16 %) and the rest of the antibiotics that were falsified only had 7 % and below.

In Zimbabwe, among the wide range of pharmaceutical products that may be found on the informal market, ibuprofen tablets, amoxicillin and doxycycline capsules are very popular. Although it is a requirement by regulatory bodies that the quality of pharmaceutical products is essential to ensure the safety of the patients, consumers resort to purchasing prescription medicines from vendors rather than registered pharmacies because they are no questions asked as to the intended use. This protects them against stigmatisation, for example, in the case of purchasing antibiotics for treatment of sexually transmitted infections. In registered pharmacies, a doctor’s prescription is required before sale and some consumers may not afford consultation fees and subsequent tests required before a prescription can be granted.

The rapid materialization of medicinal products on the informal market has been caused to a large extent, by consumers’ dissatisfaction with medical costs thus shifting to unregulated products for treatment. This is a time bomb to the public health, particularly; the young, old, pregnant and the immune-compromised that use these products for medical treatments as the same products may find their way onto the formal market without being detected. These chemicals have become of major concern to the public because of their storage conditions and handling of these products. Some of these products may be sold after they have reached their
expiry dates and this has detrimental health effects as some degradation products (impurities) may have formed, the quantity of the active pharmaceutical ingredient may also be affected.

Most of the pharmaceutical products available on the market in Zimbabwe (both formal and informal) are from the international manufacturers and a number of consumers are not versatile in the names and labels on these products. The majority of these drugs (both from formal and informal market) are not sold in their original containers and therefore do not come with the package insert that contains directions on use, side effects and precautions to take during use.

Lack of information dissemination from the vendors to consumers and self-preservation of antibiotics is a health hazard to the end user and maybe the cause of strains like drug resistant tuberculosis. According to a report by Teklu et al. (2014), the problem of pharmaceutical counterfeiting is very common in many developing countries that are faced with an increased burden of both infectious and chronic diseases among other numerous public health issues. In the same report, they went on to explain that the health workforce in developing countries is overburdened, in short supply and are faced with the problem of poor quality medicines (Teklu et al., 2014).

1.1 Statement of the problem

Recent studies show that adults are self-prescribing with over-the-counter (OTC) medications in record numbers (Blandino et al., 2008). In some cases such as the recent report by a local paper in Zimbabwe, medications not restricted to OTCs are sold by vendors on the informal market. Since products are sold on the streets and by the roadsides, storage conditions of temperature and humidity stated on the product labels are compromised causing deterioration of the product. When patients choose their own drugs, they may lack the specialized knowledge to detect

***
whether the product they are buying is of good quality let alone are able to detect whether the product is forged or not (Teklu et al., 2014). Counterfeit drugs, usually illegally marketed and most probably illegally manufactured have become a noticeable problem in most countries recently (Filajek et al. 2011).

On a daily basis, many individuals around the world risk death or serious illness when they unknowingly use and consume counterfeit drugs and products manufactured, supplied and sold outside effective regulatory regimes (Filajek et al. 2011). A recent report by WHO (2016) confirmed what Teklu et al. (2014) had earlier noted that there is apparently a high incidence of the availability of substandard drugs in most developing countries.

1.2 Research question

How comparable is the quality of selected medicinal products available on the market in Harare to the WHO set standards and are consumers of these medicinal products in Zimbabwe at any health risk due to the quality of the pharmaceutical products available to them?

1.3 Aim of the study

The main goal of the research is to investigate, compare and evaluate the quality of different brands of ibuprofen tablets and an antibiotic in the form of amoxicillin capsules available on the local market in Harare as a form of post-market surveillance.

1.4 Specific objectives of the study

- To collect fourteen samples ibuprofen tablets and eight samples of amoxicillin capsules (each sample comprising of sixty tablets/capsules) over a period of six months.
To carry out quality control tests such as physical appearance, uniformity of mass, disintegration test, friability, in-vitro dissolution test, quantification of potency of the samples by HPLC, spectroscopic(UV-Vis and FTIR) and HPLC profiling of the samples.

To compare the results obtained with the limits set by the WHO standards

1.5 Rationale

The researcher is inspired by the rate at which medicinal products and cosmetics are being sold on the informal market without being controlled or tested for compliance with the stated WHO regulations. The consumers’ inability to judge the quality of medicines they take becomes a big public health problem as such drugs can be ineffective and harmful. Fake drugs have capacity to deceive, particularly if they are copied to make it look like the original product so that purchasers are unlikely to be suspicious (Nsimba, 2008).

In recent years, there has been an increase in the amount of drugs being smuggled into the country and although some of these products are already registered in their countries of origin, they are finding their way onto the informal market and therefore their quality cannot be ascertained (Filajek et al. (2011). Continuous quality control checks of these products for their safety and effectiveness is not feasible on the wide range of products available on this market. Besides the fear that the nation may be consuming substandard products, there is greater concern that since most of these products are flooding on the informal market they might find their way to registered pharmacies and be sold to unsuspecting consumers without their knowledge which exposes them to health risks.
According to a report by WHO (2007), the prevalence of fake medicines is higher in countries with weak regulations, enforcement, and scarcity of supply of basic medicines, unregulated markets and unaffordable prices. Nasri et al. (2011) sites these reasons in his report as the explanation why the quality, safety and efficacy of drug products especially in developing countries cannot be guaranteed:

1.6 Significance of the study

This research is meant to provide some quantitative data as a form of quality indicator to randomly selected ibuprofen tablets and amoxicillin capsules available on the market in Zimbabwe as they are common pharmaceutical products sold on the informal market. Only a few adverse drug reactions due to use of pharmaceutical drugs are reported to the appropriate enforcement agencies and therefore numbers of those affected by use of substandard, spurious, falsely labelled, falsified and counterfeit medicines is hugely underestimated (Hall et al., 2006). It is in cases such as these that the need of fast, easy and reliable methods of drugs screening is essential so that the risk the public is exposed to may be estimated.

1.7 Limitations

- Most manufacturing sites for the products sampled are based in China and India; therefore onsite product sampling and production system assessment could not be carried out at the time of study.
- Due to limited resources, quantification of degradation products could not be carried out which may also be a quality indicator on storage conditions.
1.8 Delimitations

- Pharmaceuticals have a wide range, the researcher had to pick a few active pharmaceutical ingredients for the research to represent the different categories of pharmaceuticals that are manufactured and analysed around the country.

- Criterion of chemical tests to be done was determined by the product label information.
CHAPTER TWO: LITERATURE REVIEW

2.0 Introduction

Ibuprofen tablets contain the active pharmaceutical ingredient ibuprofen, \((\pm) - 2 - (p\text{-isobutylphenyl})\) propionic acid with a structure as the one below,

![Chemical structure of Ibuprofen](image)

Figure 2.1 Chemical structure of Ibuprofen

In Zimbabwe, the price ranges for Ibuprofen tablets are around $0.50 - $1 for a blister pack of ten tablets (containing 200mg API per tablet) in registered pharmacies whereas on the informal market they range from $0.20 - $0.50 for the similar blister pack. These differences in prices may be one of the many reasons why most consumers in Zimbabwe may end up purchasing ibuprofen tablets from the informal market rather than registered pharmacies.

Amoxicillin is an antibiotic that is a prescription drug. The same prices prevail for both formal and informal market with a blister pack of ten capsules (containing 250mg of API per capsule) being sold for $1. The chemical structure of amoxicillin is shown in Figure 2.2,
Figure 2.2 Chemical structure of Amoxicillin

According to a report by the WHO (2016), substandard and falsified medical products are mainly prevalent in Africa with the distribution illustrated below,

![Distribution of substandard and falsified medicines globally (WHO, 2016)](image)

**Substandard and falsified medical products**

- Africa: 48%
- Europe: 24%
- America: 14%
- Eastern Pacific region: 5%
- Western Pacific region: 8%
- South East Asia: 1%

Figure 2.3 Distribution of substandard and falsified medicines globally (WHO, 2016)
Erhun et al. (2001) suggested a number of common reasons for the dominance of counterfeit medicines in many developing countries. These include:

- High cost of medicines on the formal market
- Chaotic drug distribution systems
- Leaky supply chain systems
- Scarcity and/or erratic medicines supply
- Vested interests both on the part of the regulatory officials and the counterfeiters
- Weak laws and lack of enforcement of existing laws
- Ignorance or low literacy rates
- Pervasive poverty
- Poorly equipped laboratories
- Underfunded regulatory authorities
- Poor handling and lack of adherence to good manufacturing practices
- High level of corruption in the health care system

These are some of the many reasons that may be linked to the high prevalence of medicinal product counterfeiting in developing countries. However, it is also important to note that counterfeiting is not only experienced in developing countries but also in developed countries (Deisingh, 2004; Deconinck et al., 2013). The medicines however may differ because of the differences in lifestyles and the market for certain drugs. In the United States of America for example, some drugs that are commonly counterfeited include those that are passed off as Viagra® (sildenafil citrate manufactured by Pfizer) for the treatment of erectile dysfunction, and
Lipitor® (atorvastatin calcium manufactured by Pfizer) for the treatment of elevated cholesterol (U.S. Food and Drug Administration, 2006).

According to a report by Deconinck et al. (2013), in industrialised countries the most prominently counterfeited therapeutic categories are lifestyle drugs that include weight loss and potency enhancement drugs. The major risk associated with these lifestyle drugs are chances of containing toxic compounds and impurities, the presence of undeclared active ingredients, too high amounts of active ingredients and inadequate information on proper use of the drugs (Blok-Tip et al., 2005).

2.1 Effects of counterfeit and substandard medicines

As many researchers agree, counterfeit pharmaceutical products are a threat to global public health, patients and the pharmaceutical industry (Dubois et al., 2007; Finlay, 2011). According to Finlay (2011), many counterfeit and substandard drugs are produced overseas, often in Southeast Asia, China, India, Nigeria, Russia, Mexico, Brazil, and Latin America. In the same report, Finlay goes on to explain that both genuine and illicit products are funnelled into the legitimate supply chain through freight forwarders, shipping companies, importers, diverters, tertiary and secondary wholesalers, and individual and online purchasers.

Consumers may be using drugs without the proper dosage or even without the proper ingredients, resulting in deterioration of their illness and potentially disability and death. Public health is threatened by the development of resistant strains of infectious agents (Dubois et al., 2007). Shakoor et al. (1997) suggested that treatment failure and drug resistance are possible consequences of the use of sub-standard drugs.
While counterfeiting incidents for medicinal drugs can potentially produce serious adverse effects, if the ingested product results in a lack of effective treatment, the patient’s life may not be in jeopardy. In contrast, the most sought-after drugs in developing countries, and therefore the most likely to be counterfeited, include those for the treatment of more serious conditions such as malaria and AIDS. The risk of taking a counterfeit medication for one of these conditions not only poses health risks based on whatever ingredients are in the product, but also a more serious outcome due to the lack of effective treatment (Nsimba, 2008).

In Kenya, for example, where malaria is a major public health concern, causing approximately 26,000 deaths in children under the age of five, counterfeit anti-malarial drugs is a major problem (Amin et al., 2005). A study that analysed samples of the two most common anti-malarial drugs in Kenya, sulphadoxine-pyrimethamine and amodiaquine found that about 40% of these drugs available for sale were of substandard quality (Amin et al., 2005).

The industry is threatened by damage to its reputation resulting from the devastation caused by counterfeit versions of medicines, as well as the reduced sales when counterfeits replace legitimate product in the supply chain. Products may end up on the shelves of local pharmacies and legitimate online retailers, or may be marketed directly to consumers via phony internet pharmacies or through personal black markets. Patients may either unknowingly purchase a counterfeit from a legitimate retailer, or knowingly purchase illicit product at cut rate prices through the informal market.
2.2 Reported cases of counterfeited medicines

A study done in Nigeria by Chinwendu (2008) recorded a dominant market of counterfeit drugs around the country. He concluded the abundance of these markets could be due to weakness in the regulatory agencies and the lack of support from the government. Even though the study did not focus on the quality of drugs, it showed the availability of counterfeit medication and their abundance in the market (Teklu et al, 2014).

One of the biggest disasters related to counterfeit pharmaceuticals was a case of vaccines given as a gift to the country of Niger from neighbouring Nigeria in 1995 to help contain the meningitis epidemic there (Corkburn, 2005). According to the report by the World Health Organisation (2006), the vaccines did not contain any active ingredient, but more than 50,000 people received the vaccine and this subsequently led to 2,500 deaths. This is one of the few documented cases of counterfeited products causing deaths on such a large scale, but there are other cases of large-scale counterfeiting schemes in which it is more difficult to estimate directly related deaths (Corkburn, 2005).

Anti-retrovirals (ARVs) used in the treatment of HIV and AIDS are targets that have the potential for the greatest global impact, but other diseases and conditions are always possible targets as well. If drugs for HIV and AIDS are counterfeited, for example, it would be difficult to directly attribute deaths to the defective products due to the relatively long course of the disease. Considering that approximately 40 million people are living with HIV today as reported by the World Health Organisation (2005), this presents enormous opportunities for drug counterfeiters.
to profit without easily being exposed especially in developing countries where stigmatisation of people living with HIV/AIDS is still prevalent (Caudron, 2008).

In October 2004, a doctor working for Medecins Sans Frontieres (MSF) in Darfur reported that a local donation of Ringer’s lactate infusions was contaminated with a fungal growth. Subsequent investigations revealed that weaknesses in the bottling and quality control procedure during manufacture led to the contamination. The product then passed through three intermediates, including one UN agency, before being offered to relief agencies in Darfur, only one of which reported the problem. The World Health Organization (WHO) and the supplier jointly issued a recall of the contaminated batches. Six months after the recall, however, less than 15% (2200 of 15 000 bottles) of the contaminated product had been located (Caudron, 2008)

According to a report by Filajek et al. 2011, in Poland, in the year 2009, 33 preparations were analysed with anabolic steroids (e.g. testosterone, methandienone, oxymetholone). It was reported that samples delivered by the governmental institutions had originated from the illegal market or were suspected of being counterfeited. It was found that 33% of tested preparations with anabolic steroids contained incorrect active ingredient, 9% had no active ingredient, 58% products complied with the declaration — they contained declared active ingredient, but they were illegally manufactured and/or illegally marketed (Filajek et al. 2011).
Table 2.1 Other examples of reported cases of counterfeited medicines

<table>
<thead>
<tr>
<th>Counterfeit medicine</th>
<th>Country/year</th>
<th>Report</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alprazolam (anti-anxiety drug)</td>
<td>Canada, 2007</td>
<td>Pills found with high levels of aluminum, titanium, arsenic, and other metals (led to Canada's first casualty on fake drugs)</td>
<td>Finlay, 2011</td>
</tr>
<tr>
<td>Xenical (obesity medication)</td>
<td>United States of America, 2007</td>
<td>Contained no active ingredient and sold via Internet sites operated outside of the United States</td>
<td>Finlay, 2011</td>
</tr>
<tr>
<td>Cavinton (cardiovascular conditions and cerebral insufficiency)</td>
<td>Russia, 2006</td>
<td>Medication included foreign substances. About 600 boxes of false Cavinton discovered in warehouse.</td>
<td>Finlay, 2011</td>
</tr>
<tr>
<td>Avastin (for cancer treatment)</td>
<td>United States of America, 2012</td>
<td>Affected 19 medical practices in the USA. The drug lacked active ingredient</td>
<td>WHO, 2010</td>
</tr>
<tr>
<td>Viagra and Cialis (for erectile dysfunction)</td>
<td>United Kingdom, 2012</td>
<td>Smuggled into the UK. Contained undeclared active ingredients with possible serious health risks to the consumer</td>
<td>WHO, 2010</td>
</tr>
<tr>
<td>Drug</td>
<td>Country, Year</td>
<td>Issue Description</td>
<td>Source</td>
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<tr>
<td>-------------------------------------------</td>
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<td>-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>--------------------------</td>
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<tr>
<td>Alli (weight-loss medicines)</td>
<td>United States of America, 2010</td>
<td>Smuggled into the USA. Contained undeclared active ingredients with possible serious health risks to the consumer</td>
<td>WHO, 2010</td>
</tr>
<tr>
<td>Anti-diabetic traditional medicine (used to lower blood sugar)</td>
<td>China, 2009</td>
<td>Contained six times the normal dose of glibenclamide (a chemical used to lower blood sugar). Two people died, nine people were hospitalized</td>
<td>WHO, 2010</td>
</tr>
<tr>
<td>Metakelfin (antimalarial)</td>
<td>United Republic of Tanzania, 2009</td>
<td>Discovered in 40 pharmacies. The drug lacked sufficient active ingredient</td>
<td>WHO, 2010</td>
</tr>
<tr>
<td>Zyprexa (biopolar disorder and schizophrenia)</td>
<td>United Kingdom, 2006</td>
<td>Detected in the legal supply chain; lacked sufficient active ingredient</td>
<td>Finlay, 2011</td>
</tr>
<tr>
<td>Nandrolone (treats osteoporosis and aplastic anaemias)</td>
<td>Spain, 2004</td>
<td>Drugs had inadequate amounts of active ingredients. Discovery led to the largest counterfeit drug bust in Spain's history.</td>
<td>Finlay, 2011</td>
</tr>
<tr>
<td>Cialis (erectile dysfunction)</td>
<td>Singapore, 2004</td>
<td>Pills included active ingredients, but also consisted of varying amounts of other medication (sildenafil) to compensate for low potency.</td>
<td>Finlay, 2011</td>
</tr>
</tbody>
</table>

2.3 Quality assessment studies on medicinal products
In a controlled study reported by Shakoor et al., 1997 in Nigeria and Thailand, samples of chloroquine and selected antibiotics from pharmacies were analysed using appropriately validated methods based on High-Performance Liquid Chromatography (HPLC). The results indicated that 36.5% of the samples were sub-standard with respect to pharmacopoeial limits. In the same report, decomposition was cited as the cause of poor quality in a number of samples but overall bad manufacturing practices appeared to be prevalent. The analyses generated little evidence to implicate fraudulent manufacturing.

In studies assessing the quality of anti-malarials (chloroquine and sulfadoxine/pyrimethamine) in Yemen, the results indicated high and low failures in ingredient content for chloroquine tablets and chloroquine syrup (Abdo-Rabbo et al., 2005). In the same research, it was discovered that there were some dissolution failures for chloroquine tablets and high sulfadoxine/pyrimethamine tablets dissolution failures. It was feared these medicaments could have reduced therapeutic effectiveness that leads to the development of drug resistance (Abdo-Rabbo et al., 2005).

There are a number of factors that affect the quality of medicinal products from the raw materials used in the manufacture, the manufacturing process, packaging and storage of the final products. Kayumb et al. (2004) studied the quality of essential antimicrobial and anti-malarial drugs; amoxicillin capsules, metronidazole tablets, sulfamethoxazole/trimethoprim tablets, quinine tablets and sulfadoxine/pyrimethamine tablets in Rwanda and Tanzania. It was established that some of these drugs were substandard at time of purchase, deteriorated during storage under simulated tropical conditions and had a poor in-vitro drug release (Kayumba et al., 2004).
In Uganda, poor quality of drugs at various distribution points has also been reported by Ogwal-Okeng et al. (2003). In their research, they noted that a significant proportion of chloroquine on the Ugandan market did not meet the required pharmacopoeia quality standards. The study pointed out that even though there was routine inspection and analysis of the incoming drugs for content of the active ingredients and other pharmaceutical properties by the National Drug Authority (NDA), more vigilance and routine checks of the drugs on the market was a necessity. Nsimba also went on to suggest that quality issues of all pharmaceutical drugs including ATVs may be observed since some batches of drugs find their way into the country unofficially and storage facilities are not up to the required standard (Nsimba, 2008).

In Tanzania, according to a report by Risha et al. (2003), it was established that some drug formulations imported into the country were not optimized for stability in a tropical country. To justify this theory, dissolution rate of two diclofenac formulations was studied under stressed conditions (accelerated stability tests). From the study of the dissolution rate, Risha et al. (2003) concluded that the rate reduced significantly during storage under class four conditions (40°C, 75% relative humidity). These are some of the few known examples of high rates of counterfeited drugs in developing countries, but represent a larger global concern.

2.4 Detection of counterfeits in pharmaceutical products

Counterfeit and substandard pharmaceutical products pose a major challenge for analytical laboratories to identify and characterize them (Deconinck et al., 2013). If fraudulent products are suspected, tests such as colour reactions, spectroscopic, isotopic characterisation and various types of chromatography, including thin-layer, gas, and high performance liquid chromatography
can be used to help determine the contents (Layloff, 2006; Deisingh et al., 2004). Some of these tests are relatively inexpensive, such as colour reactions and thin-layer chromatography, but as the technology of the counterfeiters increase, more expensive tests may be needed to detect a fraud.

2.4.1 Visual inspection

According to Nsimba (2008), the current first step towards identifying counterfeit medicines is the routine checking of packaging and use of covert markers and security features such as holograms. Manufacturers have specific descriptions and markers for their products. In performing visual inspection of products, consistency of adherence to good manufacturing practices is assessed. As soon as suspect counterfeit medicines have been sighted in the marketplace, they are further analyzed in the laboratory to confirm that they are counterfeit as sometimes pharmaceuticals are made to be so similar to brand name products that it is virtually impossible to prove that they are not authentic, sometimes even by the brand name manufacturer (Nsimba, 2008).

2.4.2 Spectroscopic techniques

Deconinck et al. (2013) reported that spectroscopic methods of detecting counterfeits such as Raman and Infrared spectroscopy have been given first priority in the past. Infrared spectrophotometry on the other hand, although having limited applicability, is a simple and reliable means of distinguishing imitated and original drugs (Ali, 2000). Standard and sample spectra are compared and a deduction can be made on originality of the claimed active pharmaceutical ingredient.
There has been quite some interest in using near-infrared (NIR) spectroscopy (Olsen et al., 2002; Yoon, 2005; Vredenbregt et al., 2006; Rodionava et al., 2005). However due to evolution in the form of counterfeiting, chromatographic methods are becoming more important. According to Deconinck et al., 2013, spectroscopic techniques, particularly NIR and Raman spectroscopy, are still very popular in the detection of counterfeited and illegal preparations. The spectroscopic methods surely have many advantages, especially for the detection of counterfeit medicines, in which the spectrum of a sample can be compared to that of the genuine product. However, in the analysis of illegal pharmaceutical preparations, like imitated medicines or adulterated dietary supplements, these methods have the disadvantage of being a whole sample approach.

For the detection of a chemical compound in a matrix, especially in an herbal matrix with spectroscopy, the compound should be present in a considerable dose and no masking effects from matrix compounds should occur. If this is not the case, quicker and portable screening techniques may be employed by the customs at border posts. Hand-held Raman spectroscopy, minilab or CD3/CD3+ may be used where it is possible to classify a sample as legal and safe based on the spectroscopic results alone, especially when the sample is not sent to a laboratory. Furthermore, in the case of adulterated dietary supplements, it is not unthinkable that illicit producers add components to the matrix to mask the synthetic compounds from detection with spectroscopy.

As suggested by Dubois (2007), the basic tool of the trade for spectroscopists, the spectrometer, imposes limitations in the analysis of manufactured products involving increasingly complex formulations and product engineering. As a result, it cannot respond to the need for a better
understanding of the physical and spatial parameters impacting the performance or quality in process or of finished pharmaceutical products.

These disadvantages of the spectroscopic techniques can be solved by applying separation methods such as chromatography, in which the synthetic compounds are first extracted from the matrix and then separated on the TLC plate, chromatographic column or capillary, depending on the chosen technique. After separation, the components are separately detected, identified and quantified if necessary. When an unknown component is detected, MS and spectroscopic techniques (FT-IR or NMR) can be applied for structural elucidation.

2.4.3 Chromatographic techniques

Traditional methods of analysis for suspect counterfeit drug products include chromatographic assays for purity, potency, and content uniformity, and the dissolution testing (which basically represent the quality assurance/quality control testing normally carried out on genuine drug products). A review of the analysis of counterfeit medicines by Olsen et al. (2006) and Deisingh (2005) showed a variety of analytical techniques being employed.

In the field of health sciences, separation methods and chromatography are preferred over spectroscopic methods. This is because it is not enough to divide samples into counterfeit and genuine; they should also be evaluated for the risk they represent to the patient or to the public health. In this case, chromatographic fingerprinting can be of interest, because it not only allows identifying and quantifying the active ingredients and detecting counterfeit products, but also provides a complete image of the product.
High performance liquid chromatography (HPLC) and gas chromatography (GC) are the reliable methods for verification of the purity of pharmaceutical preparations. Most substandard and counterfeited drugs are manufactured using cheaper and in most cases low quality raw materials and these will be detected using either HPLC or GC reliably (Arzamastsev et al. 2004). Potency of the API will be quantitatively determined concurrently with impurities and related compounds. Profiles of samples and standards can be compared to detect any variation. Many impurities in the fingerprint, mean that it is a counterfeit of low quality, and thus, potentially dangerous. Additionally, as described by earlier work reported by Zhou et al. (2011), a chromatographic fingerprint can reveal the presence of a chemical substance (concentration 0.1%) in a dietary supplement presumed to be of herbal origin.

In previous work carried out by Kumasaka et al. (2005), thin layer chromatography and liquid chromatography were successfully used for the screening and quantitative analysis of six sulfonylurea type oral antidiabetic drugs used as adulterants in health food.

2.4.4 Hyphenated techniques

Liquid chromatography can be coupled with mass spectroscopy to enhance detection of compounds in the sample. According to a report by Zhou et al. (2011), liquid chromatography and mass spectroscopy have been successfully used for the detection of undeclared synthetic compounds in herbal medicines or dietary supplements with complex ingredients. LC and LC/MS are most widely used because of their high separation capacity, high sensitivity and are selective with identification of the peaks of main molecular ions (Rudzki, 2010). The tandem arrangement of detectors is mainly to get maximum qualitative and quantitative data from a single sample by fully utilising the advantages of each detector (Mesmer et al., 1997).
The main disadvantage of LC and LC/MS is that they are time consuming for the quality control and marketing criteria of numerous herbal products that they are widely used for. LC-MS-MS configurations are not very popular in Africa because of their cost. These configurations can be used for the analysis of the secondary quadrupole fragmentation of the drug, and comparison with the mass spectra of standards. In recent years liquid chromatography coupled with mass spectrometry (LC-MS) has become the most popular technique applied in the pharmacokinetic studies such as bioavailability and bioequivalence investigations.

Earlier work published by the USFDA (Westenberger et al., 2005) pointed to the additional information contained in NIR chemical images of tablets purchased on the internet, and the potential value of this additional knowledge in qualifying both the potency and the quality of the formulation as a whole. In a study done by Dubois et al. (2007), it was shown that a low-magnification measurement was ideal for the authentication of tablets based upon the active ingredient. From the same work, the researchers concluded that a higher magnification such as use of Near-infrared chemical imaging (NIR-CI) could provide additional information that we associate with formulation and process differences, and ultimately to the origin of the counterfeit tablet. Selecting the appropriate magnification ensures both a relevant answer to a particular question and optimal use of analysis time (Dubois et al., 2007).

Near-infrared chemical imaging (NIR-CI) is equally applicable to the detection of products in which incorrect excipients have been used. As the name suggests, it is the combination of near infrared spectroscopy and chemical imaging and therefore may be used to visualise the spatial
distribution of the chemical compounds in a sample (Ravn et al., 2008). Where a suspected counterfeit product contains the correct active ingredient, or low levels of active ingredient, together with common excipients that match a genuine product composition, alternative analytical techniques should be considered in addition to NIR-CI for determining the provenance of the product.

For simple counterfeit detections spectroscopic methods are very useful, but they have some disadvantages in the detection of adulterations and the evaluation of the risk for public health. Separation techniques have the advantage to allow a complete analysis of the sample: detection/identification of active substances, classification as counterfeit, imitation or genuine and risk evaluation. The disadvantages are the limited possibilities form miniaturization and application in portable devices. In conclusion, all techniques, spectroscopic and chromatographic, have their uses in the detection and analysis of illegal pharmaceutical preparations, depending on the purpose of the study.
CHAPTER THREE: MATERIALS AND METHODS

3.0 Introduction

This chapter describes how samples were collected, analysed and how results were analysed. The research focused on two products namely, ibuprofen tablets and amoxicillin capsules.

3.1 Sample collection

Fourteen samples of ibuprofen tablets and eight samples of amoxicillin capsules (each sample containing sixty tablets/capsules) were collected over a period of six months. Sampling was carried out in Harare, the capital city of Zimbabwe and has the greatest population compared to other cities hence represents the greater population of Zimbabwe. It was also discovered that a higher concentration of informal sale of pharmaceutical products is experienced in the market areas of Zimbabwe. For this research, samples were collected from Mbare Musika bus terminus, Market Square bus terminus, ‘Mupedzanhamo’ market and control samples were collected from the formal and registered pharmacies.

Mbare Musika is one of the biggest bus terminuses in Zimbabwe and also has a section that operates as a fruits and vegetable market. Most informal traders sell produce to travellers who board buses and passers-by. Market square is also a popular bus terminus as it is located on the ‘downtown’ side of town. Mupedzanhamo is well known for sale of second hand clothes. As all these three areas are well known for being crowded most of the times during the day and also for active traders, they were chosen to represent the general population of other markets in the country.
### Table 3.1  List of ibuprofen samples analysed

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Batch Number</th>
<th>Date Manufactured</th>
<th>Expiry Date</th>
<th>Labelled strength</th>
<th>Country of Origin</th>
<th>Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>131162</td>
<td>10/2013</td>
<td>09/2017</td>
<td>200mg</td>
<td>Kenya</td>
<td>Informal</td>
</tr>
<tr>
<td>B</td>
<td>998</td>
<td>01/2015</td>
<td>12/2016</td>
<td>200mg</td>
<td>India</td>
<td>Informal</td>
</tr>
<tr>
<td>C</td>
<td>57397</td>
<td>10/2013</td>
<td>10/2018</td>
<td>200mg</td>
<td>Cyprus</td>
<td>Formal</td>
</tr>
<tr>
<td>D</td>
<td>130911</td>
<td>Not given</td>
<td>09/2017</td>
<td>200mg</td>
<td>China</td>
<td>Informal</td>
</tr>
<tr>
<td>E</td>
<td>131170</td>
<td>11/2013</td>
<td>10/2017</td>
<td>200mg</td>
<td>Kenya</td>
<td>Informal</td>
</tr>
<tr>
<td>F</td>
<td>1012</td>
<td>05/2015</td>
<td>04/2017</td>
<td>200mg</td>
<td>India</td>
<td>Informal</td>
</tr>
<tr>
<td>G</td>
<td>65101</td>
<td>07/2015</td>
<td>07/2020</td>
<td>200mg</td>
<td>Cyprus</td>
<td>Formal</td>
</tr>
<tr>
<td>H</td>
<td>150402</td>
<td>Not given</td>
<td>04/2019</td>
<td>200mg</td>
<td>China</td>
<td>Informal</td>
</tr>
<tr>
<td>I</td>
<td>ITG4012</td>
<td>01/2014</td>
<td>12/2016</td>
<td>200mg</td>
<td>India</td>
<td>Formal</td>
</tr>
<tr>
<td>J</td>
<td>IDG4032</td>
<td>Not given</td>
<td>02/2017</td>
<td>400mg</td>
<td>India</td>
<td>Formal</td>
</tr>
<tr>
<td>K</td>
<td>120657</td>
<td>Not Given</td>
<td>11/2016</td>
<td>200mg</td>
<td>Not Given</td>
<td>Formal</td>
</tr>
<tr>
<td>L</td>
<td>IBU207</td>
<td>01/2015</td>
<td>12/2017</td>
<td>400mg</td>
<td>India</td>
<td>Formal</td>
</tr>
<tr>
<td>M</td>
<td>1013</td>
<td>05/2015</td>
<td>04/2017</td>
<td>200mg</td>
<td>India</td>
<td>Informal</td>
</tr>
<tr>
<td>N</td>
<td>1014</td>
<td>05/2015</td>
<td>04/2017</td>
<td>200mg</td>
<td>India</td>
<td>Informal</td>
</tr>
</tbody>
</table>

### Table 3.2 List of amoxicillin samples analysed

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Batch Number</th>
<th>Date Manufactured</th>
<th>Expiry Date</th>
<th>Labelled strength</th>
<th>Country of Origin</th>
<th>Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1098</td>
<td>02/2015</td>
<td>01/2017</td>
<td>250mg</td>
<td>India</td>
<td>Informal</td>
</tr>
<tr>
<td>B</td>
<td>150351</td>
<td>Not given</td>
<td>03/2019</td>
<td>250mg</td>
<td>China</td>
<td>Informal</td>
</tr>
<tr>
<td>C</td>
<td>150351</td>
<td>Not given</td>
<td>03/2019</td>
<td>250mg</td>
<td>China</td>
<td>Informal</td>
</tr>
<tr>
<td>D</td>
<td>AX 327</td>
<td>08/2014</td>
<td>07/2016</td>
<td>250mg</td>
<td>India</td>
<td>Informal</td>
</tr>
<tr>
<td>E</td>
<td>Not given</td>
<td>Not given</td>
<td>Not given</td>
<td>250mg</td>
<td>Not given</td>
<td>Formal</td>
</tr>
<tr>
<td>F</td>
<td>150351</td>
<td>Not given</td>
<td>03/2019</td>
<td>250mg</td>
<td>China</td>
<td>Informal</td>
</tr>
<tr>
<td>G</td>
<td>150351</td>
<td>Not given</td>
<td>03/2019</td>
<td>250mg</td>
<td>China</td>
<td>Informal</td>
</tr>
</tbody>
</table>
3.2 Reagents

Chemicals used for various experiments were of analytical reagent grade unless otherwise stated. Ibuprofen and Amoxicillin reference standards were obtained from United States Pharmacopoeia Laboratories (Rockville, USA), Lot Numbers KOJ009 and KOH332 respectively. Water for HPLC analysis was Millipore purified. Table 3.2 lists the reagents used and their respective manufacturers.

Table 3.3 List of reagents used

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Purity grade</th>
<th>Manufacturer</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>Analytical reagent</td>
<td>Associated Chemicals Enterprises (Pty) Ltd</td>
<td>32477</td>
</tr>
<tr>
<td>Hexane</td>
<td>Analytical reagent</td>
<td>Associated Chemicals Enterprises (Pty) Ltd</td>
<td>12158/3783</td>
</tr>
<tr>
<td>Acetic acid glacial</td>
<td>Analytical reagent</td>
<td>Glassworld</td>
<td>2010/05/05</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Analytical reagent</td>
<td>Romil Ltd</td>
<td>P640461</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Analytical reagent</td>
<td>Romil Ltd</td>
<td>A658421</td>
</tr>
<tr>
<td>Sulphuric acid</td>
<td>Analytical reagent</td>
<td>Minema</td>
<td>S9510</td>
</tr>
<tr>
<td>Orthophosphoric acid</td>
<td>Analytical reagent</td>
<td>Minema</td>
<td>P2570</td>
</tr>
<tr>
<td>Potassium hydrogen phosphate (monobasic)</td>
<td>Analytical reagent</td>
<td>Surechem Products Ltd</td>
<td>P5302</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>Analytical reagent</td>
<td>Surechem Products Ltd</td>
<td>S4602</td>
</tr>
<tr>
<td>Potassium hydroxide</td>
<td>Analytical reagent</td>
<td>Surechem Products Ltd</td>
<td>P6122</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>HPLC Grade</td>
<td>Romil Ltd</td>
<td>Q649451</td>
</tr>
<tr>
<td>Methanol</td>
<td>HPLC Grade</td>
<td>Merck</td>
<td>1746607 431</td>
</tr>
</tbody>
</table>
3.2.1 Preparation of Potassium Phosphate buffer pH 7.2

Monobasic potassium phosphate (40.85 g) and 9 g of sodium hydroxide were dissolved in a litre of HPLC grade water. The solution was diluted to 6 litres using HPLC grade water and the pH of the solution adjusted 7.2 ± 0.1 using sodium hydroxide solution (1 M).

3.2.2 Preparation of Potassium Phosphate buffer pH 5.0

Monobasic potassium phosphate (6.8 g) was dissolved in a litre of HPLC grade water. A 45% w/w solution of potassium hydroxide (prepared by dissolving 45g of potassium hydroxide in 100g of purified water) was used to adjust the pH of the buffer to pH 5.0 ± 0.1.

3.3 Equipment

A Shimadzu LC-20AD HPLC (Japan) equipped with a SPD-2AD UV/Vis detector was used for HPLC profiling and quantitative determination of API. A Thermo Fisher Scientific Nicolet FTIR (Japan) spectrophotometer equipped with iAD7 accessory and OMNIC software was used for spectrophotometric profiling. A Distek apparatus (USA) was used for dissolution tests and a Shimadzu UV1800 UV/Vis spectrophotometer (Japan) was used for quantitative determination of API dissolved in the media. For the friability test, an Erweka friabilator (Germany) was used. An Adwa pH meter was used for measurement of the buffer pH. The Sartorius A200S and AnD GH-202 analytical balances were used in this research. Annexure 1 provides a comprehensive list of the equipment used.
3.4 Sample characterisation methods

3.4.1 Appearance

Visual inspection of samples collected was carried out on twenty units each for uniformity of shape and colour. Samples were also checked for any chipping, cracking and packaging used. Details on the packaging were checked for manufacturing dates, expiry dates, batch numbers and country of origin.

3.4.2 Uniformity of weight

The method for uniformity weight determination was adapted from Okunlola et al. (2009). Ibuprofen tablets (20) were weighed individually and the percentage relative standard deviation from the mean of these tablets used to determine the uniformity. For the amoxicillin capsules, the method was adapted from Avbunudiogba et al. (2013). Twenty capsules were weighed with the powder, emptied and then the shell (without contents) weighed to obtain the mass of fine powder in each of them. The percentage relative standard deviation from the mean of these capsules was used to determine the uniformity.

3.4.3 Friability

The friability test method was adapted from Giri et al. (2013). Twenty tablets were weighed initially and then placed in a friabilator for 4 minutes and then the final weight determined. The friability of the tablets was calculated using equation (3.1),

\[
\% F = \frac{W_1 - W_2}{W_2} \times 100 \quad \text{(Equation 3.1)}
\]

Where \( \% F \) is the \% friability, \( W_1 \) is the initial weight and \( W_2 \) is the final weight. According to Giri et al. (2013), the friability value of tablets should be less than 1.0 \%.
3.4.4 Disintegration time

Disintegration test method was adapted from Dewan et al. (2013). Six tablets were individually placed in 800mL of water equilibrated to and maintained at 37 ± 0.5º C and the disintegrator operated for 30 minutes. The time taken for each tablet or capsule to completely disintegrate was recorded.

3.4.5 Identification

HPLC profiling of the assay samples was used for identification purposes. The standard and sample solutions were injected and the retention times of the peaks compared using the equation (3.2),

\[ \text{Compatibility} = \frac{\text{mean standard retention time}}{\text{mean sample retention time}} \times 100 \% \]  

(Equation 3.2)

UV-Vis profiling was also carried out on the dissolution samples. The spectrum of the standard and sample solutions were read in the range 200 – 300 nm for ibuprofen samples and 222-322 nm for amoxicillin samples. Comparability of the maximum peak wavelengths (\( \lambda_{\text{max}} \)) of standard and sample solutions was also calculated using equation (3.3),

\[ \text{Compatibility} = \frac{\lambda_{\text{max of standard}}}{\lambda_{\text{max of sample}}} \times 100 \% \]  

(Equation 3.3)

where \( \lambda_{\text{max}} \) is the maximum peak wavelength.

FTIR profiling of the samples was carried out using a ThermoScientific ATR-FTIR (Nicolet iS5, iD7 ATR) instrument, with an IR source, KBr beamsplitter and DTGS KBr detector between 400 – 4000 cm\(^{-1}\) for both ibuprofen and amoxicillin samples.
3.4.6 Quantitative HPLC Analyses

An HPLC method adapted from Sunaric et al. (2013) was used for the determination API in the ibuprofen samples. A Shimadzu LC-20AD HPLC (Japan) equipped with a SPD-2AD UV/Vis detector was used for this test. Twenty tablets were weighed and ground into a fine powder. A quantity of the powdered tablets containing 200 mg of ibuprofen was weighed into a 100 mL volumetric flask, shaken with about 30 mL of the mobile phase and sonicated for 30 minutes. The suspension was allowed to cool to room temperature and made up to volume with the mobile phase. This suspension was then centrifuged for 5 minutes at 2500 rpm. The supernatant liquid was used as the sample solution. The chromatographic conditions were as follows: Mobile phase - Methanol: Water: Orthophosphoric acid (750:247:3), C18, 5 µm Luna column: 4.6 mm x 15 cm, flow rate: 1.0 mL/min, injection volume: 10 µL, detection: 264 nm. The percentage released API was calculated using equation (4),

\[
\text{% Content} = \frac{A_s \times \text{Potency} \times \text{sample dilution factor}}{A_u \times \text{specific gravity} \times \text{label claim}} \times 100\% \quad \text{(Equation 3.4)}
\]

Where \(A_u\) is the mean area response of the sample and \(A_s\) is the mean area response of the standard, \(C_{std}\) is the concentration of standard in µg/mL.

An HPLC method adapted from United States Pharmacopoeia, 2015 was used for the determination API in the amoxicillin capsules samples. The contents of 20 capsules were emptied and homogenised. An amount equivalent to 200mg of anhydrous amoxicillin was weighed into a 200mL volumetric flask. The diluent (100 mL of monobasic potassium phosphate buffer pH 5.0) was added to the flask and sonicated for 10 minutes to ensure complete
dissolution in an ultrasonic bath. Amoxicillin reference standard (12 mg) were weighed into a 10mL volumetric flask and dissolved in the diluent (monobasic potassium phosphate buffer pH 5.0). The solution was sonicated for 10 minutes to ensure complete dissolution and then cooled and made up to the mark using the diluent.

The chromatographic conditions were as follows: Mobile phase–Acetonitrile: Buffer (1:24), C\textsubscript{18}, 5 µm Luna column: 4.6 mm x 15 cm, flow rate: 1.5 mL/min, injection volume: 10 µL, detection: 230 nm. The percentage released API was calculated using equation (3.4).

**3.4.7 Dissolution**

The dissolution test method for ibuprofen tablets was adapted from Giri et al., (2013). 900ml of phosphate buffer solution of pH 7.2 that had been brought to an equilibrium temperature of 37 ± 0.5º C was placed in each of the six vessels. To each of the vessels, one tablet was placed and the dissolution process (using paddles rotated at 50 revolutions per minute) was monitored over a 90-minute period. 5mL solutions were withdrawn at the following intervals with replacement, 5, 10, 15, 20, 30, 45, 60 and 90 mins and filtered through a 0.45 µm filter. 1 mL of the filtrate was pipetted into a 20 mL volumetric flask and made up to volume with the dissolution media. The amount of API was determined using a UV/Vis spectrophotometer set at 220 nm.

Ibuprofen reference standard (10mg) was weighed into a 50mL volumetric flask and dissolution medium added to this flask. The solution was sonicated for 15 minutes until dissolved, cooled and made up to volume with medium. 1mL of the solution was pipetted into a 20mL volumetric flask and made up to volume with the dissolution media. The percentage API released was calculated using equation 3.5,

\[
\% \text{ Content} = \frac{A_{\text{sample}} \times \text{specific gravity} \times \text{label claim}}{A_{\text{reference}} \times \text{sample dilution factor}} \times 100\% \quad (\text{Equation 3.5})
\]
Where $A_u$ is the mean area response of the sample and $A_s$ is the mean area response of the standard, $C_{\text{std}}$ is the concentration of standard in µg/mL.

For the amoxicillin capsules, the dissolution test method was adapted from the United States Pharmacopoeia, 37 NF 32. 900mL of purified water that had been brought to an equilibrium temperature of $37 \pm 0.5^\circ C$ was placed in each of the six vessels. To each of the vessels, one capsule was placed and the dissolution process (using baskets rotated at 100 revolutions per minute) was monitored over a 90-minute period. 5mL solutions were withdrawn at the following intervals with replacement, 5, 10, 15, 20, 30, 45, 60 and 90 mins and filtered through a 0.45 µm filter. The amount of API was determined using a UV/Vis spectrophotometer set at 272 nm.

Amoxicillin reference standard (13 mg) was accurately weighed into a 50mL volumetric flask and water medium added to this flask. The solution was sonicated for 15 minutes until dissolved, cooled and made up to volume with water. The percentage API released was calculated using equation (3.5).

3.5 Statistical analysis of results

One-way analysis of variance (ANOVA) was applied to the results of assay and dissolution in order to find variation between brands and batches for both amoxicillin capsules and ibuprofen tablets. Student’s t-test was used to analyse batch to batch consistency of different batches of the same product by the same manufacture. In cases where a manufacturer had more than two batches that were sampled, ANOVA was used to test batch to batch consistency.

Results will presented in tabular form, with the following definitions;

$H_0$: Null hypothesis

$H_1$: Alternative hypothesis
SSB: sum of squares between samples

SSW: sum of squares within samples

Df: degrees of freedom

MSB: Mean square between samples

MSW: Mean square within samples

$F_{cal}$ - value calculated from the formula

$$F_{cal} = \frac{\text{Mean Square Between samples}}{\text{Mean Square Within samples}}$$  \hspace{1cm} (Equation 3.6)

$F_{crit}$ - value obtained from the tables.

An online calculator was used for the calculation of the $F$ value \(\text{(http://www.easycalculation.com/statistics/one-way-anova-matrix.php)}\) and the value was verified by hand. The critical tables were obtained online from the statistical website \(\text{(http://web.mst.edu/~psyworld/virtualstat/anova/criticaltable.html)}\).
CHAPTER FOUR: RESULTS

4.0 Introduction

This chapter highlights the results that were obtained in the research study.

4.1 Appearance

Packing of samples and the contents were visually inspected. Figure 4.1 and 4.2 are an illustration of the samples analysed. Samples were removed from their original containers for individual inspection.

Figure 4.1 Ibuprofen samples analysed
4.2 Uniformity of mass

Figures 4.3 and 4.4 below illustrate brand comparison of amoxicillin capsules and ibuprofen tablets respectively.

**Figure 4.3** Brand comparison of amoxicillin capsules’ uniformity of weight
The results of the failing ibuprofen samples are illustrated in Figure 4.4.

![Figure 4.4: Uniformity of Mass results for Ibuprofen Tablets samples](image)

4.3 Friability

Results of % Friability of samples collected are illustrated in table 4.1:

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>% Friability</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.003 %</td>
</tr>
<tr>
<td>B</td>
<td>0.003 %</td>
</tr>
<tr>
<td>C</td>
<td>0.003 %</td>
</tr>
<tr>
<td>D</td>
<td>0.003 %</td>
</tr>
<tr>
<td>E</td>
<td>0.003 %</td>
</tr>
<tr>
<td>F</td>
<td>0.002 %</td>
</tr>
<tr>
<td>G</td>
<td>0.003 %</td>
</tr>
<tr>
<td>H</td>
<td>0.004 %</td>
</tr>
<tr>
<td>I</td>
<td>0 %</td>
</tr>
<tr>
<td>J</td>
<td>0 %</td>
</tr>
<tr>
<td>K</td>
<td>0.004 %</td>
</tr>
</tbody>
</table>
4.4 Disintegration time

Disintegration results are illustrated in Figure 4.5:

**Figure 4.5: Disintegration time results for ibuprofen tablets**

- **Samples from the informal market**
- **Samples from the retail pharmacies**
4.5 Identification

Amoxicillin capsules HPLC profiles are illustrated in figure 4.6;

![Amoxicillin Std](a)
![Sample A](b)
![Sample B](c)
![Sample C](d)
![Sample D](e)
![Sample E](f)

Figure 4.6 Amoxicillin HPLC profiles
Figure 4.7 Amoxicillin HPLC comparability results

Ibuprofen HPLC identification profiles are illustrated in figure 4.8:

(a) Ibuprofen std 1

(b) Sample A

(c) Sample B

(d) Sample D

(e) Sample K

Figure 4.8 Ibuprofen HPLC profiles
Ibuprofen UV profiles are illustrated in the diagrams below:

(a) Ibuprofen Std
(b) Sample A
(c) Sample B
(d) Sample C
(e) Sample D
(f) Sample I

Figure 4.9 Ibuprofen UV profiles
Ibuprofen comparability results are shown in Figure 4.10 and 4.11 below:

**Figure 4.10 Ibuprofen HPLC Comparability results**

**Figure 4.11 Ibuprofen UV comparability results**
Amoxicillin UV profiles are presented in the diagrams below:

Figure 4.12 Amoxicillin UV profiles.

Appendix 23-26 provides comprehensive results for the ibuprofen and amoxicillin HPLC and UV profiles
Amoxicillin UV Comparability results:

Figure 4.13 Amoxicillin UV comparability results

Amoxicillin FTIR profiles are illustrated in Figure 4.14, full spectrums in Appendix 16-22,

(a) Amoxicillin samples A, B, C

(b) Amoxicillin samples D, F, G
Amoxicillin samples E, H

**Figure 4.14 Amoxicillin FTIR identification profiles**

FTIR spectra for Ibuprofen samples are illustrated in Figure 4.15, full spectrums in Appendix 2-15;

(a) Ibuprofen samples A, B, C
(b) Ibuprofen samples D, E, F
(c) Ibuprofen samples G, H, I
(d) Ibuprofen samples J, K, L
(e) Ibuprofen samples M, N

**Figure 4.15 Ibuprofen FTIR identification profiles**
Appendix 2-22 gives the comprehensive FTIR results of the samples analysed.

4.6 Quantitative HPLC test results

Table 4.2: Ibuprofen quantitative HPLC test results

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>105.47 %</td>
<td>104.62 %</td>
<td>103.91 %</td>
<td>104.66 %</td>
<td>0.74 %</td>
</tr>
<tr>
<td>B</td>
<td>100.33 %</td>
<td>101.03 %</td>
<td>99.57 %</td>
<td>100.31 %</td>
<td>0.73 %</td>
</tr>
<tr>
<td>C</td>
<td>101.68 %</td>
<td>101.89 %</td>
<td>101.45 %</td>
<td>101.67 %</td>
<td>0.22 %</td>
</tr>
<tr>
<td>D</td>
<td>104.86 %</td>
<td>103.77 %</td>
<td>103.21 %</td>
<td>103.95 %</td>
<td>0.81 %</td>
</tr>
<tr>
<td>E</td>
<td>99.97 %</td>
<td>98.23 %</td>
<td>99.71 %</td>
<td>99.30 %</td>
<td>0.95 %</td>
</tr>
<tr>
<td>F</td>
<td>96.63 %</td>
<td>96.05 %</td>
<td>97.11 %</td>
<td>96.60 %</td>
<td>0.55 %</td>
</tr>
<tr>
<td>G</td>
<td>100.71 %</td>
<td>102.39 %</td>
<td>101.20 %</td>
<td>101.43 %</td>
<td>0.85 %</td>
</tr>
<tr>
<td>H</td>
<td>123.67 %</td>
<td>121.63 %</td>
<td>123.22 %</td>
<td>122.84 %</td>
<td>0.87 %</td>
</tr>
<tr>
<td>I</td>
<td>100.65 %</td>
<td>100.67 %</td>
<td>101.76 %</td>
<td>101.03 %</td>
<td>0.63 %</td>
</tr>
<tr>
<td>J</td>
<td>101.06 %</td>
<td>99.10 %</td>
<td>100.12 %</td>
<td>100.09 %</td>
<td>0.98 %</td>
</tr>
<tr>
<td>K</td>
<td>97.27 %</td>
<td>96.69 %</td>
<td>96.43 %</td>
<td>96.80 %</td>
<td>0.45 %</td>
</tr>
<tr>
<td>L</td>
<td>98.93 %</td>
<td>100.34 %</td>
<td>99.23 %</td>
<td>99.50 %</td>
<td>0.75 %</td>
</tr>
<tr>
<td>M</td>
<td>96.90 %</td>
<td>96.24 %</td>
<td>97.24 %</td>
<td>96.79 %</td>
<td>0.53 %</td>
</tr>
<tr>
<td>N</td>
<td>95.28 %</td>
<td>96.09 %</td>
<td>97.23 %</td>
<td>96.20 %</td>
<td>1.02 %</td>
</tr>
</tbody>
</table>

Table 4.3: Amoxicillin capsules quantitative HPLC test results

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Average</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100.28 %</td>
<td>98.62 %</td>
<td>100.29 %</td>
<td>99.73 %</td>
<td>0.97 %</td>
</tr>
<tr>
<td>B</td>
<td>93.43 %</td>
<td>96.06 %</td>
<td>92.98 %</td>
<td>94.15 %</td>
<td>1.77 %</td>
</tr>
<tr>
<td>C</td>
<td>97.99 %</td>
<td>96.75 %</td>
<td>96.88 %</td>
<td>97.21 %</td>
<td>0.70 %</td>
</tr>
<tr>
<td>D</td>
<td>96.57 %</td>
<td>96.76 %</td>
<td>94.73 %</td>
<td>96.02 %</td>
<td>1.17 %</td>
</tr>
<tr>
<td>E</td>
<td>98.73 %</td>
<td>98.10 %</td>
<td>95.43 %</td>
<td>97.42 %</td>
<td>1.80 %</td>
</tr>
<tr>
<td>F</td>
<td>93.86 %</td>
<td>94.17 %</td>
<td>91.75 %</td>
<td>93.26 %</td>
<td>1.41 %</td>
</tr>
<tr>
<td>G</td>
<td>96.05 %</td>
<td>96.78 %</td>
<td>96.91 %</td>
<td>96.58 %</td>
<td>0.48 %</td>
</tr>
</tbody>
</table>
4.7 Dissolution test results

Dissolution results at 60 minutes for both ibuprofen and amoxicillin samples are illustrated in Table 4.4:

Table 4.4 Dissolution test results at 60 minutes

<table>
<thead>
<tr>
<th>Ibuprofen Tablets Sample</th>
<th>% Dissolved ± % RSD</th>
<th>Amoxicillin Capsules Sample</th>
<th>% Dissolved ± % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>105.49 ± 3.68 %</td>
<td>A</td>
<td>103.67 ± 0.99 %</td>
</tr>
<tr>
<td>B</td>
<td>107.47 ± 3.78 %</td>
<td>B</td>
<td>105.39 ± 3.53 %</td>
</tr>
<tr>
<td>C</td>
<td>100.20 ± 2.05 %</td>
<td>C</td>
<td>104.57 ± 1.94 %</td>
</tr>
<tr>
<td>D</td>
<td>102.75 ± 4.71 %</td>
<td>D</td>
<td>98.95 ± 1.91 %</td>
</tr>
<tr>
<td>E</td>
<td>108.22 ± 4.75 %</td>
<td>E</td>
<td>105.91 ± 1.42 %</td>
</tr>
<tr>
<td>F</td>
<td>119.61 ± 5.49 %</td>
<td>F</td>
<td>96.41 ± 5.79 %</td>
</tr>
<tr>
<td>G</td>
<td>112.94 ± 2.23 %</td>
<td>G</td>
<td>97.42 ± 2.81 %</td>
</tr>
<tr>
<td>H</td>
<td><strong>97.38 ± 13.58 %</strong></td>
<td>H</td>
<td>106.41 ± 0.89 %</td>
</tr>
<tr>
<td>I</td>
<td>108.82 ± 0.87 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>J</td>
<td>106.56 ± 7.31 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>106.28 ± 5.20 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>109.16 ± 4.71 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>118.84 ± 3.84 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>122.61 ± 4.20 %</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
From the comparison of the profiles of seven different brands of ibuprofen tablets analysed, the following results illustrated in Figure 4.16 were obtained.

Figure 4.16 Brand comparison of ibuprofen dissolution profiles

Batch consistency of different manufacturers was also analysed and the following figure 4.17 (a) to (d) illustrates the results obtained,
Figure 4.17 Batch consistencies of ibuprofen samples

Figure 4.18 Brand comparison of amoxicillin samples
4.8 Statistical analysis of results

Analysis of results was used to analyse the significance of the results obtained. 95% level of confidence was applied in all cases and Equation 3.6 was used to calculate F values.

4.8.1 ANOVA for ibuprofen quantitative chemical test results

ANOVA was used to analyse if there was a significant difference in the ibuprofen quantitative chemical test results between all the fourteen samples, in the different ibuprofen brands sampled and in the different batches by the same manufacturer. The following results were obtained.

4.8.1.1 ANOVA for quantitative chemical test for all ibuprofen samples

H₀: There is no significant difference between the content of API in all ibuprofen samples (from both informal and formal retailers)

H₁: There is a significant difference between the content of API in all ibuprofen samples (from both informal and formal retailers)

Acceptance criteria: Reject H₀ if \( F_{\text{cal}} > F_{\text{crit}} \). The following results were obtained:

Table 4.5 ANOVA for quantitative chemical content of API in all ibuprofen samples

<table>
<thead>
<tr>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>1740.8788</td>
<td>13</td>
<td>133.9138</td>
</tr>
<tr>
<td>Within</td>
<td>16.5455</td>
<td>28</td>
<td>0.5909</td>
</tr>
<tr>
<td>Total</td>
<td>1757.4243</td>
<td>41</td>
<td></td>
</tr>
</tbody>
</table>

4.8.1.2 ANOVA for quantitative chemical test for the different ibuprofen brands

H₀: There is no significant difference between the content of API in the different ibuprofen brands sampled (from both informal and formal retailers)
H₁: There is a significant difference between the content of API in the different ibuprofen brands sampled (from both informal and formal retailers)

Acceptance criteria: Reject $H_0$ if $F_{\text{cal}} > F_{\text{crit}}$. The following results were obtained:

Table 4.6 ANOVA for the content of API in the different ibuprofen brands sampled

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>$F$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>128.564</td>
<td>6</td>
<td>21.4273</td>
<td>$F_{\text{cal}} = 49.4058$</td>
</tr>
<tr>
<td>Within</td>
<td>6.0715</td>
<td>14</td>
<td>0.4337</td>
<td>$F_{\text{crit}} = 2.8477$</td>
</tr>
<tr>
<td>Total</td>
<td>134.6355</td>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.1.3 ANOVA for quantitative chemical test for ibuprofen batch consistency

$H_0$: There is no significant difference between the content of API in the different ibuprofen batches by the same manufacturer (Samples B, F, M and N).

$H_1$: There is a significant difference between the content of API in the different ibuprofen batches by the same manufacturer.

Acceptance criteria: Reject $H_0$ if $F_{\text{cal}} > F_{\text{crit}}$. The following results were obtained:

Table 4.7 ANOVA for the quantitative chemical test for different ibuprofen batches by the same manufacturer.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>$F$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>32.6969</td>
<td>3</td>
<td>10.899</td>
<td>$F_{\text{cal}} = 21.4421$</td>
</tr>
<tr>
<td>Within</td>
<td>4.0663</td>
<td>8</td>
<td>0.5083</td>
<td>$F_{\text{crit}} = 4.0662$</td>
</tr>
<tr>
<td>Total</td>
<td>36.7632</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.1.4 ANOVA for ibuprofen batch consistency for products manufactured in the same month

$H_0$: There is no significant difference between the content of API in the different ibuprofen batches by the same manufacturer manufactured in the same month (Samples F, M and N).
H₁: There is a significant difference between the content of API in the different ibuprofen batches by the same manufacturer in the same month.

Acceptance criteria: Reject \( H_0 \) if \( F_{\text{cal}} > F_{\text{crit}} \). The following results were obtained:

Table 4.8 ANOVA for quantitative chemical content of ibuprofen samples manufactured in the same month

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>0.548</td>
<td>2</td>
<td>0.274</td>
<td>( F_{\text{cal}} = 0.548 )</td>
</tr>
<tr>
<td>Within</td>
<td>2.9999</td>
<td>6</td>
<td>0.5</td>
<td>( F_{\text{crit}} = 5.1433 )</td>
</tr>
<tr>
<td>Total</td>
<td>3.5479</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.2 ANOVA for amoxicillin quantitative chemical test results

ANOVA was used to analyse if there was a significant difference in the amoxicillin quantitative chemical test results between all the eight samples and in the different batches by the same manufacturer. The following results were obtained.

4.8.2.1 ANOVA for quantitative chemical test for all the amoxicillin samples analysed

\( H_0 \): There is no significant difference between the content of API in the different amoxicillin samples analysed (from both formal and informal retailers).

\( H_1 \): There is a significant difference between the content of API in the different amoxicillin samples analysed (from both formal and informal retailers).

Acceptance criteria: Reject \( H_0 \) if \( F_{\text{cal}} > F_{\text{crit}} \). The following results were obtained:
Table 4.9 ANOVA for all quantitative chemical content of all amoxicillin samples

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>89.8645</td>
<td>7</td>
<td>12.8378</td>
<td>$F_{cal} = 8.7421$</td>
</tr>
<tr>
<td>Within</td>
<td>23.4967</td>
<td>16</td>
<td>1.4685</td>
<td>$F_{crit} = 2.657$</td>
</tr>
<tr>
<td>Total</td>
<td>113.3612</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.2.2 ANOVA for quantitative chemical test for amoxicillin batch consistency

$H_0$: There is no significant difference between the content of API in the different amoxicillin samples from the same manufacture.

$H_1$: There is a significant difference between the content of API in the different amoxicillin samples from the same manufacture.

Acceptance criteria: Reject $H_0$ if $F_{cal} > F_{crit}$. The following results were obtained:

Table 4.10 ANOVA for quantitative chemical content for different amoxicillin samples by same manufacturer

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>32.2279</td>
<td>3</td>
<td>10.7426</td>
<td>$F_{cal} = 8.2935$</td>
</tr>
<tr>
<td>Within</td>
<td>10.3621</td>
<td>8</td>
<td>1.2953</td>
<td>$F_{crit} = 4.066$</td>
</tr>
<tr>
<td>Total</td>
<td>42.5900</td>
<td>11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.3 ANOVA for ibuprofen dissolution test results

ANOVA was used to analyse if there was a significant difference in the ibuprofen dissolution test at 60 minutes between all the fourteen samples, in the different ibuprofen brands sampled and in the different batches by the same manufacturer. The following results were obtained.
4.8.3.1 ANOVA for quantitative chemical test for all ibuprofen samples

H₀: There is no significant difference between the content of API dissolved in 60 minutes in all ibuprofen samples (from both informal and formal retailers)

H₁: There is a significant difference between the content of API dissolved in 60 minutes in all ibuprofen samples (from both informal and formal retailers)

Acceptance criteria: Reject H₀ if $F_{\text{cal}} > F_{\text{crit}}$. The following results were obtained:

Table 4.11 ANOVA for dissolution test for all the ibuprofen samples

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>4141.4473</td>
<td>13</td>
<td>318.573</td>
<td>$F_{\text{cal}} = 9.3739$</td>
</tr>
<tr>
<td>Within</td>
<td>2378.9465</td>
<td>70</td>
<td>33.985</td>
<td>$F_{\text{crit}} = 1.863$</td>
</tr>
<tr>
<td>Total</td>
<td>6520.3938</td>
<td>83</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.3.2 ANOVA for ibuprofen dissolution test for the different brands

H₀: There is no significant difference between the content of API dissolved 60 minutes in the different ibuprofen brands sampled (from both informal and formal retailers)

H₁: There is a significant difference between the content of API in the different ibuprofen brands sampled (from both informal and formal retailers)

Acceptance criteria: Reject H₀ if $F_{\text{cal}} > F_{\text{crit}}$. The following results were obtained:

Table 4.12 ANOVA for the content of API dissolved in 60 minutes in the different ibuprofen brands sampled

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>384.4836</td>
<td>6</td>
<td>64.0806</td>
<td>$F_{\text{cal}} = 3.8306$</td>
</tr>
<tr>
<td>Within</td>
<td>585.5062</td>
<td>35</td>
<td>16.7287</td>
<td>$F_{\text{crit}} = 3.368$</td>
</tr>
<tr>
<td>Total</td>
<td>969.9898</td>
<td>41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.8.3.3 ANOVA for ibuprofen dissolution test batch consistency

H₀: There is no significant difference between the content of API dissolved in 60 minutes in the different ibuprofen batches by the same manufacturer (Samples B, F, M and N).

H₁: There is a significant difference between the content of API dissolved in 60 minutes in the different ibuprofen batches by the same manufacturer (Samples B, F, M and N).

Acceptance criteria: Reject H₀ if F_{cal} > F_{crit}. The following results were obtained:

Table 4.13 ANOVA for the dissolution test for different ibuprofen batches by the same manufacturer.

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>794.8792</td>
<td>3</td>
<td>264.9597</td>
<td>F_{cal} = 9.9128</td>
</tr>
<tr>
<td>Within</td>
<td>534.5828</td>
<td>20</td>
<td>26.7291</td>
<td>F_{crit} = 4.938</td>
</tr>
<tr>
<td>Total</td>
<td>1329.462</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.4 ANOVA for amoxicillin dissolution test results

ANOVA was used to analyse if there was a significant difference in the amoxicillin dissolution test results between all the eight samples and in the different batches by the same manufacturer.

4.8.4.1 ANOVA for dissolution test for all the amoxicillin samples analysed

H₀: There is no significant difference between the content of API dissolved in 60 minutes in the different amoxicillin samples analysed (from both formal and informal retailers).

H₁: There is a significant difference between the content of API dissolved in 60 minutes in the different amoxicillin samples analysed (from both formal and informal retailers).

Acceptance criteria: Reject H₀ if F_{cal} > F_{crit}. Table 4.14 illustrates the results obtained.
Table 4.14 ANOVA for dissolution test for all amoxicillin samples

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>697.5344</td>
<td>7</td>
<td>99.6478</td>
<td>(F_{\text{cal}} = 12.3841)</td>
</tr>
<tr>
<td>Within</td>
<td>321.8579</td>
<td>40</td>
<td>8.0464</td>
<td>(F_{\text{crit}} = 2.249)</td>
</tr>
<tr>
<td>Total</td>
<td>1019.3923</td>
<td>47</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.8.4.2 ANOVA for dissolution test for amoxicillin batch consistency

\(H_0\): There is no significant difference between the content of API dissolved in 60 minutes in the different amoxicillin samples from the same manufacture.

\(H_1\): There is a significant difference between the content of API dissolved in 60 minutes in the different amoxicillin samples from the same manufacture.

Acceptance criteria: Reject \(H_0\) if \(F_{\text{cal}} > F_{\text{crit}}\). The following results were obtained:

Table 4.15 ANOVA for dissolution test of different samples of amoxicillin by the same manufacturer

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>Df</th>
<th>MS</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>395.2485</td>
<td>3</td>
<td>131.7495</td>
<td>(F_{\text{cal}} = 9.3166)</td>
</tr>
<tr>
<td>Within</td>
<td>282.8282</td>
<td>20</td>
<td>14.1414</td>
<td>(F_{\text{crit}} = 3.098)</td>
</tr>
<tr>
<td>Total</td>
<td>678.0767</td>
<td>23</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER FIVE: DISCUSSION

5.0 Introduction

This chapter discusses the results presented in the previous chapter. Recommendations can be made based on the findings and the research will be concluded.

5.1 Appearance

Visual appearance of tablets and capsules may serve as the primary test as to whether products may be substandard or counterfeit (Nsimba, 2008). According to the United States Pharmacopoeia (2015), tablets or capsules by the same manufacturer are supposed to be of the same size, shape and colour (http://www.fip.org/counterfeitmedicines.com). Appearance results are based on samples presented in Figures 4.1 and 4.2 that show the ibuprofen and amoxicillin samples analysed. No cracks, breaks or splits, foreign particles embedded were observed on all samples. The amoxicillin samples were free from empty capsules.

Ibuprofen samples B, E, M and N were of the same brand name. However, sample B had a different packaging as compared to the other three batches from the same manufacturer. The two packaging used had variation in the way the batch number and date of expiry were printed. Sample B had the batch details impressed on the blister pack whereas the other three batches had the details imprinted on top.

There was also a difference in the font used to label the brand name with ibuprofen sample B having small letters throughout whereas the other three batches had capital letters at the beginning and at the end of the product name, for example one sample being branded as ‘sample-
ab’, whereas the other three samples were labelled as ‘Sample-AB’. The dimensions of the blister packs used also differed with ibuprofen sample B being 9.5 cm x 3.7 cm whilst the other three batches had 8.8 cm x 3.7 cm. It is possible for a manufacturer to change packaging for the same product in the same year but it also has an effect of introducing room for counterfeiting as there are chances that it will look like the original. Differences of this nature gives room for counterfeits as a fake product may be marketed so this sample was suspected to be a counterfeit where the branding may be falsified.

Ibuprofen samples D and H also differed in appearance although they were manufactured under the same brand name. Shape, size and colour of the tablets differed as sample H was smaller in diameter and more intense in colour. This raises suspicion of counterfeiting as sample H failed the assay and dissolution tests that followed. Both samples were different batches from the informal market.

All samples from both informal and formal market did not contain a leaflet or package insert to explain dosage, medicine content, adverse effects, medicine action and how the medicine should be taken. This observation has serious health effects as not all patients ask for this information before use of medication and there is no guarantee on how accurate the information may be disseminated from supplier to end user.

5.2 Uniformity of mass

According to Karmakar et al., 2012, the variation in individual weights of tablets from the mean is an indication of the corresponding variation in the drug content. Higher weight variation is an indication of poor manufacturing practices. If the same compressors are being used, have been calibrated and are well maintained, then uniform powder is expected to be filled during the
compression process consistently. Higher percentage variations in tablet weight may mean the active pharmaceutical ingredient will differ from one tablet to the next which is detrimental to a patient who may end up being under dosed and overdosed on different occasions (Karmakar et al., 2012).

Variation of weight limits as stated by the International Pharmacopoeia are that not more than two of the individual capsule weight out of the twenty tested should have a percentage deviation from the average by $\pm 7.5\%$ (indicated as the lower and upper control limits in the chart- LCL and UCL respectively) and none of the individual capsule weight should have a percentage deviation of more than $\pm 15\%$ (indicated as the lower and upper action limits in the chart- LAL and UAL respectively) for amoxicillin capsules. Figure 4.3 illustrates results obtained from the different brands of amoxicillin capsules analysed. All the amoxicillin samples passed the test.

WHO (2014) gives the limits for tablets as not more than two of the individual tablet weight out of the twenty tested should have a percentage deviation from the average by $\pm 5\%$ (indicated as the lower and upper control limits in the chart- LCL and UCL respectively) and none of the individual tablet weight should have a percentage deviation of more than $\pm 10\%$ (indicated as the lower and upper action limits in the chart- LAL and UAL respectively) in Figure 4.4. Of the fourteen ibuprofen samples analysed, eleven samples passed and three samples failed the uniformity of mass test according to the WHO set standards (International Pharmacopoeia, 2015). The researcher concluded these three samples were substandard as they failed to meet the WHO set standards for uniformity of mass.

Two of the three ibuprofen samples that failed were different batches from the same manufacturer, that is, sample D and sample H. As can be derived from the figure 4.3, seven out of twenty tablets of ibuprofen sample D failed the first condition of not more than $\pm 5\%$ whilst one
of the tablets had a deviation more than $\pm 10\%$. Three tablets from ibuprofen sample H had variation more than $\pm 5\%$ whilst one had more than $\pm 10\%$. These results may be in agreement with what was suggested by Avbunudiogba et al., (2013) that weight variation is a strong indication of lack of adherence to good manufacturing practises and may lead to different dosages on different occasions if the mass of the API differs from tablet to tablet.

Karmakar et al., (2012) went on to explain that high variation in weight may be an indication of corresponding variation in the drug content. However, it is also possible that since these ibuprofen tablets are film coated, poor manufacturing practices during the coating process may lead to high inconsistency in the amount of coating applied per tablet. This may lead to large variations in the weight but not necessarily affecting the amount of API available per tablet but probably affect the rate at which the API is released.

5.3 Friability

Friability is the test that is done to check the resistance of a tablet to external pressure in order to withstand chipping, abrasion or breakage it may be exposed to before it reaches the consumer either from manufacturing, storage, handling during shipping and transportation (Teklu et al., 2014).

All samples passed the friability test as illustrated in Table 4.1. This could have been attributed to the film coating that has resistance to abrasion and therefore protects the contents of the tablets. Generally, from the results, all samples had a very low % friability which is a good sign for the samples that come from different continents and are transported from one place to the other as they can maintain their shape and do not crack during handling and transportation.


5.4 **Disintegration**

Disintegration is the break down process of tablet into smaller particles and is the first step towards dissolution. The time taken for each tablet or capsule to completely disintegrate was noted. According to the international pharmacopoeia, all film coated tablets or capsules should completely disintegrate within 30 minutes. Figure 4.5 illustrates the results obtained in the disintegration test.

For the samples analysed, all fifteen samples passed the disintegration test. However, it can be noted that some samples had relatively high disintegration times. Notably, samples A, D, E, H, I and J had disintegration times ranging from 15 minutes 26 seconds to 22 minutes 56 seconds. Samples A, D, E and H were from the informal market while samples I and J were from the formal market.

According to a report by Cardot et al., (2007), disintegration is the break down process of tablet into smaller particles and is the first step towards dissolution, used to determine the disintegration time of the medication in the human body. It is therefore expected by some workers that the longer the disintegration time, the longer the time taken before the API is dissolved and absorbed by the body (Esimone et al., 2008; Avbunudiogba et al., 2013).

The researcher observed a trend that samples A and E were manufactured by the same manufacturer, samples D and H were products by one manufacturer while samples I and J were also by the same manufacture. The researcher also concluded that the relatively high disintegration times observed could be related to the processes applied by these manufactures. Excipients such as binders and type of coating using play a major role in the rate at which a tablet breaks down into smaller particles and the way the API is released. If the binders have
affinity for the API then more time will be required before the API may be released for absorption.

5.5 Identification

In this research study, three forms of identifications were applied. HPLC profiling of all samples was carried out using the quantitative chemical assay test. In this case, the standard and sample preparation techniques were similar. Identification was based on comparison of the retention time of the API in the standard to that of the sample.

Figure 4.6 (a) – (i) represent the results obtained in amoxicillin HPLC profiling test. As can be observed from the results, all the samples had similar profiles with only one peak that was identified as amoxicillin by comparing sample to standard appearing. Comparability studies of these said peaks were illustrated in Figure 4.7 where the comparability was noted as ranging from 99.86 % and 100.11 % showing matching of the API peaks as very strong.

Ibuprofen HPLC profiles illustrated in Figure 4.8 (a) – (q) showed variation in the profiles. Only one peak appeared in the ibuprofen standard profiles (Figures 4.8 (a)) whereas profiles for ibuprofen samples A, B, E, F, K, M and N had more than a single peak (Figures 4.8 (b), (c), and (e); Appendix 23). All these samples except sample K were from the informal market. Samples B, F, M and N were samples by one manufacture and samples A and E were by another manufacturer. Although sample K was from a formal market, it was not sold in the original packaging and therefore did not contain the brand name of the product, date of manufacture and country of origin for the product. The two peaks that were detected in these samples could have been excipients that are soluble in the mobile phase, impurities (either intentional such as
undeclared APIs) or degradation products. Due to the limitation of standards, these peaks could not be assigned an identity.

Figure 4.7, 4.10, 4.11 and 4.13 relate to the comparability study of the HPLC and UV identification test calculated using Equation 3.4 and 3.5. All the samples showed relatively high comparability as the retention time of API detected in the samples was at the same retention time as that detected in the standard for both amoxicillin and ibuprofen respectively. This positive identification verified that all the samples contained the API stated on the labels.

Figures 4.10 and 4.12 were UV identification profiles for ibuprofen and amoxicillin samples respectively. As can be shown by the comparability graphs illustrated in Figure 4.11 and 4.13, there was a strong correlation in the $\lambda_{\text{max}}$ of the standard and the sample. Again these samples agreed with what was suggested in literature that $\lambda_{\text{max}}$ is around 221 nm for ibuprofen (Eraga et al., 2015) and 272 nm for amoxicillin (Bronnikova et al., 2008).

FTIR identification was also used in this research. Figure 4.14 and 4.15 illustrates the results obtained for amoxicillin and ibuprofen respectively. According to Kulkarni et al., (2011), in the ibuprofen spectra the following peak numbers are expected,

1. 1721 cm$^{-1}$ due to carbonyl stretching of the isopropionic acid (–COOH) group
2. 1268 cm$^{-1}$ due to aromatic distribution
3. 1232 cm$^{-1}$ due to the hydroxyl group bending vibration
4. 1273 cm$^{-1}$, 1185 cm$^{-1}$, 870 cm$^{-1}$ and 779 cm$^{-1}$ due to the aromatic structure bending vibrations.

Niharika et al., (2013) further mentions a wavenumber 2951 cm$^{-1}$ that is due to a peak caused by the hydroxyl group stretching linked to the carbonyl group. All these peaks were detected in the
ibuprofen sample that is in agreement with previous work done by Matkovi et al., (2005) and Mallah et al., (2012). In samples E and F however, all the other peaks were detected except for $1268 \text{ cm}^{-1}$ that corresponded to the aromatic distribution. Peaks due to aromatic structure bending vibrations were however detected in these samples showing presence of the aromatic structure.

According to Songsurang et al., (2011), functional groups may show band shifts and broadening due to chemical interactions. This may explain why certain bands do not appear as there may be interactions of API with excipients or degradation of the product confirming the other peaks obtained on the HPLC profile of ibuprofen sample E.

Figure 4.15 illustrates the spectra obtained from the amoxicillin samples. According to the previous works reported in literature (Songsurang et al., 2011; Angadi et al., 2012; Narkar et al., 2010), the expected wavenumbers for amoxicillin are given as,

i. $1480 \text{ cm}^{-1}$ due to the NH, CN stretch combination band  
ii. $1500 \text{ cm}^{-1}$ due to the benzene ring C = C stretch  
iii. $1583 \text{ cm}^{-1}$ due to the asymmetric stretching of carboxylate.  
iv. $1613 \text{ cm}^{-1}$ due to the C = O stretching  
v. $1684 \text{ cm}^{-1}$ due to the C = O stretching of the amide group.  
vi. $1775 \text{ cm}^{-1}$ due to the C = O stretching of $\beta$ – lactam  
vii. $3020 \text{ cm}^{-1}$ due to the benzene ring CH stretch  
viii. $3440 \text{ cm}^{-1}$ due to the OH, NH stretching vibration (Songsurang et al., 2011; Angadi et al., 2012; Narkar et al., 2010)

All these peaks were detected in all the amoxicillin samples stated in literature therefore showing there were no well defined interactions between amoxicillin trihydrate and excipients. The
samples passed the identification test as the API was compatible with the formulation components. The comprehensive spectra for ibuprofen and amoxicillin samples are attached as Appendix 2-23. From these three identification tests the researcher deduced that all the samples contained the API stated on the specific labels of the samples.

5.6 Quantitative HPLC assay

Avbunudiogba et al., (2013) explains the importance of determining the content API in a pharmaceutical preparation as its ability to successfully delivering the drug to its site of action in the right amount and at the time. The assay test determines the amount of the Active Pharmaceutical Ingredient (API) in a sample (Teklu et al., 2014). According to the WHO Model list of essential medicines, ibuprofen tablets contain not less than 90.0% and not more than 110.0% of the amount of Ibuprofen ($C_{13}H_{18}O_2$) stated on the label.

From Table 4.2 that illustrates the results obtained in the quantitative chemical assay test for ibuprofen, it can be deduced that all except ibuprofen sample H passed the test. Sample H contained $122.84 \pm 0.87$ % of ibuprofen. This means there is a high possibility that any patient who uses this sample will be overdosed by more than 12 % of the maximum allowed ibuprofen per tablet.

This sample was collected from a vendor therefore no questions are asked during sale and no information may be offered as to how the medication is taken. If ibuprofen is taken in overdose, chances are the patient may suffer heart strokes and this may be fatal. The same sample H failed the dissolution test and had a very low dissolution rate. The effects of a low dissolution rate is that the tablet may take too long to reach its site of intended action and since the patient would have bought the medication from an unregistered source, they may be forced to take another
dosage yet the API contained is already high. These results deviate from the trend in literature where most ibuprofen tablets tested passed the content API tests (Eraga et al., 2015; Giri et al., 2011). This could have been attributed to the fact that in most of the previous works reported in literature, samples were from formal and registered pharmacies. In this research, all the samples from the formal pharmacies passed the tests done.

Testing using ANOVA at 95 % confidence level using results illustrated in Tables 4.5, 4.6, 4.7 and 4.8 the following is deduced;

i. Referring to Table 4.5, since $F_{\text{cal}} (226.6268) > F_{\text{crit}} (2.089)$ for all the ibuprofen samples, we reject $H_0$ and conclude that there’s a significant difference between the quantitative chemical content of API of all ibuprofen samples. This means the fourteen samples analysed have a significant difference in the amount of ibuprofen contained by each sample.

ii. Comparing the seven different brands of ibuprofen tablets analysed (Table 4.6), since $F_{\text{cal}} (49.4058) > F_{\text{crit}} (2.8477)$, we reject $H_0$ and conclude that there’s a significant difference between the brands (Samples A, B, C, D, I, K and L).

iii. To check batch consistency of manufacturers (Table 4.7), since $F_{\text{cal}} (21.4421) > F_{\text{crit}} (4.0662)$, then $H_0$ can be rejected and conclude that there’s a significant difference between the four batches of ibuprofen by the same manufacturer (Samples B, F, M and N). This means the manufacturer was not consistent during the manufacturing process over different months.

iv. From table 4.8, three samples out of the four analysed in Table 4.7 from the same manufacture were produced in the same month. To check if there is a significant
difference in the API contained these samples, ANOVA was also used and since $F_{\text{cal}} (0.548) < F_{\text{crit}} (5.1433)$, then we fail to reject $H_0$ and conclude that there’s no significant difference between the three batches of ibuprofen by the same manufacturer (Samples F, M and N) manufactured in the same month.

Table 4.3 illustrates the results from amoxicillin quantitative chemical assay test. All the samples passed the test at the time of testing. However, according to a report by Kiron et al., 2013, at times antibiotics cannot remain stable unless the labelled storage conditions are adhered to. In their comparative study on the influence of storage condition on the shelf life of amoxicillin tablets, samples were placed at different conditions and tested periodically for twenty four mounts. They concluded that storage conditions of antibiotics should be based on the labelled conditions and should not be exposed to light as results showed there was a significant difference in the results obtained initially and those after twenty four mounts. Most of the pharmaceutical products sold by informal vendors are exposed to light and this may affect the potency of the medication if storage conditions are not adhered to.

Testing using ANOVA to see if there is a significant difference between the amoxicillin samples analysed, at 95 % confidence level gives the following results illustrated in Tables 4.9 and 4.10;

i. As illustrated in Table 4.9, since $F_{\text{cal}} (8.7421) > F_{\text{crit}} (2.657)$ for all the amoxicillin samples, we reject $H_0$ and conclude that there’s a significant difference between the quantitative chemical content of API in the amoxicillin samples. This means that although all the eight samples analysed passed the test, there is a significant difference in the amount of amoxicillin contained by each sample.
Results from Table 4.10 were used to check batch consistency of manufacturers, samples B, C, F and G were considered since they were from the same manufacturer. Since $F_{\text{cal}}(8.2935) > F_{\text{crit}} (4.0662)$, then we reject $H_0$ and conclude that there’s a significant difference between the four batches of amoxicillin by the same manufacturer.

These results show that even if samples do pass the quantitative chemical assay test, there is lack of adherence to good manufacturing practices as samples are not equivalent as expected in literature (United States pharmacopoeia, 2015).

### 5.7 Dissolution

Results obtained in the quantitative chemical assay test provide initial information on the presence or absence of the API and the potency of the product as a whole. However, the dissolution test gives more important information as it gives the identity of the API, the potency of API per tablet, and also information on the rate at which the API is released into the body for absorption (Esimone et al., 2008).

According to Avbunudiogba et al., (2013), a dissolution test is intended to determine the percent release of API in samples after a specified time in a specified media. WHO requirements are that for both ibuprofen tablets and amoxicillin capsules, all samples should release not less than 85 % of the API in 60 minutes. Table 4.4 gives the results obtained after 60 minutes dissolution time. From the table, all samples except sample H pass the dissolution test as more than 85 % of the API is released within the stipulated time of 60 minutes (International Pharmacopoeia, Fifth Edition). Although the average content of API released for sample H is 97.38 %, the % RSD of 13.58 means some tablets within the sample had a % API dissolved of below $(Q) = 85 \%$. Therefore this sample was declared as substandard as it failed the dissolution test.
Although only one sample failed the dissolution test (ibuprofen Sample H), testing at 95 % confidence interval if there is a significant difference between brands or between different batches of the same brand, it can be concluded that,

i. From Table 4.11, since $F_{\text{cal}} (9.3739) > F_{\text{crit}} (1.863)$ for all the ibuprofen samples analysed, we reject $H_0$ and conclude that there’s a significant difference between the amount of API dissolved in 60 minutes for the different ibuprofen samples.

ii. For brand comparison of the seven ibuprofen samples analysed, in Table 4.12, since $F_{\text{cal}} (3.8306) > F_{\text{crit}} (3.368)$ then $H_0$ can be rejected and conclude that there’s a significant difference between the seven different brands analysed.

iii. To check batch consistency of manufacturers, since $F_{\text{cal}} (9.913) > F_{\text{crit}} (4.938)$, then $H_0$ can be rejected and conclude that there’s a significant difference between the four batches of ibuprofen by the same manufacturer (Samples B, F, M and N).

From the dissolution profiles, Figure 4.16; Figure 4.17 and Figure 4.18, there is a significant difference in the dissolution rates of the samples from the formal market and those from the informal market. High dissolution rate means high bioavailability of the drug content. From the comparison of the profiles of seven different brands of ibuprofen tablets analysed and represented in Figure 4.16, Samples A, B and D where from the informal market whereas Samples C, I, K and L where from the formal market (registered pharmacies) and had the highest dissolution efficiencies after 15 minutes ($T_{15}$). Samples A, B and D only had dissolution efficiencies comparable to those of the latter samples after 30 minutes.

These results may show the implications of purchasing a pharmaceutical product from an informal market. Since most of the people who purchase these products use them for the relief of
pain and inflammation, the longer it takes for the drug to be bioavailable, the higher the chances of an overdose or improper drug interactions when products containing different APIs are ingested. Improper drug interactions arise when a consumer takes medication containing different APIs that may react such as ibuprofen taken simultaneously with aspirin which may lead to heart attack of the patient (www.drugs.com/ibuprofen.html).

A pharmaceutical product of oral dosage form normally contains a drug substance known as the active pharmaceutical ingredient (API) and its excipients. According to Esimone et al., 2008, the proportion between the excipients, the type of excipients (grade) and the manufacturing process may affect the bulk properties and absorption properties of the product. This gives each product a general dissolution pattern as is illustrated in the Figure 4.16.

Although the same API - ibuprofen was analysed, differences in the manufacturing process, type and proportions used for different brands this led to different dissolution profiles with the efficiencies increasing in the order Samples D and H< Samples A and E <Samples I and J< Samples B, F, M and N< Samples C and G at T₅. It can also be derived from the results that samples from the retail pharmacies had high dissolution efficiencies than those from the informal market except for Sample J that had efficiencies less than that of Samples F, M and N.

If a manufacturer is using a validated manufacturing process, results from one batch to the next are expected to be comparable such as those illustrated in figure 4.17 (a) showing results of samples from a retail pharmacy. More than 85% of the API had been released in 15 minutes for both batches. The same can be said for the samples D and H. Although very low dissolution efficiencies are noted at T₂₀(at 20minutes), the dissolution profiles are similar in the rate at which the API is released.
The same however cannot be said for Samples B, F, M and N illustrated in Figure 4.17 (b). Four different batches were analysed. Three of these samples were manufactured in the same month according to the labels (all manufactured in May 2015), and gave similar results but these three results differed from the results of the batch that had been produced earlier, (Manufactured January 2015). Sample B showed a different profile from the other three batches that released more than 100% of the API in 15 minutes whereas only 49.9% of the API had been released by Sample B in the same time. The former batch only had results comparable to the other three after 45 minutes.

Using the results of the ANOVA test to see if there’s a significant difference between the API released after 60 minutes illustrated in Table 4.13, it can be observed that since $F_{\text{cal}} (9.9128) > F_{\text{crit}} (4.938)$ then we reject $H_0$ and conclude that there’s a significant difference between the four batches by the same manufacturer.

Figure 4.17 (d) shows the dissolution profiles for Samples D and H. The illustration seems to be in agreement with what some workers suggested that there is a strong correlation between disintegration times and dissolution (Esimone et al., 2008). In the same report, Esimone and colleagues suggested that the higher the disintegration times the lower the dissolution rate (dissolution efficiencies). This means the longer the time it takes for a tablet to break into smaller particles the longer it will take to release the API. Referring to Figure 4.16, it can be noted that in both cases Samples D and H had the lowest dissolution efficiencies at $T_{15}$. This strongly correlates with Figure 4.5 that shows sample H having the highest disintegration time as suggested by Esimone et al., (2008).
This was not the case with the results represented in Figure 4.17 (b). The results agree with what was reported by Eraga et al., (2015) as the disintegration time did not have a relationship with the percentage drug release. As illustrated by Figure 4.5, sample B may have the least disintegration time of the four samples but it may not release the API as efficiently as the other three batches.

Table 4.4 also gives the results obtained after 60 minutes dissolution time of amoxicillin capsules. In Figure 4.18 the dissolution profiles of these amoxicillin samples are illustrated. All the eight samples had similar profiles with all the eight samples releasing more than 60% of the API in the first 5 minutes. However testing if there is a significant difference between the API released by these samples at 60 minutes, using ANOVA at 95% confidence level, it can be shown from table 4.14 that since $F_{cal} (12.3841) > F_{crit} (2.249)$, we reject $H_0$ and conclude that there is a significant difference between the amount of API that would have dissolved in 60 minutes between the samples.

To check for batch consistency between the amoxicillin samples, samples B, C, F and G were considered. Testing using ANOVA at 95% confidence level as illustrated in Table 4.15, since $F_{cal} (9.3166) > F_{crit} (3.098)$ we reject $H_0$ and conclude that there is a significant difference in the API released by different batches of amoxicillin samples from the same manufacturer.

5.8 Conclusion

Three out of the fourteen samples of ibuprofen tablets failed the uniformity of weight test. These three samples were different batches from the informal market. One out of these three that failed the uniformity of weight test also failed the quantitative chemical assay test and the dissolution test. Four other ibuprofen samples were from the same manufacturer but had differences in the
packaging used therefore raising suspicion of the possibility of counterfeiting. Two more ibuprofen samples were from the same manufacturer but the appearance of these samples differed and therefore failed the appearance test. The other samples of ibuprofen tablets passed all the tests done. All the amoxicillin capsules samples passed all the tests done however ANOVA tests at 95% confidence level showed that there’s a significant difference between the content API and percentage drug release therefore samples did not contain equivalent amounts of API therefore brand substitution on the assumption that since the same amount of API is declared on the label may not give the desired onset of action and subsequent therapeutic effectiveness. All the samples tested contained the active pharmaceutical ingredient (API) ibuprofen and amoxicillin as stated on the sample labels.

5.9 Recommendations

From the results obtained in this research, it can be concluded that consumers of these pharmaceutical products purchased from vendors on the streets may be at a health risk as different API may be administered at any given time, at times with chances of being overdosed. There is need for a large scale study of different medicines found in Zimbabwe to properly quantify the extent of the problem.

The lack of strict monitoring and regulatory mechanisms allows for easy access to legitimate channels of distribution, making counterfeiting an appealing source of illicit revenue. Regulatory authorities and the police workforce are encouraged to work together to improve forces on monitoring the products that are being imported into the country especially through informal traders. Besides the fear that the nation may be consuming substandard products, there is greater concern that since most of these products are flooding through the informal market, the same products may find their way onto the formal market without being detected.
Governments and regulatory authorities may need to focus on certain key areas to strengthen effective post market surveillance and monitoring to prevent distribution of substandard medicines. In many countries, including many across the developed world, weak or insufficient enforcement has contributed to the steadily rising trends of importation of SSFFCs. According to Finlay (2011), drug regulatory systems in most countries, including in developed countries, focus more on pre-marketing approvals than on post-market monitoring. No matter how thoroughly pre-marketing assessment is conducted; it is only one of the functions necessary for ensuring the efficacy and safety of drugs (Finlay, 2011).

Most of the trading of fake drugs occurs between national borders. Therefore if there is effective exchange of information between pharmacies, regulatory authorities and other points in the supply chain between nations-it will be easier to communicate if there is information of suspected counterfeits. Inter-nation relations may also help in redefining and updating legislations based on positive results obtained by other nations. This includes collaboration between countries, tightening of legislations and engagement of the consumers (the general public) in making them understand the risk they expose themselves to their health by buying medicines from informal markets.

It is of great importance to invest in technologies that can effectively detect counterfeit drugs. These include the use of hand-held Raman spectrophotometers especially at the boarder entry points and use of Near Infrared Chemical imaging and Chromatography in the laboratories. Competent manpower that can use these technologies is also a necessity so that quality work may also be produced. Holograms, tracers and taggants and electronic tracking systems have also
been reported by Deisingh et al., (2004) as being effective. Although most of the techniques reported above are out of reach for most developing countries due to cost of acquiring, it is a worth long term investment to consider especially with the evolution of counterfeit and substandard pharmaceutical products.
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http://www.fda.gov/cder/OPS/PAT.html
http://www.fip.org/counterfeitmedicines.com


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The United States Pharmacopoeia and National Formulary USP 37 NF 32, The USP Convention Inc. 2015


World Health Organisation Fact Sheet No.275, 2012


### Appendix 1: Equipment register

<table>
<thead>
<tr>
<th>Instrument ID</th>
<th>Instrument</th>
<th>Manufacturer</th>
<th>Model</th>
<th>Serial Number</th>
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<td>Beckmann</td>
<td>GS-6</td>
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<td>AND GH-202</td>
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<td>USC600T</td>
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Appendix 2
Appendix 3
Appendix 4
Appendix 5
Appendix 7
Ibuprofen sample F

Mon Feb 29 11:20:16 2016

Collection time: Mon Feb 29 11:19:00 2016
Number of sample scans: 16
Number of background scans: 16
Resolution: 4.000
Sample gain: 1.0
Optical velocity: 0.4747
Aperture: 100.00

Detector: DTGS KBr
Beamsplitter: KBr
Source: IR

Spectrum: Ibuprofen sample F
Region: 3496.20-4501.13
Search type: Correlation

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<th>Compound name</th>
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<th>Library</th>
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<td>ET-3SILON-CAPROLACTAM, 69%</td>
<td>30.65</td>
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<td>13</td>
<td>BARBITAL FREE ACID CRYSTALLINE-D</td>
<td>35.08</td>
<td>Sigma Biological Sample Library</td>
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<td>188</td>
<td>ICOPANIC ACID IN KBR</td>
<td>33.12</td>
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<td>99</td>
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<td>LEVORPHANOL HCL IN KBR</td>
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Appendix 9
Ibuprofen sample H

Collection time: Mon Feb 29 11:29:12 2016
Number of sample scans: 16
Number of background scans: 16
Resolution: 4.000
Sample gain: 1.0
Optical velocity: 0.4747
Aperture: 100.00

Detector: DTGS KBr
Beamsplitter: KBr
Source: IR

Appendix 10
Ibuprofen sample I

Collection time: Mon Feb 29 11:32:37 2016
Number of sample scans: 16
Number of background scans: 16
Resolution: 4.000
Sample gain: 1.0
Optical velocity: 0.4747
Aperture: 100.00

Detector: DTGS KBr
Beamsplitter: KBr
Source: IR

Index | Compound name                        | Library |
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12    | NONANOIC ACID, 98%                  | Aldrich Condensed Phase Sample Library |
94    | EPSILON-CAPROLACTAM, 96%           | Aldrich Vapor Phase Sample Library    |
968   | Poly(acenaphthylene)                | HK Norel Sample Library               |
168   | IOPANCIC ACID IN KBR               | Georgia State Crime Lab Sample Library|
13    | BARBITAL FREE ACID CRYSTALLINE-D   | Sigma Biological Sample Library       |
93    | (15S)-2-METHYL-4-VERBERENONE, 94%  | Aldrich Vapor Phase Sample Library    |
20    | DL-DESTOPHIBOTIN                    | Sigma Biological Sample Library       |
10    | 3-NONANONE, 95%                    | Aldrich Condensed Phase Sample Library|
42    | PROSTAGLANDINS A1 SYNTHETIC         | Sigma Biological Sample Library       |
35    | NAPHTHALENE                         | Aldrich Vapor Phase Sample Library    |
Appendix 13
Appendix 14
Ibuprofen sample M

Collection time: Mon Feb 29 11:45:25 2016
Number of sample scans: 16
Number of background scans: 16
Resolution: 4.000
Sample gain: 1.0
Optical velocity: 0.4747
Aperture: 100.00
Detector: DTGS KBr
Beamsplitter: KBr
Source: IR

Library
Aldrich Condensed Phase Sample Library
Aldrich Vapor Phase Sample Library
Sigma Biological Sample Library
Georgia State Crime Lab Sample Library

Library
Aldrich Condensed Phase Sample Library
Aldrich Vapor Phase Sample Library
Sigma Biological Sample Library
Georgia State Crime Lab Sample Library

Appendix 15
Appendix 16
Appendix 20
Appendix 21
Amoxicillin sample G

Mon Feb 29 12:30:04 2016

%Transmittance

Wavenumbers (cm⁻¹)

Collection time: Mon Feb 29 12:27:38 201
Number of sample scans: 16
Number of background scans: 16
Resolution: 4.000
Sample gain: 1.0
Optical velocity: 0.4747
Aperture: 100.00

Detector: DTGS KBr
Beamsplitter: KBr
Source: IR

Spectrum: Amoxicillin sample G
Region: 3465.20-455.13
Search type: Correlation
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Appendix 22
Appendix 23
Amoxicillin HPLC profiles

(a) Amoxicillin Std
(b) Sample A
(c) Sample B
(d) Sample C
(Amoxicillin HPLC profiles continued)
Appendix 24
Ibuprofen HPLC profiles

(a) Ibuprofen std 1
(b) Sample A
(c) Sample B
(d) Sample C
(e) Sample D
(f) Ibuprofen Std 2
(Ibuprofen HPLC profiles continued)
Appendix 25
Ibuprofen UV profiles

(a) Ibuprofen Std
(b) Sample A
(c) Sample B
(d) Sample C
(e) Sample D
(f) Sample E
(m) Sample L  

(n) Sample M  

(o) Sample N  

(Ibuprofen UV profiles continued)
Appendix 26
Amoxicillin UV profiles

(a) Amoxicillin Std
(b) Sample A
(c) Sample B
(d) Sample C
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(Amoxicillin UV profiles continuation)