CHAPTER ONE

1.0 INTRODUCTION

The production of substandard and counterfeit drugs is a vast and underreported problem, particularly affecting poorer countries. It is an important cause of unnecessary morbidity, mortality, and loss of public confidence in medicines and health structures. The prevalence of counterfeit drugs appears to be rising, for instance, it has been estimated that of up to 15% of all sold drugs are counterfeit, and in parts of Africa and Asia the figure exceeds 50% (Robert et. al. 2007).

Counterfeit drugs are products deliberately made to resemble a brand name pharmaceutical. They may contain no active ingredient or contain ingredients inconsistent with the package description. Substandard drugs are found even among cheaper products, than counterfeit drugs which are common among more expensive drugs, because some manufacturers wish to avoid costly quality control and good manufacturing practices (CDC 2006).

The quality of commercially available drugs varies greatly among countries. Due to lack of regulations and poor quality control practices in some countries, the amount of active ingredients can be inconsistent. Poor formulation techniques can affect the release of active ingredients from a tablet, with some tablets releasing very little, if any drug. Some drugs may be contaminated with other substances. Poor storage conditions, especially in warm and humid tropical environment may contribute to chemical degradation of many pharmaceuticals (CDC 2006).

The Government's commitment to improving the quality of life of all Zambians is demonstrated through its efforts in reforming the health sector. In 1991, the Government of the Republic of Zambia articulated radical health policy reforms characterized by a move from a strong centralized health system in which the central structures provided support and national guidance to the peripheral structures. An important component of health policy reform was the structured Primary Health Care (PHC) programme

(Dzekedzeke and Mulenga ZDHS 2001-2002).The reformulated PHC programme aimed at, among other things, to deal with the main health problems in the community, focusing on the needs of the underserved, high risk, and vulnerable groups. Thus, attention is paid to the rural and peri-urban areas where the health needs of the people are greatest, with particular emphasis placed on maternal and child care, family planning, nutrition, control of communicable diseases (e.g. diarrhoea, cholera, dysentery, sexually transmitted infections, HIV/AIDS, malaria, etc.), immunization, and environmental sanitation in order to secure adequate health care for all Zambians (Dzekedzeke and Mulenga ZDHS 2001-2002).

In ensuring that all Zambians have adequate health care, the Ministry of Health, in 1992, formulated a reform vision which aimed at improving the health status of all Zambians through provision of cost effective quality health services as close to the family as possible in order to ensure equity of access in health service delivery and contribute to the human and socio-economic development of the nation (MOH STRATEGIC PLAN 2005-2009). In order to address developmental challenges, millennium development goals (MDGs), which are eight goals to be achieved by 2015, were formulated from the actions and targets contained in the Millennium Declaration that was adopted by 189 nations and signed by 147 heads of state and governments during the UN Millennium Summit in September 2000, and Zambia was a signatory. The eight MDGs are further broken down into 21 quantifiable targets that are measurable by 60 indicators (UN Millennium Summit 2000). Of much concern to this study is Goal number 6 which aims at combating HIV/AIDS, malaria and other diseases. Combating the above mentioned communicable diseases requires the use of quality, safe and efficacious drugs.

Factors that have been suggested to contribute to the production of counterfeit and substandard drugs include the following;

(a) Lack of political will and commitment to fight the scourge.
(b) Lack or inadequate legislation prohibiting counterfeiting of drugs.
(c) Absence of or weak national drug regulatory authorities.
(d) Weak drug laws enforcement and weak penal sanctions.

(e) Shortage or erratic supply of drugs.
(f) High cost of medicines.
(g) Inefficient cooperation among stakeholders.
(h) Trade involving several intermediaries.
(i) Inadequate skilled human resource to run the system.
(j) Corruption and conflict of interest (WHO 2006).

Some of consequences of using counterfeit and substandard drugs are;

(1) Lacking of therapeutic effect and treatment failure.
(2) Complications and mortality due to the disease and toxic components of the drugs.
(3) High burden of the disease leading to mortality and morbidity (Kelesidis et. al. 2007).

Malaria surged in sub–Saharan Africa in the 1990s due to increased resistance to chloroquine and sulfadoxine–pyrimethamine (SP). Exposure to substandard antimalarial drugs likely exacerbated this trend (Bate, Coticelli, Tren and Attaran, 2008). This scenario could not be different from the Zambian situation, as there has been an increase in malaria cases and resistance to chloroquine has been documented. As a result of the increased resistance to chloroquine, the drug is no longer used in Zambia for both malaria treatment and prevention.

In recognition of the dangers and consequences of using counterfeit and substandard drugs, and the threat this poses to public health, the Government of the Republic of Zambia established the Pharmaceutical Regulatory Authority (PRA) in 2006 to fight the scourge. In addition, as a way of strengthening the fight against counterfeiting of drugs, government formed a taskforce on the 11th day of November, 2008. However, it is still felt that the PRA and other law enforcement agencies have not developed enough capacity to fight the scourge.

There is almost complete absence of both qualitative and quantitative data on the prevalence of counterfeit and substandard drugs, antimalarial drugs inclusive, in Zambia. Most of the drugs used in Zambia come as imports from other countries because the few local pharmaceutical manufacturing companies could not meet the demand. Access to these drugs is through the public institutions such as the government run hospitals and clinics, the private institution – hospitals, clinics, surgeries and pharmacies/chemists owned by individuals or corporations, and the vendors who are not even registered with the regulatory authority. It is most likely that not all drugs that are found on the Zambian market come through the normal channels, as some are just smuggled into the country by trying to evade the registration fees and other fees charged by PRA. What worries the citizens, especially the medical professions, is the quality of the drugs used by the people of Zambia. Lack of post – marketing surveillances on drugs is another concern.

This study will endeavour to evaluate the quality of antimalarial drugs in Lusaka so as to find ways of strengthening the fight against counterfeiting as well as production and sale/use of substandard drugs, in turn contributing to the fight and eradication of malaria, which is one of the major causes of morbidity and mortality in Zambia.

1.2 STATEMENT OF THE PROBLEM

Counterfeit and substandard medicines present an enormous public health challenge (WHO 2006). No area of the world is unaffected, but mounting evidence shows that the problem is disproportionately severe in developing and emerging market countries, which also have a high burden of infectious diseases. In poor countries, like Zambia, essential and life-saving drugs used to treat infectious diseases such as tuberculosis and malaria are often the drugs under threat (Bate and Boateng 2007). According to CDC 2006, in 1999, in Cambodia counterfeit antimalarial drugs were responsible for the deaths of at least 30 people.

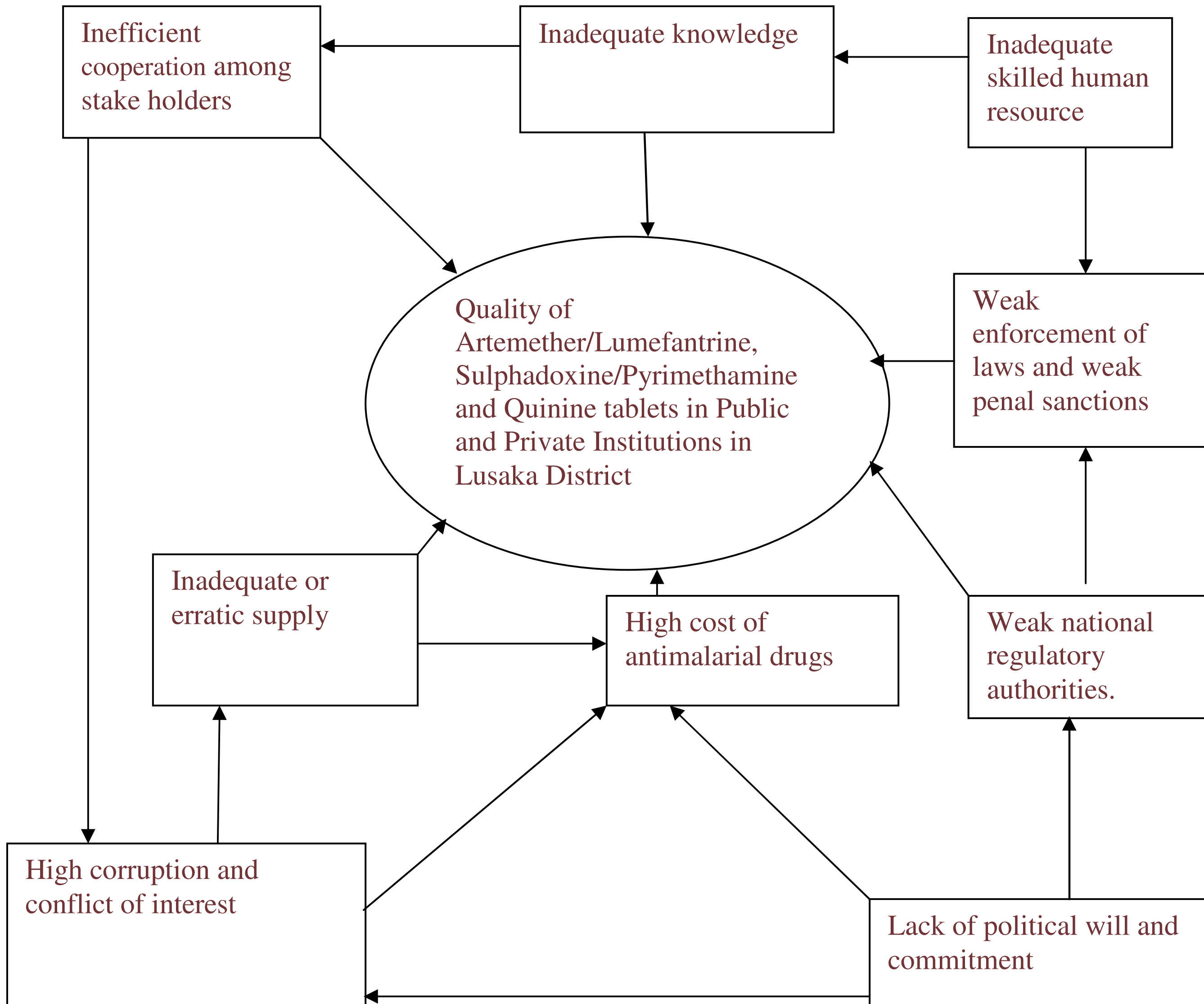
In Zambia, no studies have been done on counterfeit and substandard antimalarial drugs; hence there is almost complete absence of both qualitative and quantitative data on the prevalence of counterfeit and substandard drugs.

85-90% of malaria fatalities occur in sub-Saharan Africa, where Zambia lies, and the malaria fatality rates for Lusaka District for the past five years were;

- Year 2003 45.3/1000 admissions
- Year 2004 41.6/1000 admissions
- Year 2005 66.0/1000 admissions
- Year 2006 38.2/1000 admissions
- Year 2007 51.0/1000 admissions (LUD, HMIS 2008).

The above stipulated figures are well above the WHO threshold of 25/1000 admissions. Lusaka ranks third in malaria case fatalities in Zambia (HMIS 2008). The malaria statistics are included to justify that malaria has remained a public health problem in Lusaka. This could be as a result of use of poor quality antimalarial drugs, and other factors such as lack of both antimalarial drugs and diagnostic equipments.

1.3 PROBLEM ANALYSIS DIAGRAM: QUALITY OF ARTEMETHER/LUMEFANTRINE, SULPHADOXINE/PYRIMETHAMINE AND QUININE TABLETS IN PUBLIC AND PRIVATE HEALTH INSTITUTIONS LUSAKA DISTRICT.



1.4 RATIONALE OF THE STUDY

Some of the consequences of using counterfeit and substandard antimalarial drugs are;

(a) Lacking of therapeutic effect and treatment failure (Bate and Boateng 2007).

(b) Complications and mortality due to malaria and toxic components of the drugs (Kelesidis et.al 2007).

(c) High burden of malaria leading to mortality and morbidity (Kelesidis at.el 2007).

Counterfeit medicines are part of a broader phenomenon of substandard pharmaceuticals – medicines manufactured below established standards of quality and therefore dangerous to patients' health and ineffective for the treatment of diseases (WHO 2006).

The malaria statistics for Lusaka district included in the statement of the problem justify that malaria has remained a public health problem in Lusaka. This could be as a result of use of poor quality antimalarial drugs, and other factors such as lack of both antimalarial drugs and diagnostic equipments.

The study is likely to generate information that will be useful to the strengthening of the regulatory and law enforcement of fighting counterfeiting of drugs and production, sale and use of substandard drugs, thereby accelerating the control and fight against malaria. Once the fight against counterfeiting of antimalarial drugs is won, control and fight against malaria is likely to succeed, the health status of the Zambians will improve, leading to increased productivity, increased economic growth and reduced poverty levels, hence attaining MDG 1.

1.5 RESEARCH QUESTION

Does the quality of Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets in Public and Private Health Institutions in Lusaka District meet the standard as prescribed in the official monographs?

1.6 STUDY OBJECTIVES.

1.6.1 GENERAL OBJECTIVE:

To evaluate the quality of Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets in selected Public and Private Health Institutions in Lusaka District.

1.6.2 SPECIFIC OBJECTIVES:

1. To verify the active ingredients contained in the collected samples of Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets with reference to the label claim using the GPHF-Minilab.

2. To determine the percentage content of the active ingredients in the Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets that will be sampled and analysed.

3. To assess if the packaging and labeling on collected samples of Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets will be in conformity with reference to Pharmaceutical Standard Reference guidelines.

4. To ascertain the proportions of Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets from the collected samples that will be substandard.

5. To establish the key supply sources of the antimalarial drugs that will be found to be substandard.

CHAPTER TWO

LITERATURE REVIEW

Counterfeit and substandard medicines represent an enormous public health challenge. Anyone, anywhere in the world, can come across medicines seemingly packaged in the right way, in the form of tablets or capsules that look right, but which do not contain the correct ingredients and, in the worst case scenario, may be filled with highly toxic substances. In some countries, this is a rare occurrence, in others, it is an everyday reality. Counterfeit medicines range from random mixtures of toxic substances to inactive, useless preparations. Occasionally, there can be ''high quality'' fakes that do not contain the declared active ingredients. In all cases, contents of counterfeits are unreliable because their source is unknown or vague and always illegal. Fake drugs can cause harm and sometimes lead to death (WHO 2006).

Literature review was done to ascertain the extent of the problem of counterfeit and substandard drugs. The information gathered through literature review flows from the global perspective, regional perspective and national perspective respectively.

Globally, in developed countries the percentage of counterfeit drugs is estimated at 1% while in specific regions of the world, e.g. Asia and Africa the overall percentage is significantly higher than the global market average (Aria 2008). In India, in 2002 pharmaceutical companies suggested that in India's major cities, 1 in 5 medicines sold was a fake (WHO 2002). A survey in Southeast Asia, showed that among 104 tablets presented as the antimalarial drug artesunate, 38% did not contain any artesunate (Newton, Proux, Green et.al 2001). According to CDC 2006, in 1999, in Cambodia counterfeit antimalarial drugs were responsible for the deaths of at least 30 people.

Regionally, a study conducted in 6 countries in most severely malarious parts of Africa revealed that 35% (73/210) of tested samples of antimalarial drugs were substandard (Bate, Coticelli, Tren and Attaran 2008). In Burkina Faso, a study on substandard antimalarial drugs showed that 32/77 (42%) samples were of poor quality, of which 28

samples failed the visual inspection, 9 samples had substandard concentrations of the active ingredients, 4 samples showed poor disintegration, and 1 sample contained none of the stated active ingredients (Tipke et.al 2008). According to the American Journal of Tropical Medicine and Hygiene 2004, in Cameroon fifty (38%) of 133 Chloroquine, 52 (74%) of 70 quinine, and 10 (12%) of 81 antifolates had either no active ingredient, an insufficient active ingredient, the wrong ingredient, or unknown ingredient(s). The study results of antimalarial drugs bought in Ghana, Kenya, Nigeria, Rwanda, Tanzania and Uganda showed that 35% contained too little active ingredient or failed to dissolve, rendering them ineffective (Times online 2008). In 6 African countries a study was conducted on the quality of antimalarial drugs which were on sale, and it revealed that 16 of 42 tested drugs (38%) on the Kenyan market were ineffective in treating the disease (IRIN Africa 2008).

In Zambia, no studies have been done on counterfeit and substandard antimalarial drugs; therefore, there is almost complete absence of both qualitative and quantitative data on the prevalence of counterfeited and substandard drugs. However, the Government of the Republic of Zambia has shown its commitment to fight the scourge by establishing institutions such as the Pharmaceutical Regulatory Authority (PRA) and the Drug Enforcement Commission (DEC). In addition, as a way of strengthening the fight, on 11th day of November 2008, the government formed the Drug Taskforce to fighting counterfeiting of drugs in the country.

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.1 STUDY SETTING

The study was conducted in Lusaka, the capital city of the Republic of Zambia. Lusaka district is also the headquarters of Lusaka province, and it shares borders with Chongwe district on the east, Kafue district on the south, Mumbwa district on the west, and Chibombo district on the north. It has an estimated population of two (2) million people (CSO 2000).

The study was undertaken in Lusaka district because this is where most of the Public and Private Health Institutions in Zambia are concentrated, hence making it easy to collect the drug samples. Moreover, Lusaka province ranks third in the malaria case fatality in Zambia.

3.2 STUDY POPULATION

The study population was the total number of antimalarial drugs, of the study interest, that were found in the randomly selected 10 Public and 10 Private institutions in Lusaka district.

The prevalence of malaria in Lusaka district still remains high at 32.88% (LUD HMIS 2010). The district receives approximately 4,615626 tablets of Artemether/Lumefantrine, 1,490962 tablets of Sulphadoxine/Pyrimethamine and 1,313362 tablets of Quinine sulphate per year (LUD drug database 2010), which are accessed by the district estimated population of approximately two (2) million through the 27 Public Health Institutions and 47 Private Health Institutions registered by the Pharmaceutical Regulatory Authority and other unregistered business entities. Of the total antimalarial drugs received, about 10% is used through self medication.

3.3 STUDY DESIGN

It was an explorative chemical analysis with a cross sectional approach in the selection of drug samples. The explorative chemical analysis involved visual inspection of drug samples, verification of the identity of the drug and drug contents via thin layer chromatography. For details of the analysis refer to annex 3.

3.3.1 Inclusion Criteria

All Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets in selected institutions not expired and with shelf-life not less than 1 year were included in the study.

3.3.2 Exclusion Criteria

Non Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets in these institutions expired and those with shelf-life less than 1 year were excluded from the study.

3.3.3 Identification variables

Independent variables

- Active ingredients
- Percentage content of active ingredient
- Labeling
- Packaging
- Manufacturer of the drug
- Business type
- Location of business

Dependent variable

Quality of Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets in Public and Private Institutions in Lusaka District.

3.4 SAMPLING METHODS

Probability, multistage sampling method was employed in the collection of antimalarial drugs for analysis.

Drug samples were not bought or collected from all Public and Private Institutions, but from only a few that were selected randomly in different clusters of the district. The analysis of antimalarial drugs was done using the Minilab at Pharmaceutical Regulatory Authority premises situated in Lusaka.

Selection of drug products for this study was based on their importance in the fight against malaria and their relevance to public health. Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets were chosen because they are the antimalarial drugs used in Zambia, and they are regarded as essential drugs. Samples were collected from randomly selected 10 Public and 10 Private Health Institutions in Lusaka District.

Artemether/Lumefantrine tablet is an antimalarial agent containing active ingredients Artemether and Lumefantrine. Artemether is a semisynthetic chiral acetal derivative from artemisinin, a bicyclic sesquiterpene lactone endoperoxide isolated from the plant Artemisia annua. Lumefantrine is a racemic mixture of a synthetic fluorine derivative (USP). The drug commonly known as Coartem is used as first line drug in treatment of malaria in Zambia.

Sulphadoxine/Pyrimethamine tablet is an antimalarial agent containing 500mg N1-(5, 6-dimethoxy-4-pyrimidinyl) sulfanilamide (Sulphadoxine) and 25mg 2, 4-diamino-5-(p-chlorophenyl)-6-ethylpyrimidine (Pyrimethamine) (BP 1993). It is commonly used for the treatment of malaria as a second line drug, and in prophylaxis of malaria in pregnancy as Intermittent Presumptive Treatment.

Quinine tablet is an antimalarial drug containing a chief alkaloid of various species of Cinchona (Rubiaceae) (BP 1993). It is an optical isomer of quinidine. It is used in treatment of complicated malaria.

DRUGS AND FORMULATIONS CHOSEN FOR THE STUDY.

DRUG CLASS	DRUG NAME	FORMULATION
Antimalarial	Artemether/Lumefantrine	Tablet
Antimalarial	Sulphadoxine/Pyrimethamine	Tablet
Antimalarial	Quinine	Tablet

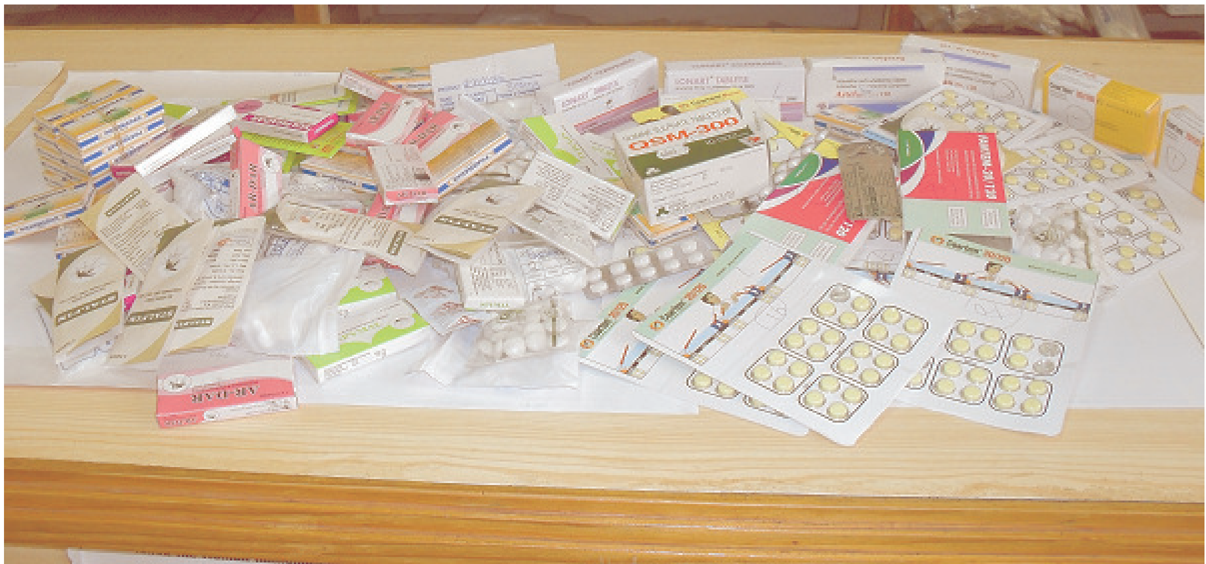


Figure 1: All antimalarial drug samples collected for quality analysis.

3.5 SAMPLE SIZE DETERMINATION

(a) **1st Stage** – random selection of 10 institutions from Public and Private. The total number of institutions was 20.

(b) **2nd Stage** – selection of at least 10% of the containers of each drug type using convenient sampling method.

(c) **3rd Stage** – selection of sampling units by simple random sampling method.

The study was designed to tolerate an absolute sampling error of up to 5 percent, with the power of the study at 95 percent.

The formula for sample size calculation was:

$$n = \frac{Z^2(100-P)}{d^2}$$

Where; Z = 1.96, factor from normal distribution

P = Expected period prevalence

d = Absolute sampling error

n = Sample size

Therefore, $n = \frac{(1.96)^2 x50(100-50)}{5^2}$

= 384 tablets of each antimalarial drug type

Adjustment for handling loses – 10%

= *422* tablets of each antimalarial drug type but rounded off to **430.**

3.6 DATA MANAGEMENT

(a) Collection *(Tools and Techniques)*

A drug collection sheet which was designed to collect information on the place of purchase, name of drug as indicated by the seller/dispenser of the drug, active ingredients contained in the drug as per label, physical appearance of the tablets, nature and material of packaging, manufacturing and expiry dates of the drug, batch number of the drug, name of manufacturer of the drug, type of business and location of business was used.

Consent was obtained from the proprietors of the Private Health Institutions before drug samples were purchased from them. Drug samples from Public Health Institutions were collected after permission was sought from the Permanent Secretary, Ministry of Health, and the Director General of the Pharmaceutical Regulatory Authority.

(b) Data entry and handling

Data was entered in SPSS version 17.0 for windows after all variables were coded. The coded variables were used to perform cross tabulation. Data were handled in strict confidence where a password was used to open the data file.

(c) Data analysis

Data was analysed using SPSS version 17.0 for windows. Cross tabulations were done to test the significance of the association between independent variables and dependent variable. Openepi was used to calculate the percentage proportions of drugs that were found to be substandard (Poor quality) drugs.

3.7 ETHICAL CONSIDERATIONS

Though the study did not involve human subjects, clearance was sought from the University of Zambia Research Ethics Committee (UNZA REC). Permission was also sought from MOH and PRA for collection of samples from Public Institutions. As for Private Institutions the investigator obtained consent from the proprietors to buy drug samples that were analysed.

CHAPTER FOUR

4.0 DATA PRESENTATION

4.1 INTRODUCTION

Drug quality was assessed by visual inspections to ascertain whether the packaging and labeling conformed to the official monographs. The nature and percentage content of the active ingredients were assessed, as a way of ascertaining the quality of drugs, by Thin Layer Chromatography (T.L.C) of the GPHF mini-laboratory based at the Pharmaceutical Regulatory Authority Quality Control Laboratory in Lusaka. Ascertaining of the proportions of drug samples that were substandard and establishing their key supply source was done.

The findings are the data obtained through analysis of study units obtained from randomly selected 10 Public and 10 Private health institutions in Lusaka district. The evaluation of the quality of drug samples was done with reference to the objectives of the study.

Data was collected over a period of three months after Biomedical Ethics Committee approval of the research proposal, and the laboratory work took a period of 1 (one) month to complete.

Data was entered in SPSS version 17.0 for windows and analysed, and presented in way of cross tabulation. Proportional percentages of substandard drugs were calculated using Openepi.

4.2 ANALYTICAL RESULTS

4.2.1 ARTEMETHER/LUMEFANTRINE TABLETS

Table 1 shows the results of the active ingredients verification assessment of the drug samples of Artemether/Lumefantrine. The sample units (tablets) were sorted out in batches according to their batch numbers, dates of manufacturing, and dates of expiry and names of the manufacturing companies. After the sorting out exercise was done, drug samples were assigned new batch numbers for the purpose of analysis, and then sample units were analysed according to batches. The assay of the Artemether/Lumefantrine drug samples revealed that all the analysed samples contained the Artemether/Lumefantrine active ingredients.

Table 1: *Results of the verification of the active ingredients contained in Artemether/Lumefantrine samples using the GPHF mini-laboratory at the Pharmaceutical Regulatory Authority Quality Control Laboratory.*

BATCH NUMBER	NUMBER OF TABLETS IN THE BATCH	ACTIVE INGREDIENTS PRESENT	ACTIVE INGREDIENTS ABSENT
1	48	Yes	No
2	36	Yes	No
3	48	Yes	No
4	72	Yes	No
5	72	Yes	No
6	49	Yes	No
7	48	Yes	No
8	23	Yes	No
9	34	Yes	No
Total tablets analysed	**430**		

Note: The assessment revealed that all the 430 drug samples of Artemether/Lumefantrine contained the active ingredients of artemether and lumefantrine.

Table 2: *Results of the percentage content of the active ingredients in the drug samples of Artemether/Lumefantrine tablets analysed using the GPHF mini-laboratory at the Pharmaceutical Regulatory Authority Quality Control Laboratory*

BATCH NUMBER	NUMBER OF TABLETS IN THE BATCH	100 PERCENT CONTENT OF ACTIVE INGREDIENTS	80 PERCENT CONTENT OF ACTIVE INGREDIENTS
1	48	Yes	No
2	36	Yes	No
3	48	Yes	No
4	72	Yes	No
5	72	Yes	No
6	49	Yes	No
7	48	Yes	No
8	23	Yes	No
9	34	Yes	No
Total tablets analysed	**430**		

Note: The assessment revealed that all the 430 tablets of Artemether/Lumefantrine which were assayed for percentage content of active ingredients had 100% (the upper limit and acceptable level according to the GPHF mini-laboratory standard).

***Table 3:** Results of the assessment on the packaging and labelling of the Artemether/Lumefantrine drug samples using the GPHF visual inspection technique.*

BATCH NUMBER	NUMBER OF TABLETS IN THE BATCH	CONFORMITY OF PACKAGING AND LABELLING WITH STANDARD REFERENCE GUIDELINES	NON-CONFORMITY OF PACKAGING AND LABELLING WITH STANDARD REFERENCE GUIDELINES
1	48	Yes	No
2	36	Yes	No
3	48	Yes	No
4	72	Yes	No
5	72	Yes	No
6	49	Yes	No
7	48	Yes	No
8	23	Yes	No
9	34	Yes	No
Total tablets assessed	**430**		

Note: The packaging and labelling of all the 430 tablet samples of Artemether/Lumefantrine conformed to the Pharmaceutical Standard Reference Guidelines.

Table 4: *Results of ascertaining the proportion of Artemether/Lumefantrine tablet samples that were substandard.*

95% Confidence Limits (CL) for proportion 0/430

NUMBER OF SUBSTANDARD TABLETS	**TOTAL NUMBER OF TABLETS ANALYSED**	**PROPORTION OF SUSTANDARD TABLETS TO TOTAL TABLETS ANALYSED % (95 CI)**
0	430	0.0%

Note: Wald's Normal Approximation was used to find the percentage proportions.

Of all the 430 tablets of Artemether/Lumefantrine analysed, no tablet was found to be substandard.

4.2.1.1 SULPHADOXINE/PYRIMETHAMINE TABLETS

Table 5 shows the results of the active ingredients verification assessment of the drug samples of Sulphadoxine/Pyrimethamine. The sample units (tablets) were sorted out in batches according to their batch numbers, dates of manufacturing, and dates of expiry and names of the manufacturing companies. After the sorting out exercise was done, drug samples were assigned new batch numbers for the purpose of analysis, and then the sample units were analysed according to batches. The assay of the Sulphadoxine/Pyrimethamine drug samples revealed that not all the analysed samples contained the Sulphadoxine/Pyrimethamine active ingredients.

Table 5: *Results of the verification of the active ingredients contained in Sulphadoxine/Pyrimethamine samples using the GPHF mini-laboratory at the Pharmaceutical Regulatory Authority Quality Control Laboratory.*

BATCH NUMBER	**NUMBER OF TABLETS IN THE BATCH**	**ACTIVE INGREDIENTS PRESENT**	**ACTIVE INGREDIENTS ABSENT**
10	30	Yes	No
11	30	No	Yes *
12	30	Yes	No
13	60	Yes	No
14	30	Yes	No
15	30	Yes	No
16	30	Yes	No
17	30	Yes	No
18	30	Yes	No
19	30	Yes	No
20	35	Yes	No
21	35	Yes	No
22	30	Yes	No
Total tablets analysed	**430**		

Note: The assessment revealed that 30 out of 430 tablets analysed did not contain the active ingredients of sulphadoxine and pyrimethamine.

Table 6: *Results of the percentage content of the active ingredients in the drug samples of Sulphadoxine/Pyrimethamine tablets analysed using the GPHF mini-laboratory at the Pharmaceutical Regulatory Authority Quality Control Laboratory*

BATCH NUMBER	**NUMBER OF TABLETS IN THE BATCH**	**100 PERCENT CONTENT ACTIVE INGREDIENT**	**80 PERCENT CONTENT OF ACTIVE INGREDIENT**
10	30	Yes	No
11	30	No	Yes *
12	30	Yes	No
13	60	Yes	No
14	30	Yes	No
15	30	Yes	No
16	30	Yes	No
17	30	Yes	No
18	30	Yes	No
19	30	Yes	No
20	35	Yes	No
21	35	Yes	No
22	30	Yes	No
Total tablets analysed	**430**		

Note: The assessment revealed that 30 tablets had less than 100 percent active ingredients (the upper limit and acceptable level according to the GPHF mini-laboratory standard).

Table 7: *Results of the assessment on the packaging and labelling of the Sulphadoxine/Pyrimethamine drug samples using the GPHF visual inspection technique.*

BATCH NUMBER	**NUMBER OF TABLETS IN THE BATCH**	**CONFORMITY OF PACKAGING AND LABELLING WITH STANDARD REFERENCE GUIDELINES**	**NON-CONFORMITY OF PACKAGING AND LABELLING WITH STANDARD REFERENCE GUIDELINES**
10	30	Yes	No
11	30	Yes	No
12	30	Yes	No
13	60	Yes	No
14	30	Yes	No
15	30	Yes	No
16	30	Yes	No
17	30	Yes	No
18	30	Yes	No
19	30	Yes	No
20	35	Yes	No
21	35	Yes	No
22	30	Yes	No
Total tablets analysed	**430**		

Note: The assessment revealed that all the 430 tablets assessed conformed to reference guidelines in terms of packaging and labelling.

Table 8: *Results of ascertaining the proportion of Sulphadoxine/Pyrimethamine tablet samples that were substandard.*

95% Confidence Limits (CL) for proportion 30/430

NUMBER OF SUBSTANDARD TABLETS	TOTAL NUMBER OF TABLETS ANALYSED	PROPORTION OF SUSTANDARD TABLETS TO TOTAL TABLETS ANALYSED % (95 CI)
30	430	6.98% (95% CI 4.57 – 9.38)

Note: Wald's Normal Approximation was used to find the percentage proportions.

6.98% of Sulphadoxine/Pyrimethamine tablets were found to be substandard.

4.2.1.1.1 QUININE SULPHATE TABLETS

Table 9 shows the results of the active ingredient verification assessment of the drug samples of Quinine sulphate. The sample units (tablets) were sorted out in batches according to their batch numbers, dates of manufacturing, and dates of expiry and names of the manufacturing companies. After the sorting out exercise was done, drug samples were assigned new batch numbers for the purpose of analysis, and then sample units were analysed according to batches. The assay of the Quinine sulphate drug samples revealed that all the analysed samples contained the Quinine sulphate active ingredient.

Table 9: *Results of the verification of the active ingredient contained in Quinine sulphate samples using the GPHF mini-laboratory at the Pharmaceutical Regulatory Authority Quality Control Laboratory.*

BATCH NUMBER	**NUMBER OF TABLETS IN THE BATCH**	**ACTIVE INGREDIENT PRESENT**	**ACTIVE INGREDIENT ABSENT**
23	30	Yes	No
24	30	Yes	No
25	30	Yes	No
26	30	Yes	No
27	30	Yes	No
28	30	Yes	No
29	60	Yes	No
30	30	Yes	No
31	30	Yes	No
32	60	Yes	No
33	40	Yes	No
34	30	Yes	No
Total tablets analysed	**430**		

Note: The assessment revealed that all the 430 tablets of Quinine sulphate that were analysed contained the active ingredient.

Table 10: *Results of the percentage content of the active ingredient in the drug samples of Quinine sulphate tablets analysed using the GPHF mini-laboratory at the Pharmaceutical Regulatory Authority Quality Control Laboratory*

BATCH NUMBER	**NUMBER OF TABLETS IN THE BATCH**	**100 PERCENT CONTENT ACTIVE INGREDIENT**	**80 PERCENT CONTENT ACTIVE INGREDIENT**
23	30	Yes	No
24	30	Yes	No
25	30	Yes	No
26	30	Yes	No
27	30	Yes	No
28	30	Yes	No
29	60	Yes	No
30	30	Yes	No
31	30	Yes	No
32	60	Yes	No
33	40	Yes	No
34	30	Yes	No
Total tablets analysed	**430**		

Note: The assessment revealed that all the 430 tablets Quinine sulphate analysed had 100 percent active ingredient (the upper limit and acceptable level according to the GPHF mini-laboratory standard).

Table 11: *Results of the assessment on the packaging and labelling of the Quinine sulphate drug samples using the GPHF visual inspection technique.*

BATCH NUMBER	NUMBER OF TBLETS IN THE BATCH	CONFORMITY OF PACKAGING AND LABELLING WITH STANDARD REFERENCE GUIDELINES	NON-CONFORMITY OF PACKAGING AND LABELLING WITH STANDARD REFERENCE GUIDELINES
23	30	Yes	No
24	30	Yes	No
25	30	Yes	No
26	30	Yes	No
27	30	Yes	No
28	30	Yes	No
29	60	Yes	No
30	30	Yes	No
31	30	Yes	No
32	60	Yes	No
33	40	Yes	No
34	30	Yes	No
Total tablets analysed	**430**		

Note: The assessment revealed that all the 430 tablets assessed conformed to standard reference guidelines in terms of packaging and labelling.

Table 12: *Results of ascertaining the proportion of Quinine sulphate tablet samples that were substandard.*

95% Confidence Limits (CL) for proportion 0/430

NUMBER OF SUBSTANDARD TABLETS	TOTAL NUMBER OF TABLETS ANALYSED	PROPORTION OF SUSTANDARD TABLETS TO TOTAL TABLETS ANALYSED % (95 CI)
0	430	0.0%

Note: Wald's Normal Approximation was used to find the percentage proportions.

No tablet of Quinine was found to be substandard.

4.3 CROSS TABULATIONS BETWEEN DEPENDENT AND INDEPENDENT VARIABLES

Table 4.3.1 *Association between type of drug and quality of drugs*

TYPE OF DRUG	QUALITY OF DRUGS			P-value
	Good	**Poor**	**Total**	<0.001
Artemether/Lumefantrine	430	0	430	
Sulphadoxine/Pyrimethamine	400	30	430	
Quinine sulphate	430	0	430	
Total	**1260**	**30**	**1290**	

Note: 30 tablets of Sulphadoxine/Pyrimethamine were of poor quality (substandard). The P-value is <0.001, which is highly significant.

Table 4.3.2 *Association between active ingredients and quality of drugs*

ACTIVE INGREDIENT	QUALITY OF DRUGS			P-value
	Good	**Poor**	**Total**	
Present	1260	0	1260	<0.001
Absent	0	30	30	
Total	**1260**	**30**	**1290**	

Note: 30 tablets of the drug samples which were found to be of poor quality (substandard) did not contain the expected active ingredients. The P-value is <0.001, which is highly significant.

Table 4.3.3 *Association between percentage of active ingredients and quality of drugs.*

PERCENTAGE OF ACTIVE INGREDIENTS	QUALITY OF DRUGS			P-value
	Good	**Poor**	**Total**	
Upper limit	1260	0	1260	<0.001
Lower limit	0	30	30	
Total	**1260**	**30**	**1290**	

Note: 30 tablets of the drug samples were of poor quality (substandard) as their active ingredients were at the lower limit. The P-value is <0.001, which is highly significant.

Table 4.3.4 *Association between business type and quality of drugs*

BUSINESS TYPE	QUALITY OF DRUGS			P-value
	Good	**Poor**	**Total**	<0.001
Public	680	0	680	
Private	580	30	610	
Total	**1260**	**30**	**1290**	

Note: 30 tablets which were of poor quality (substandard) were obtained from the Private Health Institutions. The P-value is <0.001, which is highly significant.

Table 4.3.5 *Association between business location and quality of drugs*

LOCATION	QUALITY OF DRUGS			P-value
	Good	**Poor**	**Total**	0.001
High density	898	30	928	
Low density	362	0	362	
Total	**1260**	**30**	**1290**	

Note: 30 tablets which were of poor quality (substandard) were obtained from high density areas of Lusaka district. The P-value is 0.001, which is highly significant.

Table 4.3.6 *Association between drug packaging and quality of drugs*

DRUG PACKAGING	QUALITY OF DRUGS			P-value
	Good	**Poor**	**Total**	No statistics computed. Drug packaging is constant
Appropriate	1260	30	1290	
Not appropriate	0	0	0	
Total	**1260**	**30**	**1290**	

Note: All drugs had appropriate packaging. The P-value was not computed as drug packaging was constant.

Table 4.3.7 *Association between drug labelling and quality of drugs*

DRUG LABELLING	QUALITY OF DRUG			P-value
	Good	**Poor**	**Total**	No statistics computed. Drug packaging is constant
Appropriate	1260	30	1290	
Not appropriate	0	0	0	
Total	**1260**	**30**	**1290**	

Note: All drugs had appropriate labelling. The P-value was not computed as drug packaging was a constant.

Table 4.3.8 *Association between drug manufacturer and quality of drug*

DRUG MANUFACTURER	QUALITY OF DRUG			P-value
	Good	**Poor**	**Total**	0.392
Local	30	0	30	
International	1230	30	1260	
Total	**1260**	**30**	**1290**	

Note: 30 tablets that were of poor quality (substandard) were from international manufacturers. The P-value is 0.392, which is not significant.

CHAPTER FIVE

5.0 DISCUSION

This explorative chemical analysis with a cross sectional approach in selection of drug samples study was conducted to evaluate the quality of Artemether/Lumefantrine, Sulphadoxine/Pyrimethamine and Quinine tablets in selected public and private health institutions in Lusaka District.

Validity for this study was ensured by covering all important variables under study. A pilot study was conducted and amendments to the instrument were done where it was necessary, and a pilot study was done to measure reliability of the instrument.

The results of this study are reported in tables labelled from **1** to **4.3.8** to provide objective information on the quality of antimalarial drugs obtainable in both public and private health institutions in terms of the type of active ingredient contained in the drug, the percentage content of the active ingredients and quality of packaging material and labelling.

The findings of this study have confirmed the reports and literature that were reviewed during the proposal of this study that the problem of counterfeit and substandard drugs, antimalarial drugs inclusive, is both global and local.

Of paramount importance to this study is the revelation of the 30 (6.98%), with 95% Confidence Interval of 4.57 to 9.38, tablets of Sulphadoxine/Pyrimethamine that were substandard according to the GPHF mini-laboratory standard. Sulphadoxine/Pyrimethamine is supposed to be a combination of two active ingredients; namely sulphadoxine and pyrimethamine in their correct percentage and proportions (B.P 1993). The rationale of combining two antimalarial active ingredients in one drug is to provide the synergistic action against the malaria parasite hence promoting appropriate and adequate treatment and prevention of malaria.

As a further analysis was carried out to ascertain the percentage content of the active ingredients in the drug samples, it was discovered that the same 30 tablets of Sulphadoxine/Pyrimethamine tablets did not contain the correct percentage content of active ingredients as required by the official monographs. According to the GPHF mini-laboratory standard, the percentage content of active ingredients must be 100%.

However, despite the 30 tablets of Sulphadoxine/Pyrimethamine containing 100% of Sulphadoxine, they did not contain the component of Pyrimethamine at all. The non inclusion of Pyrimethamine active ingredient in the 30 Sulphadoxine/Pyrimethamine could have been done deliberately, because even with the absence of the Pyrimethamine active ingredient the drug will still retain its antimalarial activity, though the intended synergistic effect of the drug will be absent. However, if this scenario is left to continue, rapid resistance to Sulphadoxine/Pyrimethamine will develop hence rendering an important and potent drug in the prevention and treatment of malaria useless.

The population of Lusaka district consumes approximately 1,490962 tablets of Sulphadoxine/Pyrimethamine per year. Therefore, with 6.98% (95% CI 4.57 – 9.38) substandard tablets, it means that the population consumes about 10% of 1,490962 (149096) tablets of Sulphadoxine/Pyrimethamine as substandard. The consequences of using substandard Sulphadoxine/Pyrimethamine and any other antimalarial drugs will be lack of therapeutic effect and treatment failure, increase in complicated malaria and high burden of malaria, as the case is for Lusaka district with prevalence of 32.88% (LUD HIMS 2010), leading to mortality and morbidity (Kelesidis at. et. 2007).

If the malaria prevalence continues to remain high, which is likely to be the case if the substandard antimalarial drugs, Sulphadoxine/Pyrimethamine inclusive, are allowed to be used in Lusaka district, the Disability Adjusted Life Years (DALYs) of the people will be increased hence resulting in reduced Quality Adjusted Life Years (QALYS) of the population, and this will lead to unproductive population that will in turn continue to live in abject poverty hence making it impossible for Zambia to meet the MDG 1.

The assay on the verification of the active ingredients contained in the drug samples with reference to their label claims revealed that all the tablets except 30 of Sulphadoxine/Pyrimethamine contained the active ingredients as per their label claim.
As a further analysis was carried out to ascertain the percentage content of the active ingredients in the drug samples, it was discovered that the same 30 tablets of Sulphadoxine/Pyrimethamine tablets did not contain the correct percentage content of active ingredients as required by the official monographs. According to the GPHF mini-laboratory standard, the percentage content of active ingredients must be 100%. However, despite the 30 tablets of Sulphadoxine/Pyrimethamine containing 100% of Sulphadoxine, they did not contain the component of Pyrimethamine at all.

The quality of a pharmaceutical product can also be affected by the quality and type of the packaging material used and the storage conditions to which they are subjected. Packaging materials for pharmaceutical products play an important role in the maintenance and preservation of the quality of the product. As most pharmaceutical products are chemical in nature, they have prescribed packaging materials in standard reference guidelines or official monographs. After thorough visual inspection of all drug samples' packaging material as per standard of the GPHF mini-laboratory standard, it was verified that the packaging materials for all drug samples conformed to the requirement of the official monographs. The packaging materials comprised paper, plastic, aluminium foil and or a combination of two or three of the above mentioned materials. These materials are pharmaceutically acceptable materials as they are capable of preserving the quality of the antimalarial drugs under this study.

Labelling of pharmaceutical products is a legal requirement so as to give information on the nature of the product, the ingredients and excipients contained in the drug, its use, its storage conditions, its manufacturing date, its expiry date and any other relevant information pertaining to the product. After a thorough visual inspection of the drug samples' labels to ascertain their conformity to the pharmaceutical and legal requirement, it was verified that the labels were in conformity with the requirement as per the official monographs according to the GPHF mini-laboratory standard. However, the labels for the

30 Sulphadoxine/Pyrimethamine tablets that failed to conform to the required active ingredients and percentage content of the active ingredients were misleading as the drug samples did not contain what the label claimed they contained.

The proportions of substandard drugs were 0%, 6.98% and 0% for Artemether/lumefantrine, Sulphadoxine/Pyrimethamine and Quinine sulphate tablets respectively. The results indicate that there were no substandard drugs for Artemether/Lumefantrine and Quinine sulphate samples that were analysed. However, 6.98% of Sulphadoxine/Pyrimethamine tablets were substandard. This percentage may seem small but since all pharmaceutical products, drugs inclusive, are required by law and Pharmaceutical Standards to be of good quality in order to assure their efficacy and safety, this result calls for serious pharmacovigilance of the pharmaceutical products obtainable on the Zambian market.

Factors that were found to be associated with poor quality or substandard drugs were drug type, active ingredients, and percentage contents of active ingredients, business type and location of business according to cross tabulation results in tables 4.3.1 through to 4.3.8. However, the confidence interval (C.I) was so wide as a result of empty cells.

The interpretation of the results is that substandard or poor quality Sulphadoxine/Pyrimethamine tablets are more likely to be found in Lusaka district than tablets of Artemether/Lumefantrine and Quinine sulphate. The reason could be that Sulphadoxine/Pyrimethamine tablets are commonly found in both registered and unregistered business premises, as the cost of Sulphadoxine/Pyrimethamine treatment course is less than that of either Artemether/Lumefantrine or Quinine sulphate, the drug is affordable to many people. As the demand for the drug is increased, this triggers high supply. In order to meet the high demand for the drug, some pharmaceutical companies engage in mass production and in the due course disregarding the Good Manufacturing Practices (GMPs).

The business type, that is private or public, also showed association with poor quality or substandard Sulphadoxine/Pyrimethamine tablets. It can be inferred from the results that substandard drugs are more likely to be found in Private Health Institutions than Public ones. The reasoning behind this could be that Private Health Institutions are more about making profits than provision of total quality health care. This could also mean that the Private Health Institutions are not strictly inspected and regulated regularly by regulatory bodies such as the Pharmaceutical Regulatory Authority and the Medical Council of Zambia.

The results also revealed an association between the locations of the business, which is high density area or low density area, and the poor quality or substandard Sulphadoxine/Pyrimethamine tablets. This means that substandard antimalarial drugs are more likely to be found in high density area than low density area. The reason could be that in low density areas of Lusaka, such as Kabulonga and Kalundu, people are educated and they are so particular about what they consume unlike in high density areas such as Chawama and Kanyama. In densely populated areas people are usually uneducated and they do not mind so much what they consume, and so unscrupulous business entities take advantage of them. It is, therefore, not uncommon to find many Private Health Institutions established in high density areas as they are mostly profit oriented.

The association between the manufacturer, local or international, and poor quality or substandard Sulphadoxine/Pyrimethamine could not show significant results. This could be because many of the drugs were manufactured from outside Zambia, and the few local pharmaceutical companies could not manufacture all the antimalarial drugs. However, the 30 tablets of poor quality or substandard Sulphadoxine/Pyrimethamine tablets were manufactured by an international pharmaceutical company.

5.1 CONCLUSION

This study provides objective evidence to answer speculations whether or not substandard antimalarial drugs exist in Lusaka. The study has revealed that substandard antimalarial drugs exist in Lusaka as evidenced by the 6.98% of Sulphadoxine/Pyrimethamine tablets that failed to comply with the required active ingredients and their percentage content. The substandard drugs could not have been counterfeited but could have been as a result of non adherence to Good Manufacturing Practice. The study shows that the antimalarial drugs conformed to the official monographs requirements in ascertaining the quality of the pharmaceutical products in terms of packaging and labelling. The existence of substandard antimalarial drugs in Lusaka, and their final use in prevention and treatment of malaria could have detrimental clinical consequences and implications to the patient, as substandard drugs are unsafe and inefficacious. The revelation of the existence of substandard drugs by this study poses a challenge to the Pharmaceutical Regulatory Authority to enhance its Post-marketing surveillance programme to ensure and assure constant quality monitoring of drugs that are found on the Zambian market, as quality, safety and efficacy are the tenets of every pharmaceutical product.

5.2 RECOMMENDATIONS

The revelation of the existence of substandard antimalarial drugs in Lusaka, though in insignificant proportions, has serious and detrimental effects on the health of the people and the fight against malarial, if the problem is not abated. Therefore, the following recommendations have been made:

- The National Drug Quality Control Laboratory should be fully equipped with necessary analytical equipments such as the High Pressure Liquid Chromatography, and adequate and qualified personnel.

- The Pharmaceutical Regulatory Authority should spread and intensify the Post-marketing surveillance programme, and establish itself in all districts of Zambia.

- The Pharmaceutical Regulatory Authority should have the mini-laboratory facilities in all districts of Zambia.

- The Pharmaceutical Regulatory Authority and the Medical Council of Zambia should intensify their inspection of Private Health Institutions.

- The Pharmaceutical Regulatory Authority should have educational programmes to educate people on the dangers of consuming poor quality or substandard drugs.

- The Pharmaceutical Regulatory Authority should strictly adhere to pre-registration and post-registration quality control of all pharmaceutical products.

5.3 STUDY LIMITATION

The absence of HPLC at the Pharmaceutical Authority Laboratory hindered further analysis of the drugs that were found to be substandard by the mini-laboratory technique of TLC.

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APPENDICES

APPENDIX I: DRUG COLLECTION SHEET

1. Identification number of the drug……………………………………….

2. Date of purchase of the drug…………………………………………….

3. Place of purchase of the drug……………………………………………

4. Condition of purchase……………………………………………………

5. Name of the drug indicated by vendor……………………………………..

6. Name indicated on the product package…………………………………

7. Active ingredient(s) contained in the product as indicated on the product….

………………………………………………………………………………….

8. Physical appearance of tablets……………………………………………

9. Nature and material of packaging material…………………………………..

10. Appearance of the label on the packaging………………………………….

11. Instructions on the label for the use of the product………………………………

12. Manufacturing date as stated on the product label………………Expiry date……..

13. Batch number of the product as stated on the label………………………….

14. Manufacturer of the product and address as stated on the label………………..

15. Price at which product is purchased…………………………………………………

16. Type of business:…………………………Location of business:……………………..

IMPLEMENTATION PLAN

APPENDIX II: GANT CHART

Description of activity	Year:2009							Year:2010		
	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
Research proposal Presentation to graduate forum	xxxx xxxx xxxx									
Research proposal Presentation to UNZA research ethics committee		xxx xxx xxx								
Training of research assistant			xxx xxx xxx							
Data collection				xxx xxx						
Data analysis					xxx xxx	xxx xxx				
Research report writing & Submission						xxx xxx	xxx xxx	xxx xxx		

Duration of study: 8 Months

APPENDIX III: STUDY BUDGET

Description	Responsible person	Daily	Numbers personnel	Working days /week	Duration of acting	Total cost (K)
Personnel emoluments	Research assistants	50,000=00	2	4	2weeks	800 000.00
	Principal investigator	250 000.00	1	2	2 weeks	2000 000.00
	Research statistician	250,000.00	1	4	2 weeks	2,000 000.00
					Subtotal	K6800 000.00
Supplies	**Item**	**Unit Pack**	**Quantity Required**	**Unit Cost**		
	Paper	Ream	3	50,000.00		150,000.00
	Ball point pens	Each	4	2000.00		8 000.00
	Pencils	Each	2	2000.00		4 000.00
	Stapler	Each	1	100 000.00		100 000.00
	Staples	Each	1	30 000.00		30 000.00
	Punch	100	1	150 000.00		150 000.00
	Laptop	Each	1	4 000 000.00		4 000 000.00
	Printer	Each	1	850 000.00		850 000.00
	Toner	Each	1	300 000.00		300 000.00
	Hiring of laboratory facility	Each	1	1500 000.00		1500 000.00
	Reagents			1000 000.00		1000 000.00
Purchase of samples					**Subtotal**	K8092 000.00
	Artemether/Lumefant -rine tablets	Each	430	2930.24		1260 000.00
	Sulphadoxine + Pyrimethamine tablets	Each	430	976.74		420 000.00
	Quinine tablets	Each	430	1465.12		630 000.00
					Subtotal	K2310 000.00
Travel expenses	From Kabwata to research areas & back					K1200 000.00
					Grand Total	**K18402 000.00**

APPENDIX IV: CONSENT FORM

To: …………………………………………

……………………………………………..

……………………………………………..

………………………………………………

………………………………………………

Dear Sir/Madam,

RE: REQUEST TO BUY ANTIMALARIAL DRUG SAMPLES FOR STUDY.

Iam a University of Zambia student pursuing a Masters Degree programme in Public Health requesting your organization to permit me to buy drug samples from your institution for my study titled "EVALUATION OF THE QUALITY OF ARTEMETHER/LUMEFANTRINE, SULPHADOXINE/PYRIMETHAMINE AND QUININE TABLETS IN SELECTED PUBLIC AND PRIVATE HEALTH INSTITUTIONS IN LUSAKA DISTRICT".

The study is purely academic for the award of the Masters Degree in Public Health, and the information that will be gathered through this study will be kept strictly confidential.

SIGNED:

Names: ……………………………………….

Signature: …………………………………….

Date: ………………………………………….

WITNESSED:

Names: ……………………………………….

Signature: ……………………………………..

Date: …………………………………………..

APPENDIX V: LETTER FOR PERMISSION TO CARRY OUT THE STUDY

THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

P.O. BOX 50110,

LUSAKA.

8TH MAY, 2009

THE DIRECTOR GENERAL

PHARMACEUTICAL REGULATORY AUTHORITY

LUSAKA

U.F.S. THE HEAD OF DEPARTMENT

THE UNIVERSITY OF ZAMBIA

COMMUNITY MEDICINE

LUSAKA

Dear Sir/Madam,

RE: PERMISSION TO UNDERTAKE A RESEARCH TITLED "EVALUATION OF THE QUALITY OF ARTEMETHER/LUMEFANTRINE, SULPHADOXINE/PYRIMETHAMINE AND QUININE TABLETS IN SELECTED PUBLIC AND PRIVATE HEALTH INSTITUTIONS LUSAKA DISTRICT".

Iam a University of Zambia student pursuing a Masters Degree programme in Public Health seeking for your permission to undertake the above stated study.

I further request your esteemed office to permit me use your Minilab facilities to analyse the samples of the drugs that will be collected in order to verify the quality of the drug samples for the study.

I will be grateful if my requests will be favourably considered.

Yours sincerely,

Alutuli Luke

APPENDIX VI: LETTER FOR PERMISSION TO COLLECT SAMPLES FROM PUBLIC INSTITUTIONS.

THE UNIVERSITY OF ZAMBIA
SCHOOL OF MEDICINE
P.O. BOX 50110
LUSAKA
8TH MAY, 2009
THE PERMANENT SECRETARY
MINISTRY OF HEALTH
NDEKE HOUSE
LUSAKA
U.F.S. THE HEAD OF DEPARTMENT
THE UNIVERSITY OF ZAMBIA
COMMUNITY MEDICINE
LUSAKA
Dear Sir/Madam,

RE: PERMISSION TO COLLECT DRUG SAMPLES FROM GOVERNMENT INSTITUTIONS IN LUSAKA.

Iam a University of Zambia student pursuing a Masters Degree programme in Public Health. As a requirement for the award of the Masters Degree certificate, iam required to undertake a research. Therefore, the research I wish to undertake is titled "EVALUATION OF THE QUALITY OF ARTHEMETHER/LUMEFANTRINE, SULPHADOXINE/PYRIMETHAMINE AND QUININE TABLETS IN SELECTED PUBLIC AND PRIVATE HEALTH INSTITUTIONS LUSAKA DISTRICT".

I will be required to collect or buy drug samples for analysis from government institutions, private pharmacies, market places or any other vendors who deal in drugs.

I will be grateful if my request will be granted.

Yours sincerely,

Alutuli Luke

ANNEX 1: OPERATIONAL PRINCIPLE OF GPHF (German Pharma Health Fund) – Minilab.

The Minilab uses the thin-layer chromatography (TLC) and colorimetric methods of drug analysis. The TLC technique consists of placing a spot of drug sample on a thin layer of silica attached to a plate of glass, aluminium, or plastic. The method is relatively inexpensive, specific, and sensitive; hence it is used to assess drug quality.

Colorimetry uses chemical reactions or characteristic acidity (pH) properties to evaluate drug quality. The method is convenient because it is very rapid and highly specific.

ANNEX 2: CODING OF VARIABLES

(i) **Drug type**:	Artemether/Lumefantrine - 1
	Sulphadoxine/Pyrimethamine - 2
	Quinine sulphate - 3
(ii) **Active ingredient**:	Present - 1
	Absent - 2
(iii) **Percentage content of active ingredient**:	Correct amount - 1
	Incorrect amount - 2
(iv) **Labelling**:	Appropriate – 1
	Inappropriate - 2
	Absent - 3
(v) **Packaging**:	Appropriate - 1
	Inappropriate – 2
(vi) **Manufacturer**:	Local - 1
	International - 2
(vii) **Business type**:	Public – 1
	Private - 2
(viii) **Location of business**:	High density area
	Low density area
(ix) **Quality of drugs**:	Good – 1
	Poor - 2

ANNEX 3: DETAILED QUALITY ANALYSIS OF DRUG SAMPLES

(A) ARTEMETHER/LUMEFANTRINE TABLETS QUALITY ANALYSIS

Figure 2: Artemether/Lumefantrine tablets collected for quality analysis.

1. VISUAL INSPECTION

Visual inspection of the drug samples was done to search for deficiencies on the labelling, packaging and dosage forms as described in the opening chapters on general methods and operations of the main manual. All product particulars were written down using the Reporting Form as a guide. Each tablet usually contains a 120mg of Lumefantrine combined with 20mg of Artemether.

2. VERIFICATION OF IDENTY AND DRUG CONTENT VIA THIN LAYER CHROMATOGRAPHY.

(a) PRINCIPLE

Extraction of Lumefantrine/Artemether from fixed-dose combination tablets using acetone and determined by TLC with reference to an authentic secondary standard.

(b) EQUIPMENT AND REAGENTS

- Pestle
- Aluminium foil
- Laboratory glass bottles with a filling capacity of 25 to 100ml
- Funnel
- Set of straight pipettes (1-25ml)
- 10ml-vials
- Label tape
- Marker pen
- Pencil
- Merck TLC aluminium plates pre-coated with silica gel 60 F254, size 5 x 10 cm
- Glass microcapillaries of 2-ul filling capacity
- Hot plate
- TLC developing chamber (jar)
- Filter paper
- Pair of scissors
- Pair of tweezers
- UV dipping chamber (Petri dish)
- Sulphuric acid 96%
- Menthol
- Acetone
- Ethylacetate
- Glacial acetic acid
- Toluene

- Authentic reference standard, that is, Lumefantrine/Artemether 120/20mg fixed-dose combination tablets.

3. PREPARATION OF THE LUMEFANTRINE STOCK STANDARD SOLUTION.

The stock standard solution was prepared using an authentic product containing 120mg of Lumefantrine combined with 20mg of Artemether. A tablet was wrapped in aluminium foil and it was crushed down to a fine powder using a pestle. The foil was emptied into a 100-ml glass bottle and the residual solids were washed down with 50 ml of acetone using a straight pipette. The bottle was closed and shook for about three minutes till most of the solids were dissolved. The solution was left to sit for a further five minutes and, the undissolved residues were allowed to settle at the bottom. In terms of Lumefantrine, the solution that was obtained contained 2.4mg of total drug per ml, and it was labelled as 'LUMEFANTRINE STOCK STANDARD SOLUTION'. This solution was freshly prepared for each test, and work continued with the hazy supernatant liquid or clear dilution that was obtained.

4. PREPARATION OF THE LUMEFANTRINE WORKING STANDARD SOLUTION 100% (UPPER WORKING LIMIT).

1 ml of the stock standard solution was pipetted into a 10-ml vial and 2 ml f acetone were added. The vial was closed and shaken. The solution that was obtained contained 0.8 mg of total drug per ml, and it was labelled as LUMEFANTRINE WORKING STANDARD SOLUTION 100%. In terms of Lumefantrine, the higher working standard solution represented a product of good quality containing 100% of total drug.

5. PREPARATION OF THE LUMEFANTRINE WORKING STANDARD SOLUTIONS 80% (LOWER WORKING LIMIT).

4 ml of the stock standard solution was pipetted into a 25-ml glass bottle and 11 ml of acetone were added. The vial was closed and shaken. The solution that was obtained contained 0.64mg of total drug per ml and it was labelled as LUMEFANTRINE WORKING STANDARD SOLUTION 80%.

In terms of Lumefantrine, the lower working standard solution represented a medicine of poor quality containing just 80% of the amount of drug as stated on the product's label. This drug represented the lower acceptable limit for a given product.

6. PREPARATION OF LUMEFANTRINE STOCK SAMPLE SOLUTIONS FROM DRUG PRODUCTS CLAIMING A POTENCY OF 120 MG OF LUMEFANTRINE PER UNIT.

One whole tablet from the drugs sampled in the field was taken and wrapped up into an aluminium foil, and then crushed down to fine powder before transferring into a 100-ml laboratory glass bottle, to which 50 ml of acetone using a straight pipette were added. The bottle was closed and shaken for about three minutes till most of the solids were dissolved. The solution was allowed to sit for a further five minutes until the undissolved residue settled below the hazy supernatant liquid. In terms of Lumefantrine, the solution that was obtained contained 2.4 mg of total drug per ml, and it was labeled as 'LUMEFANTRINE STOCK SAMPLE SOLUTION'. The solution was freshly prepared for each test. Work continued with the hazy supernatant liquid or clear dilution that was obtained.

7. PREPARATION OF LUMEFANTRINE WORKING SAMPLE SOLUTIONS

1 ml of the stock sample solution was pipetted into a 10-ml vial, and 2 ml of acetone were added. The vial was closed and shaken; this was labeled 'LUMEFANTRINE WORKING SAMPLE SOLUTION'. The concentration of Lumefantrine in the solution was 0.8 mg per ml, and this matched the concentration of Lumefantrine of the higher working standard solution.

8. SPOTTING

A mark was made on an origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate and 2 ul of each test and standard solution were applied onto the chromatoplate using the microcapillary pipettes.

Four spots were placed on the plate. The uniformity of all spots was checked using UV light of 254 nm. All spots were circular in shape and equally spaced across the origin line. The intensities of the spots differed but their diameter was the same. Difference in intensities was due to residual amounts of tablet excipients and difference in drug concentrations in the sample solutions. A difference in spot size relates to poor spotting. Where a homogeneous spotting was not achieved at first spotting, a repeat was done.

9. DEVELOPMENT

4 ml of ethylacetate, 2 ml of glacial acetic acid and 18 ml of toluene were pipetted into the jar which was being used as TLC developing chamber. The chamber was closed and mixing was done thoroughly. The chamber wall was lined with filter paper, and then waited for about 15 minutes to ensure saturation of the chamber with the solvent vapour. Carefully the loaded TLC plate was placed into the jar. The jar was closed and the chromatoplate was developed until the solvent front moved about three-quarters of the length of the plate, and the developing time was about 15 minutes. The plate was removed from the chamber, the solvent front was marked and excess solvent was allowed to evaporate using a hot plate.

10. DETECTION

Residual solvent was dried off and the chromatoplate was observed with UV light of 254 nm using the battery-driven fluorescent lamp. This method of detection was used for quantification of Lumefantrine. Further verification of Lumefantrine's identity and content was achieved when the same plate was observed in daylight after iodine staining. For detection of the Artemether portion, the plate was dipped into methanolic-sulphuric acid solution 5% and dried on the hot plate.

11. OBSERVATIONS

The presence of Lumefantrine was indicated by a strong blue-violet spot at a travel distance of about 0.16 when the chromatoplate was observed at 254 nm with the UV lamp. In preparations where Lumefantrine was presented in a fixed combination with Artemether, a second principal spot was observed at a travel distance of about 0.56 after the plate had been exposed to sulphuric acid and heat. As Artemether's dosage strength is six times lower than that of Lumefantrine, the spots were looking weak but they were not missed. Some fainter spots which were emerging near or on the origin of the chromatoplate were normally caused by auxiliary agents that were incorporated in the different finished product formulations.

12. ARTEMETHER CONTENT VERIFICATION

For Artemether content verification, the upper working standard solution contained 2 mg of Artemether per ml. From one fixed-dose combination tablet, Artemether was extracted with 10 ml of acetone. The concentration was adjusted to 1.6 mg of Artemether per ml by mixing 4 ml of the upper working standard solution with 1 ml of acetone. 2 ul of each working standard and sample solution were spotted on the chromatoplate and developed as for Lumefantrine.

13. RESULTS AND ACTIONS TAKEN

The principal spot (s) in the chromatogram that was obtained with the test solution corresponded in terms of colour, size, intensity, shape and traveled distance to that in the chromatogram that was obtained with the lower and higher standard solution. This result was obtained for each method of detection. Where this was not achieved the run was repeated with the second sample from the scratch. The batch was rejected if the drug content could not be verified in a third run. This required a second opinion by referring additional samples to a fully equipped drug control laboratory. However, there was no facility were the samples could be referred to, as HPLC was yet to be established at the Pharmaceutical Regulatory Authority drug quality control laboratory.

(B) SULPHADOXINE/PYRIMETHAMINE TABLETS QUALITY ANALYSIS

Figure 3: Sulphadoxine/Pyrimethamine tablets collected for quality analysis.

1. VISUAL INSPECTION

Visual inspection of the drug samples for Sulphadoxine/Pyrimethamine tablets was done to search for deficiencies on labeling, packaging and dosage form as described in the

opening chapters on general methods and operations of the main manual. All product particulars were written down using the Reporting Form as a guide. Each tablet usually contained 500 mg of Sulphadoxine and 25 mg of Pyrimethamine in a fixed combination.

2. VERIFICATION OF IDENTITY AND DRUG CONTENT VIA THIN LAYER CHROMATOGRAPHY

(a) PRINCIPLE

Extraction of Sulphadoxine/Pyrimethamine from fixed-dose tablets combination using menthol and determined by TLC with reference to an authentic secondary standard.

(b) EQUIPMENT AND REAGENTS

- Pestle
- Aluminium foil
- Laboratory glass bottles with filling capacity of 25 to 100 ml
- Funnel
- Set of straight pipettes (1 to 25 ml)
- 10-ml vials
- Label tape
- Marker pen
- Pencil
- Merck TLC aluminium plates pre-coated with silica gel 60 F 254, size 5 x 10 cm
- Glass microcapillaries of 2 ul filling capacity
- Hot plate
- TLC developing chamber (jar)
- Filter paper
- Pair of scissors
- UV light of 254 nm
- Methanol
- Ethylacetate
- Sulphadoxine 500 mg/Pyrimethamine 25 mg reference tablets

3. PREPARATION OF THE STOCK STANDARD SOLUTION

The stock standard solution was prepared using a whole reference tablet that contained 500 mg of Sulphadoxine plus 25 mg of Pyrimethamine, which was crushed prior to extraction. The precise procedure was as follows: One tablet was wrapped in aluminium foil and the crushed down to a fine powder using a pestle. The aluminium foil was emptied over a 25-ml laboratory glass bottle, and all residual solids were washed down with 20.0 ml of menthol using a straight pipette. The bottle was closed and shaken for about three minutes till most of the solids dissolved. The solution was allowed to stand for further five minutes until the undissolved residue settled below the clear supernatant liquid. The solution was labelled as 'SULPHADOXINE/PYRIMETHAMINE STOCK STANDARD SOLUTION', and it contained 25.0/1.25 mg of total drug per ml. The standard solution was freshly prepared for each test.

4. PREPARATION OF THE WORKING STANDARD SOLUTION 100% (UPPER WORKING LIMIT)

1 ml of the clear stock standard solution was pipetted into a 10-ml vial and 3 ml of methanol were added. The vial was closed and shaken. The solution that was obtained was labelled as 'SULPHADOXINE/PYRIMETHMINE WORKING STANDARD SOLUTION 100%' and it contained 6.25/0.3125 mg of total drug per ml.
The higher working standard solution represented a drug product of good quality containing 100% of Sulphadoxine and Pyrimethamine respectively.

5. PREPARATION OF THE WORKING STANDARD SOLUTION 80% (LOWER WORKING LIMIT)

1 ml of the clear stock standard solution was pipetted into a 10-ml vial and 4 ml of methanol were added. The vial was closed and shaken. The solution that was obtained was labelled as 'SULPHADOXINE/PYRIMETHAMINE WORKING STANDARD SOLUTION 80%' and it contained 5.00/0.25 mg of total drug per ml.

The lower working standard solution represented a drug of poor quality containing just 80% of the total amount of Sulphadoxine and Pyrimethamine as was stated on the product's label. This drug level represented the lower acceptable limit for a given product.

6. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A DRUG PRODUCT CLAIMING A POTENCY OF 500 MG SULPHADOXINE AND 25 MG OF PYRIMETHAMINE PER UNIT

The preparation of the sample solution was done using one whole tablet from the drug product sampled in the field.

Sulphadoxine and Pyrimethamine were extracted completely from the sample using the same procedure as for the authentic reference standard: One tablet was wrapped up into aluminium foil and crushed down to a fine powder prior to transfer into a 25-ml laboratory glass bottle. 20 ml of menthol were added using a straight pipette, and then the bottle was closed and shaken for about three minutes till most of the solids were dissolved. The solution was allowed to stand for further five minutes until the undissolved residue settled below the clear supernatant liquid. The solution was labelled as 'SULPHADOXINE/PYRIMETHAMINE STOCK SAMPLE SOLUTION' and it contained 25.0/1.25 mg of total drug per ml. The sample solution was freshly prepared for each test.

7. PREPARATION OF THE WORKING SAMPLE SOLUTION

1 ml of the stock sample solution was pipetted into a 10-ml vial and 3 ml of methanol were added. The vial was closed and shaken. The solution that was obtained was labelled as 'SULPHADOXINE/PYRIMETHMINE WORKING SAMPLE SOLUTION'.

The expected concentrations of both drug compounds in the working sample solution were 6.25 and 0.3125 mg per ml respectively, and this was to match the concentration of Sulphadoxine and Pyrimethamine of the higher working standard solution.

8. SPOTTING

The origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate was marked and 2 ul of each test and standard were applied.
Four spots were placed on the plate. The uniformity of all spots was checked using UV light of 254 nm. All spots were circular in shape and equally spaced across the origin line. Although their intensity differed, their diameters did not. Differences in intensities were due to residual amounts of excipients of the tablet, and difference in drug concentrations in the sample solution. A difference in spot size related to poor spotting, and a repeat was done when a homogeneous spotting was not achieved first time.

9. DEVELOPMENT

15 ml of ethylacetate and 5 ml of methanol were pipetted into the jar that was used as TLC developing chamber. The chamber was closed thorough mixing was done. The chamber wall was lined with filter paper and waited for about 15 minutes to ensure saturation of the chamber with the solvent vapour. Carefully the loaded TLC plate was placed into the jar. The jar was closed and the chromatoplate was developed until the solvent front had moved about three-quarters of the length of the plate, and the developing time was about 15 minutes. The plate was removed from the chamber, the solvent front was marked and the excess solvent was allowed to evaporate using a hot plate.

10. DETECTION

All residual solvent was dried off and the chromatoplate that was obtained was observed with UV light of 254 nm using the battery-driven fluorescent lamp. Also the plate was observed in daylight after iodine staining.

11. OSERVATIONS MADE AT 254 NM

The presence of Sulphadoxine and Pyrimethamine was indicated by two principal spots, the one that represented Sulphadoxine was in front at a travel distance of about 0.68, and then followed by a second spot at about 0.44 which represented Pyrimethamine. Additional strong spots generated by the test solution indicated drug degradation especially when associated with a smaller principal spot. Some fainter spots which emerged near or on the origin line on the chromatoplate were normally caused by auxiliary agents that were incorporated in the different tablet formulations.

12. OSERVATIONS MADE IN DAYLIGHT AFTER IODINE STAINING

Only spots that represented Sulphadoxine became visible for further evaluation purposes on quantities present.

13. RESULTS AND ACTIONS TAKEN

The principal spots in the chromatogram obtained with the test solution corresponded in terms of colour, size, shape and travel distance to that in the chromatogram obtained with the lower and higher standard solutions. This result was obtained for each method of detection. If the result was not achieved, the test run was repeated with a second sample from scratch. If the drug content could not be verified in a third test run, the batch was rejected.

(C) QUININE SULPHATE TABLETS QUALITY ANALYSIS

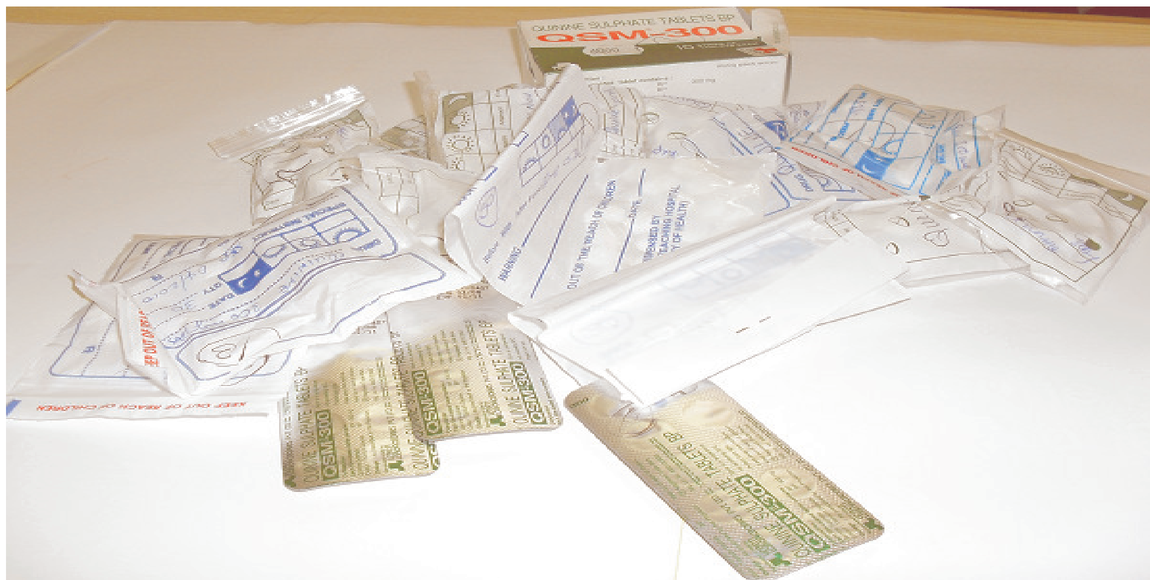

Figure 4: Quinine sulphate tablets collected for quality analysis.

1. VISUAL INSPECTION

Visual inspection of drug samples was done in order to search for deficiencies on labelling, packaging and dosage forms as described in the opening chapters on the general methods and operations of the main manual. All product particulars were written down using the Reporting Form as a guide. Each tablet usually contained 300 mg of Quinine sulphate.

2. VERIFICATION OF IDENTITY AND DRUG CONTENT VIA THIN LAYER CHROMATOGRAPHY

(a) PRINCIPLE

Extraction of Quinine from tablets with aqueous methanol solution and determined by TLC with reference to an authentic secondary standard.

3. EQUIPMENT AND REAGENTS

- Pestle
- Aluminium foil
- Laboratory glass bottles with a filling capacity of 25 to 100 ml
- Funnel
- Set of straight pipettes (1 to 25 ml)
- 10-ml vials
- Label tape
- Marker pen
- Pencil
- TLC silica gel plates
- Glass microcapillaries
- Hot plate
- TLC developing chamber
- Filter paper
- Pair of scissors
- Pair of tweezers
- UV lamp of 254 nm
- Iodine chamber
- Water
- Methanol
- Ammonia solution, concentrated
- Quinine sulphate 300 mg reference tablets

4. PREPARATION OF THE STOCK STANDARD SOLUTION

The stock standard solution was prepared using the whole reference tablet containing 300 mg of Quinine sulphate. The precise extraction procedure was as follows; the tablet was wrapped in the aluminium foil and then crushed down to a fine powder using a pestle. The foil was emptied into a 50-ml glass bottle and the residual solids were washed down

with 3 ml of water using a straight pipette. The bottle was closed and shaken for about one minute, and then 27 ml of methanol was added for further extraction. The bottle was closed and shaken again for about three minutes till most of the solids dissolved. The solution was allowed to sit for a further five minutes until the undissolved residues dissolved below the clear or almost clear supernatant liquid. The solution that was obtained contained 10 mg of total drug per ml and was labelled as 'QUININE STOCK STANDARD SOLUTION'. The solution was freshly prepared for each test.

5. PREPARATION OF THE WORKING STANDARD SOLUTION 100% (UPPER WORKING LIMIT)

1 ml of the stock standard solution was pipetted into a 10-ml vial and then 7 ml of methanol was added. The vial was closed and shaken. The solution that was obtained contained 1.25 mg of total drug per ml and was labelled as 'QUININE WORKING STANDARD SOLUTION 100%'.

The higher working standard solution represented a drug product of good quality containing 100% of total Quinine.

6. PREPARATION OF THE WORKING STANDARD SOLUTION 80% (LOWER WORKING LIMIT)

1 ml of the stock standard solution was pipetted into a 10-ml vial and then 9 ml of methanol was added. The vial was closed and shaken. The solution that was obtained contained 1.00 mg of total drug per ml and was labelled as 'QUININE WORKING STANDARD SOLUTION 80%.

The lower working standard solution represented a drug product of poor quality containing just 80% of the amount of Quinine as was stated on the product's label, and this level represented the lower acceptable limit for a given product.

7. PREPARATION OF THE STOCK SAMPLE SOLUTION FROM A DRUG PRODUCT CLAIMING A POTENCY OF 300 MG OF SALT PER ML OR UNIT RESPECTIVELY

The stock sample solution was prepared using the whole tablet from the drug samples which were sampled in the field. The tablet was wrapped up into aluminium foil and crushed down to a fine powder prior to transfer into a 50-ml laboratory glass bottle. 3 ml of water were first added and then 27 ml of methanol were added after about 1 minute. The bottle was closed and shaken for about three minutes till most of the solids were dissolved. The solution was allowed to sit for a further five minutes till the undissolved residue settled below the hazy supernatant liquid.

The solution that was obtained contained 10 mg of total drug and it was labelled as 'QUININE SAMPLE SOLUTION'. The solution was freshly prepared for each test.

8. PREPARATION OF THE WORKING SAMPLE SOLUTION

1 ml of the stock sample solution was pipetted into a 10-ml vial and 7 ml of methanol were added. The vial was closed and shaken, and labelled as 'QUININE WORKING SAMPLE SOLUTION'.

The concentration of the total drug that was expected in the solution was 1.25 mg/ml and was to match with the concentration of Quinine of the higher working standard solution.

9. SPOTTING

The origin line parallel to and about 1.5 cm from the bottom edge of the chromatoplate was marked and 2ul of each test and standard solution were applied using the microcapillary pipette.

Four spots were placed on the plate, and the uniformity of all spots was checked using UV light of 254 nm. All spots were circular in shape and equally spaced across the origin line. Although their intensities could differ, their diameter never did. Different intensities were due to residual amounts of tablet excipients or different drug concentrations in the

sample solutions. A difference in spot size related to poor spotting. If homogeneous spotting was not achieved first time, the procedure of spotting was repeated.

10. DEVELOPMENT

20 ml of methanol and 0.5 ml of concentrated ammonia solution were respectively pipetted into the jar that was used as TLC chamber. The chamber was closed and the mixing was done thoroughly. The chamber's wall was lined with filter paper and then waited for about 15 minutes to ensure saturation of the chamber with the solvent vapour. The loaded TLC chromatoplate was carefully placed into the jar. The jar was closed again and the chromatoplate was developed until the solvent front had moved about three-quarters of the length of the plate. The developing time was about 20 minutes. The plate was removed from the chamber, the solvent front marked and then excess solvent was allowed to evaporate using the hot plate.

11. DETECTION

All residue solvent were dried off and the chromatoplate was observed at 254 nm using the battery-driven UV lamp. Further verification of drug identity and content was achieved when the same plate was observed in daylight after iodine staining.

12. OBSERVATIONS MADE AT 254 NM

When the chromatoplate was exposed to UV light of 254 nm, the presence of Quinine was indicated by a vivid blue spot at a travel distance of about 0.58. Additional strong spots were generated by the test solution indicated drug degradation especially when they were associated with a smaller principal spot. Some fainter spots that emerged near or on the origin of the chromatoplate were normally caused by auxiliary agents incorporated in the different tablet formulation.

13. OBSERVATIONS MADE IN DAYLIGHT AFTER IODINE STAINING

When the chromatoplate was exposed to iodine vapour, several orange-brown spots were generated matching the pattern of spots that were already observed on the plate when it was exposed to 254 nm UV light.

14. RESULTS AND ACTIONS TAKEN

The principal spot in the chromatogram that was obtained with the test solution corresponded in terms of colour, size, intensity, shape and travel distance to that in the chromatogram that was obtained with the lower and higher standard solution. The result was obtained for each method of detection. If the result was not achieved at first, a repeat test was done with a second sample from scratch. If the drug content could not be verified in the third run, the batch was rejected.