QUALITY OF SULFADOXINE-PYRIMETHAMINE (SP) TABLETS SOLD IN DRUG OUTLETS IN ARUA DISTRICT- UGANDA

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SEPTEMBER, 2010

DECLARATION

I, Vudriko Patrick declare that this work is original and the information contained in this dissertation has never been presented for an award of a Diploma or a Degree in any higher institution of learning.

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DEDICATION

This dissertation is dedicated to my beloved parents, Mr Ivu Luka and Mrs Ivu Sylvia for their sacrifice towards my education and all those who supported me in my career development.

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LIST OF ABBREVIATIONS

ACT	Artemisinin based Combination Therapy
AFM	Africa Fight Malaria
CDC	Centre for Disease Control
CHS	College of Health Sciences
DOLTs	Drug outlets
DRC	Democratic Republic of Congo
et al	and others
FIP	International Pharmaceutical Federation
GDP	Gross Domestic Product
GMP	Good Manufacturing Practice
GSP	Good Storage Practice
HIV	Human Immune-deficiency Virus
IPT	Intermittent Preventive Treatment
L	Liters
ml	Millimeter

Mg	Milligram
MoH	Ministry of Health of Uganda
MSH	Management Science for Health
®	Registered trade name
NDA	National Drug Authority
NDP	National Drug Policy
NDQCL	National Drug Quality Control Laboratory
QA	Quality Assurance
QCA	Drug Quality Control and Assurance Department
RUFORUM	Regional Universities Forum for Capacity Building in Agriculture
SBS	School of Biomedical Sciences
SP	Sulfadoxine/ Pyrimethamine
TLC	Thin Layer Chromatography
USP	United State Pharmacopeia
USP DQI	United State Pharmacopeia Drug Quality Information
µg	Microgram
WHO	World Health Organization

ABSTRACT

Background: Sulfadoxine-pyrimethamine (SP) remain a corner stone in intermittent preventive treatment (IPT) of malaria in pregnancy. Reports of substandard and counterfeit antimalarial drugs in Uganda and neighbouring countries like DRC and Sudan continue to raise fears over the quality of SP in drug outlets in Arua District which neighbours and trades with the above countries. This study was designed to assess the post market quality of SP tablets in drug shops and clinics in Arua Municipality, Arua District.

Methods: The study involved sampling various batches of SP tablets from Arua Hill and Oli divisions in Arua municipality. The laboratory analysis involved: physical/ visual inspection, weight uniformity test, physicochemical assay for dissolution and quantitative content analysis performed according to USP and BP (2009) methods.

Results: A total of 19 SP batches were purchased from 8 drug shops and 11 clinics in the municipality. Majority (73.7%) of the samples were obtained from Arua Hill Division while 26.3% of the samples were procured from Oli Division. The SP tablets originated from various countries; Uganda (40%), India (30%), Kenya (20%) and Cyprus (10%). The country of origin of one sample could not be determined because it was sold in a plastic tin for Albendazole. Fifty percent (50%) of the brands were non existent in the December 2008 and September 2009 human drug register in NDA. One out of the ten batches failed dissolution test due to low amount of pyrimethamine (56.1±3.1). However, all the ten batches passed the USP and BP tolerance limits for content.

Conclusion: The current study indicates low level of substandard SP tablets in Arua Municipality thus the need for sustained antimalarial drug quality surveillance programme in the country by National Drug Authority.

CHAPTER ONE: INTRODUCTION

1.0 Background

Globally, malaria remains the most important parasitic disease and about 350–500 million clinical disease occur annually. Around 60% of the cases and over 80% of the deaths occur in Africa, mainly in children under the age of five years and pregnant women (WHO, 2005). In Uganda, 93% of the total population is at risk of malaria and it contributes to by far the major share of the disease burden, with 39% of outpatient visits and 35% of inpatient admissions being due to the disease (MoH, 2007; CDC, 2008). The disease is estimated to reduce GDP growth by a factor of 1.3% per annum in Uganda (MoH, 2004).

In sub-Saharan Africa, malaria affects an estimated 24 million pregnant women; malaria prevalence may exceed 50% among primigravid and secundigravid women in malaria-endemic areas (Steketee *et al*., 2001). More recent case-control (Francesconi *et al.*, 2001) and longitudinal (Whitworth *et al.*, 2000; French *et al.*, 2001) studies on the clinical pattern of malaria in HIV-infected, non-pregnant women have shown HIV infection to be associated with an increased frequency of clinical malaria and parasitemia, particularly among persons with advanced HIV disease. Infants exposed in-utero to both placental malaria and maternal HIV infection have an increased risk for postneonatal death three- to eightfold higher than infants born to mothers with either infection alone (Bloland *et al.*, 1995).

The World Health Organization (WHO) designated Intermittent Preventive Treatment (IPT) using Sulfadoxine/ Pyrimethamine (SP) as the preferred approach to reduce the number of malaria parasites in pregnant women during the critical period of greatest fetal gain (WHO,

2000). Uganda adopted the use of SP for IPT in 2005 as part of the current malaria treatment policy (MoH, 2005). During pregnancy, IPT provides significant protection against low birth weight, maternal anemia, preterm delivery and maternal mortality (Schultz *et al.*, 1994., Brabin *et al.*, 1997., Parise *et al.*, 1998).

Drug shops have been recognized as important sources of antimalarial drugs to people's home (Foster, 1991), since formal health service establishments are difficult to access and often have poor performance (Gyapong and Garshong, 2007). Of great concern though, is the quality of SP formulations sold in these drug shops. It is estimated that more than 10% of the globally traded medicines are counterfeit (Newton *et al.*, 2002; WHO, 2006). Fifty percent of the global fake drugs circulate in less developed countries. Previous studies done in some African countries like Kenya (Maponga and Ondari, 2003; Keshi, 2008), Tanzania (Minzi *et al.*, 2003), DR Congo (Atemnkeng, D Cock and Plaizer, 2007) and Sudan (Maponga and Ondari, 2003) indicate that the level of substandard and / or counterfeit SP in the market is high. A recent study by Africa Fighting Malaria (AFM) reported that 35% of antimalarials sold in six major African cities (including Kampala) failed at least one critical quality control test (Bate *et al.*, 2008). Previously, Ogwal-Okeng, Okello and Odyek (1998) reported that up to 30% of Chloroquine tablet and 33% of the injection sampled in Uganda contained less than the stated amount of the active ingredient. Similar studies done by Obua, Ogwal-Okeng and Owino (2003) also reported that 39% and 51% Chloroquine tablets and injectables respectively failed quality test in Uganda. The presence of poor quality antimalarials in Ugandan market has been attributed to the liberalization of trade, difficulty in monitoring the porous borders (Obua, Ogwal-Okeng and Owino, 2003),

counterfeiting, insufficient skilled man power to enforce the National Drug Policy (NDP) of Uganda and poor drug management practices in drug outlets.

Poor quality SP formulations (with poor bioavailability) have been partly blamed for the development of resistance by *Plasmodium falciparum* against the drug (Petralanda, 1995; Kun, 1999). The development of resistance to SP by *P. falciparum* in Africa is particularly serious, because the drug is the only affordable, safe, practical, and well-tolerated alternative to 4-aminoquinolines in addition to its sole use in IPT (Chansuda *et al.,* 2002). It is on this ground that this study was designed to assess the post-market quality of various brands of SP antimalarial tablets sold in drug shops and clinics in Arua Municipality, Arua District-Uganda.

1.1 Problem statement

Sulfadoxine-Pyrimethamine (SP) is currently the only drug recommended for IPT of malaria in pregnant women. The drug is also widely used for self medication of uncomplicated malaria given the low supply coverage and the comparative high cost of Artemether-Lumefantrine antimalarials in Uganda. Private drug shops and clinics by their proximity to the consumers provide an alternative avenue through which communities and particularly pregnant women can access SP. However, there has been recent concern on the quality of antimalarial drugs sold in drug outlets in Uganda. In addition, the level of substandard and fake drugs is reported to be very high in conflict prone neighbouring countries such as Democratic Republic of Congo and Southern Sudan. With the porous borders between Uganda and the above countries coupled with free movement of goods, Arua District stands at a higher risk of infiltration of smuggled and substandard and fake antimalarial drugs including SP. Poor quality SP predisposes to emergence

of resistance by *Plasmodium falciparum* and treatment failure. However, limited information is available on the post-market quality of SP in drug shops in Arua District.

1.2 Justification

Currently, SP is the only antimalarial drug recommended for IPT in pregnant women. Therefore, the quality of SP in the market determines the success of IPT. Thus the need to urgently determine the post market quality of SP in drug shops in Arua District that may serve as a surrogate for SP quality in the Ugandan market.

1.3 Significance of study

The findings from this study will contribute to the body of knowledge on post-market quality of SP in Uganda. The study also identified SP sources, brands and batches that do not meet pharmacopeial quality standards for possible affirmative action by the regulatory authorities, NDA and MoH. Through its recommendation, the study will help in strengthening the implementation of the National Drug Policy of Uganda. For any substandard or fake products found during this study, withdrawal of such poor quality SP from the market will improve IPT outcomes thus reduction of maternal mortality and subsequently, achievement of Millennium Development Goal number five.

1.4 Research questions

1. What are the different brands, sources (country of origin) and shelf-life of SP tablets sold in drug shops in Arua Municipality?

2. Do the different brands of SP tablets sold in drug shops in Arua Municipality meet established pharmacopeial quality standards?

1.5 Objectives of the study

1.5.1 General objective

To assess the post-market quality of the various brands of Sulfadoxine-Pyrimethamine antimalarial tablets sold in drug shops and clinics in Arua Municipality-Arua District.

1.5.2 Specific objectives

i. To establish the various generics, sources (country of origin), shelf-life, registration status and visual quality of SP packaging materials and tablets sold in drug shops and clinics in Arua Municipality.

ii. To determine the weight uniformity of the SP tablets in the various brands and batches sold in drug shops and clinics in Arua Municipality.

iii. To determine the dissolution profile of the various brands of SP tablets sold in Arua Municipality.

iv. To determine the content (quantity) of the active pharmaceutical ingredients (Sulfadoxine and Pyrimethamine) in the various brands of SP tablets sold in Arua Municipality.

1.6 Conceptual frame for the quality of sulfadoxine- pyrimethamine in drug outlets

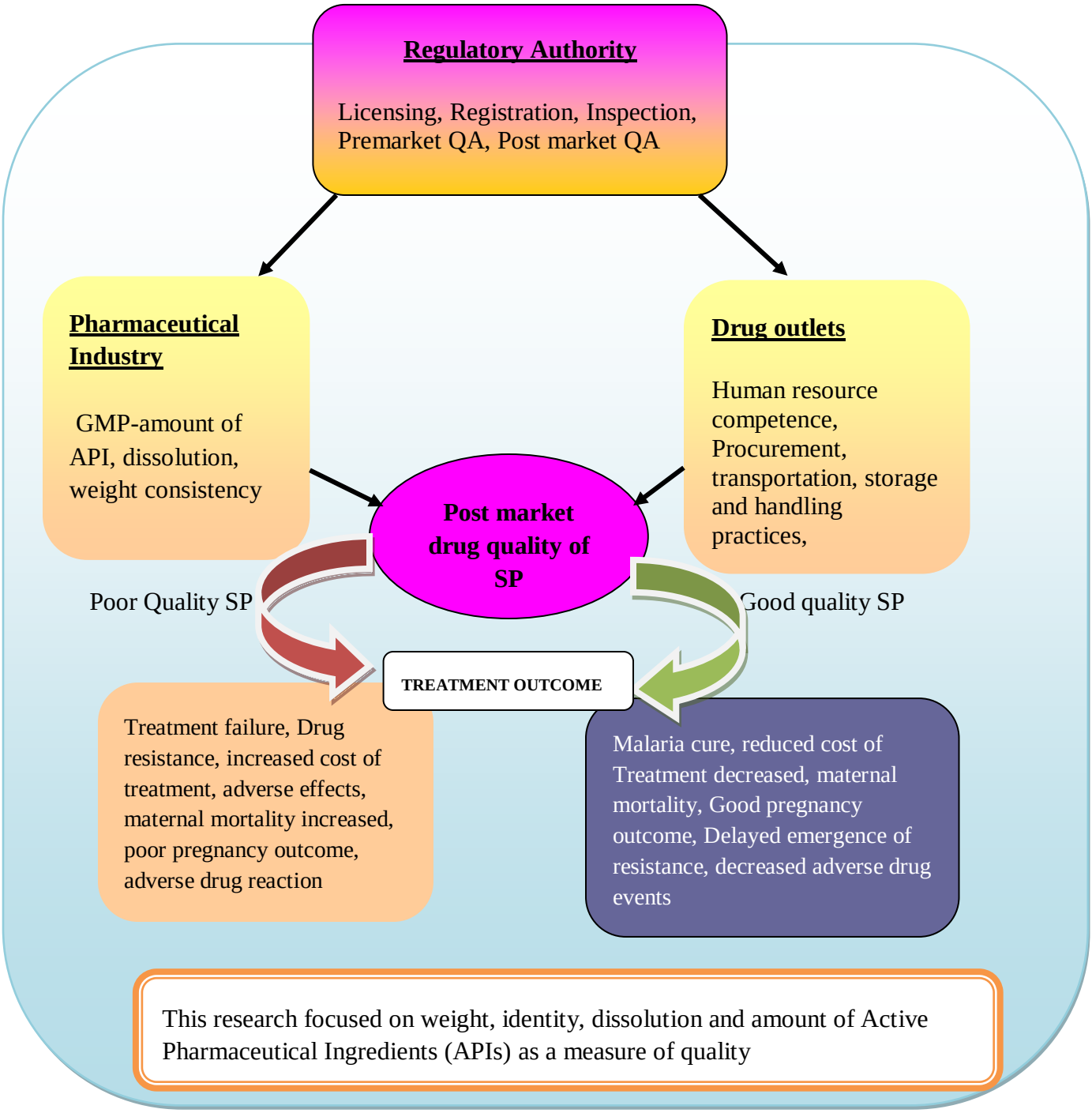

Figure 1: Research conceptual framework for the post-market quality of drugs

CHAPTER TWO: LITERATURE REVIEW

2.0 Malaria in pregnancy

Malaria causes serious complications in pregnant women, especially in those who have a low level of acquired immunity before pregnancy (McGregor, Wilson, Billewicz, 1983; Brabin, 1991; Menendez, 1995). Among women who live in areas with high rates of transmission of malaria, the susceptibility to malaria is highest during the second and third trimesters of pregnancy and the early postpartum period (Nafissatou *et al.,* 2000). In these women, the rates of maternal mortality, stillbirth, and premature delivery are high. Therefore, the WHO (2005), recommended the use of SP in the intermittent preventive treatment (IPT) of malaria in pregnant women in endemic areas. The important benefit of antimalarial treatment may be to reduce the likelihood of women having high-density placental malaria and in also helping reduce the other known adverse effects of malaria during pregnancy, including anemia, low birth weight, and prematurity (Steketee, Nahlen, Parise, Menendez, 2001).

2.1 Sulfadoxine-Pyrimethamine

Sulfadoxine (N-[5,6-dimethoxy-4-pyrimidinyl] sulfanilamide) is a sulfonamide with a particularly long half-life (7 to 9 days). It is used in combination with Pyrimethamine (500mg of sulfadoxine plus 25mg of pyrimethamine) for prophylaxis and treatment of malaria caused by chloroquine resistant strains of *Plasmodium falciparum* (Willian and Petri 2001). Sulfadoxine and pyrimethamine combination is an antimalarial agent which acts by reciprocal potentiation of its two components, achieved by a sequential blockade of two enzymes involved in the biosynthesis of folinic acid within the parasites.

Sulfadoxine, like other sulfonamides, is a structural analog of p-aminobenzoic acid (PABA) and competitively inhibits dihydrofolic acid synthesis by inhibiting dihydropteroate synthetase, which is necessary for the conversion of PABA to folic acid. Pyrimethamine is a folic acid antagonist and has a mechanism of action similar to that of trimethoprim. By binding to and reversibly inhibiting dihydrofolate reductase, pyrimethamine inhibits the reduction of dihydrofolic acid to tetrahydrofolic acid (folinic acid). Pyrimethamine interferes with the synthesis of tetrahydrofolic acid in malarial parasites at a point immediately succeeding that where sulphonamides act. The combination of sulfadoxine and pyrimethamine results in a synergistic action against susceptible plasmodia. Sulfadoxine and pyrimethamine are blood schizonticidal agents and are active against the asexual erythrocytic forms of susceptible plasmodia (www.malaria-ipca.com).

2.2 Sulfadoxine-pyrimethamine in IPT of malaria in pregnancy

Owing to the severity of malaria in pregnancy the WHO (2005) recommended the use of SP in the intermittent treatment of malaria in pregnant women in endemic areas. Only recently has Uganda accepted the intermittent preventive treatment (IPT) with SP as its strategy for malaria prevention in pregnancy, recommending two doses, one between weeks 12 and 24 and the second between weeks 24 and 36 of pregnancy (CDC, 2008). In 2002 and 2003, major efforts have been undertaken to train all health workers in government and NGO facilities in IPT and to ensure consistent supply of drug and information materials. This effort is however, compromised due to increased prevalence of drug resistance in most parts of the malaria endemic countries

including Uganda. Poor quality antimalarial drugs have been reported among others as the most important reason for the wide spread drug resistance (Bate *et al.,* 2008).

2.3 Quality of antimalarial drugs

About 15% of all drugs in circulation worldwide are believed to be counterfeit, with the figures rising to as high as 50% in some parts of Africa and Asia (Cockburn *et al.*, 2005). Drug quality is a source of great concern worldwide, particularly in many developing countries (USP, 2004). There are several reports of increase in the level of substandard and counterfeit drugs in resource poor countries in Africa and Asia (Bate *et al.,* 2008; USP, 2004). Poor drug quality can be the result of poor manufacturing practices, counterfeiting, or inappropriate drug storage in excessive heat, moisture, or light (Pecoul *et al,,* 1999). Substandard drugs are genuine products that do not conform to the pharmacopeial standards set for them (Behrens, Awad and Taylor, 2002). Use of poor quality drugs bears serious health implications such as treatment failure, adverse reactions (Pecoul, 1999), drug resistance (Taylor, Shakoor and Behrens, 1995), increased morbidity, and mortality (Alubo, 1995). Amin and Kokwaro, 2008 reported that in sub-Saharan Africa, a balance has to be struck between the need to make affordable antimalarials available close to where the majority of the people live, and ensuring that the quality of the drugs is not compromised.

2.4 Regulatory systems for medicines

The existence of adequate legislation and regulations concerning pharmaceutical activities, including drug registration, licensing of pharmaceutical establishments, inspection, control of drug production, importation, and exportation is critical. Since they are the legal tools used by

regulatory authorities to ensure the quality assurance and control of drugs circulated in any country (USP, 2004). Proper registration of a drug by a drug regulatory agency provides some guarantee to the user of its quality, safety and efficacy (USP DQI, 2004). To ensure quality standards and safety of pharmaceuticals, the National Drug Authority (NDA) of Uganda was formed by the Act of Parliament (MoH, 2004). The National Drug Quality Control Laboratory (NDQCL) constitutes the Drug Quality Control and Assurance Department (DQCA) of National Drug Authority of Uganda. Its mandate is premarket and post-market quality assurance of all pharmaceutical products and ensuring compliance with GMP by pharmaceutical industries in the country through regular supervision (www.nda.or.ug).

2.5 Drug management for quality assurance

Drug management is organized around the four basic functions of the drug management cycle: selection, procurement, distribution and use. Effective distribution management is achieved by designing an efficient network of storage facilities appropriate to the country's geography, appropriate transportation strategy, record keeping and managing the storage facility to maintain quality (MSH, 1997). This takes into account, protection of pharmaceuticals against adverse climatic hazards such as moisture, temperature, light, pressure and atmospheric gases (Dean, 1988).

2.6 Drug quality assessment

Testing enables assurance on the safety, efficacy, and quality of the drugs to be used by patients. Several physicochemical and quantitative methods of drug quality assessments are found in pharmacopeial monographs (USP, 2004). Some of these can be performed at facilities with

modest infrastructure while others require more substantial investment (Amin and Kokwaro, 2008). They can broadly be classified as physical tests (those that include visual inspection), chemical tests (for content of active ingredients and impurities under normal and simulated storage conditions), *in vitro* disintegration and dissolution tests and *in vivo* bioavailability studies (Amin and Kokwaro, 2008). Thus, physical tests may include tests done on liquid, semi-solid and solid pharmaceutical dosage forms. For instance, for tablets, uniformity of weight, friability (how well a tablet holds under normal conditions of transportation, measured by the proportion of the tablet that is lost as powder), tablet hardness, and so on, can all be carried out as part of the quality tests (Aulton, 2001). Conventionally, drug quality tests are performed using procedures outlined in *official monographs* such as the European, British and the United States Pharmacopoeia (USP). Such monographs state the most basic aspects of drug quality that need to be assessed for a particular drug and formulation, and the criteria to be used in the assessment (USP, 2000 and BP, 2000). For instance, the test for content determines the amount of active ingredient in a product, which is expressed as a percentage of the label claim, while the dissolution test determines the amount of active ingredient that is released from the dosage form and available for absorption, and is used as surrogate marker of *in vivo* bioavailability for oral dosage forms containing poorly aqueous-soluble drugs such as sulfadoxine-pyrimethamine (Amin and Kokwaro, 2005).

A recent review of articles published on the quality of antimalarial drugs in Africa (Amin and Kokwaro, 2008) revealed that there were 30 studies which reported on the dissolution of the tablet form analysed. In general, most antimalarial solid drug products, especially those containing SP have been found to have problems of dissolution but not content of the active

ingredient (Kibwage and Ngugi , 2000, Risha, 2002, Amin and Kokwaro, 2005. Most of the SP samples which failed the dissolution test as reported in the various studies did so with respect to the pyrimethamine component (Amin and Kokwaro, 2008).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area and setting

This study was carried out in Arua Hill and Oli Divisions of Arua Municipality, Arua District. The municipality was particularly selected for this study because it is where most drug outlets like Pharmacy, Drug shops and Clinics are clustered in Arua District. In addition, the district was purposively selected because it borders Democratic Republic of Congo (DRC) and it is also in close proximity with Southern Sudan and provides strategic and lucrative location for pharmaceutical business with the neighbouring countries. Previous studies done on the post market quality of antimalarials in DRC (Atemnkeng, De Cock and Plaizier, 2007) and Sudan (Maponga and Ondari, 2003) reported a high level of circulation of substandard and counterfeit SP.

3.2 Study design

This was a cross sectional study that involved sampling various batches of SP tablets in drug shops and clinics in March, 2009 in Arua Municipality, followed by laboratory analysis in Kampala Pharmaceutical Industries (1996) Limited and Department of Pharmacology and Therapeutics, College of Health Sciences. The laboratory analyses involved: visual inspection of the packaging materials and individual tablets, weight uniformity and dissolution tests and assay for quantity of active ingredients in the various generics of SP tablets sampled. The quality of the packaging materials and the individual tablets in each batch were assessed using a standard checklist provided by the International Pharmaceutical Federation (FIP) and USP. The

dissolution profile and assay for content (quantity of API) was carried out according to methods for analysis of SP tablets described in the USP (2009).

3.3 Sampling design

3.3.1 Sample size of drug outlets

The number of drug outlets required for this study was calculated using the formula for sample size determination for drug quality study provided by WHO (1999) below.

$$S_b = p \times 20 \iff S_b = (n_1/n) \times 20$$

Where $\mathbf{S_b}$ is the number of drug shops required for the study, $\mathbf{p=n_1/n}$; **p** is proportion of licensed drug shops in Arua Municipality to the total in Arua District; $\mathbf{n_1}$ is the number of licensed drug shops in Arua municipality; **n** is the total number of licensed drug shops in Arua District.

$$S_b = [36/45] \text{ x}20 = 16$$

Although the initial plan was to sample drug shops only, it was later realized in the field that there were very few drug shops in the municipality compared to the rampant clinics. This necessitated including clinics in the sampling frame. Hence, all the drugs shops and clinics (drug outlets) encountered in various blocks of Arua Hill and Oli Divisions were included in the study. The calculations above were done according to estimates provided by the drug inspector Arua District (Personal communication).

3.3.2 Sampling procedure

a) Sampling of drug outlets

The drug shops and clinics included in this study were conveniently sampled based on the ease of their accessibility in the two divisions of Arua Municipality. This method was adapted because it became difficult to obtain the lists of registered drug shops and clinics in the area. In Arua Hill division, drug outlets were sampled from Awindiri, Bursar and Ochiba coast blocks. While in Oli Division, Mvaradri, Kebiri and Tanganyika blocks were identified for sampling SP tablets.

b) Sampling of SP for quality analysis

The sampling of drugs was carried out using WHO (1997) guidelines. Sixty (60) SP tablets were anonymously and purposively purchased per brand and/ or batch from each of the selected drug outlets visited. In cases where a drug outlet had more than one brand and/ or batch of SP tablets, all were sampled (Obua, Ogwal-Okeng and Owino, 2003). Upon purchase, the name of the drug outlet (where possible), date of sampling, brand/ trade name and batch number of the sampled SP tablets were recorded in sampling form (shown in Appendix I). Each SP sample was then packed in dark water proof polythene bag to protect the drugs from sunlight and moisture. The packs were identified by codes assigned according to order of sampling, batch number, brand/ trade name, place and date of sampling as shown in the figure 1 below.

Figure 2: SP samples packaged in black polyethene bag

The samples were then transported in a closed roof vehicle to the Department of Pharmacology and Therapeutics, School of Biomedical Sciences (SBS) – College of Health Sciences (CHS), Makerere University where they were stored in dry room at 25 ^{o}C and minimal light intensity until laboratory analysis commenced in April 2009.

The standard SP/ chemical reference (*USP Sulfadoxine RS* and *USP Pyrimethamine RS*) was purchased from Sigma Aldrich (Steinheim, German). The reference SP was also kept under the same storage condition as the test SP samples prior to laboratory analyses.

3.4 Laboratory analysis

Out of the 60 SP tablets sampled per batch, 6 were randomly picked from each container or blister pack for dissolution test. An additional 20 tablets were used for visual inspection, weight uniformity test, identity test and assay for content. The remaining 34 tablets were kept as reserve

for future use in case of any need. The different analytical procedures undertaken are described below.

3.4.1 Visual inspection of packaging materials and tablets

Each brand or batch of SP tablets sampled was analysed through visual inspection of the packaging material and the individual drug in order to identify suspicious and potentially counterfeit drugs for further examination (USP DQI, 2007). The checklist Tool for Visual Inspection of Medicine (TVIM) provided by the International Council of Nurses in partnership with the United States Pharmacopeia (USP) and Military and Emergency Pharmacists Section of the International Pharmaceutical Federation (FIP) was used for inspection (see appendix V).

The packaging materials were inspected for label information such as trade/ brand name, scientific/ generic name of active ingredients, manufacturers' details, medicine strength (mg/unit tablet), dosage statement, batch/lot number, storage information, dates of manufacture and expiry. Individual tablets per batch were also analysed for physical characteristics of the tablets. This involved randomly sampling 20 tablets from each batch and visually inspecting them for uniformity of shape, size, colour and texture, markings (scoring and letters), breaks/ cracks/ splits, embedded surface spots/ contamination and smell. In addition, the SP brands and their country of origin were authenticated using the Human Drug Register (September 2008 and September 2009 versions) obtained from NDA for establishment of the registration status of each brand in Uganda. Such verification also helped in identifying the extent of leakage of smuggled SP drugs into Uganda from neighbouring countries.

3.4.2 Weight uniformity determination

The 20 tablets (from each batch) previously used for visual inspection were weighed individually using analytical balance (*Adam, AEA 250g [250g x 0.1 mg] Model, USA*) to determine their weight. The individual tablet weight, average, standard deviation and percentage deviation were calculated and used to establish weight uniformity among the SP tablets within each brand and/ or batch. The weight uniformity was considered acceptable when the percentage standard deviation per batch did not exceed ±5% (USP, 2009). After weight uniformity test, the tablets were put into a weighing cup, labeled and later used for the subsequent content analysis.

3.4.*3 In-vitro* dissolution test for SP samples

The SP tablets from the different brands were subjected to dissolution test performed according to the method described in the USP (2009) monograph.

3.4.3.1 Equipment

The equipment used in the dissolution test included Dissolution Tester QD014 (Electrolab-Tablet Dissolution Tester-USP, TDT-06P Model, USA) for agitating the tablets; Analytical balance QD 008 (Schimadzu, Au x 220, Japan) for weighing the SP tablets and the salts used for preparation of the dissolution medium; Digital pH Meter (QD012) for determining the pH of the dissolution medium; HPLC Column (ZORBRAX ODS 4.5 x 250mm, 5 µm, SN USF0059832, Agilent Technologies, USA), SCHIMADZU HPLC System: Pump (LC-10AT *vp*, Schimadzu Liquid chromatography, Detector (SPD-10A *vp*, Schimadzu UV-VIS Detector), Oven (CT0-10ASP *vp* Shimadzu), Software (Schimadzu Class VPTM, Chromatography Data System, Version 6.1 Kyoto, Japan) were used for chromatographic quantification of sulfadoxine and pyrimethamine

in the dissolution medium; Nylon membrane filter (Whatman®, 47mm x 0.45µm, Whatman international Ltd, England) for filtering the dissolution medium after the agitation; Beakers, Pipette, Measuring cylinder and syringes were also used in volumetric measurements during dissolution profiling.

3.4.3.2 Reagents

Dibasic Potassium Hydrogen Orthophosphate GR (Merck Specialties Private Ltd, India) and Sodium hydroxide pellet AR (Sd-fine Chem Ltd-India) were used for preparation of the dissolution medium; Acetonitrile HPLC grade (Merck Specialties Private Ltd, India) was used as a mobile (A) phase; Triethyl amine (Sd-fine Chem Ltd-India) for enhancing separation and elution of sulfadoxine and pyrimethamine in the dissolution medium; Glacial Acetic acid AR (Merck Specialties Private Ltd, India) used as part of the mobile phase in the isocratic system; HPLC Water (Lichrosolv® , 5K85F81149, Merck Specialties Private Ltd, India) used as a solvent; Sulfadoxine reference standard (20030621242, % Purity 99.46), Pyrimethamine reference standard (20080722, % Purity of 99.9) used for obtaining the standard chromatograms were subsequently used for calculating the quantity of SP released by each batch analysed.

3.4.3.3 Preparation of dissolution medium

To prepare 18 liters (L) of 0.2 M Dibasic Potassium Hydrogen Orthophosphate and 0.2 M Sodium hydroxide (dissolution medium), 122.49 g and 16.92 g of the above salts respectively were dissolved in 18 L of distilled and deionised water. Then 500 ml of the dissolution media was transferred into a beaker and the pH was determined using Digital pH Meter QD012, a pH reading of 6.81 was recorded and considered suitable for the experiment.

3.4.3.4 Preparation of mobile phase

The mobile phase used was a mixture of Acetonitrile and Glacial acetic acid in a ratio of 3:7 for the isocratic system. The Glacial acetic was prepared by measuring 20 ml of Acetic acid into a 2000 ml measuring cylinder and diluted to the mark with distilled water. Then 1400 ml of the resultant solution was transferred into a 2000 ml volumetric flask and 600 ml of Acetonitrile was added to make 2 L of the mobile phase solution. One liter of the mobile phase was transferred into a reservoir bottle and loaded to the HPLC system ready for running.

3.4.3.5 Preparation and assay of standard reference solution

Five hundred milligram of sulfadoxine and 25 mg of pyrimethamine reference substances were weighed using analytical balance QD 008 (Schimadzu, Au x 220, Japan) into a 100 ml volumetric flask (Actual weights taken were 500.5 mg and 25.3 mg of sulfadoxine and pyrimethamine respectively). Then 35 ml of Acetonitrile was added, shaken and sonnicated for 10minutes and toped to 100 mark using mobile phase. Ten milliliter of the above solution was pipetted into 100 ml volumetric flask and diluted to the mark using mobile phase. The resultant solution was filtered using membrane filter paper (Whatman®, 47mm x 0.45 µm, Whatman international Ltd, England) and degassed using a sonicator.

The chromatographic conditions for the assay were set as follows; Oven temperature of 40 ^{o}C, Detection wavelength of 254 nm, Flow rate of 1.5 ml/minute, Pressure of 219 kg/force/cm^2 and Run time of 8.5 minutes.

Twenty (20) microliter (µl) of the standard solution was injected into the HPLC system 5 times and resultant chromatograms for sulfadoxine and pyrimethamine were recorded. The average,

standard deviation and percentage relative standard deviation for the areas of the five chromatograms were recorded for subsequent calculations shown in appendix VI.

3.4.3.6 Dissolution analysis of the SP tablets

The dissolution conditions of the tester were sets as follows: Temperature of 37±5 ^{o}C, agitation speed of 75 RPM and agitation time of 30 minutes. One thousand milliliter of the dissolution medium was introduced into each of the 6 vessels of the dissolution tester QD014 (Electrolab-Tablet Dissolution Tester-USP, TDT-06P Model, USA). The medium was allowed to attain a temperature of 37±5 ^{o}C. One tablet was carefully introduced in each of the 6 vessels to minimize air bubbles from forming on the surface of the tablet. The paddles were lowered into the vessels and the tester was immediately started and let to run for 30 minutes, after which 20 ml was withdrawn from each of the vessels at a position midway between the surface of the solution and top of the paddle and not less than 10 mm away from the wall of the vessel.

The samples from each vessel were then filtered using Nylon membrane filter (Whatman®, 47 mm x 0.45 µm, Whatman international Ltd, England) into sample tubes and labeled (D1 to D6 corresponding to the dissolution vessel number). Twenty microliters (20 µl) of the filtrates were separately injected into the HPLC system using a micro-syringe (Hamilton 80465, Nevada). The resultant chromatograms were recorded (Appendix VIII (b)) and later used for calculating the percentage of sulfadoxine and pyrimethamine dissolved in 30 minutes in the dissolution medium. The chromatographic conditions for the assay were set as follows as described for the standard reference drug above.

The percentage of sulfadoxine and pyrimethamine dissolved was calculated using the formula below;

$$\%\text{Dissolution (Sulfadoxine)} = \frac{As \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$$

Where As=Area of test solution, SA= Average area of reference sulfadoxine solution, P= Purity of sulfadoxine, Ws= Actual weight of sulfadoxine feference standard.

$$\%\text{Dissolution (Pyrimethamine)} = \frac{Ap \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$$

Where Ap =Area of test solution, SA= Average area of pyrimethamine reference solution, P= Purity of Pyrimethamine, Ws= Actual weight of pyrimethamine reference standard.

To increase the accuracy of calculations, the above formulae were fed into Microsoft Excel, 2007 template availed by Kampala Pharmaceutical Industries (1996) Limited as shown in appendix VI.

USP limits for relative standard deviation of tablets in each batch is not more than (NMT) 2.5%; USP Pharmacopeia acceptance specification for the dissolution of sulfadoxine and Pyrimethamine in dissolution medium is not less than (NLT) 60% for each tablet. Therefore, a batch would fail if less than 60% of the labeled amount of sulfadoxine and pyrimethamine has dissolved in the dissolution medium in 30 minutes.

3.4.4 Quantitative content analysis for SP samples

Identification and quantification of sulfadoxine and pyrimethamine in the different brands and/ or batches of SP tablets was carried out using High Performance Liquid Chromatography described USP and BP 2009 monographs.

3.4.4.1 Equipment

Besides the dissolution tester and the pH meter, the rest of the equipment described above were used for quantitative and volumetric measurements in assay of content in the various SP batches.

3.4.4.2 Reagents

Triethyl amine AR (Sd-fine Chem Ltd-India), acetonitrile HPLC grade (Merck Specialties Private Ltd, India), glacial acetic acid AR (Merck Specialties Private Ltd, India), HPLC water (Lichrosolv®, , 5K85F81149, Merck Specialties Private Ltd, India), sulfadoxine reference standard (20030621242, % Purity 99.46), pyrimethamine reference standard (20080722, % Purity of 99.9) (Donation by KPI), 85% Orthophosphoric acid AR (Scharlau Chemie S.A., Spain) were used for assay for content in the various batches of SP tablets analysed.

3.4.4.3 Preparation and assay of standard reference solution

Sulfadoxine (500mg) and pyrimethamine (25mg) reference substances were carefully weighed using analytical balance QD 008 (Schimadzu, Au x 220, Japan) into a 100 ml volumetric flask (actual weights taken were 500.2 mg and 25.1 mg of sulfadoxine and pyrimethamine respectively). 35 ml of acetonitrile was added, shaken and sonnicated for 10 minutes and toped

to 100 mark using mobile phase. 10 ml of the above solution was pipetted into 100 ml volumetric flask and diluted to the mark using mobile phase.

The resultant solution was filtered using membrane filter paper (Whatman®, 47 mm x 0.45 µm, Whatman international Ltd, England) and degassed using a sonicator. The chromatographic conditions for the assay were set as follows; Oven temperature of 40 ^{o}C, detection wavelength of 254 nm, flow rate of 1.5 ml/minute, pressure of 219 kg/force/cm^{2} and run time of 8.5 minutes. Twenty microliters of the standard solution was injected into the HPLC system 5 times and resultant chromatograms for sulfadoxine and pyrimethamine were recorded (Appendix VIII (c)). The average, standard deviation and percentage relative standard deviation for the areas of the five chromatograms were recorded for subsequent calculations.

3.4.4.4 Preparation and assay of SP samples for content (quantity of APIs)

For each brand of SP, 20 SP tablets were weighed using analytical balance [*AE Adam, 100L (100g x 0.1 mg) Model*] individually and their average weight determined. The twenty tablets were pounded using a motor and pestle into a fine powder as shown in figure 2 below.

Figure 3: Fine powder of the various SP samples used in assay for content

An accurate average weight (that contains 500 mg of sulfadoxine and 25 mg of pyrimethamine) of the finely ground SP was carefully transferred to a 100 ml volumetric flask, 35 ml of acetonitrile was added and shaken and sonicated for 15 minutes. The resultant solution was dilute with mobile phase to volume (100 ml mark), mixed and filtered using Nylon membrane filter ((Whatman®, 47 mm x 0.45 µm, Whatman international Ltd, England), the initial 5 ml of the filtrate was discarded. 10 ml of the filtrate was pipetted into a 100 ml volumetric flask and diluted to the mark with mobile phase. Using a microsyringe, 20 µl of the standard solution was injected into the HPLC system 3 times for each brand and resultant chromatograms (Appendix VIII (a)) for sulfadoxine and pyrimethamine were recorded. The average, standard deviation and percentage relative standard deviation for the areas of the five chromatograms were recorded for subsequent calculations. The values recorded were entered into a Microsoft Excel 2007 formula designed for calculation of the percentage of sulfadoxine and pyrimethamine in the assay as shown appendix VII. The formulae for calculation of percentage of the active ingredients in the test assay are shown below.

$$\% \text{ Sulfadoxine in test Assay} = \frac{TA \times Ws \times Av.\ Wt. \times P \times 100}{SA \times WT \times 500 \times 100}$$

Where TA is area of sulfadoxine in test assay, Ws is weight of sulfadoxine reference drug taken, Av.Wt is Average weight of SP tablets, SA is Area of sulfadoxine in standard/ reference solution, WT is actual weight of SP sample (powder) taken, P is percentage purity of sulfadoxine reference/ standard.

$$\% \text{ Pyrimethamine in Assay} = \frac{TA \times Ws \times Av.\ Wt. \times P \times 100}{SA \times WT \times 25 \times 100}$$

Where TA is Area of Pyrimethamine in Test assay, Ws is weight of Pyrimethamine reference/ standard taken, Av.Wt is Average weight of SP tablets, SA is Area of Pyrimethamine in standard/ reference solution, WT is Actual weight of SP sample (powder) taken and P is percentage purity of pyrimethamine reference/ standard.

3.4.4.5 Identity test for SP tablets sold out of the original packaging tin

This was carried in the pharmacokinetics laboratory, Department of Pharmacology and Therapeutics, School of Biomedical Sciences, College of Health Sciences (Makerere University). The method described by NDQCL (2005) for analysis of sulfadoxine-pyrimethamine tablets was used. The identity (retention time) test for the APIs (sulfadoxine and pyrimethamine) in the claimed SP tablets was carried out using HPLC. To determine the identity of the tablets, the corresponding retention time of sulfadoxine and pyrimethamine in the assay was compared to that of the standard solution. The HPLC system used was SHIMADZU model (Auto-injector, SIL-10ADvp, Shimadzu Corporation, Kyoto, Japan); with Ultra-Violet detector, 3.9 mm x 30 cm column that contains packing L1.The details for preparation of the mobile phase, standard sulfadoxine and pyrimethamine reference substances and test assay are described in appendix II.

3.5 Quality assurance

Expired SP tablets were not analysed for dissolution profile and quantity of APIs. To ensure reliability, calibrated equipment and validated standard operating procedures (SOPs) for dissolution test and HPLC for Sulfadoxine/ Pyrimethamine were used. For the assay and standard drugs, three and five injections respectively were made to optimize the validity of the resultant data. To minimize calculation errors, formulae for determining the percentage of

sulfadoxine and pyrimethamine were designed using Microsoft Excel, 2007. Water and solutions used in the assay and dissolution was filtered using Nylon membrane filter so that heavy metals were removed from the water to avoid blockade of the column. In addition, clean and dry glassware were used in sample preparation to avoid contamination of the solutions prepared. The data generated was entered in a laboratory log book and later transferred in computer and safely stored in 3 different locations.

3.6 Inclusion criteria

Only tablets claimed to be SP were included for sampling in this study. The SP tablets were strictly sampled from drug shops and clinics randomly selected in Arua Municipality. Only SP brands and/ or batches with at least 3 months left to their expiry date were sampled.

3.7 Measurable variables

This included the source (country of origin), shelf-life, weight uniformity, identity, dissolution rates and quantity of active APIs (sulfadoxine and pyrimethamine) in the sampled SP tablets.

3.8 Data management

The laboratory results generated were entered in a log book for safety and inference. Resultant chromatograms from HPLC analyses were printed and filed. Soft copies of the data were stored in more than three locations for safety.

3.9 Data analysis

Data generated through visual inspection were analysed descriptively. For the weight uniformity data, average weight, standard deviation and percentage relative standard deviations were calculated for each batch. The uniformity of weight was considered acceptable if the standard deviation of the tablets in a batch does not exceed ±0.05. The weight and average dissolution variations were analysed using two-tail ANOVA using MINITAB (Version 12) statistical package, with significance assumed at p value of 0.05. The mean dissolution rate was considered acceptable if not less than 60% of the labeled amount of sulfadoxine and pyrimethamine had dissolved in 30 minutes. The amount of API in the test assay was deemed acceptable if the ratio of the test against the reference at 95% CI falls within 90-110%.

3.10 Ethical considerations

The research work proceeded after seeking approval and clearance from the Department of Pharmacology and Therapeutics, Ethics and Research Committee (College of Health Sciences and the Uganda National Council of Science and Technology. The identity of drug shops and clinics sampled were protected through coding. Not more than half the total stock of SP tablets was purchased per drug outlet.

CHAPTER FOUR: RESULTS

The different batches of SP brands were purchased from 19 drug outlets (clinics and drug shops) in Arua Hill and Oli divisions' of Arua Municipality, Arua District. In Arua Hill division, SP tablets were sampled from a total of 14 drug outlets while in Oli Division, the tablets were obtained from 5 drug outlets. Of the 14 drug outlets in Arua hill, 8 were clinics while 6 were drug shops. In Oli division, 3 of the drug outlets were clinics while 2 were drug shops. A total of 10 SP brands and/ or 18 batches were sampled in the two divisions of the municipality. In addition, one sample that was claimed to be SP tablets yet sold in a tin for albendazole was also obtained for further laboratory investigation. The various brands of SP tables were mainly sampled from clinics (57.1%), while 42.1% were sampled from drug shops in the municipality. In all the areas visited within each division, there were more numbers of clinics than drug shops. The distribution of the SP samples by the nature of drug outlets from which they were obtained is shown in table 1 below.

Table 1: Distribution of the SP samples by drug outlets

S/N	*Drug outlet*	*No. of SP samples*	*%*
1	Clinics	11	57.9
2	Drug shops	8	42.1
	Total	19	100.0

The majority of SP batches (73.7%) were sampled from clinics and drug shops in Arua hill Division, while only 26.3% were obtained from Oli Division. Within Arua hill Division, most of the drug outlets were sampled from Awindiri (26.3%) and Arua town (26.3%) while only 21.1% of the total SP samples were purchased from Ochiba coast. In Oli division, SP batches were sampled from clinics and drug shops in Mvaradri (10.5%), Kebiri (5.3%) and Tanganyika (10.5%) blocks. The distribution of the samples by the two divisions and corresponding blocks in Arua Municipality is shown in table 2 below.

Table 2: Distribution of SP samples by zones in Arua Municipality

Divison	***Area/block***	***No. of drug outlet***	***%***	***% by division***
Arua hill	Awindiri	5	26.3	
	Bazar	5	26.3	
	Ochiba coast	4	21.1	
				73.7
Oli	Mvaradri	2	10.5	
	Kebiri	1	5.3	
	Tanganyika	2	10.5	
				26.3
	Total	19	100.0	

Majority (60%) of the SP brands sampled were manufactured in East Africa, considering 40% were made locally in Uganda and 20% were imported from Kenya. The rest of the generics were imported from India (30%) and Cyprus (10%). Of the total SP batches sampled, 31.58% were manufactured in Uganda, 31.58% from Kenya, 26.32% from India and 5.26% from Cyprus (United Kingdom). The country of origin of 5.26% of the samples could not be traced because

they were sold in a container meant for storage of albendazole. The distribution of SP tablets/ samples by country of origin is shown in table 3 below.

Table 3: Distribution of SP batches and brands by country of origin

SN	*Country of origin/source*	*Per batch basis*		*Per generic basis*	
		No. of SP batch	*%*	*No. of SP brand*	*%*
1	Cyprus	01	5.26	01	10
2	Kenya	06	31.58	02	20
3	India	05	26.32	03	30
4	Uganda	06	31.58	04	40
5	Unknown*	01	5.26	-	-
	Total	**19**	**100**	**10**	**100**

** Sold in a container for albendazole thus details on its origin could not be obtained*

SP Brand registration status

A total of 19 SP batches were sampled from 10 brands in the municipality. Verification of the registration status of the 10 brands revealed that only 50% appeared in the NDA drug registry. All the SP brands manufactured in Uganda and one from India were registered. On the other

hand, all the SP brands from Kenya, four from India and the lone brand from Cyprus were not in both December 2008 and September 2009 human drug registry as shown in table 4 below.

Table 4: The various brands and their registration status

S/N	*Trade/ Brand Name*	*No. of batches*	*Reg. No.*	*Origin*	*Registry**
1	Malaren	2	4672/06/04	Uganda	Yes
2	Agosidar	2	5354/06/06	India	Yes
3	Orodar	4	-	Kenya	No
4	Malamox	2	-	India	No
5	Malagon	2	3094/06/98	Uganda	Yes
6	Falcidin	2	-	Kenya	No
7	Falcistat	1	-	Cyprus	No
8	Kamsidar	1	0745/06/97	Uganda	Yes
9	Neosidar	1	4632/06/04	Uganda	Yes
10	Nopyrin	1	-	India	No

***Yes**-if trade name is available; **No**-if trade name is not available in 2008/09 drug register*

Of the 19 SP batches sampled, 18 samples were claimed to be containing 500mg sulfadoxine and 25mg pyrimethamine by their manufacturers. However, the strength of the tablets (SP14) sold

outside its original packaging material could not be established since the vital information on the packaging material was lost. Two similar cases of transferring drugs into another packaging material were also encountered in Awindiri and Mvaradri blocks in Arua Hill Division. The shelf life of the samples varied from 2 years for locally manufactured SP to over 4 years for the imported SP tablets. Further sample (product) details are provided in table 5 below.

Table 5: The product details for the various batches of SP brands sampled

Sample ID	*Trade/ Brand Name*	*Batch No.*	*Manuf. Date*	*Exp. Date*	*Type of Drug outlet*	*Country Origin*	*Pharmc. Status*	*NDA Reg. Status*
SP01	MALAREN	00308	Jan-08	Dec-09	Clinic	Uganda	USP	Yes
SP02	ORODAR	6E65	May-06	Apr-10	Clinic	Kenya	BP	No
SP03	AGOSIDAR	T7654	Dec-07	Nov-10	Clinic	India	USP	Yes
SP04	ORODAR	6E64	May-06	Apr-10	Clinic	Kenya	BP	No
SP05	FALCIDIN	051042	Aug-05	Dec-09	Clinic	Kenya	USP	No
SP06	MALAMOX	ME17-82	Jun-06	May-09	Clinic	India	USP	No
SP07	MALAGON	SP429	Feb-09	Jan-11	Clinic	Uganda	USP	Yes
SP08	KAMSIDAR	1308	Dec-08	Nov-11	Drug shop	Uganda	USP	Yes
SP09	ORODAR	7E02	May-07	Apr-11	Clinic	Kenya	USP	No
SP10	FALCISTAT	30758	Sep-06	Sep-11	Drug shop	Cyprus	BP	No
SP11	NOPYRIN	165	Nov-06	Oct-09	Drug shop	India	USP	No
SP12	MALAGON	SP908	Oct-08	Sep-10	Drug shop	Uganda	USP	Yes
SP13	MALAMOX	ME17506	Dec-06	Nov-09	Drug shop	India	USP	Yes
SP14	None*		-	-	Drug shop	-	-	-
SP15	AGOSIDAR	T7258	Apr-07	Mar-10	Clinic	India	USP	Yes
SP16	FALCIDIN	062605	Sep-06	Aug-10	Clinic	Kenya	USP	No
SP17	ORODAR	7E04	May-07	Apr-11	Clinic	Kenya	BP	No
SP18	NEOSIDAR	0408	Nov-08	Oct-10	Drug shop	Uganda	USP	Yes
SP19	MALAREN	00908	Nov-08	Oct-10	Drug shop	Uganda	USP	Yes

**SP14 was sold in a plastic tin for albendazole hence vital information about the product could not be traced*

Visual inspection of the packaging materials and the individual table

Of the 19 SP batches sampled, nine (47.4%) were packaged in blister packs and ten (52.6%) batches were in plastic tin (of 1000 tablets). The 9 blister packs for the SP batches sampled all met the quality standard requirements provided by the International Council of Nurses in partnership with the United States Pharmacopoeia (USP), Military and Emergency Pharmacists Section of the International Pharmaceutical Federation (FIP). Visual analysis of the individual tablets from each batch using the above tool showed that all the tablets had uniform shape, size and scorings. Majority (18) of the samples had uniform colour except SP01 that had minor spots on three out of the ten tablets randomly selected for colour uniformity assessment. However, these spots were tinny and few to significantly affect the physical quality of the product. Thus all the SP samples passed the visual analysis test for packaging, tablet and batch integrity.

Weight uniformity

The relative standard deviation of all the 19 batches of SP tablets fell within the USP and BP pharmacopeial tolerance limit of ±5%, thus all passed weight uniformity test although statistical analysis revealed that there was significant ($p<0.0001$) difference in weight of all the batches of SP tablets sampled. Similarly, the relative standard deviation (RDS) for the twenty tablets weighed per batch varied from 0.38% (SP13) to 2.36% (SP07).The weight of the individual SP tablets sampled varied between 0.5949 g (SP17) and 0.7031 g (SP08) with the median weight being 0.6338 g. The weight variations within each SP batch is shown in appendix III and IV while the average weight and percentage standard deviation of the SP tablets within each batch is shown in the table 6.

Table 6: Weight variations within specific batches of SP brands

Sample ID	*Average weight (g)*	*Standard deviation*	*%Standard deviation*	*Verdict**
SP01	0.628905	0.013563	1.36	Pass
SP02	0.617995	0.00837	0.84	Pass
SP03	0.644685	0.007163	0.72	Pass
SP04	0.61278	0.006679	0.67	Pass
SP05	0.641245	0.006315	0.63	Pass
SP06	0.625525	0.005678	0.57	Pass
SP07	0.62037	0.023626	2.36	Pass
SP08	0.67578	0.011013	1.10	Pass
SP09	0.624465	0.00491	0.49	Pass
SP10	0.64402	0.004766	0.48	Pass
SP11	0.66652	0.011721	1.17	Pass
SP12	0.62268	0.008808	0.88	Pass
SP13	0.628175	0.003792	0.38	Pass
SP14	0.676415	0.009299	0.93	Pass
SP15	0.63438	0.004518	0.45	Pass
SP16	0.64016	0.019247	1.92	Pass
SP17	0.60996	0.01206	1.21	Pass
SP18	0.673715	0.011371	1.14	Pass
SP19	0.635405	0.01952	1.95	Pass

** Pharmacopeial acceptance limit is ≤5%*

Dissolution profile of the various brands of SP tablets sampled

Ten randomly selected batches of the various SP brands were analysed for dissolution in Kampala Pharmaceutical Industries (1996) Limited (KPI). The percentage of sulfadoxine released in the dissolution medium from the ten SP brands varied from 85.1% to 101.2%. On the other hand, the percentage dissolution of pyrimethamine ranged from 50.2% to 96.9%. Thus, all the sulfadoxine released by the ten SP brands complied with the USP and BP acceptance limits. However, this was not the case with pyrimethamine since one batch of SP brand (SP15) consistently yielded low level of pyrimethamine in the dissolution media from the six vessels as shown in table 7.

Table 7: Percentage dissolution of sulfadoxine and pyrimethamine in the various brands in the six dissolution vessels

Sample ID	*% Dissolution of Sulfadoxine and Pyrimethamine in the six vessels (V1-V6)*												*Verdict**
	V1		*V2*		*V3*		*V4*		*V5*		*V6*		
	Sdx	*Pyr*	*Sdx*	*Pyr*	*Sdx*	*Pyr*	*Sdx*	*Pyr*	*Sdx*	*Pyr*	*Sdx*	*Pyr*	
SP01	96.6	86.0	96.7	95.4	101.2	94.7	98.1	96.9	95.0	84.0	94.8	95.6	Pass
SP04	94.0	88.0	94.8	94.6	91.7	85.9	91.5	84.9	93.6	93.3	95.7	96.7	Pass
SP05	98.7	76.0	96.8	78.3	95.7	78.2	97.0	81.2	97.9	76.6	98.3	79.9	Pass
SP08	91.8	80.8	86.8	73.6	85.6	77.0	86.1	73.0	85.1	79.5	86.7	78.3	Pass
SP10	95.9	84.0	95.2	94.4	94.8	96.1	95.0	96.7	93.8	88.5	94.8	95.5	Pass
SP11	100.1	79.1	101.2	78.9	99.0	71.9	100.9	78.6	99.4	77.7	98.5	74.6	Pass
SP12	94.5	70.7	92.1	70.8	92.8	74.1	91.1	67.5	91.2	69.8	94.0	75.8	Pass
SP13	90.3	82.3	90.1	90.7	90.6	91.9	90.6	94.2	90.8	93.4	91.4	92.2	Pass
SP15	80.1	**50.2**	87.0	**57.8**	87.9	**58.2**	87.1	**55.0**	87.2	**57.7**	87.4	**57.7**	**Fail**
SP18	86.3	84.3	87.0	94.2	87.8	97.0	86.5	87.5	85.8	93.4	87.2	96.6	Pass

**USP Pharmacopeial tolerance limit is NLT 60%*

Therefore, 90% of the SP brands analysed passed USP and BP pharmacopeial tolerance limit of NLT 60% sulfadoxine and pyrimethamine released in the dissolution medium in 30 minutes.

However, analysis of variance for the dissolution profile revealed a significant ($p<0.001$) difference in the amount of sulfadoxine and pyrimethamine released in each of the six dissolution vessels. The average percentage of sulfadoxine and pyrimethamine released by the 10 batches is shown in table 8 below.

Table 8: Showing average percentage dissolution of sulfadoxine and pyrimethamine in 30 minutes (mean±SD) per brands

Sample ID	***% of Sulfadoxine***	***% of Pyrimethamine***	***Verdict****
SP01	97.1±2.4	92.1±5.6	Pass
SP04	93.6±1.7	90.6±4.9	Pass
SP05	97.4±1.1	78.4±2.0	Pass
SP08	87.0±2.4	77.0±3.2	Pass
SP10	94.9±0.7	92.5±5.1	Pass
SP11	99.9±1.1	76.8±2.9	Pass
SP12	92.6±1.4	71.5±3.0	Pass
SP13	90.6±0.5	90.8±4.3	Pass
SP15	86.1±3.0	**56.1±3.1**	**Fail**
SP18	86.8±0.7	92.2±5.1	Pass

**USP Pharmacopeial tolerance limit is NLT 60%*

Assay for content

Representative samples of 10 brands were randomly selected from the 19 SP batches of tablets purchased. The percentage of sulfadoxine in the assay for quantity of API ranged between 91.97% (SP01) and 101.53% (SP10), while pyrimethamine varied from 91(SP13) % to 102.06% (SP15). All (100%) the brands assayed passed US pharmacopeial acceptance limit for Sulfadoxine and Pyrimethamine (90% -110 %.). The percentage of Sulfadoxine and pyrimethamine in the 10 SP generics was tabulated as Mean±%RDS as shown in table 9.

Table 9: Average percentages of sulfadoxine and pyrimethamine in the brands assayed for content of API

Sample ID	*% Sulfadoxine of label claim*	*% Pyrimethamine of label claim*	*Verdict**
SP01	91.97±0.13	98.03±0.11	Pass
SP04	94.57±0.09	101.16±0.63	Pass
SP05	94.91±0.17	95.98±0.65	Pass
SP08	95.93±3.33	96.42±2.52	Pass
SP10	101.53±0.17	97.25±0.28	Pass
SP11	102.45±0.03	92.38±0.25	Pass
SP12	98.83±0.37	103.06±0.30	Pass
SP13	92.88±0.17	91.00±0.09	Pass
SP15	94.22±0.09	102.06±0.31	Pass
SP18	99.15±0.04	98.43±0.30	Pass

**USP and BP acceptance limit for SP is 90-110 of label claim*

Identity test for SP14 that was sold out of its original packaging material

This particular drug was amongst the three such cases encountered during the study. Upon analysis using HPLC, sulfadoxine and pyrimethamine eluted after 3.992 and 9.142 minutes respectively. This elution time was commensurate to that of the sulfadoxine and pyrimethamine reference substance (standard). Therefore, SP14 tablets passed USP identity test for sulfadoxine

and pyrimethamine. However, dissolution profile and content assay for the sample could not be determined due to lack of information on the strength of the product. Similarly, the brand name of the sample could not be determined because the individual tablets lacked embodiment of the manufacturer's logo on them.

CHAPTER FIVE: DISCUSSION

Principally, the Malaria treatment policy of Uganda (2004) recommends the use of SP strictly for Intermittent Preventive Treatment (IPT) during pregnancy. However, irregularity in supply of Artemether-Lumefantrine formulations (first line drug for treatment of uncomplicated malaria) in government health centers may account for the demand for SP in private drug outlets. This demand is further consolidated by the drive for self medication by individuals and affordability of the drug compared to ACTs whose prices only favours the minority high income earners.

In this study, SP tablets were more encountered in clinics (57.9%) as opposed to drug shops (42.1%). This could probably be due to the fact that SP is currently recommended in IPT during pregnancy. Clinics provide immediate alternative avenue for pregnant women seeking for antenatal care, and this may explain the frequency at which SP samples were purchased from clinics as opposed to drug shops.

The study also reveals that 40% of the SP brands sampled were manufactured in Uganda. This may be attributed to the ban imposed on importation of SP by the national drug regulatory authority following malaria treatment policy change. Whereas all the products manufactured in Uganda were registered, only one product amongst the imported brands appears in the NDA drug registry. This literally means that other than Agosidar (a product from India) the rest of the imported SP brands sampled could not be traced in the official compendium that contains the list drugs registered for sale and/ or use in Uganda. Sources in NDA revealed that the products were initially registered but when policy on malaria treatment changed in 2005, importation of SP was

banned thus accounting for the deletion of the 5 brands from the drug registry. Another school of thought may probably attribute the unregistered SP products to smuggling of the drugs from neighbouring countries. This is may be reaffirmed by the fact that Agosidar which is also an imported SP from India appeared in the September 2008 human drug register.

It is also very important to note that three cases of SP tablets being sold in tins that were for other pharmaceutical products were encountered. It was suspected that such drugs might have reached their expiry dates and by transferring them to another container, the information on their expiry dates are automatically lost. When asked to give reasons why they changed the packaging materials, all the personnel selling such drugs declined to give reasons for their action but pleaded that their drugs were genuine. Although HPLC analysis of one of the samples revealed that the tablets were indeed SP, such practices need to be discouraged since they reduce consumer confidence and precipitate sale of expired and suspicious drugs. Tipkel *et al*., (2008) in their study on standard antimalarial drugs in Burkina Faso also reported that one sample that was repackaged and claimed to be SP failed identity test since it did not have sulfadoxine and Pyrimethamine. They suspected the above tablets were metronidazole since they had the label METRO on them. Transferring one product into the packaging material for another drug may be attributed to employing under qualified human resource in drug outlets. This was also reported by Tipket *et al.*, (2008) in Bukina Faso. Therefore, NDA needs to create more public awareness on the importance of keeping drugs in their original containers; the public also needs to be sensitized on their rights to seek information on any pharmaceutical product they purchase.

Apparently, all the SP batches that were sampled passed visual inspection tests for authenticity of packaging materials except the sample that was sold in albendazole tin. This increases consumer confidence on the product. The USP drug quality information also noted that the quality of packaging provides clues on counterfeited products. No major discrepancies were observed when physical appraisals of the individual tablets in each batch were done for uniformity of shape, size, colour and texture. This may be attributed to good manufacturing practices by the pharmaceutical companies that manufactured the various generics sampled in this study. In addition, premarket quality analysis of the drugs before registration may probably account for the quality of the packaging and individual tablets sampled. USP DQI (2004) also agrees that proper registration of drugs by regulatory agencies provide some guarantee to the user on its quality, efficacy and safety.

The dissolution profile of the 10 brands analysed in this study showed that 90% of the SP batches passed the test. The bioavailability of oral dosage forms is determined by its ability to dissolve in the intestinal fluid and release the active ingredients. Whereas bioequivalence studies would be more appropriate, in-vitro dissolution tests also provide a quick alternative assessment tool for the bioavailability of fast release oral dosage forms and are directly correlated to the *in-vivo* bioavailability of the drug (Amin and Kokwaro, 2008). In a review of antimalarial drug quality in Africa, Amin and Kokwaro (2008) observed that most antimalarial solid drug products, especially those containing SP have been found to have problems of dissolution but not content of the active ingredient (Amin, Snow and Kokwaro 2005; Risha *et al.*, 2002; Kibwage and Ngugi, 2000). This is consistent with the current study which also showed that the single batch that failed the dissolution test was due to low (56.1%) amount of Pyrimethamine released

in the dissolution media. Odeniyi *et al.,* (2003) reported that this may be attributed to the nature of excipients used or the formulation process. It has been shown by Abdu (1986) that dissolution rate of a pure drug can be altered significantly when mixed with various adjuncts during the manufacturing process of solid dosage forms. This could also be due to the fact that the disintegrated particles may have retained the active drug within their hard cores and did not release the drug into the dissolution medium (Odeniyi *et al.,* 2003). Amin and Kokwaro (2008) equally noted that SP tablets have notoriously poor *in vitro* dissolution profiles, especially with regard to the Pyrimethamine component. This is mostly a problem with the generic products rather than the originator, attributed to the poor aqueous solubility of pyrimethamine occasioned by the use of poor quality raw materials or poor choice of excipients in the formulation (Risha, 2002; Kibwage and Ngugi, 2000). The clinical implications of a poor dissolution profile of SP products are not hard to fathom; the pharmacopoeia assume a good *in vitro-in vivo* correlation such that a product which failed *in vitro* dissolution will most likely fail in an *in vivo* (bioavailability) test and therefore result in a low plasma level of sulfadoxine and pyrimethamine with the attendant risks of therapeutic failure (Amin and Kokwaro, 2008). This in clinical terms will have a grave implication in pregnancy outcome in women using such substandard SP products for intermittent preventive treatment of malaria.

This study also revealed that 100% of SP brands passed Pharmacopeial acceptance range of 90-110% for both sulfadoxine and pyrimethamine in the tablets. This agrees with Amin and Kokwaro (2008) that most problems encountered in quality analysis of SP tablets are due to dissolution but not content. Overall, only 10% of the SP batches and/ or generics failed quality test in the current study. This is quite remarkable since Bate *et al.,* (2008) reported that 35% of

antimalarials sold in six major African cities, Kampala inclusive, failed at least one critical quality control test a year ago.

In Arua Municipality, NDA is a popular body amongst owners of drug outlets. Through their regular post market surveillance activities, they have been able to suppress vices that predispose to sale of poor quality medicines. Routine inspection of drug outlets for good storage and dispensing practices as well as field analysis of antimalarials using Mini-LAB Kit could have probably greatly contributed to the significant observation made in this study. However, the ban on importation of SP formulations in Uganda since the MoH announced phasing out SP and the assessment of only representative batches for brands that had more than one batches might also have contributed to the low percentage of poor quality SP in the market. Since entry of new product has been regulated, the SP products in circulation are mainly those manufactured by the local industries like Kampala Pharmaceutical Industries (1996) Limited, Uganda Pharmaceuticals (1996) Ltd and Rene Industries Ltd among others. Needless to say, NDA closely monitors the operations of the above pharmaceutical industries hence there is little room for errors in GMPs.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

CONCLUSION

- The current study revealed low level of substandard SP tablets sold in Arua Municipality although cases of drugs that failed authentification against the national human drug register was encountered.

RECOMMENDATION

- There is need for further study on SP that failed dissolution test by the NDA for appropriate action.

- Further investigation is required on the five SP products that failed authentication for registration status in Uganda using the human drug register (September, 2008/09).

- Future studies should focus on the quality of SP in Pharmacies and established government health facilities and drug outlets that are away from the Municipality.

- The routine post market quality surveillance of drugs in the country needs to be maintained in order to continually assure the public on the effectiveness and safety of antimalarial drugs.

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APPENDICES

Appendix I: Drug (SP) sampling form

Name of company/ enterprise (selected) . . . . ………………….

Address/ Code . . . . …………...

Date of sampling . . . . ………………………………………….............................

Trade name of the drug…. . . . . …………………...

Dosage form . . . . ……………………………………………………………...

Batch no. . . . . ………………………………………………………………

Date of manufacture… . . . . ……………………………………………..

Date of expiry………………………………………………………………………………..

No. of samples taken (tins, packets, etc.) . . . .

………………………….   …………………..

Name of Sampler   Sign

Appendix II: Protocol for identification of SP14

Mobile phases: Phase A: Acetonitrile; Phase B: Phosphoric acid, **Ratio of mobile phase**: 55 (B): 45 (A)

Chromatographic Conditions

Temperature:40^{o}C, **Retention time**: 6 minutes (Sulfadoxine 2.3 and Pyrimethamine 4) **Detection wavelength**: 230nm, **Column type**: Water Spherisorb, ODS, 250x4.6mm (5µm particle size), **Pressure**: 78 bars, Injection volume = 20µl, Run time =10 minutes.

Preparation of B Phase for the mobile phase

Carefully measure 10 mls of 85% Orthophosphoric acid by use of pipette & dilute to 1560 mls with distilled water.

A phase: Acetonitrile HPLC grade

Preparation of standard solution

Accurately weigh **0.1000g** of **Sulfadoxine** chemical reference substance into a **50 ml volumetric** flask. Dissolve with **Methanol** & make to the mark at 20oC (**Solution 1**).

Accurately weigh **0.0100g** of **Pyrimethamine** & put into a **50 ml volumetric flask**. Dissolve with **methanol** & make to the mark at 20^{o}C (**Solution 2**).

Pipette 2.0 ml of solution 1 and 1.0 ml of solution 2 into a 250 ml volumetric flask. Add mobile phase up to the mark and shake. Sonicate for 2 minutes.

Create a sequence run and inject 20µl of resulting solution in the SHIMADZU HPLC and chromatograph six 2 replicates.

Identification

Peaks of Sulfadoxine and Pyrimethamine in sample solution must have the same retention times as the corresponding peaks in standard solution. Sulfadoxine =2.7 minutes, Pyrimethamine =8.6 minutes

Appendix III: Weight variation of individual tablets in each batch of SP generics

	Weight of individual tablets (g)									
Tab. No.	**SP06**	**SP03**	**SP07**	**SP011**	**SP10**	**SP04**	**SP01**	**SP012**	**SP08**	**SP15**
1	0.6354	0.6469	0.6001	0.6814	0.6443	0.6131	0.6298	0.6261	0.6866	0.6389
2	0.6254	0.6493	0.5813	0.6496	0.6452	0.6189	0.6338	0.6265	0.6692	0.6362
3	0.6267	0.6467	0.6248	0.6897	0.6476	0.6245	0.613	0.6313	0.6651	0.6355
4	0.6231	0.6457	0.6513	0.6591	0.6461	0.6161	0.6566	0.6227	0.6629	0.637
5	0.626	0.6413	0.6374	0.669	0.6461	0.6071	0.6284	0.6018	0.6712	0.642
6	0.6208	0.6473	0.5603	0.6627	0.6439	0.6089	0.6238	0.6288	0.6778	0.6329
7	0.6349	0.6456	0.6292	0.6829	0.6469	0.5989	0.6135	0.6127	0.6682	0.6307
8	0.6238	0.649	0.6304	0.666	0.6439	0.6102	0.6423	0.6114	0.6938	0.6295
9	0.6237	0.6462	0.6092	0.6847	0.6441	0.6135	0.6003	0.6195	0.6923	0.6257
10	0.6279	0.6396	0.6051	0.6597	0.645	0.6096	0.6295	0.6226	0.6809	0.6362
11	0.6163	0.6415	0.6216	0.6579	0.6453	0.6217	0.6375	0.6343	0.6829	0.6342
12	0.6258	0.6269	0.6127	0.6537	0.6449	0.6139	0.6268	0.6399	0.6776	0.637
13	0.6266	0.647	0.6272	0.6688	0.6243	0.6137	0.6337	0.6126	0.673	0.6263
14	0.6345	0.6444	0.6223	0.6543	0.644	0.6194	0.6182	0.6214	0.7031	0.64
15	0.6285	0.6322	0.6672	0.6535	0.6437	0.6059	0.6252	0.6197	0.6676	0.6274
16	0.6236	0.6457	0.6445	0.6754	0.6443	0.6124	0.6234	0.6324	0.6651	0.6347
17	0.6271	0.664	0.6243	0.6574	0.6464	0.615	0.6451	0.6263	0.667	0.637
18	0.6122	0.6421	0.6348	0.66629	0.6451	0.5991	0.6501	0.6216	0.6686	0.6388
19	0.6274	0.6477	0.6128	0.6764	0.6443	0.6186	0.6325	0.6194	0.672	0.6339
20	0.6208	0.6446	0.6109	0.6619	0.645	0.6151	0.6146	0.6226	0.6707	0.6337
Total wt	12.5105	12.8937	12.4074	13.33039	12.8804	12.2556	12.5781	12.4536	13.5156	12.6876
Av. wt	0.625525	0.644685	0.62037	0.66652	0.64402	0.61278	0.628905	0.62268	0.67578	0.63438

Appendix IV: Weight variation of individual tablets in each batch of SP generics

	Weight of individual tablets (g)								
Tab. No.	**SP09**	**SP18**	**SP16**	**SP17**	**SP13**	**SP14**	**SP02**	**SP05**	**SP19**
1	0.625	0.6787	0.6551	0.6136	0.629	0.6799	0.6263	0.6415	0.6248
2	0.6244	0.6637	0.6324	0.5988	0.6279	0.6723	0.6127	0.6445	0.6031
3	0.6191	0.6604	0.6376	0.6147	0.6295	0.6644	0.6212	0.6398	0.6757
4	0.622	0.673	0.6311	0.6132	0.64	0.6732	0.6088	0.6413	0.6382
5	0.6203	0.6713	0.6364	0.6264	0.6298	0.6854	0.6268	0.6405	0.6217
6	0.6227	0.6469	0.6409	0.5879	0.6245	0.6633	0.6107	0.6398	0.6255
7	0.6195	0.6738	0.6305	0.6046	0.6296	0.675	0.6167	0.6338	0.6188
8	0.6278	0.6568	0.631	0.6169	0.6234	0.6712	0.6023	0.6384	0.6421
9	0.6189	0.6726	0.6353	0.5986	0.6289	0.6904	0.6132	0.6426	0.6325
10	0.6291	0.6814	0.6213	0.6063	0.6278	0.6684	0.6215	0.6385	0.6829
11	0.6196	0.6833	0.6387	0.5974	0.6292	0.671	0.6107	0.6394	0.6402
12	0.6295	0.6662	0.6348	0.5979	0.6261	0.6855	0.6256	0.6452	0.6203
13	0.6274	0.6751	0.6454	0.6298	0.6232	0.6675	0.637	0.6372	0.6267
14	0.6367	0.6802	0.6484	0.614	0.6306	0.6772	0.624	0.6392	0.6461
15	0.6222	0.6794	0.7143	0.6274	0.6248	0.6744	0.6101	0.6375	0.6252
16	0.6219	0.6782	0.6244	0.6289	0.6296	0.6932	0.6214	0.6659	0.6145
17	0.6182	0.6951	0.6472	0.6087	0.6295	0.6709	0.6242	0.6402	0.6373
18	0.6278	0.6709	0.6332	0.5949	0.6297	0.6953	0.62	0.6406	0.6597
19	0.6263	0.6743	0.635	0.612	0.6222	0.6798	0.608	0.6399	0.6352
20	0.6309	0.693	0.6302	0.6072	0.6282	0.67	0.6187	0.6391	0.6376
Total	12.4893	13.4743	12.8032	12.1992	12.5635	13.5283	12.3599	12.8249	12.7081
Av. wt	0.624465	0.673715	0.64016	0.60996	0.628175	0.676415	0.617995	0.641245	0.635405

Appendix V: Tools for Visual Inspection of Medicine

A checklist for visual inspection of medicines in order to identify suspicious products for further examination.

This document has been produced by the International Council of Nurses in partnership with the United States Pharmacopoeia (USP) and modified by the Military and Emergency Pharmacists Section of FIP. The tool is designed to help health professionals carry out a visual inspection of medicines for signs of counterfeiting such as improper packaging, labelling or description of dosage. All suspicious products with incorrect labels, missing information about the strength, dosage, or expiration date should be reported to the appropriate national authority or to the WHO or FIP.

1. PACKAGING

Any medicine should be packaged in a container, which can be anything from a glass bottle to a blister pack, to a tube of glass, plastic or metal. A folding carton bearing the label very often protects the container. Check the type of packaging and compare it to known containers for the same product from the same manufacturer. The packaging and the labelling of pharmaceutical products is a very complex and an expensive business. Thus, the process and the quality of packaging material are difficult to counterfeit. This is why a thorough visual inspection could be an important screening step for product quality control. However, producers of counterfeit products are quick to copy special labelling and holograms

	Yes	No	Other Observations
1.1 Container and Closure			
Does the container and closure protect the product from the outside environment; e.g. is the container properly sealed?			
Do they assure that the product will meet the proper specifications throughout its shelf life?			
Are the container and the closure appropriate for the product inside?			
Is the container safely sealed?			

1.2 Label The information written on the label is very important. The information can be printed on a label adhered to the container, or printed directly onto the container itself, but all information must be legible and indelible.			
If there is a carton protecting the container, does the label on the carton match the label on the container?			
Is all information on the label legible and indelible?			

1.2.1 The trade (brand) name	Yes	No	Other Observations
Is the trade name spelled correctly?			
Is the medicinal product (trade name) registered in the country by the Drug Regulatory Authority)? Is the product legally sold in the country?			
Does the symbol ® follow the trade name?			
For blister or foil strip packed products, is the trade name indelibly impressed or imprinted onto the strip?			
1.2.2 The active ingredient name (scientific name/generic name):			
Is the active ingredient name spelt correctly?			
Do the trade name and the active ingredient names correspond to the registered product?			
1.2.3 The manufacturer's name and logo:			
Are the manufacturer's name and logo legible and correct?			
Does the logo or hologram (if applicable) loo authentic?			
Does the logo or hologram (if applicable) change colour when viewed from different angles?			
1.2.4 The manufacturer's full address: All manufacturers are required by international law to print their complete address on the label. Many companies making substandard or counterfeit products do not have a traceable address on the label.			
Is the manufacturer's full address legible and correct?			
Has this company or its agent registered the product in the country?			
1.2.5 The medicine strength (mg/unit):			
Is the strength - the amount of active ingredient per unit - clearly stated on the label?			
For blister or foil strip packed products, is the medicine strength indelibly impressed or imprinted onto the strip?			
1.2.6 The dosage form (e.g., tablet/capsule):			
Is the dosage form clearly indicated on the container label?			
Does the dosage form stated on the label match the actual dosage form of the medication?			
Is the indicated medicine under this dosage form registered and authorised for sale in the country?			
1.2.7 The number of units per container:			
Does the number of dosage units listed on the label match the number of dosage units stated on the container?			

1.2.8 Dosage statement (if appropriate)	Yes	No	Other Observations
Is the dosage clearly indicated on the label?			
Is the dosage stated on the label appropriate for the medicine in this form and strength?			
Is the product registered and authorised for sale in the country with this dosage?			
1.2.9 The batch (or lot) number: Medicines with the same batch/lot number are expected to be equivalent. In a continuous process, a batch corresponds to a defined portion of the production, based on time or quantity. Products from the same batch number should have the same history of manufacturing, processing, packing, and coding. All product quality control testing should be based on batch/lot numbers.			
Does the numbering system on the package correspond to that of the producing company?			
For blister or foil strip packed medicines, is the batch number indelibly impressed or imprinted onto the strip?			
1.2.10 The date of manufacture and the expiry date: An expired product should not be sold under any circumstances.			
Are the manufacture and expiry dates clearly indicated on the label?			
For blister or foil strip packed products, is the expiry date indelibly impressed or imprinted onto the strip?			
1.2.11 Storage information:			
Are the storage conditions indicated on the label?			
Has the product been properly stored?			

1.3 Leaflet or package insert: All product packs contain a leaflet explaining dosage, the medicine content, the adverse affects, the medicine's actions, and how the medicine should be taken. The only exceptions are where the packaging includes all the information that would otherwise be in the leaflet			
Is the package insert printed on the same coloured or same quality paper as the original (If available to compare) or does it look familiar?			
Is the ink on the package insert or packaging smudge-proof?			
Does the information on the package insert match the information on the product container?			

2. PHYSICAL CHARACTERISTICS OF TABLETS/CAPSULES

All types of medicines can be and have been counterfeited from cough syrups to injections. As mentioned above, it is important to check the packaging of these medicines. Additionally, tablets or capsules can be checked for signs of moisture, dirty marks, abrasion erosion, cracks, or any other adulteration.			
2.1 Uniformity of Shape:	**Yes**	**No**	**Other Observations**
Are the tablets/capsules uniform in shape?			
2.2 Uniformity of Size:			
Are the tablets/capsules uniform in size?			
2.3 Uniformity of Colour:			
Are the tablets/capsules uniform in colour?			
2.4 Uniformity of Texture: Tablets can be film-coated, sugar-coated or enteric-coated.			
Do the tablets have a uniform coating?			
Is the base of the tablets fully covered?			
Are the tablets uniformly polished, free of powder, and non-sticking?			
2.5 Markings (scoring, letters, etc):			
Are markings uniform and identical?			
Does the logo (if present) match that of the manufacturing company?			
2.6 Breaks, Cracks and Splits:			
Are the tablets/capsules free of breaks, cracks, splits or pinholes?			
2.7 Embedded surface spots or contamination:			
Are the tablets/capsules free of embedded surface spots and foreign particle contamination?			
2.8 Presence of empty capsules in the case of a sample of capsules:			
Is the sample examined free of empty capsules?			
2.9 Smell			
Does the medicine smell the same as the original (If available)? Does it smell peculiar?			

Reporting Counterfeit Medicines

If, after carrying out the above visual inspection, you suspect you have discovered a counterfeit medicine, you should report this immediately to your local health authority. Alternatively, you can contact WHO's Department of Quality Assurance and Safety of Medicines (QSM):

http://www.who.int/medicines/organization/qsm/activities/qualityassurance/cft/CounterfeitReporting.htm

or to FIP. A simple reporting card can be found on the FIP website:

http://www.fip.org/counterfeitmedicines.com

Appendix VI: Dissolution calculation sheets for the various generics of SP tablets

Sample ID: SP10	Batch No30758	
TEST	**DISSOLUTION**	**DATE:07/08/09**
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN	500.5mg	25.30mg
% PURITY	99.46	99.97
No. of HPLC injection	***Standard area (SULFADOXINE)***	***Standard area (PYRIMETHAMINE)***
1	17216749	815950
2	17214686	817853
3	17250003	814742
4	17285167	819244
5	17316629	819030
Average	**17256646.8**	**817363.8**
STD.DEV	**44181.30**	**1963.63**
%RSD	**0.2560**	**0.2402**
SULFADOXINE		
No. of HPLC injection	***Sample Area***	***% DISSOLUTION***
1	16744693	96.61
2	16756081	96.67
3	17539520	101.19
4	17002144	98.09
5	16467466	95.01
6	16437607	94.83
PYRIMETHAMINE		
No. of HPLC injection	***Sample Area***	***%DISSOLUTION***
1	694859	86.01
2	770692	95.39
3	765202	94.71
4	782799	96.89
5	678735	84.01
6	739772	91.57

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP13	**BATCH NUMBER:**	**ME17506**	**DATE:07/09/2009**
TEST:		**DISSOLUTION**	
STANDARD		***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN		500.5mg	25.30mg
% PURITY		99.46	99.97
No. of HPLC injection		***Standard area (SULFADOXINE)***	***Standard area (PYRIMETHAMINE)***
1		17216749	815950
2		17214686	817853
3		17250003	814742
4		17285167	819244
5		17316629	819030
AVERAGE		**17256646.8**	**817363.8**
STD.DEV		**44181.30**	**1963.63**
%RSD		**0.2560**	**0.2402**

SULFADOXINE

No. of HPLC injection	***Sample Area***	***DISSOLUTION***
1	15646040	90.27
2	15621419	90.13
3	15697744	90.57
4	15701009	90.58
5	15736294	90.79
6	15780487	91.04

PYRIMETHAMINE

No. of HPLC injection	***Sample Area***	**%DISSOLUTION**
1	665199	82.34
2	732830	90.71
3	742195	91.87
4	760833	94.17
5	754722	93.42
6	744687	92.17

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP12:	**BATCH No.**	**DATE:07/09/2009**
TEST:	**DISSOLUTION**	
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN	500.5mg	25.30mg
% PURITY	99.46	99.97
No. of HPLC injection	***Standard area* (SULFADOXINE)**	***Standard area* (PYRIMETHAMINE)**
1	17216749	815950
2	17214686	817853
3	17250003	814742
4	17285167	819244
5	17316629	819030
AVERAGE	17256646.8	817363.8
STD.DEV	44181.30	1963.63
%RSD	0.2560	0.2402
	SULFADOXINE	
No. of HPLC injection	***Sample Area***	**% DISSOLUTION**
1	16384215	94.53
2	15964005	92.10
3	16089058	92.82
4	15793596	91.12
5	15806391	91.19
6	16284417	93.95
	PYRIMETHAMINE	
No. of HPLC injection	***Sample Area***	**%DISSOLUTION**
1	571062	70.68
2	572175	70.82
3	598857	74.12
4	544936	67.45
5	563685	69.77
6	612419	75.80

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP11:	**Batch No.165**	**DATE:07/09/2009**
TEST:	**DISSOLUTION**	
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN	500.5mg	25.30mg
% PURITY	99.46	99.97
SERIALNO	***Standard area* (SULFADOXINE)**	***Standard area* (PYRIMETHAMINE)**
1	17216749	815950
2	17214686	817853
3	17250003	814742
4	17285167	819244
5	17316629	819030
AVERAGE	**17256646.8**	**817363.8**
STD.DEV	**44181.30**	**1963.63**
%RSD	**0.2560**	**0.2402**
	SULFADOXINE	
No. of HPLC injection	***Sample Area***	***% DISSOLUTION***
1	17343807	100.06
2	17548131	101.24
3	17155050	98.97
4	17486435	100.89
5	17219761	99.35
6	17070443	98.49
	PYRIMETHAMINE	
No. of HPLC injection	***Sample Area***	***%DISSOLUTION***
1	638690	79.05
2	637590	78.92
3	580489	71.85
4	635322	78.64
5	627386	77.66
6	602987	74.64

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP04	**Batch No. 6E64**	**Date:07/09/2009**
TEST:	**DISSOLUTION**	
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN	500.0mg	25.00mg
% PURITY	99.46	99.97
SERIALNO	***Standard area (SULFADOXINE)***	***Standard area (PYRIMETHAMINE)***
1	17767856	806208
2	17848658	805543
3	17787579	805336
4	17863077	809949
5	17955051	809752
AVERAGE	**17844444.2**	**807357.6**
STD.DEV	**73651.55**	**2299.44**
%RSD	**0.4127**	**0.2848**
	SULFADOXINE	
No. of HPLC injection	***Sample Area***	***% DISSOLUTION***
1	16847158	93.90
2	17014634	94.83
3	16459612	91.74
4	16416792	91.50
5	16794268	93.61
6	17164411	95.67
	PYRIMETHAMINE	
No. of HPLC injection	***Sample Area***	%DISSOLUTION
1	710809	88.01
2	765192	94.75
3	693562	85.88
4	685376	84.87
5	753321	93.28
6	780618	96.66

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP05	**Batch No. 051042**	**Date:07/09/2009**
TEST:	**DISSOLUTION**	
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN	500.0mg	25.00mg
% PURITY	99.46	99.97
No. of HPLC injection	***Standard area (SULFADOXINE)***	***Standard area (PYRIMETHAMINE)***
1	17767856	806208
2	17848658	805543
3	17787579	805336
4	17863077	809949
5	17955051	809752
AVERAGE	**17844444.2**	**807357.6**
STD.DEV	**73651.55**	**2299.44**
%RSD	**0.4127**	**0.2848**
	SULFADOXINE	
No. of HPLC injection	***Sample Area***	**% DISSOLUTION**
1	17703456	98.67
2	17362776	96.78
3	17170184	95.70
4	17405811	97.02
5	17566117	97.91
6	17637140	98.30
	PYRIMETHAMINE	
No. of HPLC injection	***Sample Area***	*%DISSOLUTION*
1	613720	75.99
2	632491	78.32
3	631135	78.15
4	655831	81.21
5	618515	76.59
6	645169	79.89

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP08:	**Batch No.1308**	**Date: 07/09/2009**
TEST:	**DISSOLUTION**	
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN	500.0mg	25.00mg
% PURITY	99.46	99.97
No. of HPLC injection	***Standard area (SULFADOXINE)***	***Standard area (PYRIMETHAMINE)***
1	17767856	806208
2	17848658	805543
3	17787579	805336
4	17863077	809949
5	17955051	809752
AVERAGE	**17844444.2**	**807357.6**
STD.DEV	**73651.55**	**2299.44**
%RSD	**0.4127**	**0.2848**
	SULFADOXINE	
No. of HPLC injection	***Sample Area***	***% DISSOLUTION***
1	16473930	91.82
2	15567630	86.77
3	15358555	85.60
4	15445804	86.09
5	15260737	85.06
6	15557751	86.71
	PYRIMETHAMINE	
No. of HPLC injection	***Sample Area***	***%DISSOLUTION***
1	652627	80.81
2	594135	73.57
3	621974	77.02
4	589689	73.02
5	641602	79.45
6	632524	78.32

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP15	**Batch No. T7258**	**Date: 07/09/2009**
TEST:	**DISSOLUTION**	
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN	500.0mg	25.00mg
% PURITY	99.46	99.97
No. of HPLC injection	***Standard area (SULFADOXINE)***	***Standard area (PYRIMETHAMINE)***
1	17767856	806208
2	17848658	805543
3	17787579	805336
4	17863077	809949
5	17955051	809752
AVERAGE	**17844444.2**	**807357.6**
STD.DEV	**73651.55**	**2299.44**
%RSD	**0.4127**	**0.2848**
	SULFADOXINE	
No. of HPLC injection	***Sample Area***	***% DISSOLUTION***
1	14366113	80.07
2	15612054	87.02
3	15762193	87.85
4	15628935	87.11
5	15644000	87.20
6	15686170	87.43
	PYRIMETHAMINE	
No. of HPLC injection	***Sample Area***	*%DISSOLUTION*
1	405354	50.19
2	466439	57.76
3	469591	58.15
4	444380	55.02
5	466163	57.72
6	465850	57.68

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP18:	**Batch No.0408**	**Date: 07/09/2009**
TEST:	**DISSOLUTION**	
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***
STD WT TAKEN	500.0mg	25.00mg
% PURITY	99.46	99.97
No. of HPLC injection	***Standard area* (SULFADOXINE)**	***Standard area* (PYRIMETHAMINE)**
1	17767856	806208
2	17848658	805543
3	17787579	805336
4	17863077	809949
5	17955051	809752
AVERAGE	**17844444.2**	**807357.6**
STD.DEV	**73651.55**	**2299.44**
%RSD	**0.4127**	**0.2848**
	SULFADOXINE	
No. of HPLC injection	***Sample Area***	**% DISSOLUTION**
1	15482033	86.29
2	15605180	86.98
3	15748067	87.78
4	15510471	86.45
5	15389089	85.77
6	15644880	87.20
	PYRIMETHAMINE	
No. of HPLC injection	***Sample Area***	%DISSOLUTION
1	680715	84.29
2	760887	94.22
3	783516	97.02
4	706766	87.51
5	754533	93.43
6	779978	96.58

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Sample ID: SP10	**Batch No.30758**	**Date: 07/09/2009**
TEST:	**DISSOLUTION**	
STANDARD	**SULFADOXINE**	**PYRIMETHAMINE**
STD WT TAKEN	500.0mg	25.00mg
% PURITY	99.46	99.97
No. of HPLC injection	***Standard area (SULFADOXINE)***	***Standard area (PYRIMETHAMINE)***
1	17767856	806208
2	17848658	805543
3	17787579	805336
4	17863077	809949
5	17955051	809752
AVERAGE	17844444.2	807357.6
STD.DEV	73651.55	2299.44
%RSD	0.4127	0.2848
	SULFADOXINE	
No. of HPLC injection	***Sample Area***	***% DISSOLUTION***
1	17207736	95.91
2	17086250	95.23
3	17007437	94.79
4	17039137	94.97
5	16822834	93.77
6	17012900	94.83
	PYRIMETHAMINE	
No. of HPLC injection	***Sample Area***	%DISSOLUTION
1	677950	83.95
2	762505	94.42
3	776101	96.10
4	780887	96.69
5	714937	88.53
6	770864	95.45

%Dissolution (SULFADOXINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 500 \times 100}$

%Dissolution (PYRIMETHAMINE) = $\frac{TA \times Ws \times 10 \times 1000 \times P \times 100}{SA \times 100 \times 100 \times 25 \times 100}$

Appendix VII: Calculation data sheet for content assay for various SP generics

Sample ID:	**SP 10**	**BATCH No. 30758**	
TEST:	**Content Assay**		
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	645.00mg		
AV. WT OF TABS	645.14mg		
SERIAL NO:	***AREA (SULFADOXINE) STANDARD***	***SERIAL NO***	***AREA (SULFADOXINE) SAMPLE***
1	18970965	1	19104277
2	18954880	2	19078759
3	18682052	3	19157841
4	18572959	**AVERAGE**	**19113625.67**
5	18499865	**STD DEV**	**32954.91**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.1724**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		
% ASSAY =	$\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 500 x 100}}$		
% ASSAY =	**101.53%**		
SERIAL NO	***AREA (PYRIMETHAMINE) STANDARD***	***SERIAL NO***	***AREA (PYRIMETHAMINE) SAMPLE***
1	834842	1	810431
2	835674	2	811597
3	831960	3	815685
4	837435	**AVERAGE**	**812571**
5	837612	**STD DEV**	**2252.80**
AVERAGE	**835504.6**	**% R.S.D.**	**0.2772**
STD.DEV	**2302.15**		
R.S.D	**0.2755**		
% ASSAY =	$\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 25 x 100}}$ = **97.25%**		

Sample ID:	**SP 11**	**BATCH No. 165**	
TEST:	**Content Assay**		
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	677.10mg		
AV. WT OF TABS	676.85mg		
SERIAL NO:	***AREA (SULFADOXINE) STANDARD***	***SERIAL NO***	***AREA (SULFADOXINE) SAMPLE***
1	18970965	1	19291216
2	18954880	2	19305188
3	18682052	3	19301607
4	18572959	**AVERAGE**	**19299337.00**
5	18499865	**STD DEV**	**5925.59**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.0307**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		
% ASSAY =	TA x Ws x Av. Wt. x P x 100 / SA x WT x 500 x 100		
% ASSAY =	**102.45%**		
SERIAL NO	**AREA (PYRIMETHAMINE) STANDARD**	**SERIAL NO**	**AREA (PYRIMETHAMINE) SAMPLE**
1	834842	1	773372
2	835674	2	769609
3	831960	3	774074
4	837435	**AVERAGE**	**772351.6667**
5	837612	**STD DEV**	**1960.42**
AVERAGE	**835504.6**	**% R.S.D.**	**0.2538**
STD.DEV	**2302.15**		
R.S.D	**0.2755**		
% ASSAY =	TA x Ws x Av. Wt. x P x 100 / SA x WT x 25 x 100		
% ASSAY =	**92.38%**		

Sample ID:	**SP 18**	**BATCH No. 0408**	
TEST:	**Content Assay**		
STANDARD	**SULFADOXINE**	**PYRIMETHAMINE**	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	673.90mg		
AV. WT OF TABS	673.90mg		
SERIAL NO:	***AREA (SULFADOXINE) STANDARD***	***SERIAL NO***	***AREA (SULFADOXINE) SAMPLE***
1	18970965	1	18674503
2	18954880	2	18658776
3	18682052	3	18676949
4	18572959	**AVERAGE**	**18670076.00**
5	18499865	**STD DEV**	**8052.46**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.0431**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		
% ASSAY =	TA x Ws x Av. Wt. x P x 100 / SA x WT x 500 x 100		
% ASSAY =	**99.15%**		
SERIAL NO	***AREA (PYRIMETHAMINE) STANDARD***	***SERIAL NO***	***AREA (PYRIMETHAMINE) SAMPLE***
1	834842	1	826140
2	835674	2	820640
3	831960	3	821192
4	837435	**AVERAGE**	**822657.3333**
5	837612	**STD DEV**	**2472.91**
AVERAGE	835504.6	**% R.S.D.**	**0.3006**
STD.DEV	2302.15		
R.S.D	0.2755		
% ASSAY =	TA x Ws x Av. Wt. x P x 100 / SA x WT x 25 x 100		
% ASSAY =	**98.43%**		

Sample ID: SP 12

TEST:	**Content Assay**	**BATCH No. SP908**
STANDARD	**SULFADOXINE**	**PYRIMETHAMINE**
STD WT TAKEN	500.2mg	25.00mg
% PURITY	99.46%	99.97%
WT OFSPL	614.00mg	
AV. WT OF TABS	614.80mg	

SERIAL NO:	***AREA (SULFADOXINE) STANDARD***	***SERIAL NO***	***AREA (SULFADOXINE) SAMPLE***
1	18970965	1	18674845
2	18954880	2	18509897
3	18682052	3	18571445
4	18572959	**AVERAGE**	**18585395.67**
5	18499865	**STD DEV**	**68058.44**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.3662**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		

% ASSAY = $\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 500 x 100}}$

% ASSAY = 98.83%

SERIAL NO	***AREA (PYRIMETHAMINE) STANDARD***	***SERIAL NO***	***AREA (PYRIMETHAMINE) SAMPLE***
1	834842	1	863033
2	835674	2	856757
3	831960	3	860892
4	837435	**AVERAGE**	**860227.3333**
5	837612	**STD DEV**	**2604.92**
AVERAGE	835504.6	**% R.S.D.**	**0.3028**
STD.DEV	2302.15		
R.S.D	0.2755		

% ASSAY = $\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 25 x 100}}$

% ASSAY = 103.06%

Sample ID:	**SP 04**	**BATCH No.6E64**	
TEST:	**Content Assay**		
STANDARD	**SULFADOXINE**	**PYRIMETHAMINE**	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	615.60mg		
AV. WT OF TABS	615.30mg		
SERIAL NO:	**AREA (SULFADOXINE) STANDARD**	**SERIAL NO**	**AREA (SULFADOXINE) SAMPLE**
1	18970965	1	17826283
2	18954880	2	17827374
3	18682052	3	17793417
4	18572959	**AVERAGE**	**17815691.33**
5	18499865	**STD DEV**	**15756.63**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.0884**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		
% ASSAY =	TA x Ws x Av. Wt. x P x 100 / SA x WT x 500 x 100		
% ASSAY =	**94.57%**		
SERIAL NO	**AREA (PYRIMETHAMINE) STANDARD**	**SERIAL NO**	**AREA (PYRIMETHAMINE) SAMPLE**
1	834842	1	852746
2	835674	2	839638
3	831960	3	845302
4	837435	**AVERAGE**	**845895.3333**
5	837612	**STD DEV**	**5367.74**
AVERAGE	835504.6	**% R.S.D.**	**0.6346**
STD.DEV	2302.15		
R.S.D	0.2755		
% ASSAY =	TA x Ws x Av. Wt. x P x 100 / SA x WT x 25 x 100		
% ASSAY =	**101.16%**		

Sample ID:	**SP 01**	**BATCH No. 00308**	
TEST:	**Content Assay**		
STANDARD	**SULFADOXINE**	**PYRIMETHAMINE**	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	620.50mg		
AV. WT OF TABS	620.70mg		

SERIAL NO:	**AREA (SULFADOXINE)**	**SERIAL NO**	**AREA (SULFADOXINE)**
	STANDARD		**SAMPLE**
1	18970965	1	17315162
2	18954880	2	17283355
3	18682052	3	17339929
4	18572959	**AVERAGE**	**17312815.33**
5	18499865	**STD DEV**	**23155.77**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.1337**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		

% ASSAY = $\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 500 x 100}}$

% ASSAY = 91.97%

SERIAL NO	**AREA (PYRIMETHAMINE) STANDARD**	**SERIAL NO**	**AREA (PYRIMETHAMINE) SAMPLE**
1	834842	1	820384
2	835674	2	818309
3	831960	3	818293
4	837435	**AVERAGE**	818995.3333
5	837612	**STD DEV**	981.96
AVERAGE	835504.6	**% R.S.D.**	0.1199
STD.DEV	2302.15		
R.S.D	0.2755		

% ASSAY = $\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 25 x 100}}$

% ASSAY = 98.03%

Sample ID:	**SP 08**	**BATCH No. 1308**	
TEST:	**Content Assay**		
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	669.60mg		
AV. WT OF TABS	669.90mg		

SERIAL NO:	***AREA (SULFADOXINE)***	***SERIAL NO***	***AREA (SULFADOXINE)***
	STANDARD		**SAMPLE**
1	18970965	1	17591539
2	18954880	2	17671011
3	18682052	3	18906035
4	18572959	**AVERAGE**	**18056195.00**
5	18499865	**STD DEV**	**601802.83**
AVERAGE:	**18736144.2**	**% R.S.D.**	**3.3329**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		

% ASSAY = $\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 500 x 100}}$

% ASSAY = 95.93%

SERIAL NO	***AREA (PYRIMETHAMINE)*** **STANDARD**	***SERIAL NO***	***AREA (PYRIMETHAMINE)*** **SAMPLE**
1	834842	1	791037
2	835674	2	791211
3	831960	3	834131
4	837435	**AVERAGE**	**805459.6667**
5	837612	**STD DEV**	**20273.82**
AVERAGE	**835504.6**	**% R.S.D.**	**2.5170**
STD.DEV	**2302.15**		
R.S.D	0.2755		

% ASSAY = $\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 25 x 100}}$

% ASSAY = 96.42%

Sample ID:	**SP 13**	**Batch No.ME17506**	
TEST:	**Content Assay**		
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	626.20mg		
AV. WT OF TABS	626.55mg		

SERIAL NO:	***AREA (SULFADOXINE)***	***SERIAL NO***	***AREA (SULFADOXINE)***
	STANDARD		**SAMPLE**
1	18970965	1	17439844
2	18954880	2	17504388
3	18682052	3	17497181
4	18572959	**AVERAGE**	**17480471.00**
5	18499865	**STD DEV**	**28877.90**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.1652**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		

% ASSAY = $\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 500 x 100}}$

% ASSAY = 92.88%

SERIAL NO	***AREA (PYRIMETHAMINE)***	***SERIAL NO***	***AREA (PYRIMETHAMINE)***
	STANDARD		**SAMPLE**
1	834842	1	760192
2	835674	2	759192
3	831960	3	761031
4	837435	**AVERAGE**	**760138.3333**
5	837612	**STD DEV**	**751.73**
AVERAGE	835504.6	**% R.S.D.**	**0.0989**
STD.DEV	2302.15		
R.S.D	0.2755		

% ASSAY = $\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 25 x 100}}$

% ASSAY = 91.00%

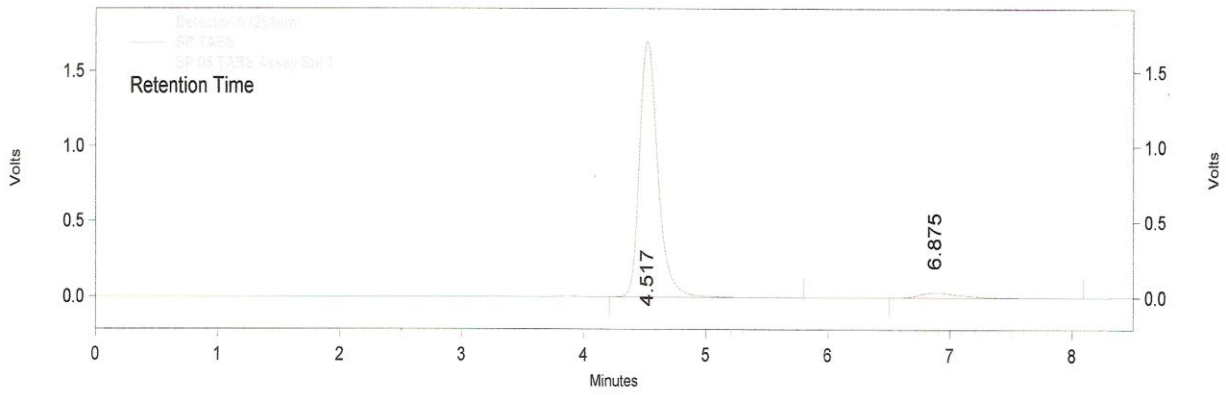
NAME OF PRODUCT:	**SP 05**	**BATCH No. 051042**	
TEST:	**Content Assay**		
STANDARD	***SULFADOXINE***	***PYRIMETHAMINE***	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	642.50mg		
AV. WT OF TABS	642.65mg		
SERIAL NO:	***AREA (SULFADOXINE)*** **STANDARD**	***SERIAL NO***	***AREA (SULFADOXINE)*** **SAMPLE**
1	18970965	1	17827685
2	18954880	2	17879382
3	18682052	3	17898481
4	18572959	**AVERAGE**	**17868516.00**
5	18499865	**STD DEV**	**29906.20**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.1674**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		
% ASSAY =	$\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 500 x 100}}$		
% ASSAY =	**94.91%**		
SERIAL NO	***AREA (PYRIMETHAMINE)*** **STANDARD**	***SERIAL NO***	***AREA (PYRIMETHAMINE)*** **SAMPLE**
1	834842	1	796269
2	835674	2	808932
3	831960	3	800747
4	837435	**AVERAGE**	**801982.6667**
5	837612	**STD DEV**	**5242.97**
AVERAGE	835504.6	**% R.S.D.**	**0.6538**
STD.DEV	2302.15		
R.S.D	0.2755		
% ASSAY =	$\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 25 x 100}}$		
% ASSAY =	**95.98%**		

Sample ID:	**SP 15**	**BATCH No.T7258**	
TEST:	**Content Assay**		
STANDARD	**SULFADOXINE**	**PYRIMETHAMINE**	
STD WT TAKEN	500.2mg	25.00mg	
% PURITY	99.46%	99.97%	
WT OFSPL	633.10mg		
AV. WT OF TABS	633.00mg		
SERIAL NO:	***AREA (SULFADOXINE) STANDARD***	***SERIAL NO***	***AREA (SULFADOXINE) SAMPLE***
1	18970965	1	17735331
2	18954880	2	17729710
3	18682052	3	17769083
4	18572959	**AVERAGE**	**17744708.00**
5	18499865	**STD DEV**	**17387.82**
AVERAGE:	**18736144.2**	**% R.S.D.**	**0.0980**
STD.DEV:	**194097.60**		
% R.S.D	**1.0360**		
% ASSAY =	$\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 500 x 100}}$		
% ASSAY =	**94.22%**		
SERIAL NO	***AREA (PYRIMETHAMINE) STANDARD***	***SERIAL NO***	***AREA (PYRIMETHAMINE) SAMPLE***
1	834842	1	852666
2	835674	2	856469
3	831960	3	850114
4	837435	**AVERAGE**	**853083**
5	837612	**STD DEV**	**2611.12**
AVERAGE	835504.6	**% R.S.D.**	**0.3061**
STD.DEV	2302.15		
R.S.D	0.2755		
% ASSAY =	$\frac{\text{TA x Ws x Av. Wt. x P x 100}}{\text{SA x WT x 25 x 100}}$		
% ASSAY =	**102.06%**		

Appendix VIII

a) Sulfadoxine and Pyrimethamine chromatograms in SP05 content assay

Method Name: C:\CLASS-VP\Group\Methods\SP Tablets .met
Data Name: C:\CLASS-VP\Data\SP-tablets\SP 05 TABS Assay Spl 1
User: William
Acquired: 8/15/2009 12:43:27 PM
Printed: 8/15/2009 7:53:00 PM

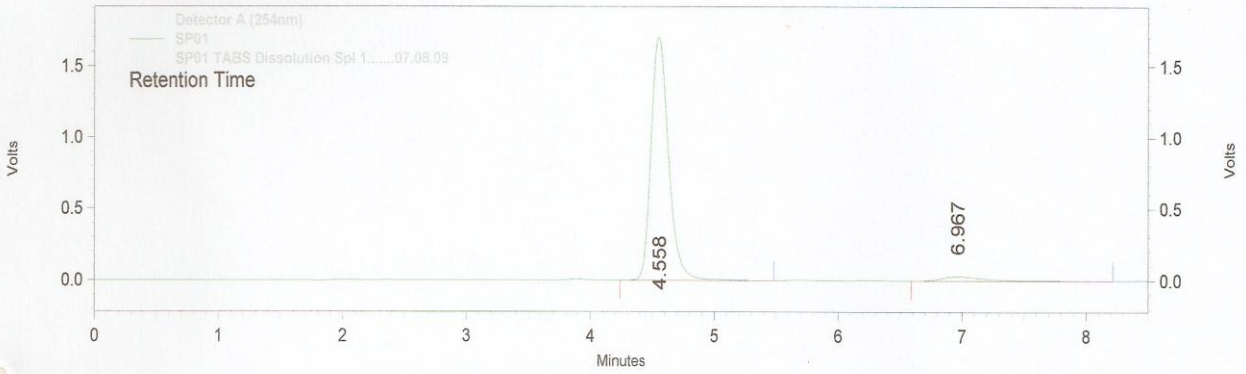


Detector A (254nm)

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Sulfadoxine	4.517	17827685	0.000	BI
2	Pyrimethamine	6.875	796269	0.000	BV
Totals			18623954	0.000	

b) Sulfadoxine and Pyrimethamine chromatograms in SP01 dissolution assay

Method Name: D:\Class-VP1\Methods\Kamsidar Tablets Diss.met
Data Name: C:\CLASS-VP\Data\SP-tablets\SP01 TABS Dissolution Spl 1.....07.08.09
User: William
Acquired: 8/7/2009 2:10:31 PM
Printed: 8/7/2009 2:21:13 PM

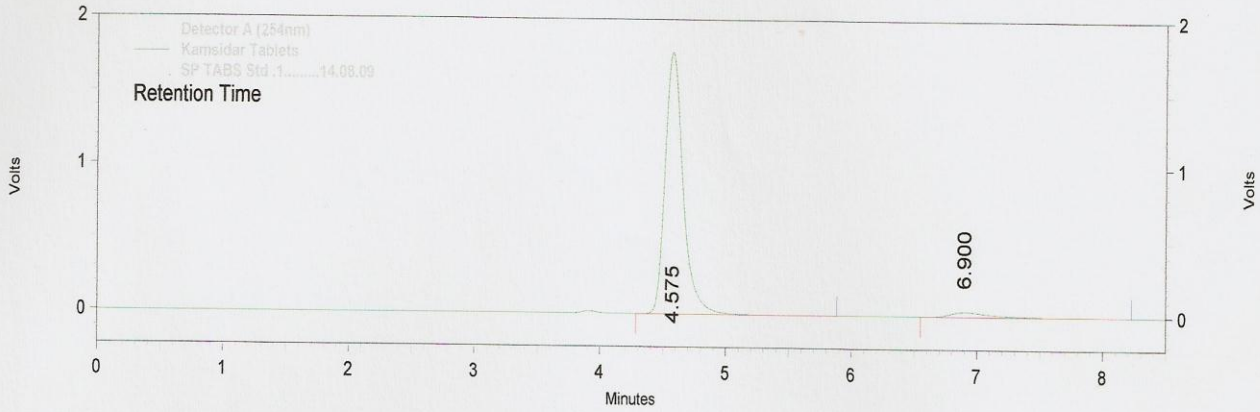


Detector A (254nm)

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Sulfadoxine	4.558	16744693	0.000	BI
2	Pyrimethamine	6.967	694859	0.000	VV
Totals			17439552	0.000	

c) Sulfadoxine and Pyrimethamine chromatograms in the standard assay

Method Name: D:\Class-VP1\Methods\Kamsidar Tablets Diss.met
Data Name: C:\CLASS-VP\Data\SP-tablets\SP TABS Std .1.....14.08.09
User: William
Acquired: 8/14/2009 1:59:44 PM
Printed: 8/14/2009 9:28:46 PM



Detector A (254nm)

Pk #	Name	Retention Time	Area	ESTD concentration	Integration Codes
1	Sulfadoxine	4.575	17767856	0.000	VI
2	Pyrimethamine	6.900	806208	0.000	VV
Totals			18574064	0.000	